POST GRADUATE DEGREE PROGRAMME
(CBCS)
IN
BOTANY

SEMESTER - II

Course: BOTCOR T205

(Palaeobotany & Palynology)

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING
UNIVERSITY OF KALYANI
KALYANI – 741235, WEST BENGAL

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Director's Messeage

Satisfying the varied needs of distance learners, overcoming the obstacle of distance and reaching the unreached students are the threefold functions catered by Open and Distane Learning (ODL) systems. The onus lies on writers, editors, production professionals and other personal involved in the process to overcome the challenges inherent to curriculam design and production of relevant Self Learning Materials (SLMs). At the University of Kalyani a dedicated team under the able guidance of the Hon'ble Vice-Chancellor has invested its best efforts, professionally and in keeping with the demands of PostGraduate CBCS Programmes in Distance Mode to devise a self-sufficient curriculum for each course offered by the Directorate of Open and Distane Learning (DODL) University of Kalyani.

Development of printed SLMs for students admitted to the DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode UGC, DEB Regulations, 2020 had been our endeavour. We are happy to have achieved our goal.

Utmost care and precision have been ensured in the development of SLMs, making them useful to the lesrners, besides avoiding errors as far as practicable. Further, suggestions from the stakeholders in this would be welcome.

During the production-process of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Manas Kumar Sanyal, Hon'ble Vice-Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticisms to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

Sincere gratitude is due to the respective chairpersons as well as each and every Members of PGBOS (DODL), University of Kalyani, Heartfelt thanks is also due to the Course Writers- faculty members at the DODL, subject-experts serving at University Post Graduate departments and also to the authors and academicians whose academic contributions have enriched the SLMs. We humbly acknowledge their valuable academic contributions. I would especially like to convey gratitude to all other University dignitaries and personnel involved either at the conceptual or operational level of the DODL of University of Kalyani.

Their persistent and co-ordinated efforts have resulted in the compilation of comprehensive, learners friendly, flexible text that meets curriculum requirements of the Post Graduate Programme through distance mode.

All the Self Learning Materials are self-writing and collected from e-Book, journals and websites.

Director
Directorate of Open & Distance Learning
University of Kalyani

SYLLABUS
COURSE – BOTCOR T205
Palaeobotany and Palynology
(Full Marks – 75)

Course	Group	Details Contents Structure		Study hour
BOTCOR T205	**Palaeobotany**	**Unit 1. Preservation of Plants as Fossils**	1. Preservation of plants as fossils: Definition; taphonomy; environment for fossilization; modes of preservation; types; major rock types, rock cycle and rocks containing Fossils; systematics, reconstruction and nomenclature.	1
		Unit 2. Geologic Time	2. Geologic Time: Geologic timescale, relative vs. numerical age, physical and biological principles for defining relative and numerical age.	1
		Unit 3. Early Life	3. Early Life: The origin of earth, earliest environment, theories on origin of life, evidences for the origin of life - prokaryotes, evolution of eukaryotes and fossil records, diversified life - algae and fungi.	1
		Unit 4. Colonization of Land by Plants	4. Colonization of Land by Plants: Geologic time, environment, vegetative and reproductive adaptations to land dwelling, fossil evidences - transitional plants with land adaptive features, early non vascular land plants (bryophytes), early vascular land plants (pteridophytes).	1
		Unit 5. Early Vascular Plants to Early Spore Producing Trees (Arborescent Pteridophytes & Progymnosperms)	5. Early vascular plants to early spore producing trees (arborescent pteridophytes & progymnosperms): Geologic time, environment, advancement in plant adaptive features for land dwelling with fossil evidences.	1
		Unit 6. Early Vascular Plants to Early Spore Producing Trees (Gymnosperms)	6. Early spore producing trees to early seed producing trees (gymnosperms): From isospores to free sporing heterospores, origin of ovule, hydrasperman reproduction with fossil evidences.	1
		Unit 7. Origin and Evolution of Flowering Plants (Angiosperms)	7. Origin and evolution of flowering plants (angiosperms): Geologic time, evolutionary trends - angiosperm derived characteristics, fossil evidences for early flowering plants, place of origin, radiation, phylogeny.	1
		Unit 8. Aspects and Appraisal of Palaeobotany	8. Aspects and Appraisal of Palaeobotany: Palaeobotanical study in exploring - mysteries in the living planet; origin, evolution, diversification and extinction of species; plant-animal interaction and co-evolution; plate movement, geological age and correlation of strata; palaeogeogrpahy, palaeoclimate; fossil fuel.	1

Course	Group	Details Contents Structure		Study hour
BOTCOR T205	**Palynology**	**Unit 9. Spore-Pollen Morphology**	1. Spore-pollen morphology: units, polarity, symmetry, shape, size, aperture; NPC system for numerical expression of apertural details; evolution of aperture types.	1
		Unit 10. Pollen Wall and Extraexinous Wall Materials	2. Pollen wall and extraexinous wall materials: Sporoderm stratification and sculptures; LO-analysis; sporopollenin; pollen wall development; Ubisch body; pollen connecting threads, perine, pollen-kit.	1
		Unit 11. Pollen Grains Adaptation	3. Pollen grains adaptation: Pollen grains adaptation in different habitats and pollination types; pollen wall adaptation and significance; Hermomegathic mechanism.	1
		Unit 12. Spore/Pollen Viability and Storage	4. Spore/Pollen Viability and Storage: Estimation; variations; responsible factors; short- and long-term storage; significance.	1
		Unit 13. Pollen Limitation and Plant Diversification	5. Pollen limitation and plant diversification: Definition; ecological and evolutionary relevance.	1
		Unit 14. Natural Spore/Pollen traps	6. Natural spore/pollen traps: Types, their implications in floristic & environment reconstruction.	1
		Unit 15. Branches of Palynology & Application	7. Branches of palynology & application: Branches of palynology; palynology in taxonomic & phylogenetic deductions; palynology in academic & applied aspects including melissopalynology, medical palynology, forensic palynology, entomopalynology & copropalynology.	1

Content

COURSE – BOTCOR T205
PALAEOBOTANY & PALYNOLOGY
Core Theory Paper **Credit: (Groups A+B) = 3**

Group – A (PALAEOBOTANY)

Content Structure

1. Introduction
2. Course Objective
3. Preservation of plants as fossils: Definition; taphonomy; environment for fossilization; modes of preservation; types; major rock types, rock cycle and rocks containing Fossils; systematics, reconstruction and nomenclature.
4. Geologic Time: Geologic timescale, relative vs. numerical age, physical and biological principles for defining relative and numerical age.
5. Early Life: The origin of earth, earliest environment, theories on origin of life, evidences for the origin of life - prokaryotes, evolution of eukaryotes and fossil records, diversified life - algae and fungi.
6. Colonization of Land by Plants: Geologic time, environment, vegetative and reproductive adaptations to land dwelling, fossil evidences - transitional plants with land adaptive features, early non vascular land plants (bryophytes), early vascular land plants (pteridophytes).
7. Early vascular plants to early spore producing trees (arborescent pteridophytes & progymnosperms): Geologic time, environment, advancement in plant adaptive features for land dwelling with fossil evidences.
8. Early spore producing trees to early seed producing trees (gymnosperms): From isospores to free sporing heterospores, origin of ovule, hydrasperman reproduction with fossil evidences.
9. Origin and evolution of flowering plants (angiosperms): Geologic time, evolutionary trends -angiosperm derived characteristics, fossil evidences for early flowering plants, place of origin, radiation, phylogeny.
10. Aspects and Appraisal of Palaeobotany: Palaeobotanical study in exploring - mysteries in the living planet; origin, evolution, diversification and extinction of species; plant-animal interaction and co-evolution; plate movement, geological age and correlation of strata; palaeogeogrpahy, palaeoclimate; fossil fuel.

11. Let us sum up
12. Suggested Readings
13. Assignments

1. Introduction

The relevance for the unit for attaining a comprehensive knowledge about the sequences of nature's own creations (origin and diversification) and disasters (extinction) occur including interrelations among different spheres geosphere-atmosphere-biosphere through millions of years in the globe. Fossil is one of the authentic evidences in bio-geo-atmospheric evolutionary history of the Earth through geological ages. By introducing this course, students would be acquainted with overall idea about fossils and different techniques for their study. The knowledge would help them to describe primordial life forms and their evolution, to determine the age of the sediments and fossils, to enlighten significance of continental drift hypothesis and plate tectonics, and to explain Indian Gondwana sequence and its importance.

2. Course Objectives

After completion of the course the learners will be able to:

- describe the general idea about fossils focusing on plant fossils and their applications in our life;
- explain the origin of life, early life forms and their evolution through geological ages;
- discuss the Indian Gondwana sequence and its importance;
- explain the concepts and validation of continental drift hypothesis and plate tectonic theory;
- determine the geological age of sediments and fossils

3. Preservation of plants as fossils: Definition; taphonomy; environment for fossilization; modes of preservation; types; major rock types, rock cycle and rocks containing Fossils; systematics, reconstruction and nomenclature.

Definition

The word 'Fossil' is derived from the Latin verb *'fodere'* which means '*to dig'*. Originally it described to anything one might remove from the Earth. Subsequently, it changed to the remains of whole or part of an organism or the evidence of former existence of an organism preserved in the consolidated sediments of the Earth. As such, the definition of fossil is **"Any direct** (*actual or imprint of body of organism as a whole or part)* **or indirect** *(tract, trail, boring, teeth mark, coprolite etc.)* **evidences of the life forms of geological past which are predominantly found in sedimentary rocks".**

Taphonomy

The process for being a fossil from a living biological organism is apparently a simple, natural phenomenon though in true sense it is an elaborate and complicated process, scientifically termed as **'Taphonomy'**.

Taphonomy involves sequential steps of biological, environmental, and sedimentological factors, namely **'Necrology'** (death/abscission/decay of organisms), **'Biostratinomy'** (burial in the sediment), **'Diagenesis'** (microbial diagenesis, or other physico-chemical changes), and '**Lithification'** (post burial changes & mineralization) (Fig. 1). The formation of a fossil begins after the death of a whole organism or any of its part(s). Gradually its body sinks through the depth of enclosed water bodies of freshwater or marine environments (Basin). Ultimately, it settles down into the bottom of the basin. The soft body parts decay quickly by microbial diagenesis or are eaten by scavengers leaving behind the hard, skeletal parts. In due course of time, the sediments comprising of sand, mud, and other debris deposits on this and continue to accumulate year after year, compressing the successive layers. The compacted sediments are gradually changed into sedimentary rocks, and finally the biological remains harden into fossils.

Environment for fossilization

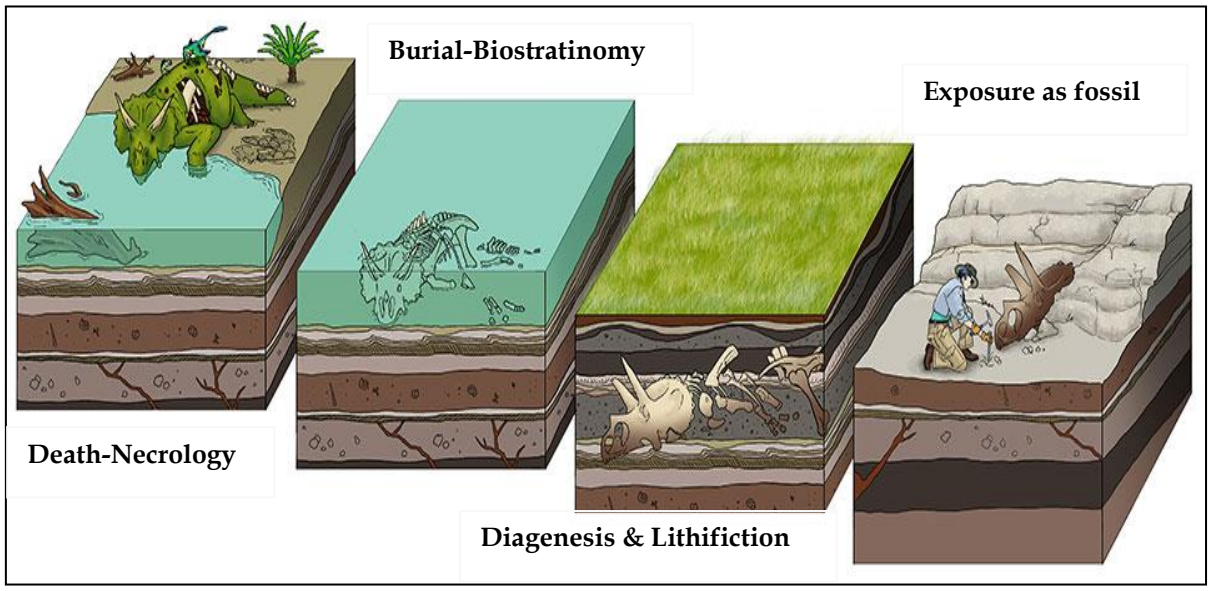
The fossilization process is dependent on a variety of internal (structural) and external (environmental) factors.

Internal (structural) factors: The tissue systems of any vascular plant are made up of different types of soft and hard cells like parenchymatous, collenchymatous, sclerenchymatous, vascular etc. Among these, the harder tissues like bark, wood, sclerenchyma and cutinized cells are usually better preserved than the softer tissues like parenchyma.

External (environmental) factors: The external factors are both mechanical and biological. The mechanical action of wind, water, rolling stones etc. influence greatly the extent of preservation. The microorganisms are responsible for the decay and decomposition of the dead plant bodies that can escape them by suitable environment like-

Fig. 1: Step wise process for fossilization

(source: http://blog.statemuseum.nd.gov/blog/life-of-fossil)



- ***Enclosed or protected water*** **(small lake, swamp etc):** the dead organism should remain in undisturbed condition

- ***Rapid burial*****:** to avoid first microbial decomposition

- ***Low O_2 content* (e.g., anaerobic sediment):** to check aerobic decomposition because most decomposers (e.g., fungi, most decomposing bacteria and invertebrates) require oxygen for metabolism.

- ***Acidity or Low pH*:** limits the activity of decomposers

- ***High concentration of toxic substances*:** to retard decay process

- ***Protection against high wind*:** to prevent mechanical destruction of organisms and from being carried away from its place of origin

- ***Absence of strong water current*:** the wave action and rolling boulders cause fragmentation of the organism

- ***Higher depth of water*:** activity of aerobic decomposition is restricted at higher depth; afford good protection from wave action

- ***Fine textured sediments*:** allow lesser amount of O_2 in inter-grain space retarding the process of aerobic decomposition

- ***Ample source of particulate sedimentary material*:** may reduce the chance of long exposure of the organisms to the decomposer and /or scavenger

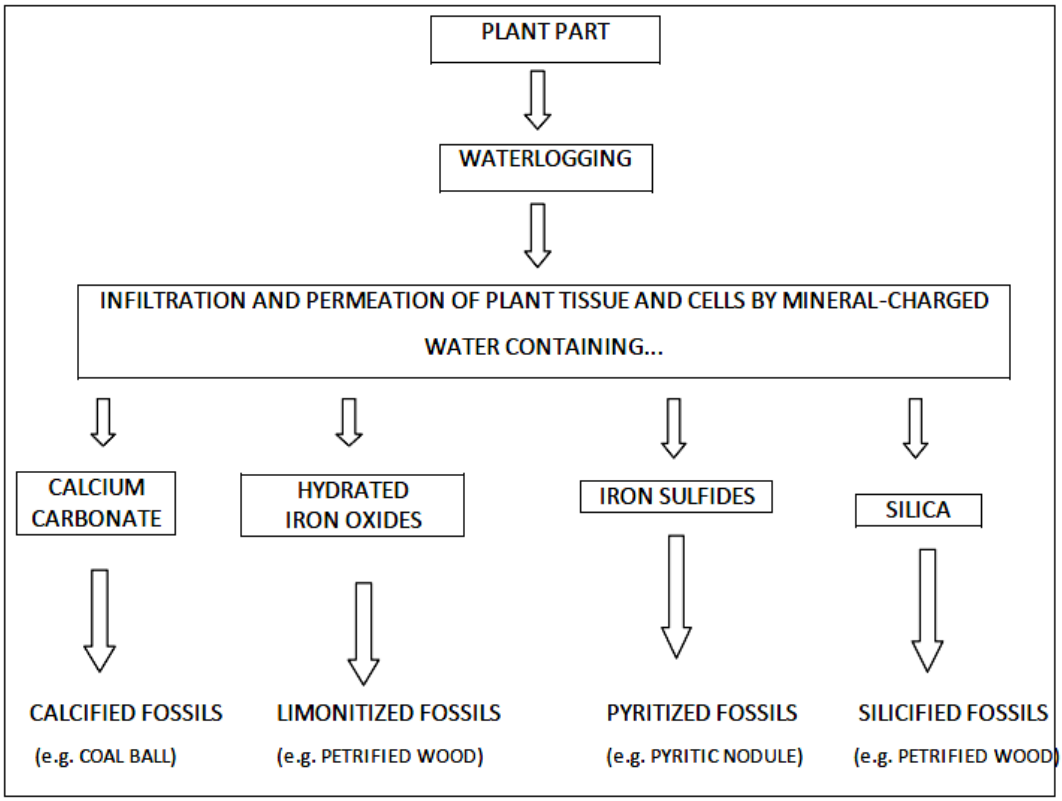
Modes of Preservation (Schopf 1975)

James M. Schopf (1975) stated that the processes of geologic preservation are important for understanding the organisms represented by fossils and the interpretation of fossils are influenced by modes of their preservation. He distinguished four modes of preservation namely **(1) Cellular permineralization ("petrifaction")** preserves anatomical detail, and, occasionally, even cytologic structures **(2) Coalified compression ("impressions")**, best illustrated by structures from coal but characteristic of many plant fossils in shale, preserves anatomical details in distorted form and produces surface replicas (impressions) on enclosing matrix **(3) Authigenic preservation (cementation)** replicates surface form or outline (molds

and casts) prior to distortion by compression (4) **Duripartic (hard part) preservation,** characteristic of fossil skeletal remains, predominantly animal.

(1) Cellular permineralization ("petrifaction"): This is the most informative fossil as because anatomical details are preserved through this mode of fossilization (Fig. 3). Here, infiltration of cells and intercellular spaces by mineral matrix occur at or very soon after deposition. Depending upon infiltrated minerals it may be of Silicified (silica), Calcified, Pyritized, Limonitized etc. (Fig. 2). Cryo-preservation is another form of permineralization, where tissue becomes permeated microcrystalline ice. However, this form of permineralization tends to contain only relatively recent fossils; for example, the Pleistocene frozen mammoths found on the plains of Siberia and dating back to approximately 10,000 years ago.

Fig.2: Different types of permineralized fossils



Most common types of permineralized plant fossils

"Coal balls" are very distinctive calcified permineralized fossil which vary considerably in size, but are usually spheroidal in shape, *c*. 10-20 cm radius, and composed of numerous plant fragments incorporated within a Calcium carbonates matrix (Fig. 3). They are found exclusively in coal deposits of Carboniferous age (~354-290 Ma) in Europe and North America, and of Permian age (~290 Ma) in China.

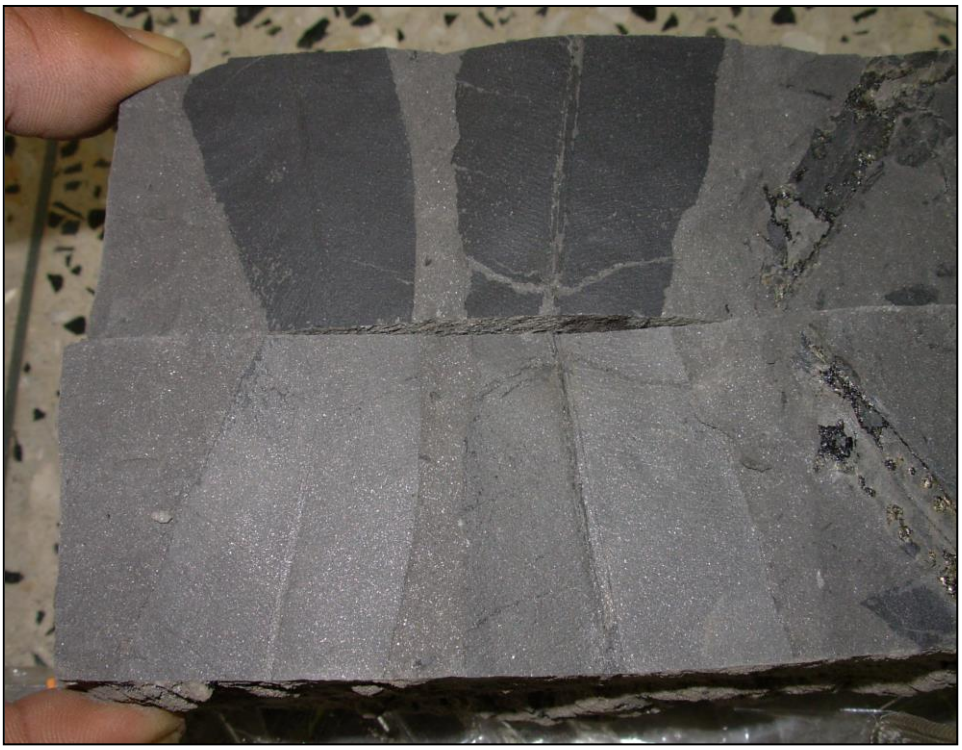
Fig. 3: Fossils preserved through Cellular Permineralization mode

Coal ball
(source: https://www.flickr.com/photos/28709338@N04/16224790745)

Petrified Wood
(source: http://www.indigenous.co.uk/contemporary-furniture/Petrified-

(2) Coalified compression ("impressions"): This mode comprises softening of cell walls, collapse of internal cell spaces, loss of volatiles matters like, gas, moisture, soluble, and finally consolidation of nonmineralized altered residues in an anoxygenic environment which in consequence, it becomes coaly deposits due to lithification. Splitting commonly yields one coalified part which becomes compression, and the counterpart becomes impression (Fig. 4).

Fig. 4: Fossils preserved through Coalified Compression mode



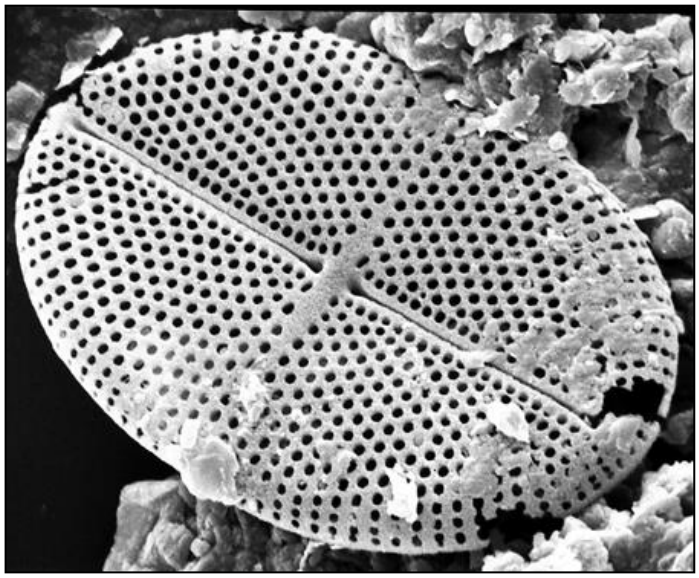
(3) Authigenic preservation (cementation): Authigenic preservation involves early cementation of dead body parts by minerals like iron and carbonate compounds during the soft-mud stage soon after burial which eventually preserves the surface of buried organic parts. Traces of internal organization usually are lost, and most tissues are degraded and thus it acts as 'Mold' and occasionally the hollow internal part is filled with bulk materials which form the 'Cast' (Fig. 5).

Fig. 5: Cast and mold as preserved through authgenic preservation

(4) Duripartic preservation (Hard part): The hard parts of both plants and animals are most readily preserved and form the most abundant class of fossils. Certain parts of organisms are durable because of their resistance to decay and oxidation and resistant to physical change. Among plants, the "skeletal" parts of lime-precipitating algae, coccoliths, and diatom frustules are usually fossilized through this mode (Fig. 6).

Fig. 6: Fossils preserved through Duripartic Preservation mode

(source: http://www.ucl.ac.uk/GeolSci/micropal/images/diat/diat006.gif)



Exceptional preservation: unaltered forms

Besides, above mentioned modes, there are three most common deposits namely, lake sediments, amber and packrat middens from where organic remains have been recovered almost in unaltered fossilized forms as because here burial occur in microbial inhibited, or at least severely restricted environment.

Many exceptional plant fossils have been recovered from lake sediments, where burial has been rapid and the environment at the bottom of the lake anoxic. An example of this type of burial includes the Clarkia beds in Idaho, northern U.S.A. where thousands of early Miocene fossil leaves (~17-20 Ma) have been recovered. Some of these leaves are in such an exceptional state of preservation that it is possible to extract strands of ancient DNA fossil leaf of *Magnolia latahensis* (Golerberg *et al*., 1990; Soltis et al., 1992) (Fig. 7).

Fig. 7: Fossil leaf of *Magnolia* from Miocene lake sediments (Clarkia beds) in Idaho, U.S.A. *(http://www.webpages.uidaho.edu/tertiary/images/dirtwo/magnolia.jpg)*

Amber is a fossilized plant resin and when the resin is formed, any animal or plant fragments (e.g. small flowers, leaves, seeds, and insects) that fall into it are incorporated due to its sticky nature. Oligocene fossil leaves contained in amber (~24-34Ma) have been recovered from several localities, and ancient DNA has been extracted successfully from the extinct plant ***Hymenaea protera*** contained in fossil amber (Fig. 8) from the La Toca mines, Dominican Republic.

Fig. 8: Exceptional preservation of spider within fossilized resin (Amber)

(source: https://www.pinterest.com/pin/493003490432865299/)

The most unusual preservation medium for unaltered fossil plants are packrat middens found in the arid areas of south-eastern North America, the Middle East and South Africa. The middens provide shelters/nests for the animals, are constructed of plant material (Fig. 9) that they collected, and which became encased in crystallized urine (amberat). The amberat combined with the extremely dry environment, provides an exceptional preservation medium.

Fig. 9: Piece of Packrat Midden showing well defined sub-fossilized plant remains

(source: https://sites.google.com/site/joywardlab/research/ice-age-plant-physiology/wells-packrat-collection)

Types of Fossils

Depending on the modes of preservation (Schopf, 1975), namely, cellular permineralization, compression-impression, authigenic, and duripartic, we can get either external or internal characteristics of fossilized organisms. Eventually, the recovered fossilized forms are the basis for segregation in different types (Frey 1975; Hayes 1983, Bateman 1991) which are as follows:

Body fossil: Actual or imprint of body of organism or part is called as 'Body Fossil'. Depending on size, it is divided into macro or *mega (body fossil beyond the size range of 200 µm)* (Fig. 10) and micro *(body fossil within the size range of 200 µm)* (Fig. 11) fossils.

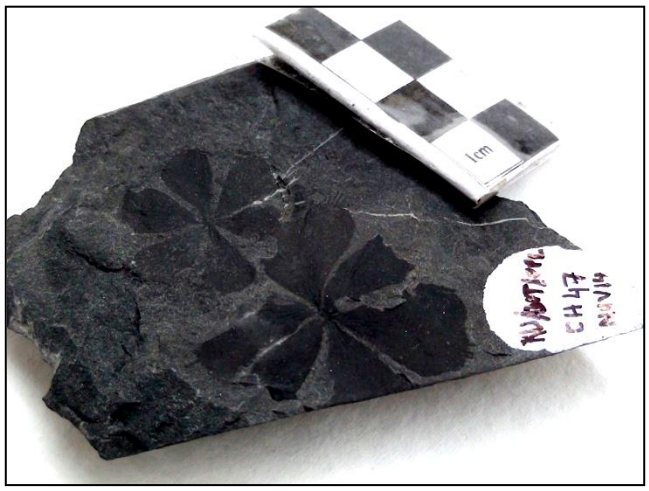


Fig. 10: Body-Megafossil of *Sphenophyllum* leaf

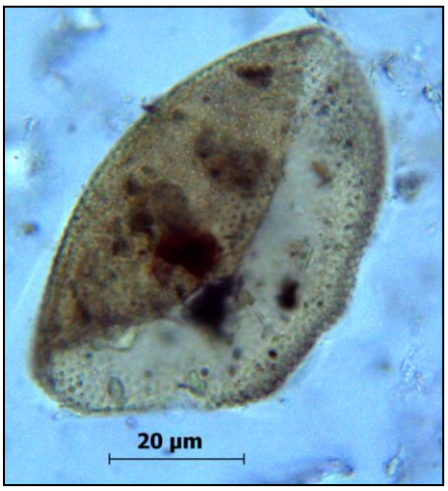


Fig. 11: Body-Microfossil of Palm pollen grains

Ichnofossil or trace fossil: It is the relic of activity or movement pattern *(footprint, track, burrow, trail, fecal matter etc.)* of organism of geological past providing indirect evidences of life (Fig. 12).

Fig. 12: Ichnofossil: Track of *Eohippus* sp.

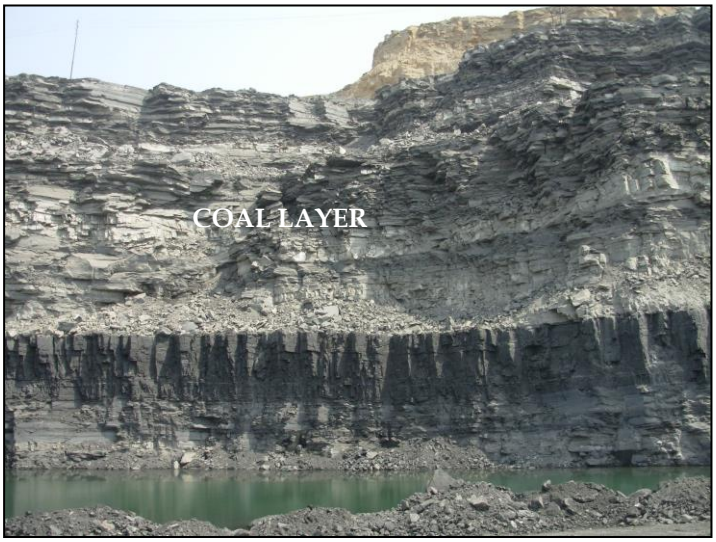
(source: http://www.fossilmall.com/Western-Fossils/Ichno7/ICH007e.jpg)

Chemical fossil: If different types of chemical namely, kerogen, amino acids, lignin etc. are found in fossilized forms are categorized in chemical fossil type.

Organic molecules: Several bio-molecules like DNA, RNA etc. may be preserved as fossils.

Organic deposits: Solid (lignite, coal etc.), and liquid (petroleum etc.) hydrocarbons are the fossilized form of organic remains of past plant (Fig. 13) and animal organisms.

Fig. 13: Coal layer-fossilized as organic deposits



Subfossil: Sometimes incomplete diagenesis process forms subfossils.

Fig. 14: Peaty clay deposition remains in subfossil state

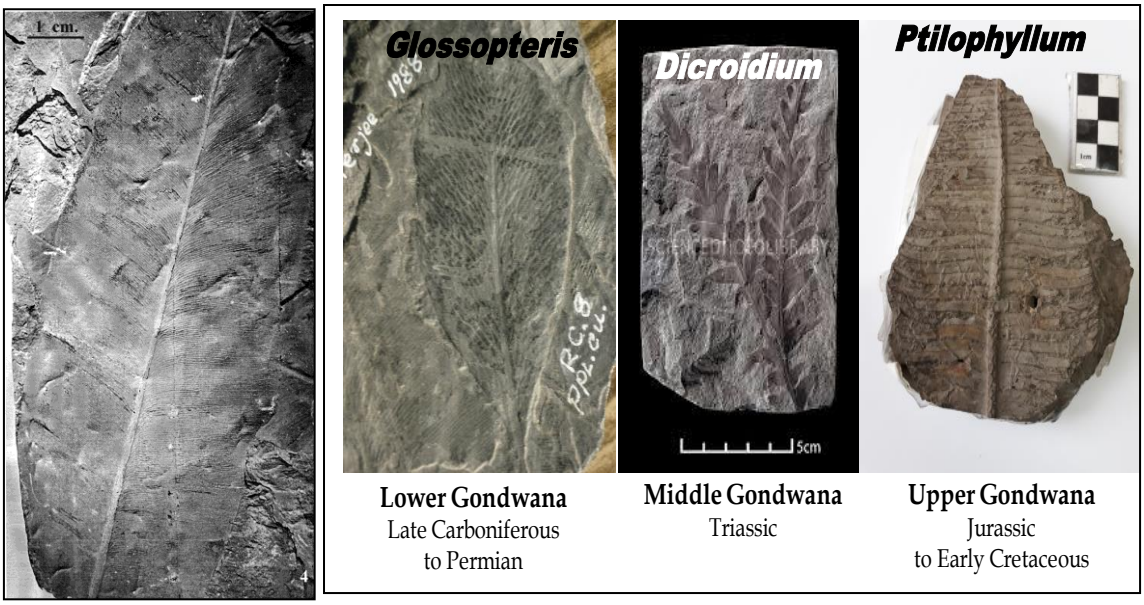
Pseudofossil: Occasionally, due to mineral deposition some rocks may mimic fossilized structure (Fig. 15) of any organism which are not a fossil in true sense. These forms are termed as pseudofossils.

Fig. 15: Pseudofossil – Iron oxide deposition on rock looks like a plant

In addition to above mentioned fossil types, Index fossil is another well-known type which is categorized by the palaeontologists on basis of the application of the fossil.

Index fossil: The index fossil is defined as *"a fossil which is useful for dating and correlating the strata in which it is found"*. An index fossil should be easily recognizable and abundant in the strata having a wide geographic distribution (broad range of geographical regions) and a narrow geological distribution (short range through geological timescale). Example: *Glossopteris* (Fig. 16, 17) is a prevalently used as an index fossil which is found abundantly in Upper Carboniferous to Early Triassic in the present-day countries (Australia, Africa, South America, Madagascar, Indian sub-continent, New Guinea, New Zealand, and Antarctica) which were belonging to the supercontinent Gondwanaland. *Dicroidium* and *Ptilophyllum* are also well-known index fossils (Fig. 17).

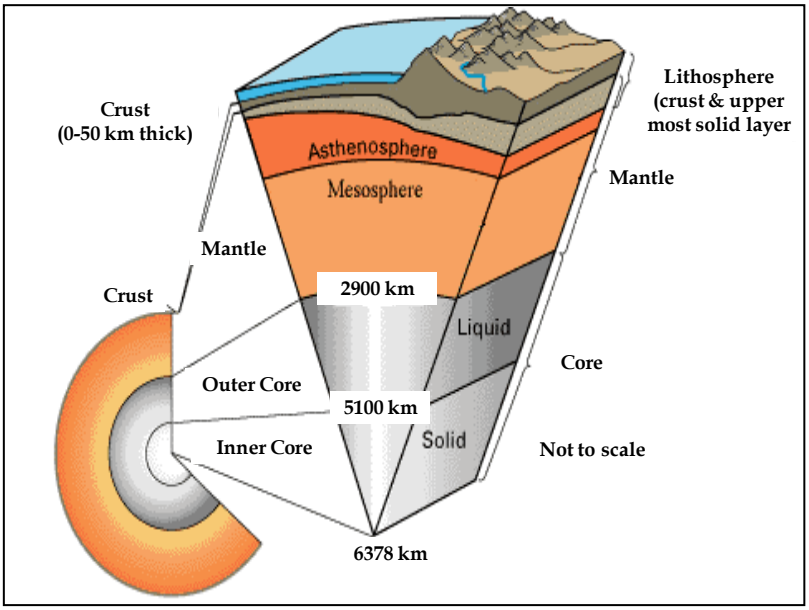
Fig. 16 & 17: Some well known index fossils of Gondwana sediments



Rock type, rock cycle and fossiliferous rock

Rock is a naturally occurring solid aggregate of minerals. The Earth's outer solid layer, the lithosphere is made up of rock (Fig. 18).

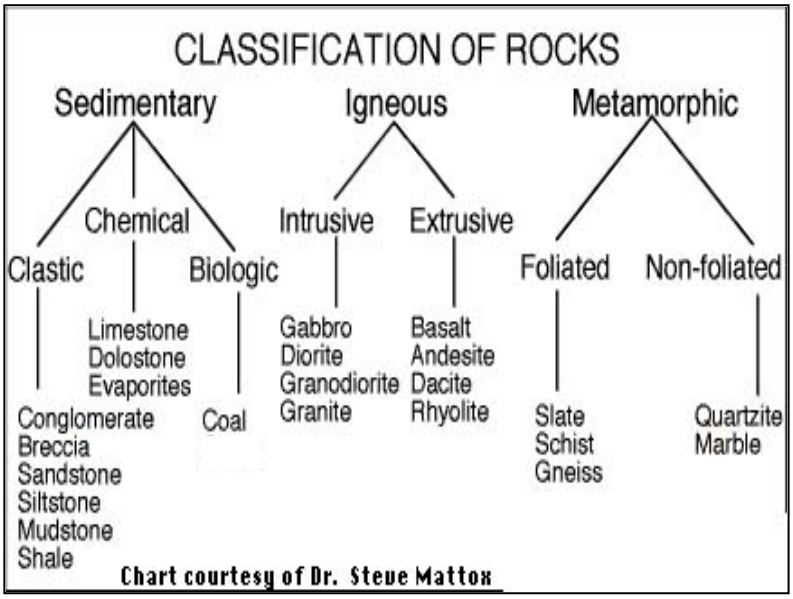
Fig. 18: Lithosphere of Earth containing of rock



Rock Type

Basic rock types are mainly three those that form from melt (**igneous** rocks), those that are deposited from air or water (**sedimentary** rocks), and those that have formed by "cooking" or otherwise altering another rock (**metamorphic** rocks). Sedimentary rocks form by breaking down other kinds of rocks into small particles and washing or blowing them away; metamorphic rocks form from other rocks and igneous rocks form by melting other rocks. These three basic types are again classified into different forms by their origin (Fig. 19).

Fig. 19: Classification of Rock



Igneous rock: Igneous rocks (from the Greek word for fire) form from when hot, molten rock crystallizes and solidifies. The melt originates deep within the Earth near active plate boundaries or hot spots, and then rises toward the surface. Igneous rocks are divided into two groups, intrusive or extrusive, depending upon where the molten rock solidifies.

Intrusive Igneous Rocks: Intrusive, or plutonic, igneous rock forms when magma is trapped deep inside the Earth. Great globs of molten rock rise toward the surface. Some of the magma may feed volcanoes on the Earth's surface, but most remains trapped below, where it cools very slowly over many thousands or millions of years until it solidify. Slow cooling means the individual mineral grains have a very long time to grow, so they grow to a relatively large size. Intrusive rocks have a coarse grained texture. Example: Granite.

Extrusive Igneous Rocks: Extrusive, or volcanic, igneous rock is produced when magma exits and cools above (or very near) the Earth's surface. These are the rocks that form at erupting volcanoes and oozing fissures. The magma, called lava when molten rock erupts on the surface, cools and solidifies almost instantly when it is exposed to the relatively cool temperature of the atmosphere. Quick cooling means that mineral crystals don't have much time to grow, so these rocks have a very fine-grained or even glassy texture. Hot gas bubbles are often trapped in the quenched lava, forming a bubbly, vesicular texture. Example: Basalt

Sedimentary Rock: Rock formed from weathering, erosion, deposition and compaction of rock material through agents like wind, water, ice and chemical precipitation. Sedimentary rocks cover about three-quarters of the land surface**.**

Clastic or detrital: These are formed from the cemented sediment grains that are fragments of preexisting rocks i.e. Shale, Conglomerate etc. Shale is the most common sedimentary rock.The type of Detrital or Clastic Sedimentary Rocks is dependent on sediment Particles (Table 1).

Table 1: Clastic or Detrital Sedimentary Rock

Diameter (mm)	**Sediment**		**Clastic or Detrital Sedimentary Rock**
256+	Boulder	Gravel	Breccia (angular particles) Conglomerate (rounded particles)
64-256	Cobble		
4-64	Pebble		
2-4	Granule		
1/16-2	Sand		Sandstone
1/256-1/16	Silt	Mud	Siltstone (mostly silt)
Less than 1/256	Clay		Shale or mudstone (mostly clay)

Chemical: are deposited by precipitation of minerals from solution

- Limestone: $CaCO_3$; Dolomite: $CaMg(CO_3)_2$;
- Evaporites: evaporation of seawater or a saline lake e.g. gypsum ($CaSO_4.2H_2O$); Chert: entirely of silica

Biologic or organic: This is composed or organic carbon compounds formed by fossilized biological organisms. For example-Coal.

Metamorphic rock: Metamorphism refers to change to existing rocks that take place in Earth's interior due to heat and pressure. The changes may be new texture, new minerals assemblages, or both. Transformations occur in the solid state (i.e. rock does not melt). The changed new rock is metamorphic rock.

REGIONAL METAMORPHISM: Metamorphism takes place at considerable depth at underground so affects a large area and it is results from plate tectonics. It is almost always foliated. "Foliated" rocks contain much mica and other rocks that produce layering or banding. Example: Slate, Gneiss etc.

CONTACT METAMORPHISM: mostly takes place not too far beneath earth's surface (less than 10 km) i.e. affects rocks on a local scale, such as "baking" of sedimentary rocks adjacent to an intrusive contact. This type is mostly non foliated in nature.

Example: Marble (from limestone), Dolomite marble (from dolomite), Quartzite (from sandstone), Hornfels (from shale and basalt) etc.

Rock Cycle:

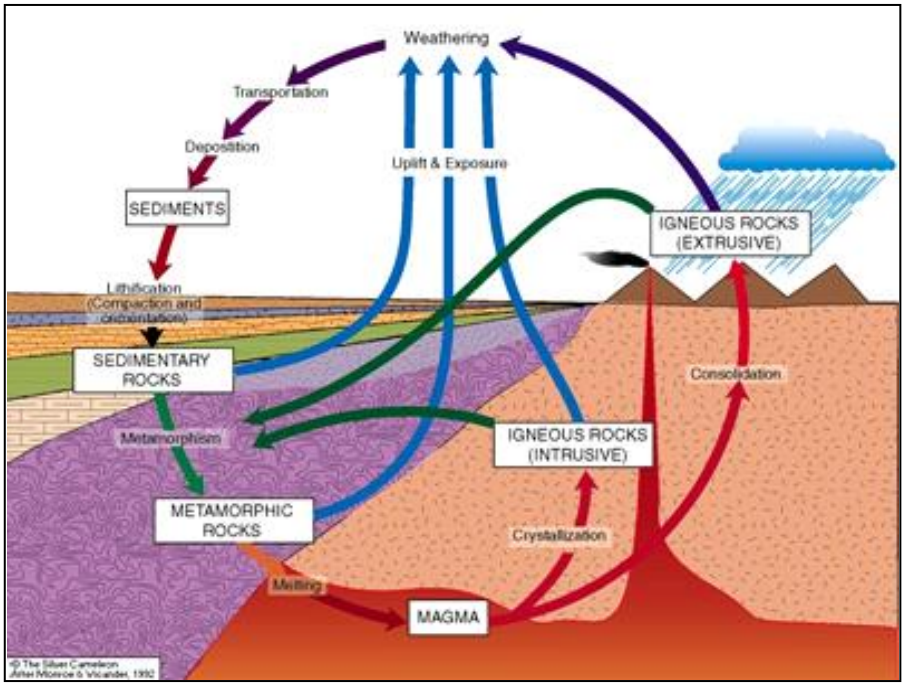
The rock cycle is a fundamental concept in geology that describes the dynamic transitions through geologic time among the three main rock types: sedimentary, metamorphic, and igneous. So the rock cycle is an illustration that explains how the 3 rock types are related to each other and how processes change from one type to another over time (Fig. 20). The original concept of the rock cycle is usually attributed to James Hutton, father of geology from the eighteenth century. Each type of rock is altered or destroyed when it is forced out of its equilibrium conditions. An igneous rock such as basalt may break down and dissolve when exposed to the atmosphere or melt as it is subducted under a continent. Igneous rock can change into sedimentary rock or into metamorphic rock. Sedimentary rock can change into metamorphic rock or into igneous rock. Metamorphic rock can change into igneous or sedimentary rock.

Igneous rock forms when magma cools and makes crystals. Magma is a hot liquid made of melted minerals. The minerals can form crystals when they cool. Igneous rock can form underground, where the magma cools slowly. Or, igneous rock can form above ground, where the magma cools quickly. When it pours out on Earth's surface, magma is called lava. Yes, the same liquid rock matter that you see coming out of volcanoes.

On Earth's surface, wind and water can break rock into pieces. They can also carry rock pieces to another place. Usually, the rock pieces, called sediments, drop from the wind or water to make a layer. The layer can be buried under other layers of sediments. After a long time the sediments can be cemented together to make sedimentary rock. In this way, igneous rock can become sedimentary rock.

Inside Earth there is heat from pressure. There is also heat from radioactive decay and this heat bakes the rock. Baked rock does not melt, but it does change. It forms crystals. If it has crystals already, it forms larger crystals. Because this rock changes, it is called metamorphic. Metamorphosis can occur in rock when they are heated to 300 to 700 degrees Celsius. When Earth's tectonic plates move around, they produce heat. When they collide, they build mountains and metamorphose the rock. The rock cycle continues. Mountains made of metamorphic rocks can be broken up and washed away by streams. New sediments from these mountains can make new sedimentary rock. The rock cycle never stops.

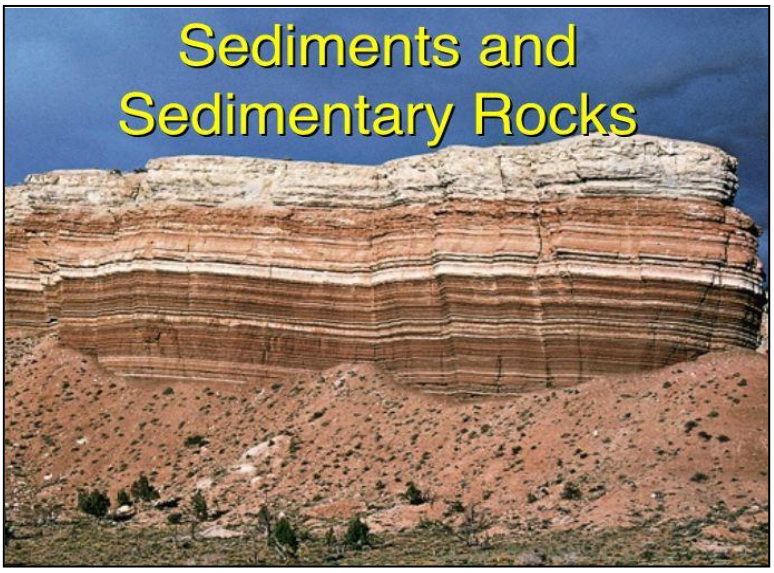
Fig. 20: Rock Cycle



Fossiliferous Rock

Most favoured site for fossil occurrence is sedimentary rock (Fig. 21) as because here layering of deposition occurred. Though fossils are most commonly found in sedimentary rocks, but all sedimentary rocks are not necessarily fossiliferous. Here are the three main reasons: Few if any creatures or plants larger than microscopic forms lived in those environments because it was toxic or otherwise unsuitable for things to live in; there was an incredible amount of biological activity such as the that of soft-bodied, bottom dwelling organisms that continually turned over the sediments and prevented things from getting trapped and preserved in the forming sediments; subtle heating of the strata by nearby volcanic activity, or altering of the rocks by chemicals, one or both destroying the fossil evidence, but not enough metamorphosis to alter the appearance of the deposits.

Fig. 21: Sedimentary rock as most favoured fossiliferous Rock



Systematic, nomenclature, reconstruction

Systematic, nomenclature

The beginning of the nomenclature of fossil plants is appeared in the year 1820 by Sternberg who first validly described *Lepidodendron obovatum* for a bark fragment. This date is chosen as the starting point for palaeobotanical nomenclature but since the publication of the Vienna Rules (1906) it has been accepted that taxonomic nomenclature of plant fossils should follow essentially the same procedure as used for living plants. But the issue was properly addressed in the year 1950 at Stockholm Congress, where the International Association for Plant

Taxonomy was established, and the Rules were recognized as the International code of botanical nomenclature (Stockholm Code 1952).

Very rarely, the palaeobotanists unearth a whole plant as fossil (fossil plant) rather they generally find detached plant parts of leaf, stem, flower, spore, pollen grains etc. (plant fossils) as fossilized through various modes of preservation. Cleal and Thomas (2010) very clearly explained two confusing concepts – **'plant fossils'** and **'fossil plants'**. They clarified that the *"plant fossils are the physical objects taken out from the ground that reveal evidence of long dead plants whereas fossil plants were the plants from which these fossilized remains were derived"*. As such, 'plant fossils' represent just fragments of the plants in which aspects of the original tissue have been at least partly lost through fossilization and they are now biologically inert objects but originated from living organisms. In contrast, 'fossil plants' were whole living organism, which in principle could be treated in the same way as living plants. As such, due to this non-entirety discovery of fossil plant, it becomes impossible to name the 'fossil plants' and 'living plants' in exactly in the same manner.

If the characteristics of the discovered plant fossils are alike to the modern plants, some taxonomists suggest that in spite of this similarity the generic name of the fossils should have a different name by adding appropriate suffix (*odendron* for stem-*Lepidodendron*; *oxylon* for wood-*Araucarioxylon*; *opteris* for leaf-*Glossopteris* and so on) or prefix (*Rhizo* for wood belongs to root; *Cormo* for stem; *Clado* for branch wood and so on) whereas other palaeobotanists prefer to use same generic name as living plant with a different species designation in the binomial.

The naming of the 'fossil plant' would only be possible after reconstruction of the whole plant from detached plant fossils. By using the rule of priority, the earliest validly published generic name applied to any one of its parts might be applicable for naming of whole fossil plant or investigator can choose a new binomial for the reconstructed fossil plant. If the whole plant is known, there is no nomenclatural difficulty in naming the parts but if there is no inevitable indication as to where a detached plant fragment belongs, it is necessary to establish a term by which one may refer to it, until and if ever its real position becomes known. This is the background for the establishment of various different nomenclatural measures over the years for inclusion within the *code* to accommodate fossils which were

started from “form and organ genera” in the Stockholm Code (1952), and subsequently “morphotaxa” in the St. Louis Code (2000), and “fossil taxa” in the Vienna Code (2006).
Since the names of the species, and consequently of many of the higher taxa of fossil plants are usually based on fragmentary specimens, and since the connection between these organs can only rarely be proved, organ genera and form genera are distinguished as taxa within which species may be recognized.

Organ-genus: An organ-genus is a genus assignable to a family whose diagnostic characters are derived from single organs of the same morphological category or from restricted groups of organs connected together. Organ genera based on detached parts may be distinguished not only by morphological characters, but also by reason of different modes of preservation.

Form-genus: A form-genus is a genus not assignable to a family, but it may be referable to a taxon of higher rank and it is maintained for classifying fossil specimens that lack diagnostic characteristics indicative of natural affinity but which for practical reasons need to be provided with binary names. Form genera are artificial in varying degree.
Examples: **organ-genera:** *Lepidocarpon* Scott (Lepidocarpaceae), *Mazocarpon* (Scott) Benson (Sigillariaceae); **form-genera:** *Dadoxylon* Endl. (Coniferopsida), *Stigmaria* Brongr. (Lepidophytales and Lepidospermales).

Morphotaxa: In St. Louis Congress Code (1999), a new nomenclatural concept, **‘morphotaxa’** was adopted replacing the two old terms ‘organ genus’ and ‘form genus’. The ‘morphotaxa’ is ***“a fossil taxon which, for nomenclatural purposes, comprises only the parts, life history stages, or preservational states represented by the corresponding nomenclatural type”***.

Fossil taxa: The Vienna Code (2006) referred one more term **‘fossil taxa’** in addition to morphotaxa for classifying plant fossils. Fossil taxa are any taxon whose type is a fossil. The International Code of Nomenclature (ICN) for algae, fungi, and plants (Melbourne Code 2011) adopted a set of proposals by which the concept of ‘morphotaxa’ was abandoned (Melbourne Code 2011) and ‘fossil taxa’ was only retained which is defined as ***“A taxon (diatom taxa excepted) the name of which is based on a fossil type”***. A fossil-taxon comprises the remains of one or more parts of the parent organism, or one or more of their

life history stages, in one or more preservational states, as indicated in the original or any subsequent description or diagnosis of the taxon"

Recent views regarding Nomenclature: The Nomenclature Section of ICN (2011) adopted a set of proposals (for details see Cleal & Thomas in Taxon 59: 261–268; 312–313. 2010) by which the concept of morphotaxa is abandoned.

Art. 1.2 A taxon (diatom taxa excepted) the name of which is based on a fossil type is a fossil-taxon. A fossil-taxon comprises the remains of one or more parts of the parent organism, or one or more of their life history stages, in one or more preservational states, as indicated in the original or any subsequent description or diagnosis of the taxon (see also Art. 11.1 and 13.3).

Art. 11.1 Each family or taxon of lower rank with a particular circumscription, position, and rank can bear only one correct name, special exceptions being made for nine families and one subfamily for which alternative names are permitted (see Art. 18.5 and 19.8). However, the use of separate names is allowed for fossil-taxa that represent different parts, life-history stages, or preservational states of what may have been a single organismal taxon or even a single individual (Art. 1.2).

Art. 13.3 For nomenclatural purposes, a name is treated as pertaining to a non-fossil taxon unless its type is fossil in origin (Art. 1.2). Fossil material is distinguished from non-fossil material by stratigraphic relations at the site of original occurrence. In cases of doubtful stratigraphic relations, and for all diatoms, provisions for non-fossil taxa apply.

Usual suffixes/prefixes used for nomenclature

- Leaf/Fronds : -phyllum (*Sphenophyllum*)
  -pteris (*Archaeopteris*)
- Secondary Xylem : -oxylon (*Araucarioxylon*)
- Microsporangiate str : -theca (*Glossotheca*)
- Ovulate structure : -carpon (*Lepidocarpon*)
  -sperma (*Archaeosperma*)
- Cone : -strobus (*Lepidostrobus*)

- Seed : -testa (*Pachytesta*)
- Spore : -spora (*Lycospora*)
  -sporites (*Laevigatosporites*)
- Pre-pollen, Pollen :-pollenites (*Palmaepollenites*)

Reconstruction: Three well known forms of evidences are present for reconstruction of a fossil which are

- Actual attachment
- Similar anatomical characters
- Frequency of association

Actual attachment

Charles Beck (1960) reconstructed *Archaeopteris*: actual attachment of Late Devonian free sporing fern like frond *Archaaeopteris* + *Callixylon* stem bearing gymnospermous secondary wood characters.

Similar anatomical characters

Oliver and Scott (1904) reconstructed *Lyginopteris oldhamia:* Similar anatomical feature, large capitate gland, on the surface of dispersed stems (*Lyginopteris*), leaves (*Sphenopteris*) and cupulate ovule (*Lagenostoma*).

Frequency of association

Reconstruction of the plant *Medullosa*: Repeated occurrence of pteridopsermous stem *Medullosa*, foliage of *Alethopteris*, seeds of *Pachytesta* and pollen bearing structure *Bernaultia* as dispersed state in assemblage.

4. Geologic Time: Geologic timescale, relative *vs*. numerical age, physical and biological principles for defining relative and numerical age

Dating is a method for age determination of rock strata and fossils. Earth scientists generally consider two methods for the purpose: Relative dating and Absolute dating and by combining these two methods they developed a standard geological time scale.

Geological Time scale

Geological timescale is a hierarchical scale in which the 4.6 Byrs history of the Earth (since the Earth Formation) is divided into time units of varying duration i.e. eons, eras, periods, and epochs. It is necessary for establishing the history of geological events and for determining the rates of geological processes.

Geological time scale is necessary for establishing the history of geological events and for determining the rates of geological processes.

Relative dating: It is a method of sequencing events in chronological order (i.e. in the order they happened through geological time scale) without exact dates. Relative dating method is based on fossil stratigraphy (biostratigraphy) and this is the basis for formation of geological time scale initially (Fig. 22). Three major Era boundaries are identified; two of which are defined by mass extinctions in the animal record and the remaining one by the rapid expansion of multicellular animals. The era boundaries are-

- **Mesozoic-Cenozoic** *(c. 65 Ma, dinosaur died out along with 75% of marine invertebrates)*

- **Palaeozoic-Mesozoic** *(c. 248 Ma, extinction of upto 96% of marine species)*

- **Precambrian-Paleozoic** *(c. 543 Ma, major increase in numbers of multicellular animals with hard parts soon after the boundary-Cambrian Explosion)*

Principles of Relative dating: Different pprinciples should keep in mind for Relative dating which are as follows:

Uniformitarianism: The theory that changes in the earth's crust during geological history have resulted from the action of continuous and uniform processes.

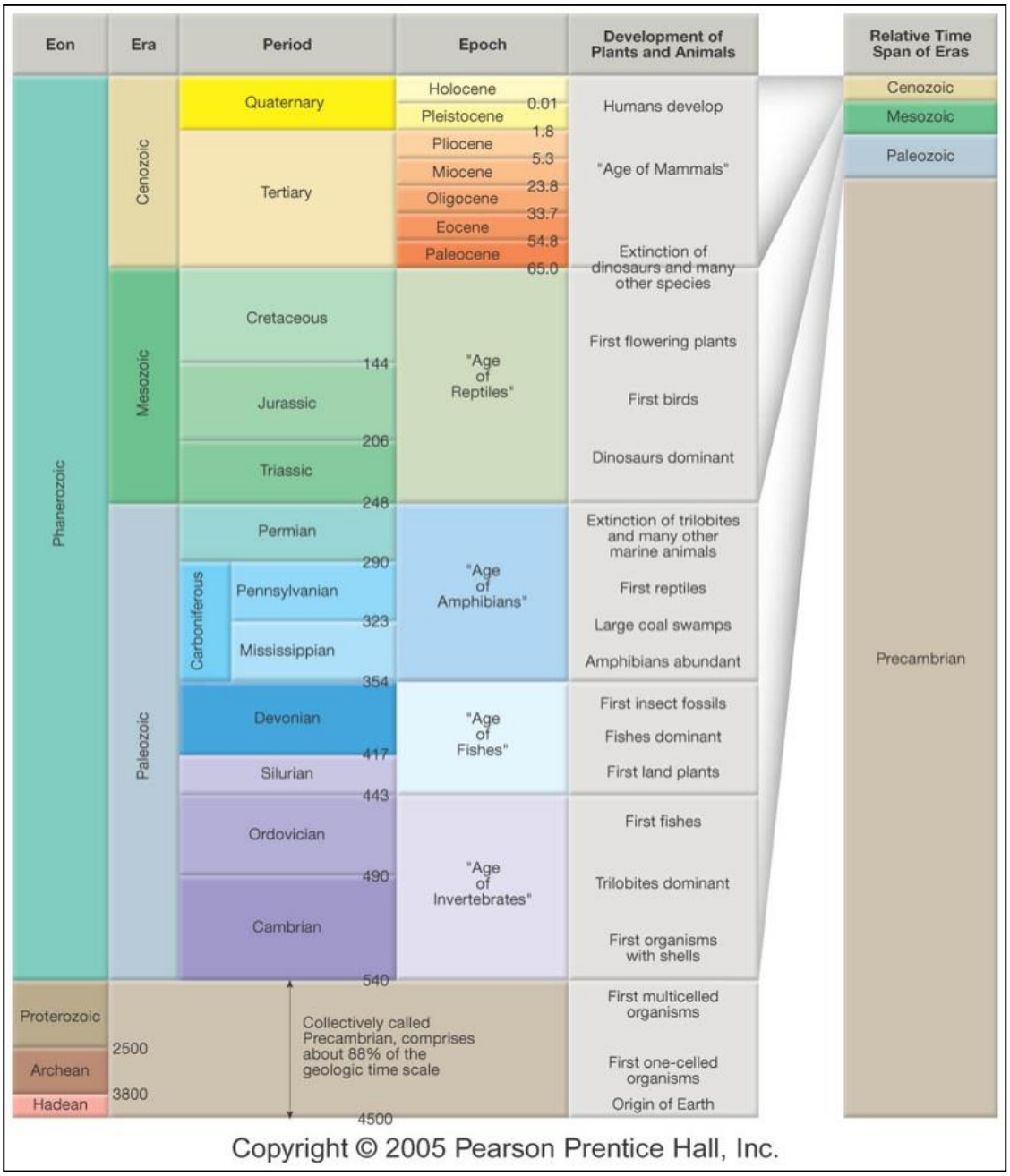
Superposition: In an undisturbed sequence the oldest rock is at the bottom and each successive layer is younger. As layers accumulate through time, older layers are buried beneath younger layers.

Original Horizontality: Sediment particles settle to the bottom of a body of water in response to gravity and thus, sedimentary rocks are deposited in horizontal layers.

Lateral continuity: Layers of sediment extend in all directions horizontally for some distance over Earth’s surface—from a few meters to hundreds of kilometers, depending on the conditions of deposition. Continuity may be interrupted unless a river erodes them or an earthquake moves them.

Faunal Succession: This principle is attributed to William Smith, an English engineer in the late 1700s. Smith noticed that the kinds of fossils he found changed through a vertical succession of rock layers, and furthermore, that the same vertical changes in fossils occurred in different places. The observation that fossils change in a consistent manner through stratigraphic successions can be extended to the entire world.

Fig. 22: Standard Geological time scale (*fossil and correlation)



Cross cutting relationships: A vein of rock (intrusion) or a fault is younger (more recent) than the rock it cuts across.

Absolute dating: It is a method of dating of rocks and fossils by using techniques especially radiometric dating to determine their actual numerical age. The initially developed geological

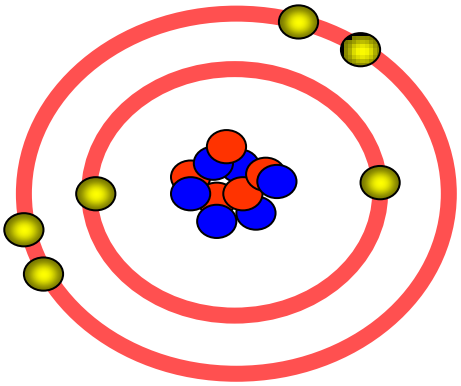
time scale is later on calibrated by radiometric method and eventually standard geological time scale was formed (Fig. 22).

The technique of radiometric dating was developed early in the 20th century after the discovery of radioactive isotopes n the laboratories of Cambridge University, England. The frequently used radioactive isotopes for absolute age determination of rock and fossils are Uranium-238, Uranium-236, Thorium-232, Potassium-40, Carbon-14.

Uranium and Thorium are mostly found in igneous rocks whereas Potassium-40 is component of some sedimentary rocks and Carbon-14 is utilized to date the past organic remains that are fossils.

The atom is a basic unit of matter that consists of a dense central nucleus surrounded by a cloud of negatively charged electrons (Fig. 23). Electrons have virtually no mass; present usually in orbit around the nucleus, and also in the nucleus as part of a neutron. The atomic nucleus contains a mix of positively charged protons and electrically neutral neutrons. Neutron is a proton with an electron inside of it; thus it is electrically neutral.

Fig. 23: Atomic nucleus



Atomic Number: Number of Protons; **Atomic Mass Number:** Combined No.s of Proton & Neutron.

Not all atoms of the same element have the same number of neutrons. These variable forms of the same element are called isotopes. Most isotopes are stable; some are unstable and spontaneously decay to a more stable form. It is the process by which the radioactive substances (an unstable atomic nucleus) are spontaneously transformed into an atomic nucleus of a different element. Three (3) types of radioactive decay are present which are-

Alpha Decay: Emission of 2 protons+2 neutrons from nucleus; Loss of 2 atomic number, 4 atomic mass number

Beta Decay: Emission of fast moving electron from a neutron in the nucleus, changing that neutron to a proton; Increase of 1 atomic number, no change in atomic mass number

Electron Capture: A proton in the nucleus captures an orbiting electron, thereby converts to a neutron; Loss of 1 atomic number, no change in atomic mass number

Some elements undergo only one decay step in the conversion from an unstable form to a stable form

Rubidium 87 decays to Strontium 87 (single beta emission)

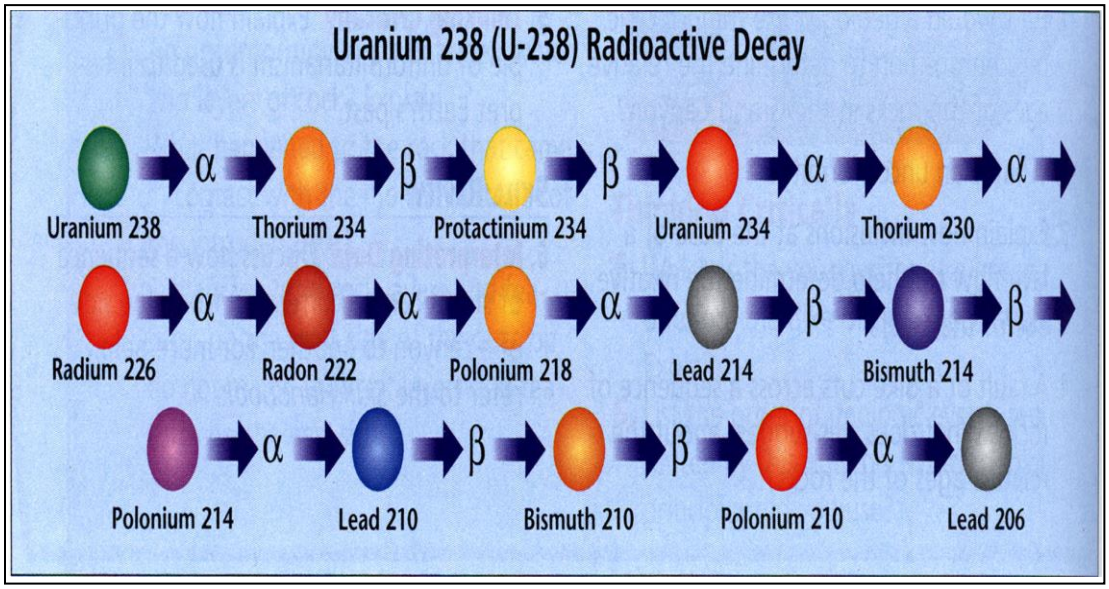
Potassium 40 decays to Argon 40 (single electron capture)

Other radioactive elements undergo several decay steps (Fig. 24)

Uranium 235 decays to Lead 207 (7 alpha, 6 beta steps)

Uranium 238 decays to Lead 206 (8 alpha, 6 beta steps)

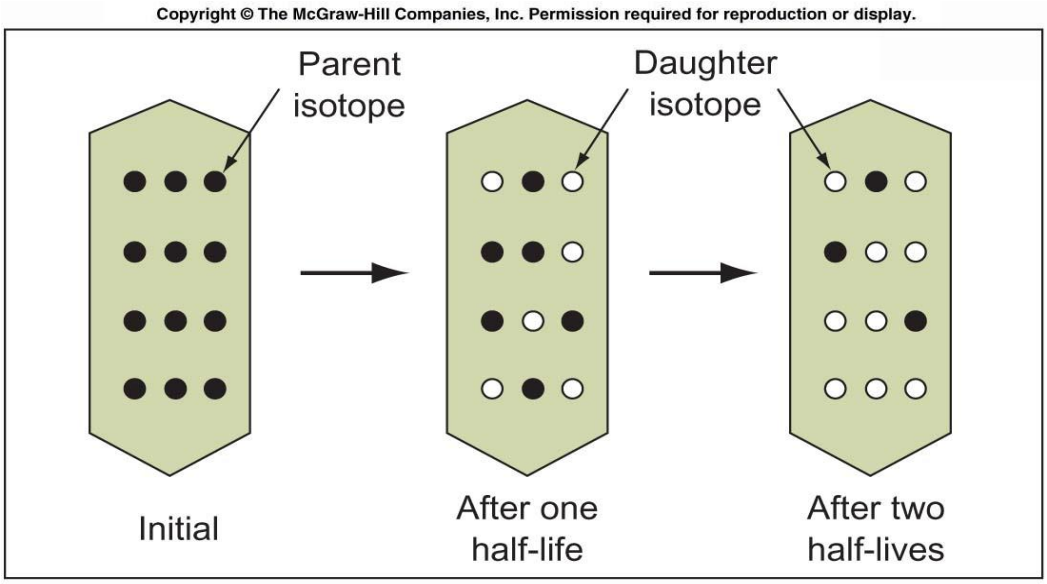
Fig. 24: Decay step of Uranium 238



Parent Isotope: The original isotope is called parent isotope (Fig. 25) e.g. Uranium 238

Daughter Isotope: The new isotope is called daughter isotope (Fig. 25) e.g. Thorium 234

Fig. 25: Schematic representation of Parent and daughter isotope

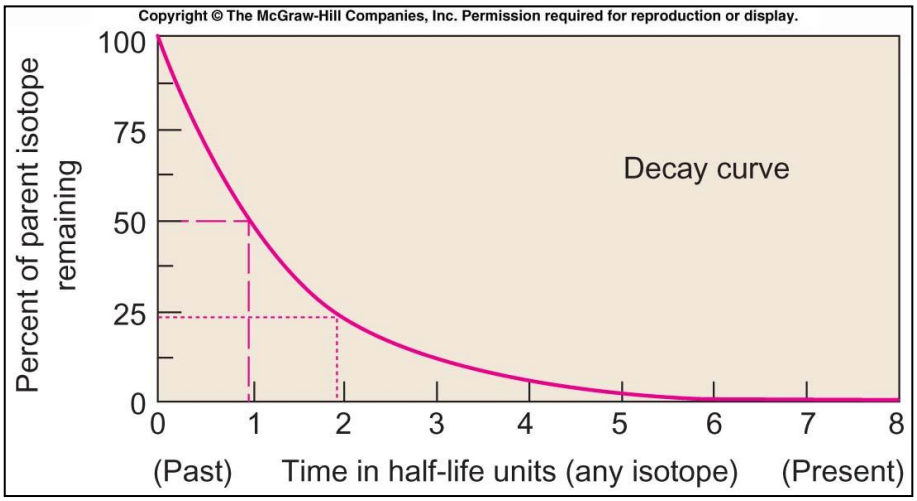


Half–life

The half life of a radioactive element is the time it takes for one-half of the atoms of the original unstable parent element to decay to atoms of a new, more stable daughter element. Since the rate of decay is constant, the ratio of parent to daughter atom is calculated to determine the age of the rock. The parent-daughter ratio is usually determined by a mass spectrometer, an instrument that measures the proportions of atoms of different masses.

Radioactive decay curve is a simple arithmetic plot of a universal isotopic decay curve. After 1 half-life 50% of parent isotope remains; after 2 half-lives, 25% remains. So, it is geometric curve rather than a linear curve (Fig. 26).

Fig. 26: Radioactive decay curve



Radiocarbon dating method

Carbon dating was developed by a team led by Willard Libby. Carbon-14 is a radioactive isotope of carbon, with a half-life of 5,730 years, which is very short compared with other radioactive isotopes. Carbon–14, though, is continuously created through collisions of neutrons generated by cosmic rays with nitrogen in the upper atmosphere and thus remains at a near-constant level on Earth. The carbon-14 ends up as a trace component in atmospheric carbon dioxide (CO_2).

An organism acquires carbon during its lifetime. Plants acquire it through photosynthesis, and animals acquire it from consumption of plants and other animals. When an organism dies, it ceases to take in new carbon-14, and the existing isotope decays with a characteristic half-life (5730 years). The proportion of carbon-14 left when the remains of the organism are examined provides an indication of the time elapsed since its death. The carbon–14 dating limit lies around 58,000 to 62,000 years.

The rate of creation of carbon-14 appears to be roughly constant, as cross-checks of carbon–14 dating with other dating methods show it gives consistent results. However, local eruptions of volcanoes or other events that give off large amounts of carbon dioxide can reduce local concentrations of carbon–14 and give inaccurate dates. The releases of carbon dioxide into the biosphere as a consequence of industrialization have also depressed the proportion of carbon-14 by a few percent; conversely, the amount of carbon-14 was increased by above-ground nuclear bomb tests that were conducted into the early 1960s. Also, an increase in the solar wind or the Earth's magnetic field above the current value would depress the amount of carbon-14 created in the atmosphere. These effects are corrected for by the calibration of the radiocarbon dating scale.

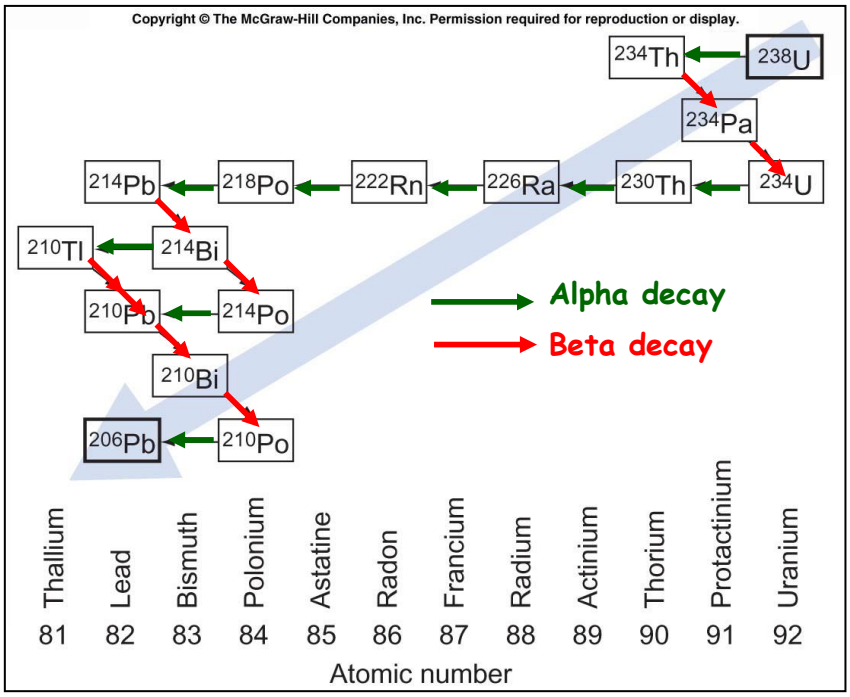
Uranium-thorium dating method

A relatively short-range dating technique is based on the decay of uranium-234 into thorium-230 (Fig. 53), a substance with a half-life of about 80,000 years. It is accompanied by a sister process, in which uranium-235 decays into protactinium-231, which has a half-life of 34,300 years. While uranium is water-soluble, thorium and protactinium are not, and so they are selectively precipitated into ocean-floor sediments, from which their ratios are measured. The scheme has a range of several hundred thousand years.

Potassium-argon dating method

This involves electron capture or positron decay of potassium-40 to argon-40. Potassium-40 has a half-life of 1.3 billion years, and so this method is applicable to the oldest rocks. Radioactive potassium-40 is common in micas, feldspars, and hornblendes.

Fig. 27: Decay steps of Uranium 238 and Uranium 234



The relationship between time and radioactive decay of an isotope is expressed by $N=N_0e^{-\lambda t}$

N=Number of the atoms of the isotope at time t, the time elapsed

N_0=No. of atoms of that isotope present when the clock was set

The mathematical constant e has a value of 2.718 is a decay constant – a proportionately constant that relates the rate of decay of an isotope to the number of atoms of that isotope remaining.

The relationship between λ and the half life (t_{hl}) is

$\lambda = \ln 2/t_{hl} = 0.693/t_{hl}$

Replacing λ in the first equation and converting that equation to natural logarithmic (to the base e) form, we get

$t=t_{hl}/.693 \ln N/N_0$

N/N_0 is the ratio of parent atoms at present to the original number of parent atoms.

5. Early Life: The origin of earth, earliest environment, theories on origin of life, evidences for the origin of life - prokaryotes, evolution of eukaryotes and fossil records, diversified life - algae and fungi.

The origin of earth, earliest environment, theories on origin of life

Earth is a system of interconnected components that interact and affect each other in many ways.

Principal subsystems:

Atmosphere **:** Gases that envelop Earth

Biosphere **:** All of the living or once living material on Earth

Hydrosphere **:** Water on or near Earth's surface

Litho or Geosphere **:** Rock & other inorganic Earth material that make up the bulk of the planet

Earth's interior **:** Mantle and Core

The complex interaction among these subsystems result in a dynamically changing planet in which matter and energy are continuously recycled into different forms

There are 3 options for Origin of the Cosmos that is

Option 1: Someone made the universe (Big Bang or creationist theory)

Option 2: The universe made itself (Inflationary or evolutionist theory)

Option 3: The universe has always been here (Steady State Theory)

Scientists now know that the cosmos had a beginning; therefore only options 1 and 2 are viable

The Big Bang/Nucleosynthesis: The singularity at the beginning of the universe.

- 13.7 billion years ago→Mass of universe→size of head of a pin (singularity)
- Unimaginable release of energy (an expansion not a traditional explosion)→trillions of degrees
- Nobody knows how/why it happened
- Created space and time
- Created the 4 main forces: gravity, strong nuclear, weak nuclear and electromagnetic forces.

Gravitational Force: *attraction of one body towards another;* ***Strong Nuclear Force:*** *binds protons & neutrons together;* ***Weak Nuclear Force:*** *responsible for the breakdown of an atom's nucleus and radioactive decay;* ***Electromagnetic Force:*** *combines electricity and magnetism into one force and binds atoms into molecules*

- Electrons, quarks and gluons created (building blocks for all atoms)
- Universe experienced enormous expansion
- About 300,000 years later, the Universe was cool enough for complete atoms of hydrogen and helium to form
- Early space: mostly hydrogen and helium atoms (He formed from H)
- Photons (the energetic particles of light) separated from matter and light burst forth for the first time
- During the next 200 million years, Universe continued expanding and cooling, stars and galaxies began to form, chemical makeup of the Universe changed.
- Initially Universe was 100% H & He, today it is 98% and 2% of all other elements by weight

How did such a change in the Universe's composition occur?

- Throughout their life cycle, stars undergo many nuclear reactions in which lighter elements are converted into heavier elements by nuclear fusion. When a star dies, often explosively, the heavier elements are returned to interstellar space and are available for inclusion in new stars. In this way, the composition of the Universe is gradually enhanced in heavier elements

How did earth form?

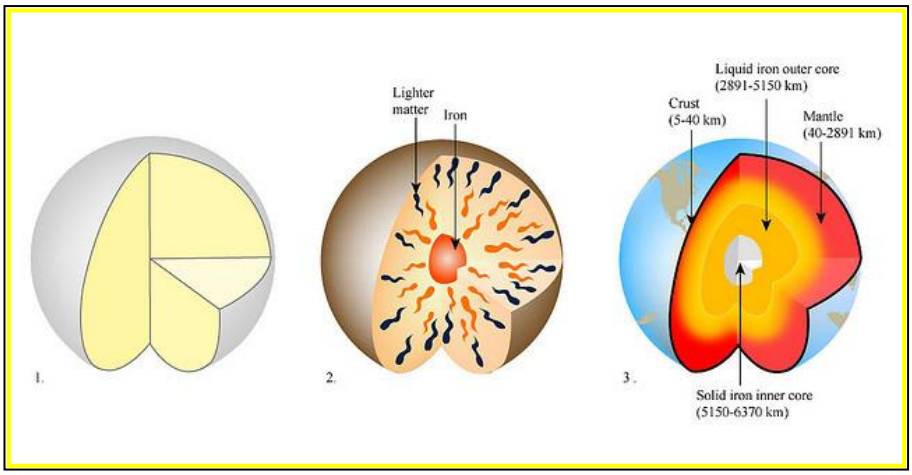
Earth is a planet of our solar system, part of the Milky Way Galaxy. **Solar Nebula Theory** is most acceptable theory for the origin of solar system-condensation and collapse of interstellar material in a spiral arm of the Milky Way Galaxy. The collapse of this cloud of gases and small grains into a counterclockwise rotating disk concentrated about 90% of the material in the central part of the disk and formed an embryonic sun-around which swirled a rotating cloud of material called a solar nebula. Within this solar nebula were localized eddies (swirling of fluid) in which gases and solid particles condensed.

During the condensation process, gaseous, liquid, and solid particles began to accrete into ever-large masses-planetesimals. Some 4.6 billion years ago, various planetesimals in our solar system gathered enough material together to form Earth and other planets.

The early Earth was probably cool, of generally uniform composition and densely throughout, and composed mostly of – silicates, iron, magnesium oxides, and small amounts of all other chemical elements.

- subsequently, temperature of Earth increased by combination of meteorite impacts, gravitational compression, and heat from radioactive decay
- the temp was enough to melt iron and nickel, then the homogenous composition of Earth disappeared and was replaced by a series of concentric layers of differing composition and density, resulting in a differentiated planet (Fig. 28)
- The differentiation not only led to the formation of a crust but also contributed to the eventual formation of Earth's oceans and atmosphere

Fig. 28: Changes of homogenous composition of Earth to series of concentric layers



The earliest environments

Between 4400-3900 Ma: Oceans formed from the condensation of atmospheric water vapour Composition (high conc. of ferrous irons, dissolved CO_2 and bicarbonate, perhaps a pH as low as 6.0) and the warm temperature (between 80-1000c) of the early oceans is strongly influenced by mantle out gassing. Other gases contributing to the overall composition of the early atmosphere included hygrogen, hydrogen cyanide, formaldehyde & hydrogen sulphide

– resulting from volcanic outgassing, photochemical reactions in the atmosphere, and cometary infall

~ 4200 Ma: continental crust started to form

Between 2200-1900 Ma: various sedimentary deposits require free oxygen for their formation – apparent in the geological record in form of-red-beds (sandstone and shale with red iron oxide) highly oxidized palaeosols; increasing amount of atmospheric O_2

By 1900 Ma: Early State of Continental Crust- a large single, lens-shaped body had accumulated around the equatorial belt –the supercontinent Rodinia

From 1000 Ma: Initial splitting up of Rodinia – three major parts-Laurasia (N. America, Greenland, Baltica, Siberia); East Gondwana (S. Africa, India, Most Australia, E. Antarctica); West Gondwana (S. America, W. Africa)

- Early continental crust was thin and 2-3 times more heat production than present due to heat flow from the mantle
- By 3000 Ma, heat had subsided to allow the development of a continental crust, up to 40 km in thickness
- Surface air temp is also influenced by the high level of atmospheric CH_4 and CO_2 from volcanic outgassing
- These high level of CO_2 & CH_4 also have blocked outgoing long-wave radiation, promoting greenhouse warming
- In contrast to the high levels of CO_2, early atmospheric O_2 was extremely low – before 2200 Ma the O_2 content of the air accounted for 1% of total atmospheric composition, and was probably a major limiting factors for organic evolution
- Most restrictive factor associated with this low O_2 content- lack of an ozone layer & therefore, no protection for terrestrial organisms from incoming solar radiation

Origin of Life

Life arose through chemical evolution at ~3500 million years ago. Life is a difficult concept to define.

Aristotle was the first to attempt to define life: *something that grows and maintains itself (he called this "nutrition"), and reproduces*

Austrian physicist Erwin Schrödinger (1944) defined: *life as that which resists decaying to disorder and equilibrium*

Some scientists proposed: *life is that which can reproduce itself;* however, this definition would exclude mules, which are born sterile, and would include nonliving things like fire

Some scientists suggested: *life is something that can metabolize — that is, take in energy to move or grow, and release waste* — but many nonliving things, like cars, can do that
"Life, because it is such a complex system of things with so many interacting parts, each of which is essential, it's really tough to make a definition" ……David Wilson Deamer

- It is generally agreed that an assemblage of molecules is 'alive' if it can capture energy from the environment, use that energy to replicate itself, and thus be capable of evolving.
- In the living things this functions are performed by nucleic acids that carry information and by proteins, which replicate nucleic acids, transduce energy, and generate the phenotype. These components are held together in compartments-CELL-formed by lipid membranes.

There are two contrasting theories for organic matter accumulation in the pre-biotic environment-

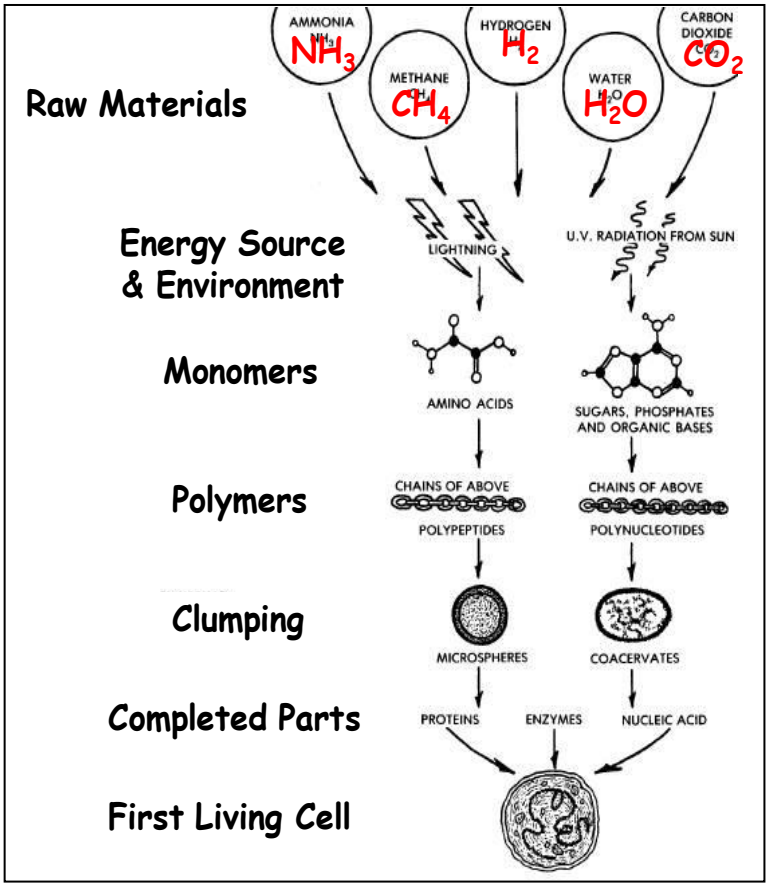
- **Cometary Infall *(proposed by McKay et al., 1996):*** There is increasing evidence to support this theory that meteoritic and cometary infall provided the first organic material on Earth (McKay et al., 1996)

  Provenance: Analysis of the present day meteorites on the ice beds of the Antarctic (carbonaceous chondrites) – typically contain between 1 and 4% carbon, mainly as graphite but also as much as 1% organic molecules which include hydrocarbons, amino acids, carbon, hydrogen cyanide and amphiphilic molecules

- **Terrestrial Accretion** *(proposed by Oparin & Haldane 1920; first experimentally proved by Miller 1953).* **First cells may have originated by chemical evolution involving 4 steps (Fig. 29):**
  - Abiotic (Non-biological) synthesis & accumulation of small organic molecules (monomers) → C + H = organic molecule
  - Monomers joined together to form polymers (proteins, nucleic acids)

- Origin of self-replicating hereditary molecules (inheritance of traits) → proteins and polynucleic acids
- Packaging of these organic molecules into cell like membrane bound droplets - protobionts (precursor to prokaryotic cell) → Aggregates of abiotically produced molecules that had an internal chemical environment differing from the external chemical environment and exhibit some of the properties associated with life (i.e. metabolism, excitability, heredity).

Fig. 29: Likely steps in the origin of Life



Evidence that supports the four-stage hypothesis for the origin of life

In 1920s, I. Oparin (Russia) and J. B. S. Haldane (Great Britain) postulated that abiotic synthesis of organic molecules is testable in the laboratory.

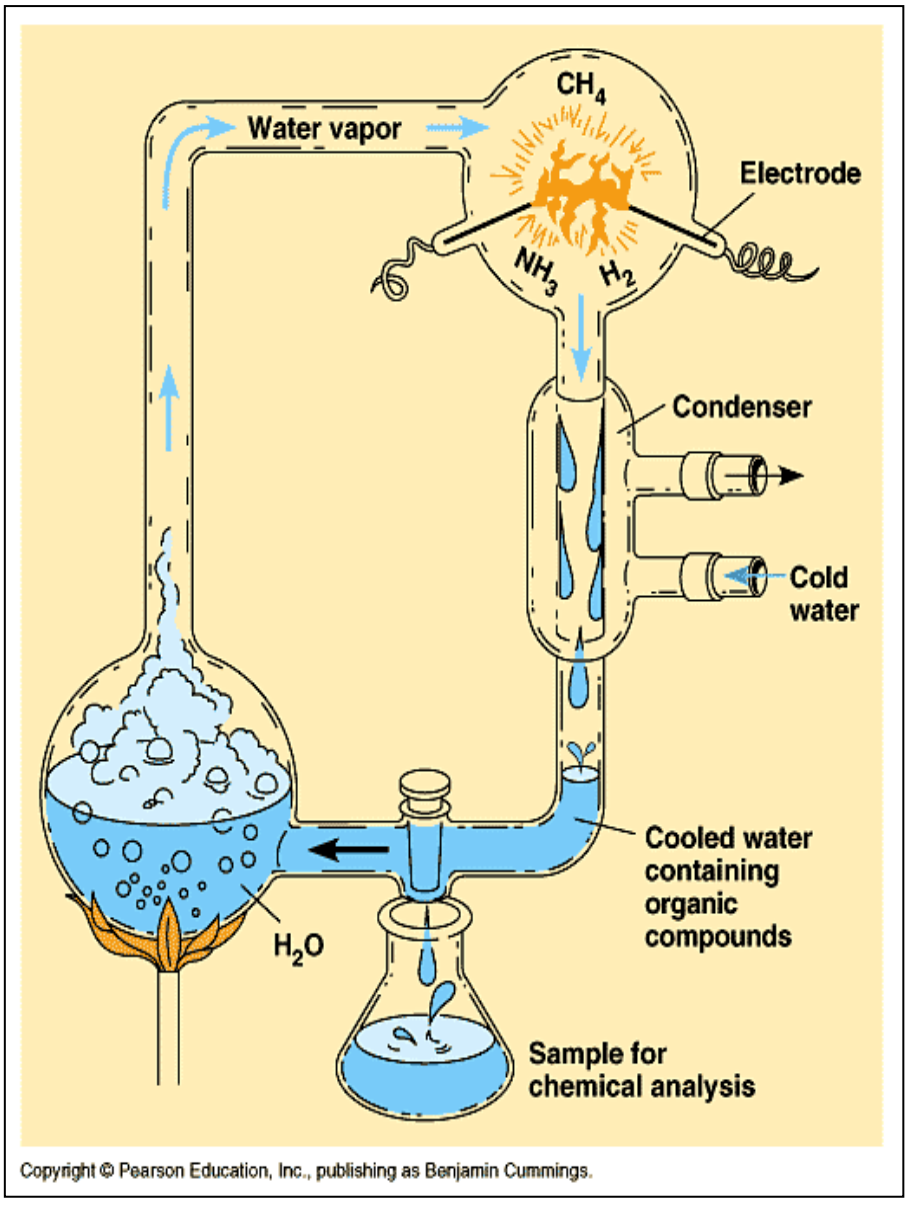
Hypothesis: Conditions on primitive earth favored chemical reactions that synthesized organic compounds from inorganic precursors. These conditions were different from present atmosphere.

As we know that earth atmosphere was

- No Free Oxygen
- More Reducing Environment than present (no free oxygen, only in form of H_2O, CH_4, NH_4, and H_2) = lots of free electrons that could be used to reduce carbon and produce organic molecules.
- Energy from lots of Lightning, UV radiation (no O_2 to block UV rays from the sun) and Volcanic Activity (heat).

This early environment was recreated in the laboratory by **Stanley Miller & Harold Urey (Fig. 30) in 1950s and tested the Oparin-Haldane hypothesis.**

Fig. 30: Urey-Miller experiment



Results of this experiment is that they were able to create Amino Acids, Urea, Simple Fatty Acids as in a week which were resultant of 15% of the conversion of carbon in the mixture to organic compounds. They concluded that **Life may have evolved in "primordial soup" of biological molecules formed in early Earth's oceans.**

The initial Miller-Urey experiment and various similar experiments succeeded in producing:

- All 20 amino acids
- Several sugars
- Lipids
- Purines and Pyrimidines
- ATP (when phosphate was added)

More recent experiments subjecting a reducing mixture of gases to a violent energy source produces:

- Formaldehyde
- Hydrogen Cyanide
- Cyanoacetylene
- All highly reactive intermediate molecules

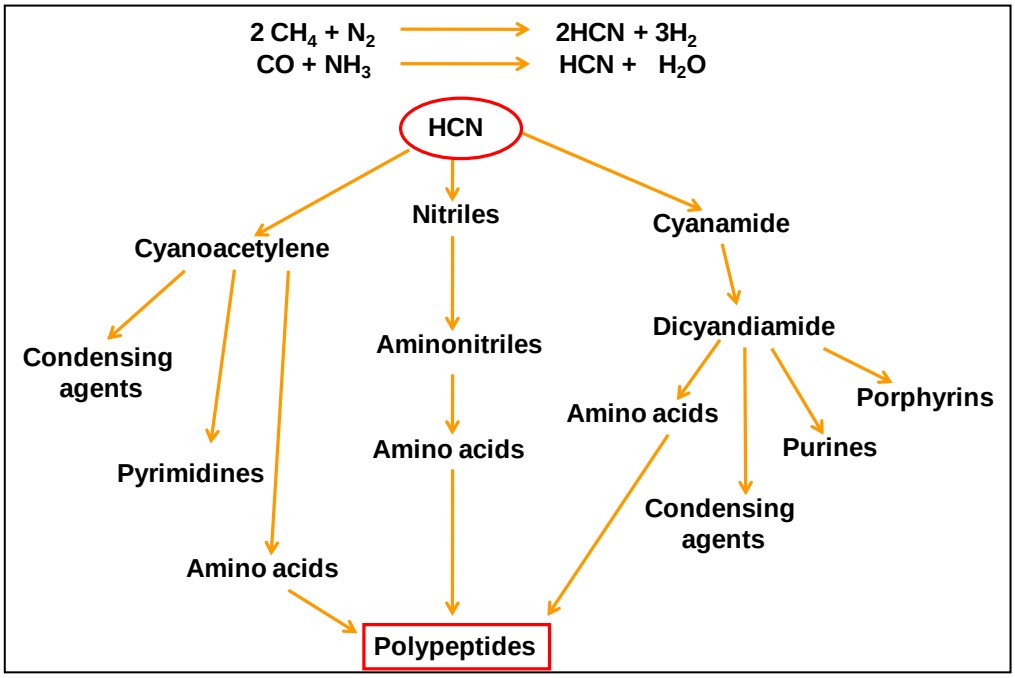
All react with water and NH_3 or N_2 to produce a variety of organic compounds:

- Amino Acids, Fatty Acids, Urea, Sugars,
- Aldehydes, Purine and Pyrimidine Bases

And it signifies the subunits for complex organic compounds.

Chemical evolution of biomolecules is supposed to produce in following pathway (Fig. 31).

Fig. 31: Chemical evolution of biomolecules



Nowadays DNA needs proteins in order to form, and proteins require DNA to form, so how could these have formed without each other? But was it the same situation during the origin of life?

Which came first- RNA?

The answer may be RNA, which can store information like DNA, serve as an enzyme like proteins, and help create both DNA and proteins. Later DNA and proteins succeeded this "RNA world," because they are more stable & efficient.

For life to originate and perpetuate, we need to have a molecule that can be used to store information and also can catalyze the synthesis of other molecules. Thomas Czech (1980) proved that

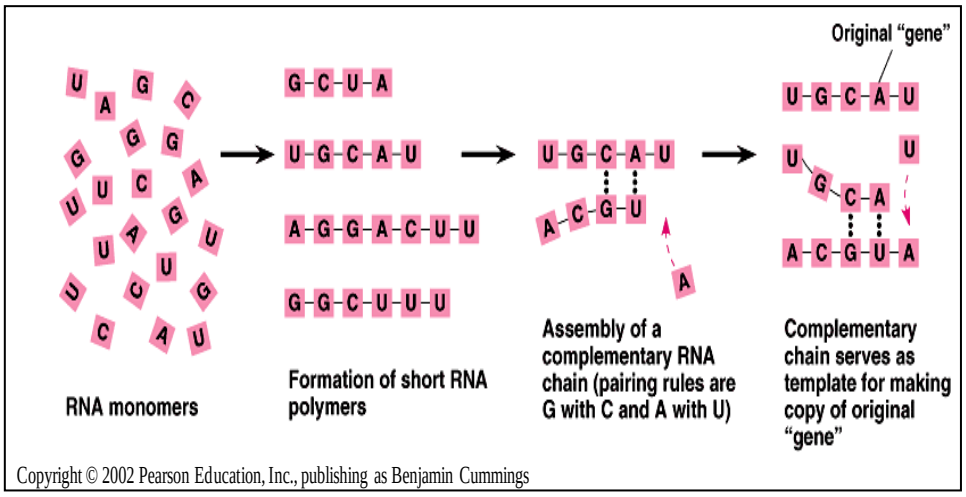
- RNA can catalyze simple reactions leading to the belief that RNA was probably the first genetic molecule to start life.
- RNA can help as a template for protein synthesis and for more RNA synthesis.
- Once proteins (enzymes) are made, they can make carbohydrates and lipids. Later on DNA evolved to be more stable molecule and proteins evolved to be more efficient enzymes.
- RNA with catalytic activity is referred to as ribozyme.

So, RNA was probably the first hereditary material which is termed as RNA World

Today some organisms such as viruses use RNA to store information.

- Short polymers of ribonucleotides can be synthesized abiotically in the laboratory (Fig. 32).
- If these polymers are added to a solution of ribonucleotide monomers, sequences up to 10 based long are copied from the template according to the base-pairing rules.
- If zinc is added, the copied sequences may reach 40 nucleotides with less than 1% error.

Fig. 32: Short polymer of RNA synthesis

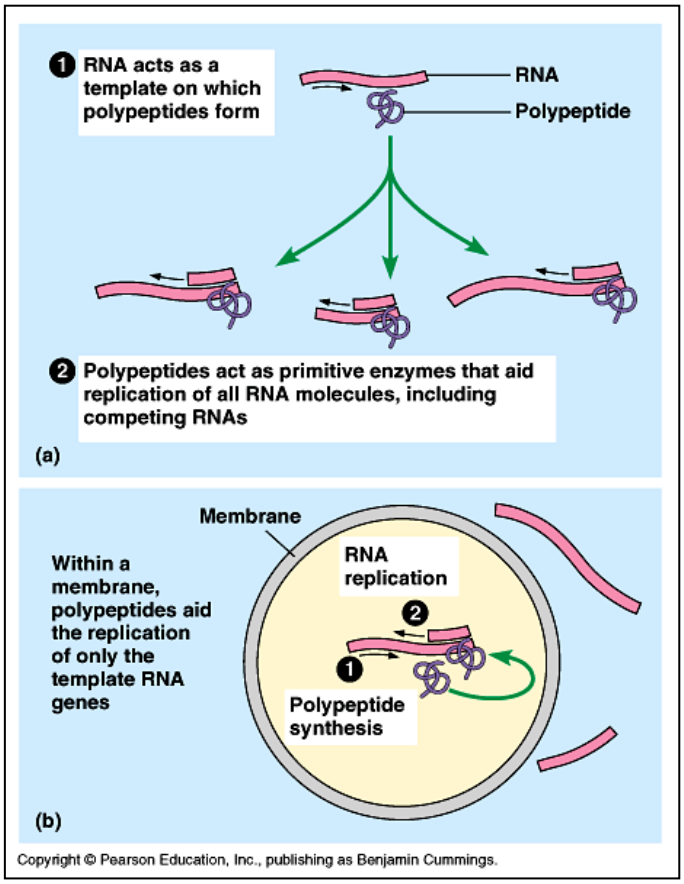


Natural selection could refine protobionts containing hereditary information

- Once primitive RNA genes and their polypeptide products were packaged within a membrane, the protobionts could have evolved as units (Fig. 33).

- Molecular cooperation could be refined because favorable components were concentrated together, rather than spread throughout the surroundings.

Fig. 33: Protobionts containing hereditary information

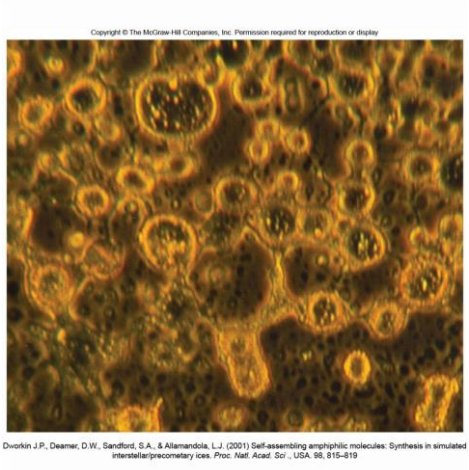


How did the membrane form?

The strongest current hypothesis for pre-biotic assembly of biologically important polymers suggests that they occurred within the boundaries of semi-permeable membranes (Fig. 34).

- Membranes were formed by aggregation of amphiphilic molecules.
- Meteorites are common sources of organic amphiphiles.

Fig. 34: Cell membrane formation



Dworkin J.P., Deamer, D.W., Sandford, S.A., & Allamandola, L.J. (2001) Self-assembling amphiphilic molecules: Synthesis in simulated interstellar/precometary ices. *Proc. Natl. Acad. Sci.*, USA, 98, 815–819

Evidences for the origin of life - prokaryotes recovered from Precambrian strata

The definite evidences of earliest life forms in the Precambrian strata were come to existence by the landmark publication of Tyler and Barghoorn (1954, 1965). A. C. Seward described the Precambrian "as an age of algae – algae with doubtful credentials." Fossil evidences of anaerobic chemo-heterotrophic bacteria, unicellular cyanobacteria are identified in the 3500 million (3.5 b.y.) year's old rocks in the Western Australia (Warrawoona Group) as the oldest structurally preserved forms of life so far recorded. Precambrian lifeforms occur principally in two types of sedimentary deposits namely 'cherts' and 'shales'.

Cherts: These rocks are composed of minute interlocking grains of silica, occurring as the mineral quartz (SiO_2), that have been deposited chemically, petrifying microscopic organisms in the place in which they live. Fossils preserved on these rocks are generally unflattened, composed of three-dimensionally preserved organic-walled cells that are thoroughly embedded in, and in filled by, the petrifying fine-grained quartz in the layers of stromalites. Stromatolites are extensive mats of microorganisms mostly comprising of cyanobacteria and green algae

Shales: This type of rocks is formed by consolidation of layers of clay or mud disseminated, along with phytoplankton and other debris, at the bottom of lakes or ocean basins. Carbonaceous microfossils of shales have been preserved by compression and flattened between thin layers of consolidated silt.

Evidences supporting the earliest life forms in the Precambrian strata are mostly recovered from the fossils sites of Onverwacht Group (Swaziland Supergroup), and Tree Group of South Africa and Warrawoona Group (Pilbara Supergroup) of Western Australia Prokaryotes dominated from 3.5 to 2 billion years ago. During this time, the first divergence occurred: Bacteria and Archaea Earliest fossil prokaryotes are mainly representative of two types of living organisms:

Blue-Green Algae (Cyanobacteria)

Bacteria

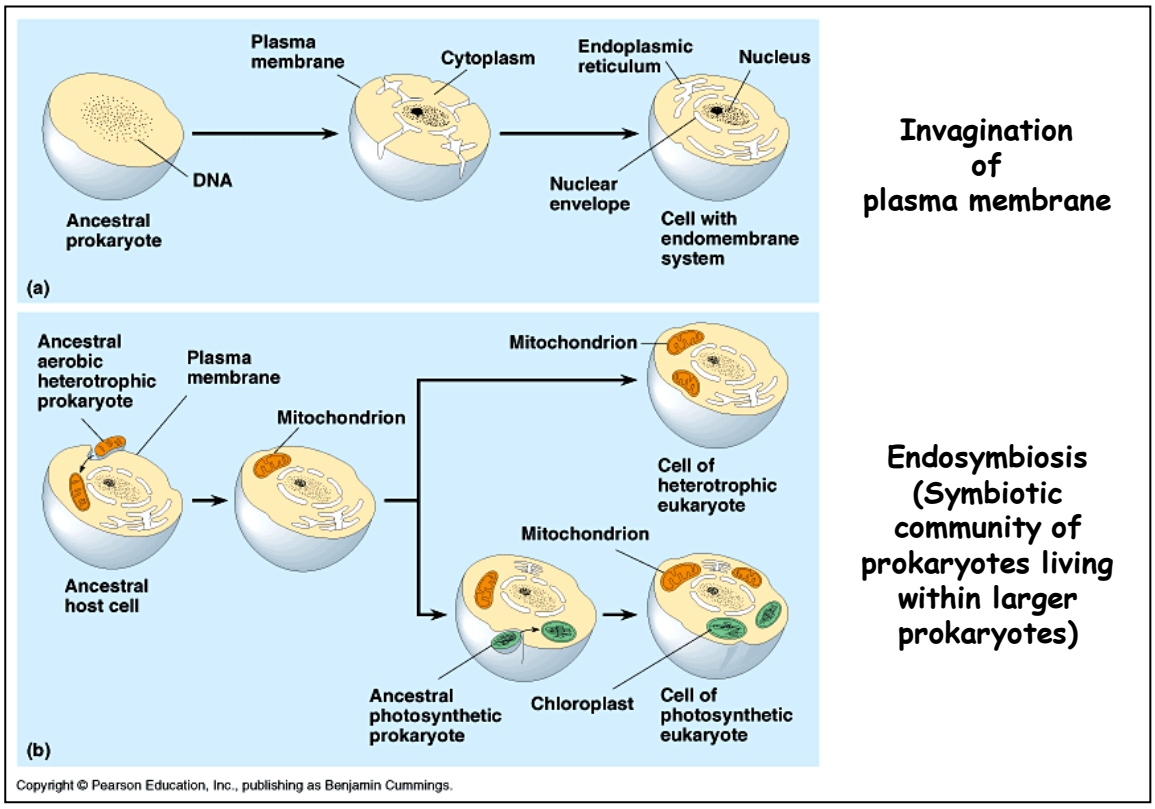
The oldest unequivocal remains of a diversity of microorganisms occur in the 2.0 BYO or 2000 my Gunflint Cherts of the Canadian Shield. It includes not only bacteria and

cyanobacteria but also ammonia consuming *Kakabekia* and some things that resemble green algae and fungus-like organisms

Eukaryotic Origins: Endosymbiotic Theory is proposed by Lynn Margulis and her associates for describing origin of eukaryotic cell. They proposed that eukaryotes resulted from a symbiotic relationship between two or more bacteria (Fig. 35):

- Mitochondria and plastids contain their own DNA.
- Nuclear, plastid and mitochondrial ribosomal RNAs show distinct evolutionary lineages.

Fig. 35: Endosymbiotic Theory



Modern-day endosymbiotic relationships

- common among protists
- similarity between eubacteria & the chloroplasts & mitochondria of eukaryotes
- size
- inner membrane systems, enzymes, electron transport systems
- reproduction resembles binary fission
- circular DNA

Three records of early eukaryotes of particular interest to the evolution of plants

- **Oldest recorded alga *Grypania***: 2100 Ma; banded iron formations of northern Michigan, USA.
- Coiled cylindrical organisms, giant unicellular alga like modern day green algae ***Acetabularia***.
- **Bangiacean red algae**: 1200 Ma; silicifed carbonate rocks of Huntington Fm in arctic Canada.
- Unbranched, uniserate, filamentous like extant red algae ***Bangia***.
- **Cladophoralean green algae**: between 800-700 Ma; shale deposit from the Svanbergfjellet Fm on Spitsbergen (island of northern Norway).
- Multicellular, branched, filamentous thalli composed of large cylindrical cell comparable to the extant cladophoranean green algae ***Cladophora***.

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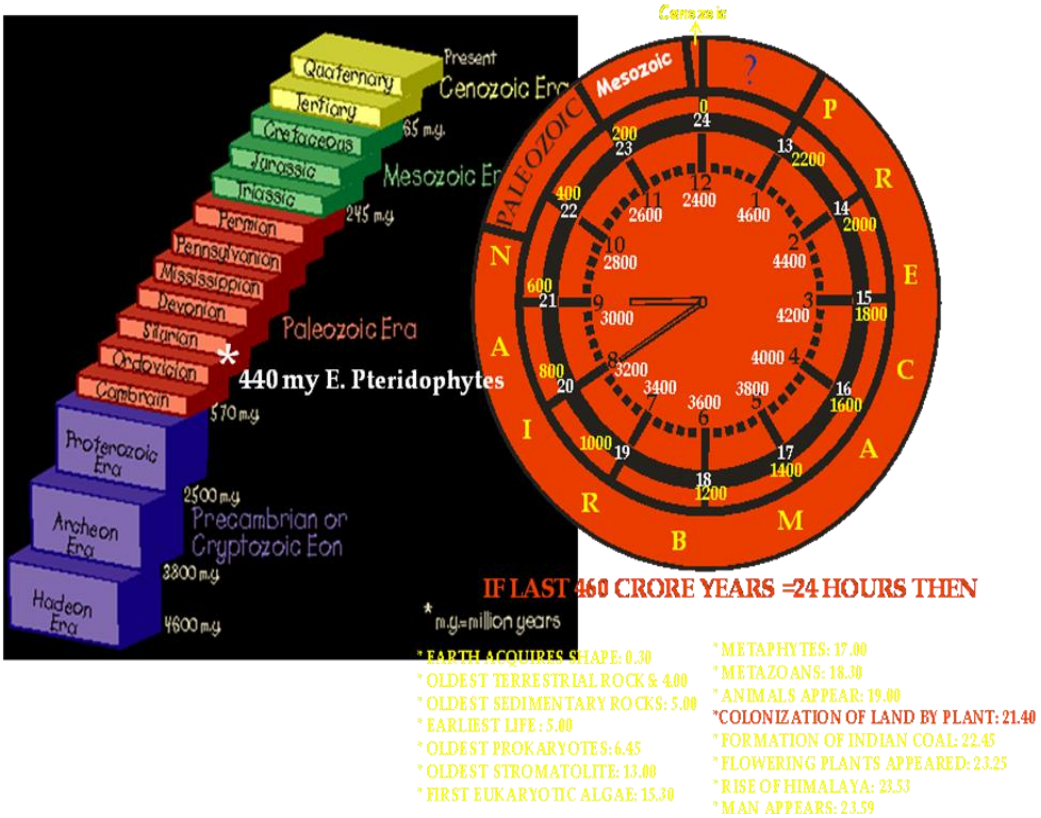
6. Colonization of Land by Plants: Geologic time, environment, vegetative and reproductive adaptations to land dwelling, fossil evidences - transitional plants with land adaptive features, early non-vascular land plants (bryophytes), early vascular land plants (pteridophytes).



Why so late?

What was the stimulus for their appearance?

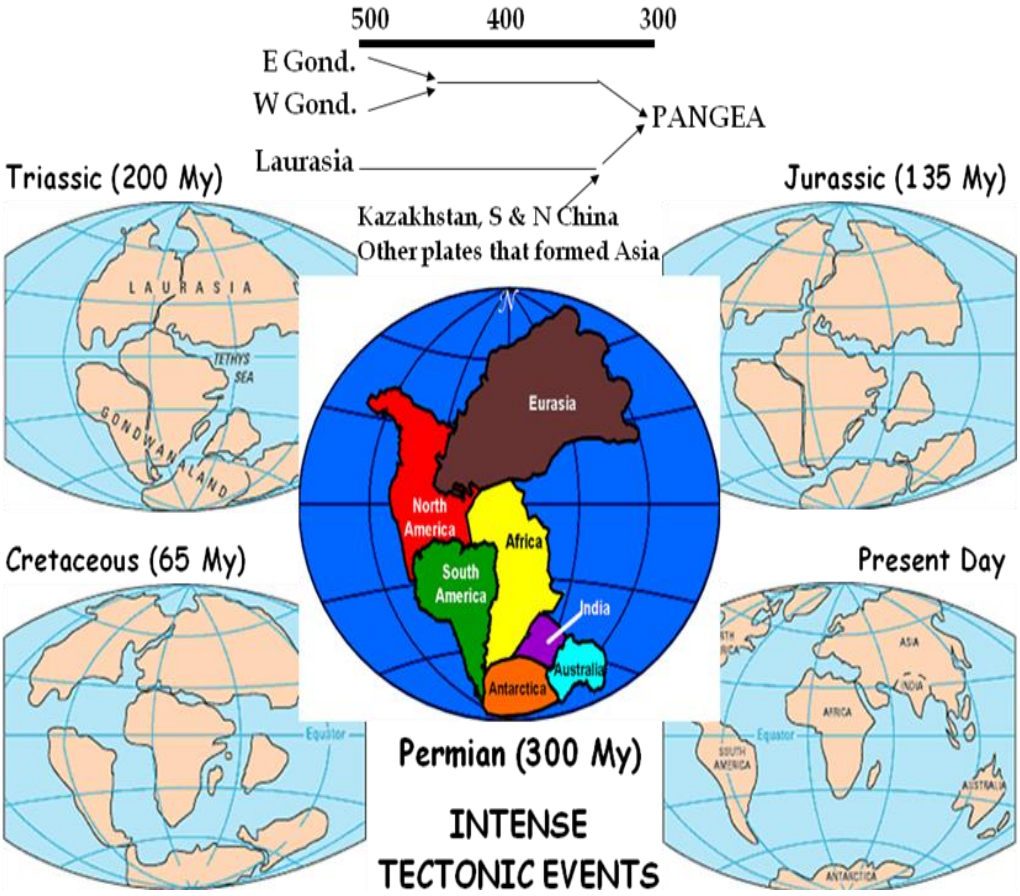
Development of essential environmental prerequisites

- Formation of new sizeable and stable near-shore environment
- Formation of soil
- Development of suitable climatic and atmospheric conditions

Major changes to early plants for land conquest

- Reduction of dependence on water for reproduction
- Protection against desiccation
- Formation of pore for gaseous exchange
- Anchoring mechanism
- Development of specialized cells for water and nutrient uptake

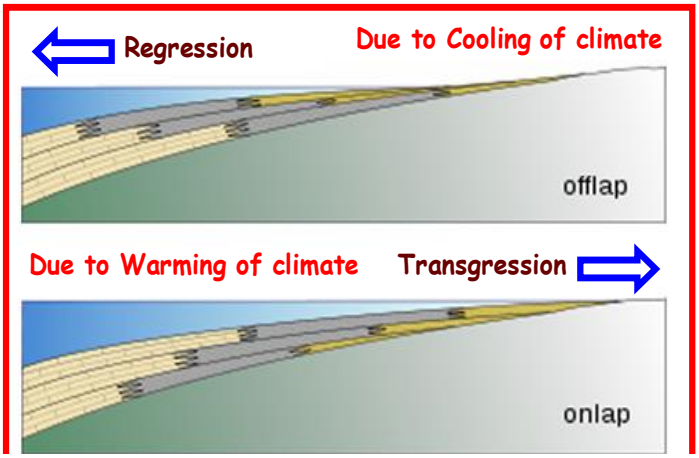
Formation of new sizeable and stable near-shore environment



Regression and transgression occur in the ice ages causing sea level change

Glaciation from approximately 440 million years ago (end of the Ordovician) led to a dramatic reduction in sea level

Melting of ice sheets during Varanger ice age in the Late Proterozoic (650-590 Ma)



Formation of soil:

Land surfaces were there but humic material or "biological available" mineral elements (e.g. N, P, Fe, S) were absent in the bare surfaces of the earliest terrestrial environments.

Various important biological and non biological processes were responsible for formation of first soils:

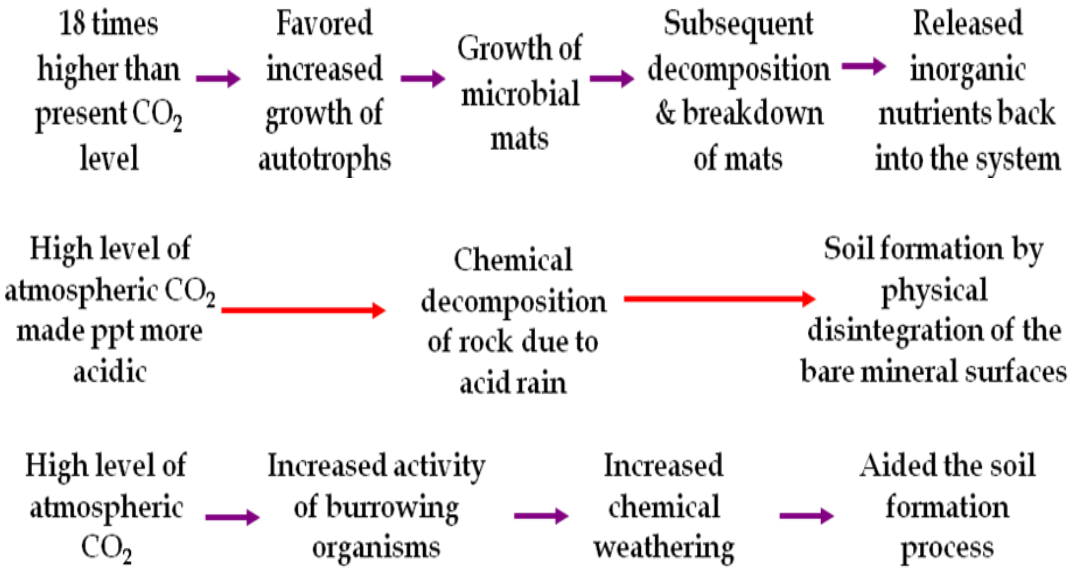
1. **Atmospheric element input:** addition of S and N via atmospheric transfer of volatile products from the metabolism of marine organism
   a. **Phytoplankton died→SO_2 volatized & Oxidized→transferred to terrestrial environment by dry deposition or in rain**
   b. **Fixing of atmospheric nitrogen→biologically usable through lightening strikes**

2. **Weathering by acid rain and organic acids produced by early microbial organisms:**

Cyanobacteria, non-photosynthetic bacteria, eukaryotic algae → Secreted organic acid → Breaking down rock material → Releasing Fe & P

How??

Chelation of Fe(III) from rock is carried out by specific organic moleccules Siderophores. These Siderophores are secreted by prokaryotic and eukaryotic organisms. These siderophores containing the Fe(III), are trhen taken up by other organisms and their breakdown results in the release of Fe.



3. **Lichens (symbiotic association of fungus and green alga or cyanobacterium)**

Colonize-Weather Rock-Soil Formation

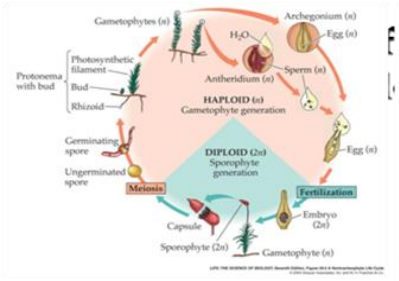
Development of suitable climatic and atmospheric conditions:

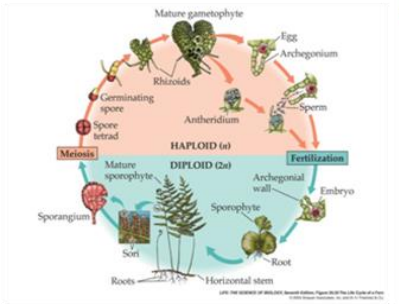
Cambrian-Early Ordovician: 18 times higher CO_2 level than present associated with high global temperature

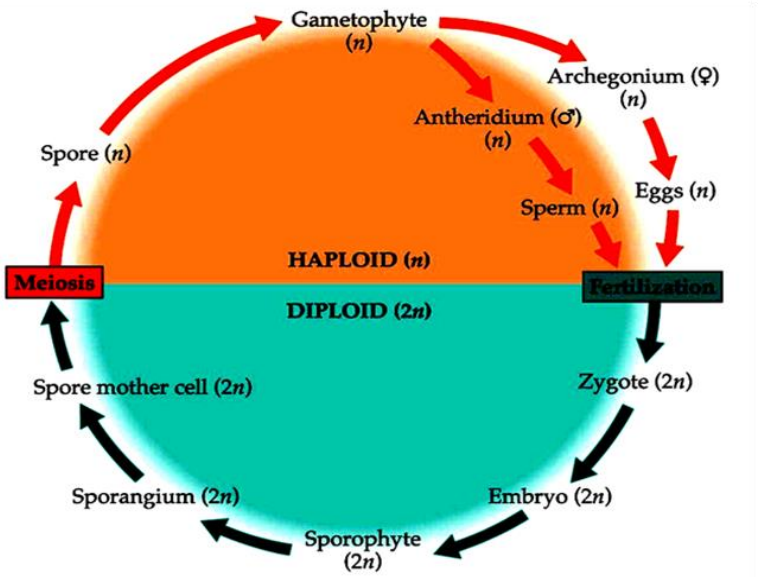
Late Ordovician (458-443 Ma): Gradual reduction of CO_2 level & global temperature because of

A. Decrease in volcanic out gassing
B. Increased burial of organic carbon
C. Late Ordovician glaciation

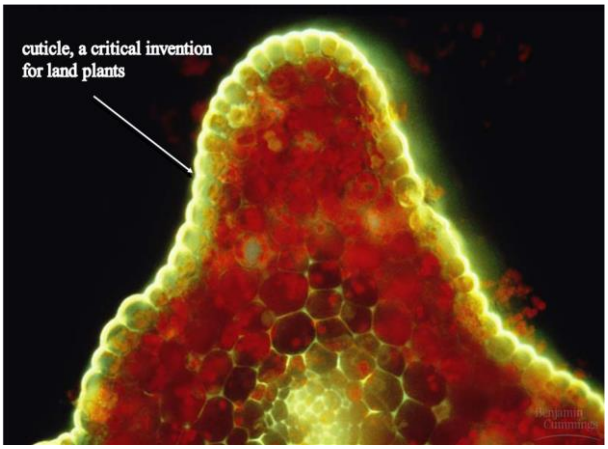
Reduction of dependence on water for reproduction:

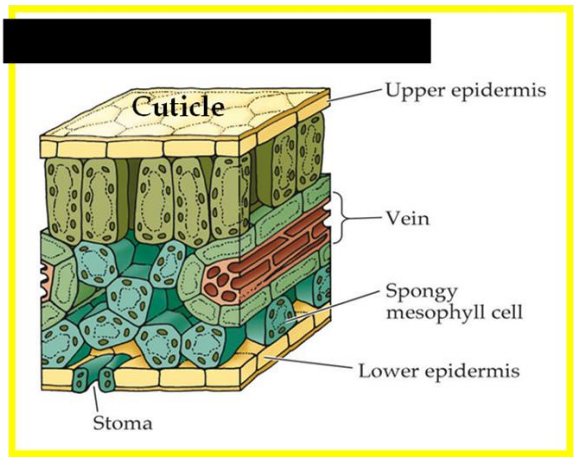






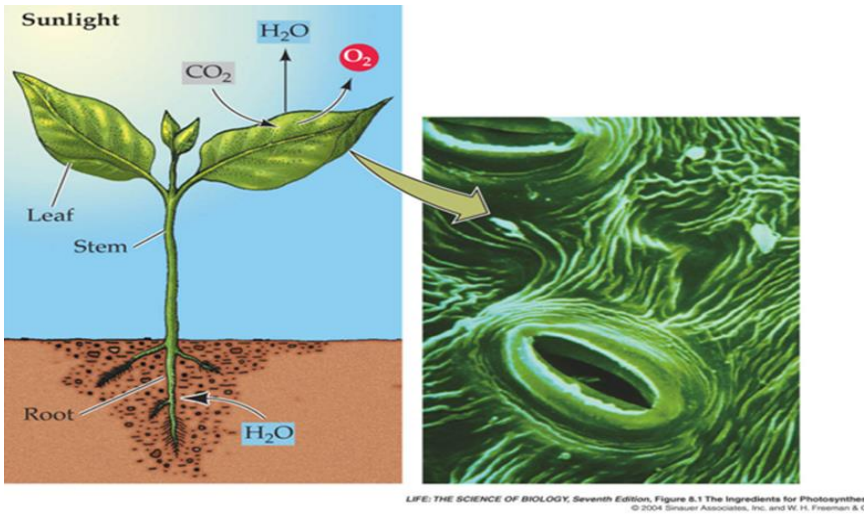
Protection against desiccation:





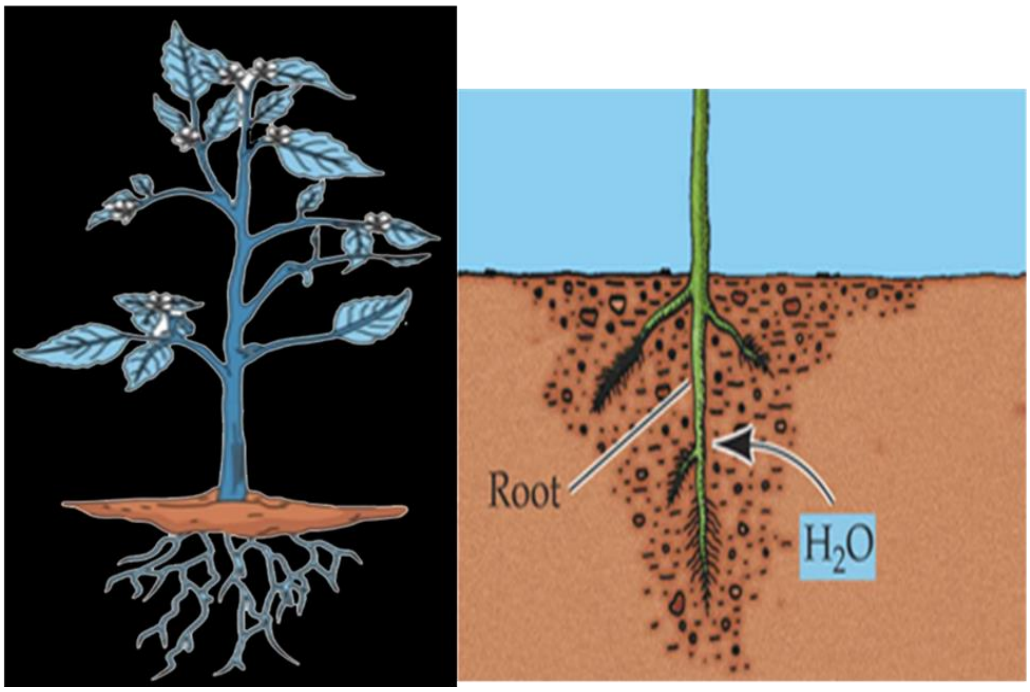
Trilete spore with sporopollenin on outer wall

Formation of pore for gaseous exchange:

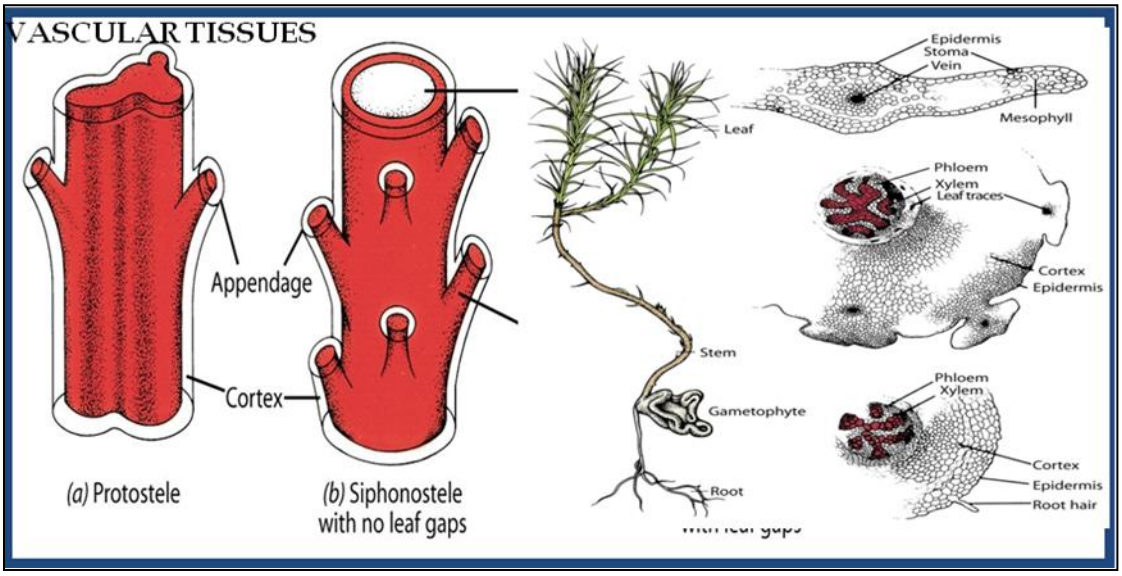


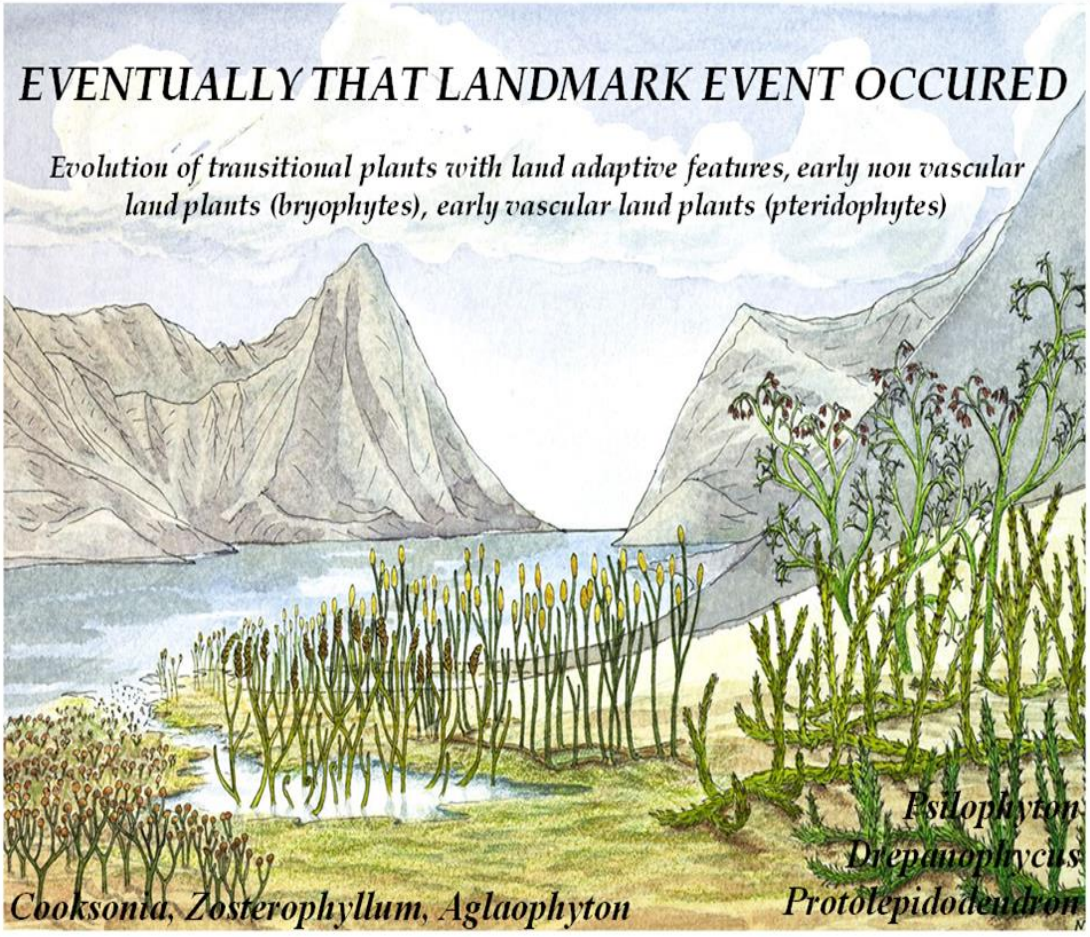
LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 8.1 The Ingredients for Photosynthes
© 2004 Sinauer Associates, Inc. and W. H. Freeman & C

Anchoring mechanism:



Development of specialized cells for water and nutrient uptake:

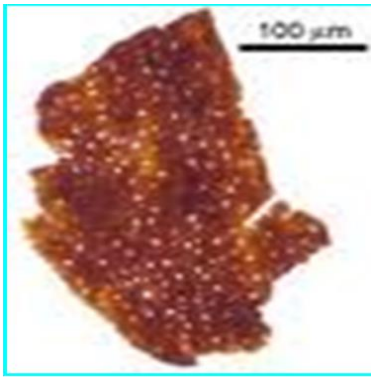




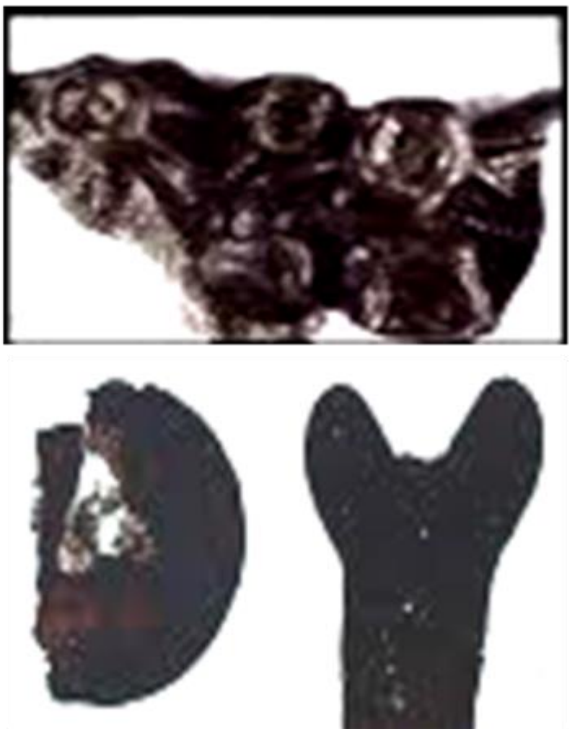
Transitional plants (Enigmatic non vascular plants) with land adaptive features:

Nematothallus (Early Silurian-Devonian)

- Plant body thalloid
- Pseudoparnchymatous cells comprised of filament and surrounded by thick cuticle
- Branching tubes with secondary wall thickening that have the appearance of tracheids
- Some produce thick walled spores



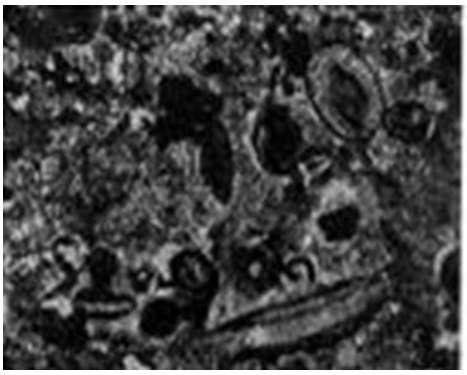
Protosalvinia (Devonian)



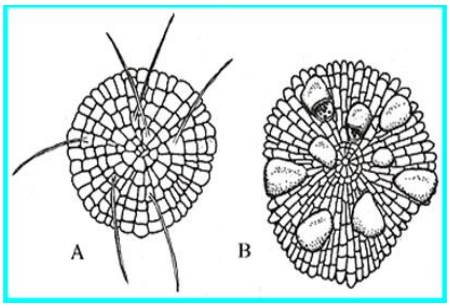
- Thalloid plant body
- Plant body developed depression within which thick walled spores present in a tetrahedral manner
- Though biochemical evidences pointed out towards the affinity with brown algae, but authentic botanical affinity unknown; thus it is considered as transitional non-vascular plants

Pachytheca (Late Silurian – Early Devonian)

- Single sphaeroidal body, 1.5-5.0 mm in diameter with outer and inner zone
- Inner zone consists of multicellular filament oriented randomly
- Radially arranged filament present in outer zone
- The plant having affinity with algae



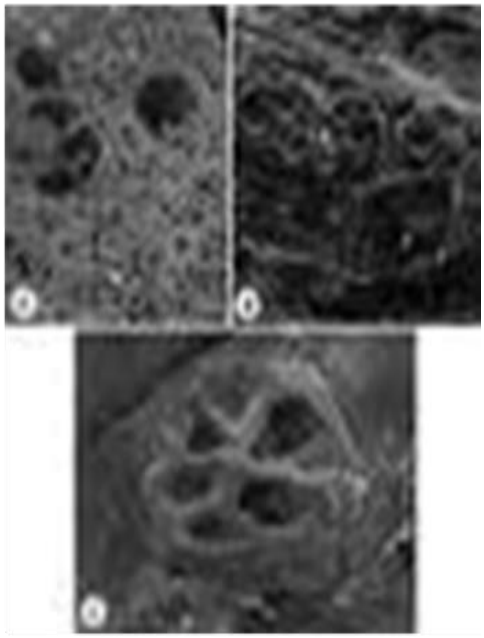
Parka (Early Devonian)



- Dorsiventral thallus like plant circular to ovoid up to 5 cm with clustered spores
- The plant body cellular, upper and lower epidermis parenchymatous attached with a ventral holdfast

- Spores appear cuticularized, 28-34 µm in size, interrupted to be in a sporangium at the surface

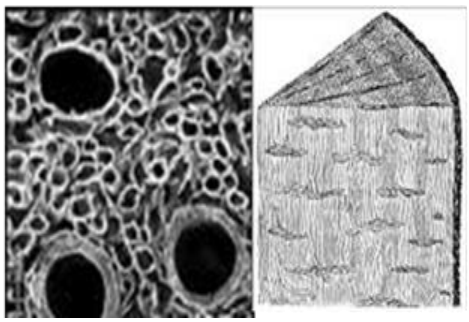
Spongiophyton (Middle Devonian)



- Thalloid, branching plant thought to have a habit similar to modern liverworts
- Typically preserved as a cuticular sheath with a porate (200-300 µm raised areas with holes) and non porate surface
- Porate cuticle are three times thicker than non porate one
- There is no evidence of vascularization
- Stein et al. (1993) suggested that thase plants are lichens

Prototaxites (Silurian-Devonian)

- Giant, terrestrial, saprotrophic organism, axes termed as pseudostem, 1.25 m in diameter and more than 8 m long



- Outer surface smooth or mildly ribbed
- Pseudoparenchymatous or plectenchymatous internal organization constructed to longitudinally oriented tubes of three different types
- In addition tubes, several lacunae are scattered throughout the pseudoparenchyma
- Previous chemosystematic data suggested an affinity with algae, where most recent interpretation (Hueber, 2001) hypothesizes that it may belong with the basidiomycetes

Early non vascular land plants (Bryophytes)

Poor record because of the delicate nature of many bryophytes

LIVERWORTS

1. Oldest fossil bryophyte: *Pallavicinites devonicus* (thallus liverwort)

Mode of preservation: Compression fossil

Age: Upper Devonian age (Paleozoic)

Preserved form: Dichotomizing axis with some cellular details

Affinity: Jungermanniales-Anacrogynae (having the female sex organs arising from any cell below the apex of the stem, thereby not terminating its growth): high degree of similarity with extant genus *Pallavicinia.*

2. *Hepaticites kidstoni* (leafy liverwort)

Age: Carboniferous (Paleozoic)

Affinity: Anacrogynous *Treubia*

At least eight *Hepaticites* and ten *Thallites* are also found from Mesozoic rocks

3. *Thallites willsi*

Age: Carboniferous

Affinity: *Metzgeria*

Preserved from: Dorsiventral dichotomizing thallus

4. *Metzgerites* (anacrogynous liverwort)

Age: Triassic (Mesozoic)

5. *Jungermannites* (leafy liverwort-Jungermanniales-Acrogyne)

Age: Tertiary

Some specimen of leafy liverworts and mosses are found preserved in famous Oligocene (Tertiary) Baltic amber deposits and are assigned to modern genera

6. *Hepaticites cyathodoides*

Age: Middle Triassic

Affinity: Marchantiales

Preserved from: Undeffertiated, dorsiventral dichotomizing thallus, pores with air chamber, small scales on the ventral surface

7. *Ricciopsis*

Age: Jurassic: Triassic rock ((Mesozoic)

Affinity: *Riccia*

8. *Marchantites sezannensis*

Age: Eocene **(**Tertiary)

Affinity: Marchantiales

Preserved from: Antheridial gametophores similar to *Marchantia*

Naiadita lanceolata: an enigma

(Discovery by T. M. Harris in 1938)

Age: Triasic

Unquestionable liverwort with unusual combination of characters of both Hepaticeae and Musci; as such it is not possible to place in a family of Hepatics and so family Naiaditaceae is suggested for this fossil genus belonging to Sphaerocarpales order of Hepatics.

- Unicellular rhizoids
- Sessile archegonia with venter wall one cell layer thick, and
- Leaf cells of a similar shapes;
- Erect gametophores with spirally arranged leaves like most of mosses;
- Leaves lack costa which is a characteristics of mosses;
- Archegonia are enclosed in a perianth of several leaf like lobes;
- Sporophytes are found at the tips of a short lateral branch of gametophore;
- Sporophyte with poorly developed bulbous foot and globose sporangium

MOSSES

1. Bogmoss

Distinct leaf cell pattern like bog moss reported from Permian

Protosphagnum:

Age: Lower Jurassic

Preserved from: Leaves with midribs and occasional lateral vein

2. True Moss (Eubrya)

***Muscites polytrichaceus*:** Carboniferous

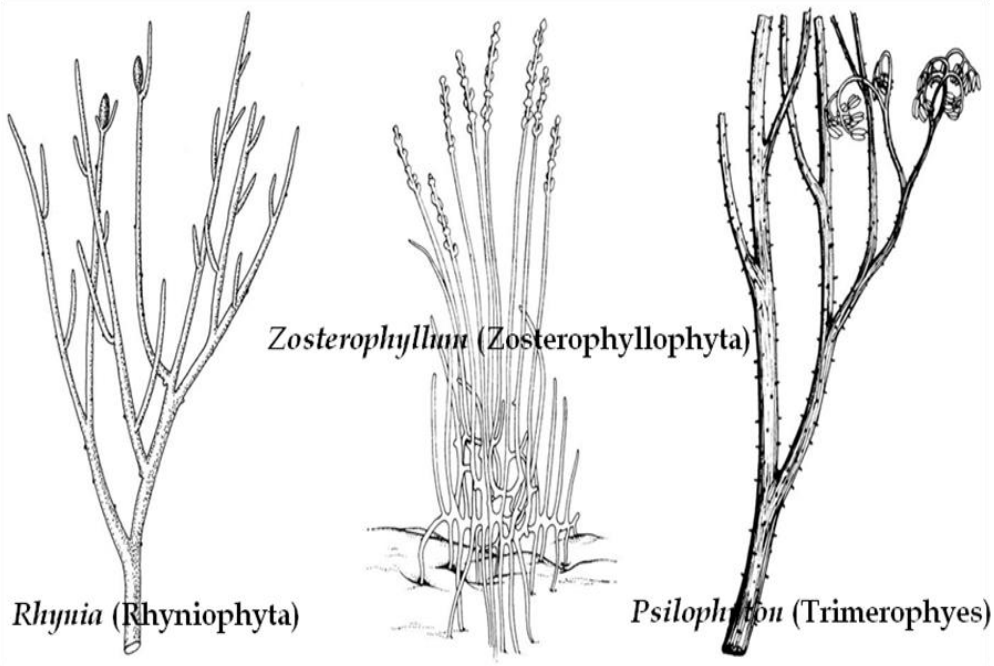
Intia: Permian; looks like modern *Mnium* and *Bryum*

Merceria: Permian; permineralized from looks like Bryales

Aulacomnium heterostichoides: Tertiary (Eocene); *Aulacomnium heterostichum*

Ditrichites: Palaeocene; assigned to Districhales

Some early land vascular plants:
Early Pteridophytes



Discovery of Early Pteridophytes

- *Psilophyton princeps* by Sir J Williams Dawson (1859) from Devonian age from Gaspe Peninsula, Quebec, Canada
- Dichotomously branched plant with lateral mass of sporangia

- Modified his description in 1870: sporangia occurred in pairs at the tips of slender branches
- It is now apparent that Dawson was examining the remains of several different taxa

Almost 60 years later the real significance of Dawson's discoveries was appreciated

- Kidston and Lang (1917-1921) described well preserved vascular plants from deposits of Lower Devonian age in the Rhynie Chert Beds, Aberdeenshire, Scotland
- Recognized similarities between *Psilophyton* and their discoveries
- The order Psilophytales were established to include all of these presumably primitive extinct vascular land plants
- Following these early studies, additional genera were discovered in other parts of the world and the Psilophytales became a catchall group for extinct Devonian plants

With continuing research on fossil plants, it has become increasingly clear that there were at least three main groups within the Psilophytales

1. Rhyniopsida: Plants had terminal sporangia, predominantly fusiform. The stems were naked. e.g. *Cooksonia*, *Rhynia*, *Horneophyton*

2. Zosterophylopsida: The sporangia were lateral on axes, predominantly globose or reniform. Each sporangium dehisced along the distal edge. The stems were either devoid of appendages or hard spine like outgrowths. e.g. *Zosterophyllum*, *Sawdonia*, *Gosslingia*

3. Trimerophytopsida: to accommodate the genera that do not fit into the other two groups. Plants had a main axis that branched pseudomonopodially. Each lateral branched a no. of times and finally terminated in several or a mass of sporangia. e.g. *Psilophyton*, *Pertica*

Some palaeobotanists suggested the Trimerophytes are natural outgrowth of the Rhyniophyta. Within the Trimerophyta the distinction between a central axis (stem) and lateral branches may represent the precursors of fronds. Through planation and development of laminar tissue, a dorsiventral leaf could be evolved.

Rhyniophyta

Salient features:

- Small plant only a few cm high
- Dichotomously branched, without appendages (lateral branches or leaves)
- The slender, naked axes are terminated by sporangia that are short and wide, fusiform or almost reniform (kidney shaped)

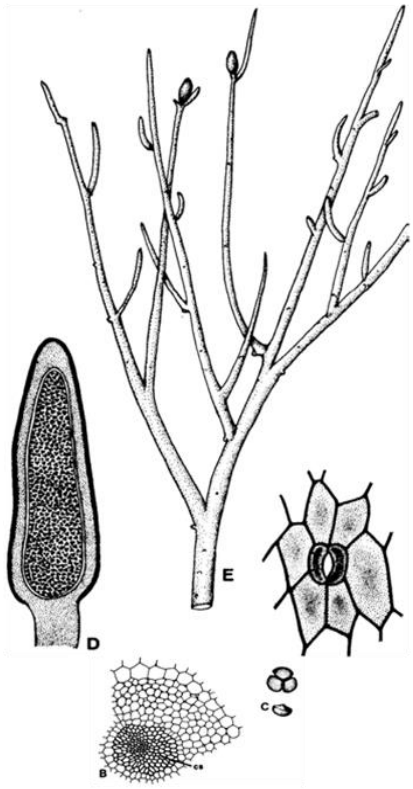
Originally Kidston and Lang described *Rhynia gwynne-vaughanii* :

- **A prostrate dichotomously branched rhizome system with rhizoids and an upright dichotomously branch aerial system with smaller lateral branches.**

- **The sporangia were fusiform and terminated some of the upright main branches, although Kidston and Lang were unable to document conclusively the connection between the stems and homosporous sporangia.**

- **Stomata and cuticle were present on the stems**

- **The stem vascular cylinder was a protostele with centrally placed tracheids that had annular thickenings, surrounded by a thin walled phloem like region.**



Edwards (1980) has shown that

- The aerial branch system was much more branched than originally suggested.
- A limited number of dichotomies near the base of the aerial system with lateral (adventitious) branches arising on the tapering main axes.
- An aerial branch system was about 20 cm in height.
- Sporangia were terminal on the main axes and probably abscised after spore dispersal and were usually overtopped by the development of lateral branches.
- No specialized dehiscence mechanism.
- She confirmed the presence of a central stand of annular or helically thickened tracheids surrounded by phloem.

Another species: *Rhynia major*

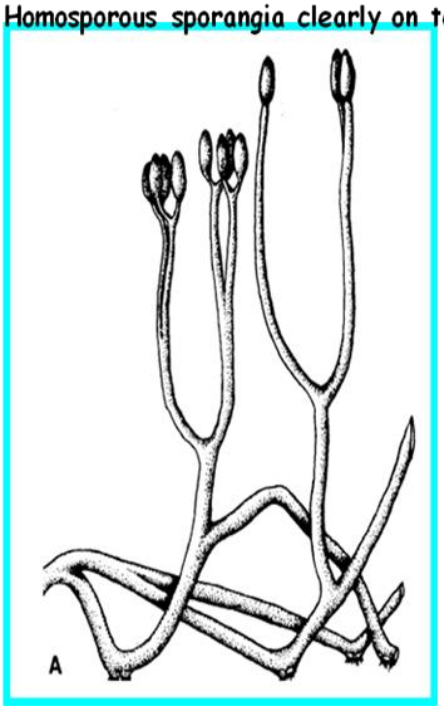
(described by Kidston and Lang from the Rhynie Chert & was suspected as gametophyte of *Rhynia gwynne-vaughanii*)

More robust than *R. gwynne-vaughanii*, about 60 cm. in height

Aerial stems branched dichotomously

Dichotomous nature of the branching is not masked

Homosporous sporangia clearly on terminal axes, not overtopped as in *R. major*



Edwards (1986) produced a reconstruction that differs from that of Kidston and Lang. According to her

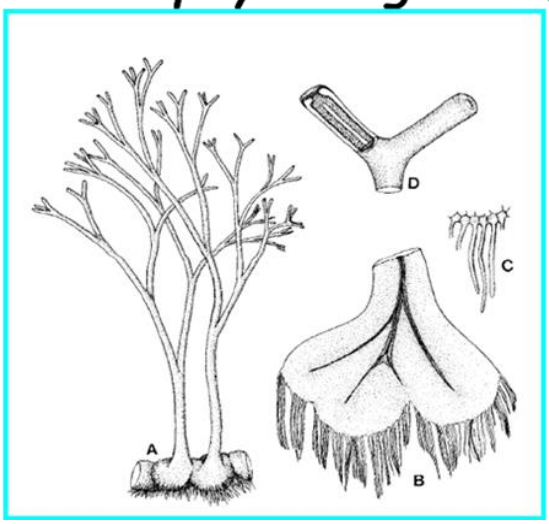
- An extensive dichotomously branched rhizome system with a limited number of upright stems that branched dichotomously at wide angles.
- All axes terminated in paired homosporous sporangia above a dichotomy.
- The upright branch system is estimated to have been about 18 cm in height- much less than that estimated by Kidston and Lang.
- The sporangia were less than 12 mm long and about 4 mm wide with stomata in the outer layer
- Dehiscence was longitudinal.
- The central conducting strand of stems consisted of elongate thin walled cells of phloem like region surrounded numerous elongate cells with uniformly thickened cell walls. The innermost cells were narrower and had thinner walls than the peripheral cells.

Nothia (found with other rhyniophytes)

Naked, invaginated axes with pear shaped (apical dehiscence) sporangia on adaxially (downward) recurved stalks

Sporangia may be helical or whorled in arrangement

Horneophyton lignieri (found with *Rhynia* in Rhynie cherts)



- The basal part consisted of a tuberous, corm like structure with numerous rhizoids
- The aerial axes were smooth and branched dichotomously
- A vascular cylinder was present in the aerial branches, but disappeared in the basal corm

Based upon detailed studies, the sporangia have been shown to be unique (Eggert, 1974):

The tips of the branches end in 2-4 cylindrical lobes, each having a sterile columella surrounded by spores

Although it has not been conclusively demonstrated, the sporangia may have opened by means of a small apical pore at the tips of the lobed sporangium

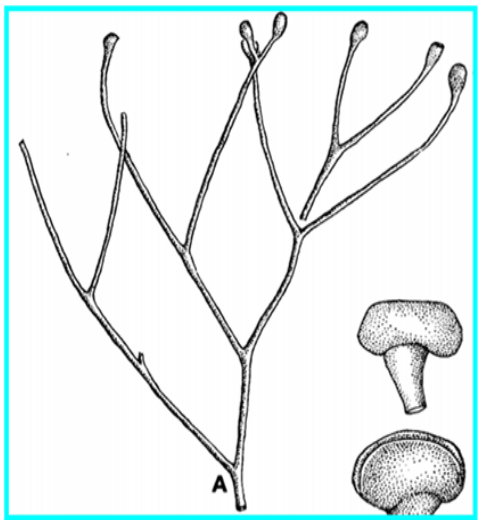
Each spore had a triradiate ridge (trilete), homosporous like *Rhynia*.

Cooksonia (oldest known vascular plant)

Specimens are from the United States, Canada, Wales, Scotland and Czechoslovakia. Those from Wales are as old as mid Silurian; others are from the Lower Devonian.

- Smallest and simplest vascular plants
- Slender, leafless, dichotomously branched plants 5-7 cm in length and from 1 mm or less to about 2 mm in diameter
- Some specimens had vascular strands with annular tracheids
- The terminal sporangia were globose to renifrom and contained trilete spore

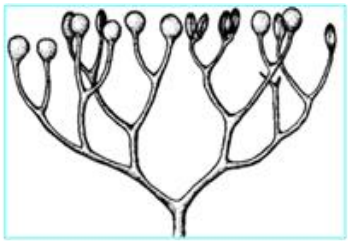
Steganotheca (Edwards, 1970)
(Upper Silurian – Lower Devonian)



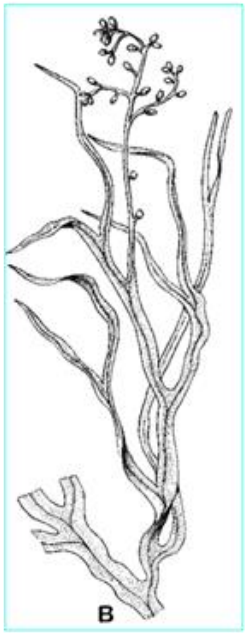
Bushy, dichotomously branched plant 5 cm high with terminal sporangia.

Sporangia truncated at the apex and taper to the base.

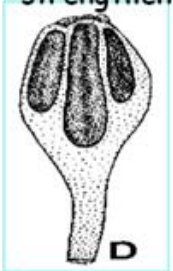
A central strand can be seen in the compressed branches suggesting the presence of a vascular system.



Uskiella – Lower Devonian of South Wales
Simple dichotomous branching of naked axes but sporangia is longer and wider than those of *Cooksonia* and possesses a more complete wall structure.



Taeniocrada – It may have been more of an aquatic member instead of marshes, bogs or mud flats habitat of most rhyniophytes. Evidence of aquatic habit comes from absence of stomata on the lower part of the plants, although the epidermis is fairly well preserved.
The stems were flat or ribbon like, exhibited dichotomous branching, and had clusters of terminal sporangia. The conducting strand may have consisted of helically strengthened tubes rather than typical tracheids.



Hedeia and *Yarravia* – Lower Devonian fragments consisting of short axes with terminal sporangia. The sporangia of *Hedeia* are similar in size and shape to those of *Rhynia* but are grouped closer to one another at the tips of naked dichotomous branches. *Yarravia* had 5-6 sporangia fused externally forming a terminal sporangia.
Other genera: *Hicklingia*, *Dutoitea*, *Eogaspesiea*

Zosterophyllophyta

1. Dichotomous branching the stems were either smooth or had multicellular spines
2. Sporangia occurred laterally on the stems and in some were aggregated into spikes
3. The presence of distal dehiscence has been well established in the group
4. The xylem strand of stems was exarch

Sawdonia ornata: Upon re-examination of Dawson's collections Hueber (1971) proposed the binomial *Sawdonia ornata* what Dawson named as *Psilophyton princeps* var *ornatum*. The genus is an anagram of the surname Dawson, so named in honour of the nineteenth century geologists

- The plant branched dichotomously from a rhizome system
- Multicellular emergences or spines,
- Reniform sporangia borne laterally and singly on short stalks that were located towards the curled (circinate) tips of branches
- The protostele was exarch with tracheids that had helical, scalariform or reticulate secondary wall thickenings

Sawdonia acanthotheca:

- Terate-solid cylinder which broad at base but tapers towards apex
- It is quite similar to *S. ornata* but differs in the nature of the emergences on the axes and their presence on sporangia

Crenaticaulis verruculosus (Lower Devonian of Gaspe Bay):

1. It is similar to *Sawdonia* but had one or two vertical series of emergences.
2. Xylem cylinder was elliptical in outline and maturation was exarch, one of the unifying characteristics of the Zosterophyllophyta.

Gosslingia brecoenensis (Lower Devonian):

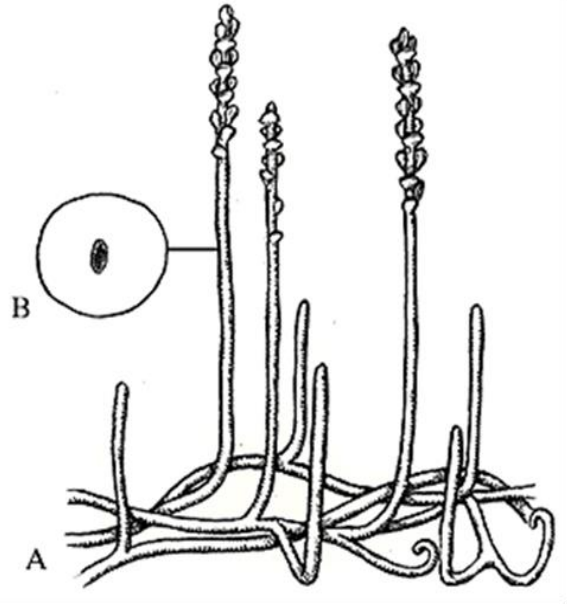
1. At least 50 cm high and branched predominantly by unequal dichotomies
2. The axis tips circinately coiled as in *Sawdonia* but the stems were smooth except for curious vascularised tubercles at points of branching
3. The oval to reniform sporangia were scattered along the main and lateral branches
4. Apparently homosporous

Zosterophyllum

Known from Lower Devonian rocks of Scotland, South Wales, Belgium, France, Russia and Australia

First described by Penhallow in 1892, then Lang (1927) reinvestigated the original as well as new specimens

1. The sporangia aggregated into a spike which differs from *Gosslingia*
2. Basically dichotomous branching but a rather unique type rhizome system present especially in *Z. myretonianum* with an upward and a downward directed system like 'H' shaped
3. The sporangia dehisced by means of a slit along the distal edge
4. The xylem strand in *Z. lanoveranum* was elliptical to terete, and the xylem was exarch



Trimerophytopsida

Established by Banks (1968) for early vascular plants that were not members of the Rhyniophyta or Zosterophylls

***Psilophyton*:** Hueber & Banks (1967) selected a type *Psilophyton princeps* during re-examination of Dawson's collections and other specimens

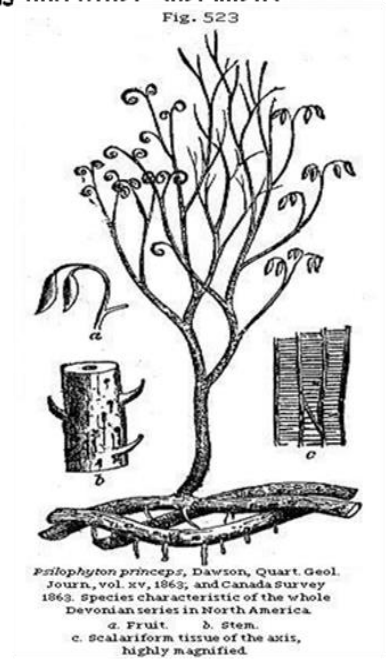


Fig. 523

Psilophyton princeps, Dawson, Quart. Geol. Journ., vol. xv, 1863; and Canada Survey 1863. Species characteristic of the whole Devonian series in North America.
a. Fruit. *b*. Stem.
c. Scalariform tissue of the axis, highly magnified.

- **Stout dichotomously branched main axes and lateral branches**
- **The axes had blunt spines and interrupted longitudinal ridges**
- **The ultimate branches ending in pendulous paired sporangia**
- **Elongate and elliptical sporangia, 7.5-8.0mm long and 1.2 mm wide**
- **Homosporous**

Psilophyton dawsonii:(L. Devonian of the Gaspe and Ontario, Canada)

- A stout main axes and smaller lateral branches were undoubtedly produced by unequal dichotomous (anisotomous) branching
- Lateral branches were arranged alternately in two rows
- Fertile lateral branches usually dichotomized six times before terminating in clusters of approximately 32 sporangia
- The axes were smooth, unlike *P. princeps*
- In main axes, the xylem cylinder occupied about 1/4th of the stem
- Terete, centarch protostele
- The protoxylem tracheids with helical and scalariform thickenings
- The metaxylem with scalariform tracheids and some tracheids with circular bordered pits

Psilophyton charientos: (New Brunswick province, Canada)

- Probably grew to a maximum height of about 50 cm
- Exhibited the common pseudomonopodial habit
- All except the most distal segments were densely covered with slender, tapered spines
- The fertile lateral branches dichotomized often terminating in gracefully recurved tips bearing sporangia that dehisced longitudinally
- The xylem was centarch, in which the protoxylem tracheids had annular thickenings and the metaxylem tracheids were scalariform pitted

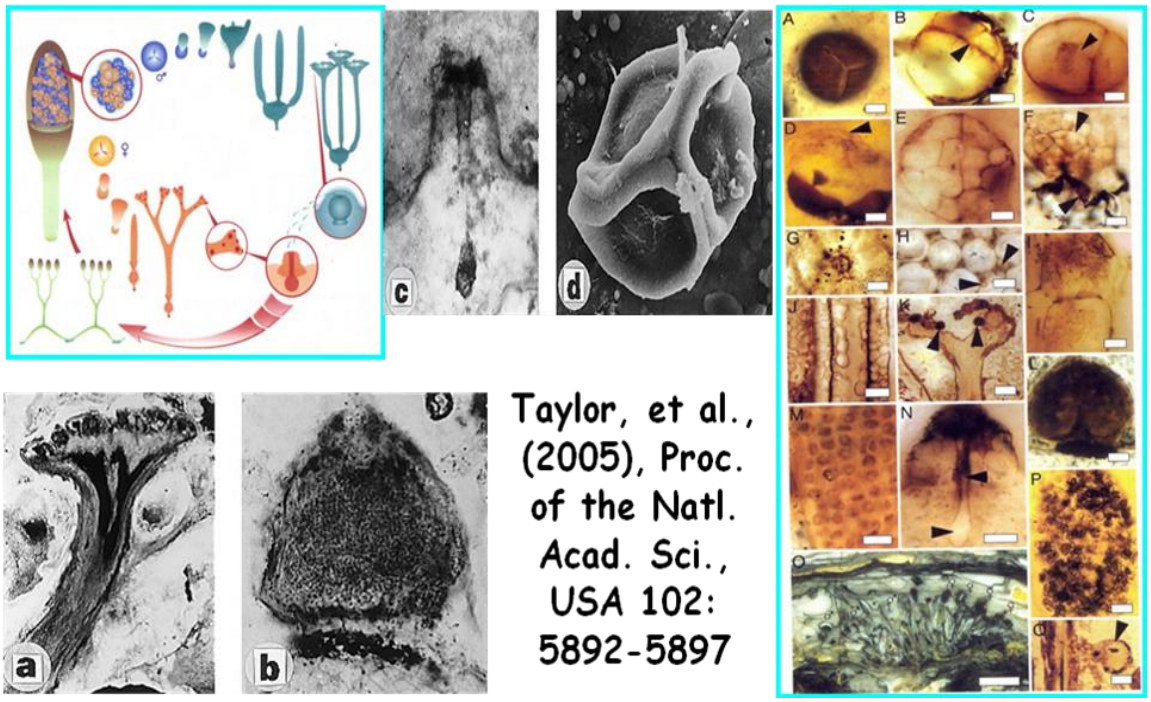
Gametophytic Generations

It has long been assumed that none of the known remains of early vascular plants are gametophytes

Pant (1962) and Lemoigne (1968, 69, 70) concluded that the vascularised axes of *Rhynia gynne-vaughanii* are probably the gametophytes of *R. (Aglaophyton) major*

This assumption was based upon several morphological features and Kidston and Lang's inability to ever prove actual connections between axes and sporangia for *R. gwynne-vaughanii*. However, the recent discovery of sporangia attached to axes is a challenge to the theory

Life history biology of early land plants : Deciphering the gametophyte phase



Taylor, et al., (2005), Proc. of the Natl. Acad. Sci., USA 102: 5892-5897

Such a life cycle strongly supports the ideas of Pant (1962), Lemoigne (1968) and others that some primitive vascular plants of the Lower Devonian had alternation of phases where gametophytic and sporophytes were isomorphic both being photosynthetic and containing a vascular tissue

Rhynia gwynne-vaughanii:	*Remyophyton delicatum*
Rhynia (Aglaophyton) major:	*Lyonophyton*
Horneophyton:	*Langiophyton mackiei*
Nothia:	*Kidstonophyton discoides* *Scidophyton,* *Calyculiphyton*

Evolution

- Zimmermann (1952):

Rhynia represents a primitive vascular plant morphology

by evolutionary ↓ modification of its parts

More highly evolved vascular plants with roots, stem, leaves, more complex vascular systems, and protected sporangia

Overtopping

❖Primitive dichotomously branched system where one branch of the pair produced by the apical meristem outgrows or overgrows the others

❖The main axes become the stems; subordinate or overtopped branches represent the beginning of leaves or lateral branches

Reduction

❖Branching systems resulting from overtopping may be pseudomonopodial. Extrapolation of the overtopping process accompanied by reduction of the laterals results in a branching system where there is a recognizable main stem, rachis or midrib

The process of reduction of an overtopped telome truss has been used by Zimmemmann to explain the origin of leaves of the Lycopsida, Sphenopsida as well as the needle like leaves of conifers

Planation

Shift from a 3-dimensional pattern to one in which the branches occur in a single plane.

Webbing

Infilling with photosynthetic and other tissues between the planated branches resulted in a flattened leaflike structure with a dichotomously veined lamina.

Syngenesis

❖A closed or reticulate venation pattern in leaves of some ferns, occasional gymnosperms, and many angiosperms resulted from syngenesis or lateral fusion of the dichotomizing veins of the primitive leaf to form the anastomoses characteristic of venation patterns of the reticulate type

❖Tangential fusion resulted in the formation of synangia when fertile trusses with their terminal sporangia became reduced.

❖Complex anastomosing vascular systems in stems were produced as a result of syngenesis of vegetative telomes and mesomes

Recurvation

Fertile or vegetative telomes may been inward towards an axis. An example would be the inward projecting sporangia on a sporangiophore of *Equisetum*

Figs. Stewart W. N. & Rothwell G. W. (1993). Palaeobotany and the evolution of plants.

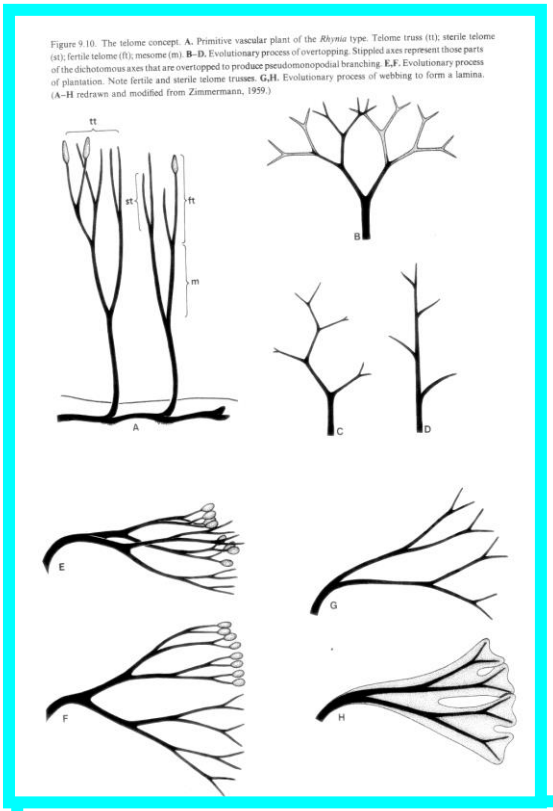


Figure 9.10. The telome concept. **A.** Primitive vascular plant of the *Rhynia* type. Telome truss (tt); sterile telome (st); fertile telome (ft); mesome (m). **B–D.** Evolutionary process of overtopping. Stippled axes represent those parts of the dichotomous axes that are overtopped to produce pseudomonopodial branching. **E,F.** Evolutionary process of plantation. Note fertile and sterile telome trusses. **G,H.** Evolutionary process of webbing to form a lamina. (**A–H** redrawn and modified from Zimmermann, 1959.)

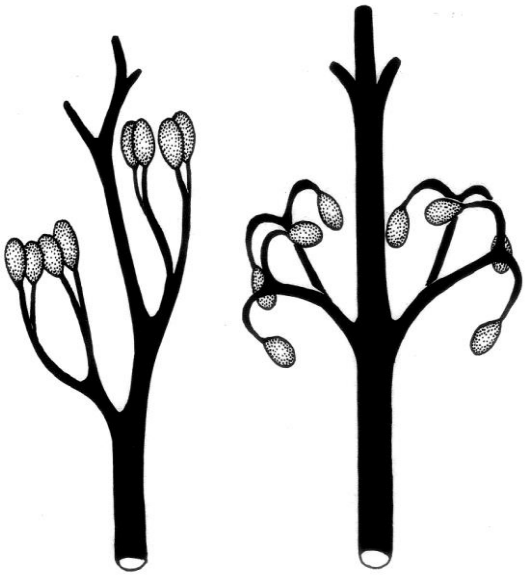


Figure 9.12. Evolutionary process of recurvation of fertile telomes so that the terminal sporangia are directed toward the axis. (Redrawn from Zimmermann, 1959.)

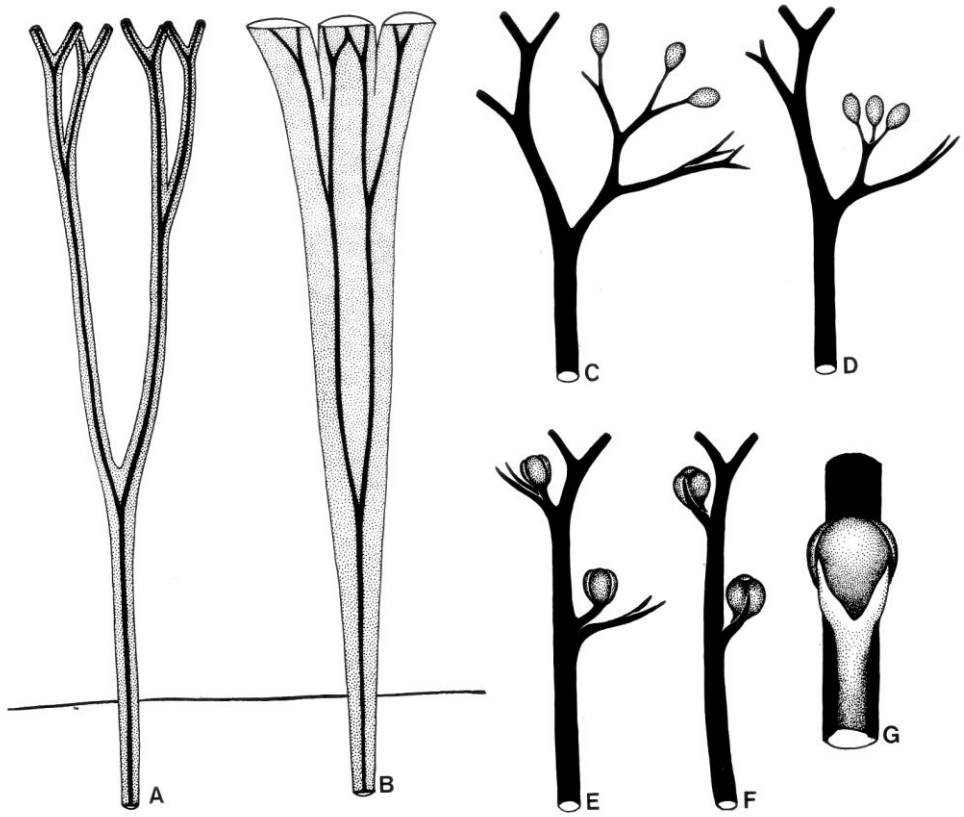


Figure 9.11. The telome concept continued. **A,B.** Evolutionary process of syngenesis, the tangential fusion of telomes and mesomes. **C–G.** Series of steps using the evolutionary process of the telome theory to depict the origin of the *Psilotum* synangium. (**A,B** redrawn and modified from Zimmermann, 1959; **C–G** redrawn and modified from Emberger, 1944.)

after that again a landmark evolutionary event… …

DEVELOPMENT OF FIRST FOREST

Reconstruction of a Carboniferous period (~340 MYA) swamp forest

Evolution of non vascular land plants

Between the first occurrences of prokaryotic algal like plants (Cyanobacteria) in the Precambrian and the first well documented terrestrial plants there is a gap of about 3 billion years. This delay in colonization of terrestrial habitats has been related to oxygen levels in the palaeo-atmosphere and specifically to the lack of a sufficient ozone shield to protect terrestrial organisms from UV radiation, which acts as a strong mutagen in organisms. In considering the history of early terrestrial plants, it is essential to distinguish between the first authentic vascular plants and the more primitive land plants that undoubtedly preceded them. In the search for the most primitive vascular plant, some palaeobotanists have called attention to certain enigmatic organisms that they believe might represent early land plants or even progenitors of the earliest vascular plants – earlier than late Silurian. Some form represented by sheet of cells, had tubes with annular or helical thickenings that resemble tracheids. Also thick walled resistant spores with tri-radiate ridges have been described, but bryophytes or some extinct algae could have produced this type of spore.

Isolated cuticle fragments, spores and tubes are found in lower to middle Ordovician rocks and extend through the Silurian into the Lower Devonian. Although even less is known about these fragments than the enigmatic fossils, they constitute an important source of

palaeobiological data because they occur at a point in geologic time that coincides with the origin of a terrestrial biota. The presence of land plants with a bryophytic level of organisation as early as the Ordovician is supported by both palaeobotanical and molecular evidence. The first evidence of plants on land comes from spores of Mid-Ordovician age (early Llanvirn, ~470 million years ago). These spores, known as cryptospores, were produced either singly (monads), in pairs (dyads) or groups of four (tetrads), and their microstructure resembles that of modern liverwort spores, suggesting they share an equivalent grade of organization. They are composed of sporopollenin – further evidence of an embryophytic affinity. A close examination of algal spores shows that none have trilete spores, either because their walls are not resistant enough, or in those rare cases where it is, the spores disperse before they are squashed enough to develop the mark, or don't fit into a tetrahedral tetrad.

The earliest megafossils of land plants were thalloid organisms, which dwelt in fluvial wetlands and are found to have covered most of an early Silurian flood plain. They could only survive when the land was waterlogged. There were also microbial mats. Once plants had reached the land, there were two approaches to dealing with desiccation. The bryophytes avoid it or give in to it, restricting their ranges to moist settings, or drying out and putting their metabolism "on hold" until more water arrives. Tracheophytes resist desiccation. They all bear a waterproof outer cuticle layer wherever they are exposed to air (as do some bryophytes), to reduce water loss, but—since a total covering would cut them off from CO_2 in the atmosphere—they rapidly evolved stomata, small openings to allow gas exchange. Tracheophytes also developed vascular tissue to aid in the movement of water within the organisms, and moved away from a gametophyte dominated life cycle. Vascular tissue also facilitated upright growth without the support of water and paved the way for the evolution of larger plants on land. The establishment of a land-based flora caused increased accumulation of oxygen in the atmosphere, as the plants produced oxygen as a waste product. When this concentration rose above 13%, wildfires became possible. This is first recorded in the early Silurian fossil record by charcoalified plant fossils. Apart from a controversial gap in the Late Devonian, charcoal is present ever since.

Some fossils with land adaptive features have been recovered from various strata and it is concluded that the structures do not represents the remains of ancient vascular plants but are parts of other plant forms and known as enigmatic plant fossil. e.g.:

Protosalvinia **(Devonian):** Thalloid plant body. Botanical affinity unknown so it is considered as transitional non-vascular plants. The plant body developed depression within which thick walled spores present in a tetrahedral manner. Biochemical evidences has pointed towards the affinity with brown algae.

Pachytheca **(Late Silurian–Early Devonian):** The plant having affinity with algae consisting of single, sphaerical body, 1.5-5.0 mm in diameter with outer and inner zone. The inner zone consists of multicellular filament oriented randomly whereas in outer zone radially arranged filament present.

Parka **(Early Devonian):** Dorsiventral thallus like plant, circular to ovoid upto 5 cm with clustered spores. The plant body cellular, upper and lower epidermis parenchymatous attached with a ventral holdfast. Spores appear cuticularized, 28-34 um in size, interpreted to be in a sporangium at the surface.

Spongiophyton **(Middle Devonian):** Thalloid, branching plant thought to have a habit similar to modern liverworts that is typically preserved as a cuticular sheath with a porate (200-300 um raised areas with holes) and non porate surface. Porate cuticle are three times thicker than non porate one. There is no evidence of vascularisation. Recently Stein et. al. (1993) has suggested that these plants are lichens.

Nematothallus **(Early Silurian–Devonian):** Plant body thalloid, characterized by pseudoparenchymatous cells comprised of filament and surrounded by thick cuticle. Branching tubes with secondary wall thickenings present in the plant body that have the appearance of tracheids. Some produced thick walled spores.

Prototaxites **(Silurian and Devonian):** Largest representative of Nematophytes known to date. It occurs in the form of compressed or silicified axes, termed as pseudostem, some of which up to 1.25 m in diameter and more than 8m long. Outer surface is smooth or mildly

ribbed. Thin section show a pseudoparenchymatous or plectenchumatous internal organisation constructed of longitudinally oriented tubes of three different types:

- Skeletal hyphae, which are thick walled, large, long, straight or flexuous, aseptate or unbranched.

- Generative hyphae, which are large and thin walled, septate with an open and occluded core and profusely branched and

- Binding hyphae, which are small, thin walled with a pore in the septum, and profusely branched. Arrangement of the tissue made up of these hyphae, along with the presence of well defined borders of growth increments marked by increased tissue density, suggests some type of periodicity in growth. In addition to the tubes, numerous lacunae are scattered throughout the pseudoparenchyma.

Nematoplexus **(Lower Devonian):** It consists of a meshwork of intertwined aseptate and septate tubes of two or three different sizes. Most of the larger aseptate tubes have annular or helical thickening; some of the narrow smooth walled tubes display distinct septa with a centrally located elliptical aperture or pore.

7. Early vascular plants to early spore producing trees (arborescent pteridophytes & progymnosperms): Geologic time, environment, advancement in plant adaptive features for land dwelling with fossil evidences.

Geologic Time:

Mid Devonian to Late Carboniferous (~395-290 Ma)

Environment:

Cooler, drier climates with extensive glaciations in the high latitudes of the southern hemisphere **which was altered from warm, humid, ice free condition;**

Although continental climates became generally drier, fossil evidence suggests that **precipitation became high in a narrow equatorial belt;** *this was due to the effect of active continental plate movement and collisions causing extensive mountain building episode at tropical latitude which had a significant impact on global circulation and precipitation*

High year round wetness in this tropical belt is thought to be partially responsible for the development of extensive lowland swamps, resulting in the formation of coal deposits in eastern North America, western Europe, and parts of Russia during the Carboniferous.

Factors affecting global climate during Mid Devonian to Late Carboniferous (~395-290 Ma)

ACTIVE PLATE MOVEMENT

Continental blocks that had formed the Gondwana and Laurussian groups during the Silurian moved northwards, resulting in the formation of supercontinent PANGEA by 300 Ma;

During E. to M. Devonian (~395-360 Ma)	• The South Pole was situated either over Central America or South Africa. This would have resulted in extensive warming and retention of heat at the pole
From the late Devonian (~360)	• South Pole became much closure to the coast and would have caused significant global cooling- how?
	• The larger heat capacity of the water would have suppressed the magnitude of warming on the adjacent land masses, and resulting summer temp. might therefore not have reached above freezing in the high latitudes of the southern hemisphere. This would have led to the formation of a large southern hemisphere ice sheet, with increasingly cool and arid conditions in continental interiors

Factors affecting global climate during Mid Devonian to Late Carboniferous (~395-290 Ma)

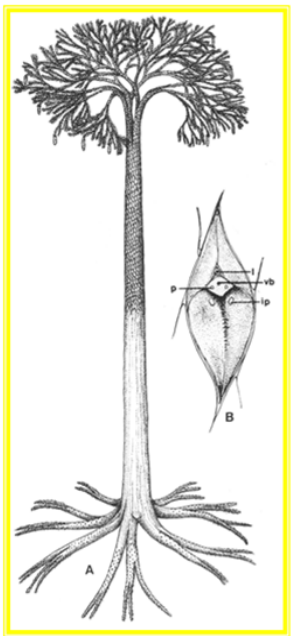
WIDESPREAD COLONIZATION OF THE LAND BY PLANTS

Atmospheric CO_2 in the Early Devonian (~395 Ma) was 8-9 times higher than present day which had a considerable effect on global warmth through an enhanced greenhouse effect;

Between 360-286 Ma modeling estimates that atmospheric CO_2 levels plummeted from 3600 p.p.m to 300 p.p.m., such that by the late Carboniferous, levels of atmospheric CO_2 were comparable to those of present day; this declining atmospheric CO_2 conc. would also have made a significant contribution to the pattern of global cooling evident from approximately 360 million years onwards

Advancement in Plant Adaptive Features

The plant fossil record indicates major innovations were occurring in land plant morphology-



1. Arborescent Habit

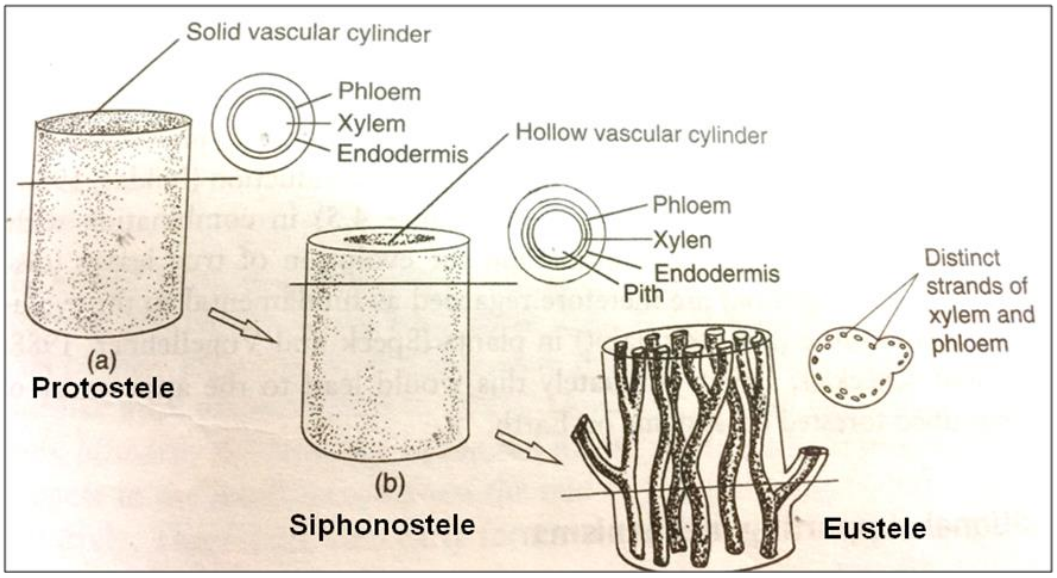
Terrestrial plant were getting much bigger and acquiring more refined reproductive structures. In order to attain greater height, most important adaptation- progressively more complex form of stele

2. Stelar Evolution

Evolution of more advanced vascular system for the transport of water and nutrients:

Siphonostele (~395 Ma-Lower Devonian) – fossil filicopsida, lycopsids and some sphenopsids

Eustele (~380 Ma-Middle Devonian)– first occurred in progymnosperms- the precursors to the seed plants



3. Additional supporting mechanisms

Thickening of trunk through the growth of wood – results from the growth of secondary xylem and phloem from lateral meristematic zones (cambium).

However, palaeobotanical evidence indicates that in other early trees, thickening of the trunk was achieved by –

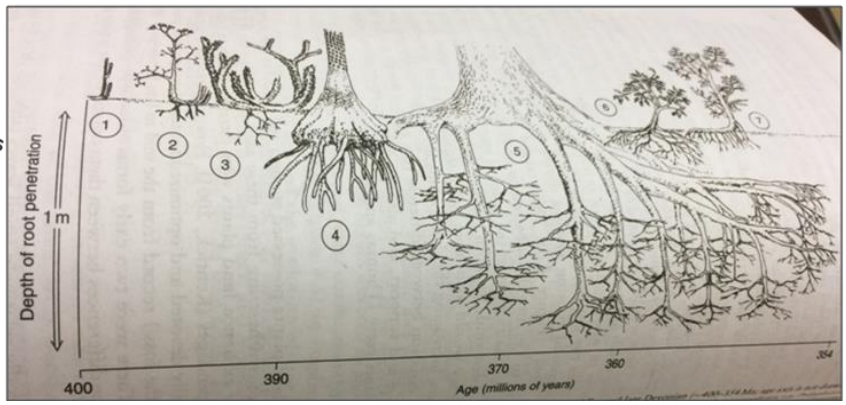
- development of secondary cortex and greatly thickened stem as a result of numerous leaf bases (arborescent Lycopsid - *Lepidodendron*) or
- development of abundant mantles of roots that were thickest at the base of the trunk (fossil fern – *Psaronius*)

4. Advanced rooting systems

Palaeosols (fossil soil) indicates the development of at least seven morphologically distinguishable below ground rooting system during the Devonian – Carboniferous ranged from highly branched systems extending upto 1 m into the substrate to small annulated – segmented roots from which long root hairs grew at the point of segmentation.
These rooting systems would have been vital, for both support and the increased uptake of water and nutrients

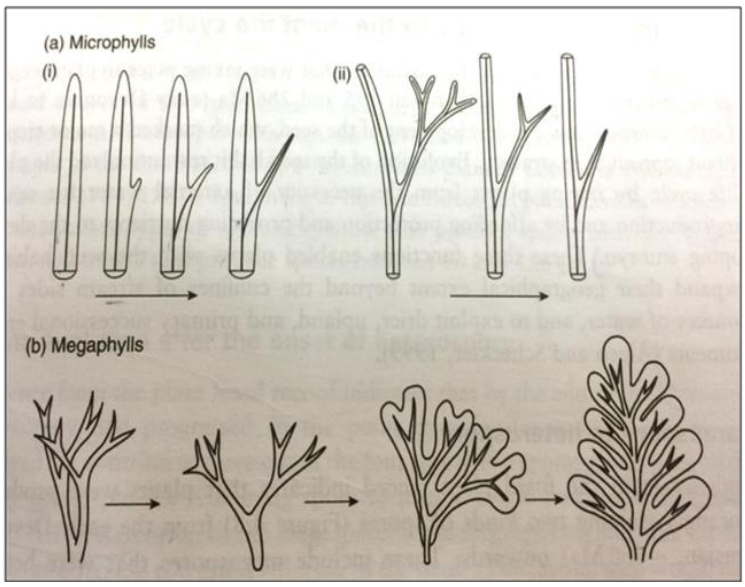
The variation between different root types is thought to reflect the need for anchorage and support, and also the different environmental conditions in which the plants grew

1. *Rhyniosida*
2. *Trimerophytes*
3. *Herbaceous lycopsids*
4. *Tree Lycopsids*
5. *Progymnosperms*
6. *Early Gymnosperms*
7. *Filicopsids*



5. Leaves

First 40 million years of the existence of land plants were leafless or had only small, spine like appendages
Mid-Late Devonian (~390-354 Ma) - True leaves (microphylls, megaphylls) appeared



Fossil record of Earliest trees

Earliest arborescent forms of plants - appeared in the Middle Devonian (~380 Ma)

Earliest spore-producing trees :

Lycopsids - *Lepidodendron (Mid Devonian – Permian* ~390-248 Ma*)*

Sphenopsids – *Calamites* (Carboniferous – Permian: ~354-248 Ma)

Filicopsids – *Psaronius* (Early Carbonifeorus: ~360 Ma)

Progymnosperms (gymnosperms like stem anatomy but reproduce by spores, like ferns and lycopods) – *Archaeopteris* (mid Devonian – Early Carboniferous: ~390-340 Ma)

Arborescent Lycopsids (Lepidodendrales)

Arborescent lycopsids lived from the Late Devonian through the Permian, with a zenith and a major extinction in the Middle-Late Pennsylvanian

In most reconstructions of Carboniferous flora, arborescent lycopods (Lepidodendrales) predominate with record of trunk fragments, abscised leaves, and cones

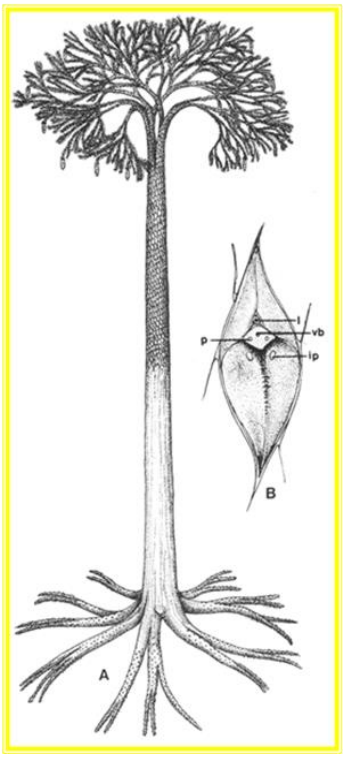
Arborescent lycopsids that dominated peat-forming and clastic swamps of the Carboniferous of Europe and North America are mostly members of Lepidodendrales which are fossilized by the variety of preservation styles (e.g., coal ball permineralizations, compressions/impressions, casts/molds)

Characteristic features

- The bases of the upright trees can be attached to the supporting and anchoring stigmarian axes.
- Dichotomizing branches of young trees with grass like leaves.
- The bark shows the spirally arranged leaf cushions.
- An arborescent growth habit associates with cambial activity and the production of abundant secondary tissues: an important evolutionary innovation.

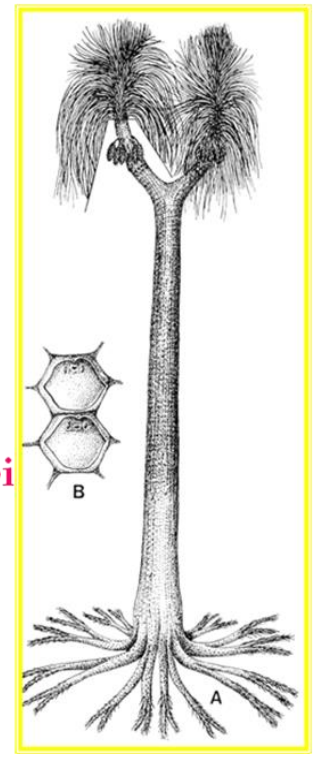
Advantage

- The greater size of these plants presents their leaves to maximum light energy
- Their spores shed from cones to wider distribution by air currents and gravity

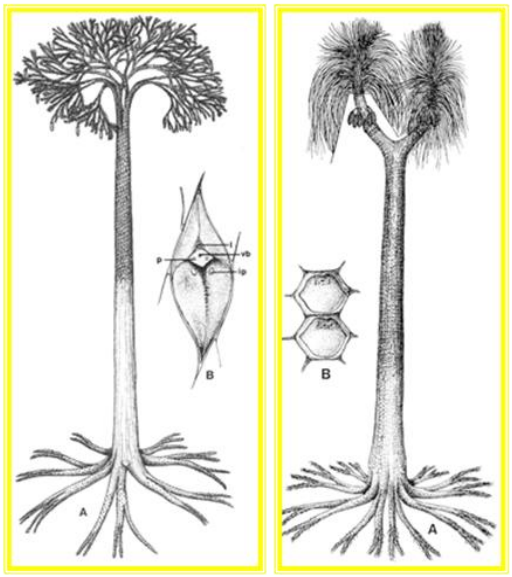


Some Genera

Lepidodendron: stem
Lepidophloios: stem
Sigillaria: stem
Lepidophylloides: leaves
Lepidostrobus:
free sporing mono/bi sporangiate cones
Lepidocarpon:
megasporangiate cones
Stigmaria: rhizophores
Lycospora: microspores



Reconstruction of an Arborescent Lycopod



- Bipolar growth : A main axis that grew and branched at both ends.
- Branches of the aerial part formed a 3-dimentional system of dichotomous or pseudomonopodial branches with spirally arranged leaves and terminal cones.
- Basal end also branched dichotomously to form the anchoring and water absorbing system comprised of rhizomorphs bearing spirally arranged roots.

❖Arborescent lycopsids began life as an embryo germinating from an indehiscent megaspore.

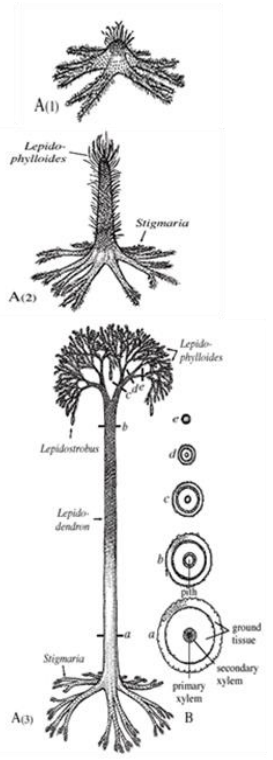
❖Early in life, the sporeling is characterized by a thick cortex surrounding a slender protostele which gave the young plant a stump-like appearance.
This pattern differs from tree-size seed plants, whose primary growth is mainly upward and girth expansion occurs over time by the accumulation of secondary xylem.

❖As the arborescent lycopsid grew taller, the cortex thinned somewhat, a pith was added to form a siphonostele, and secondary growth was initiated from a unifacial vascular cambium.

❖At some point in development, the apical meristem dichotomized and continued to do so at intervals.

❖However, past the point of first branching, axes began to thin, the pith decreased in diameter, cortex thinned and growth terminated in slender branch tips vascularized by only a handful of tracheids arranged in a protostele.

❖Commonly, the apical meristems differentiated into reproductive structures, making these plants monocarpic and determinant in their growth pattern.



Stem Genera

Lepidodendron, Lepidophloios and *Sigillaria*

❑Stem genera show raised leaf bases (also known as leaf "cushions") with a small leaf scar at the apex of the elevated cushion: the point of attachment of abscised leaves.

❑Within the leaf scar, there are three other scars: The middle scar representing the leaf's vascular trace, flanked by two parichnos scars.

❑Parichnos scars mark the position of parichnos tissue (parenchymatous with lots of air space between cells) within the leaf and stem which aided photosynthetic gas exchange much like the lenticels of modern trees (e.g., *Betula*).

❑Above the leaf scar, the position of the ligule can be noted which is marked by a small pit known as ligule pit.

Lepidodendron: Diamond shaped, taller than width, spirally arranged

Lepidophloios: Diamond shaped, wider than height, spirally arranged

Paralycopodites: Shape intermediate

Sigillaria: square or rectangular, with hexagonal leaf scars, vertically arranged

Anatomical feature of Stem Genera

❖An exarch siphonostele surrounded by secondary xylem.

❖Primary xylem is composed of metaxylem tracheids (scalariform) and have fimbrils traversing the apertures between adjacent wall thickenings.

❖The small protoxylem tracheids form vertical ridges at the periphery of the primary xylem cylinder. In cross section, the ridges appear as undulations or small radiating teeth of protoxylem, named the structure as corona.

❖The secondary xylem was produced by a unifacial vascular cambium. Unlike the vascular cambium of arborescent seed plants, the vascular cambium of a lepidodendrid did not produce secondary phloem.

❖A continuous band of cortical parenchyma immediately outside the secondary xylem.

❖Departing leaf traces and strands of primary phloem are embedded in this tissue.

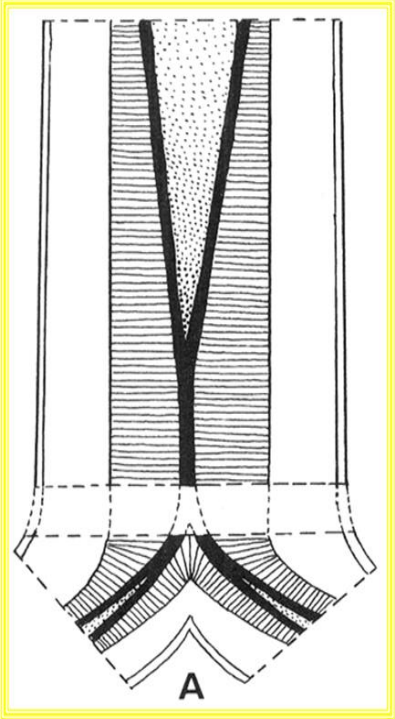
❖Cells of the cortical parenchyma can assume a meristematic function to produce cells of secondary cortex or the so called periderm. One of the distinctive features of this stem is the production of copius amount of this tissue, play major role for mechanical support of the plants.

Epidogenesis

The primary vascular system at the base of a mature tree was protostelic

The primary cylinder became medullated, with the resulting pith increasing in size at higher levels in the trunk.

This aspect of development, where there is progressive enlargement and increase in complexity of the primary vascular system, has been termed epidogenesis.

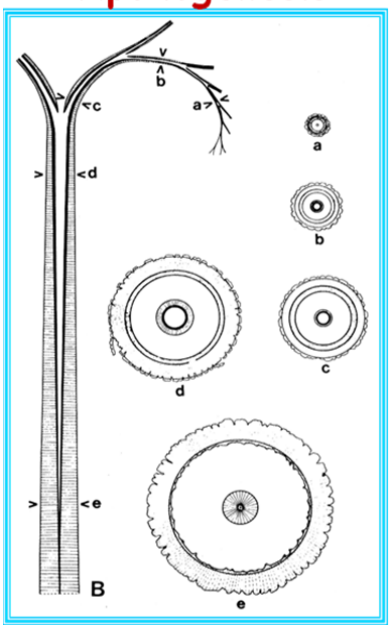


Where dichotomous branching occurred in the aerial axes, it is apparent that the resulting branches were smaller than the axis from which they are formed. Thus, with each successive dichotomy, there was an ever-decreasing size of branches.

This is reflected in a decrease in size of the stele so that in penultimate branches, the vascular cylinder is only slightly medullated while in the ultimate branches only a tiny protostele remains.

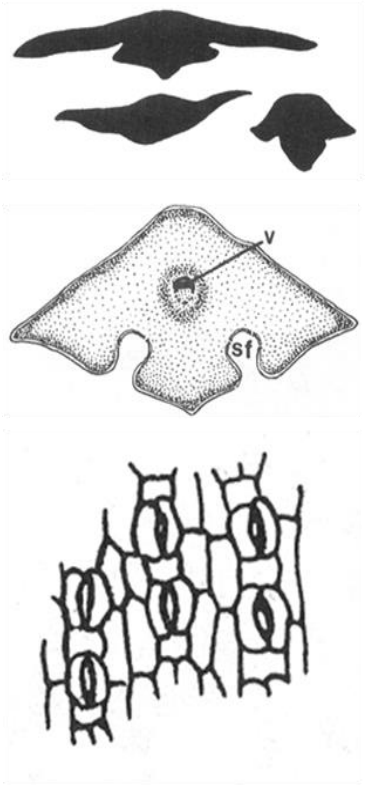
Concomitant with the diminution of the primary vascular system was a decrease in the amount of secondary xylem and secondary cortex. This type of development, which involves the production of progressively smaller and simpler anatomy, is called apoxogenesis.

Apoxogenesis



Leaves

- Two sizes of leaves: short, thick scale-like leaves & slender grass-like leaves up to one meter long.
- The grass-like leaves are commonly found as dispersed organs, while the scale-like leaves commonly are preserved attached to their axes.
- Both types are known as *Lepidophylloides* although the name refers primarily to the dispersed grass-like leaves.
- The grass-like leaves were mostly associated with juvenile, unbranched individuals, where scale-like leaves grew on branches after branching had begun.
- In the detached, compressed state, one cannot be sure whether a lepidodendrid type leaf belongs to *Diaphorodendron*, *Lepidodendron*, *Lepidophloios*, *Sigillaria*, or some other arborescent lycopod. For this reason, a form genus, *Cyperites*, has been adopted.



T.S: 2 furrows on lower surface containing several rows of stomata parallel to the long axis of leaf, well developed hypodermis, thin walled mesophyll surrounding the single sheathed (composed in part of parenchymatous transfusion cells with reticulate 2ndary wall) vein

Rhizomes or Rhizophores (*Stigmaria*)

❖"Rootlets" are borne in a spiral on the underground rhizome.

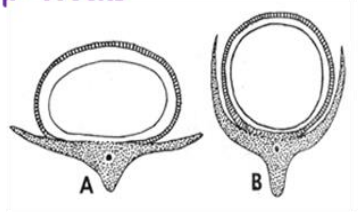
❖Anatomy and morphology of *Stigmaria* has been homologized with stem structures, which leads to the interpretation that the shoot meristem in arborescent lycopsids bifurcates early in development.

❖This differs from the bipolar development of seed-plant embryos and means that it is technically inappropriate to call *Stigmaria* a "root" or its appendages "rootlets" because they are anatomically, morphologically and developmentally distinct from underground structures of seed plants.

In arborescent lycopsids, free-sporing heterospory clearly precedes the endosporic condition and enclosure of the megasporangium in sporophytic tissue

Sporangia (some with spore) are born on modified leaves called sporangiophores.

In *Lepidocarpon*, the megasporangium is surrounded by the sporangiophore, which has grown to almost completely enclose the sporangium like ovule of seed-plant.



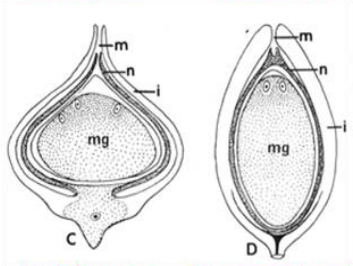
Lepidocarpopsis lanceolatus *Lepidocarpopsis semialata*

But these are not true ovules, although moving toward the "seed habit" by

(1) developing heterospory

(2) reducing the number of megaspores to one

(3) beginning to surround their megasporangia in a protective layer of tissue



Lepidocarpon lomaxi Gymnospermous ovule

Microspores and Microsporangia

- The microspores of *Lepidodendron and Lepidophloios:* dispersed spore genus *Lycospora*
- *Lycospora* spores are trilete and zonate
- Strobili bearing microspores are called *Lepidostrobus*
- *Flemingites* has been proposed for cones containing both microspores and megaspores

Megaspores and Megasporangia

- Dispersed megaspores of the arborescent lycopsids commonly go by the name *Cystosporites*
- Cones that produce only megaspores are called *Lepidocarpon*
- Arborescent lycopsids had not evolved bipolar embryo independently, rather, their embryo apical meristem bifurcates at a very early stage of development.
- This also accounts for the anatomical and morphological similarity between above-ground shoot and stigmarian system.

Variations in reproductive structure

Genus	Cone Type	Name of cone megasporangiate cone	Name of cone microsporangiate cone	Name of spore
Lepidodendron	Monosporangiate	*Achlamydocar-pon takhtajanii*	*Achlamydocar-pon takhtajanii*	*Lycospora*
Paralycopodi-tes	Bisporangiate	*Flemingites*		*Cystosporites*
Diaphoroden-dron	Monosporangiate	*Achlamydocar-pon varius*	*Achlamydocar-pon varius*	*Capposporites*
Lepidophloios	Monosporangiate	*Lepidocarpon*	*Lepidostrobus*	*Lycospora*
Sigillaria	Monosporangiate	*Mazocarpon oedipternum*	*Mazocarpon oedipternum*	*Crassispora kosankei*

Mazocarpon: Monosporangiate cone of *Sigillaria*

- Monosprangiate
- A distinct columella is present within the sporangium.
- The lower heal of the sporophyll is very well developed, but the upper part of the distal lamina is short and barely cover the distal end of sporangium.
- Entire cone was shed at maturity unlike *Lepidocarpon* and *Achlamydocarpon* where only sporophyll were shed.

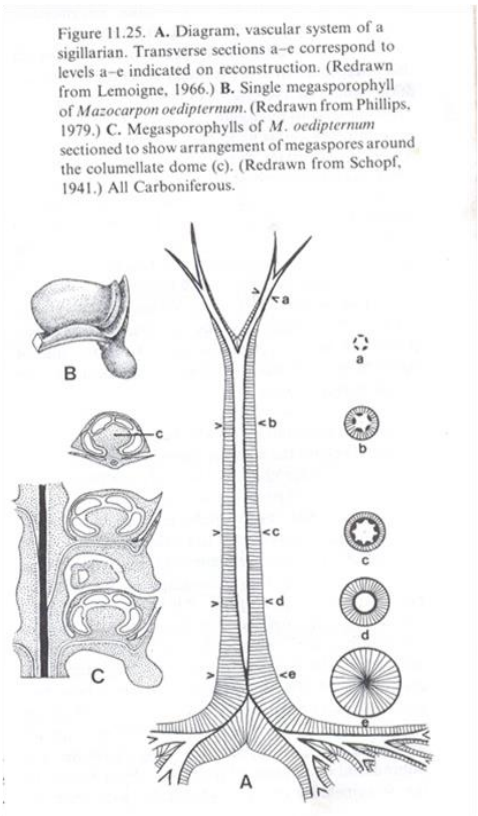


Figure 11.25. **A.** Diagram, vascular system of a sigillarian. Transverse sections a–e correspond to levels a–e indicated on reconstruction. (Redrawn from Lemoigne, 1966.) **B.** Single megasporophyll of *Mazocarpon oedipternum*. (Redrawn from Phillips, 1979.) **C.** Megasporophylls of *M. oedipternum* sectioned to show arrangement of megaspores around the columellate dome (c). (Redrawn from Schopf, 1941.) All Carboniferous.

Evolution proceeds in the elaboration of the reproductive structure

1. The most notable features is the decrease in the no. of megaspores per megasporangium.
   Flemingites noii: 100 of megaspores per megasporangium
   Flemingites foliaceous: only 4 megaspores in each megasporangium.
   Several other species of *Flemingites* : represent the intermediate no.
   Lepidocarpon: only a single megaspore in each megasporangium.

2. Monosporangiate Cone (*Achlamydocarpon, Lepidocarpon*) excepting *Fleminzytes* and Bisporangiostrobus which produce bisporangiate strobili in the same cone.

3. The organization of sporophyll, closely approximates the function of integument in seed plant.

4. The endospory has evolved along with the development of protecting covering around the megasporangium.

5. Unlike the sporophylls of bisporangiate *Flemingites,* the megasporophyll units in *Lepidocarpon* and in others are shed from the cones and are modified into a sail boat like structure to facilitate efficient dispersal and fertilization.

6. Evolved structures in both *Achlamydocarpon* and *Lepidocarpon* gave protection to the developing megaspore and megagametophyte. The megasporangiaum wall is several layers thick, the cells are thin walled.

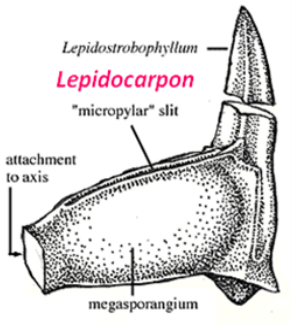
Lepidocarpon is well on the way, evolutionary speaking to fulfilling the characteristic of an ovule structure but it is not true ovule- why?

Megasporangium dehisce to expose the archegonia on the surface of mega-gametophyte.

Achlamydocarpon

Fleminzytis

The so called micropyle is an radially elongated slit and so the flagellated sperms has direct access to female reproductive organ



True integument is formed by modification of telome truss but here 'integument like structure - covering of megasporangium' is formed from prolongation of sporophyll.

So, unlike gymnospermous ovule megasporangium of *Lepidocarpon* is always dehiscences. Siphonogamy is not present. True micropyle and integument are absent

DiMichele (1981) speculated that most speciation in arborescent lycopsids took place outside what he considered to be the evolutionary refugia of the coal swamps. Since lycopsids today are represented only by herbaceous species, all arborescent lycopsids were ultimately "unsuccessful" – why?

CAUSES FOR EXTINCTION

❖Functionally the copious amount of secondary cortex must have provided support for stems and branches with their leaves and cones. But, the amount of support provided by the relatively small amount of xylem tissue would not have been sufficient for trees of such magnitude. So the Carboniferous arborescent lycopods did not have sufficient hardiness to withstand the rigorous climate that prevailed in Permian time due to tremendous glaciations.

❖Moreover, some dry spells in the Upper Carboniferous were unsuitable for their growth. Accordingly, they could not tolerate that dry spells as they inhabited typically in swampy condition.

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8. Early spore producing trees to early seed producing trees (gymnosperms): From isospores to free sporing heterospores, origin of ovule, hydrasperman reproduction with fossil evidences.

Further Adaptations to the Plant Life Cycle

Development of the seed

It marked a major step in plant reproductive strategy

Geological Time for morphological innovation towards seed formation

Early Devonian to Late Carboniferous (~395-286 Ma)

Advantages

Evolution of the seed habit revolutionized the plant life cycle

*by freeing plants from the necessity of external water for sexual reproduction

*by affording protection against predators and desiccation

*by providing nutrients to the developing embryo

Step 1:

One of the most evolutionary trends in the development of seed bearing plants was noticed in the

Transition from homosporous to heterosporous plants

Homospory (sporangia producing one kind of spores) to Heterospory (sporangia producing two kinds of spores)-

from the early Devonian (~400 Ma) onwards

Fossil evidences indicate that

Megaspores size were approx. between 150 and 200 µm in dia
Microspores size were usually <50 µm in dia

It is hypothesized that

Larger spores of heterosporous plants were the precursor of ovules, and the small spores, the precursor to pollen

Reasons behind the formation of megaspores

Most widely accepted theory is -

Mutation *(evidence from the geological record supports this theory by recording dispersed spores of two size classes (anisospory) which indicates a gradual increase in occurrence of heterospory through Devonian)*

Stages in evolution of heterospory

**Decrease in no. of spores in some sporangia*
**Increase size in remaining spores*
**Spore content of some sporangia remaining constant size and number of spores*
**Change from monoecious to dioecious gametophytes*
**Change from exosporic to endosporic gametophytes*

First evidence of free sporing heterosporous plant:

Chaleuria (this Mid. Devonian plant had two types of sporangia, those that produce spores between 30 and 48 µm and others that produce spores between 60 and 156 µm)

Step 2:

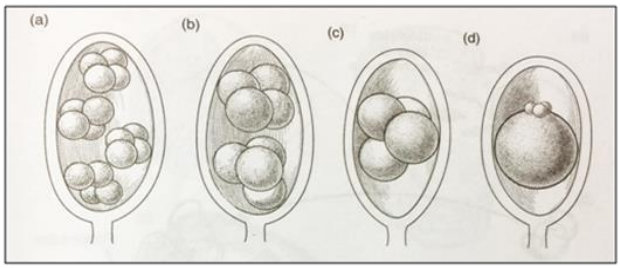
Ovule evolution after the onset of heterospory-

By the end of Devonian, heterospory had progressed to the point that-

development of megaspores involved the abortion of three out of four spores in a sporangium, with all the energy going into the remaining spore to form a single functional megaspore

Ovule evolution occurred by two steps-

*Retention of the megaspore in the megasporangium



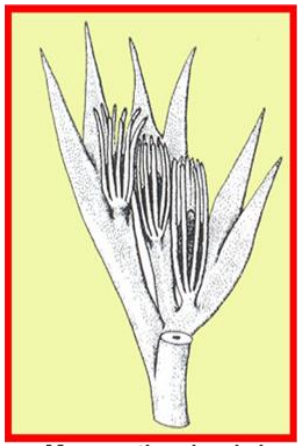
* Development of integument i.e. seed coat

Development of integument (seed coat) – Late Devonian (370-354 Ma)

Analysis of Late Devonian fossil megaspores revealed an outer protective coating which could have protected the megasporangium from desiccation and attack.

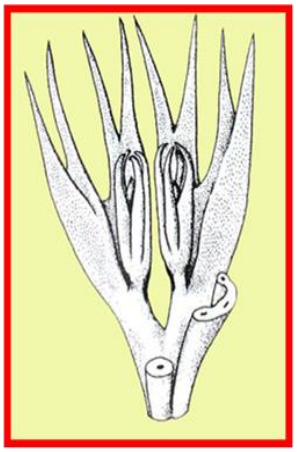
Seed coat probably resulted from the envelopment of the megasporangium by sterile telome trusses which fused around the megasporangium to encase it, so forming an ovule

Fossil Evidences: During Upper Devonian



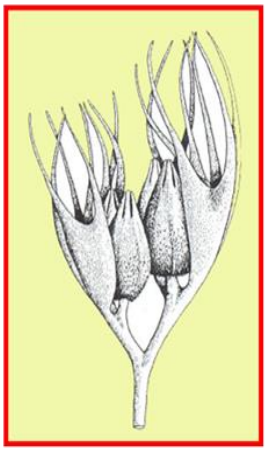
Moresnetia zalesskyi

Cupule containing preovules with one quarter of cupule removed *(fused only at the base)*



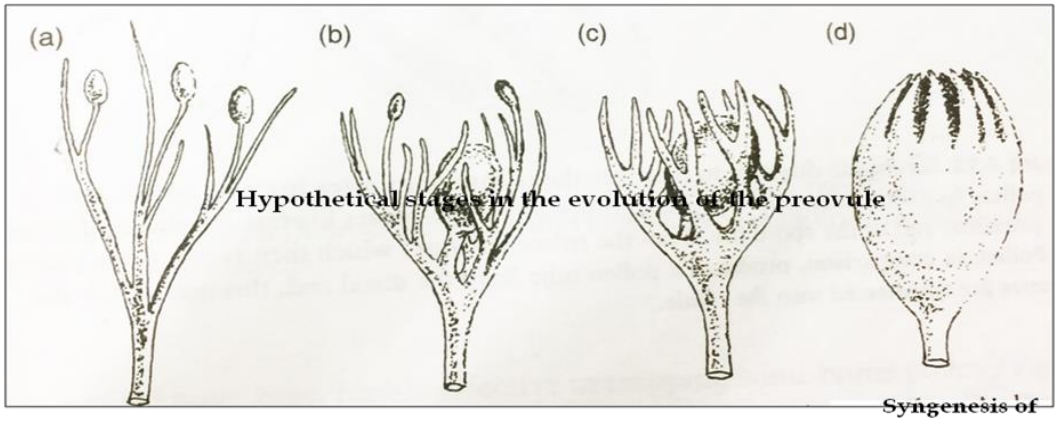
Elkinsia polymorpha

Cupule containing preovules showing forking branching pattern *(fused to basal 1/3rd)*



Archaeosperma arnoldii

Restoration of Cupule Complex (*greatest amount of fusion)*

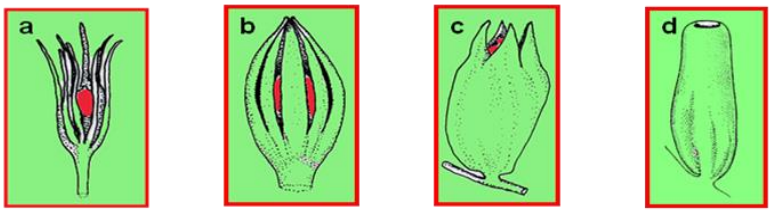


Telome trusses – fertile & sterile

Heterangy & Heterospory: Micro & Mega- sporangia

Dioecious habit: Enveloped Megasporangium by sterile telome

Syngenesis of sterile telome around megasporangium to form integument



Fossil Evidences: During Early Carboniferous

Fossil Evidences: a. *Genomosperma kidstonii* **– unfused telome; b.** *G. latens* **– partial fusion of telomes; c.** *Eurystoma angulare* **– fusion of telomes, except upper 3rd of preovule; d.** *Stamnostoma huttonense* **– complete fusion of telomes to form integument and micropyle**

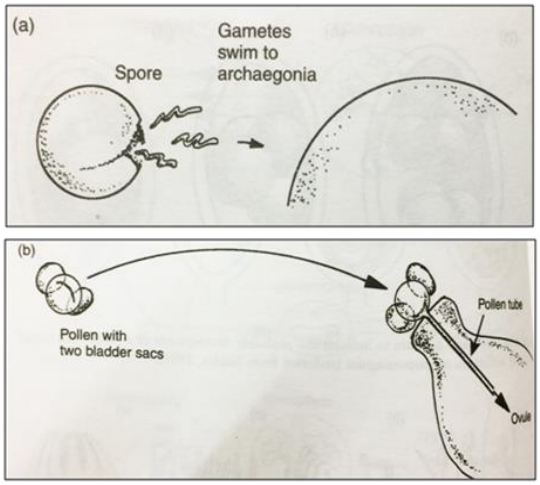
Step 3:

Evolution of pollen grains (microspores-Prepollen-Pollen)- Upper Devonian (~364 Ma) onwards

Microspores: produce endosporic gametophytes that liberate flagellated gametes from the proximal end of the spore, which then swim to the archegonia for fertilization.

Pollen (Late Carboniferous ~310 Ma) – produce a pollen tube from the distal end, through which the gametes are transferred directly into the ovule

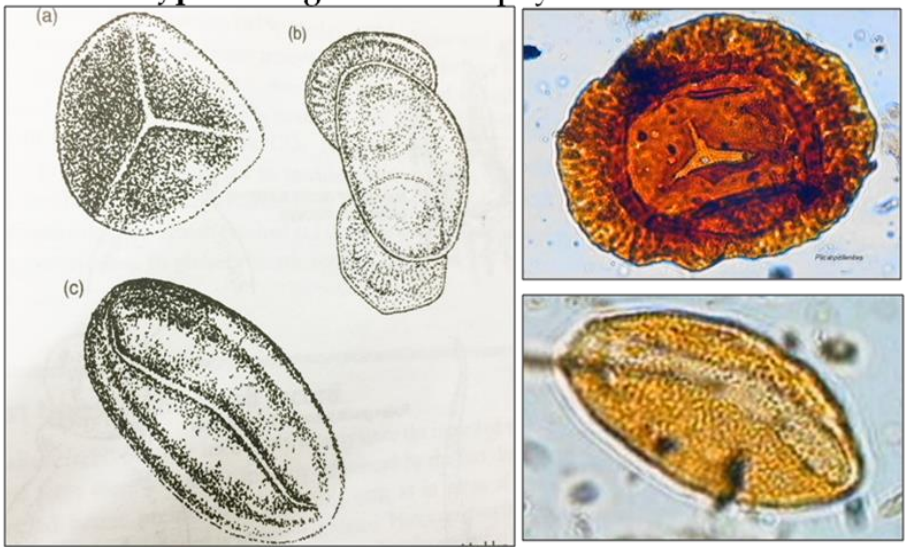
Earliest pollen grains evident in the fossil record termed as prepollen which are halfway between spore and pollen.



Prepollen– morphological features as like of spores, such as presence of monolete or trilete scar on proximal surface (characters of isospores) but absence of a preformed germination region on the distal surface of the grain (essential feature of pollen), but evidence suggests that germination occurred on or very near to the opening of the megasporangium e.g. *Monoletes* (Medullosan prepollen); *Crossotheca* (Lyginopterid)

By the late Carboniferous – Four common morphologically distinct **pollen and prepollen types** were present in the fossil record –

a. Small trilete forms of the Lyginopterid seed fern
b. Saccate grain of Cordaits and Conifers
c. Large monolete grains of the Medullosan pteridosperms
d. Monosaccate type belongs to Callistophytalean seed fern



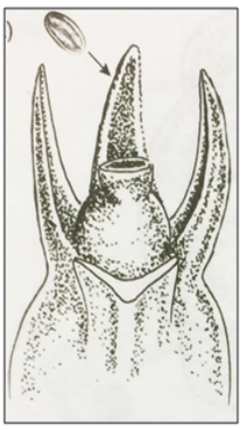
Step 4: Development of pollen reception mechanisms –

with the encasement of ovule in the seed coat, it would have been necessary to develop a mechanism by which the pollen could reach the egg contained within the ovule, for germination.

There is evidence for evolution of various elaborate structure at the neck of the megasporangium – in effect, these acted as a funnel to channel the pollen towards the ovule.

Pollen reception in preovules is the concept of hydrasperman reproduction – first described by Long (1961) from Early Carboniferous strata – he assigned these specimens to a new genus *Hydrasperma*

***Hydrasperma* & most other archaic gymnosperm preovules contained a characteristic feature called lagenostome**

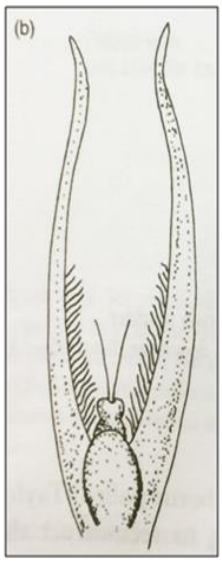


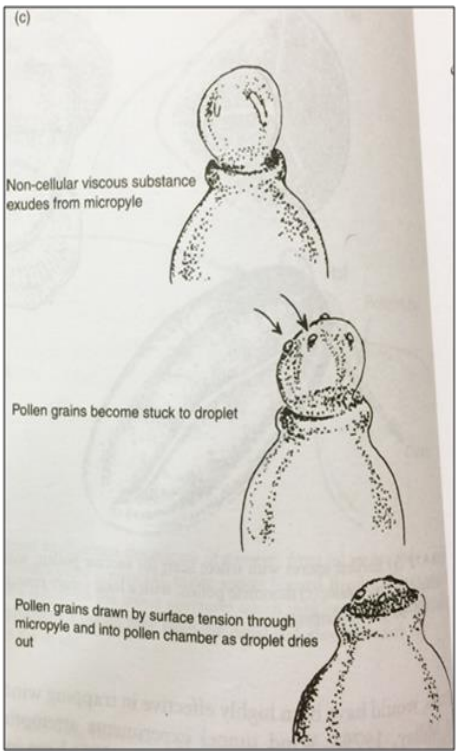
Lagenostome – a inverted funnel, ring, or cup like str. rests on top of a hemispherical pollen chamber formed by the lysigenous disintegration of nucellar cells at the apex.
Pollen chamber: A membranous floor with a prominent column
Inverted funnel shaped: Physostoma elegans
Cup shaped: Eurystoma angulare

This pollen receiving structure, in combination with the lobes of the seed coat and cupules, would have been highly effective in trapping wind-borne pollen

Some of the earliest ovules had much simpler mechanisms for attracting pollen -

Presence of hairs that lined both the inside and outside of the integumentary lobes





Pollination droplets (*sticky material that exuded from tentacle like projections situated on the outside of the integument*) **to which pollen became stuck**

e.g. *Callospermarion* **type ovule contained** *Vesicaspora* **type of pollen grains germinated to form branched pollen tube**

Extant seed-

***Seed coat is fully enclosed, resulting from the fusion of the integumentary lobes with each other and the nucellus.**

***There is clearly defined hole in the top of the seed called the micropyle, through this that pollen or the pollen tube extends to deposit the male gametes near or in the archegonia**

***In extant gymnosperms, e.g., a sugary pollination drop exudes from the micropyle in which wind borne pollen becomes trapped. As the pollination drop is withdrawn back into the nucleus, the pollen is taken in with it**

Major Emergences of the Seed Plants

During the Period Permian (290-248 Ma) a major transformation occurred in global vegetation, with the emergence and widespread radiation of the seed plants

Although two seed plants groups evolved earlier, the new seed plant groups to emerge between 280 and 260 Ma included the cycads, ginkgos, bennettites and glossopterids

This time of major floral evolution occurred against a backdrop of increasing global warmth and aridity – often described as a change from a global 'icehouse' to a global greenhouse

Evolution and radiation of the conifers occurred between ~248-206 Ma (Triassic). Nine conifer families that radiated at this time still have widespread global coverage today

By the early Cretaceous, global floras, for the first time, contained a significant component of vegetation with recognizable present day forms

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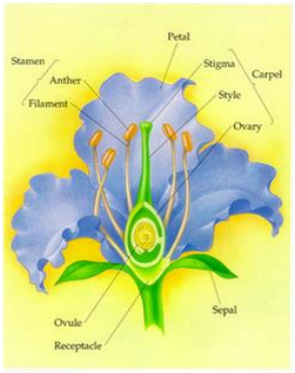
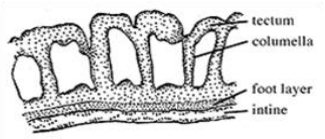
9. Origin and evolution of flowering plants (angiosperms): Geologic time, evolutionary trends -angiosperm derived characteristics, fossil evidences for early flowering plants, place of origin, radiation, phylogeny.

What to call an angiosperm?



Plants with----

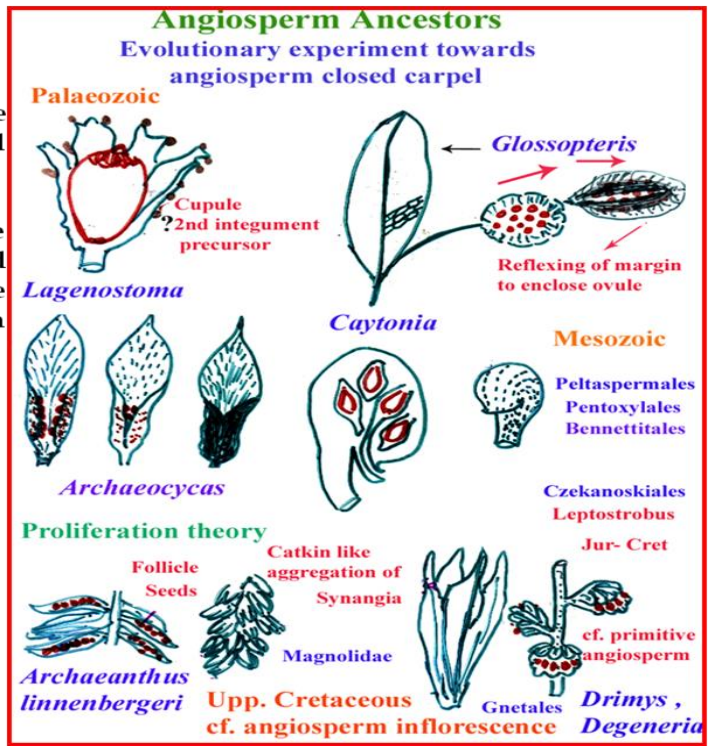
- ❑Enclosed nature of ovary (closed carpel)
- ❑Bisexual Flower
- ❑Specialized conducting cells in xylem and phloem
  - *Vessels in xylem*
  - *Sieve-tube and companion cells in phloem*
- ❑Reticulate leaf venation
- ❑Double layered seed coat (two integument)
- ❑Eight-celled female gametophyte
- ❑Double-fertilization
- ❑Tectate columellate pollen
- ❑Germination of pollen grains on stigmatic surface
- ❑Alkaloids differ from non angiosperm plants



Evolution of Angiosperm carpel

Cupule was the precursor to the caepel (ovary wall) or second integument

Many of the early ovules were further developed by stalked cupule was thought to have developed from fusion of a vegetative branching system



*First appearance:
At around 140 million years ago in the early Cretaceous

*Rapid diversification and radiation:
In the mid-Cretaceous

*Ecological dominance:
Early Tertiary (65 Ma)

So, angiosperms evolved some 300 million years later than the first vascular plants and 220 million years later than the first seed plants

MESOZOIC	65	Upper (Late) Cretaceous	Senonian	Maastrichtian	
				Campanian	
				Santonian	
				Coniacian	
	97.5				
			Barremian	Turonian	
				Cenomanian	Major radiation & global distribution
		Lower (Early) Cretaceous		Albian	
				Aptian	
				Barremian	
			Neocomian	Hauterivian	
				Valanginian	First appearance
	144			Berriasian	

Place of Origin and Radiation

The most favoured hypothesis suggests that angiosperms originated in the Palaeotropics ($0\text{-}30^0$), radiating out to colonize higher latitude environments some 20-30 million years later

Evidences:

The earliest well dated angiosperm pollen has been found in late Valanginian (135 Ma) fossil localities in Israel and Morocco. During early Cretaceous these regions lay between the Palaeoequator and 25^0N

Angiosperms then appear to have spread relatively rapidly into the higher latitudes, with evidence for slightly younger pollen grains from fossil localities in England and China

By the Barremian (127 Ma), angiosperms appear to have been widespread, with fossil localities in central Africa, Australia, Europe, and China

There are also reported occurrences of angiosperm pollen from older sediments in Libya (Berriasian, 140 Ma) and China (Hauterivian), but the ages of these sediments are controversial

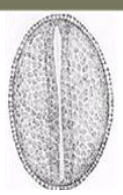
All the earliest angiosperm pollen grains are small, between 10-50 micrometer in diameter. 4 morphological groups have been identified:

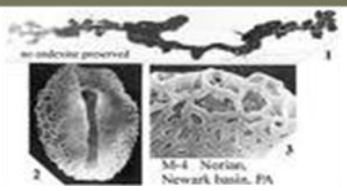
Clavatipollenites: characteristic collumelate wall with 1 furrow, affinity with chloranthaceae

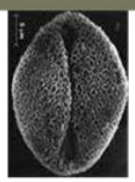
Pre-Afropollis: inaperturate, grain wall pattern is wedge shaped with fluted walls

Spinatus: distinguished by short spines on the margin

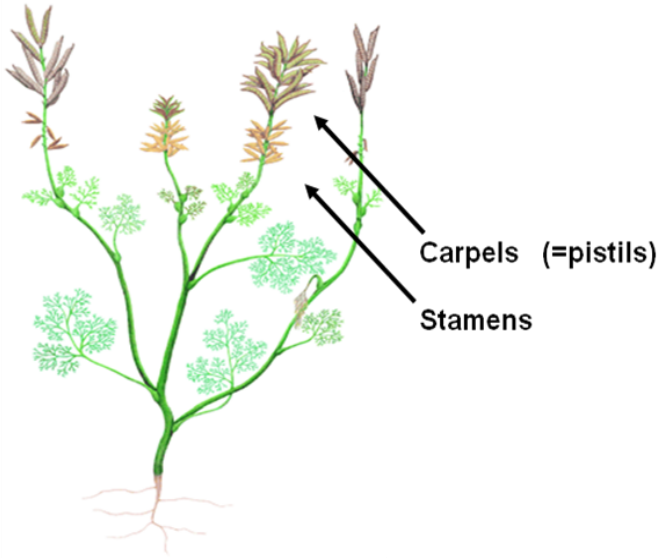
Lilacidites: distinguished by large size, single germinal furrow and cell wall composed of very high columellae, similarity with Liliaceae







Earliest Flower (125mys old) *Archaefructus sinensis*- an intact specimen



Surprisingly, it was an herbaceous plant- only about 25 cm tall

Sun, G, Ju, Q, Dilcher, D, Zheng, S, Nixon, K, Want, X. 2002. Archaefructaceae, a new basal angiosprem family. Science 296: 899-904
Stokstad, E. 2002. Fossil plants hint how first flowers bloomed. Science 296:821

Archaefructus liaoningensis

- 120 million year old fossil from northeast China. The leafy, seed-containing pods (carpels) are the defining characteristic of angiosperms.
- Petals are apparently absent, but leaf-like structures subtending each fruiting axis define them as flowers.
- Previously the age of the fossil deposits strata was dated as late Jurassic (145 Ma) by biostratigraphical correlation (Sun et al, 1998) but radiocarbon dating and other palaeontological evidence suggest late Cretaceous age (125-120 Ma) (Luo, 1999; Swisher et al, 1999, Barrett, 2000)

Enlarged view of the carpels (each is about 1 mm long) showing seeds in carpel (Sun, Dilcher, Zheng & Zhou. 1998. Science 282:1692)

Evolutionary trends: gymnosperms to angiosperms?

Earlier suggestion that angiosperms were of a polyphyletic origin (a number of different ancestors)

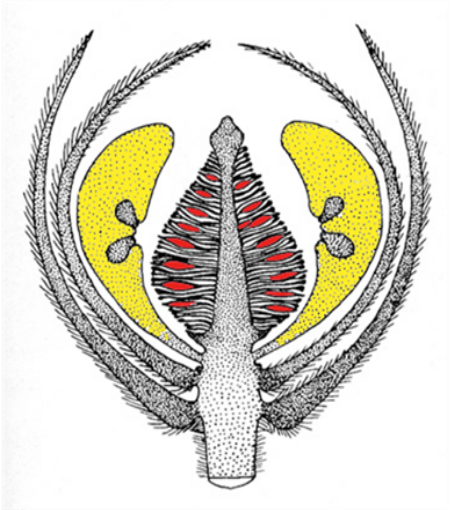
Almost all recent morphological and molecular evidences suggest that angiosperms were derived from a single common ancestors (monophyletic origin)

Possible evolutionary pathways between gymnosperms and angiosperms were via

Bennettitales (Arber and Parkin 1907, 1908) and

Gnetales (Von Wettstein 1907)

Bennettitales



Flower like bisexual reproductive organs and similar wood anatomy

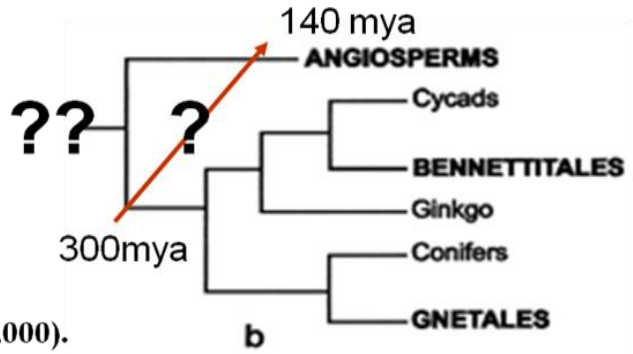
e.g. *Williamsoniella* – bisexual reproductive axis bearing naked ovules above a series of pollen bearing structure, the whole enclosed by large bracts which is thought to be the equivalent to petals in an angiosperms flower

Also, the position of the reproductive organ, erect and at the end of the branch, bore some similarities to extant insect pollinated flower structure

Gnetophytes

Morphological similarities between many species of Gnetales and angiosperms first led to the suggestion that they were probably close in evolutionary terms e.g.

* *bisexual reproductive organ*
* *presence of vessels*
* *leaves with a venation pattern closely that of dicotyledons*
* *tectate pollen wall in certain species of Ephedra*
* *phylogenetic analysis also confirmed a close relationship*



This figure is from Crepet (2000).

Which of the earliest angiosperms forms an evolutionary link with the Gnetales?

Earlier suggestions

•Most suggest that early members of the Nymphaeales and Piperales were the evolutionary link (Taylor and Hickey, 1992; Doyle et al, 1994)

•Others have suggested a single genus, *Ceratophyllum*, within the Nymphaeales (Les et al, 1991) and members of the Laurales (Loconte and Stevenson, 1991; Locontem 1996)

•There is also strong support for woody Magnoliales (Wing and Boucher, 1998)

Most recent and extensive molecular studies of angiosperm phylogenetics indicate

***Amborella trichopoda*, only extant species of Amborellaceae, represents the most primitive (basal group) of all flowering plants (Qui et al, 1999; Soltis et al, 1999)**

This is followed by the Nymphaeales, and then a group including Illiciaceae, Trimereniaceae, Austrobaileyaceae, and Schisandraceae. Together they have been referred to as ANITA

Division: Tracheophyta			Age of Occurrence	Significant Characters
Class- Spermatopsida	Subclass-Angiospermae		Early Cretaceous - Recent	Ovules are enclosed in carpels, within a flower, and fertilization is double
	Subclass - Gymnospermae	Gnetales	Triassic - Recent	Leaves opposite each other, and vessels in the wood; male and female cones are flower like
		Coniferales	Mississippian - Recent	Trees with resin canals, and needle or scale like leaves
		Ginkgoales	Late Triassic - Recent	Trees with seed bearing shoots and with fan shaped or more divided leaves
		Cycadales	Mississippian - Recent	Bushy to tree like plants with leaf traces that girdle the stem; frond like leaves; seeds attach to a megasporophyll stalk below a leaf like structure
		Bennettitales	Late Triassic-Late Cretaceous	Bushy to tree like plants with sterile scales between the seeds; frond like leaves; flower like cones with enclosing structures that surrounds ovules and pollen sacs
		Medullosales	Mississippian - Permian	Primitive seed plants with large pollen grains and unusual stem anatomy
Class-Progymnospermopsida			Late Devonian	Plants with gymnosperms like wood but free sporing (fern like) reproduction
Class-Filicopsida			Mid Devonian - Recent	Ferns, flat leaves which uncurl as they develop; sporangia are grouped in clusters usually on the underside of leaves
Class-Equisetopsida			Late Devonian - Recent	Vertical stems with jointed structure and a whorl of fused leaves at the nodes, sporangia grouped in cones
Class-Lycophyta			Late Silurian - Recent	Small to large plants with lateral sporangia and small leaves
Class-Rhyniopsida			Mid Silurian – Early Devonian	Simple vascular plants with dichotomously branching stems and terminal sporangia

Indian Gondwana system: Indian Gondwana sequence, classification and megafloristics assemblages in Gondwana sequence

Indian Gondwana sequence

World's earliest forests appeared during Permo-Carboniferous Periods that is ~345-245 million years ago and those forests of the geological past eventually transformed into the coal deposits of the modern world. Depending on the typical floral assemblages of the earliest forests, four distinct palaeo-phytoprovinces were categorized which are 1. Euramerian

phytoprovince, 2. Angara phytoprovince, 3. Cathaysian phytoprovince and 4. Gondwana phytoprovince and the corresponding floral assemblages are Lepidodendron, Angara, Cathaysian and Glossopteris respectively. The first three phytoprovinces (Euramarian, Angara, Cahaysian) were placed at the equatorial belt of the Northern Hemisphere continents whereas the Gondwana province extended at the higher latitudes of the Southern Hemisphere region.

The term 'Gondwana' or 'Gondwanaland' is derived from the ancient territory of the 'Gond' (a tribal community inhabiting the area south of the Narmada River in Central India), from where Medlicott and Oldham, staff of Geological Survey of India, first recognized well exposed distinctive sedimentary strata during the 1870s (Lele 1964, Maheswari 1992). Some workers applied the term 'Gondwana' only to the region hosting the classical Permian-Cretaceous sequences of India and also employed a broader term 'Gondwanaland' to denote the Southern Hemisphere supercontinent that existed from Palaeozoic to mid-Mesozoic times (Wadia 1957, Metcalfe 1991, Li and Powel 1993). The supercontinent Gondwanaland eventually broke apart by the influence of Plate Tectonics (continental drift) to form the modern continents of Australia, Africa, South America, Madagascar, Indian sub-continent, New Guinea and New Zealand.

Classification and megafloristics assemblages in Indian Gondwana sequence

The earlier studies (Oldham 1893, Cotter 1917, Fox 1931) mostly employed a two-fold subdivision of the Indian Gondwanan strata- the 'Lower' and 'Upper' Gondwanas for Late Palaeozoic (*Glossopteris* bearing) and Mesozoic (*Ptilophyllum* bearing) sequences, respectively. Later on, the *Dicroidium* dominated fossil plant bearing Triassic (a Period under the geological Era Mesozoic) sequence was documented in between *Glossopteris* and *Ptilophyllum* fossil assemblages and accordingly a three-fold subdivision of the Gondwanan sequence was adopted by the workers (Feistmantel 1882, Vredenburg 1910, Wadia 1957, Lele 1964). This three-part scheme remains a useful subdivision of the Indian Upper Carboniferous-Permian to Early Cretaceous succession although geologists and palaeontologists subsequently recognized numerous distinct litho- and bio-stratigraphic units within these sequences (Sukh-Dev 1987, Maheswari 1992).

The dominant member of the Lower Gondwana flora is recognized under a single genus *Glossopteris*, accommodating many species exhibiting wide diversity in their morphological characters which flourished during Upper Carboniferous-Permian for about fifty million years. Origin of the Glossopteris flora occurred during the phases of climatic amelioration in the intermittent interglacial phases. By the end of the Upper Carboniferous glacial phase, the Glossopteris flora emerged as a distinct and dominant vegetation in the Southern Hemisphere continents and Indian subcontinent and gradually started to diversify since Early Permian constituting the earliest forest. In Indian subcontinent, the flora is recorded from the Upper Carboniferous to Permian rocks of extra-Peninsular region whereas from Early Permian to Early Triassic rocks in the Peninsular region.

Studies on the Glossopteris flora started about 200 years ago since the *Glossopteris* leaves were first discovered by Adolph Brongniart (1822, 1828) from the 'Gond' tribe inhabited areas in Central India (now in Madhya Pradesh), India from which the name of the supercontinent Gondwanaland was proposed by Eduard Suess (1855, 1898) although Medlicot (1872) first formally utilized the term Gondwana. Since then, many leaves, stems, roots, fertile organs belonging to glossopterid group are described by many workers from different Lower Gondwana sediments. Besides, different plant remains belonging to bryophytes, pteridophytes, and other gymnosperms are also recovered from the Indian Lower Gondwana sediments in Glossopteris flora. The Triassic aged Middle Gondwana sediments recorded Dicroidium flora comprising with *Lepidopteris, Cladophlebis, Taeniopteris, Cyclopteris, Neomariopteris, Algacites, Hepaticites*, *Sphagnophyllites, Bosea, Nidpuria* etc. and the Pitlophyllum flora is documented from Upper Gondwana sediments of Late Cretaceous–Jurassic age with the discovery of fossil plants *Williamsonia, Pentoxylon* etc. A comprehensive list of some megafloral assemblages recovered from Indian Gondwana sequences is represented in Table 2.

Table 2: Characteristics megafloral assemblages in Indian Gondwana sequences

Stratigraphic Age	**Megafloral assemblages**		
UPPER GONDWANA			
Early Cretaceous - Jurassic	*Ptilophyllum, Pterophyllum, Pagiophyllum, Elatocladus, Marattiopsis, Brachyphyllum, Araucarioxylon, Hausmannia, Pachypteris, Dictyozamites, Gleichenia, Williamsonia, Weltrichia, Pentoxylon, Carnoconotes, Nipaniophyllum* etc.		
MIDDLE GONDWANA			
Triassic	***Lepidopteris, Cladophlebis, Taeniopteris, Cyclopteris, Neomariopteris, Algacites, Hepaticites, Sphagnophyllites, Bosea, Nidpuria*** **etc**		
LOWER GONDWANA			
Permian - Late Carboniferous	***Bryophytes***	*Dwakea, Buthelezia, Bryothallites, Hepaticites, Talchirophyllites*	
	Pteridophytes	***Lycopsids***	Cyclodendron, Boldihadendron
		Sphenopsids	Phyllotheca, Schizoneura, Raniganjea, Trizygea, Lelstotheca (Stelotheca), Annularia**,** Notocalamites, Giridia, Gondwanostachys, Tulsidabaria, Rajmahaliyastachys
		Filicopsids	Neomariopteris, Dizeugotheca, Damudopteris, Cuticulatopteris, Searsolia, Botrychiopsis
	Glossopteridales	**Leaf**	*Glossopteris, Gangamopteris, Euryphyllum, Rubidgea, Palaeovittaria, Macrotaeniopteris*
		Root	*Vertebraria*
		Stem	*Araucarioxylon, Dadoxylon, Damudoxylon*

		Fertile organ	*Ottokaria, Dictyopteridium, Scutum, Lidgettonia, Eretmonia, Lanceolatus, Glossotheca, Senotheca, Veekaysinghia, Birbalsahnia*
		Seedling	*Diphyllopteris, Deogharia*
	Cordaitales	*Noeggerathiopsis*	
	Coniferales	*Buriadia, Feruglíocladus,*	
	Ginkgoales	*Ginkgophyton, Psygmophyllum, Rhipidopsis, Polyspermophyllum, Ginkgophyllum*	
	Cycadales	*Pteronilssonia, Pseudoctenis*	
	Incertaesedis	*Sharmastachys, Saharjuria, Chitraphyllum, Santhalitheca, Pachwarophyllum*	

10. Aspects and Appraisal of Palaeobotany: Palaeobotanical study in exploring - mysteries in the living planet; origin, evolution, diversification and extinction of species; plant-animal interaction and co-evolution; plate movement, geological age and correlation of strata; palaeogeogrpahy, palaeoclimate; fossil fuel.

Plant fossils have enormous applications in both academic and applied aspects which are enumerated below-

Mysteries in the living planet; origin, evolution, diversification and extinction of species

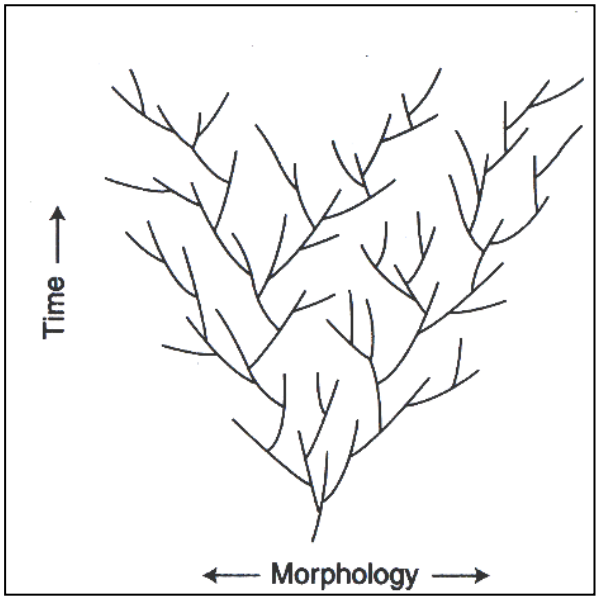
Plant fossil record in deciphering evolutionary history and mass extinction event:

Two evolutionary theories Phyletic gradualism and Punctuated equilibrium are established based on fossil record.

Phyletic gradualism (Darwin, 1859): Evolutionary change results from descent with modification and accomplished by 'natural selection'. Species are therefore temporary stages in continuing gradual change. Ecological interactions are ultimate driving mechanism behind macroevolution. So, this theory supports the view that speciation is a gradual and continuous process (Fig. 36). "If variations useful to any organic being do occur, individuals thus characterized will have the best chance of being preserved in the struggle for life; and from

the strong principle of inheritance, they will tend to produce offspring similarly characterized"

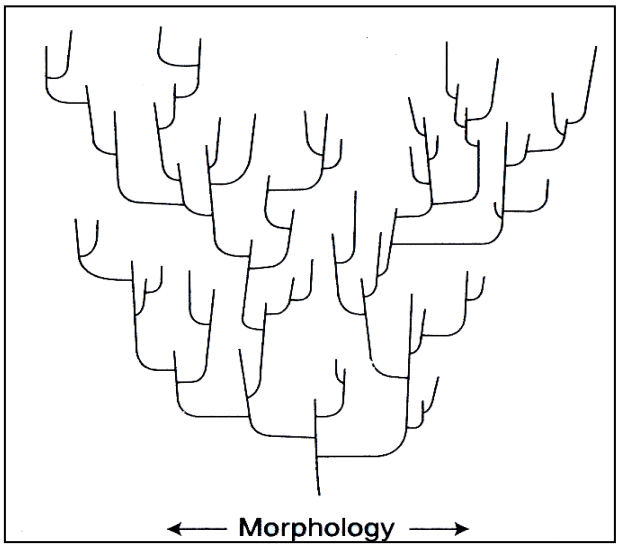
Fig. 36: Phyletic gradualism



Punctuated equilibrium (Eldredge & Gould, 1972):

Geological record does not indicate a long sequence of continuous intermediate species from the parental to the descendant species as expected if the phyletic gradualism was occurring. Rather there are long periods where there appears to be little morphological change or speciation, followed by periods of relatively rapid change (Fig. 37). This pattern of abrupt speciation in the geological record has been attributed to gap in the fossil record- the 'breaks' could represent periods of rapid speciation. A phyletic line that was usually in stasis disturbed only rarely by rapid and episodic events of speciation. There are relatively rapid periods of speciation interspersed by long periods where there appears to be little morphological change or speciation, referred to as stasis.

Fig. 37: Phyletic gradualism (cone of increasing diversity, but with vertical branches representing periods of stasis, interspersed by horizontal lines representing periods of rapid change)



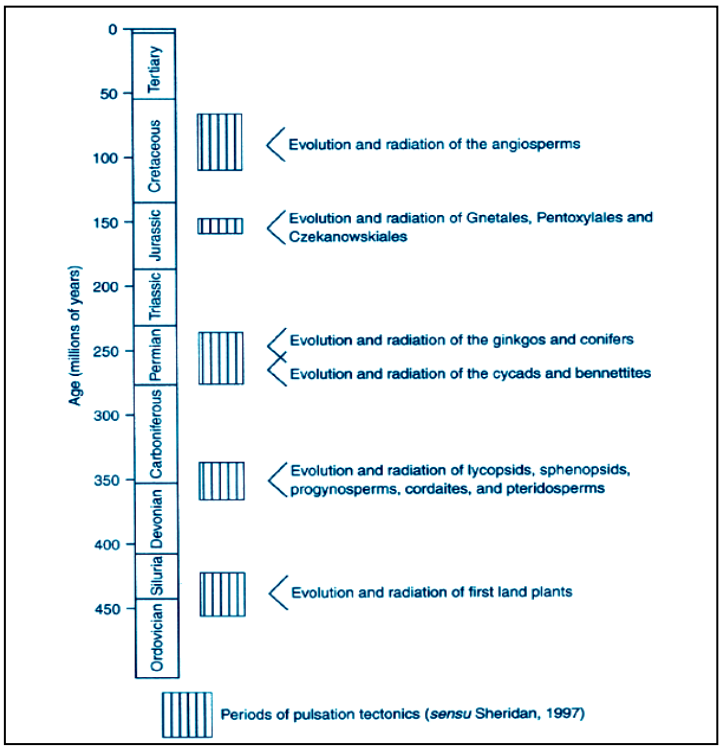
Mass extinctions (physically or biologically mediated) are the punctuating events leading to the apparent sudden speciation events. It effectively wipes the slate clean by clearing away the accumulated evolution of a lineage, and thereby allows a new lineage to evolve rapidly to fill the niche. Evolutionary time is a hierarchical system of distinct tiers, with the uppermost tier of mass extinctions representing the ultimate driving mechanism behind macroevolution.

Recent survey concluded that the 'palaeontological evidence overwhelming supports a view that speciation is sometimes gradual and sometimes punctuated and that no one mode characterizes this very complicated process in the history of life'. Existence of living fossils (*Ginkgo biloba*) show morphological 'stasis' since the Jurassic (~200 Ma) and supports punctuated equilibrium theory. It is established that changes in the environment are largely responsible for the radiation and extinction of taxa (Fig. 38) for which **Turnover-pulse hypothesis is** proposed.

Turnover-pulse hypothesis

Evolution is normally conservative, and speciation does not occur unless forced by changes in the physical environment. So, it supports the concept of punctuated equilibrium suggesting that periods of speciation are largely a function of changes in the physical environment, especially climatic change, possibly of tectonic origin, but most probably of astronomical origin.

Fig. 38: Periods of pulsation tectonics in plant fossil records indicating evolution and radiation of major plant groups (Sheridan, 1997)



The model also proposes that extinction may result directly from climatic change or from ecological interactions among newly associated species. In broader scale, two factors are responsible which are Orbital variations and Plate tectonics.

The patterns of major events occurred in geological past (Table 3), evolutionary history (Fig. 39) and their changes (Fig. 40) are best explored through plant fossil records.

Fig. 39: Major Evolutionary history through geological time scale

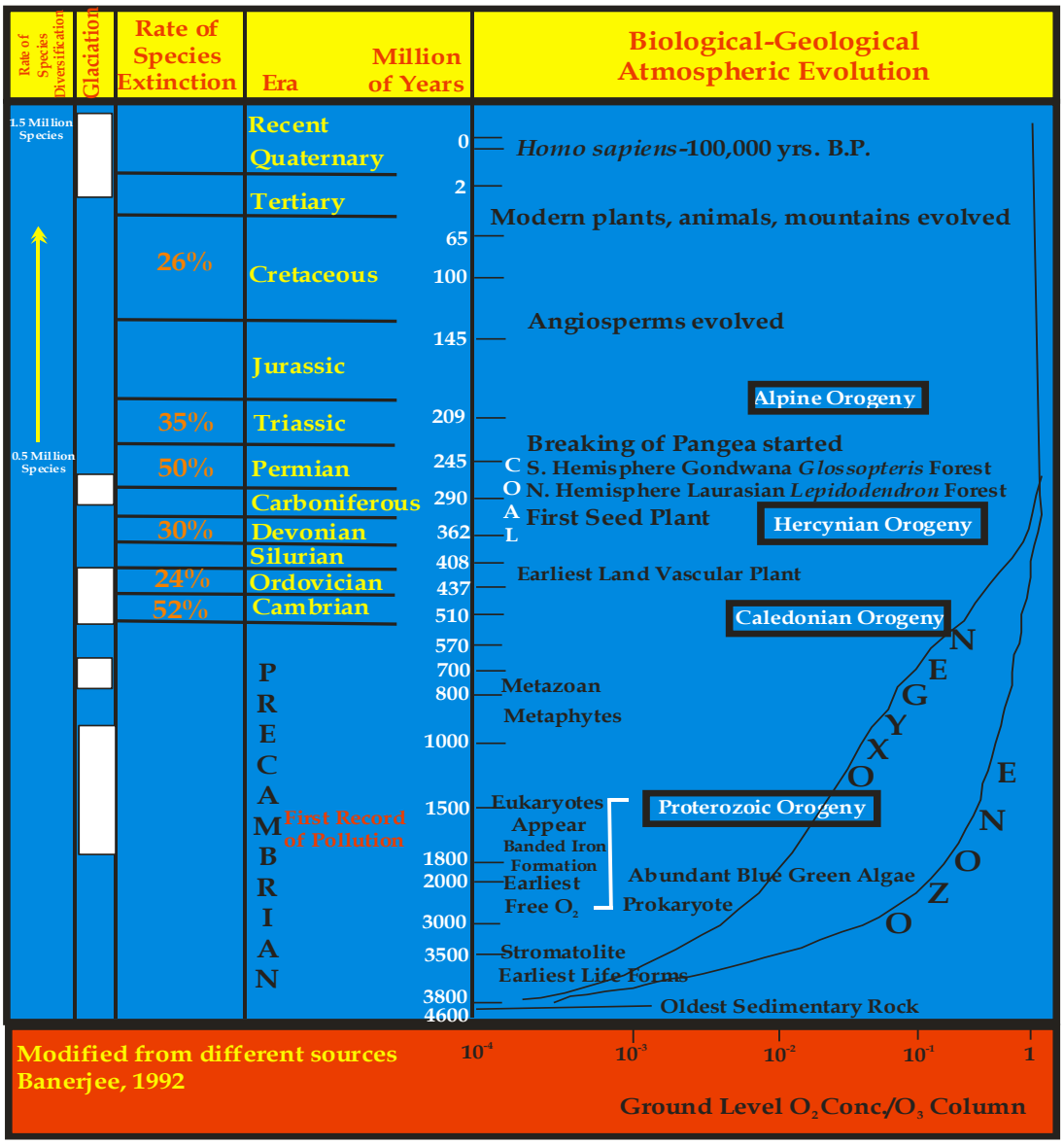
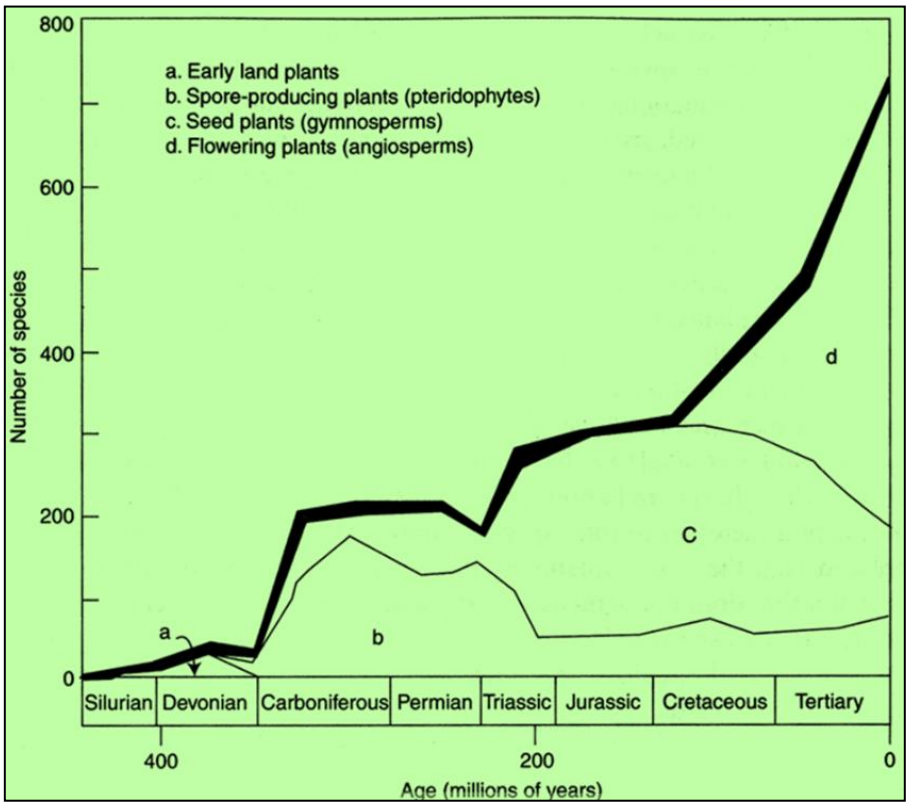


Table 3: Major events in evolutionary history occurred in geological past

Geological Period	**Major events in evolutionary history**
Quaternary	Modern humans
Tertiary	Primitive humans & modern mammals
Cretaceous	The first flowering plants; placental mammals
Jurassic	Giant dinosaurs & first birds
Triassic	Diversification of ferns & conifers, first dinosaurs & small mammals

Permian	Diversification of glossopterids & reptiles
Carboniferous	The first conifers and reptiles; seed ferns, arborescent lycopods and calamites
Devonian	The first amphibians, diversification of vascular plants
Silurian	First vascular plants & breathing animals
Ordovician	First vertebrates
Cambrian	First trilobite, abundant marine invertebrates & BGA
Precambrian	Primitive life forms

Fig. 40: Patterns of evolutionary change in the plant fossil record



Mass extinction

Mass extinction is a real catastrophe brought about by extraordinary environmental factors & involves substantial biodiversity losses that are global in extent, taxonomically broad & rapid. It describes a relatively short intervals of geological time when a high proportion of diverse and geographically wide spread taxa underwent extinction. It differs from normal 'background' species level extinction in that it is characterized by the relatively rapid extinction of groups of organisms, usually at a higher taxonomic level, that is families and even orders. There is different earth bound and extra-terrestrial causes behind the mass

extinction event through environmental changes like shock-waves, heat-waves, impact winters (long periods of darkness shutting down photosynthesis), super-acid rains, toxic oceans, super waves & super floods etc.

Earth bound causes (physical & biological):

- Global cooling and glaciations
- Massive volcanism
- Reduction of salinity & Oxygen in the ocean
- Sea-level changes

Extra-terrestrial causes:

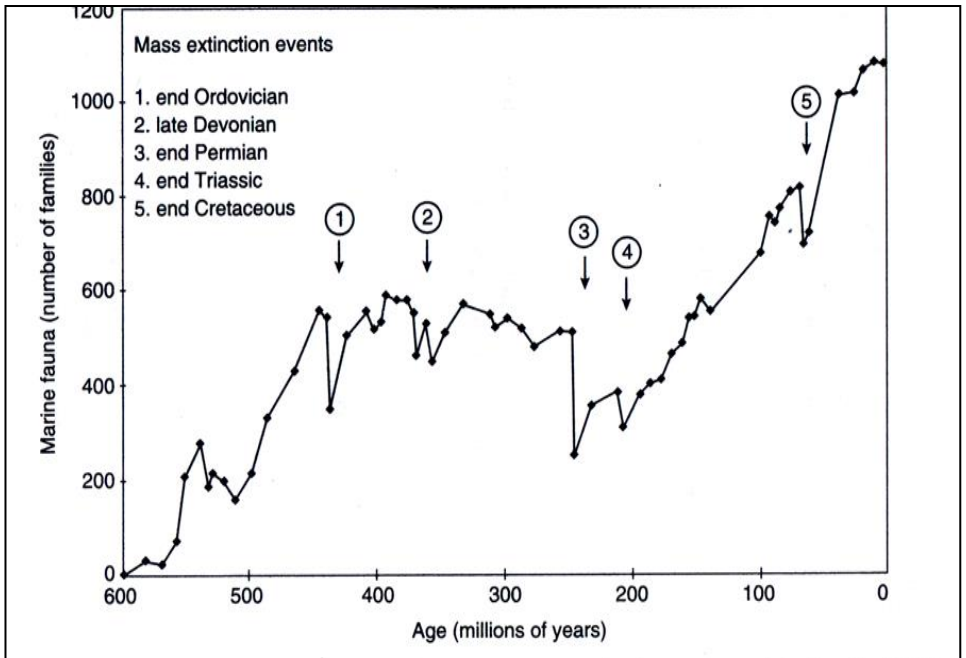
- Impacts from meteorites
- Comet storms
- Radiation from supernovae
- Large solar flares

Fossil study (mainly marine fossil record) is one of the reliable methods for establishing mass extinction event. Five major extinction events (Table 4; Fig. 41) are recorded in geological history.

Table 4: Magnitude in the major mass extinction (Erwin 1993)

Geological Period	Geological Time	Percentage (%) of family extinction	Percentage (%) of generic extinction
End Cretaceous (K-T extinction)	65 Ma at the Cretaceous-Tertiary transition	17	50
End Triassic	206 Ma at the Triassic-Jurassic transition	23	48
End Permian (P-T extinction)	248 Ma at the Permian-Triassic transition	57	83
Late Devonian	364 Ma near the Devonian-Carboniferous transition	19	50
End Ordovician	443 Ma at the Ordovician-Silurian transition	27	57

Fig. 41: Five mass extinction events in the fossil record



Five big mass extinctions in the fossil marine record (after Raup and Sepkoski, 1982).

Plant-animal interaction and co-evolution

Plant animal interaction in geological past is determined through fossil study and it is important to infer the co-evolution of these two biological organisms in geological past.

The evolution of plant-animal interactions goes back to the Early Archean, where the first signals of photosynthesis may have been detected in the Isua Peninsula in Greenland, a phenomenon that is related to the isotopic anomalies of carbon. The first evidence of reliable fossils of photosynthetic microorganisms has been identified by micropaleontologists in the Late Archean and Early Proterozoic. A closely related topic in this geologic time interval is the evolution of trophic relations and metabolic diversification in the microbial world. In the context of the three domains of life, Archaea, Bacteria and Eucarya, the bifurcation of multicellular organisms into plant and animals becomes evident only during the Paleozoic. Cell evolution also leads up to the unicellular dichotomy of autotrophs and heterotrophs. Symbiosis has a strong role to play in the transition to plants and animals in the Phanerozoic. A special case of plant-animal interaction is the evolution and dispersal of hominins, including their impact on the ecosystems.

There are a number of types of evidence *(animals on land, early pant-animal associations, herbivory, interaction with vertebrates, plants as habitats, other plant-anima interaction etc.)*

that can be cited as example of plant-animal interactions in the fossil record. Incomplete leaf margins, holes in leaves of various types, missing tissue in permineralized specimens, and coprolites are but a few of the examples that can be used to assess levels of plant-animal interaction. Plant-animal interactions can also be examined as indicators of climate change.

Fossil evidence of herbivory

Coprolites

Some of the oldest coprolites have been described from Ordovician rocks, and by the Late Silurian-Early Devonian there are numerous examples of fossil feces that are composed of spores, cuticle, and macerated plant materials. Well preserved coprolites are a component of the Rhynie Chert ecosystem and represent an important segment of the food web at this time.

Insect evolution

Information about the distribution of fossil insect families through time is another important component of studying herbivory. Examining the diversity of insects in the fossil record together with data about mouthpart morphology, represent important avenues of research, useful in documenting feeding guilds and mouthpart evolution during the evolution of herbivory. e.g., some tree ferns in the Late Pennsylvanian show evidence of piercing and sucking by insects, based on the wounds present in the plant tissues.

The presence or absence of insect damage on a larger number of fossil plant specimens can be used to examine herbivore changes and even extinction, and to track specialized feeding associations, recovery rates, and the appearance of new co evolutionary associations between plants and insects.

Wood boring is a common feeding mode in the modern flora, and the fossil record provides evidence of this interaction as well. In the wood of *Premnoxylon,* a presumed cordaitean root, the wood was apparently digested to form a complex series of ramifying tunnels that contain the fecal remains of the organism.

Gut contents

One of the most accurate methods of determining precisely what organism is feeding on which plant is by analysis of the gut contents of fossil animals. e.g., presence of Carboniferous lycophyte tracheids in the presumed gut of *Arthropleura*, and spores have been

extracted from several mason Creek invertebrates. Pollen grains have been reported as the primary diet of some insects in the Permian and of sawflies from the Lower Cretaceous of the Transbaikalian, Russia. These reports suggest the presence of pollinivory in aome groups. Such studies can be useful in tracing co-evolutionary patterns and provide insight into fossil community structure. In another example, the pollen found inside each of five Cretaceous xyelid species is of a single type, suggesting feeding preferences, or that the plants produced pollen over a short a short period of time. Several different pollen morphotypes in a primitive Permian booklouse suggest that these species were not dietary specific.

Fossil seeds in stomach contents can also be used as indirect evidence about the dispersal agent of a particular plant species. e.g., the presence of whole seeds might suggest that the vertebrates were acting as a dispersal agent, whereas fragments of seeds would suggest they merely served as a food source. Gnaw marks and perforations on seeds also provide evidence of animals seeking plants as a source of food.

Marginal feeding

Another more direct method of measuring the effects of herbivory is by directly observing leaves and other plant parts that have been damaged by phytophagous organisms. This type of damage was once considered to be a rare feature in fossil foliage. e.g., marginal feeding on Carboniferous Neuropteris pinnules, Mississippian Triphyllopteris pinnules and Permian gigantopterid leaves.

Defoliation

In modern ecosystem, one of the most dramatic examples of herbivory involves the defoliation of forest trees. By reducing photosynthetic surface area, leaf-feeding herbivores reduce tree growth by depleting carbohydrate reserves. A variety of interrelated biological and physical parameters influence the development of insect populations that are involved in defoliation. Although it has been hypothesized that such interactions have existed throughout the geologic history of plants and animals, no data from the fossil record are yet available to document this interaction.

Leaf mines or Skeletonization

In addition to the general feeding of phytophagous insects, there are other, more specific types of herbivories on plants. e.g., some larvae feed selectively on the mesophyll tissues of

leaves. This activity results in the formation of feeding channels or mines, as well as disklike excavations or holes that can be identified in the leaf. The herbivore removes the intervein tissues, leaving primary and secondary veins generally untouched. The presence of a reaction rim outlining the damaged area indicates that the leaf was alive when the mining took place. e.g., Triassic Triassohyponomous, a leaf mine in the form of a series of tightly curved coils on Heidiphyllum leaves. Mines have also been reported from the Mesozoic seed fern foliage type *Pachypteris*.

Wound tissue

In addition to arthropods with chewing mouthparts in the Devonian, there were others from the Lower Devonian that appears to have possessed piercing mouthparts. One line of evidence for this type of feeding behaviour is the presence in the Carboniferous and Permian of two orders of insects, the *Palaeodictyoptera* and *Megasecoptera*, which had mouthparts modified for piercing and sap sucking. The second line of evidence involves the identification of wound responses in plants formed as a result of tissue penetration. e.g., the presence of wound tissue attributed to arthropods with piercing mouthparts in the Carboniferous fern *Etapteris*.

Plate movement, geological age and correlation of strata

Plate Movement

The rigid lithosphere of our planet Earth consists of numerous variable sized pieces called plates. At present, 7 large lithospheric plates and a few dozen smaller plates make up the outer shell of Earth (Fig. 42). These plates move slowly over the hotter and weaker semiplastic asthenosphere (*uppermost mantle underlying the lithosphere)* and change in size because of some type of heat transfer system within the asthenosphere.

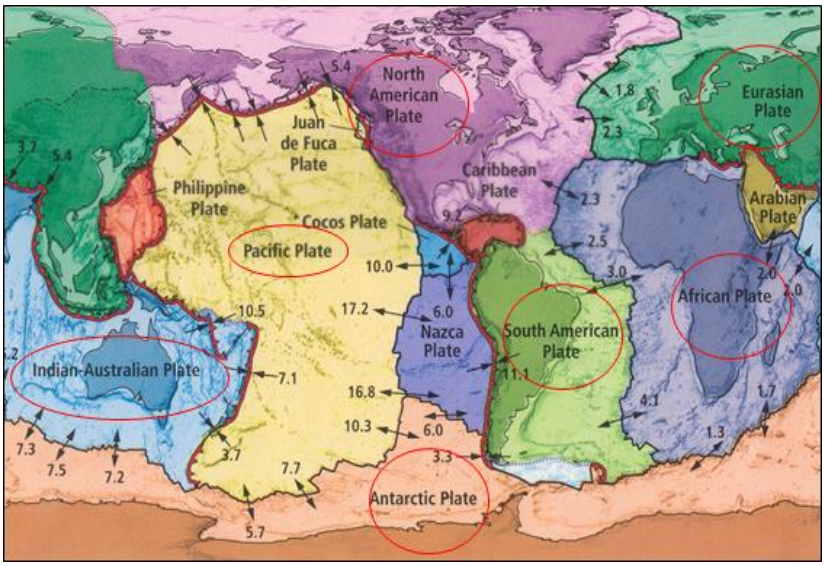


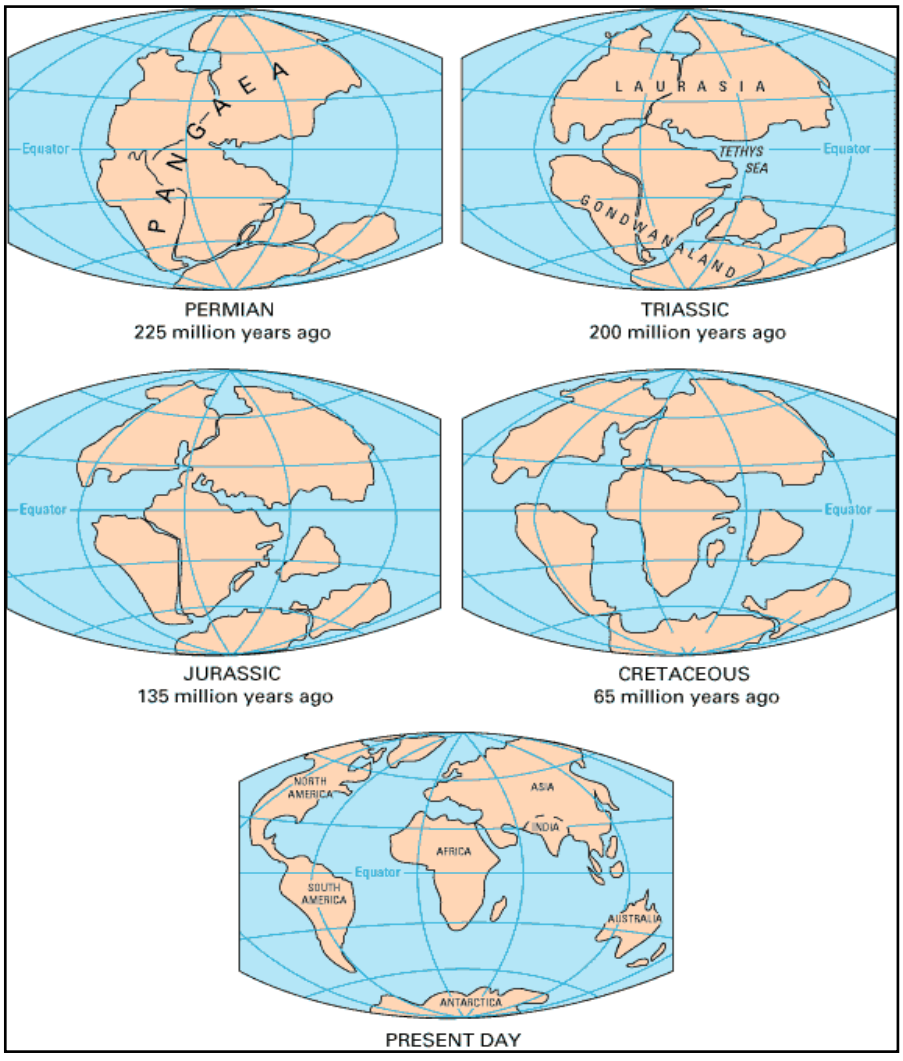
Fig. 42: Lithospheric plates of outer shell of modern Earth
(Source: https://geology.com)

Consequently, plates separate, mostly at oceanic ridges *(underwater mountain system formed by plate tectonics)*, and/or collide, usually at oceanic trenches *(depressions of the sea floor)*, where they are sub-ducted back into the mantle

Concept of Continental drift hypothesis

The continental drift hypothesis was proposed by Alfred Wegener in 1915. He proposed that all the land masses were originally united in a single supercontinent – Pangaea (Greek meaning all land). The supercontinent Pangaea started to break up about 200 million years ago by the movement of crustal plates over the mantle and uultimately continents "drifted" to their present positions (Fig. 43).

Fig. 43: Changing position of crustal plates of Earth over millions of years
(Source: https://futuremaps.com/blog/plate-tectonics

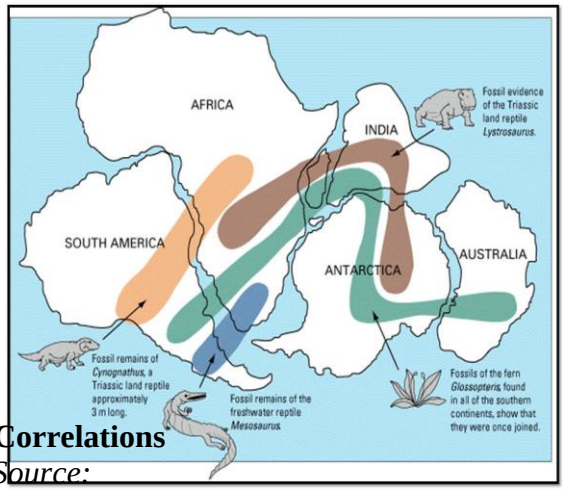


Evidences in support of Continental drift hypothesis

This hypothesis was supported by fossils, rock types and by matching the coastline shapes. Importance of the Gondwanaland concept and the present distribution of the Glossopteris flora was realized by Wegener (1915), Du Toit (1932) and other geoscientists while they were advocating the famous Continental drift hypothesis.

A. Fossil correlations: Identical members of Glossopteris flora including fertile organs and some reptiles distributed in all the Southern hemisphere continents and Indian subcontinent (Fig. 42). Important examples are *Senotheca murulidihensis* fertile organ in organic connection with *Glossopteris clarkei* (Fig. 44) are recorded from Lower Gondwana sediments of both India and Australia. Restricted occurrence of the small fresh water aquatic reptile *Mesosaurus* (Fig. 44) in the eastern part of Brazil, South America and western part of Africa (Fig. 44).

Fig. 44: Fossil evidences in support of continental drift hypothesis



Fossil Correlations
(Source: http://www[illegible]lt.org/Fossils_2.jpg)

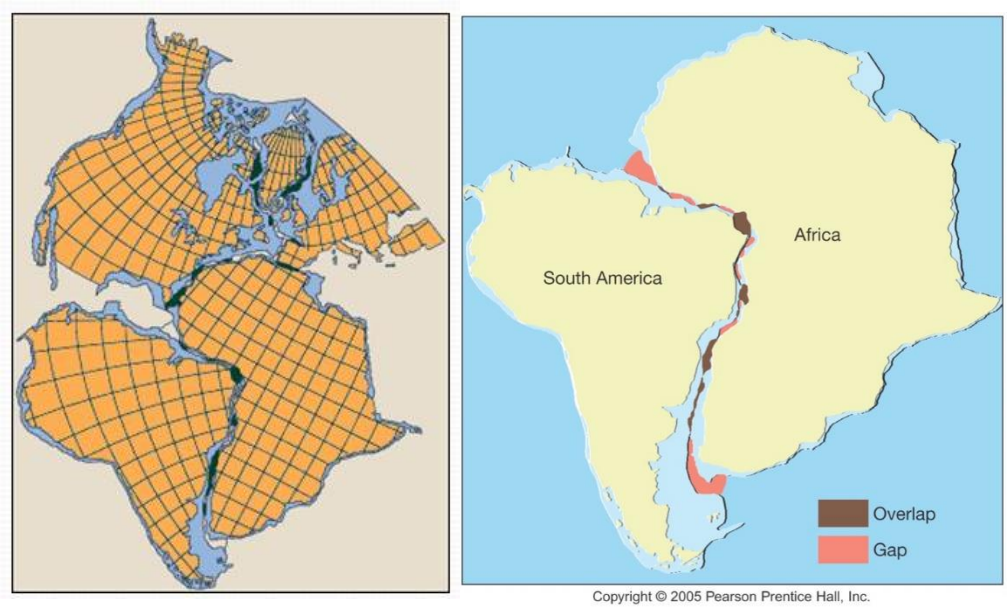
Reconstruction of *Senotheca*

Mesosaurus
(Source: https://www.busaccagallery.com/catalog.php?itemid=5231)

***Senotheca*-fertile organ of *Glossopteris clarkei* leaf**
(Source:https://www.sciencedirect.com/science/artic

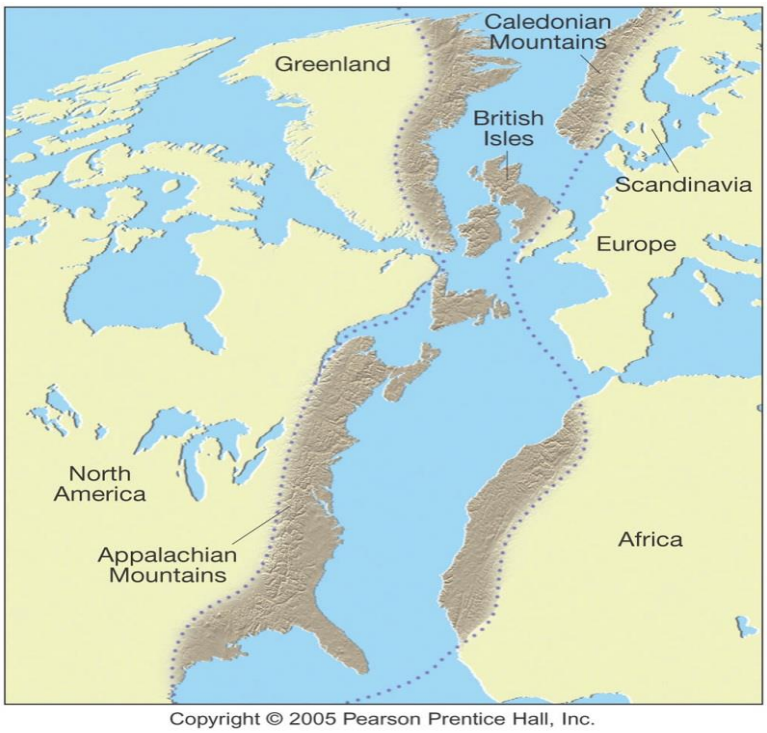
B. Tight fit of the Continents: Shape of the present-day continents look like jigsaw puzzle pieces especially the coastal contours between eastern South America and western Africa suggesting that once they fitted together geographically (Fig. 45) and drifted apart later.

Fig. 45: Geographical fit of the Continents



C. Correlation of mountains: The mountain range with nearly identical rocks and structures are found in the eastern USA and north-west Europe (Fig. 46).

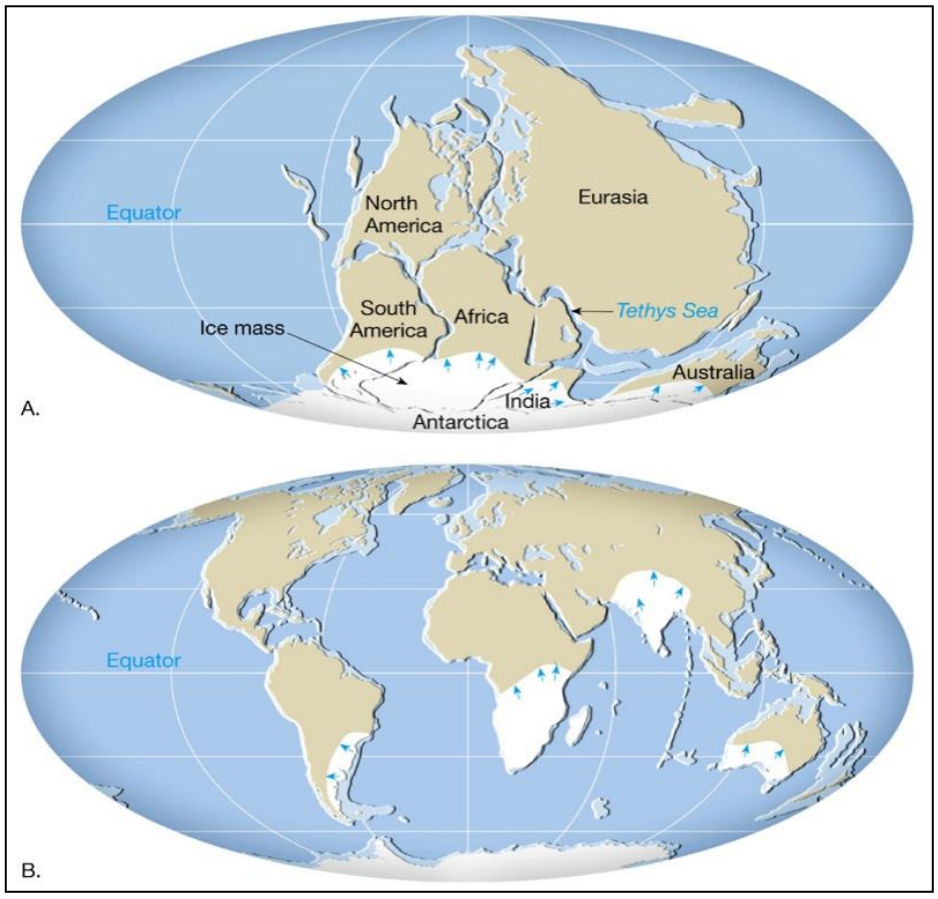
Fig. 46: Correlation of mountains with nearly identical rocks and structures



D. Glacial features: Similar glacier deposits are found in Antarctica, South America and India which are now thousands of km apart (Fig. 47A). The identical glacial features of the same age establish a tight polar distribution of the Gondwana continents (Fig. 47B).

Fig. 47: Identical glacial features in Gondwana continents

A: Tight polar distribution during past B: Present-day distribution



E. Similar climate change event: Similar sequence of climate change from glacial climate to warm humid climate occurred in the entire Gondwanaland as revealed from the lithological, and fossil evidences.

Validation of Continental drift hypothesis

The Continental drift hypothesis was received well in Europe and southern hemisphere continents but rejected in U.S.A, as because of lack of a suitable mechanism to explain how continental crusts, composed of granitic rocks, could seemingly move through the denser basaltic oceanic crust. But, this conflict remained unresolved because seafloors were almost

completely unexplored until 1960s, when Military Spending U.S. Navy mapped seafloor with echo sounding to find and hide submarines during the World War II and the Cold War. This detailed oceanographic research provided convincing evidence that the continents had once been joined together and subsequently separated, and finally the hypothesis of continental drift became widely accepted.

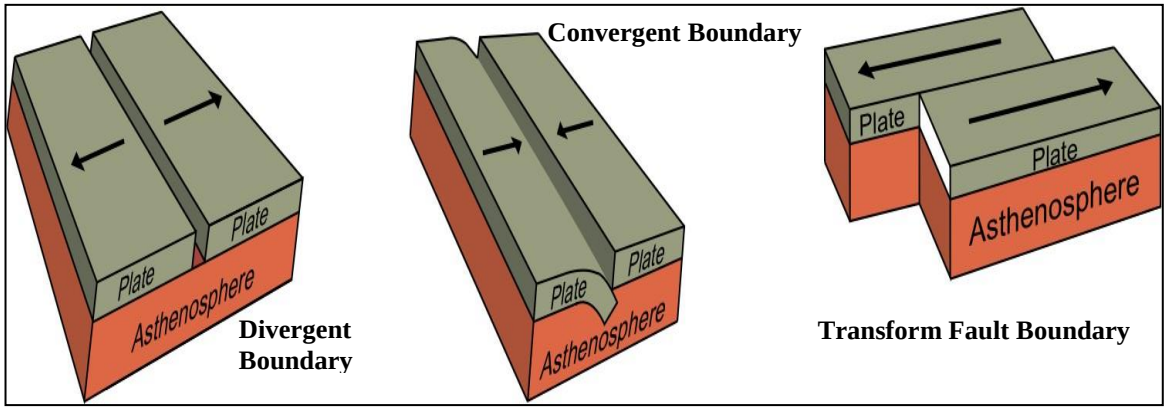
Concept of Plate Tectonics

Seafloor mapping is the basis for rising of the Plate Tectonic theory. Plate Tectonic is a unifying theory of Geology which combines the earlier hypotheses of 'continental drift' (moving continent) and 'seafloor spreading' (moving sea floor). The generalized map of seafloor explores different unique structures like-

- **Oceanic ridges**—submerged mountain ranges
- **Fracture zones**—cracks perpendicular to ridges
- **Trenches**—narrow, deep gashes (cuts)
- **Abyssal plains**—vast flat areas
- **Seamounts**—drowned undersea islands

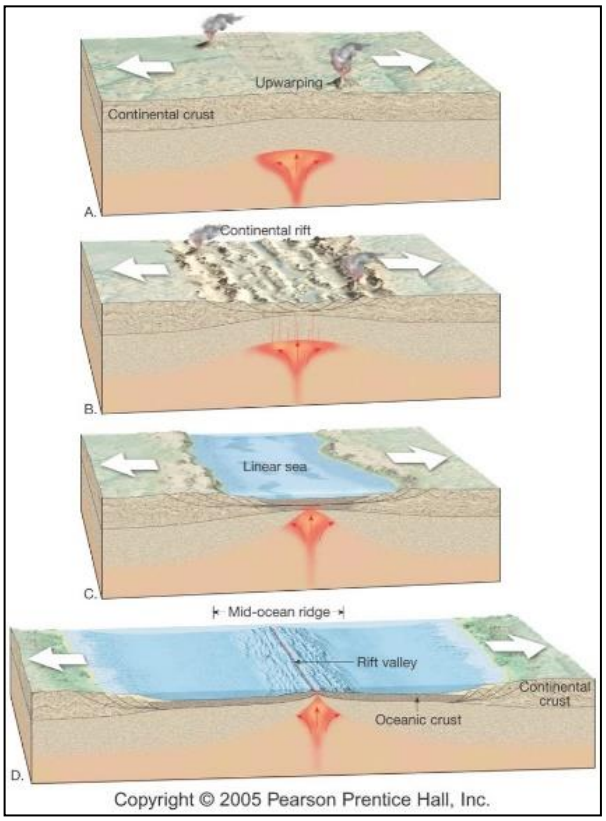
Furthermore, marine geologists found that seafloor magnetism has a striped pattern which is completely unlike patterns on land and they hypothesized that these stripes are indicatives of periodic reversal of the direction of Earth's magnetic field. Scientists validated the hypothesis by determining the eruptive ages and the polarity of young basalts using the K-Ar (Potassium-Argon) radiometric dating. Afterwards (1962-1963) geologists realized that the patterns are symmetrical across oceanic ridges and the K-Ar dating determines the age of rocks at the ridges as youngest. They also resolved that bands of seismicity chiefly exist at trenches and oceanic ridges which are the origins of different plate boundaries where earthquakes and volcanic eruptions happen. Three types of relative motions like divergent, convergent, and transform (Fig. 48) exist between plates.

Fig. 48: Different boundaries between the tectonic plates



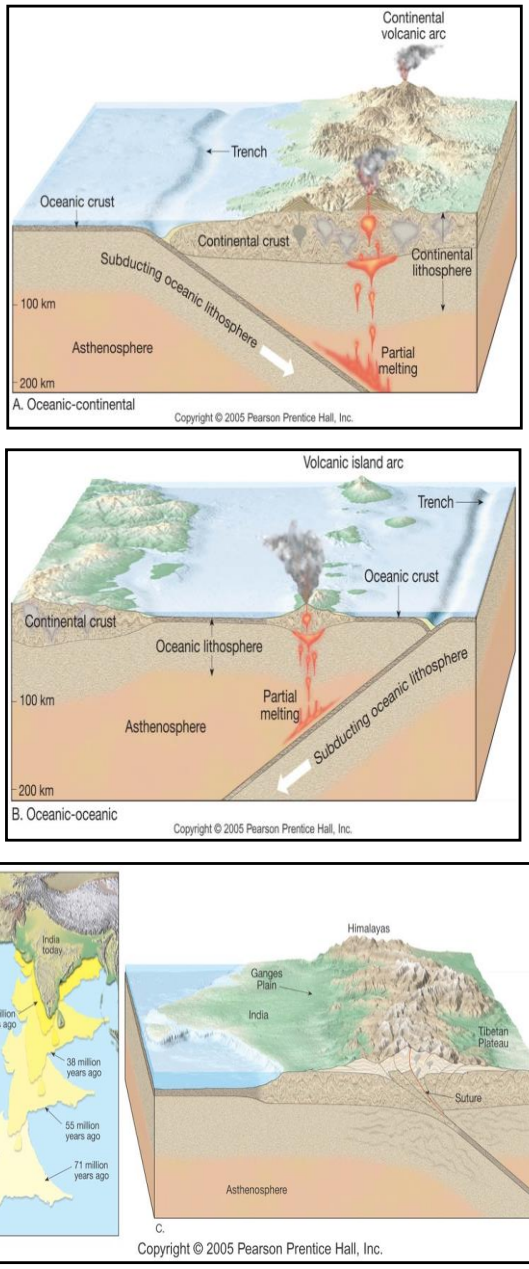
Divergent boundary: Here, the plates move apart from each other. This boundary chiefly occurs at oceanic ridges and it also can rip apart ("rift") the continents leading to formation of rift valley (Fig. 49) in oceanic lithosphere (example: East Africa rift, Red sea, Atlantic Ocean). Presumably, Pangaea was ripped apart by such continental rifting and drifting.

Fig. 49: Divergent boundaries leading to formation of oceanic lithosphere



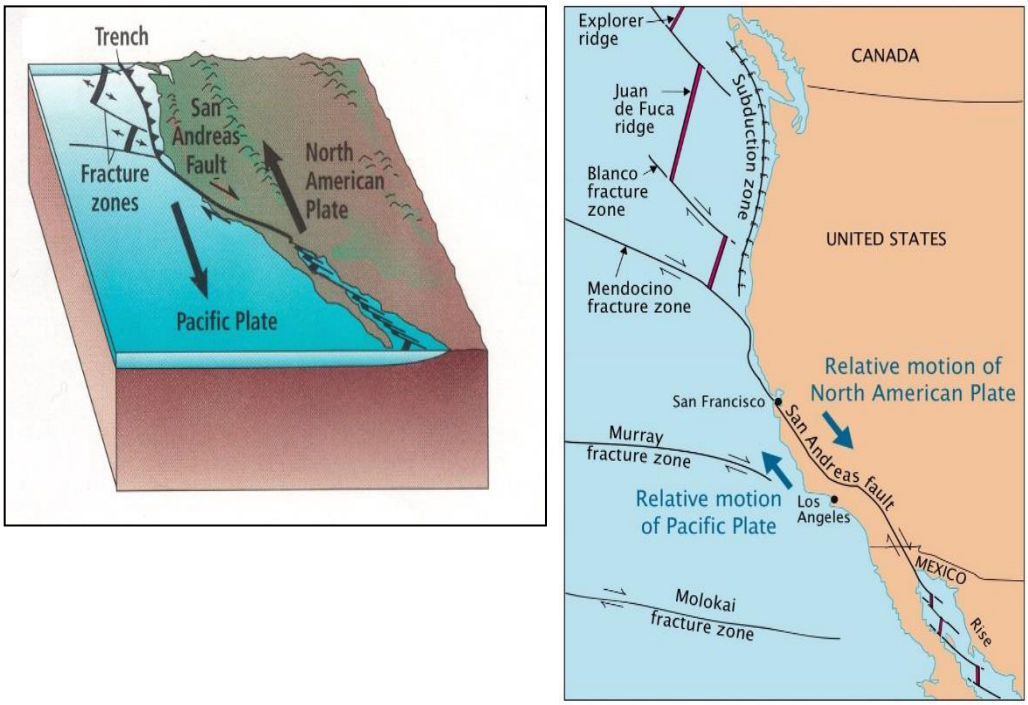
Convergent Boundary: Convergent boundary causes movement of plates together and it causes for creation of either *'subduction zones'* or *'collision zone'* (Fig. 50). *Subduction zone* may form continental volcanic arc (example: Andes, Cascades) if at least one side has denser oceanic material or volcanic island arc (example: Japan) and trenches if both the sides have oceanic materials whereas the *Collison zones* form mountain range (example: Himalayas) where both sides of a convergent boundary consist of continental (buoyant) material.

Fig. 50: Different types of convergent boundaries



Transform fault boundary: Most transform boundaries are present in the ocean moving the plates sideways past each other (example: San Andreas Fault where the North American and Pacific plates moving past each other (Fig. 51).

Fig. 51: Transform fault boundaries

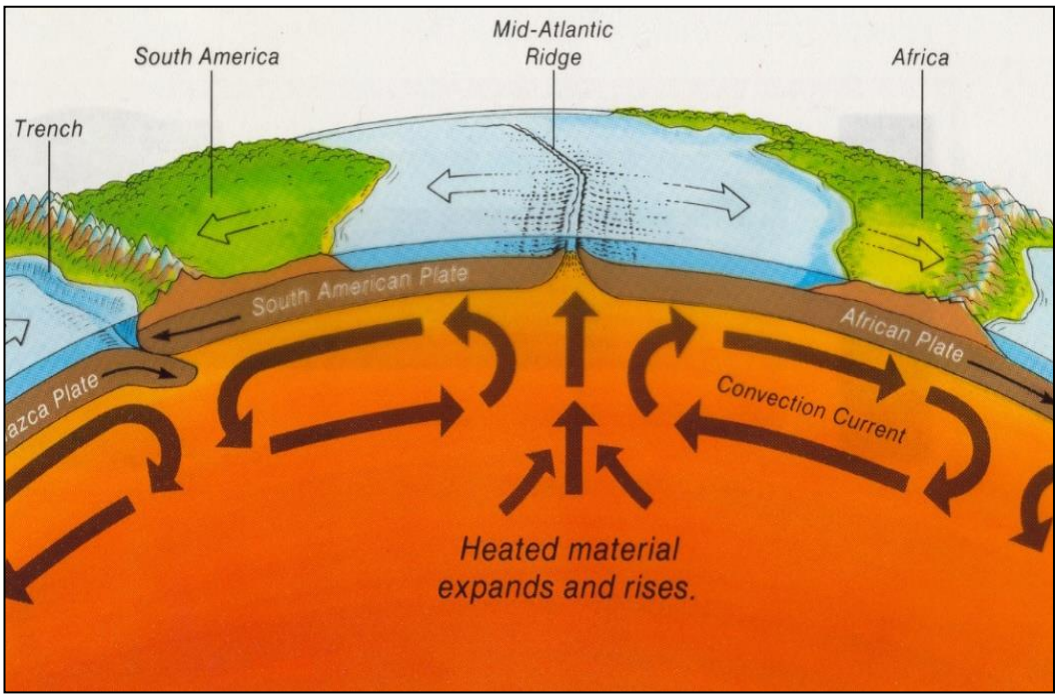


Scientists eventually succeeded to elucidate the reasons behind the plate movement by gaining detailed knowledge about seafloor and suggested two related ideas namely **'Slab pull'** and **'Mantle convection'** which are widely accepted for explaining drifting of plates.

Slab pull: Denser, colder plate sinks at subduction zone, pulls rest of plate behind it.

Mantle convection: Hotter mantle material rises beneath divergent boundaries and cooler material sinks at subduction zones (Fig. 52). As such, plate movement, earthquakes, and volcanic eruptions are due to Earth's loss of internal heat.

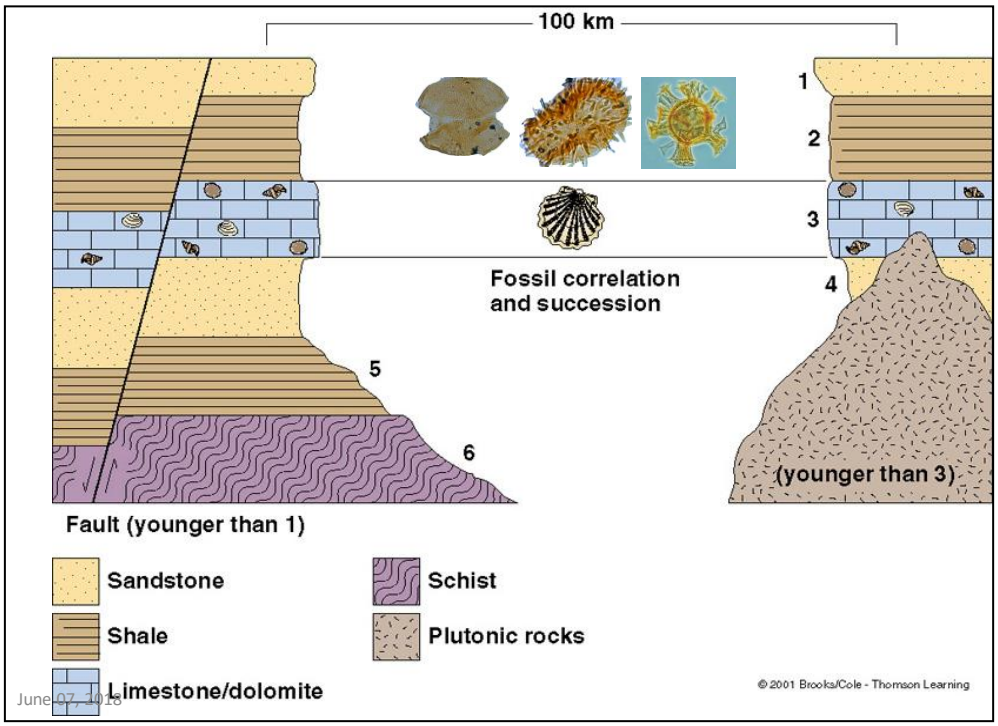
Fig. 52: Convection current



Geological age and correlation of strata

Biostratigraphy is the branch of stratigraphy which focuses on correlating and assigning geological ages (relative age) of rock strata and correlation between and among the strata by using the fossil assemblages contained within them. The methods by which the age relationship between various strata of Earth's crust is established is called correlation (Fig. 53). Such relationships can be established, in general, in one of two ways: by comparing the physical characteristics of strata with each other (physical correlation through lithostratigraphy); and by comparing the type of fossils found in various strata (fossil correlation through biostratigraphy). It provides information about changes that have taken place at various times in Earth history.

Fig. 53: Correlation of rock strata



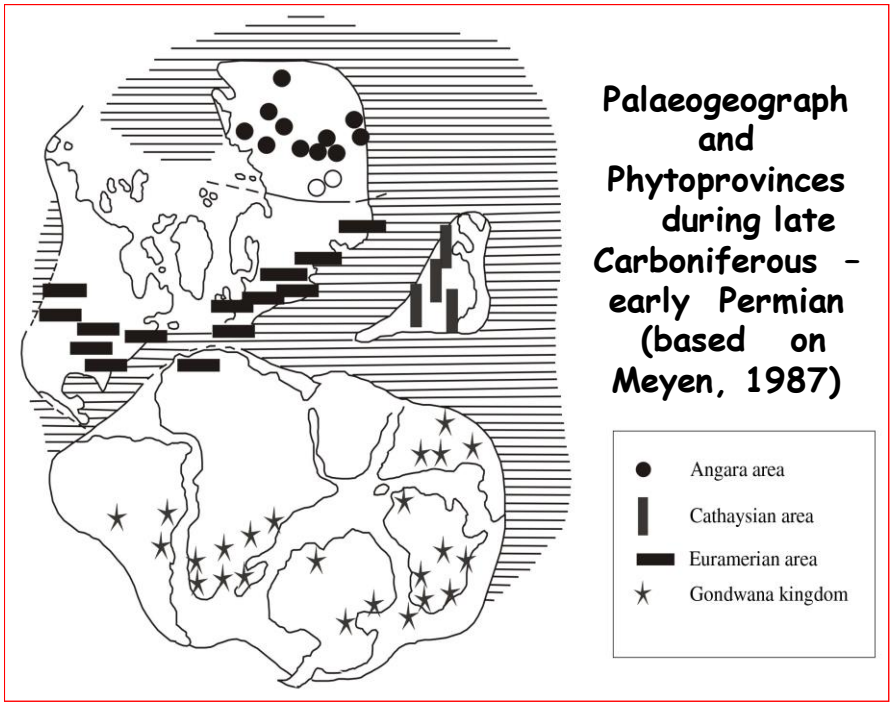
Palaeogeography, palaeoclimate; fossil fuel.

We know flroistics is the geographical distribution of taxa, the plotting of the plant system against the geographical background and palaeofloristics is the study of the distribution of the taxa — in time and space. In palaeofloristics both the spatial distribution and the chronological ranges of the taxa are considered.

The concentration of the boundaries in certain areas of the Earth can be observed by plotting the taxa areas on maps. This implies that the taxa form natural associations, each of which is confined to a definite territory. It can also be seen that the associations are in lain one in another, forming a system of subordination. Each association corresponds to a territorial floristic unit — the phytochorion. Phytochorion is a geographic area with a relatively uniform composition of plant species. Adjacent phytochoria do not usually have a sharp boundary, but rather a soft one, a transitional area in which many species from both regions overlap. Phytochoria may be of different ranks. In descending order, they are as follows: kingdom, area, province, district, region. Each is denoted by a specific geographical name. The taxa list of a phytochorion or its part is termed flora.

Plant Fossil evidences show that apparently uniform pattern of pre-Carboniferous vegetation of the globe was changed by the influence of Glacial cycle of mid Carboniferous and thus the floral diversification in the late Palaeozoic was initiated with changed climatic and geographic conditions of the globe. The diversified late Palaeozoic flora was designated as four geographic regions specific namely, **Angara flora** (in modern Siberia), **Cathaysian Flora** (in modern China & South East Asia), **Euramarian flora** (in Western part of Laurasia-North eastern North America, Western Europe) and **Gondwana flora** (in South America, Africa, India, Australia, Antarctica) (Fig. 54).

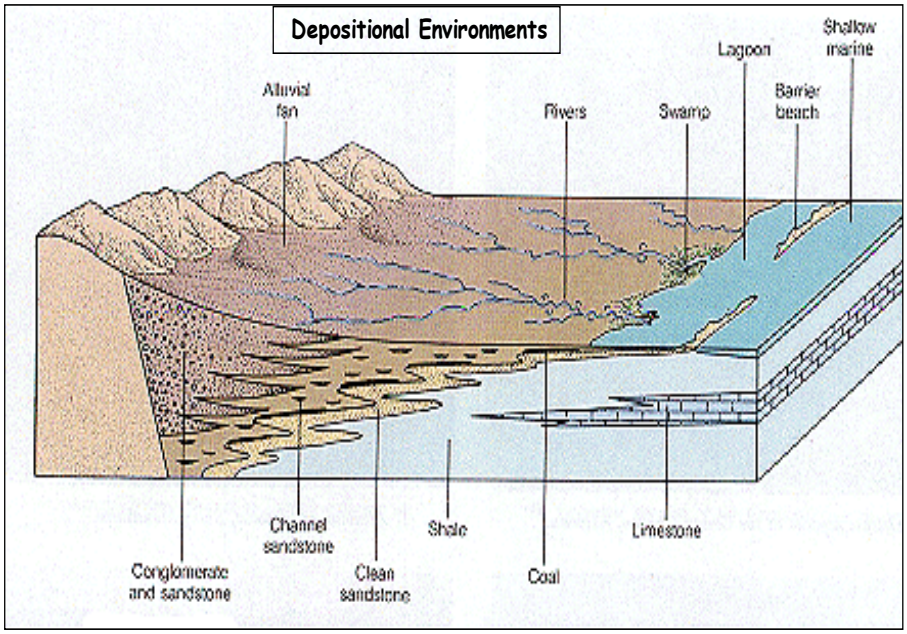
Fig. 54: Floral diversification in late Palaeozoic (Late Carboniferous – Early Permian)



Facies analysis for determination of depositional environment: Facies is a body of rock with specified characteristics. Ideally, facies are distinctive rock unit that forms under certain conditions of sedimentation, reflecting a process or environment. Thus, sedimentary facies refer to all the characteristics of a rock unit which come from the depositional environment (Fig. 55). In this regard, **Walther's Law of Facies** is well known which describes the vertical succession of facies reflects lateral changes in environment. Conversely, it states that when a depositional environment "migrates" laterally, sediments of one depositional environment

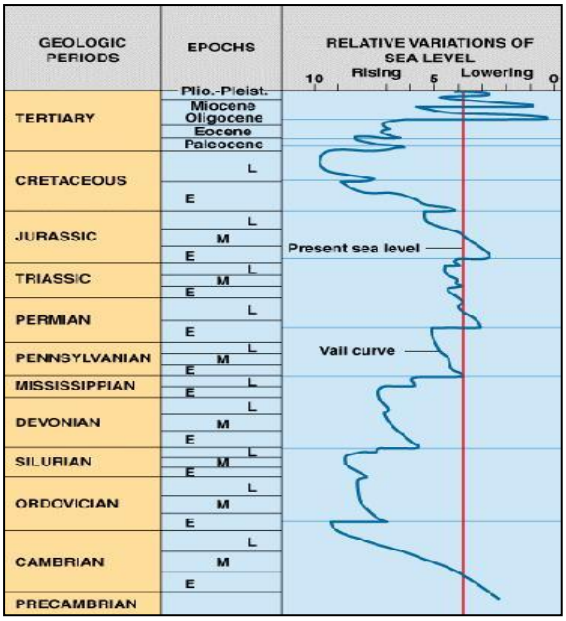
come to lie on top of another. A classic example of this law is the vertical stratigraphic succession that typifies marine transgressions (sea level rise) and regressions (sea level fall).

Fig. 55: Sedimentary Facies showing depositional environment



Determination of sea level change: The sea level throughout the geological history is changed by transgression and regression which is represented by the sea-level curve (Fig. 56). The first such curve is the **Vail curve or Exxon curve**. The names of the curve refer to the fact that in 1977 a team of Exxon geologists from Esso Production Research headed by Peter Vail published a monograph on global eustatic sea-level changes. Their sea-level curve was based on seismic and biostratigraphic data accumulated during petroleum exploration.

Fig. 56: Vail Curve



Plant fossils in analysis of palaeoenviroment, and palaeoclimate: Environment is the natural environment encompasses all living and non-living things occurring naturally on Earth or some region thereof. The biophysical environment is the biotic and abiotic surrounding of an organism, or population, and includes particularly the factors that have an influence in their survival, development and evolution.

Palaeoenvironment is the past environment of an area during a given period of its history. Paleoenvironmental records are derived from a wide variety of natural archives, such as:

- Fossils both mega and micro
- Tree rings
- Lake and ocean sediments
- Wind-blown deposits
- Coral
- Ice cores
- Historical documents

Plant fossil is one of the most reliable tools for deciphering past environment because of their ecological and geographical specificities. In their structure & distribution, extant organisms often reflect the environmental composition in which they grow and reproduce. In the same ways as those of the present, we make assumption that organism of the past became adapted to their environments. As for example-

- the presence of growth rings in petrified fossil wood suggests seasonal variations in the availability of water usually accompanied by temperature changes in the palaeoenvironment.
- fossil woods lacking growth rings suggest an equable environment with a continuous supply of water and a more uniform temperature regimen.
- thick cuticles and sunken stomata of fossil leaves suggest lack of available water
- roots and stems with spongy tissues indicate an aquatic or swamp environment.

Information of this kind gleaned from the morphology and anatomy of the fossil plants provides part of the basis of palaeoecology and palaeoclimatology. Chemical, isotopic, and ecological analyses of these records have demonstrated that the natural climate system has varied locally and globally over a far greater range.

Climate is the statistical data of temperature, humidity, atmospheric pressure, wind, rainfall, atmospheric particle count and numerous other meteorological elements in a given region over long periods of time. It can be contrasted to weather, which is the present condition of these same elements over periods up to two weeks.

Paleoclimate is the past climate and it changes taken on the scale of the entire history of Earth. It uses a variety of proxy methods from the Earth and life sciences to obtain data previously preserved within rocks, sediments, ice sheets, tree rings, corals, shells, mega & microfossils. It then uses these records to determine the past states of the Earth's various climate regions and its atmospheric system. Studies of past changes in the environment and biodiversity often reflect on the current situation, and specifically the impact of climate on mass extinctions and biotic recovery.

Few basic approaches used by paleobotanists to reconstruct ancient climate from fossil assemblages are:

- Nearest living relative (NLR) or the coexistence model
- Leaf Physiognomy: Leaf Area & Leaf Margin Analysis
- Palaeodendrology: Tree ring analysis
- Stomatal Index

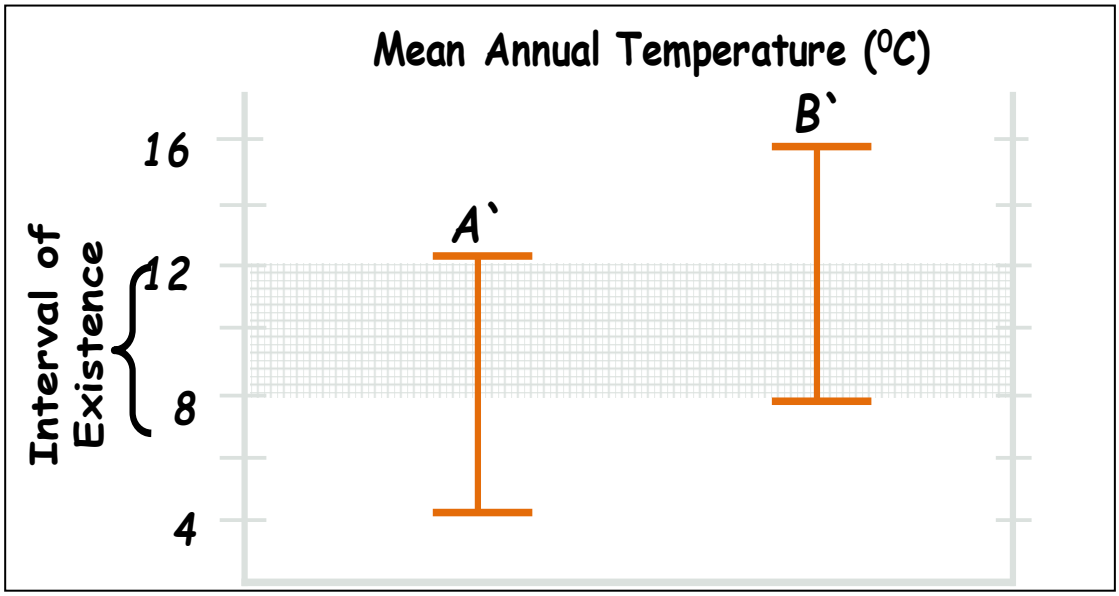
Nearest living relative or the coexistence model

For a given fossil flora and a given climatic parameter the climatic interval in which all nearest living relatives (NLR) of the fossil flora can co-exist. It assumes that fossil plant taxa have similar climatic requirements as their nearest living relatives. The coexistence approach (Mosbrugger and Utescher, 1997) is an efficient and reliable method for quantitative terrestrial palaeoclimate reconstructions from the Cenozoic palaeobotanical record.

Suppose a fossil flora with two taxa, A and B, is given and that the nearest living relatives of these fossil taxa, A' and B', are known, along with their climatic requirements (Fig. 57). It is assumed that the modern taxa A' and B' require a mean annual temperature between 6-12^{0}C and 8-16°C, respectively. Obviously, there exists a mean annual temperature interval between 8°C and 12°C in which A' and B' can (or could) coexist (N.B.: it is not required that A' and B' do really coexist in a modern phytocoenosis). According to the coexistence approach this interval of coexistence is taken as the best estimate of the mean annual temperature under which the fossil taxa A and B once lived. In a similar way estimates (coexistence intervals)

can be obtained for other climatic parameters, such as temperature of the coldest and warmest month or mean annual precipitation, etc.

Fig. 57: Graphical representation of co-existence approach for deciphering past climate



The list of climate parameters considered for co-existence model is

- MAT [Mean Annual Temp (°C)]
- WMT [Mean temp of the warmest month (°C)]
- CMT [Mean temp of the coldest month (°C)]
- RH [Mean relative humidity (%)]
- PE [Potential evaporation (mm)]
- MAP [Mean annual precipitation (mm)]
- MMaP [Mean maximum monthly precipitation (mm)]
- MMiP [Mean minimum monthly precipitation (mm)]
- MWP [Mean precipitation of the warmest month (mm)]
- AI [Aridity index MAP/PE)]

Leaf Physiognomy: Leaf Area & Leaf Margin Analysis

Analyses of leaf physiognomy (characteristic external features of extant vegetation) have been used to obtain more precise information, which in turn can be extrapolated when preparing reconstructions of climatic and vegetational changes of the past. Wolf (1979) established a classification of vegetational types based on the physiognomic characteristics of extant angiosperm leaves that occur within the temperature parameters.

Three categories of temperature parameters

- microthermal vegetation growing within a range of -5°C to 13°C
- mesothermal vegetation growing within a range of 13°C to 20°C
- megathermal vegetation growing within a range of 20°C to 30°C

A correlation exists between leaf size and decreasing temperatures and /or precipitation which are as follows:

Serrate (toothed) leaf margins: are typical of humid microthermal vegetation. Show a marked decrease in abundance in forests where higher temperatures prevail (replaced by entire margin species)

Thick leaf texture: is typical of evergreen angiosperm that predominate in megathermal and mesothermal vegetation where precipitation may be limited

Usually deciduous plants with thin leaves: predominate in microthermal or successional mesothermal vegetation

Evergreen leaves in humid environments: usually have attenuated apices (drip-tips)

Compound leaves: tend to occur on plants that are frequently associated with deciduous plants.

Narrow leaves: are often found on plants inhabiting stream banks where the substrate is disturbed

Broader heart-shaped leaves, usually with palmate venation: are produced by lianus, sprawling shrubs in successional vegetation, or colonizers in open areas of the forest

Lobed leaves: are often found on successional or understory species

Palaeodendrology

It is the branch of paaleobotany that studies fossil trees rings (Fig. 58). Tree rings are an important source of information about past climates and fossil environments. Fossil tree rings

are unique source of detailed information about seasonality, annual growing conditions, water availability, limiting temperatures and forest productivity in the geological past.

Fig. 58: Fossil tree ring

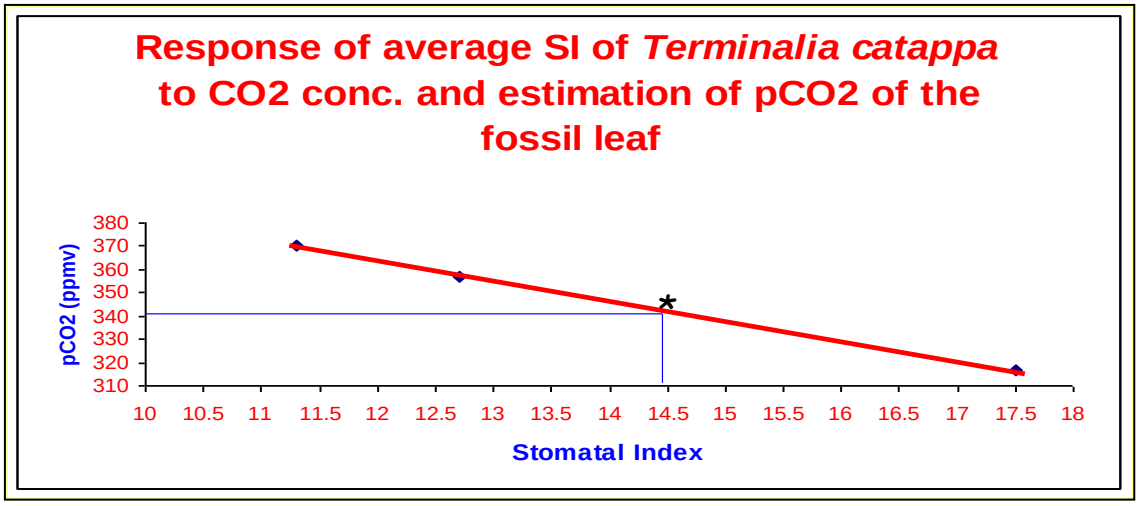
Stomatal index (SI) study

Stomatal index study is utilized for determining the pCo_2 Concentration by using the principle '**Stomatal frequency is inversely proportional to atmospheric CO_2 conc.'.** The pCO_2 could be estimated by plotting the data of extant SI and corresponding CO2 conc. in graph (Table 5; Fig. 59).

Table 5: Stomatal Index (SI) & pCO_2 data for extant and fossil leaves of *Terminalia catappa* *(after Van der Burgh et al., 1993; Mehrotra et al., 2003)*

Extant Leaf	**Stomatal Index (SI)**	**CO_2 conc (in ppmv)**
2002	11.3	370
1992	12.7	357
1960	17.5	317
Fossil Leaf	**14.5**	**342 [pCO_2 conc (in ppmv)]**

Fig. 59: pCO_2 estimation through plotted data of extant SI and correspondence CO_2 conc.



Determination of past climatic data from various sources is prerequisite in modern day aspect for climatic simulation study for future trend analysis of climate.

Fossil Fuel

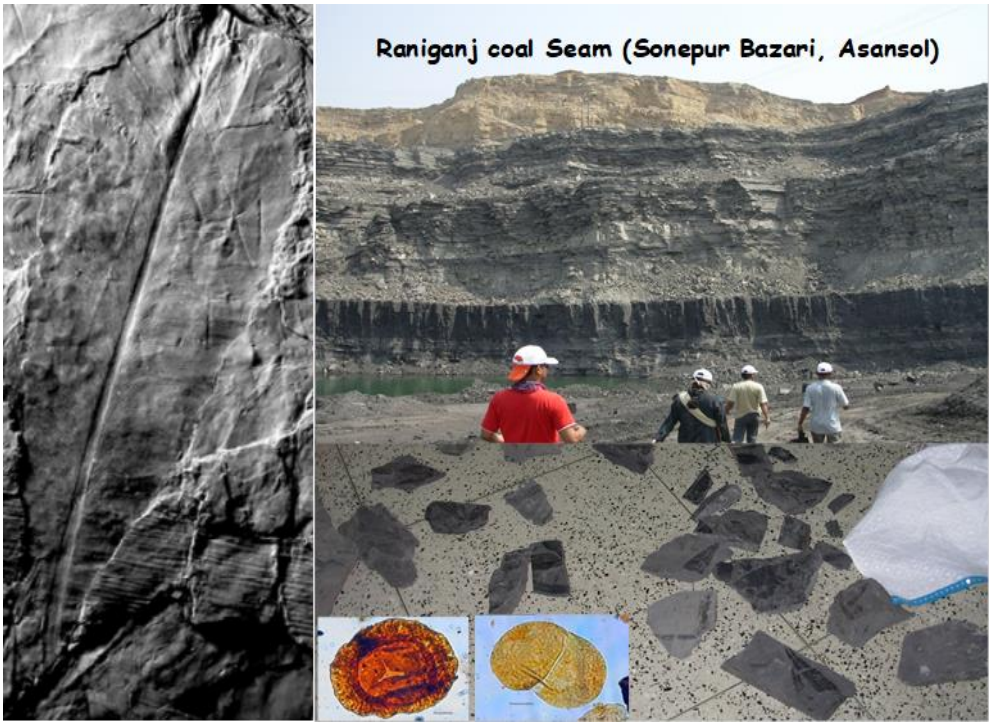
Palaeoecology is the study of the interrelationships between ancient organisms and the palaeoenvironemnts in which they lived. Fossil fuels in form of solid (peat, lignite, coal), liquid (petroleum), gas, and other hydrocarbons, limestone, algal stromatolite, phosphorites, diatomaceous Earth etc. are the different energy resources i.e. biogenic substances fossilized in different ecological conditions in geological past. Fossil fuels are, in effect, stored ancient sunlight through carbon captured by plants and stored by geology and we now rely on them for so much of our energy needs.

It was during the Cool, wet and glacial Carboniferous Period that an unusual and fortuitous set of ecological conditions prevailed which began a process of mass sequestration of terrestrial carbon, resulting in the oil, gas and coal deposits we enjoy burning so much today. Thus, coal deposits were formed around 300 Ma ago in a geological period known as the **CARBONIFEROUS** (meaning coal bearing) in terrestrial ecological conditions through fossilization of *Lepidodendron* flora in northern hemisphere and *Glossopteris* flora in southern hemisphere continents.

Coal is older than petroleum oil and gas, which were deposited in organic rich marine strata of Jurassic and Cretaceous age (65-200 Ma ago). Petroleum is made from the remains of phytoplanktons and zooplanktons which fell to the bottom of seas where this is little or no oxygen. The organisms were buried under sediments through repeated ice age style rises and falls of sea level flooding and then retreating from coastal swamps and there slowly cooked at high temperature and pressures to produce oil. The higher the temperature and pressures and the longer the process, the lighter the oil becomes as it continues to evolve chemically, some turning into associated natural gas.

Being the fossilized product, fossil fuel rich palaeoecological conditions could easily be identified through fossil study. As for example study of *Glossopteris* flora (Fig. 60) is important in coal exploration in Gondwanaland continents including India.

Fig. 60: *Glossopteris* fossils explored from an Indian coal deposits



Discovery of new fossils may change the conventional ideas of taxonomists and evolutionary biologists: Reconstructed Plant *Archaeopteris* of Devonian age introduced a new group-Progymnosperms by Charles Beck in 1960 after the discovery of fossil of *Callixyon* wood (like gymnosperm) and *Archaeopteris* leaf (like fern) connected organically.

Palaeogenetics and Palaeophytochemistry: Study of chemical fossils and isolation of a-DNA developed new branches namely 'palaeophytochemistry' and 'palaeogenetics' respectively for exploring new vistas in phylogenetic and climatic research.

Study of sub fossils from Archaeological sites: It helps to detect the Socio-cultural evolution of ancient people and the Agricultural practices and other uses of plants (Fig. 61).

Fig. 61: Charred seeds recovered from archaeological site

11. Let's sum up

Carbonate: a salt or ester of carbonic acid (H_2CO_3); carbonates can combine with other elements to form minerals, for example with calcium (calcium carbonate) or with iron (iron carbonate)

Cast: opposite of mold; a preservation type that forms within a mold; casts are usually three-dimensional and therefore preserve form

Chert: a type of rock; chert can form as a primary deposit preserving fossils within it, or replace organic materials in fossils embedded in different rock material

Coal: a general name given to stratified accumulations of carbon-rich material derived from vegetation. The starting point for coal formation is usually peat or some similar accumulation of partially decayed plant matter. By the process of compaction, heating, and chemical alteration, the peat is converted by a series of stages into coal. The type of coalification corresponds to the amount of heating that a peat has undergone:

- Peat
- Lignite (brown coal)
- Bituminous coal
- Anthracite

Coal balls: a type of preservation known from Carboniferous and Permian coal seams, in which plants are preserved by calcium carbonate and other minerals; see petrifaction and permineralization.

Compaction: in sedimentation: the formation of massive rock from loose sediment, normally brought about by the weight of the overlying sediments; in preservation: organic preservation in three dimensions, for example in peat or clay

Compression: a fossil preservation type in which a thin film of organic matter is preserved; compare to impression

Cuticle: an impermeable layer of cutin on the outer walls of epidermal cells

Cutin: the waxy substance of which a cuticle is composed

Fossil: any evidence of prehistoric life; any remains of any once living organism preserved in the Earth's rocks

Lignite: see coal

Midden, packrat midden: midden: garbage or refuse heap; packrats (*Neotoma* sp.) collect and store plant and animal parts in middens; plant material is preserved and cemented together by urine and feces from the packrats; packrat middens are only found from the later part of the Pleistocene through the present, but their fossils have been instrumental in reconstructing Pleistocene and Holocene vegetational changes in the North American southwest and have also been important in archeological studies

Mold: a three-dimensional preservation type that represents a negative image of the plant; compare to cast

Silicification: a type of fossilization in which silica (SiO_2) infills intercellular spaces (permineralization) or replaces the cell walls (petrifaction)

Silica: SiO_2; occurring in crystalline (quartz), cryptocrystalline (very finely crystalline; crystals are very, very small) (opal) and non-crystalline (chert) forms; one of the most common minerals in the crust of the earth; an important mineral in the process of silification; also see permineralization

12. Suggested Readings

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2. Golenberg, E. M., D. E. Giannasi, M. T. Clegg; C. J. Smiley, M. Durbin; D. Henderson and G. Zurawski, 1990. Chloroplast DNA sequence from a Miocene Magnolia species. Nature 344: 656-658.
3. Kumar, R. 1985 Fundamentals of Historic al geology and stratigraphy, Wiley Eastern Ltd.
4. Meyen, Sergei, V. 1987 Fundamentals of Palaeobotany. Chapman and Hall
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6. Stewart W. N. & Rothwell G. W. (1993). Palaeobotany and the evolution of plants. 2nd edn. Cambridge: Cambridge University Press.

7. Surange, K. R., Lakhanpal, R.N., Bharadwaj, D. C. 1974 Aspects and appraisals of Palaeobotany Edited Volume, Birbal Sahni Institute of Palaeobotany, Lucknow
8. Taylor Thomas N., Taylor Edith L. & Krings Michael. (2009). Palaeobotany: The biology and Evolution of fossil plants. 2nd edn. Elsevier Publication.
9. Willis K. J. & McElwain J. C. (2002). The evolution of plants. 1st edn. New York: Oxford University Press.

13. Assignments

1. What is Palaeobotany?
2. Define Fossil.
3. What is Taphonomy?
4. Enumerate the types of fossils.
5. What is index fossil? Give example.
6. Briefly describe different modes for preservation of fossils.
7. Mention the differences between coal and coal ball.
8. What are the external and internal factors responsible for fossilization?
9. Mention the difference between amber and amberat.
10. What is the difference between ‘plant fossils’ and ‘fossil plant’?
11. How does the naming of fossils occur?
12. Describe any one technique for studying anatomical details of plant fossils.
13. What is peat? How does it analyze for extracting fossil?
14. Mention the time of first appearance of Prokaryotes.
15. Name commercially important organic deposits.
16. Name the dominant plant of Indian Lower Gondwana coal forest.
17. Mention the forces responsible for movement of tectonic plates.
18. State the differences between relative and absolute dating method.
19. Define geological time scale.
20. Which mode of evolution (gradualism or punctuation & stasis) is predominates in the fossil record?
21. How does the fossil plant record fit into either of these two evolutionary theories?
22. Enumerate the pattern of evolutionary change in plant fossil record.
23. What is meant by Turnover-pulse hypothesis?

24. Describe the mechanisms driving the evolutionary change.
25. Describe the causes behind mass extinction events in evolutionary history.

COURSE – BOTCOR T205

PALAEOBOTANY & PALYNOLOGY

Core Theory Paper **Credit: (Groups A+B) = 3**

Group – B (PALYNOLOGY)

Content Structure

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1. Introduction
2. Course Objective
3. Spore-pollen morphology: units, polarity, symmetry, shape, size, aperture; NPC system for numerical expression of apertural details; evolution of aperture types.
4. Pollen wall and extraexinous wall materials: Sporoderm stratification and sculptures; LO-analysis; sporopollenin; pollen wall development; Ubisch body; pollen connecting threads, perine, pollen-kit.
5. Pollen grains adaptation: Pollen grains adaptation in different habitats and pollination types; pollen wall adaptation and significance; Hermomegathic mechanism.
6. Spore/Pollen Viability and Storage: Estimation; variations; responsible factors; short- and long-term storage; significance.
7. Pollen limitation and plant diversification: Definition; ecological and evolutionary relevance.
8. Natural spore/pollen traps: Types, their implications in floristic & environment reconstruction.
9. Branches of palynology & application: Branches of palynology; palynology in taxonomic & phylogenetic deductions; palynology in academic & applied aspects including melissopalynology, medical palynology, forensic palynology, entomopalynology & copropalynology.
10. Let’s sum up
11. Suggested Readings
12. Assignments

1. Introduction

Palynology is the study of plant pollen, spores and certain microscopic plankton organisms (collectively termed palynomorphs) in both living and fossil form. Botanists use living pollen and spores (actuopalynology) in the study of plant relationships and evolution, while geologists (palynologists) may use fossil pollen and spores (paleopalynology) to study past environments, stratigraphy (the analysis of strata or layered rock) historical geology and paleontology. Palynology is a useful tool in many applications, including a survey of atmospheric pollen and spore production and dispersal (aerobiology), in the study of human allergies, the archaeological excavation of shipwrecks, and detailed analysis of animal diets. Entomopalynology is the study of pollen found on the body or in the gut of insects. It is useful for determining insect feeding and migratory habits, especially as it involves economically important insects (as the boll weevil, or earwigs). Forensic palynology, or the use of pollen analysis in the solving of crimes, is used by Crime Scene Investigators around the world.

2. Objectives

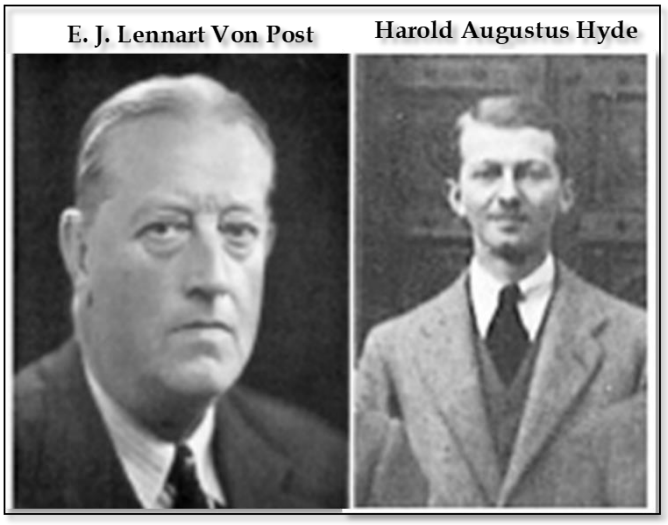
It is the branch of biology which has important contribution in both classical and applied aspects which encompassing wide spectrum of life, earth and biomedical science. Due to immense contribution in immerging field of both advanced as well as classical studies it may represent itself as sphere of knowledge of science and technology. Being an interdisciplinary science, it has diverse range of application. By studying this branch of science student get knowledge of -

- Palynotaxonomy and evolutionary studies
- Aerobiology
- Melissopalynology
- Forensic palynology
- Biostratigraphy and geochronology
- Paleopalynology
- Archaeopalynology

3. Spore-pollen morphology: units, polarity, symmetry, shape, size, aperture; NPC system for numerical expression of apertural details; evolution of aperture types.

Palynology is the branch of science concerned with the study of pollen grains, spores, and similar palynomorphs, both living and fossil. H. A. Hyde & D. A. Williams (1944) coined the term Palynology but the study of pollen analysis was started in 1916 by E. J. Lennart Von Post (Fig.1). **Pollen grains** are male reproductive unit of the flowering plants and **Spores are** various types of reproductive bodies in non-flowering plants formed by sexual and asexual methods.

Fig. 1: Renowned Palynologists



Spore-pollen morphology: units, polarity, symmetry, shape, size, aperture

UNIT

Pollen unit is the product of microsporogenesis (Fig. 2) through which a single pollen mother cell forms pollen units (Fig. 3A-K) by meiotic division either singly (monad- Fig. 3a) or aggregation of two (dyad- Fig. 3B), four (tetrad- Fig. 3C-I), and more (polyad- Fig. 3J).

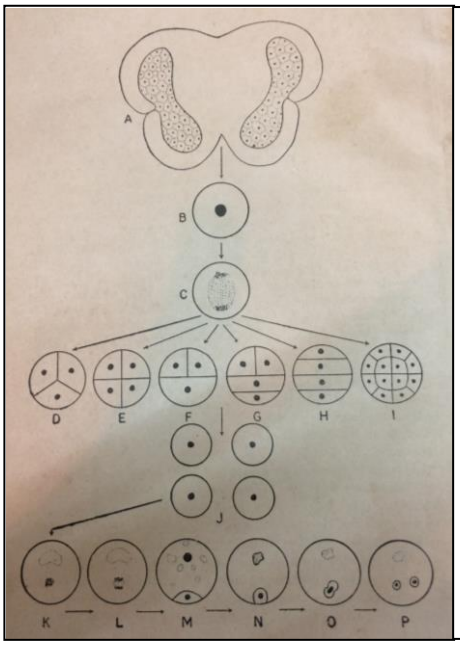


Fig. 2: Microsporogenesis

A. Cross Section of anther showing the pollen sacs with the sporogenous tissue

B. Pollen mother cell

C. Meiosis stage

D. – H. Tetrad type

I. Polyad

J. Individual pollen grains

K. – P. Post meiotic stage

Dyads:

Pollen grains which are united in pairs and shed from the anthers as doubles are called dyads (Fig. 3B). Dyads are present in *Scheuchzeria palustris* and other members of Podostemonaceae. The dyads are formed due to the incomplete break up of individual grain or monad.

Tetrads:

Four pollen grains are united to form tetrad. Tetrads are product of non-separation of meiotic cell. Tetrads maybe categorized into different types based on their arrangement (Fig. 3C-I).

Tetrahedral tetrad:

Pollen grains are arranged in two different planes (Fig. 3C). Three grains are in one plane and one lies centrally over the other three. In some cases, the pollen grains are released from the anther in the tetrad condition. These types of tetrads are called obligate or permanent tetrads, namely, *Drymis* (Winteraceae), *Drosera* (Droseraceae), *Rhododendron* Ericaceae).

Tetragonal tetrad:

All the four pollen grains are arranged in one plane (Fig. 3D), as for example, *Typha latifolia* (Typhaceae), *Hedycaria arborea* (Monimiaceae).

Rhomboidal tetrad:

All pollen grains are arranged in one plane forming rhomboidal shape (Fig. 3E) as for example, *Annona muricata* (Annonaceae).

Decussate tetrad:

Pair-wise the pollen grains are at right angle to each other (Fig. 3F), as for example, *Magnolia grandiflora* (Magnoliaceae).

T-Shaped tetrad:

The first division of pollen mother cell is transverse to form a dyad. The upper or lower cell of dyad undergoes a vertical or longitudinal division instead of transverse, yielding either straight or inverted T-shaped configuration (Fig. 3G), as for example, *Aristolochia* sp. (Aristolochiaceae), *Polyanthes* sp; (Amaryllidaceae).

Linear tetrad:

The first division of pollen mother cell is transverse, and a dyad is formed. Each cell of the dyad again divides transversely to form a linear tetrad (Fig. 3H), as for example, *Mimosa pudica*.

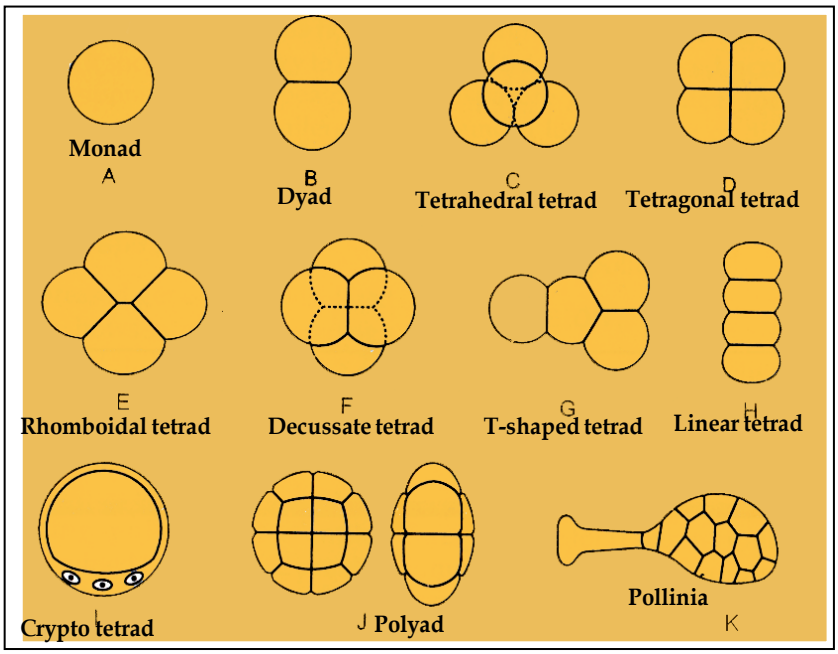
Cryptotetrad or Pseudomonad:

Here tetrads are formed without partition walls between the four compartments. One out of the four nuclei develops normally and the rest three obliterate. Thus, an apparent monad but homologous to the tetrad is formed (Fig. 3I), as for example in Cyperaceae.

Polyads:

In most of the Mimosaceae

Fig. 3: Different types of pollen unit



members each of the tetrad cells divides once or twice or more, yielding a group of 8 to 64 cells which remain together after maturity. These compound grains are usually held together in small units and are called polyads (Fig. 3J) as for example, *Acacia auriculiformis*, *Adenanthera pavonina*, *Calliandra hematocephalla*, *Samania saman*, *Albizzia lebbeck.*

Pollinia:

In Orchidaceae and Asclepiadaceae the whole contents of an anther or anther locule which shed as one united mass of pollen are called Pollinia (Fig. 3K). The pollinium (singular) apparatus is the functional unit of a "corpusculum" with its two attached arms (translator) and Pollinia. As for example, *Calotropis* sp., *Daemia* sp., etc., of the Asclepiadaceae and majority of the family Orchidaceae

POLARITY

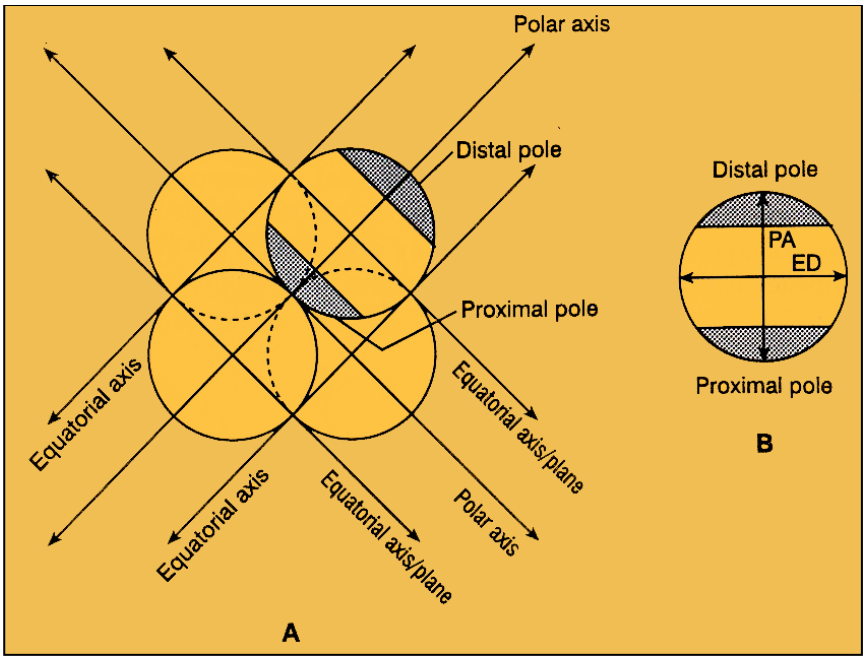
Polarity of individual pollen grains is expressed in terms of their arrangement in the tetrad; end of the grains directed towards the centre of the tetrad is proximal pole & towards the outside is the distal pole. The orientation of polarity is an important criterion in identification and description of pollen grains, as apertural position is of primary phylogenetic and functional significance. All pollen grains are in tetrad stage during development and the polarity is determined in this stage, prior to their separation.

The part of the pollen grains which is nearest to the centre of the tetrad is the proximal pole and that towards the opposite side is the distal pole (Fig. 4). The imaginary line between the proximal and distal pole of the grain is called the Polar Axis (PA) which passes through the centre of the spore to the centre of the tetrad.

The plane perpendicular to the polar axis through the middle of the grain is the equatorial plane (equatorial diameter). Positions on the surface of the grain may be determined by their latitude comparing to the latitude on a regular sphere. Similarly, surface features in a pole to pole direction at right angles to the equatorial plane are called meridional.

Fig. 4: Polarity in tetrad and monad

A= Polarity in tetrad stage; B= Length of polar axis (PA): *hypothetical line connecting two poles; perpendicular to it is the equatorial axis;* ED: Breadth of equatorial diameter in a monad



The pollen grains maybe either apolar or polar. In apolar spores, poles or Polar Regions cannot be distinguished in individual spore (monad) after separation from tetrad. Among the polar types the pollen grains are either isopolar or heteropolar depending upon the demarcation between two equal or unequal polar faces, respectively (Fig. 5).

Apolar spore: In individual spore, poles or Polar Regions cannot distinguished after separation from tetrad stage

Isopolar grain: If equatorial axis demarcates two equal polar faces

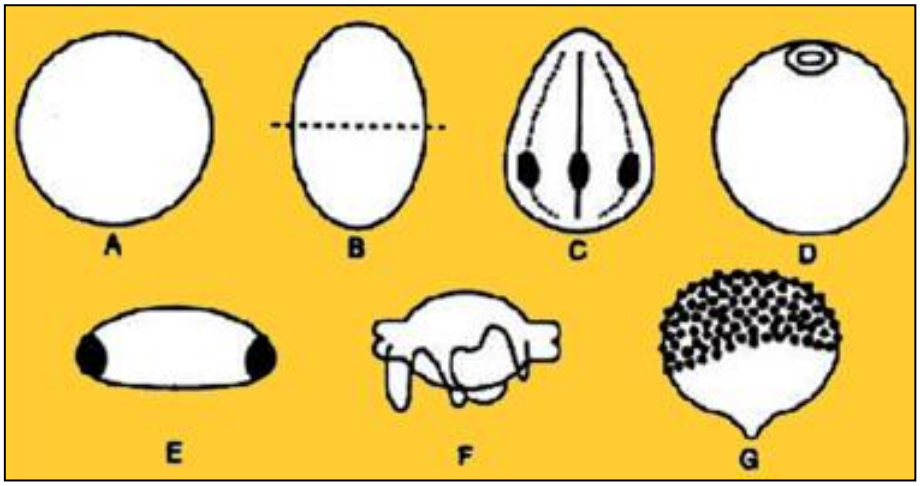
Paraisopolar or subisopolar: slight differences exist between the distal and proximal faces for example *Carya*, *Ulmus*, etc.

Heteropolar grain: If equatorial axis demarcates two unequal polar faces so that two faces are distinctly different either in shape, ornamentation of apertural system

Cryptopolar: Distal and proximal faces have dissimilar sculpturing and lack tetrad mark for example *Calobryum dentatum, Haplomitrium hookeri*

Fig. 5: Different types of Polarity

A. Apolar; B. Isopolar; C & D. Heteropolar; E & F. Paraisopolar; G. Cryptopolar



SYMMETRY

Pollen grains or spores may be of symmetric or asymmetric (Fig. 6).

Asymmetric grain: It has no plane of symmetry and it is rare in occurrence.

Nonfixiform: not having fixed shape

Fixiform: having fixed shape

Symmetric grain:

Radiosymmetric- having a radial symmetry

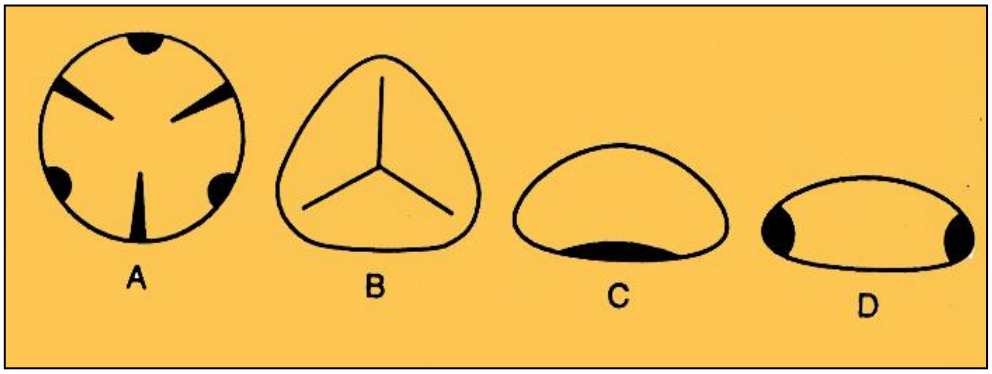
Bilateral symmetry-having a single plane of symmetry

In radiosymmetric grain the shape is such that any plane including the polar axis that passes through will produce identical halves. So the radiosymmetric grains have more than two vertical planes of symmetry. Radial symmetric isopolar grains have one horizontal and two or more vertical planes of symmetry. Radial symmetric heteropolar grains have no horizontal plane of symmetry. Bilateral heteropolar pollen grains have two vertical planes of symmetry. Bilateral isopolar grains have three planes of symmetry, one horizontal and two vertical. In

some bean- shaped or boat-shaped spore/pollen there is only one vertical plane of symmetry with an opening towards the end of the grain.

Fig. 6: Pollen Symmetry

A. & B. Radial symmetry; C. & D. Bilateral symmetry

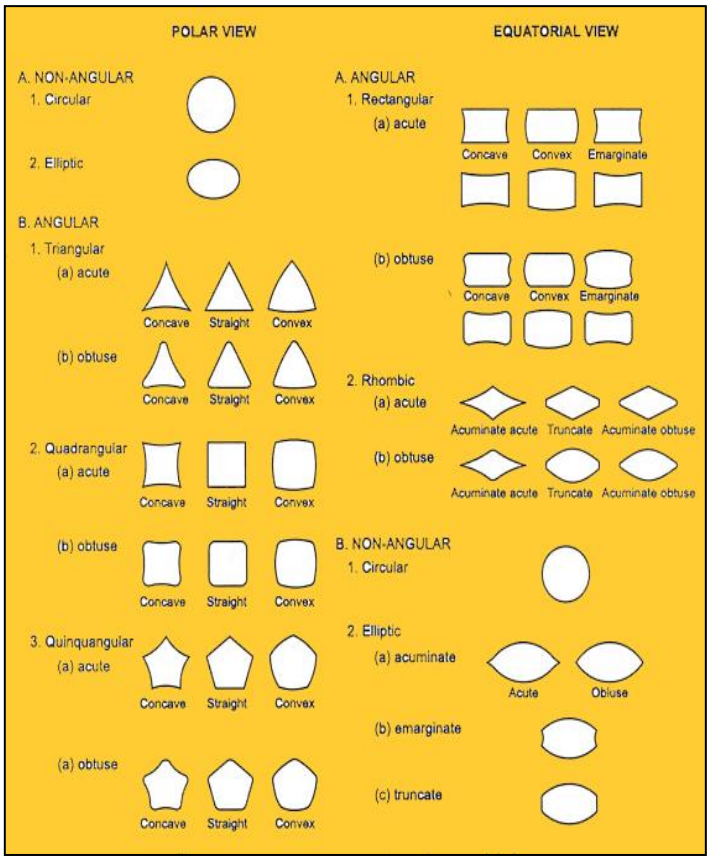


SHAPE

The shape of the pollen grains varies from species to species. Shape of the grains is found to be useful in spore/pollen identification. However, the shape may vary considerably within one grain type or even within one species.

Pollen grains and spores are often described by the shape (non-angular and angular) of their outline both in polar and equatorial views. The shape of the pollen/spores may be circular, elliptical, triangular, rectangular, and quadrangular or in other geometrical shapes (Fig. 7).

Fig. 7: Shape of grains in polar and equatorial views



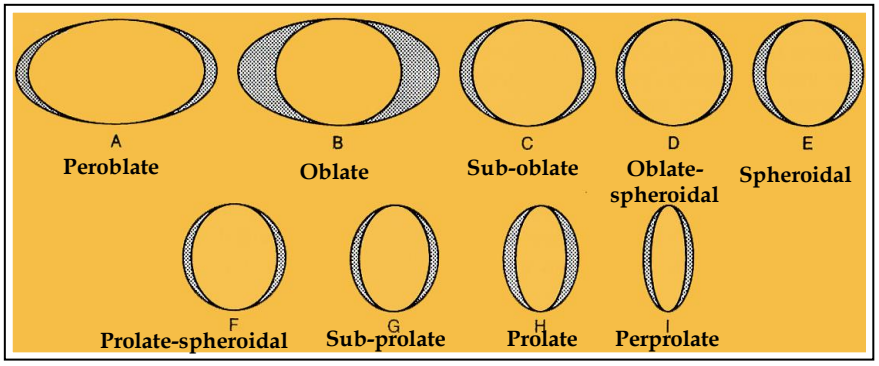
Determination of Shape: Various shape classes (Table 1; Fig. 8) can be determined by calculating the ratio between the length of polar axis (PA) and equatorial diameter (ED) multiplied by 100. In bilateral grains, pollen grains are plano-convex, concavo-convex or biconvex in lateral view.

Table 1: Shape class

Shape Class	**(PA/ED) X 100**
Per-oblate	<50
Oblate	50-75
Sub-oblate	75-88
Oblate-Spheroidal	88-99
Spheroidal	100
Prolate-Spheroidal	101-114
Sub-prolate	115-133
Prolate	134-200

Shape Class	(PA/ED) X 100
Per-prolate	>200

Fig. 8: Different shape classed as categorized by PA/ED ratio



SIZE

Pollen grains show a great variety in their sizes. Smallest pollen grains of about 5 x 2.4 µm is noted in *Myosotis palustris* and some members of Boraginaceae, while the largest pollen grains (> 200 µm in diameter) are observed in Curcurbitaceae, Nyctaginaceae and *Orectanthe ptaritepuiane* (Abolbodaceae). In taking measurements of size the length of polar axis (PA), equatorial diameter (ED) and sometimes equatorial breadth (EB) are considered in bilateral grains.

In radial symmetric pollen grains the PA and the greatest ED can be measured in equatorial view, while the EB can be measured in polar view only. It is also necessary to measure exine elements, taking into consideration the thickness of exine, sexine/nexine thickness ratio and the thickness of the exine projections greater than 0.5 µm if any.

Erdtman (1945) categorized the different pollen size classes based on the size expressed as length of the longest axis (Table 2).

Table 2: Pollen size class

Pollen size class	Length of longest axis (in um)
Very small grain	<10
Small grain	10-25
Medium sized grain	26-50
Large grain	51-100

Pollen size class	Length of longest axis (in um)
Very large grain	101-200
Gigantic grain	>200

APERTURE

Aperture is a specialized region of the sporoderm that is thinner than the remainder of the sporoderm and generally differs in ornamentation and/or in structure. Morphologically aperture is an opening or thinning of the exine where the in tine is usually thick; physiologically it is a germination zone or a harmomegathus (A mechanism accommodating changes in volume of the semi-rigid pollen exine) or both.

Regarding their position, the apertures are polar, global or equatorial. The polar apertures are either monopolar (either in proximal or in distal pole) or bipolar (both in proximal and distal face). Global apertures are uniformly distributed over the pollen/spore surface. Equatorial apertures are meridionally arranged.

Some taxa have ‘atreme’ (trema, a Greek word means aperture) pollen/spore, i.e., they seem to have no special aperture, are termed as ‘inaperturate’ or non-aperturate.

Majority of the pollen grains described as ‘inaperturate’ seem to be ‘omniaperturate’, that is, the entire pollen wall is made up of a thin exine and a thick intine or at least thick as the exine, for example *Canna* sp. of Cannaceae. There are two types of apertures known as Pores (Porus, in plural Pori) and furrows (Colpus, in plural Colpi. or Sulcus, in plural Sulci). In most cases the furrows act as harmomegathi.

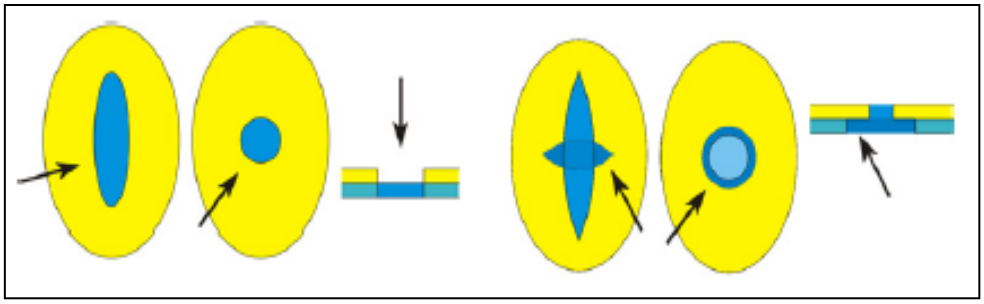
In living pollen grains or spores the apertures usually function as

- sites of germination;
- they may also provide routes for transfer of water and other substances;
- play a part in harmomegathy

Aperture types

The term is often used in conjunction with a prefix or suffix, as for example in, ectoaperture, endoaperture, inaperturate, omniaperturate, pseudoaperture, triaperturate. Depending upon the position of the aperture on the pollen wall it is categorized as simple and compound aperture (Fig. 9)

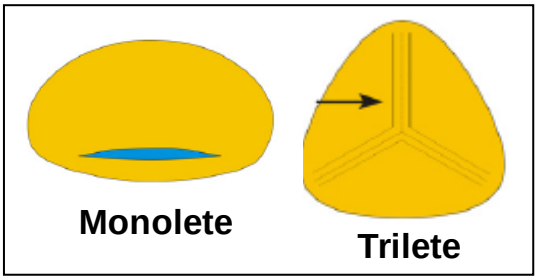
Fig. 9: Position of aperture on pollen wall



Simple Aperture: Apertures are described as simple if they are present in only one wall layer. Different types of simple apertures (Fig. 10, 11) are presented below-

Lete: Slit like; present on proximal end; depending on the number of lete it is either monolete (1 lete) or trilete (3 letes). These types of apertures are found in spores of lower group of plants like pteridophyte.

Fig. 10: Lete type of simple aperture



Other various types of simple apertures are present on pollen grains of plants belong to gymnosperms and angiosperms.

Porus: circular or elliptic aperture with a length/breadth ratio less than 2

Ulcus: A rounded ectoaperture situated at the distal or proximal pole of a pollen grain. Examples: *Sparganium (Sparganiaceae), Typha* (Typhaceae). Pollen grains are called ulcerate.

Ulculus: A rounded ectoaperture which is not situated at a pole. Example: Poaceae.

Colpus: An elongated, aperture with a length/breadth ratio greater than 2.

Sulcus: An elongated latitudinal ectoaperture situated at the distal or proximal pole of a pollen grain. A sulcus has the same shape as a colpus, but differs in orientation. Sulci are essentially latitudinal apertures whereas colpi are essentially longitudinal apertures.

Trichotomosulcate: pollen grain with a three-armed sulcus. Example: some Palmae

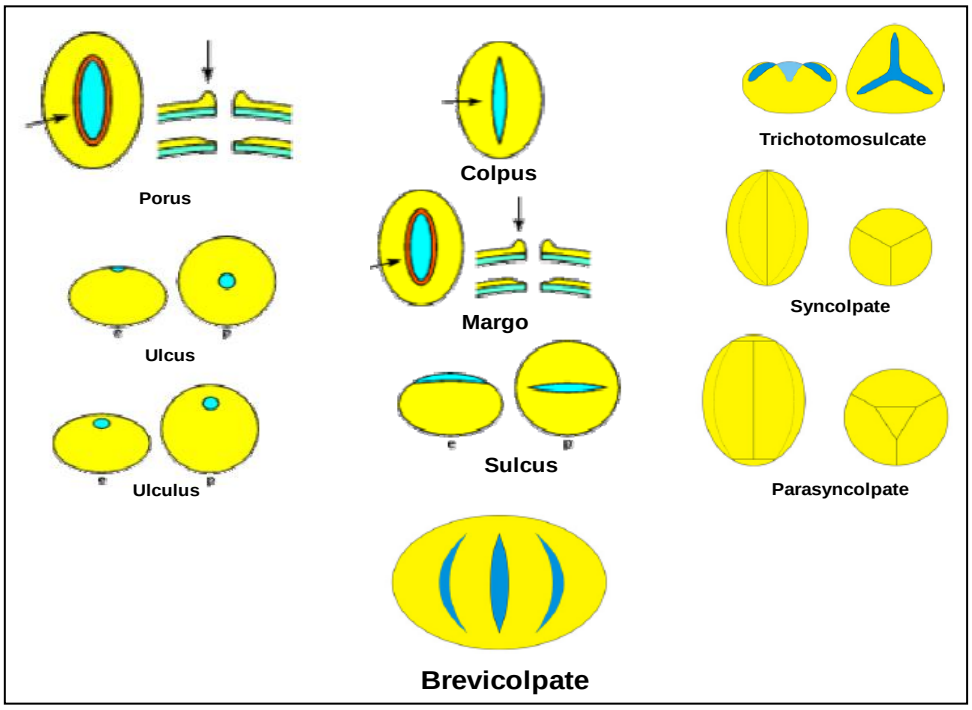
Syncolpate: pollen grain with two or more simple colpi the ends of which anastomose at the pole. Example: *Primula farinosa*

Parasyncolpate: syncolpate pollen grains in which the apices of the colpi divide into two branches and anastomose towards the poles, delimiting an isolated area known as the apocolpial field. Examples: *Nymphoides peltata*

Brevicolpate: brevi is a prefix for short; it is pollen grain with shorter length of colpi.

Breviaxial: pollen grains with a polar axis that is shorter than their equatorial diameter.

Fig. 11: Different types of simple apertures present in pollen grains

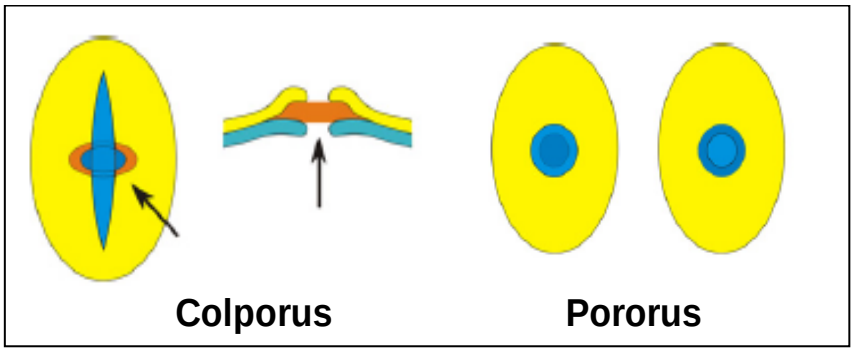


Compound Aperture: Compound aperture is combination of two simple apertures present both at ektexine- (ektoaperture-Colpi or pore) and endexine (endoaperture-ora) so that they affect more than one layer of the wall. In compound apertures the shape of apertures may differ between layers. Ectoapertures occur in the sexine/ectexine, endoapertures in the nexine/endexine, and mesoapertures are sometimes found in an intermediate position between an ecto- and endoaperture. Below the types of compound apertures (Fig. 12) –

Colporus: A compound aperture consisting of an ectocolpus with one or more endoapertures; aperture type colporate; example Asteraceae, Fabaceae etc.

Pororus: pollen grain with compound apertures in which both the ectoaperture and the endoaperture are pores and the two are not congruent; aperture type pororate; *Example: Myrica gale* (Myricaceae) etc.

Fig. 12: Different types of compound apertures



Besides simple and compound apertures sometime other conditions are also found.

Pseudoaperture: A thinning of the exine which, although superficially resembling an aperture, is not associated with a thickening of the intine and is presumed not to function as an exitus *(the site of exit of the pollen tube from the sporoderm)*

Pseudoacolpus – colpus like pseudoaperture;

Pseudopore – pore like pseudoaperture

Spiraperturate: It is 'anomotreme' aperture, referred to as N_8 in the NPC classification. Here the spiral apertures are irregularly arranged over the surface of the pollen, irrespective of their number, for example *Thunbergia* (Acanthaceae), *Eriocaulon* (Eriocaulaceae).

Pollen grains with other intermediate types are also noted, and they include:

Synorate or Svnclinorate: Pollen with lalongate ora anastomose latitudinally, for example, *Polygala*, *Solanum*.

Multi orate: Pollen with two or more ora found latitudinally along the length of the colpus, for example, *Congea*, *Viticipremna*.

Colporoidate: Pollen have well developed colpi with weakly developed ora (= Oroids) for example, *Rex canariensis*, *Salix myrsinites*, *Phalline lucida*.

Colpoidorate: Pollen have weakly developed colpi (= Colpoids) with well developed ora, for example, *Alangium villosum*.

Pororoidate: Pollen have well developed pores with weakly developed ora (=Oroids).

Poroidorate: Pollen have weakly developed pores (= Poroids) with well developed ora.

Colpororate: A compound aperture characterized by an ectoaperture, a shorter lolongate mesoaperture and a lalongate endoaperture for example, *Sonchus* (Compositae).

Heterocolpate: Pollen grains have both simple and compound colpi for example, *Lythrum*, *Peplis* (Lythraceae), *Myosotis* (Boraginaceae).

Porocolpate: Pollen grains with an arrangement of apertures in which colpi alternate with pores round the equator, for example, *Pardoglossum* (Boraginaceae).

Edges of apertures

Annulus: A distinct ring-like thickening or thinning of the ektexine bordering the pore is called annulus (Fig. 13A). A halonated area around a pore is found in Poaceae.

Costa: A distinct rib-like thickening of the endexine bordering the aperture is called Costa (Fig. 13B). Costae colpi maybe transverse i.e. Costae – transversales, or, if they are

continuous around the equator in uninterrupted, equatorial rings, they are called Costae-equatorials.

Margo (margin): An area of exine around an ectocolpus that is differentiated from the remainder of the sexine, either in ornamentation or by difference in thickness (Fig. 13C).

Operculum: The thick membrane, either of ektexinous, endexinous or both, covering the aperture is called Operculum (Fig. 13D). This may be circular, elliptical, annular or bridge-like. The panto-opercular nature of the pollen may be ascertained by studying the intine thickening under the panto-operculum.

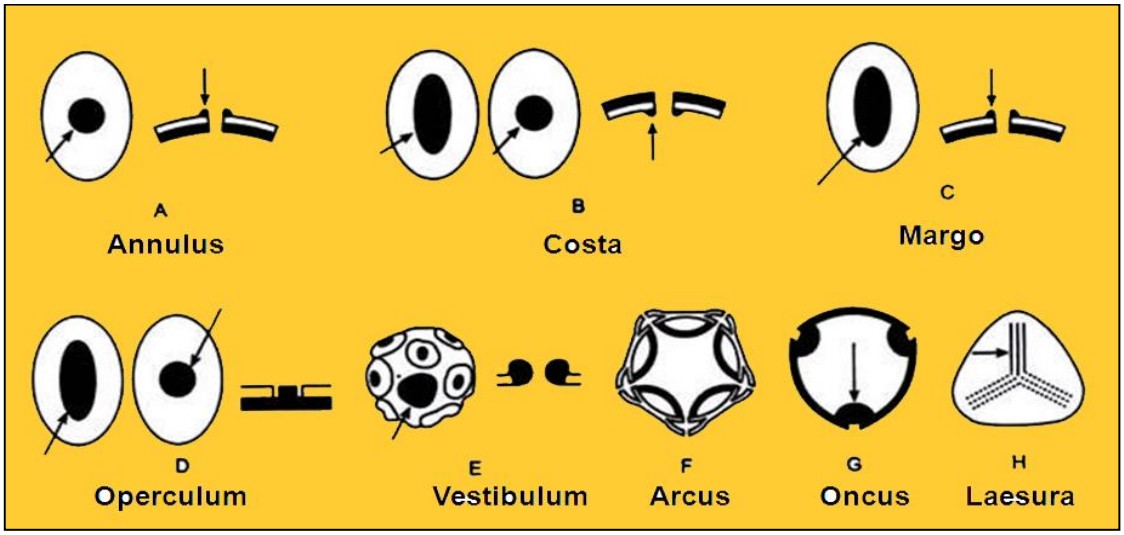
Vestibulum or Aspidote: The apertures or at least their outermost parts are borne on small, more or less circular, shield-shaped areas (aspidote), protruding as round domes from the general surface of the grains for example *Betula*, Dorstenia (Fig. 13E). If the chamber (space) is formed between an outer (ectopore) and inner (endpore) aperture opening, it is called an atrium. Here endopore is much larger than ectopore. In this condition apertures are also referred to as oriferous colpi for example, *Myrica*.

Arcus: In *Alnus*, *Rhoiptelea* pollen grains, band-like locally thickened parts of ektexine extending in a sweeping curve (arcus) from one aperture to another. Pollen having such arcus is referred to as arcuate type (Fig. 13F).

Oncus: Oncus is a lens-shaped structure that is not resistant to acetolysis and occurs beneath the aperture of many kinds of pollen grains (Fig. 13G), for example *Corylus* (Betulaceae).

Laesura (in plural Laesurae): Laesurae (Fig. 13H) are the tetrad scars of moss and fern spores which show the contact of spores with their neighbours in the original tetrad from which they were separated. Laesura may be trilete or monolete. A laesura has a centre suture or commissure which serves the purpose of providing a zone of weakness for rapture upon germination. The separated arms of trilete laesura are called radii, and the terminal ends of radii are forked.

Fig. 14: Edges of apertures

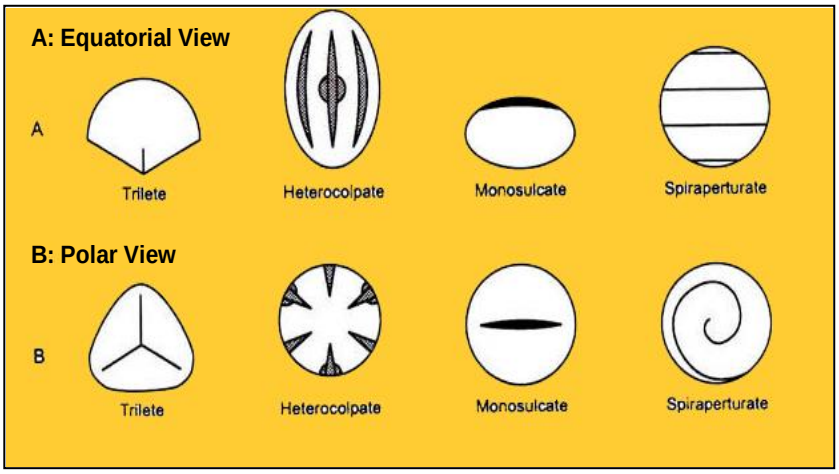


Views of grains with reference to apertures:

Polar view: In this view the pollen/ spores are viewed with one of the poles exactly uppermost i.e. with the polar axis directed straight towards the observer (Fig. 15). In polar view lete/sulcus/ulcus type of apertures will be observed centrally as they are situated at polar region. The polar axis will not be viewed here. The zonal apertures like colpi, pore, etc. will be viewed in the circumference of the pollen grains.

Equatorial view: In this view the apertures are arranged meridionally-pole to pole at right angles to the equator (Fig. 15).

Fig. 15: Views of grains with reference to apertures



Both polar axis and equatorial diameter will be viewed, and they can be measured in this view.

Amb: Outline i.e. contour or circumference of the grain in polar view is called amb (ambit, L. ambitus) which is viewed with one of the poles exactly uppermost directed towards the observer. In isopolar, equatorially non-constricted grain amb is as same as equator, while in equatorially constricted grain amb does not coincide with equator. Amb of radiosymmetric pollen grains are of different types based on the nature of circumference and arrangement of apertures (Fig. 16):

Peritreme: Apertures are more or less uniformly distributed along a circular amb for example, *Solarium*.

Goniotreme: Amb is angular and the apertures lie at the angles of the grain, for example, Myrtaceae, Proteaceae, etc.

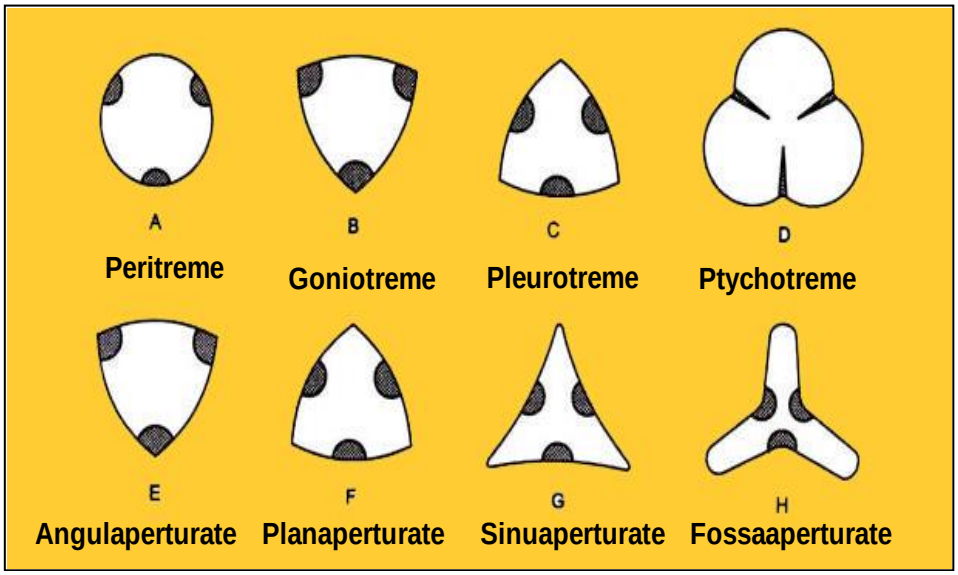
Pleurotreme: Amb is angular like the goniotreme, but the apertures are situated at the mid-points of the sides and the sides are more or less straight, for example, *Bombax*, *Ceiba* of Bombacaceae.

Ptychotreme: The sides of the amb are concave or lobate and the apertures are situated half way between the angles, for example, *Gunnera*.

Pollen grains with equatorial apertures and angular amb may be categorized (Fig. 16) into following types:

i. Angulaperturate: Apertures are situated at the angles of the amb (sides of amb convex, straight or concave) for example, Proteaceae, Olacaceae.

Fig. 16: Different Ambs (Outline that is contour or circumference of the grain in polar view)



Planaperturate: Apertures are situated at the mid-points of the sides of the amb (sides of amb straight), for example, *Bombax*.

iii. Sinuaperturate: Apertures are situated equally halfway between the angles (side of the amb concave).

iv. Fossaperturate: Apertures are situated at the ditch-like indentations between the lobes of lobate amb, for example, *Anomopanax* (Araliaceae).

NPC SYSTEM FOR NUMERICAL EXPRESSION OF APERTURAL DETAILS

Gunner Erdtman (1969) proposed NPC-System pollen/spore classification based on the apertures, their Number (N-whether single or two or many), Position (P- polar: distal or proximal; global; meridional) and Characters (C – circular or elongated) regarding microspore tetrad (Fig. 17). Under this system the term 'treme' (aperture) has been used for preparing keys for the classification of the pollen grains/spores.

The pollen number (N) groups are of nine types. The grain without aperture is named "Atreme" and is designated as No. Depending upon the number of apertures, the types of pollen are Monotreme (N_1) with one aperture, Ditreme (N_2) with two apertures; Tritreme (N_3)

with three apertures, Tetratreme (N_4) with four apertures, Pentatreme (N_5) with five apertures, Hexatreme (N_6) with six apertures and Polytreme (N_7) having more than six apertures. Irregularly arranged spiral apertures over the surface of the pollen irrespective of their number are designated as 'Anomotreme' (N_8).

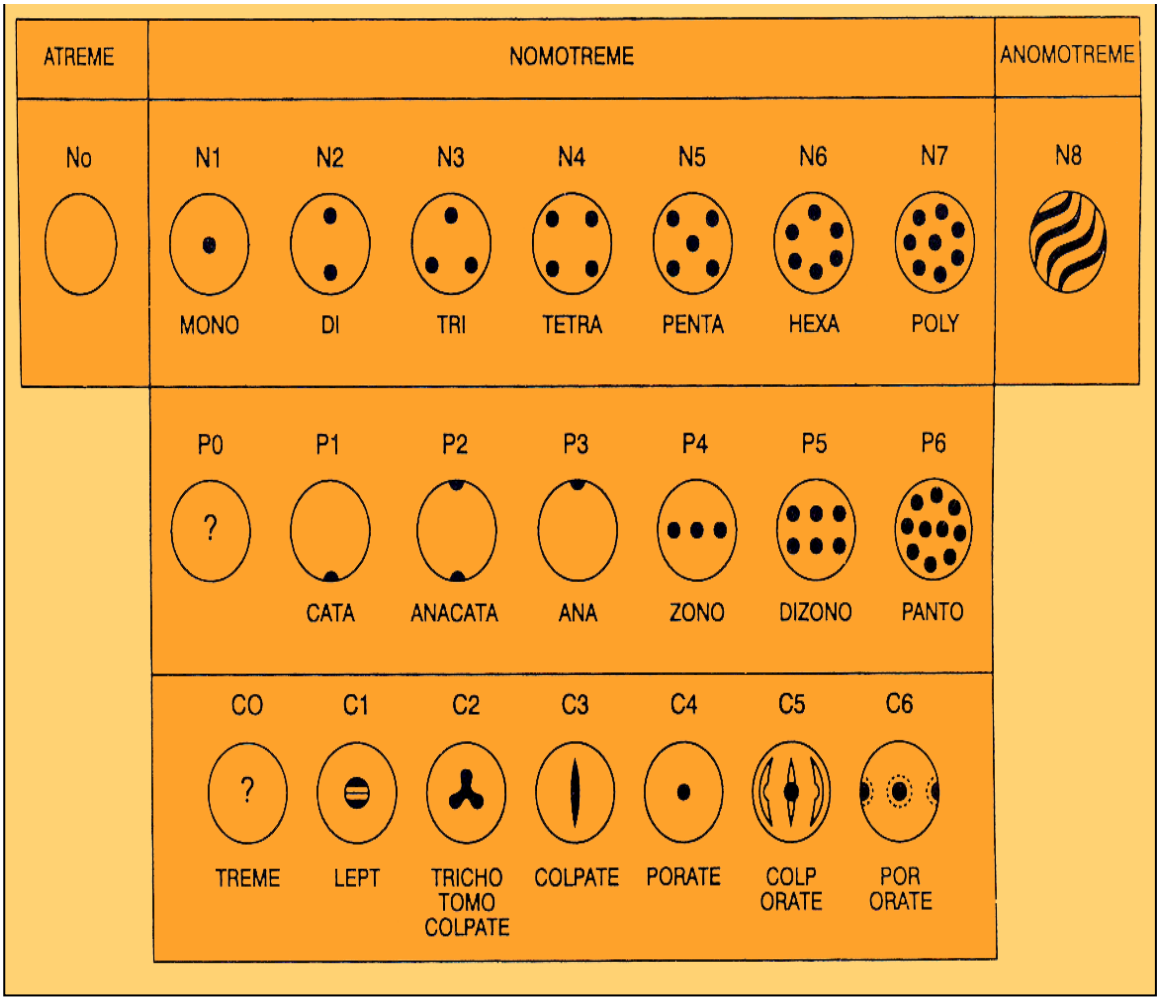
On the basis of the position (P) of apertures, pollen grains are categorized into seven groups (P_0 to P_6). In 'Catatreme' (P_1) pollen aperture is in proximal face, while in 'Anatreme' (P_3) it is in distal face. The pollen grains are designated as Anacatatreme' (P_2) where apertures are both in proximal and distal faces.

The pollen grains are referred to as 'Zonotreme' (P_4), when the apertures are located on the equatorial zone. 'Dizonotreme' (P_5) are like zonotreme, but with two rows of apertures on the equatorial region. In 'Pantotreme' (P_6), apertures are globally distributed all over the pollen surface.

Like position groups the character (C) groups are of seven types ($C_0$to C_6). If the character of the aperture is not known, it is designated as C_0. Pollen having an aperture like thin area or Leptoma is designated as C_1. Pollen with one leptoma is called Monolept, it may be called Cataleft if present in the proximal face, or Analeft if in the distal face.

Pollen with three- slit like colpus are called Trichotomocolpate which belongs to C_2 category. The remaining character classes i.e., C_3, C_4, $C_5$and C_6 include Colpate (with colpa i.e. furrow), Porate (with pore i.e. circular aperture), Colporate (both with colpa and pore/ora apertures), Pororate (aperture with pore and ora) respectively.

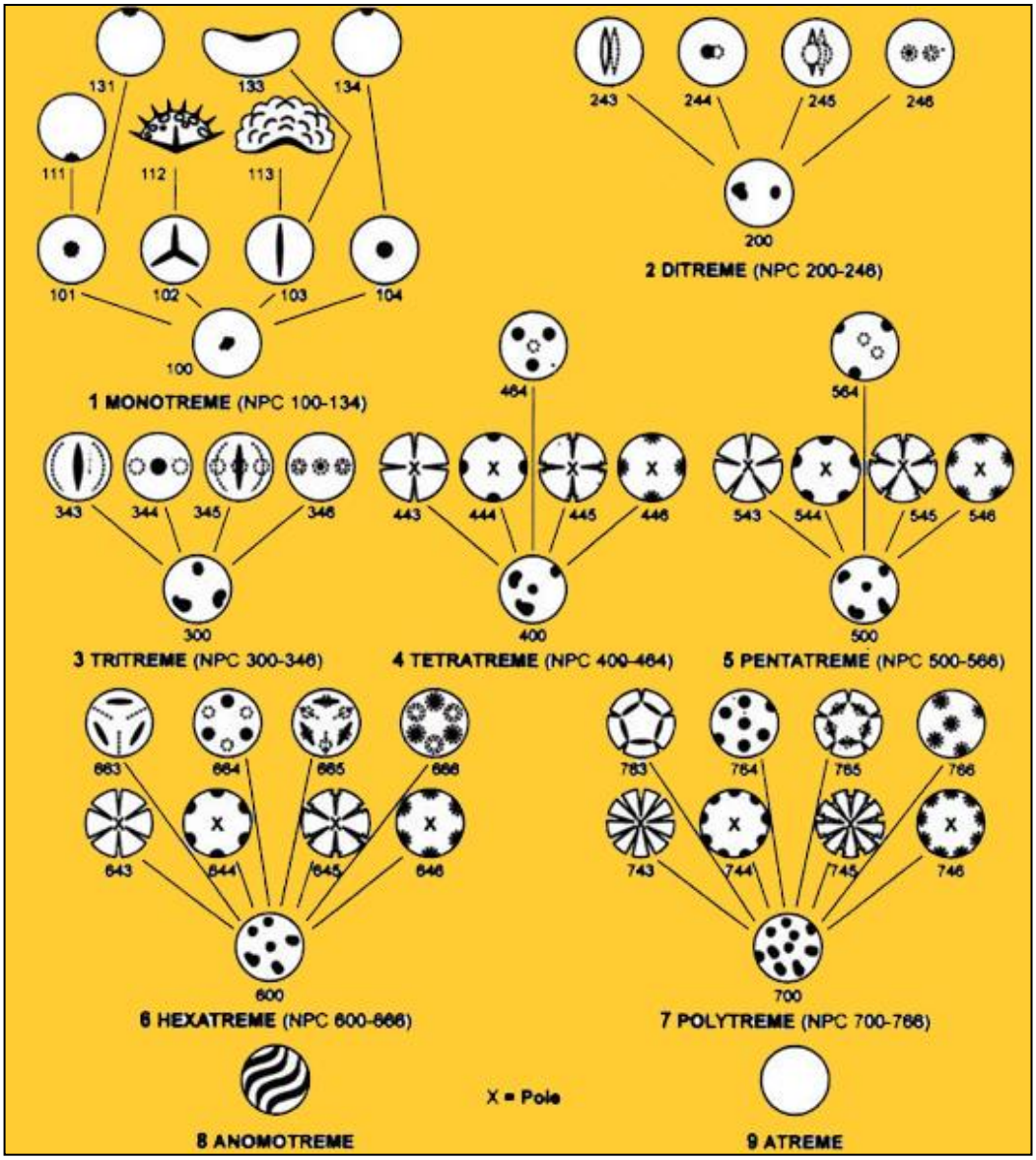
Fig. 17: NPC classification of pollen grain/spore



Based on NPC classification, each pollen type is designated by using a three digit number (Fig. 18). The first digit denotes the number of aperture, for example, 100 is assigned to monotreme, 200 to ditreme, 300 for tritreme, 400 for tetratreme, 500 for pentatreme, 600 for hexatreme, 700 for polytreme, and 8 for anomotreme and 9 for atreme.

The second digit denotes the position of the aperture, e.g. 010 to proximal aperture, 030 for distal aperture, 040 for equatorial aperture, and 060 for global aperture. The third digit denotes the characters of the aperture, e.g., 002 for trilete, 003 for colpate, 004 for porate, 005 for colporate. Therefore, the number 112 is assigned to trilete grains, similarly 133 to monosulcate grains, 343 to tricolpate and 345 to tricolporate grains, etc. (Fig. 18).

Fig. 18: Pollen grain/spore types designated by three-digit numbers



EVOLUTION OF APERTURE TYPES

Bryophytes, Pteridophytes: Aperture *(either trilete or monolete)* is proximal in position

Primitive extinct gymnosperm like pteridosperm *(*Lyginopterids-*trilete,* Medullosans-*monolete),* Cordaitales *(trilete),* voltziales *(trilete)*: Pre-pollen which is characterized by proximal aperture presuming proximal germination rather than distal as in case of other seed plants.

Extant gymnosperm: either inaperturate or if aperture present, it is in distal position

Angiosperm: along with distal and zonal position, global distribution of apertures is also found which increase the probability of pollen germination in any part of the pollen surface and its adherence to stigmatic surface may accelerate pollen germination

Positional change of aperture from proximal to distal: required to meet up the physiological and ecological needs that occurred due to diversification of new plant taxa.

APERTURE TYPES IN EXTINCT PRE-ANGIOEPERMIC PLANTS

Most primitive trilete spores: Mid. Silurian to Early Devonian vascular plants- *Rhyniales, Zosterophyllales, Trimerophytales, Lepidodendrales, Sphenophyllales, Calamitales, Primofilicales, some Cycadeoids*

Pre-pollen: *Pteridosperms, Cordaitales, Voltziales*

Monosulcate pollen: Bennettitales

APERTURE TYPES IN EXTANT PRE-ANGIOEPERMIC PLANTS

Monolete spore: *Psilotales and some members of Filicales*

Inaperturate spore: *Equisetales*

Monosulcate pollen grains: *Cycadales, Coniferales, Ginkgoales*

As such, pre-angiospermic plants have following morpho-forms:

a. Basic form-trilete, mon-perinous, non-saccate

b. Derived form-i. Trilete, perinuous non-saccate

ii. Trilete, saccate

iii. Monolete, non perinous, non-saccate

iv. Monolete, perinous

v. Monosulcate, saccate

vi. Inaperturate

Perine *(occurs especially in certain pteridophytes. It seems to develop after the exine proper and forms a usual wrinkle, loosely attached covering of the mature spore)* **and saccus** *(occurs in most of the gymnospermous pollen grains; winged like expansion made as a windborne mechanism)* **developed later during the morphological evolution of spore and pollen which indicates**

Origin of monosulcate and inaperturate forms **-** *reduction of the basic structural organization i.e. trilete*

Origin of perine and sacci - *modification in form of expansion of the basic structural organization*

Angiospermic Plants: Earliest record is from Lower Cretaceous

Most primitive angiosperms: monosulcate aperture *(pollen grains were named as **Clavatipollenites, Retimonocolpites, Liliacidites** – these may have originated from a common Mesozoic ancestor Benettitales)*

This monosulcate aperturate condition is distributed among followings:

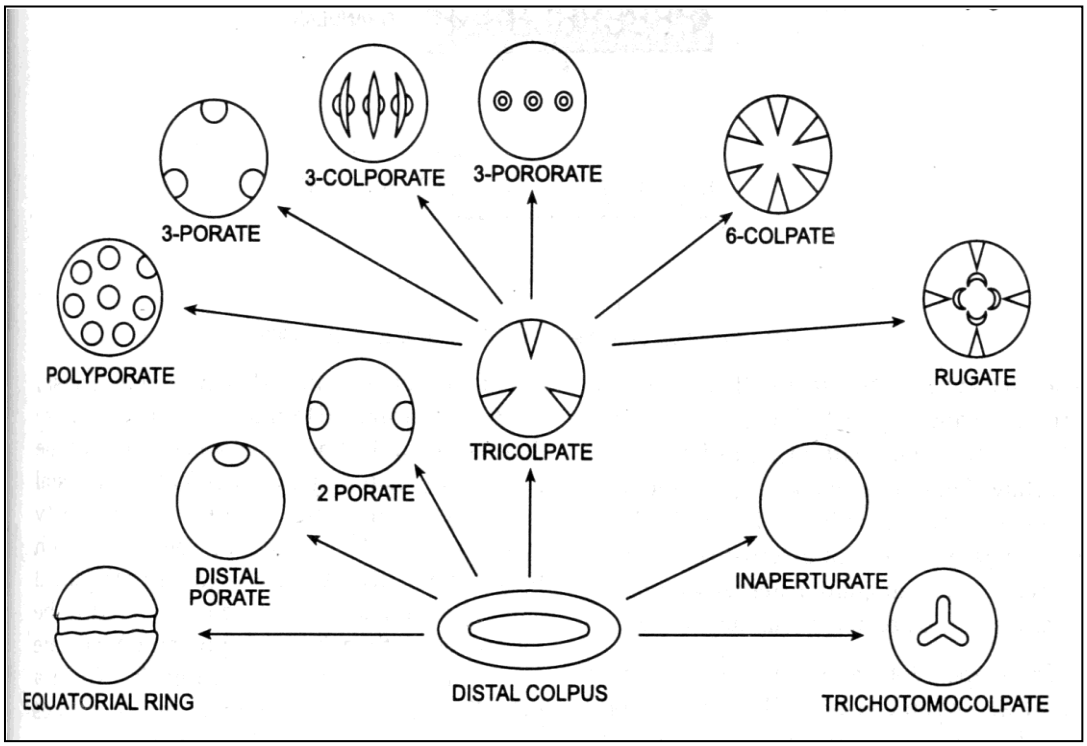
- **Most gymnosperms:** *Cycas* (Cycadales); *Pinus, Abies, Cedrus* (Coniferlales); *Ginkgo* (Gikgoales)
- **Primitive dicotyledoous angiosperms:** *Magnolia, Degeneria* etc.
- **Many monocotyledonous angiosperms:** members of Arecaceae

Distal sulcate aperture then modified to a few other types –

- distal porate (some monocots-poaceae);
- distal trichotomosulcate (both in dicots & monocots);
- equatorial ring like aperture (Nymphaeaceae);
- Inaperturate (by having reduced thin exine and thick intine)

As such, the main trend of evolution of pollen aperture is the transformation of the distal monosulcate (monocolpate) into tricolpate form. Takhtajan (1980) hypothesized (Fig. 19) that tricolpate condition developed because of evolutionary deviation of the primitive stage of sporoderm development and this is derived form of monocolpate condition. Tricolpate type independently given rise to other types namely polycolpate, triporate, pantoporate. Highest stage of evolution of pollen aperture: compound apertures like tricolporate or tripororate etc. are form simple tricolpate type.

Fig. 19: Evolution of apertures in pollen (Takhtajan 1980)



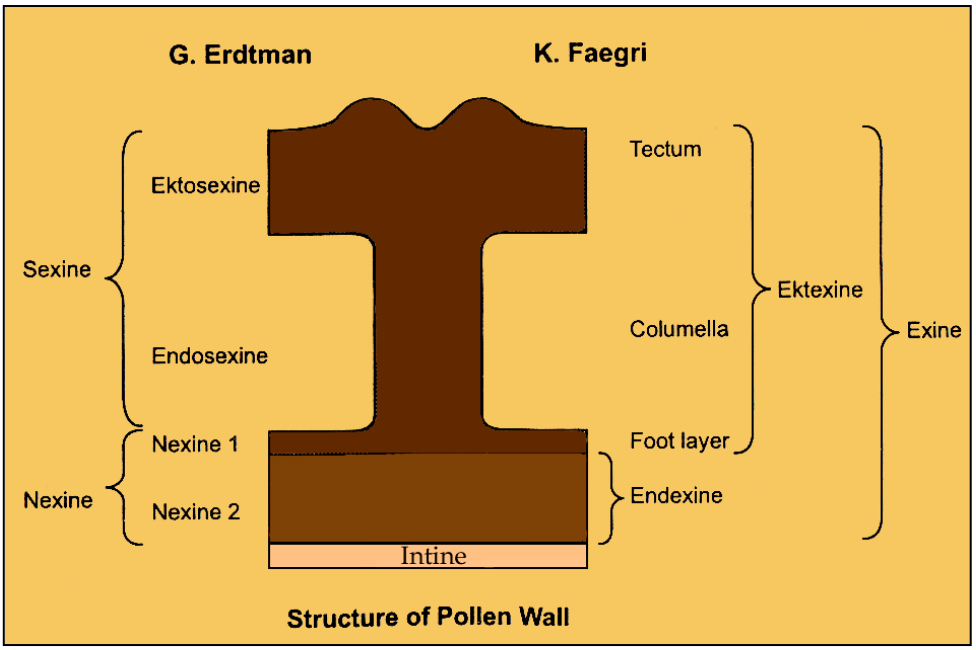
4. Pollen wall and extraexinous wall materials: Sporoderm stratification and sculptures; LO- analysis; sporopollenin; pollen wall development; Ubisch body; pollen connecting threads, perine, pollen-kit.

Sporoderm Stratification:

The pollen wall, the sporoderm is generally stratified i.e. layered (Fig. 20). The walls of the mature pollen, at least in angiosperms, consist of two fundamentally different layers, intine and an outer acetolysis resistant layer exine composed of sporopollenin.

The exine covers the entire pollen surface except germinal apertures where it is absent or greatly reduced. The exine of pollen grains can be divided into an outer sculptured sexine and an inner unsculptured nexine (Fig. 20).

Fig. 20: Sporoderm stratification



Sexine again consists of two layers: the outer, ectosexine and inner, endosexine. The sexine is generally constituted of a set of radially-directed rods supporting a roof-like structure (tectum or tegillum), which may be partially perforated or completely absent.

Rods supporting the tectum are known as columella, and rods not supporting anything but standing vertically on the nexine are called bacula. Columella are usually simple but may be branched. In Compositae the columellae are either distally branched (digitate) or proximally branched (conjunctate) or sometimes the columella hangs down from the tectum, e.g., Caryophyllaceae (Fig. 21).

The nexine has been divided into two layers namely nexine I and nexine II. Knut Faegri (1964) proposed an alternative terminology for exine stratification (Fig. 20). He recognized two layers of exine, the outer ektexine (including sexine and nexine I) and endexine (nexine II). He designated nexine I as foot layer and considered it to be the basal part of ektexine for its identical chemical composition and staining property as that of sexine. Faegri's ektexine is quite different from the endexine because the former contains denser sporopollenin and stains more deeply. The ektexine may be regarded as a three-layered structure in which the granules form small columns, columella, thus dividing an outer tectum and inner foot layer strata. The

endexine is often well developed in dicots but is virtually absent or have it only in the apertural region in monocots.

In some pteridophyte spores (e.g. Polypodiaceae) and a few gymnosperms pollen (e.g., *Taxodium*) there is a hyaline loosely organized sporopolleninous envelope called Perine covering the exine (Fig. 22). The perine maybe continuous or sometimes folded in various ways. In some gymnosperms, especially among conifers, the ektexine enlarges to form bladdery wings (Saccus, in plural Sacci) generally two (*Pinus, Cedrus, Abies, Picea* etc.) or one (*Tsuga*) in number.

Types of Pollen in respect to Sporoderm

The pollen grain with a tectum which covers most of the surface of the grain is called tectate. Here the structural elements fused distally forming a continuous roof (Tectum). **Faegri and Iversen (1964) divided tectate grains into three categories (Fig. 21):**

(a) Tectum solidum (unbroken tectum with or without supratectal processes viz., *Betula, Zea*).

(b) Tectum perforatum (perforated tectum, viz., *Stellaria*).

(c) Tectum perfossulatum (fossulated tectum viz., *Saxifraga oppositifolia*)

Pollen grains without an apparent tectum are called intectate (Fig. 21). Here the structural elements are free or absent. Intectate grains may be either (a) without granules (e.g., *Juncus*) or (b) with granules (e.g., *Ilex*). The granules maybe more or less crowded (e.g., *Populus trimula*), or somewhat scattered (e.g., *Callitriche*).

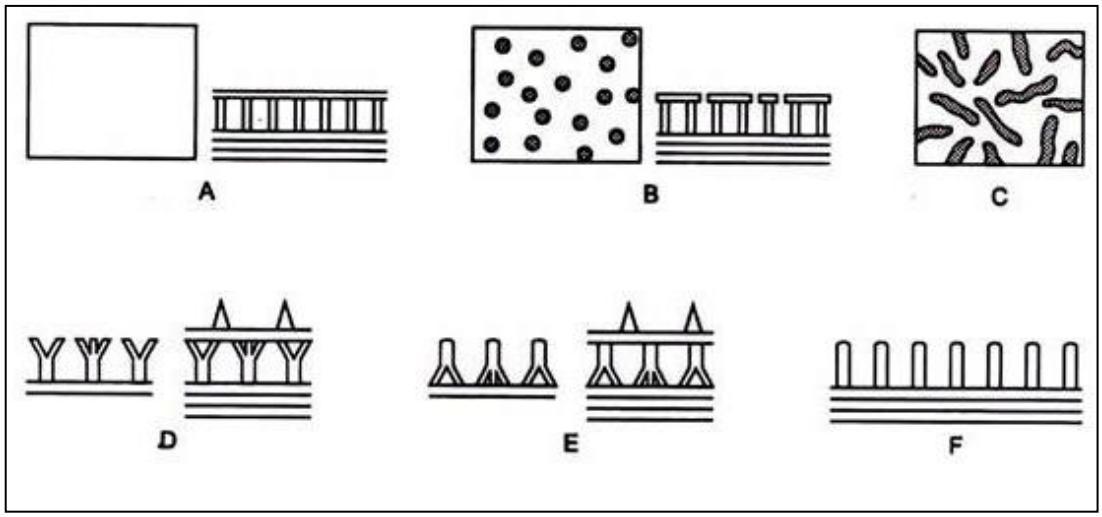
They may be evenly dispersed or form patterns (e.g., *Croton*) with a great variation in their shape, club-shaped type (e.g., *Ilex, Croton* etc.) being the predominant. The graules are generally simple, but occasionally branched and anastomose (e.g., *Geranium*).

Some of the pollen grains are an intermediate between tectate and intectate grains. They are called semitectate or sub- tectate (Fig. 21) in which tectum is not continuous i.e., tectum is partially absent. Here columellae are generally found underneath the fragmentary tectum, but they may also exist as free granules that do not support a tectum.

Semitectate grains maybe (a) Per-reticulate (where structural elements fused distally forming an open reticulum, e.g., *Salix),* (b) Frustillate (synonym of areolate) (where structural elements fused distally forming isolated frustillae, e.g. *Populus balsamifera*).

Fig. 21: Nature of tectum

A: Tectum imperforatum/solidum (Tectate/Eutectate); B: Tectum perforatum (Semi-tectate); C: Tectum perfossulatum; D: Digitate columella; E: Conjunctate; F: Intectate

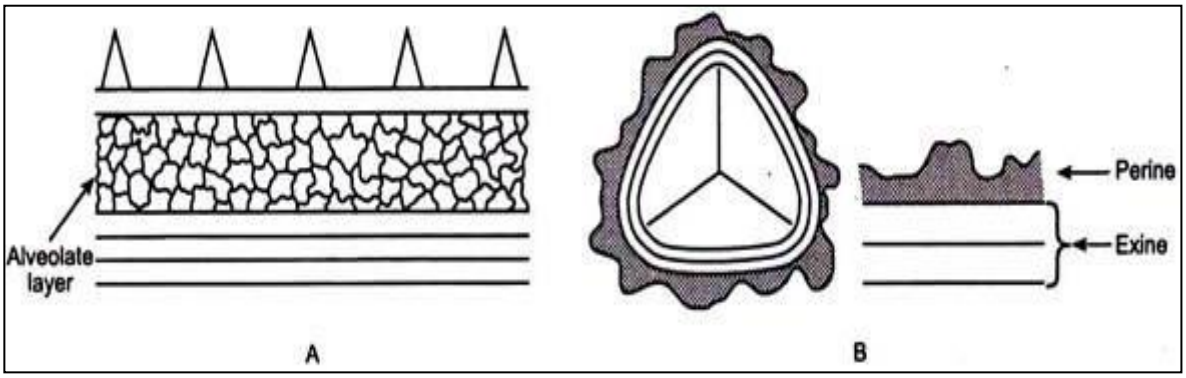


Angiosperm exines show a middle layer of distinct, well-defined columella. Gymnosperm pollen can be distinguished from the angiosperm pollen due to the presence of an irregular spongy, alveolate middle layer instead of columella (Fig. 22). In addition, the endexine of angiosperm pollen is relatively homogenous, whereas in gymnosperm it is typically laminate. Morphologically the spores of bryophytes and pteridophytes can easily be distinguished from the pollen of gymnosperms and angiosperms. The walls of bryophyte and pteridophyte spores often appear to be laminated throughout their thickness and without having wall stratification (there is no ektexine/endexine division in spore).

The spores have either one long slit-shaped aperture (monolete) or with a trifurcated slit (trilete) forming 'Y' shape. In some pteridophytes a loose outer layer called perine or perispore surrounding the exine is often noticed (Fig. 22).

Fig. 22: Gymnosperm and Pteridophytic sporoderm

A: Alveolate layer of Gymnosperm pollen grain; B: Perine and exine of Pteridophyte spore



Sporoderm Sculpture- Exine Ornamentation

There are two different types of exine ornamentation, the structure or texture and the sculpturing. The structure comprises of all the internal (infratectal) baculae of various form and arrangements. All the ektexine characters belong to the structural features, while the sculpturing comprises external (supratectal) geometric features without reference to their internal construction.

1. Supratectal sculpturing:

There are several external textural modifications of the exine of pollen grains and spores (Fig. 23). The sculpture is a usual feature of the ektexine but may be a perine character. Tectum may be smooth i.e., psilate or with processes or excrescence of various kind like spinules, spines, pila, verruca, gemma, clava, granules, etc. Tectum with the processes is referred to their respective terminology (Fig. 23a, b).

2. Sculpturing on subtectate sexine:

In subtectate or semitectate grains the tectum may be provided with minute perforations having a diameter of 1.0 µm called puncta and the tectum is referred to as punctate (Fig. 23). If the width of such perforation is more and the sexine displays a net-like pattern usually forming a honey-comb like hexagonal meshes, it is called a reticulum (Fig. 23).

Fig. 23a: Supratectal sculpturing *(Gemmate, Clavate, Verrucate, Baculate, Echinate, Spinulose)* and **sculpturing on subtectate sexine** *(Punctate, Reticulate, Striate)*

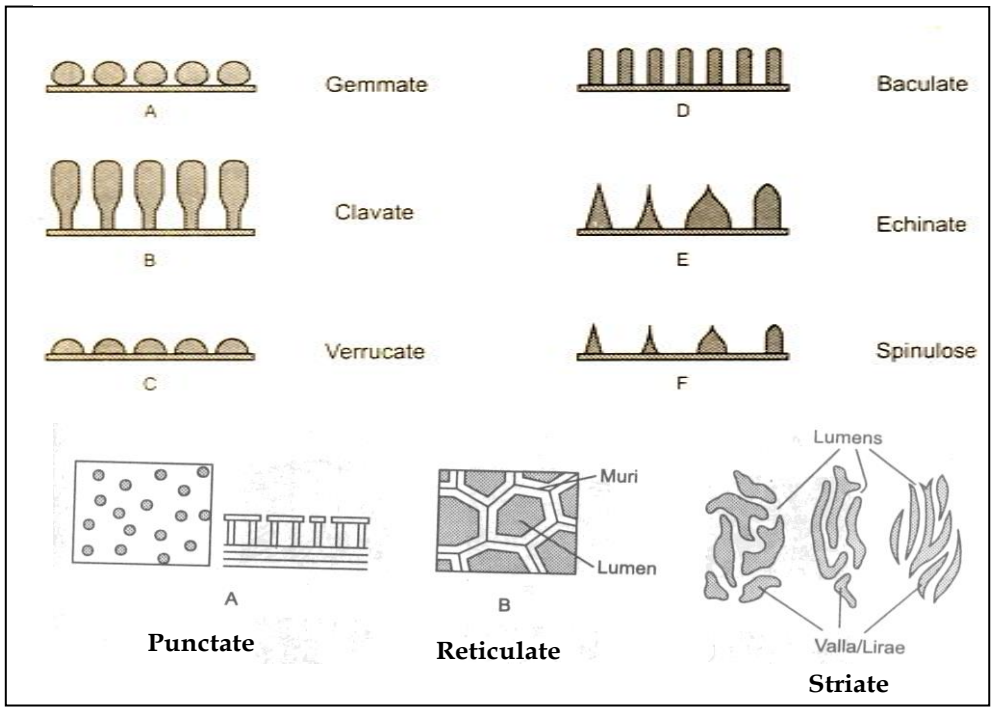
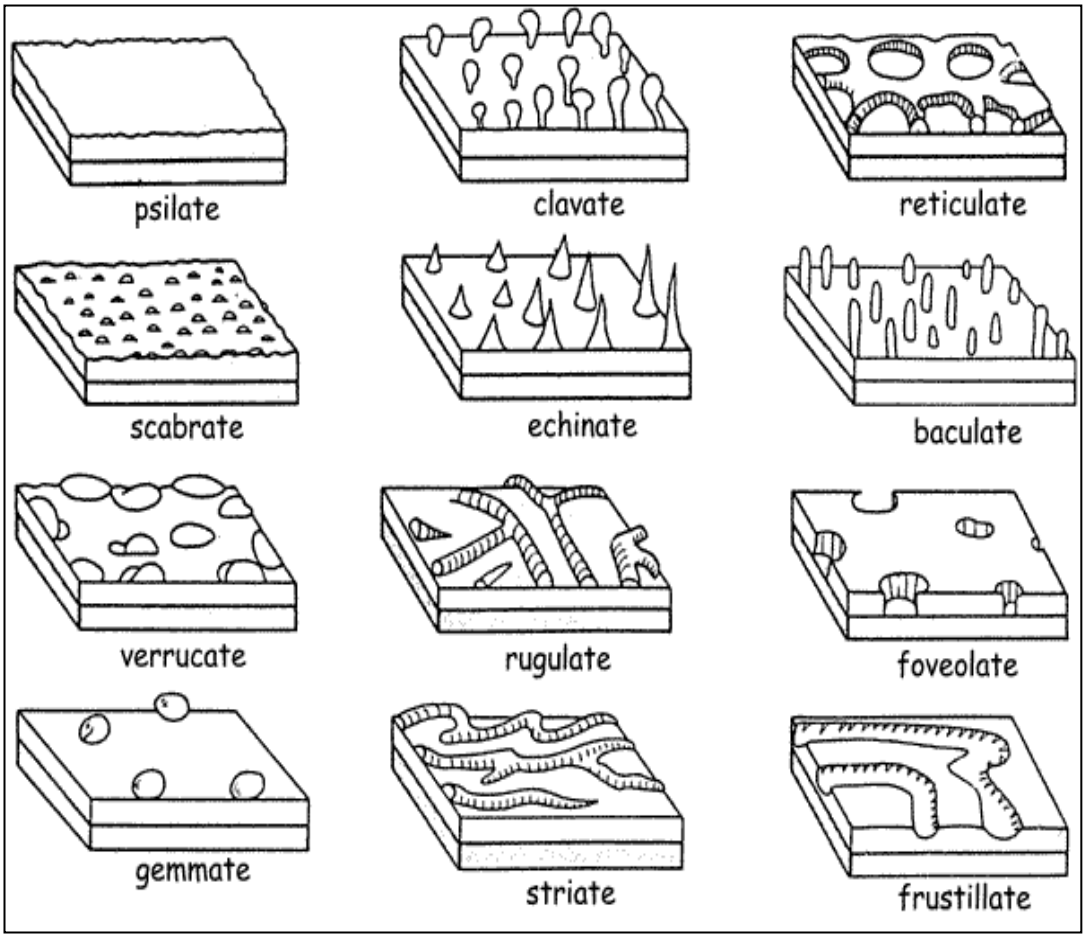


Fig. 23b: Supratectal sculpturing



Faegri and

Iverson (1964) proposed a key for identification of various sculpturing types (Table 4). In tectate grains the sculpturing types are given the prefix supra-. So the supra-reticulate grain means a reticulation on the outside of the tectum, while in infra-reticulate grain columellae form a reticulation beneath the tectum.

A typical reticulum comprises of a system of ridges called muri (singular murus), separated by empty roofless interspaces called lumina (Fig. 23). The lumina have the nexine as floor and muri as walls. The muri have an upper tectal part and a lower part consisting of baculae (infratectal) standing between the outer surface of nexine and the lower surface of tectum.

A mesh consists of a lumen and the adjoining half of the muri which separate that lumen from other lumina. A mesh is referred to as brochus (plural brochi). A reticulum may be homobrochate (Fig. 24) (with brochi of the same size) or heterobrochate (Fig. 24) (with brochi of distinctly different sizes).

The muri in which the upper tectal part is supported by a single row of baculae is called simplibaculate (Fig. 24) and the muri supported by two rows of baculae is called duplibaculate (Fig. 24) (e.g., *Avicennia*). In some cases, a negative reticulum is observed where sexine areas are separated by narrow reticulately arranged grooves (Fig. 24).

Table 4: Key to the sculpturing types of pollen grains

A. Sculpturing elements absent	
B. Surface even or diameter of pits <1µm	**Psilate**
BB. Surface pitted, diameter of pits >=1µm	**Foveolate**
BBB. Surface with grooves	**Fossulate**
AA. Sculpturing element present	
B. Radial portion of sculpturing elements ± isodiametric *(sphaerical or polyhedral)*	
C. No dimensions >=1µm	**Scabrate**
CC. At least one dimension >=1µm	
D. Sculpturing elements not pointed	
E. Lower part of element constricted	
F. greatest dia of radial projection is more of equal to height of element	**Gemmate**
FF. height of element is more than greatest dia of projection, club shaped	**Clavate**
EE. Lower part of element not constricted	
F. greatest dia of radial projection >=height of element; wart like	**Verrucate**
FF. height of element >greatest dia meter of projection, cylindrical	**Baculate**
DD. Sculpturing element pointed	
E. Spines long, > 3µm	**Echinate**
EE. Small spines, <3µm in length	**Spinulate**
BB. Radial projection of sculpturing elements elongated	
C. Elements regularly distributed	**Rugulate**
CC. Elements parallel	**Straite**
BBB. Sculpturing elements forming a reticulate pattern	**Reticulate**

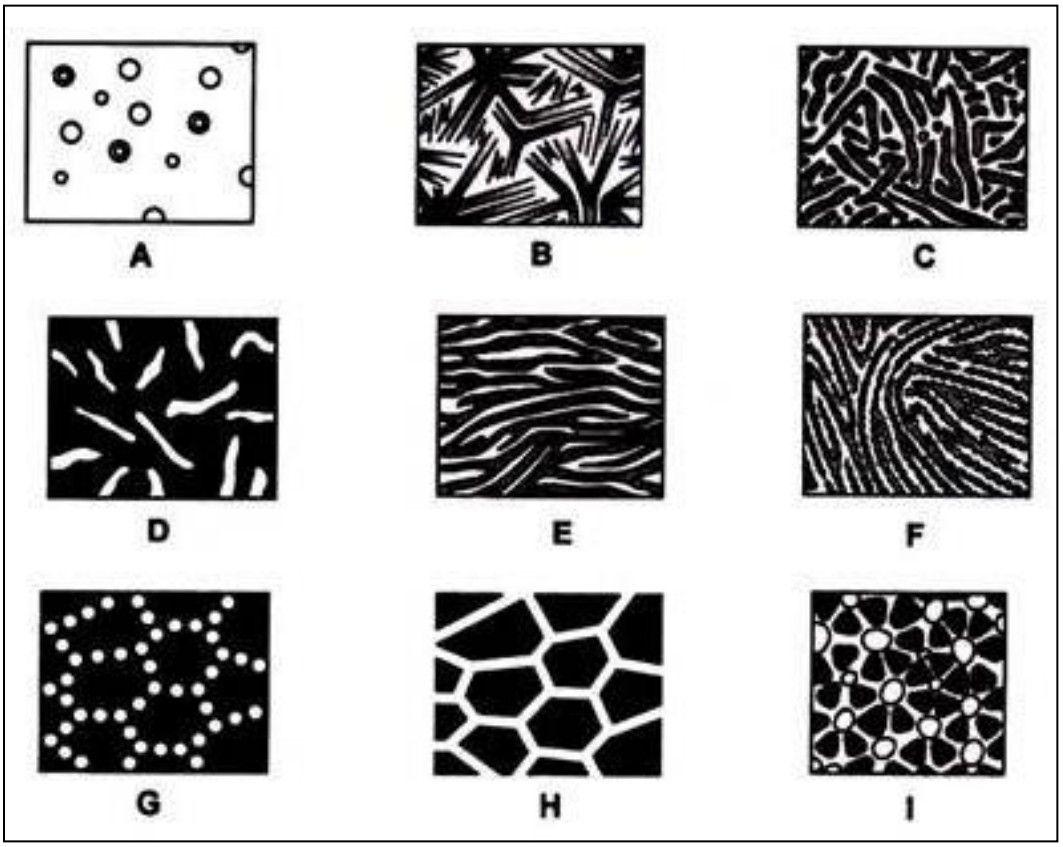
Based on the nature of lumina, the surface ornamentations are of different types (Fig. 25) namely, scrobiculate (very small circular, distantly placed lumina separated by sexinous streaks, scrobiculate is a synonym of punctate), foveolate (circular, closely placed lumina,

diam. of pits 1.0 µm), rugulate (ridges run irregularly, lumina parallel and anastomosing), fossulate (elongated lumina, surface with irregular grooves).

In striate ornamentation, the ridges run parallel instead of forming a network. In this case the ridges are often called lirae (singular lira) or valla (singular vallum) and the streak-like furrows between them are called striae (sing, stria) (Fig. 25). If the lira forms a definite reticulation, the pattern is called striato- reticulate. Elements are very irregularly distributed. If pila instead of muri form a reticulum, then the sexine pattern is called retipilate (Fig. 25).
Crotonoid pattern is a characteristic type of ornamentation comprising of five or six (sometimes more) raised, often triangular, sexine elements arranged around a circular area, usually formed by pila (Fig. 25). For example, *Croton*, *Jatropha* (Euphorbiaceae), *Callitriche antarctica* (Callitrichaceae), *Pimelea arenaria* (Thymelaceae).

Fig. 25: Sculpturing types

A: Foveolate; B & C: Rugulate; D: Fossulate; E & F: Striato-Reticulate; G: Retipilate; H: Negative reticulum; I: Crotonoid pattern

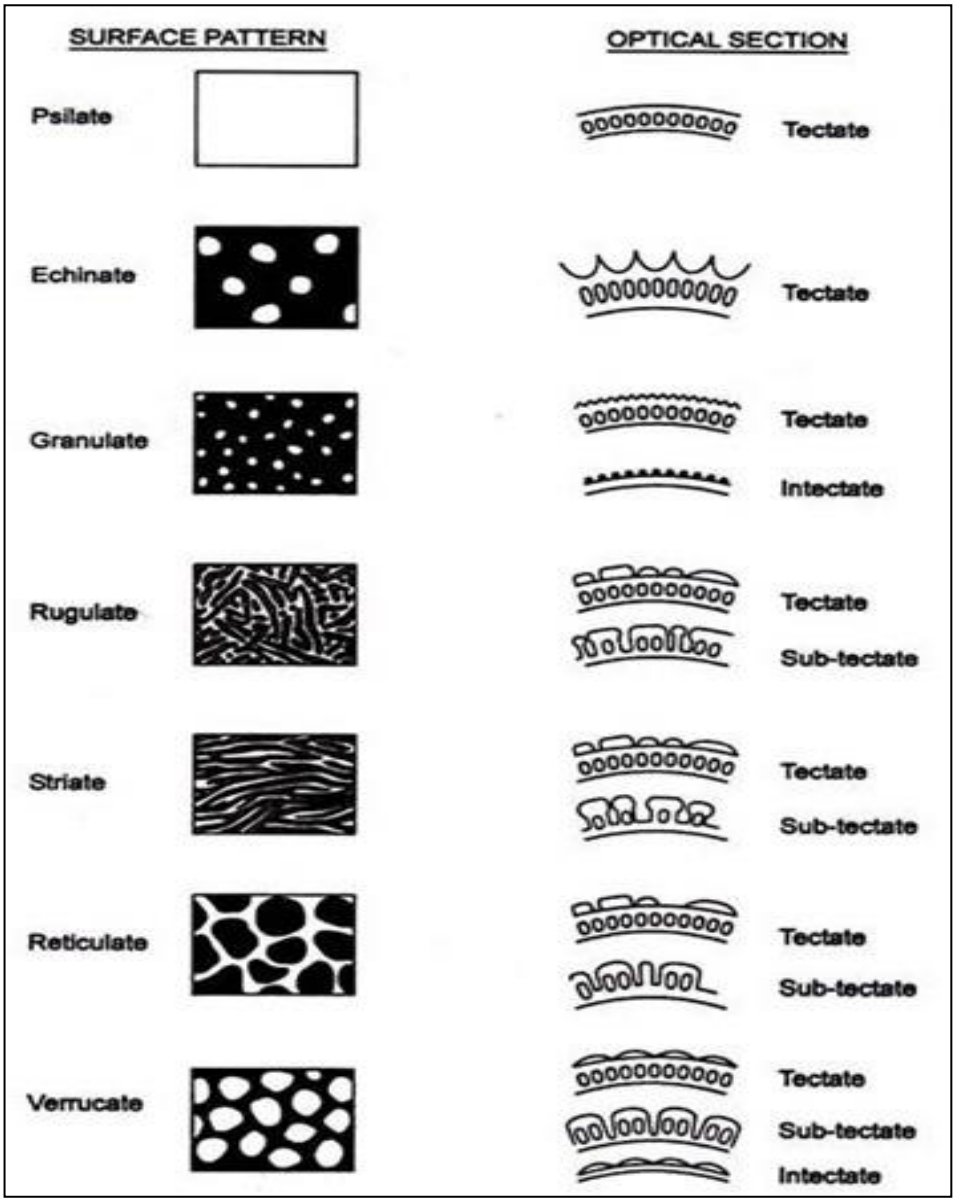


It is observed that two or more grain types show same surface ornamentations (Fig. 26). Say for example, regulate, striate or reticulate can have very different fine structure. One grain type is tectate with ornamentation on the top of the tectum, and the other is sub-tectate where surface ornamentations are formed of columellae.

The verrucae sculpturing may be produced by three different exine structures (tectate, semitectate and intectate) (Fig. 26). The optical section would determine the type of the grain (whether tectate or semitectate). The other sculpturing types namely, psilate or echinate are produced only by tectate grains, while granulate type is formed of by two different exine types (tectate and intectate) (Fig 26).

Fig. 26: Sculpturing pattern visible in surface view and optical section

(all raised areas are shown light and lower areas or holes are shown dark)



3. Other exine patterns (Fig. 27):

Areolate: An ornamentation feature where the ektexine is composed of circular or polygonal areas separated by grooves which form a negative reticulum. For example, *Phyllanthus* (Euphorbiaceae), *Apama* (Aristolochiaceae).

Caveate: In some Compositae like Ambrosia, a cavity is formed between two layers (ektexine and foot layer) of the exine extending to the colpus margin where the layers meet. The cavity is called cavea or cavum and the pollen of such type is termed as caveate or cavate. When cavea is highly developed the pollen grain is said to be vesiculate or saccate.

Lophate: Describing a pollen grain in which the outer exine is raised in a pattern of ridges (lophae) surrounding depression (lacunae). For example, *Taraxacum*, *Hieracium* (Compositae). A lophate pollen grain with echinate (spines) ridges is called echinolophate, while a lophate pollen grain which lacks spines is known as psilolophate.

Fenestrate: Describing a class of pollen grains characterized by large, window-like spaces lacking a tectum. The term is accepted as a category in the classification of Iversen and Troels-Smith (1950) which includes lophate pollen grains. This term is not recommended in descriptions and such pollen grains can be described as lophate.

Halo: A clear zone around a well-defined feature such as spine or an aperture. For example, *Ranunculus* (Ranunculaceae), *Valeriana* (Valerianaceae).

Hamulate: Describing a form of rugulate ornamentation consisting of irregularly arranged, winding, or angular rounded muri of varying thickness, which do not form a distinct reticulum, but rather a maze-like pattern. For example, *Lycopodiella inundata* (Lycopodiaceae). This term is mostly used in spore terminology.

Haploxylonoid (= Haploxylon-type): Describing a bisaccate pollen grain in which the outline of the sacci in polar view is more or less continuous with the outline of the corpus, so that the grains appear a more or less smooth ellipsoidal form. For example, *Pinus*, *Picea* (Pinaceae).

Diploxylonoid (= Sylvestris-type): Describing a bisaccate pollen grains in which the outline of the sacci in polar view is discontinuous with the outline of the corpus, so that the grains seem to consist of three distinct, oval parts.

Metareticulate: A reticulum which is characterised by the consistent presence of one porate aperture in each lumen. For example, *Kallstroemia mexicana* (Zygophyllaceae), *Froelichia floridana* (Amaranthaceae).

Urceolate: Describing a type of ornamentation consisting of urn-shaped elements situated on the foot layer. For example, *Pinanga aristata* (Arecaceae).

Polumbra: A darkened triangular or subcircular area centred on the proximal pole. This feature appears to be most commonly observed in specimens that have lost a perisporal outer exoexinal layer. For example, *Retusotriletes distinctus*.

Bireticulate: A two layered reticulum consisting of a suprareticulum supported by a microreticulate tectum. For example, *Entelea arborescens* (Tiliaceae), *Phyllanthus oppositifolius* (Euphorbiaceae), *Salvia azurea* (Lamiaceae).

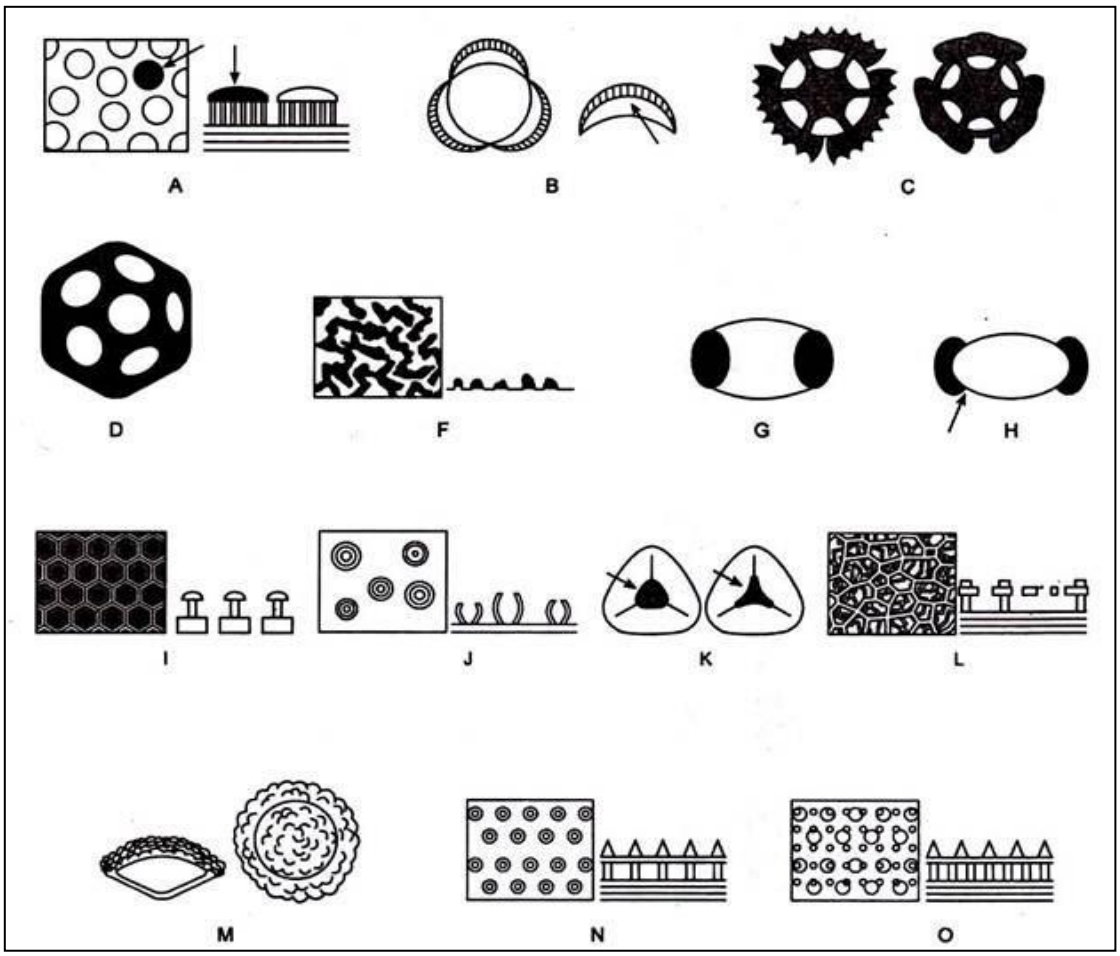
Velum: A feature of a monosaccate pollen grain in which the saccus is convoluted. For example, *Tsuga* (Pinaceae). The pollen having this feature is called velate.

Concordant pattern: A pattern in a tectate pollen grain in which the arrangement of the columellae is the same as that of the elements upon the tectum. For example, *Lilium* (Liliaceae).

Discordant pattern: A pattern in a tectate pollen grain in which the arrangement of the columellae is different from that of the elements on the tectum. For example, *Geranium* (Geraniaceae).

Fig. 27: Other exine pattern

(A: Areolate; B: Caveate; C: Lophate; D: Fenestrate; F: Hamulate; G: Haploxylonoid; H: Diploxylonoid; I: Metareticulate; J: Urceolate; K: Polumbra; L: Bireticulate; M: Velum; N: Concordant pattern; O: Discordant pattern



'LO' Analysis:

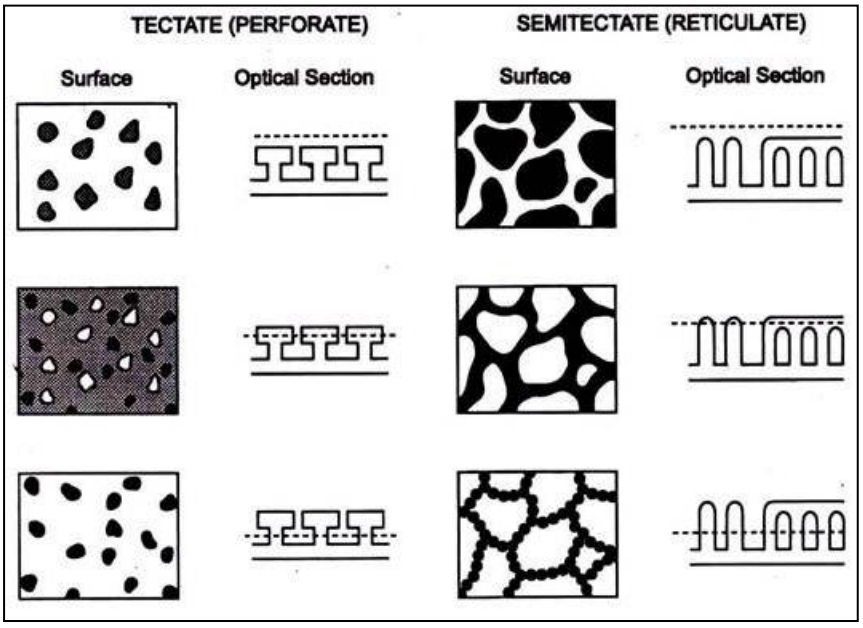
An optical section does not always make the fine structure of the sexine as clear as one might expect. A careful focusing through the sculpturing and patterning presented in a surface view of the grain provide a good deal of information.

Erdtman (1952) proposed the term LO-analysis (derived from two Latin words: lux means light and obscuritas means darkness) which is a method for analysing patterns of sexine organisation by means of light microscopy. This method is valuable for elucidating exine patterns. The surface types show the holes or lower areas to be dark and any raised areas or projecting elements to be light (Fig. 28).

On focusing carefully down through the exine their appearance would change due to a changing that diffraction images produced. For example, when focused at high level, raised sexine elements appear bright, whereas holes in the tectum are relatively dark (Fig. 28).

At lower focus holes become lighter and the sexine elements become darken (Fig. 28). If a reverse sequence occurred i.e. a pattern of ornamentation that appears to show "dark islands" at high focus and that become bright at low focus, it is given the term "OL- pattern". This system works very well if the pollen grains are embedded in such a medium having lower refractive index.

Fig. 28: 'LO' Analysis



Sporopollenin

Sporopollenin forms the basic structure of the resistant wall of most palynomorphs, like spores, pollens, dianoflagellates and acritarchs. It has also been recorded from spores of *Aspergillus niger*, sexual spores of *Mucor mucedo*, asexual spores of *Pithophora oedogonia*, cell wall of *Phycopeltis epiphyton*, *Chara corallina*, cyst of *Presinocladus marinus*, and spores of Bryophytes, Pteridophytes, pollen of Gymnosperms and Angiosperms. Besides exine, it is found in perispore, viscin threads, elaters, Ubisch bodies.

Its natural colour is pale yellow, but with thermal maturation it loses O and H, increases percentage of C, the colour deepens through dark yellow, orange, reddish brown, finally to black. The specific gravity is about 1.4 and the index of refraction is 1.48. It is also sensitive to high pH over prolonged period of time.

It is not affected by enzymes so pollen and spore exine pass through most animal guts unchanged.

Functions

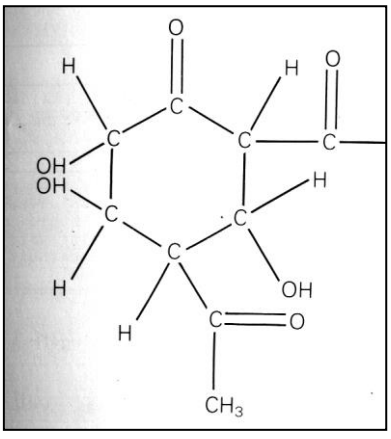
- It played the role of protector of protoplasm against UV radiation and also acts as a sheild against oxidation and desiccation.
- Due to its resistance property it remains intact in the various palynomorph and thus helps in reconstruction of past vegetation, predicting climate changes, understanding the evolution of plant life and in the exploration of hydrocarbons.

Chemical nature

Sporopollenin is highly resistant to physical, chemical and biological degradation procedures. Consequently, its precise chemical composition, structure and biosynthetic route have not yet been ascertained.

It is highly inert compound having empirical formula $C_{90}H_{144}O_{27}$ in spores of *Lycopodium*. It resists acetolysis, but degrades in strong oxidants like H_2O_2 or CrO_3 and exhibits secondary fluorescence when stained with primuline. Zetzsche et al. (1937) determined that the sporopollenin is an oxygeneted hydrocarbon and contains hydroxyl and C-methyl groups and substantial level of unsaturation. Shaw and Yeadon (1966) proposed that the sporopollenin is composed of a lipid fraction of 55-65%, consisting of molecules with a chain length of up to C_{16} and a lignin fraction representing 10-15% of the total mass. Heslop-Harrison (1968) and Brooks et al. (1969) suggested that sporopollenin is a copolymer of β-carotene and xanthophylls such as antheraxanthin and fatty acids. By applying ^{13}C NMR spectroscopy it is seen that sporopollenin contains a series of related biopolymers derived from largely saturated precursors such as long chain fatty acids and oxygenated aromatic rings.

An approximate empirical formula: $C_{90}H_{142}O_{27}$ for *Lycopodium, Picea, Pinus, Corylus* sporopollenin



As such, traditional convention asserts that sporopollenin is a polymer of carotenoid esters. However, modern purification, degradation and analytical techniques have shown that it is comprised of polyhydroxylated unbranched aliphatic units with small quantities of oxygenated aromatic rings and phenylpropanoids.

Pollen wall development

Pollen wall development may be considered under exine and intine growth phases.

1. Exine Growth Phase:

After meiosis of microsporocyte (microspore mother cells) the tetrads of haploid microspores are enveloped by a callosic wall. The callose wall can be detected around the microspore mother cells during initiation of meiosis (Figs. 29a and 30a). It forms a layer between the cytoplasm and pollen mother cell wall.

Additional callose is formed after the second meiotic division which isolates the young microspores from each other. The callose special cell envelope, persists until it is enzymetically digested at the end of the tetrad stage. The pollen wall formation is discussed under two models, viz., the primexine model and the undulation model.

a) Primexine Model:

Heslop-Harrison (1971) considers the primexine as the blue print for the exine. The primexine has a matrix presumably made of cellulose microfibrils, and is deposited in between the spore and the callose wall. Scott (1994) believes that **"the primexine acts as a loose scaffold on to which sporopollenin monomers (fatty acids and phenols) are covalently attached by the localized action of super oxide radicals generated at the plasmalemma".**

Beside its function as a controlled deposition of sporopollenin it also functions as a pathway for the diffusion of other substances such as enzymes, including those responsible for digestion of the callose wall.

In the beginning the primexine is discontinuous at certain specific regions of the plasmalemma (Figs. 29b and 30b) which marks the position of the future germ pore. During the later period of primexine deposition additional gaps appear and these are occupied by columns of electron dense intricate tubular lamellae of 70 nm diameter, called probaculae that rests on the plasmalemma (Figs. 29c and 30c).

The probaculae appear to condense round the plasmalemma and become more evident by the deposition and polymerization of the sporopollenin precursors. Later during the late tetrad stages, the probaculae become disjointedly differentiated into electron dense baculae.

The baculae become laterally expanded at the base to form the foot layer (Figs. 29d and 30d).

Fig. 29: Ubisch bodies formation and the development of pollen wall

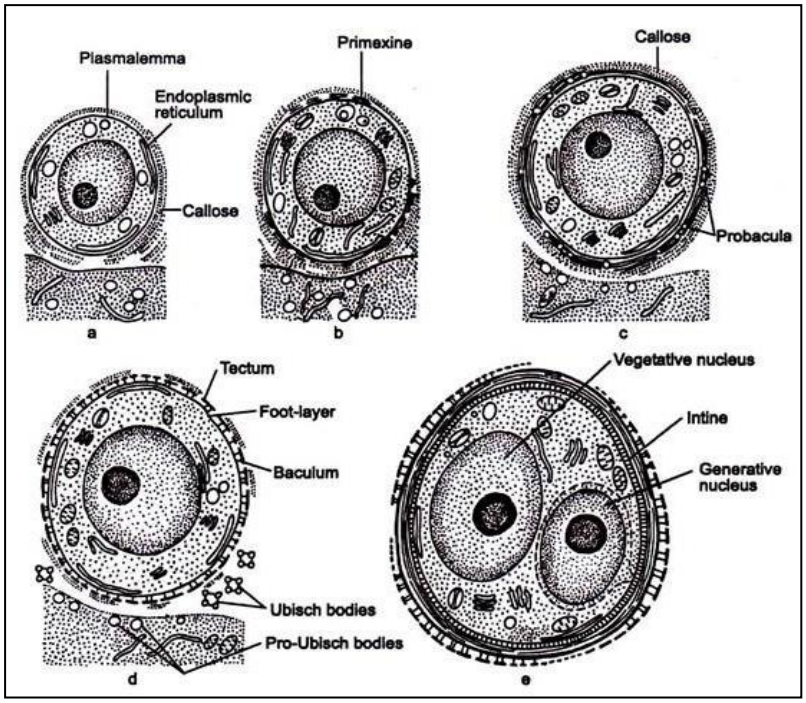
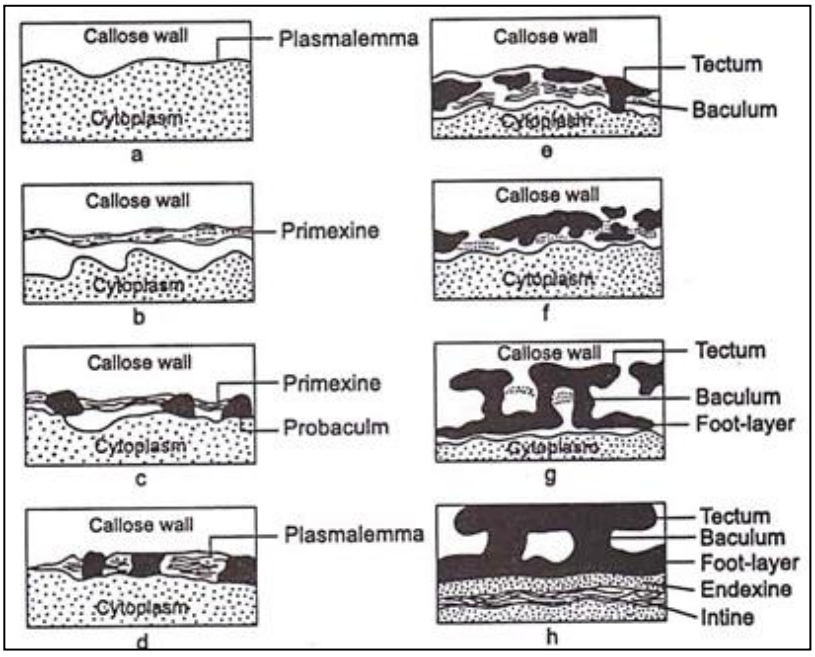


Fig. 30: Diagrammatic development of the pollen wall.



Depending upon the taxon they may remain free above or increase in electron density due to rapid deposition of sporopollenin, and their heads expand laterally to form the tectum (roof), over the primexine matrix.

The foot layer represents the future nexine 1, while the baculae and the roof layer, the future sexine. Further deposition of sporopollenin continues and the whole pollen grain expands laterally and radially as the pollen grain enlarges.

The chemical constituent of the probaculae and the foot layer is not clear and their electron opacity is different from that of the sporopollenin of mature pollen, for which it has been described as protosporopollenin. In the initial stages these layers are not resistant to acetolysis, however, they become resistant with the development of the tetrad stage.

In many marine angiosperms primexine is absent and consequently do not develop normal exine. The nexine2 is deposited below the sexine. In the initial process a number of very thin electron transparent lamellae appear to arise from the cytoplasm and provide a locus around which sporopollenin is deposited. With the progress of deposition the lamellae thicken and merge with each other to from the nexine2.

The presumptive germinal apertures of pollen grain are already demarcated during the microspore tetrad stage. The sites of pre-aperture are distinguished by the absence of the primexine matrix and is associated with an underlying plate of endoplasmic reticulum oriented parallely to the plasma membrane.

This endoplasmic reticulum may physically prevent the movement of membranous structures (coated with primexine material) to the cell surface. Sheldon and Dickinson (1986) believed that the meiotic spindle plays a role in aperture positioning.

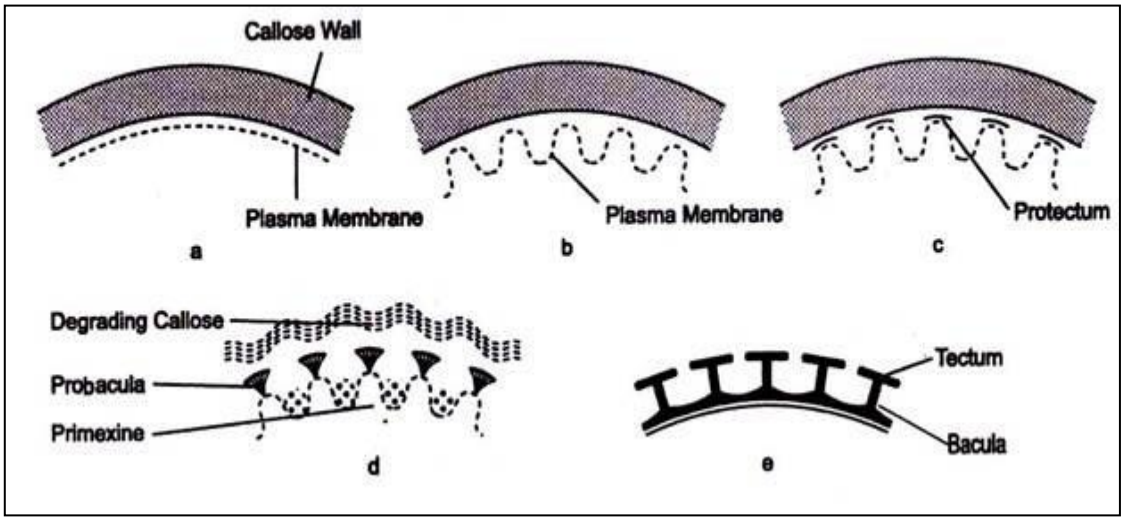
Later the callose wall dissolves, thus releasing the tetrads. The spores now expand rapidly and the primexine matrix is largely disrupted, and only the residue can be observed between the baculae of the mature exine. A rapid conversion of the protosporopollenin occurs, and the primexine acquires the staining properties of the exine.

b) Undulation Model:

Studies on exine development by Takahashi, (1989, 1993, 1995) in *Caesalpinia* and *Lilium* gives little support to the primexine model. It explains that the exine formation commences with the tetrad stage by the invagination or undulation of the plasma membrane which is possibly under the control of cytoskeleton elements. These invaginated localized regions match to the regions of future lumina and distensions that correspond to the muri of the mature exine.

Takahashi (1995) observed that in Lilium the plasma membrane assumes a reticulate pattern which matches the pattern of the mature exine. Fibrous threads (10–20nm diameter) together with granules (10 nm diameter) aggregate at the regions of protuberances of the plasma membrane and slowly these aggregates develop into a smooth protectum of 0.5 to 0.7 µm diameter (Fig. 31).

Fig. 31: Diagrammatic representation of pollen wall development based on Undulation Model



During the later part of the tetrad stage the probaculae and the protectum are more distinguishable beneath the callose wall and the plasma membrane assumes a smooth outline. Probaculae are later formed in between the plasma membrane and the protectum.

At this time the cellulosic fibrous primexine is distinguishable in spaces between the probaculae and the callose wall dissolves, thus releasing the microspores and further

differentiation of the exine continues. Thus the undulated plasmamembrane plays an important role in pollen wall development and in fact the protectum is the first exine layer that is deposited on this membrane.

Christensen (1972) outlines the following steps in the development of the pollen wall:

a) In the tetrad stage a new wall called primexine, is deposited around the microspore protoplast within the wall of callose. The primexine appears to contain cellulose microfibrils.

b) In the transition of primexine to exine elements of primexine produces precursors of rod like bacula, which form the sexine. As a result of rapid deposition of sporopollenin the baculae enlarge in electron density and their heads expand laterally to form the tectum.

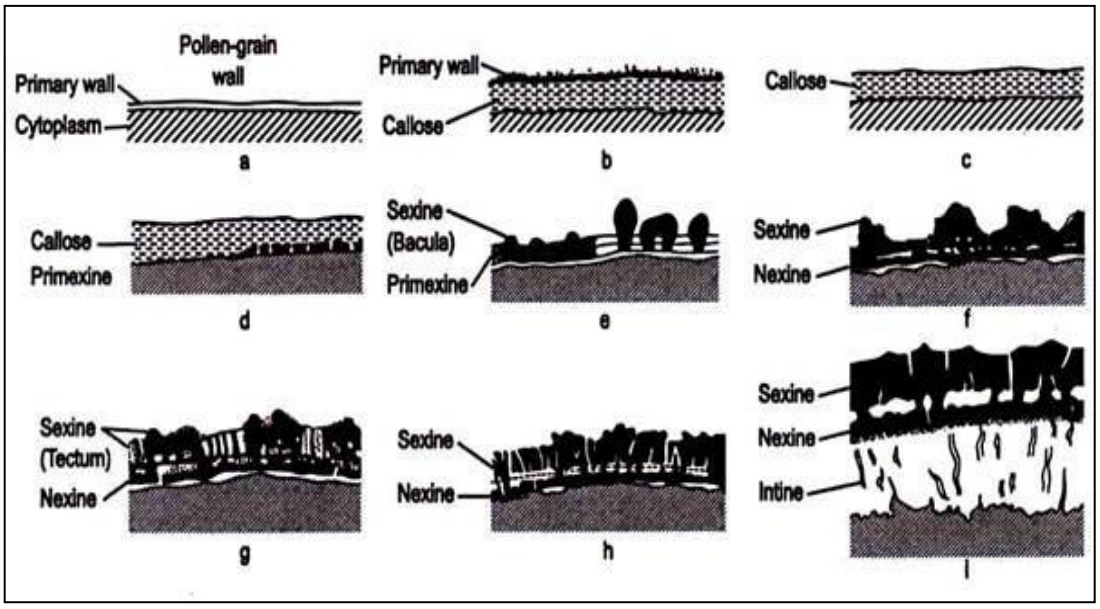
This is simultaneously followed by the lateral expansion of the base of the bacula to form the foot-layer. Deposition of sporopollenin continues and the whole wall expands laterally and radially. It also witnesses the dissolution of the cellulose wall as a consequence the pollen grain lie free in the pollen sac.

c) Nexine is deposited below the sexine. This is initiated by the deposition of number of electron transparent lamellae originating from the cytoplasm, around which sporopollenin is deposited. Deposition is followed by the thickening of the lamellae which unite with each other to form the nexine (Fig. 32).

d) In the aperturate region the sexine has very short bacula, while the nexine becomes much thicker and discontinuous in this region than the rest of the pollen wall.

e) At later stage cellulosic intine is formed inside the nexine.

Fig. 32: Diagrammatic representation of the pollen wall development



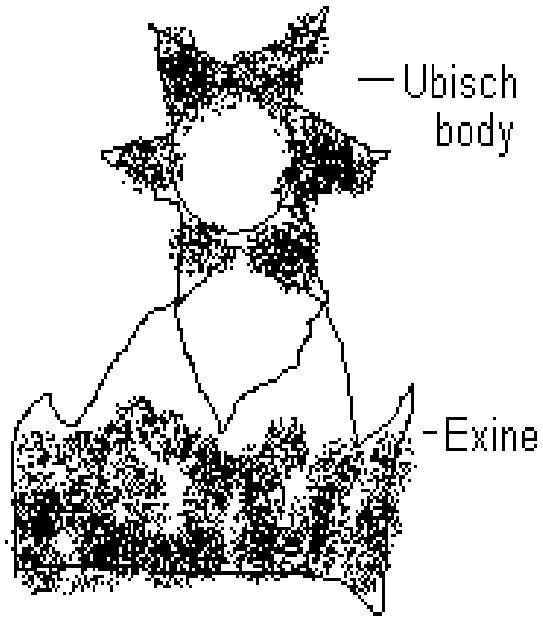
2. Intine Growth Phase:

The intine usually starts to develop at the vacuolated stage, beneath the apertures. It increases in thickness under the pores and later on starts to develop under the interapertural parts as a thin layer.

Golgi bodies are frequent during intine synthesis e.g., Ranunculaceae while in others, E.R. and polyribosomes are abundant e.g. *Cosmos*. The intine beneath the pore become comparatively very thick and is provided with fibrillar material and radially arranged membranous units.

In the final period of wall development, additional lipoidal and pigmented substances may accumulate on or/and within the outer exine. This material called the pollenkitt, imparts colour or odour to the pollen and may cause the pollen grains to adhere together during dehiscence.

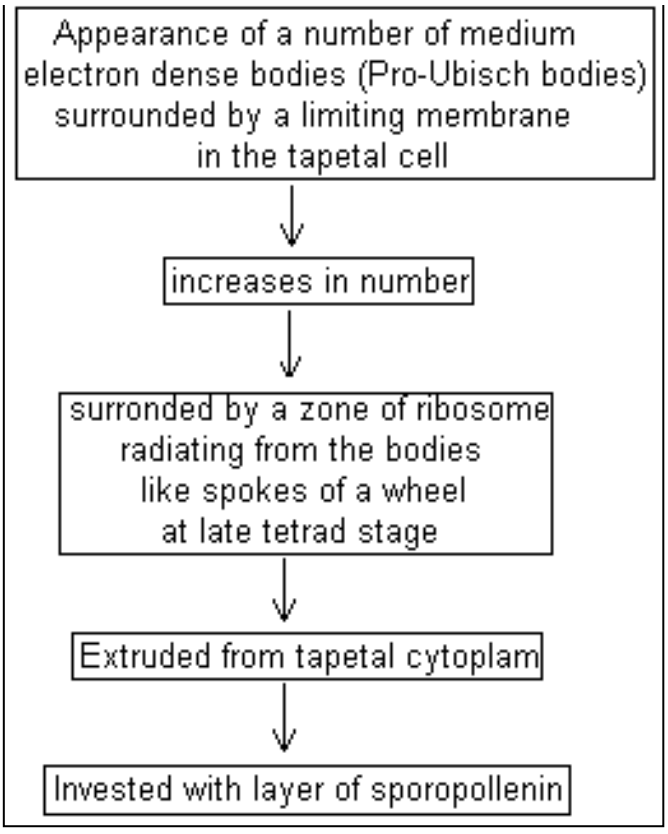
Ubisch bodies



These are spherical structure (2-5 µm) found in the anthers of many genera of angiosperms, both monocotyledons, e.g. *Triticum aestivum* and dicotyledons, e.g. *Betula pendula* and many gymnosperms, e.g. *Ephedra foliata*. They may also oval, rounded, triangular, rod like etc. They frequently fuse into large compound aggregates as, in *Euphorbia caputmedusae*. They generally have a central core and thick wall with microchannels. They are a distinctive feature of secretary or glandular tapetum. Their walls apparently consist of sporopollenin.

It was first discovered by Rosanoff (1865) in close association with the tapetum. Erdtman *et al* (1961) proposed the term 'orbicules' for these bodies.

Development of Ubisch bodies



Functions:

- Orbicules have no specific function; they are a byproduct of tapetal cell metabolism.
- They might actively partcipate in lysis and degradation of tapetal cell.
- They prevent osmosis and collapses of the developing microspores.

Pollen connecting threads

The selective factors in pollination biology include both structural and functional efficiency. It is observed that anemophilous angiosperms produce mostly small, single and dry pollen grains whereas, in zoophilous angiosperms, flower visitors transfer large clusters of pollen grains that are certainly a positive selective factor in pollination biology. There are three different types of pollen clusters occur in angiosperms: a. sticky pollen grains b. polyads and c. pollen connected by threads.

Types and Function of pollen connecting threads

Previously most of the known pollen connecting threads were grouped under the term "viscin threads". But recent knowledge interprets that principally there are two main types of adhesion threads, either containing sporopollenin or not. The adhesion strings containing sporopollenin is named as 'viscin threads' whereas threads lacking sporopollenin cannot be called as viscin threads.

In modern day, 5 different types of pollen connecting threads are categorized on the basis of nature and ultrastructure, which are as follows:

- Filiform sporopollenin threads
- Non Sporopollenin threads of uncertain origin
- Cellular threads
- Elastoviscin threads of tapetal origin
- Threads from degenerated materials

Sporopollenin Threads: It occurs only in three zoophilous unrelated families of angiosperm viz., Onagraceae, in many Ericaceae and in a single genus *Jacqueshuberia* of Caesalpiniaceae but the nature and function of the threads is same.

Patel et. al. (1985) described the ultrastructure of sporopollenin threads and added some interesting characters:

Often four grains are found connected by threads – this indicates that four microspores of post-meiotic tetrads remain connected.

- Before acetolysis the *Jacqueshuberia* threads and pollen grains are widely covered and sculptured by some granular tapetal debris. After acetolysis the pollen grains and threads are found free of debris with smooth surfaces.

- The sporopollenin threads are very flexible but apparently not elastic, their average diameter is similar to that of columella.

- The columellae are pilar like and distally branched; with tangled ribs forming a network.Some enormously elongated ribs of the columellae form the pollen connecting threads, which are extension of ektexine.

- The sporopollenin threads of Ericaceae and Onagraceae pollen also represent distal elongations of sculpture elements i.e. branched columellae. The ektexine characters are strikingly similar to that of viscin threads.

- In Ericaceae and Onagraceae, like *Jacqueshuberia*, the ektexine of viscin thread pollen consists of distally branched and vaulted columellae forming a peculiar type of tectum, while some extremely elongated, not vaulted and not thickened columellar branches form sporopollenin threads.

- In onagraceae and Mimosaceae, in addition to long sporopollenin threads, small filiform bridge like threads and fragile, short non-sporopollenin fibres are present.

Function:

i. Strictly ektexinous viscin threads connect the neighboring pollen grains or polyads and help in easy dispersal of large quantity of pollen grains by flower visitors.
ii. Extremely short bridge like threads act as additional cohesion elements in polyads and does not connect neighboring polyads. These are formed from endexinous material.

Non Sporopollenin threads: These types of threads are formed by:

- Cells – in only one zoophilous angiosperm family
- Exclusively by 'dead cell' secretions – in most of the zoophilous angiosperm families

Cellular threads: This type of threads is rough and massive and formed by modified anther cells (e.g. *Strelitzia* of Musaceae). The threads are covered by thin, lipid coating and stick on the pollen grains. These large, massive and rough threads connected the pollen for carrying by heavy pollinators while flexible threads seem to be associated with small insect pollinators.

Non-Celllular threads: It is found in several zoophilous angiosperm taxa.

In Orchids, there are at least two different types of threads are present:

- **Elastoviscin threads** – These are produced by specialized tapetum cell cytoplasm and not by plastids. Threads are elastic, non sticky, often non homogenous, mostly lipid materials in and outside the pollinia connecting the individual pollen grains. When the pollen grains are physically separated, the elastoviscin forms highly elastic threads.

- **Lipid threads** – This type of threads are sticky, highly viscous but inelastic substances originate from some immature, degenerating cells in the transition region between the caudicle *(a slender, elastic appendage, to which the masses of pollen in orchidaceous plants are attached)* and the massulae *(=polyad)* (e.g. *Habenaria*).

- **Slimy, viscous fibres** (containing protein and lipid) connect individual pollen grains in some zoophilous angiosperm families. Very little is known about their nature and origin. Slimy threads connecting pollen are either coated with small droplets or resemble a rather rope like fluid (e.g. Aristolochiaceae, Annonaceae). These slime threads may act as special pollen cement or may play some role in pollen germination.

Perine

It is a hyaline loosely organised sporopollenin envelope covering exine and occurs in some pteridophytes (Polypodiaceae) and a few gymnosperms (e.g. *Taxodium*). It is secondary in origin and developed after differentiation of exine. It may be continuous or sometimes folded

in various ways. Well-developed, complex and highly ornamented perine is found in advanced groups of ferns. The presence of fold and well developed excrescences is possibly a special advancement of retention of moisture and adherence to the substrate to enhance healthy germination. Sometime it is difficult to decide whether a layer is perinous or exinous. In such case, Erdtman suggested the term 'Sclerine' (Greek *Skleros*, hard) can be used as a comprehensive name for perine and exine. Generally, perine is used for pollen, and perispore for spore.

Pollen-kit

In the final period of pollen wall development, additional lipoidal and pigmented substance may accumulate on or/and within the outer exine. This material is called pollen kitt.

Originally the term was used to describe the whole content of the mature anther. However, the term is now referred as an oily often pigmented layer found coating the exine. In entomophious flower, it is electron-dense homogenous films on the pollen surface; in anemophilous flower it is present as small, heterogenous lumps in the cavity of exine.

Chemical nature- It is fluid, viscous, sticky and partially with crystalline inclusions. It contains carotenoid and other lipid; its principal pigment is carotene 5, 6-epoxide.

Ontogeny- It is synthesized from plastids of anther tapetum. Within the tapetal plastid, osmiophilic globuli appear as small droplets; they coalesces upon contact and attain certain size; finally they occupy the whole plastid and interact with plastid membrane; after breaking the membrane they release in cytoplasm; degeneration of cytoplasm results in discharge of globules which is deposited as pollen kitt on surface of pollen grain.

Functions-

- Act as insect attractant;
- Sticky nature would serve as adherent to insect's body and because of its hydrophilic nature might even be associated with dispersal of the pollen grain;
- Its carotenoid can protect the genetic content of the pollen against radiation damage during dispersal;
- Imparts colour and odour to the pollen.

5. Pollen grains adaptation: Pollen grains adaptation in different habitats and pollination types; pollen wall adaptation and significance; Hermomegathic mechanism.

Pollen grains adaptation in different habitats and pollination types

Adaptation to different habitats:

Xeric condition

The high temperature of the arid zones prefers to select pollen with thicker exine and/ or fewer and smaller apertures to check excessive loss of water. This is supported by the pollen morphology of few Apiaceae and Boraginaceae. As a result of thick exine, the form and size of pollen remains the same. In multiaperturate operculate pollen grains, the thick exine has given a selective advantage to them by virtue of a mechanism, to resist desiccation and quick germinability thus ensuring rapid fertilization in the arid zones, where the species have a short life span, like members belonging to Cactaceae.

Hydrophytic condition

In the aquatic angiosperms most of the pollen grains are with a less rigid, discontinuous or very thin exine thus permitting volume changes. The aquatic species of Utricularia have large number of apertures with an equatorial harmomegathus, whereas the terrestrial species of Utricularia have 3-5 apertures, which not being synorate (pollen with lalongate ora anastomose latitudinally), lack the equatorial harmomegathus. In most of the epiphytic species, the brevicolpate pollen has restricted apertural areas and same is also seen in few terrestrial species, but never in hydrophytic species.

Halophytic condition

The pollen of several mangrove species inhabiting tidal zones of alternating salinity shows remarkable harmomgathic adaptations, like extension of apertures, multiplication of aperture and transfer of harmomgathic functions to non-apertural regions. Harmomegathic adaptation of few halophytes are presented in Table 5-

Table 5: Harmomegathic mechanisms in halophytes

Genera	**Adaptation**
Aegialitis	Wide colpi; thin non apertural exine

Avicennia	Wide endo and ecto apertures
Bruguiera, Ceriops, Kandelia, Excoecaria	Equatorially extended large endoaperute
Rhizophora	Endoaperture often fused
Nypa	Meridionosulcus
Heritiera	Thin non apertural nexine
Lumnitzera	Pseudocolpi fused at poles
Sonneratia	Thin non apertural nexine; pseudocolpi sometime present

Adaptation to different pollination types

The size and surface of the pollen and apertures are usually selected on the basis of the pollinating agents, like air, water and insects. Such a correlation with the agents is exemplified by the significant association between the structure of viscin threads in Onagraceae and the pollen vector. The beaded viscin threads are associated with moth and bird pollinated taxa, while smooth viscin threads occur in bee pollinated taxa. Among the tropical woody taxa of Caesalpiniaceae and Fabaceae there is a remarkable similarity between regulate verrucate pollen and the large pollinators, like bats and birds. At the same time there is no correlation between exine sculpturing and pollination in Cactaceae and Polemoniaceae.

Adaptation in entomophilous pollen

The pollen grains of entomophilous species are heavier, relatively larger, and are with various types of exine ornamentation among which reticulate pattern predominates. This pattern helps in the adherence of the grains to the appendages of the pollinating agents. The pollen wall protein provides a greater genetic specificity than the exine morphology. Some of the flavonoids that contribute to the viable colour of the pollen grains provide a fragrance or taste, which either encourages or discourages the pollinating insects. The pollen transported by insects has rich electron dense homogenous pollenkitt spread all over the exine surface and this adhesive factor has an adaptive value. Further the cohesion of monads into tetrads and polyads has a functional advantage, since they often behave as a single harmomegathic unit.

Adaptation in anemophilous pollen

The anemophilous flowers are usually small, non-attractive due to reduced perianth, neither produce nectar nor they emit any fragrance, have dangling anther lobes, and feathery stigmas. Pollen produced by such flowers is small and produced in large quantities, and have a high surface to volume ratio. Further, the exine surface is generally smooth. They have little amount of pollen kitt and that is mostly locked in the exine cavities. Thus, such grains are less adhesive and often are without distinct sculptural elements.

Pigments especially carotenoids have the potentiality to screen UV radiation. Air borne pollen contain higher amount of UV screening pigments than entomophilous pollen. Since they have a longer ambience, such pigments make them more adaptive.

Adaptation in hydrophilous pollen

It has been seen that the reduction in thickness of exine is linked to the origin of the species in aquatic e.g., Zosteraceae or highly moist e.g., Heliconiaceae habitats. Plants have unique devices to make their pollen waterproof or to adapt them for an aquatic environment. Such taxa have omniaperturate pollen grains with thin elastic exine that could stretch easily and accommodate the increase in size due to imbibition of water. However, there are aquatics like *Aponogeton* and *Pontederia* that overcome this problem by flowering above the water surface. In marine angiosperms like the filiform pollen of *Amphibolis* is covered by droplets of lipids and mucilage which is so important that it regulates the cohesion and the water relations of the grains when they are dispersed in sea water for pollination. The exine reticulum of *Ruppia* is believed to play an important role in keeping the pollen afloat. The linear tetrads of *Halophila* and the dyads of Podostemonaceae with elliptic to cylindrical forms may be the product of selection for dispersal by water.

Pollen wall adaptation and significance

Presence of a complex mixture of lipid substances (pollen kitt) on the surface of mature pollen grains

which provide the necessary shield against the harmful effects of the environment especially radiation hazards *(as because ultra violet ray absorbing pigments are present in pollenkitt)*

which protect the pollen grains from desiccation due to its impermeability to water and water vapour

Presence of a mucilage coat around the pollen of some aquatic taxa

which protects the cytoplasm from the chances of desiccation and from fungal and microbial attacks

Stratified structure of pollen exine

which is most difficult to bend. During hydration and dehydration, the radial and tangential differentiation of exine layers absorb the bending stresses.

Early delimitation of apertures

which is considered to be the most justified first direct pressure sensitive control. For example-

Triaperturate: an expression of mechanical stress mode

Periaperturate: an equal spacing mode

Multiaperturate: law of equidistance causes proportional alteration of apertural and non-apertural regions

Stratification of apertural intine in pollen grains

which has special functional significance

Enzymatic inclusion in this layer is sealed above and below by a continuous polysaccharide layer until hydration. Following hydration, the outer layer is loosened and disrupted releasing the underlying enzymes. The inner layer then becomes the precursor of the pollen tube. Since the enzymes of the apertural intine are lytic they are associated with the digestion of stigmatic substrates, thus providing the initial nutrition to the pollen tube. The compatibility reactions are also determined by the enzymes of the apertural intine.

This explains the reasons for the absence of an enzymatic layer in the intine of the spores of pteridophytes and bryophytes.

Omniaperturate pollen grains (exine thin or absent and intine thick, so that no specific apertural region is distinguished and thus the whole surface can be considered apertural in nature)

where the entire sporoderm represents the potential site for pollen tube emergence and clearly the entire intine of the grain is loaded with enzymes

Increase in exine thickness at the non-apertural areas in case of multi-aperturate pollen grains

Multi-aperturate pollen grains have the selective value of rapid germination and early fertilization but increase in aperture logically reduces the mechanical efficiency of the wall and this lacuna is compensated by increase in exine thickness at the non apertural areas e.g. *Hibiscus*, *Ipomoea* etc.

Presence of tapetal proteins in the chambered exine

which acts as a recognition substance that is responsible for the acceptance or rejection of the pollen by the stigma

This functional outcome means that the pollen lacking a chambered exine would be incapable of sporophytic incompatibility

Harmomegathic mechanism

It is described by Wodehouse (1935). Pollen grains can close off their apertures to avoid losing water in arid environments by a particular mechanism. It prevents water loss until it arrives in a wetter environment.

Harmomegathus: as an organ or mechanism which accommodates a semi-rigid exine to change in volume

Harmomegathy: volume-change accommodation

Pollen grains possess certain harmomegathic mechanisms to accommodate volume changes associated with the phases of desiccation and rehydration. Such contrivances help to avoid plasmolysis of the cytoplasm. As such, Harmomegathy is one kind of adaptive mechanism associated with the phases of desiccation and rehydration and the contrivances needed for this mechanism help to avoid plasmolysis of the cytoplasm.

The different hermomegathic mechanisms are enumerated below-

a. The invagination of the aperture membrane in colpate and colporate grains, this is comparable to the meridional folding.

b. Extensive invagination in synorate endoapertures, comparable to equatorial folding wherein the equatorial belt acts as a hinge zone.

c. Invagination of the polar regions which becomes prominent by the thinning of exine at the polar region or by the fusion of the apertures at the poles (syncolpy).

d. In order to control the direction of folding, there is a development of striate ornamentation, linear operculum, conduplicate margins and periapertural thinning.

e. There is an invagination of the aperture membrane of the porate grains. However, in porate grains with distinct thickening the interpore invaginates mainly due to the plasticity of the exine.

f. The flexibility of the pollen grains is enhances by the discontinuity of the sexine layers and by the absence or reduction of the nexine to a lamellar condition.

g. In some members of Asteraceae with immobilized apertures, the interapertural cavea develops to act as a buffer zone between the environment and the cytoplasm. Further in such cases the movement of the lacunae floor compensates the immobility of apertures.

h. In the turgescent stage due to stretching the pollen grains are susceptible to breakage, and desiccation in non-turgescent stage. This is compensated by the presence of band-like sexinal elements that spreads over the aperture membrane, e.g., *Crocus*, *Calectasia*, etc.

i. In Morinaceae there is a development of protruding apertures with oncoid plugs to function as harmomegathic processes.

j. The thinning or development of perforations in the proximal part of the monads of pollen dyads, pollen tetrads and polyads enable them to function as a single harmomegathic unit, e.g., Annonacéae, Asclepiadaceae, Burmanniaceae, Mimosaceae and Orchidaceae.

6. Spore/Pollen Viability and Storage: Estimation; variations; responsible factors; short- and long-term storage; significance.

Pollen viability and storage

The evaluation of pollen viability and its germination capacity are two essential criteria for pollinator's characterization. Viability means ability to live; but pollen viability connotes the ability of pollen to complete post-pollination events and to effect fertilization. Thus, pollen

viability refers to the ability of the pollen to deliver functional sperm cells to the embryo sac following compatible pollination.

Mature pollen grains are relatively quiescent, and contain small quantities of reserve food, usually in the form of starch or sugars, lipids and proteins. The cytoplasm of viable and mature pollen is non-vacuolate, granular and contains many plastids, mitochondria, golgi derived vesicles, lipid droplets and a large population of quiescent dictyosomes.

The changes which proceed and accompany germination are rapid and dramatic. The permeability of the pollen plasmalemma is altered during hydration of pollen at the time of germination. Water uptake and activation or synthesis of enzymes is the basic initiating factors for germination. Respiration and metabolism of external sucrose by germinating pollen is evident long before tube growth becomes visible. An initial high rate of respiration coincides with rapid starch accumulation in germinating pollen. During the activation phase, before the emergence of pollen tube, protein and nucleic acid synthesis takes place.

Many enzymes are present in pollen grain wall and these readily diffuse out into the surrounding medium before the pollen tubes are formed. Many of these are hydrolyzing and cell wall softening enzymes. Free amino acids are released rapidly when pollen grains are placed in a germination medium.

Pollen grains of a wide variety of species germinate successfully in sugar solutions. Sucrose is probably the best and most commonly used source of carbon and energy for pollen. It provides and maintains a proper osmotic environment for germination of pollen and continued growth of pollen tubes. Supplementing the medium with boron stimulates germination and pollen tube growth. Boron is an essential element for plant growth and must be present in adequate amounts to ensure optimal plant growth and productivity. Boron plays a role in flowering and fruiting process of plants and its deficiency results in low pollen viability, poor pollen germination and reduced pollen tube growth. Boron takes part in pollen germination and style tube formation and therefore has a vital function in fertilization of flowering crops. Boron regulates the hydration of colloids, is associated with polyhydroxyl compounds of the pollen membrane, and is involved in the synthesis of pectic substances for

the tube wall. Boron added in the form of boric acid, is also essential for the *in vitro* culturing of pollen from most species.

In nature, water, sugar and amino acids are supplied by the style to nourish the growing pollen tube. For many species, boron and calcium are also required for pollen tube growth. Boron, which is provided by stigmas and styles, facilitates sugar uptake and has a role in pectin production in the pollen tube.

Pollen grains of many species are known to contain relatively large amounts of plant growth substances, particularly auxins, gibberellins (GAs) and cytokinins. Depending on the species examined and the concentration used, GAs can promote, inhibit, or have no effect on pollen germination and tube elongation *in vitro*.
The pollen tube grows as a result of stretching of the wall caused by various factors including turgor pressure. Such a stretching is restricted to the tip region where new material is being deposited via fusion of vesicles with the plasmalemma. The non-vacuolated and agranular cytoplasm of the cap block is devoid of cell organelles like mitochondria, golgi bodies, ER, amyloplasts and lipid bodies and shows no cytoplasmic streaming in growing pollen tubes.
The activation of the relatively quiescent pollen grain during germination is accompanied by protein synthesis, which is followed by RNA synthesis. The greatest concentration of RNA in pollen tubes is found in the growing tip region, which lacks ribosomes but is packed with smooth membranes that are sensitive to RNase treatment. Proteins synthesized during germination of pollen are required for germination and early pollen tube growth.

Thus, the spore/pollen viability is an index of is quality and vigour. This viability or longevity varies between minutes and years, and which primarily depends on the taxonomic status of the plant on the abiotic environmental conditions. In order to maintain the viability and fertilizing ability of the pollen grains for a long period of time special storage conditions are needed.

Cryopreservation is the most efficient methods for long-term preservation of partly dehydrated pollen grains. *In-vitro* biotechnological techniques like isolation and fusion of reproductive cells, and DNA transformation of artificially produced zygotes and embryos, have opened new prospects for germplasm storage.

Sophisticated methods such as Nuclear Magnetic Resonance (NMR) spectrometry, Fouriere transform infrared spectroscopy (FTIR), and different ultra-micro-techniques for electron microscopy have helped to carry out for precise studies on the water regime in pollen, together with molecular changes occurring in membranes during pollen dehydration and redydration.

Pollen viability:

It refers to the ability of the pollen grains to perform its function of delivering male gametes to the embryo sac. This functional property of the pollen after their release from the anther varies greatly from species to species and its quality is assessed on the basis of its viability. Several reasons have been assigned for the loss of viability, like deficiency of respiratory substrate, inability to withstand desiccation and the loss of membrane integrity.

Variation in the longevity of pollen

The life span of pollen is primarily determined by the plant genome but is also influenced by external environmental conditions. Plant taxa can be grouped into three main groups based on their pollen longevity-

a) Long lived pollen (six months to a year), example-Ginkgoaceae, Pinaceae, Arecaceae, Saxifragaceae, Rosaceae, Fabaceae, Anacardiaceae, Vitaceae and Primulaceae.
b) Pollen with a medium life span (approximately 1-5 months) – examples, Liliaceae, Amaryllidaceae, Salicaceae Ranunculaceae, Brassicaceae, Rutaceae, Scrophulariaceae, Solanaceae
c) Short lived pollen (from few minutes to a couple of days) – examples, Alismataceae, Poaceae, Cyperaceae, Commelinaceae, and Juncaceae

Causes for the loss of pollen viability

Deficiency of respiratory substrates or/and inactivation of certain specific enzymes or growth hormones that are likely to affect the viability of the pollen. This idea is however, untenable when it is seen that the pollen of cereals (short lived) in spite of having abundant metabolites quickly lose their viability. Similarly changes in amino acid composition of stored pollen fail to explain the loss of viability. There are variable reasons to explain such inactivity which are stated below-

Biochemical alteration

The major biochemical cause for the loss of pollen viability during storage is basically due to the deficiency of respiratory metabolites, which is the result of continuous metabolic activity by the pollen. As a result of long-term storage, considerable changes in the amount of carbohydrate, amino acids and organic acid level have been occurred in the pollen of different species. A higher respiratory rate in the three celled pollen leads to the scarcity of respiratory substrate that strongly contribute to their rapid loss of pollen viability. It is reported that stored pollen grains require a higher concentration of sugar for germination in vitro tan fresh pollen. Higher relative humidity also plays an active role in decreasing germinability by rapidly degrading endogenous substrates essential for germination.

Desiccation and loss of membrane integrity of pollen

The regulation of pollen water content is an important adaptive mechanism for survival after pollen dispersal and accordingly pollen grains tat remain viable after dehydration are called desiccation tolerant, and those that lose viability parallel to dehydration are called desiccation sensitive. The water content of living pollen grains in different families vary between 15% and 35% of fresh weight at the time of shedding, which is however, very high in Poaceae pollen between 35-60 %. The original pollen moisture to some extent depends upon temperature, air humidity, and the water supply to the pollen donor plant.

An investigation on the membrane state of pollen grains from different taxa exposed to dry conditions indicated that most of the samples had lost their membrane integrity, it has also been observed that the plasma membrane may undergo gel phase transition during water loss by increasing van der Waals interaction or free radical damage. Thus, water plays an important role in maintaining the structural integrity and the stability of the pollen membrane, by acting through hydrophobic and hydrophilic interactions. A positive correlation has been established between the loss of viability and a reduction in the amount of membrane phospholipids irrespective of the storage conditions.

Factors affecting pollen viability

Pollen viability after is influenced by a number of biotic and abiotic factors. The longevity of pollen, as influenced by the bi-nucleate or trinucleate conditions at the time of dispersal was attributed to various structural and physiological changes; further the low moisture content,

more resistant cell wall and other features of the two-celled pollen reduce the loss of viability which naturally have a higher life-span as compared to the tri-nucleate pollen.

Pollen cytology

There exists a close relation between the cytology of pollen and its viability. Studies on pollen morphology and physiology have shown that the binucleate and trinucleate pollen grains show differences in their physiological and structural characters at the time of pollen dispersal. The two celled pollen grains have a longer life span because of their more resistant wall structure, low plasma water content and reduced metabolic activity, whereas the trinucleate pollen grains are short lived due to their less resistant wall and high moisture content, which can easily be lost by desiccation. This trinucleate pollen has a high rate of metabolism, respiring two or three times more than the binucleate pollen grains.

Humidity and temperature

Environmental factors especially humidity and temperature greatly affect pollen viability. This relationship has been investigated by many authors and it transpired that pollen of majority of the species retained viability best at low relative air humidity (0%-30% RH) and temperature (between 0 and 10 °C).

In most of the cases it is possible to standardize the conditions (low temperature and /or low humidity) for extending pollen viability of two celled taxa. However, on the other hand little progress has been made with the preservation of the three celled pollen taxa under Poaceae, Brassicaceae, Caryophyllaceae, Apiaceae, and Chenopodiaceae families. The longevity of Poaceae appears to be short under all conditions. Low relative humidity is harmful and pollen stored at 0° -10 °C remains viable only for a couple of days. Under high relative humidity (80% to 100 %) also the viability can be prolonged to 1-3 weeks at the most.

Estimation of pollen viability

An assessment of pollen viability is imperative factor in the study of reproductive biology, pollen storage and hybridization. Thus, there must be a quick and dependable method to determine the viability of pollen grains. Several tests have been standardized from time to time which are as follows-

***In vitro* germination test**

t is the most popular and quantitative, simple speedy test for assessing pollen viability. A small sample of the pollen is observed under microscope and the percent of grains producing tubes after a given time is determined. This percent is considered an index of viability of the pollen sampled. Such tests assume that the optimum conditions have been established for the *in vitro* test so that germination approximates that on the plant. However, pollen tubes of most of the grains cultured in vitro stop growing before they reach the size normally attained in the style and the rate of tube growth is seldom as rapid as *in vivo*. This suggests that optimum growth conditions are not always established *in vitro* media. Pollen grains of *Antirrhinum* remain viable up to 670 days, but show only 180 days of viability *in vitro,* this however, can be extended if a piece of stigma is placed in the medium. This again suggests that the inability to germinate *in vitro* following storage due to deficiency of some substances is compensated by the stigma. Thus pollen, which loses germinability following storage, cannot be considered completely nonviable, and needs to be tested by supplementing *in vivo* pollination.

Pollen germination on stigma

By controlled pollination and observing the growth of the pollen tube through the style it is possible to some extent in assessing the viability of a pollen grain. At least 70 pollen tubes in the style of *Brassica oleracea* are considered to indicate total viability of the pollen grains. In spite of the limitations of available techniques (fluorescent method) for observing the pollen tube through the style, it is better than the laborious and time-consuming method of fruit and seed set technique.

Enzyme assay method

It demonstrates cytochemically the activity of certain enzymes in living pollen, like dehydrogenases by the triphenyl tetrazolium chloride (TTC) test; catalases by the benzidine test or esterases by the fluorescein diacetate (FDA) test, etc. It has to be remembered that the presence of a functional enzyme in the cell itself does not guarantee that the pollen is still viable and functional.

Fruit and seed set

It is the most genuine way of testing viability and is expressed by the ability of the pollen to effect fertilization resulting in seed and fruit set. The system has many limitations like,

receptivity and incompatibility of the stigma; post fertilization determinants related to seed development may thwart seed-set; not applicable in apomixis reproduction; tedious and time-consuming system; and applicable only at the time of flowering period and thus cannot be used as routine practice.

Tetrazolium Test

The most commonly used tetrazolium salt in pollen viability test is 2, 3, 5-triphenyltetrazolium chloride. Tetrazolium test is based on the reduction of soluble colourless tetrazolium salt to reddish insoluble formazan in the presence of dehydrogenase. If the pollen grains are incubated in tetrazolium solution (0.1-1%) for at 30-37 C, viable 30-60minutes pollen grains take a reddish colour due to the formation of formazan. Since the tetrazolium salts can be easily reduced by light, it is necessary to keep the solution and the pollen grain in dark.

***Fluorochromatic reaction test (FCR) or Fluorescein diacetate test* (FDA)**

The Fluorescein di-O-acetate is a non-polar, nonfluorescent compound that can be rapidly hydrolyzed by acetyl esterase activity to fluorescein, a polar, fluorescent compound that is retained by the living cells only. FDA test for pollen viability assesses two properties of the pollen, viz., the integrity of the plasma membrane of vegetative cell; and the activity of esterase capable of cleaving the fluorescein di-O-acetate. The test has been widely accepted for its better resolving power than the other existing tests of viability. Viable pollen grains when mounted in a suitable osmoticum FDA solution, non-polar, nonfluorescent FDA penetrates the plasma membrane of the vegetative cell and enters the pollen cytoplasm. Hydrolytic cleavage of the FDA by esterases produces fluorescein, which accumulates in the pollen cytoplasm, as the intact plasma membrane is impermeable to it. This fluorescein gives bright fluorescence when observed under fluorescent microscope.

In non-viable pollen the plasma membrane is generally disrupted as a result it allows the fluorescein to move out readily and produce uniform background fluorescence. Further, in the absence of active esterases in the pollen cytoplasm the fluorescein is not formed and thus pollen grains do not fluoresce.

Storage of pollen

Horticulturists and plant breeders have long been interested in crossing varieties, species and even genera to produce new and improved types of plants better suited to human requirements. However, many of these attempts have failed, due to the barriers to crossability, e.g., flowering of the selected parents at different times or at different places, failure of the pollen grains to germinate on the stigma, bursting of pollen tubes in the style, failure of tubes to grow through the style, and slow growth of the tubes so that they do not reach the ovules before abscission of the flower. Occasionally the pollen tubes no doubt enter the embryo sac, but sterility results either from failure of the male gamete to fuse with the egg nucleus or from subsequent failure or arrested development of the embryo and the endosperm. The most efficient technique to overcome this barrier, imposed by time and space, is the use of stored pollen. Pollen storage also eliminates the need to continuously grow lines frequently used in crosses.

Short-Term Storage

Storage of pollen solvents avoids the problem of maintenance of relative humidity and may be a useful technique for transporting pollen refrigeration. The efficacy of organic solvents for storing pollen of different taxa has been systematically studied by the researchers indicating the species-specific responses. The effects of these solvents on leaching of phospholipids and their relation to viability resulting through their implications on membrane integrity. It has been reported that the polar solvents like hexane and diethyl ether cause very little leaching of substrates like sugars, phospholipids and amino acids as compared to pollen stored in non-polar solvents. The studies on the moisture content and the dehydration and rehydration effects on pollen indicate that many of the structural and functional changes in the cell are quite irreversible; as such the pollen viability of many taxa could be prolonged for shorter durations through appropriate manipulations of water content and humidity of the storage conditions. The relative roles of moisture and storage temperatures on the period of storage are highly variable for a number of plants. In many of the taxa of Poaceae with tri-nucleate pollen, the viability is reported to be very short under natural conditions. Even the short-term storage conditions are not as effective as in other angiosperm families. It was shown that the wheat pollen with viability up to 1- 3 h at 20°C storage may retain it up to one day at 4°C. In maize, the estimated pollen viability is up to 9 to 11 days at 4°C and 90% relative humidity; however, this has reduced to six days with a further increase of relative

humidity by 5-10%. In Secale cereale, the 12 h storage period at 17-21°C storage temperature could be enhanced upto 4 to 7 days by lowering the temperature to 4 °C at similar humidity levels. However, the information obtained using techniques like spin probe, electron paramagnetic resonance (EPR) and saturation transfer paramagnetic resonance (ST-EPR) have shown that partially hydrated pollen is commonly devoid of mechanisms to conserve pollen viability suggesting that optimum levels of moisture and humidity have to be assessed properly for each taxon.

Long - Term Storage

For a limited number of taxa, the storage of pollen above 0°C, although enhanced the period of longevity, the results are not always encouraging; the success is much more limited in Poaceaeous members. In addition, the application of freezing and cryogenic conditions has become a successful event in the subsequent studies. Use of Freezing Temperatures Under this category of preservation, long-term storage could be achieved through exposure of pollen to temperatures between -10 to 34°C. The vast amount of information from large numbers of taxa indicate that the method is more suited for improving the longevity of bi-nucleate pollen with low water content rather than trinucleate ones.

Pollen viability of most of the species was enhanced to significantly longer periods with a range of a few months to as high as three years in some cases. Different studies have recorded that pollen of annual soybean retain their viability for four months when exposed to - 20°C; however, the germination *in vitro* was a failure with the wild relatives as against near normal germination of the stored pollen of cultivars. Similar studies in lily resulted in a delay in germination by one hour, in case of stored pollen (at - 20°C) as compared to fresh pollen; degradation of some proteins during storage was presumed and will be synthesized during the lag period and was confirmed in their studies on the qualitative and quantitative differences in the proteins of fresh and stored pollen.

Freeze-Drying and Vacuum-Drying

This kind of approach of pollen preservation is also referred to as lyophilization. Since the earlier reports of applications of freezing and drying, it has become a potential tool for safe storage of a number of biological materials. It has also been extended for preservation of pollen of many species to satisfactory levels. In freeze-drying method, the pollen would be

initially exposed to rapid freezing temperatures (-60 to - 80°C) followed by gradual removal of water under vacuum sublimation.

In the vacuum-drying, on the other hand, the pollen is directly exposed to vacuum and simultaneous cooling while the moisture is withdrawn by evaporative cooling and does not include the initial freezing step of freeze-drying method. The method is further influenced by the conditions of the temperature, humidity and pressure as well as the agents used for attaining pressure. The presence of inert gases (like helium and nitrogen) or simply vacuum in the pollen atmosphere profoundly influence the duration of storage.

Freeze-drying of pollen for various periods had not changed the seed set, germinability and the seedling vigour of Douglas-fir pollen indicating its potential for safe and prolonged storage. Since water molecules sublime with minimum change of molecular configuration in this method, the influence of pre-freezing as well as freezing temperatures of various periods need to be established for improving the storage conditions of pollen for each taxon.

The rate of removal of water appears to be a function of water content of the pollen and was slower for cold-stored pollen. Researchers claimed that air-drying of pollen for 4 hours or slight refrigeration of the air-dried pollen for several weeks before freeze-drying for 30-60 minutes was sufficient to remove free water and to retain higher viability.

The impact of temperature, humidity and pressure on storage ability has also been emphasized by many workers. Such methods are more effective for taxa with desiccation - tolerant pollen to achieve fairly longer periods of viability.

Cryopreservation

The fact that pollen could be preserved in unaltered conditions by exposure to extremely low temperatures (-70 to – 196 °C) has become evident when the method has been first used for the pollen of *Antirhinum majus*. The potential of the technique is evident from the quite many applications of it in the preservation of pollen of diverse taxa since 1950. The results of utility of cryopreservation of pollen from agronomic species using liquid nitrogen has followed further substantiation in a number of species, including many crop species of the family Poaceae. Their implications on storage and germinability and on the

pollen of *Pyrus malus* and *Pyrus communis* indicated the practical utility of it. In these plants, the viability was extended up to 1 to 2 years whereas the pollen remains viable for as many as 1062 days in the case of *Lycopersican esculentum* by exposing pollen to -190°C and up to as many as 5 Years in *Vitis vinifera*. However, the method may not be equally effective for all species. There are reports of preservation for only shorter periods as in *Glycine max* (21 days) and only 10 days in *Gossypium hirsutum* and to about one month in *Vicia faba*. In *Solanum tuberosum*, the exposure to -196°C led to the viability of stored pollen from 9 months to 24 months. In Capsicum, cryogenic method has not only extended the longevity but also the stored pollen retained their fertilization ability.

7. Pollen limitation and plant diversification: Definition; ecological and evolutionary relevance.

Plants are immobile, and therefore rely on abiotic and/or biotic vectors to transport pollen (gametes) for sexual reproduction. An inadequate quantity or quality of pollen can reduce plant reproductive success (seed quantity or quality). The term that has been used to describe this phenomenon is ‘‘pollen limitation’’ (‘‘PL’’). For instance, in animal-pollinated plants, pollen quantity may be reduced as a result of fewer pollinator visits or less pollen delivered per visit, and pollen quality can be reduced if self or otherwise incompatible pollen is delivered.

Over the last two decades, determining whether seed production is pollen limited has been an area of intensive empirical study. However, these empirical studies have been only weakly connected to contemporary theory developed to explain the ecological or evolutionary causes of PL or its expected evolutionary consequences (i.e., character and mating system evolution). In addition, the effects of PL on plant demography and population persistence, plant species coexistence, and community structure and ecosystem functioning have not been fully evaluated or incorporated into our understanding of the long-term consequences of PL.
Pollen limitation has both ecological and evolutionary causes and consequences. For instance, ecological context, plant life history, mating system, and phylogenetic history may all influence or be associated with the probability or strength of pollen limitation. In turn, reproductive characters can evolve in response to pollen or pollinator limitation (i.e., plants

may evolve mechanisms of reproductive assurance), reducing the potential for PL in the future. If a species' population size is limited by seed production, PL may also differentially decrease the absolute or relative abundance of that species and shift the community to one dominated by species less prone to PL, such as autogamous species. Such changes in species composition could have consequences at the ecosystem scale, and for the frequency of pollen limitation among species. Chronic PL, in particular, can have several outcomes for plant populations—reproductive or life history characters can evolve that minimize it (or its effects), their population abundances can decline leading local extinction, or they can evolve but not at a rate fast enough to forestall extinction. Thus determining 1) whether populations can evolve to reduce PL, and (2) whether and how PL influences population dynamics are central to our understanding of the importance of PL in the maintenance of plant diversity.

Many plant species often produce more flowers and ovules than fruits and seeds. Several hypotheses have been presented to explain this phenomenon, including pollen and resource limitation. Pollen and resource limitation have received special attention because inadequate pollen and an insufficient availability of resources can reduce the reproductive success of plants. An insufficient amount of pollen and compromised pollen quality have been demonstrated to result in reduced fruit and seed set, a phenomenon referred to as pollen limitation. Many studies have also indicated that plants are assumed to be limited by resources if the addition of resources increases fruit or seed set. In most flowering plants, pollen limitation and resource limitation are important constraints on reproduction.

Pollination is the first stage in sexual reproduction, and pollination traits have an important influence on plant reproductive success. Plant–pollinator interactions have been viewed as a key process in most flowering plant species. In animal-pollinated plants, pollen delivery and the visiting frequency and activity of pollinators are major biotic factors influencing pollination success. Pollinator abundance and activity decline as a result of a reduction in floral rewards if they then cannot meet the nutritional requirements of pollinators. The majority of pollen limitation occurs in cases when there are either not enough pollinators, or they are ineffective. A recent meta-analysis showed that estimates of pollen limitation are often biased when the flowers of a plant are manipulated, due to reallocated resources. Many studies have indicated that pollen supplementation experiments may overlook the potentially confounding effects of reallocated resources on seed production. However, most studies only

examine the consequences of pollen limitation on seed set, and few studies have measured the possible effects of resource allocation on the success of pollen supplementation.

Many studies have indicated that pollen limitation is strongly correlated with pollinator visitation. Pollinator visitation and activity have pervasive effects on pollination success or failure. A reduction in pollinators causes a decline in the amount of pollen delivered to the stigmas and reduces the probability of the transfer of cross pollen, resulting in reduced seed set. In addition, low-quality pollinator activity can bring about pollen limitation by causing limited pollen availability and inefficient pollen transfer. Pollen limitation may be caused by quantity and quality limitation. In animal-pollinated plants, insufficient pollen deposition is mostly caused by pollinator assemblage characteristics, such as pollinator visitation and abundance. Moreover, pollen quantity limitation is related to both pollinator frequency and pollination effectiveness.

Causes of pollen limitation

There are at least two ultimate causes of PL: (1) the population is not at its Haig and Westoby (1988) equilibrium; and (2) the population is at equilibrium

Ecological perturbations lead to non-equilibria situations—

Recent ecological changes in the pollination or resource environment may cause populations to diverge from their Haig and Westoby equilibrium, resulting in chronic PL. For example, introduction of highly attractive non-native plants, invasion of a new habitat or habitat fragmentation, decrease in population size, or loss of native pollinators, may reduce pollen receipt and increase PL of seed production. Some of the most extreme effect sizes are reported for plants in disturbed systems. For example, Parker (1997) found effect sizes of over 2000% in an invasive shrub, *Cytisus scoparius*. Anthropogenic fragmentation of habitats, climate change, and alteration of species distributions all may have wide-ranging effects on the degree to which plants exhibit optimal allocation for their resource–pollen environment.

If most habitats today are influenced by some anthropogenic disturbance, or if ecosystems are naturally in rapid flux, then most plants will not be at their Haig and Westoby equilibrium.

Bet hedging in a stochastic pollination environment—

Stochastic variation among flowers in pollen receipt is a bet-hedging strategy that commonly leads to low fruit set or PL. This hypothesis predicts that flowers should be ‘‘oversupplied’’ with ovules (or plants oversupplied with flowers) relative to the average pollen load received. Oversupply is adaptive in an environment with stochastic pollen receipt because plants can profit from occasional unpredictable arrival of unusually high quantity (or high-quality) pollen on stigmas, or occasional visits from heavily laden pollinators that could pollinate many flowers. Although stocking every flower with many ovules, or every inflorescence with many flowers, entails a loss of reproductive resources to the plant, the fitness gains from the conversion of occasional ‘‘jackpot’’ visits into extra seeds may outweigh the cost of unutilized ovules or flowers. Burd’s (1995) model of ovule packaging suggests that the fitness benefit of oversupply is greater when variation in stigmatic pollen loads is higher, and when the cost of ovules is lower. Studies suggest that there is substantial variation in stigmatic pollen loads among flowers in natural populations, indicating that there is ample opportunity for bet-hedging strategies to be adaptive. Burd’s model is also appealing because it offers an adaptive explanation for what might otherwise be considered to be a maladaptive allocation of resources.

Ecological and evolutionary consequences of pollen limitation

Population size may also affect PL in ways that are independent of population density. In particular, small population size can reduce the number of compatible mates, especially for plants with barriers to selfing (e.g., self-incompatibility alleles, dioecy, heterostyly), and increase the relatedness among possible mates. For example, A°gren (1996) found that the degree of PL increased as the size of the population decreased in island populations of heterostylous *Lythrum salicaria*. Thus, populations with fewer individuals may experience more PL, even if they receive adequate pollinator visits, owing to incompatibility or greater relatedness among individuals.

Pollen limitation of a focal species could also affect other members of the community. PL can affect other species, as for example, reduced seed production owing to PL will lower resource availability for seed predators and pathogens. When PL affects the abundance of the focal species, not only is it possible that interacting species (e.g., mutualists, antagonists) will be influenced, but other community-structuring processes may also be altered. For example, if

interspecific competitive interactions at the seedling stage are important among co-occurring species, reductions in the seed rain of the focal species may lead to altered competition. In addition, seed-sowing experiments show that plant community structure (e.g., species richness, evenness) is altered by seed additions, suggesting that PL may affect plant community structure when colonization of local patches is seed limited.

Population and community effects of PL may scale up to affect ecosystem processes. To the extent that the focal species uses or provides a resource disproportionately relative to its biomass, changes in its abundance ensuing from PL may have ecosystem-level effects. For example, reductions in the abundance of a nitrogen-fixing species may have strong effects on nitrogen availability for multiple species. Negative effects of PL on species that provide other ecosystem services such as water filtration or soil stabilization will also have serious ramifications. In addition, it has recently been shown that at low levels of species richness both species diversity and species composition can strongly influence a variety of ecosystem processes, such as primary productivity or invasibility. If PL leads to extinction of some key species from local communities, or alters community composition, then it might directly influence these ecosystem-level processes.

Many evolutionary arguments regarding the diversity of plant reproductive strategies incorporate pollen limitation (PL) as an important mediator of selection, and predict that plants will evolve to reduce PL. For instance, it has been argued that traits that enhance pollinator attraction, or lessen reliance on pollinators (i.e., obligate or delayed self-pollination), or on sexual reproduction (i.e., increased clonal growth) may evolve. Haig and Westoby's (1988) graphical model was a pioneering effort in this pursuit, but the exclusion of many biological features and the lack of a mathematical formulation have limited its ability to guide empirical investigations into these expected evolutionary consequences of PL. For instance, the fitness gain and resource-cost functions represented in their model are fixed in both ecological and evolutionary time, and there is no indication of how the mechanistic representation of these functions might evolve. In addition, life-history characters that could be expected to influence pollen or resource limitation of female fitness are not included.

8. Natural spore/pollen traps: Types, their implications in floristic & environment reconstruction

The study of natural spore/pollen traps demonstrates that the trapped palyno-assemblage reliably reflects the modern flora of the studied areas. Different types of natural traps used in studies of modern spore/pollen rain are lake sediments, soil surface, moss and lichen cushions, bark and leaves, spider webs, seed hairs of some plants e.g. *Populus* etc. Much of the pollen found in these natural traps was produced by anemophilous plants in the surrounding area. It was carried by air currents and fell like rain onto the earth's surface. The composition of this pollen rain should reflect the composition of the vegetation that produced it. Assuming the fossil pollen assemblage to be indicative of the vegetation that produced it, a study of modern pollen rain in relation to present vegetation should aid in interpreting the environments responsible for the pollen recorded in pollen diagrams.

LAKES: Lakes are ideal natural traps as their surface samples provide an exact analogue of the depositional environment of any fossil pollen cores taken from a lake. Palynologists would attempt to correlate present vegetation with the pollen assemblages found in the uppermost sediments of the lakes being sampled. However, the spatial distribution of lakes rarely matches that of the ecosystems under study and the extent to which bioturbation has occurred is usually unknown. Samples that have been contaminated through stream action or erosion processes should be avoided. Furthermore, variations in lake size and basin morphometry can be major complicating factors.

SOIL: Soil samples have been more widely used than other types of natural traps in the tropics because of the ease of sampling and their ubiquitous occurrence. Samples may be taken from the surface of materials not generally incorporated in deposits analyzed for pollen; chiefly these include forest litter and mineral soils. The main problems with the use of soil samples are that in most environments oxidation, microbial degradation occur resulting in poor pollen preservation and rates of sedimentation are not ordinarily known. The compactness of the soil or litter will probably also influence differential vertical migration of the spores.

BRYOPHYTIC POLSTERS: Analysis of polsters of mosses, liverworts and lichens has been frequently employed to sample modern pollen rains. It has been found that the more compact growth forms are superior to the loose, many-branched forms as pollen traps. The polster collections should be representative of the pollen rain over the last few seasons. Moss polsters are not an appropriate method of sampling in much of the tropics as many tropical ecosystems, such as savannas and deciduous dry forests, contain few mosses. However, Weng *et al*. (2003) and Grabandt (1980) have shown that pollen from moss polsters provide a very effective means of distinguishing plant communities along the eastern flank of the tropical Andes above 500 m elevation.

BARKS: Bark may be used as an easily available pollen trap in the reconstruction of the vegetation. Samples, a few millimetres in diameter, taken from bark of beech, birch, hophornbeam, oak, pine, sycamore and willow contained enough pollen grains for reliable counts (between 200 and 600 pollen grains per slide). Pollen deposition at various levels in the vegetation and at different times of the year provides useful information on vegetation composition, and the possible effect of wind direction. Analysis of archaeological bark samples forms another promising application besides pollen data from other samples.

LEAVES: Leaf surfaces are differential spore/pollen traps. Their efficiency as traps depends upon whether they are horizontal or vertical, wet or dry, hairy or glabrous, glossy or mat, waxy or non-waxy and so on. Not all spores that land become securely attached. Some are washed off by rain, blown off by wind or redistributed by dew. Virtually any spore which may become air-borne can be found on leaves.

SPIDER WEBS: Spider webs are used to trap insects and provide useful environmental information on caves. Bera et al. (2002) and Song et al. (2007) have shown that spider webs have also proved to be an efficient natural trap of airborne spores and pollen grains. Pollens collected from these samples have been also used to interpret about the vegetation and environment of the area.

SEED HAIRS OF POPLAR: The seed hairs and pericarp of poplar (*Populus* spp.) is efficient natural trap of airborne spores and pollen grains. In a study, these were collected from the trees and on the ground in Beijing Botanical Garden of Chinese Academy of

Sciences and around Miyun Reservoir during May 2005 for pollen analysis. Different pollen spectra are recorded from different samples and are characterized by dominant occurrence of pollen grains of arboreal and anemophilous plants. In addition, pollen grains of non-arboreal plants including grasses are also found trapped. Among the 46 trapped pollen grains, 26 are known to be allergenic. This study also suggested that poplar seed hairs possibly make people feel uncomfortable due to the presence of allergenic pollen trapped in the hairs.

9. Branches of palynology & application: Branches of palynology; palynology in taxonomic & phylogenetic deductions; palynology in academic & applied aspects including melissopalynology, medical palynology, forensic palynology, entomopalynology & copropalynology

Palynology is the science that studies contemporary and fossil palynomorphs, including pollen, spores, orbicules, dinoflagellate cysts, acritarchs, chitinozoans and scolecodonts, together with particulate organic matter (POM) and kerogen found in sedimentary rocks and sediments. Palynology does not include diatoms, foraminiferans or other organisms with silicaceous or calcareous exoskeletons. Palynomorphs are broadly defined as organic-walled microfossils between 5 and 500 micrometers in size.

The term *palynology* was introduced by Hyde and Williams in 1944, following correspondence with the Swedish geologist Antevs, in the pages of the Pollen Analysis Circular (one of the first journals devoted to pollen analysis, produced by Paul Sears in North America). Hyde and Williams chose *palynology* on the basis of the Greek words *paluno* meaning 'to sprinkle' and *pale* meaning 'dust' (and thus similar to the Latin word *pollen*).
Palynology is an interdisciplinary science and is a branch of earth science (geology) and biological science (biology), particularly plant science (botany). Palynology can broadly categorize into paleopalynology and neopalynology. Paleopalynology deals mainly with study of fossilized spores and pollen grains and other palynomorphs. Neopalynology is the study of concerned spores and pollen grains of extant plants. Palynological investigations are mainly confined to the exomorphic characters of spores and pollen grains as well as the structural details of exine and their implications in plant systematics and evolution. Sophisticated approaches like use of SEM and TEM provided palynologists with valuable

insight into the study of pollen grains with increased accuracy. Palynology is used for a diverse range of applications, related to many scientific disciplines:

APPLICATION OF PALAEOPALYNOLOGY

Origin and evolution of plant through ages:

Earth originated 4.5 billion year ago. At the time, the earth was full of volcanoes and the temperature was so high that life could not exist. This unfavourable condition continued for 1.5 billion years. Thereafter, some macromolecules were formed by reacting different compounds. This chemical evolutionary phase continued until the protenoids were produced. One of the oldest known rocks from where earliest life forms (e.g. *Eobacterium, Eosphaera, Palaeolyngbya, Kakabekia*) were recorded is Fig Tree formation in South Africa. The palaeontological records of spores began in the Cambrian rocks, but fossil spores were not abundant until Devonian time. The most primitive trilete sporoforms (e.g. *Retusotriletes*) were observed in mid Silurian–Early Devonian vascular plants. In the extinct groups like Rhyniales, Zosterophyllales, Trimerophytales, Lepidodendrales, Sphenophyllales, Calamitales and Primofilicales, the spores are always trilete. Pre-pollen in early gymnosperms like Pteridosperms, Cordaitales were nearly always trilete and found in the Devonian rock. Monolete (monosulcate) pollen grains were identified in Bennettitales during late Triassic period. Monosaccate pollen of *Felixipollenites* (Cordaitales) was recorded in Carboniferous sediments and disaccate pollens of *Straitopodocarpites, Vesicaspora* were found in Permian rock. The earliest record of angiosperm pollen was from lower Cretaceous. The most primitive angiosperm pollen grain (e.g. *Clavatipollenites*) was boat-shaped monosulcate type with clavate ornamentation. Other types of pollen were derived from this primitive sporoform. Observation on sudden appearance of new types of spores and pollen grains, gradual modification of the existing types and the extinction of such types in sedimentary matrix are found very useful to reach conclusion on the evolutionary aspects of present day's plants, when such information are correlated with geological times.

Reconstruction of past vegetation

The exine of the spore and pollen are highly resistant to decay due to presence of sporopollenin. Variation in exine structure and sculpture together with the number and distributional pattern of apertures provides the morphological diversity of the grains. These phenomena enable palynologists to reconstruct the past vegetation and to interpret the

depositional environment in terms of the present day known ecological status. Since pollen grains are indicators of species components in vegetation, dominance or absence of pollen grains can be correlated to deduce a general picture of dominant habitats prevailed during a particular period. Pollen analysis may also throw light on the afforestation or deforestation phenomena in relation to prehistoric life form reflected by agricultural activities.

Reconstruction of palaeoclimate and palaeoenvironment

Vegetation reacts sharply to changes in temperature and other related ecological factors. Vegetation is a definite indicator of climate. Palynomorphs are also sensitive to any minor fluctuation in their surroundings, and they are highly indicative of the environment in which they were deposited. Presence of bisaccate and monosaccate pollen grains in a region confirmed its upland nature. Geothermal alteration studies examine the colour of palynomorphs extracted from rocks to give the thermal alteration and maturation of sedimentary sequences, which provides estimates of maximum palaeotemperatures. Information gathered from fossil pollen grains have made possible to reconstruct the relationship of vegetation to climate changes and finally reconstruction of paleoenvironment for many localities, countries and phytogeographical zones.

Indication of geological age

Geologists use palynological studies to determine the relative age of rock. Palynomorphs are great indicators of narrow time ranges because of the rapid evolution of the samples. The earliest known angiosperm pollen, *Clavatipollenites* was found only in lower Cretaceous rock.

Biostratigraphy through correlation

The fundamental principle in stratigraphy is that the sedimentary rock in the earth's surface accumulated in layers, with the oldest at the bottom and youngest on the top. Biostratigraphy is the differentiation of rock units based on the fossils contained in them. Various fossil groups can be found in different sedimentary environments, namely, terrestrial and marine. Spore/pollen has big advantage for biostratigraphy over marine fossils, since they occur in all sedimentary environments. Biostratigraphy plays a critical role in the building of geological models for hydrocarbon exploration.

Determination of ancient shore lines

Most fossil spore/pollens in sediments have been transported sometimes very long distances, by streams and by ocean currents. It is possible to determine distance and direction of ancient shorelines by the kinds and quantities of microfossils, as pollen and spores will decrease in density in a seaward direction, with a corresponding increase in marine forms. According to Hoffmeister (1954), proximity of an ancient shoreline is indicated where the concentration of spores/pollens is about 7500 per gram of sediment.

Petroleum exploration

The rich presence of fossil pollen grains and spores has been detected in oil shales. Since they are >50 μm in diameter, they are not destroyed or damaged by drilling action. Pollen analysis has yielded good results in petroleum geology, especially in areas of brackish water deposits where correlation by means of even other microfossils and megafossil is practically difficult.

Demarcation of coal layer

Most bituminous coal contains abundant plant spores and majority of the spores are believed to be autochthonous in origin. The spore/ pollens in coals occur in a characteristic association, implying the occurrence of different plant communities associated with the deposition of the peat. Spores/pollens in finer sediments of coal deposit bear a variety of shapes, sizes, organization and ornamentation. These characteristics together with their quantitative data have helped in demarcating coal layers and seams in coalfield. The oldest coal deposits have been dated by palynology to 200-180 million years.

Archaeological palynology

Archaeologists examine human uses of plants in the past by using palynological data. This can help determine seasonality of site occupation, presence or absence of agricultural practices or products and plant-related activity areas within an archaeological context. Archaeological objects are mostly dated by pollen analysis. Intensive paleopalynological investigations have yielded much information about different aspects of human civilization like domestication of plants, nature of prehistoric agriculture, the cereals used by pre-neolithic settlement, the plant economy of prehistoric and historic time.

APPLICATION OF NEOPALYNOLOGY

Palynotaxonomy

The study of pollen morphology is of great significance to the disputed problems of higher plant taxonomy and phylogeny, since the pollen grains posses distinctly unique characters which are genetically lebelled. Palynologically plant families can be segregated into two groups:

Stenopalynous, where taxa of the family display more or less same type of pollen grains; e.g. *Poaceae, Casuarinaceae, Chenopodiaceae, Amaranthaceae* etc.

Eurypalynous, where taxa are characterised by an obvious difference in pollen types; e.g. *Rubiaceae, Acanthaceae, Verbenaceae, Solanaceae, Convolvulaceae, Fabaceae* etc.

Based on stenopalyny or eurypalyny the inter- or intra- family affinity and phylogeny of taxa may be determined. The knowledge of pollen morphology has been to substantive many taxonomic revisions sometimes even up to the formation of new taxa. Segregation of Bombacaceae from Malvaceae*;* Trapaceae from Onagraceae*;* amalgamation of Cannaceae, Musaceae and Zingiberaceae into Scitamineae*;* Moraceae and Cannabinaceae into Urticaceae, are a few examples of the contribution of the pollen morphology to taxonomy. With evidences from other areas like anatomy, embryology, cytology, chemotaxonomy, the conclusions on the basis of palynology would become more reliable and convincing.

Pollen biotechnology

This is the branch of biology which deals with the management or manipulation of pollen grains for the production and improvement of crops and related economic products. The first successful induction of haploids (pollen embryo) in cultured anthers of *Datura innoxia* was made by Guha and Maheswari (1964, 1966). Since then this technique has widely been used for induction of haploids of several crop plants and is found to be an effective measure of plant breeding programme.

Aeropalynology

Studies on atmospheric pollen (aerospora) are of great interest because of their application in paleobotany, medicine and forestry. ‘Pollen rain’ studies of today furnish a key to those of the past, especially the quaternary deposits. Many air-borne pollen and spores cause allergic

reaction in human being especially when they enter into respiratory tracts. As the pollen output and its dispersal in the atmosphere are closely related to seed production in trees, investigations on the pollen content of the atmosphere are providing a new approach in the practice of foresty. The bulk of the atmospheric pollen flora is supplied by anemophilous plants which are prolific producers of pollen which is discharged into the air in enormous quantities. Most of the wind borne pollen grains range from 20 to 50 µm in diameter and some may possess special devices such as air sacs as in *Pinus*.

Iatropalynology

This branch of applied palynology deals with the medical aspects specially the allergies (Greek *Iatro* = healing). The allergic response to pollen is called 'Pollinosis' and may involve any part of the body, the most frequently in nose, eye and chest with resultant symptoms of **hay fever, rhinitis or asthma**. Hay fever is characterized by intense sneezing, watery eyes, nasal obstruction, itchy eyes and nose, and often coughing. If the lower respiratory tract, lungs or bronchi are affected, the clinical reaction will be that of asthma. Other reacting organs may be the skin, the gastrointestinal tract, the central or peripheral nervous system or the cardiovascular system. Pollinosis may be caused by pollen grains produced by one or more plant (e.g. *Ambrosia* sp.). Pollen grains causing allergy are quite variable in different ceozones and also in a particular place from season to season, year to year depending on changes in ceological and climatic conditions. Pollen calender may be prepared to correlate the seasonal occurrence of the pollen types to the patients' allergic symptoms.

Pharmacopalynology

The medicinal properties of spore and pollens have been recognised since a long time. Pollen grains contain proteins, carbohydrates, vitamins, enzymes, trace elements etc. The pollen grains of *Cycas circinalis* are narcotic and those of *Typha laxmanii* are used as a stringent and styptic. Spores of *Lycopodium selago* are employed as a powerful cathartic and that of *L. clavatum* are supposed to be diuretic, demulcent and antispasmodic. The spores of *Adiantum philippense* are effective in the treatment of leprosy and other skin disease. Now-a-days pollen grains are sold in tablets or liquid form as a nutritional supplement. Pollens that form a critical component of the honey have antibiotic qualities, intestinal functions, combat respiratory problems, balance endocrine system etc.

Melissopalynology

This is the study pollen and spore found in honey. While collecting nectar from flowers in the field, pollen grains are occasionally swallowed by bees and carried into the hive. Analysis of pollen grains in honey provides information about the geographical origin and period of honey production as pollen grain in honey indicate the plants visited by the bees. Many of the identified honey plants are important ecological indicator types in the area of honey production. Based on the quantity of pollen grains in a given honey sample, the degree of its purity in commercial honey can be tested. Palynology plays an important role in bee-keeping industry. It is calculated that about 50-70 lbs of pollen grains are required by a fair sized colony of bees for a single year.

Entomopalynology

It is the study of pollen found on the body or in the gut of insects. It is useful for determining insect feeding and migratory habits especially as it involves economically important insects. Adults of numerous insect species feed on nectar, pollen and other plant exudates that are frequently associated with flowers. As a result of this feeding activity, these adults become contaminated with pollen. From the pollen found on several species of Lepidoptera, Mikkola (1971) determined that some of the insects examined migrated to Finland. Hendrix & Showers (1992) found that black cutworm [*Agrotis ipsilon* (Hufmagel)] and armyworm [*Pseudaletia unipuncta* (Haworth)] adults captured in Iowa and Missouri contained pollen from plants that only grew in South and Southwest Texas. The identification of this pollen indicated that these insects migrated over 1300 km. The most sophisticated pollination mechanisms have been evolved in the orchid family (Orchidaceae). Some bee orchids are pollinated by male wasps because the orchid flowers evolved to mimic female wasps.

Forensic palynology

This is the science of applying modern and fossil pollen and spores to help solve legal problems. In its broader application, the field of forensic palynology also includes legal information derived from the analysis of a broad range of organic walled microscopic organisms such as pollen grains, dinaflagellates, acritarchs and chitinozoans that can be found in both fresh and marine environments. Pollen grains and spores from plants with restricted distribution may, if found in dirt on shoes, clothes etc. indicate more or less clearly where the contamination occurred. The analysis of soil and mud samples containing the pollen grains of

the vegetation of that area can provide sufficient information for determination of their source and led to their use in forensic science.

Erdtman (1969) first reported two criminal cases, one in Sweden and other in Austria. In the Swedish case the pollen from the body of a murdered woman did not correspond with the area in Sweden, where the body was found. Thus the murder was committed elsewhere, far from where the corpse was found. In the Austrian case, a man disappeared during a trip near Vienna on a journey down to Danube. A pair of muddy shoes belonging to his suspected killer was examined and the soil revealed much pine and alder pollen along with some early tertiary (oligocene) pollen grains. Fortunately, only one area along the Danube velley, 20 km north of Vienna, contained this precise mixture of pollen. When the suspect was confronted with this fact, he confessed and showed authorities where he buried the body as pinpointed by pollen.

Copropalynology

Analysis of pollen grains contained in faecal materials provides interesting insights into the feeding habits of many herbivorous animals. Pollen analysis of coprolites (fossilized faeces) ranging in age from the middle Paleolithic period to late prehistoric times is providing remarkably specific evidences on diets and seasonal activities of early hunters and farmers and their domesticated animals. The sheep excrements from a Swiss lake dwelling that were found to contain pollen grains of a large number of species indicated the composition of the meadows grazed by sheep several thousand years ago. Guano deposits in caves inhabited by bats may – particularly in tropical and subtropical countries – contain a record of entomophilous plants visited, century after century by moths that were subsequently devoured by bats. Reptile coprolites (fossilized feces) recovered from Jurassic sediments indicate that these animals browsed on the pollen-bearing plant structures (Harris 1945, 1956). It has been suggested that reptiles, not beetles, were the original pollinators of angiosperms (Hughes 1976).

10. Let's sum up

- Palynology is the branch of science concerned with the study of pollen grains, spores, and similar palynomorphs, both living and fossil. H. A. Hyde & D. A. Williams (1944) coined the term Palynology but the study of pollen analysis was started in 1916 by E. J. Lennart Von Post.
- Pollen unit is the product of microsporogenesis through which a single pollen mother cell forms pollen units by meiotic division either singly or aggregation of two, four, and more.
- Gunner Erdtman proposed NPC-System pollen/spore classification based on the apertures, their Number, Position and Characters regarding microspore tetrad.
- Harmomegathy is one kind of adaptive mechanism associated with the phases of desiccation and rehydration and the contrivances needed for this mechanism help to avoid plasmolysis of the cytoplasm.
- The pollen wall, the sporoderm is generally stratified i.e. layered. The walls of the mature pollen, at least in angiosperms, consist of two fundamentally different layers, intine and an outer acetolysis resistant layer exine composed of sporopollenin.
- LO-analysis is a method for analysing patterns of sexine organisation by means of light microscopy. Development of a durable spore wall is essential for terrestrialization as it enables the spore to withstand physical abrasion, desiccation and UV-B radiation.
- Pollen wall development may be considered under exine and intine growth phases. Sporopollenin forms the basic structure of the resistant wall of most palynomorphs, like spores, pollens, dianoflagellates and acritarchs. It is highly inert compound having empirical formula $C_{90}H_{144}O_{27.}$
- In modern day, 5 different types of pollen connecting threads are categorized on the basis of nature and ultrastructure. Perine is a hyaline loosely organised sporopollenin envelope covering exine.

- Different types of natural traps used in studies of modern spore/pollen rain are lake sediments, soil surface, moss and lichen cushions, bark and leaves, spider webs, seed hairs of some plants e.g. *Populus* etc.

- Palynology is an interdisciplinary science and is a branch of earth science (geology) and biological science (biology), particularly plant science (botany). Palynology can broadly categorize into paleopalynology and neopalynology.

11. Suggested Readings

1. Bhattacharya, K., Majumdar, M.R. & Gupta Bhattacharya, S. (2006). A Text Book of Palynology, New Central Book Agency.
2. Bryant, V.M. Jr. and S.A. Hall. 1993. Archaeological palynology in the United States: a critique. American Antiquity, volume 58, pages 416-421.
3. Jarzen, D.M. and McAllister, D.E. 1994. Eco-services: l. Pollination - love in the plant world benefits the whole world. Wildflower, North America's Magazine of Wild Flora, volume 10, number 3, pages 24-27.
4. Jarzen, D.M. & Nichols, D.J. 1996. Chapter 9 Pollen. In: Jansonius, J. & McGregor, D.C. (eds.) Palynology: principles and applications. American Association of Stratigraphic Palynologists Foundation, volume 1, pages 261-291.
5. Erdtman, G. (1986). Pollen Morphology and Plant Taxonomy, Latest Ed., Lelden, E.G. Brill.
6. Faegri, K. and Iversen, J. (1964). Text Book of Pollen Analysis, Munksgor, Copenhagen.
7. Heslop- Harisson (1971). Pollen: Development and Physiology, Butterworth
8. Nair, P.K. K. (1970). Pollen Morphology of Angiosperms, Latest Ed., Scholar Publications.

12. Assignments

1. Distinguish between microspore and pollen.

2. Pollen has equatorial distance 125 µm and polar axis 150 µm. Categorize the pollen based on its size and shape.
3. Name two extraexinous wall materials. Mention their functions.
4. Mention the chemical nature and functions of sporopollenin.
5. Describe different types of pollen connecting threads with examples.
6. Distinguish between ‘Lalongate’ and ‘Lolongate’ types of ora.
7. Draw and describe the pollen grain having NPC number 345.
8. Distinguish between exospore and perispore.
9. State the physical and chemical properties of sporopollenin.
10. Mention one adaptive character of pollen grains for anemophily.
11. What is pollen kitt? Mention its functions.
12. Describe different types of pollen connecting threads with examples.
13. What is meant by viability of pollen? What are the factors affecting pollen viability.
14. Enumerate the role of primexine in pollen wall development.
15. What are natural spore/pollen traps? Mention their implications?
16. Mention the chemical nature and functions of sporopollenin.
17. Enumerate different types of pollen units with examples.
18. Illustrate different types of sporoderm sculpturing with examples.
19. Discuss the morphological changes of pollen wall during its development.
20. Describe different types of harmomegathic mechanisms in pollen grains with examples.
21. Enumerate the application of palynology in human welfare.
22. Explain the significance of the following studies: Melissopalynology, Medical palynology, Forensic palynology, Entomopalynology and Copropalynology.

POST GRADUATE DEGREE PROGRAMME (CBCS) IN

BOTANY

SEMESTER-II

Course:BOTCOR T206

(Plant Physiology and Biochemistry)

Self-LearningMaterial

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Director's Message

Satisfying the varied needs of distance learners, overcoming the obstacle of distance and reaching the unreached students are the threefold functions catered by Open and Distance Learning (ODL) systems. The onus lies on writers, editors, production professionals and other personnel involved in the process to overcome the challenges inherent to curriculum design and production of relevant Self Learning Materials (SLMs). At the University of Kalyani a dedicated team under the able guidance of the Hon'ble Vice-Chancellor has invested its best efforts, professionally and in keeping with the demands of Post Graduate CBCS Programmes in Distance Mode to devise a self-sufficient curriculum for each course offered by the Directorate of Open and Distance Learning (DODL), University of Kalyani.

Development of printed SLMs for students admitted to the DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode DEB Regulations, 2020 had been our endeavour. We are happy to have achieved our goal.

Utmost care and precision have been ensured in the development of the SLMs, making them useful to the learners, besides avoiding errors as far as practicable. Further suggestions from the stakeholders in this would be welcome.

During the production-process of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Manas Kumar Sanyal, Hon'ble Vice- Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticism to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

Sincere gratitude is due to the respective chairpersons as well as each and every member of PGBOS (DODL), University of Kalyani. Heartfelt thanks are also due to the Course Writers-faculty members at the DODL, subject-experts serving at University Post Graduate departments and also to the authors and academicians whose academic contributions have enriched the SLMs. We humbly acknowledge their valuable academic contributions. I would especially like to convey gratitude to all other University dignitaries and personnel involved either at the conceptual or operational level of the DODL of University of Kalyani.

Their persistent and co-ordinated efforts have resulted in the compilation of comprehensive, learner-friendly, flexible texts that meet the curriculum requirements of the Post Graduate Programme through Distance Mode.

All the Self Learning Materials are-self writing and collected from e-book, journals and websites.

Director
Directorate of Open and Distance Learning
University of Kalyani

SYLLABUS
COURSE–BOTCOR T206
(Plant Physiology and Biochemistry) (FullMarks–75)

Group A (Plant Growth & Biochemical Processes)

Course	Group	Details Contents Structure		Study hour
BOTCOR T206	Group A (Plant Growth & Biochemical Processes)	**Water and Plant Cells And Water Balance of Plants:**	**Unit 1.**Water in Plant life, chemical properties of water, water potential of Plant Cells, major factors contributing cell water potential, cell wall and membrane properties and Water in the Soil, Water absorption by roots, transport through Xylem, movement from leaf to atmosphere, Soil-Plant-atmosphere continuum.	1
		Cell wall and Early Growth in Plants:	**Unit 2.** Structure, biogenesis and growth. Embryogenesis and differentiation of plant organs, Seed germination and seedling growth.	1
		Photosynthesis	**Unit 3.** Light reactions, organization of light absorbing system, mechanism of electron and proton transport. Carbon concentrating mechanisms.	1
		Transport Processes:	**Unit 4.** Solute transport across membranes; Phloem translocation of photoassimilates.	1
		Stress physiology	**Unit 5.** Response and adaptation to abiotic stress: water stress, temperature stress (heat and cold stress) and Gene regulation and proteomics of stress tolerance; Development of transgenic plants to stress tolerance.	1
		Principle of biochemistry	**Unit 6.** pH, buffer, reaction kinetics, thermodynamics, law of mass action, acid base reactions, bond energy, energy rich compounds, redox potential, free energy.	1
		Enzyme	**Unit 7.** Enzyme kinetics, catalytic reactions and regulatory properties, inhibitions, isoenzymes, allosterism, ribozyme and abzymes, vitamins as coenzymes.	1

Content

COURSE–BOTCOR T206

Group A: Plant Growth & Biochemical Processes

Hard Core Theory Paper Credit: =3

Content Structure

1. Introduction
2. Course Objective
3. Water and Plant Cells: Water in Plant life, chemical properties of water, water potential of Plant Cells, major factors contributing cell water potential, cell wall and membrane properties.
4. Water Balance of Plants: Water in the Soil, Water absorption by roots, transport through Xylem, movement from leaf to atmosphere, Soil-Plant-atmosphere continuum.
5. Cell Walls: structure, biogenesis and growth.
6. Early Growth in Plants: Embryogenesis and differentiation of plant organs, Seed germination and seedling growth.
7. Photosynthesis: Light reactions, organization of light absorbing system, mechanism of electron and proton transport, Carbon concentrating mechanisms.
8. Transport Processes: Solute transport across membranes; Phloem translocation of photoassimilates.
9. Stress Physiology: Response and adaptation to abiotic stress: water stress, temperature stress (heat and cold stress); Gene regulation and proteomics of stress tolerance; Development of transgenic plants to stress tolerance.
10. Principle of Biochemistry: pH, buffer, reaction kinetics, thermodynamics, law of mass action, acid base reactions, bond energy, energy rich compounds, redox potential, free energy.
11. Enzyme: Enzyme kinetics, catalytic reactions and regulatory properties, inhibitions, isoenzymes, allosterism, ribozyme and abzymes, vitamins as coenzymes.
12. Letus sumup
13. SuggestedReading
14. Assignment

1. Introduction

Plant physiology is a subdiscipline of botany concerned with the functioning, or physiology, of plants.Closely related fields include plant morphology (structure of plants), plant ecology (interactions withthe environment), phytochemistry (biochemistry of plants), cell biology, genetics, biophysics andmolecular biology. Fundamental processes such as photosynthesis, respiration, plant nutrition, planthormonefunctions,tropisms,nasticmovements,photoperiodism,photomorphogenesis,circadianrhythms, environmental stress physiology, seed germination, dormancy and stomata function andtranspiration, both parts of plant water relations, are studied by plant physiologists. The field of plantphysiology includes the study of all the internal activities of plants-those chemical and physicalprocesses associated with life as they occur in plants. This includes study at many levels of scale ofsize and time. At the smallest scale are molecular interactions of photosynthesis and internal diffusion of water, minerals, and nutrients. At the largest scale are the processes of plant development, seasonality, dormancy, and reproductive control. Major subdisciplines of plant physiology include phytochemistry (the study of the biochemistry of plants) and phytopathology (the study of disease inplants). The scope of plant physiology as a discipline may be divided into several major areas of research. Finally, plant physiology includes the study of plant response to environmental conditions and their variation, a field known as environmental physiology. Stress from water loss, changes in air chemistry, or crowding by other plants can lead to changes in the way a plant functions. These changes may be affected by genetic, chemical, and physical factors.

2. CourseObjectives

- The objective of this module is not only to develop a better aptitude towards the knowledge ofphysio-biochemicalmetabolismofplantsystem,butalsotounderlinetheneedforbettermanagementwith knowledge and skills to handle the task.
- Oncompletionofthisstudymaterialwillbebetterknowledge–
  - Waterpotentialactivityanditssignificanceinplantsystem
  - Differentmetabolicanalysisofplants
  - Adaptationofplantsandregulationofhormonalsystem
  - Energyconservation,signalingsystem

3. Water and Plant Cells: Water in Plant life, chemical properties of water, water potential ofPlant Cells, major factors contributing cell water potential, cell wall and membrane

1. properties.

Water plays a crucial role in the life of the plant. For every gram of organic matter made by the plant,approximately 500 g of water is absorbed by the roots, transported through the plant body and losttothe atmosphere. Even slight imbalances in this flow of water can cause water deficits and severemalfunctioningof manycellular processes.

Thus, every plant must delicately balance its uptake and loss of water.This balancing is a seriouschallenge for land plants. To carry on photosynthesis, they need to draw carbon dioxide from theatmosphere, but doing so exposes them to water loss and the threat of dehydration. A major differencebetween plant and animal cells that affects virtually all aspects of their relation with water is theexistence in plants of the cell wall. Cell walls allow plant cells to build up large internal hydrostaticpressures, called **turgor pressure**, which is a result of their normal water balance. Turgor pressure isessentialformanyphysiologicalprocesses,includingcellenlargement,gasex**c**hangeintheleaves, transportinthephloem,andvarioustransportprocessesacrossmembranes.Turgorpressurealso contributestotherigidityandmechanicalstabilityofnon-lignifiedplanttissues.Inthischapterwewill consider how water moves into and out of plant cells, emphasizing the molecular properties ofwater and the physical forces that influence water movement at the cell level. But, first we willdescribethe major functions of water in plant life

Water in plant life:

Water Makes up most of the Mass of plant cells, as we can readily appreciate if we Look at Microscopic sections of mature plant cells:Each cell contains a large water-filled vacuole. In such cells the cytoplasm makes up only 5 to 10% of the cell volume; the remainder is vacuole. Water typically constitutes 80 to 95% of the mass of growing plant tissues. Common vegetables such as carrots and lettuce may contain 85 to 95% water. Wood, which is composed mostly of dead cells, has lower water content; sapwood, which functions in transport into xylem, contains 35 to 75% water; and heartwood has slightly lower water content. Seeds, with a water content of 5 to 15%, are among the driest of plant tissues, yet before germinating they must absorb a considerable amount of water. Plants continuously absorb and lose water. Most of the water lost by the plant evaporates from the leaf as theCO_2neededforphotosynthesisisabsorbedfromtheatmosphere.Onawarm,dry,sunnydayaleafwill exchange up to 100% of its water in a single hour. During the plant's lifetime, water equivalent to100 times the fresh weight of the plant may be lost through the leaf surfaces. Such water loss is called **transpiration**.

Transpirationis an important means of dissipating the heat input from sunlight. Heat dissipates because the water molecules that escape into the atmosphere have highert han-average energy, which breaks the bonds holding them in the liquid. When these molecules escape, they leave behind a massofmoleculeswithlower-than-averageenergyandthusacoolerbodyofwater.Foratypicalleaf, nearlyhalfofthenetheatinputfromsunlightisdissipatedbytranspiration.Inaddition,the treamof water takenupbythe roots isanimportantmeansof bringing dissolved soilminerals totherootsurfacefor absorption.

ThePolarityofWaterMoleculesGivesRisetoHydrogenBonds:

Thewatermoleculeconsistsofanoxygenatomcovalentlybondedtotwohydrogenatoms.Thetwo O—H bondsform anangleof105°.Becausetheoxygenatom ismore**electronegative**than hydrogen, it tends to attract the electrons of the covalent bond. This attraction results in a partialnegative charge at the oxygen end of the molecule and a partial positive charge at each hydrogen.Thesepartialchargesareequal,sothewatermoleculecarriesno*net*charge.Thisseparationofpartial charges, togetherwiththeshapeofthewatermolecule,makes watera*polarmolecule*, andthe opposite partial charges between neighboring water molecules tend to attract each other. The weakelectro static attraction betweenwatermolecules,known as a **hydrogen bond**, is responsible formany of the unusual physical properties of water. Hydrogen bonds can also form between water

andother molecules that contain electronegative atoms (O or N). In aqueous solutions, hydrogen bondingbetween water molecules leads to local, ordered clusters of water that, because of the continuousthermalagitationofthewater molecules, continuallyform,breakup,and re-form.

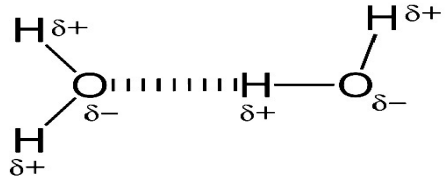


Figure 1. Diagram of the water molecule. The two intramolecular hydrogen–oxygenbondsforman angleof 105°.

ThePolarityofWaterMakesItanExcellentSolvent

Water is an excellent solvent: It dissolves greater amounts of a wider variety of substances than doother related solvents.This versatility as a solvent is due in part to the small size of the water moleculeand in part to its polar nature.The latter makes water a particularly good solvent for ionic substancesandformoleculessuchassugarsandproteinsthatcontainpolar—OHor—NH_2groups.Hydrogen bonding betweenwatermoleculesandions,andbetweenwater andpolar solutes,in solution effectivelydecreasestheelectrostaticinteractionbetweenthechargedsubstancesandthereby increasestheirsolubility.Furthermore,thepolarendsofwatermoleculescanorientthemselvesnextto charged or partially charged groups in macromolecules, forming **shells of hydration**. Hydrogenbondingbetween macromoleculesand waterreducestheinteractionbetweenthose.

TheCohesiveandAdhesivePropertiesofWaterAreDuetoHydrogenBonding

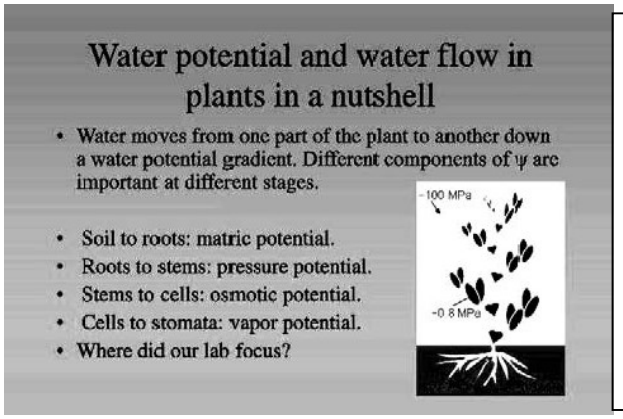
Water molecules at an air–water interface are more strongly attracted to neighboring water moleculesthan to the gas phase in contact with the water surface. As a consequence of this unequal attraction, anair–water interface minimizes itssurface area. To increase the area of an air–water interface, hydrogenbonds must be broken, which requires an input of energy. The energy required t**o** increase the surfacearea isknown as **surface tension**. Surface tension not only influences the shape of the surface but alsomay create a pressure in the rest of the liquid. As we will see later, surface tension at the evaporativesurfaces of leaves generates the physical forces that pull water through the plant's vascular system.The extensive hydrogen bonding in water also gives rise to the property known as **cohesion**, themutual attractionbetween molecules. Arelated property, called **adhesion**, is the attraction of water to asolid phase such as a cell wall or glass surface. Cohesion, adhesion, and surface tension give rise to aphenomenonknownas**capillarity**,themovementof wateralonga capillarytube.

Watertransportprocesses

When water moves from the soil through the plant to the atmosphere, it travels through a widelyvariable medium (cell wall, cytoplasm, membrane, air spaces), and the mechanisms of water transportalso vary with the type of medium. For many years there has been much uncertainty how water movesacrossplantmembranes.Specificallyitwasunclearwhetherwatermovementintoplantcellswas limited tothediffusionofwatermoleculesacrosstheplasmamembrane's lipidbilayer oralso

involveddiffusionthroughprotein-linedpores.Somestudiesindicatedthatdiffusiondirectlyacross thelipidbilayerwasnotsufficienttoaccountforobservedratesofwatermovementacross membranes,buttheevidenceinsupportofmicroscopicporeswasnotcompelling.

This uncertainty was put to rest with the recent discovery of **aquaporins**. Aquaporins are integralmembrane proteins that form water-selective channels across the membrane. Because water



Tble 1.:Comparison of units ofpressure
1tmosphere=14.7pounds persquareinch
=760mmHg(atsealevel,45°latitude)
=1.013bar
=0.1013Mpa
=1.013·10⁵Pa
Acartireistypiallyinflatedtoabout 0.MPa.
Thewaterpressureinhomelumbingistyically 0.2–0.3MPa.
The water pressure under 15 feet (5 m) ofwteris about 0.05MPa.

diffusesfaster through such channels than through a lipid bilayer, aquaporins facilitate water movement into plantcells.Notethatalthoughthepresenceofaquaporinsmayalterthe*rate*ofwatermovementacross the membrane, they do not change the direction of transport or the driving force for watermovement. Now consider the two major processes in water transport: molecular diffusion and bulkflow.

Osmosisisdrivenbyawaterpotentialgradient

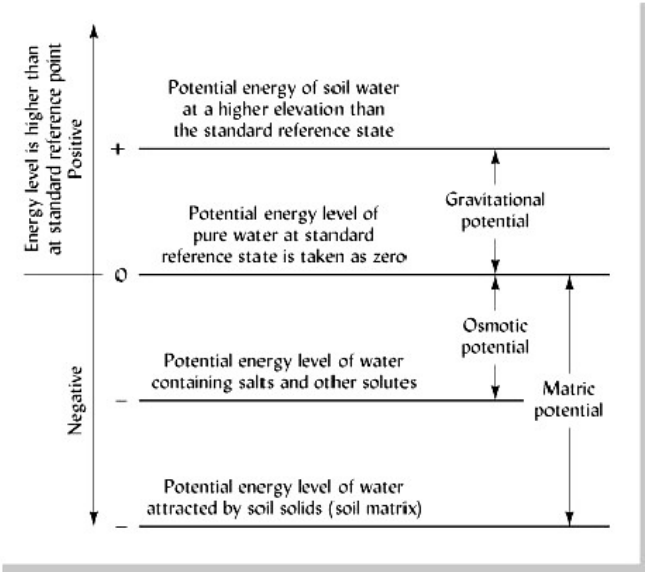
Membranesofplantcellsare**selectivelypermeable**;thatis,theyallowthemovementofwaterandothersmallu nchargedsubstancesacrossthemmorereadilythanthemovementoflargersolutesand chargedsubstances.Likemoleculardiffusionandpressure-drivenbulkflow,**osmosis**occurs spontaneouslyinresponseto adriving force.Insimplediffusion,substancesmove downa concentration gradient; in pressure-driven bulk flow, substances move down a pressure gradient; inosmosis, bothtypes of gradients influence transport (Finkelstein 1987). The direction and rate of waterflow across a membrane are determined not solely by the concentration gradient of water or by thepressuregradient, butbythe sumofthese two drivingforces.

TheChemicalPotentialofWaterRepresentstheFree-EnergyStatusofWater:

Alllivingthings,includingplants,requireacontinuousinputoffreeenergytomaintainandrepairtheirhighlyor ganizedstructures,aswellastogrowandreproduce.Processessuchasbiochemical reactions,soluteaccumulation,andlong-distancetransortarealldrivenbyaninputoffreeenergy intothe plant.The **Chem Potential**of waterisa quantitativeexpression ofthefreeenergy associated with water. In thermodynamics, free energy represents the potential for performing work. Itis expressed as the difference between the potential of a substance in a given state and the potential ofthe same substance in a standard state. The unit of chemical potential is energy per mole of substance(J mol–1). Thus **water potential**, defined as the chemical potential of water divided by the partialmolalvolumeof water.

Three Major Factors Contribute to Cell Water Potential

The major factors influencing the water potential in plants are *concentration*, *pressure*, and *gravity*.Water potential is symbolized by *Y*w (the Greek letter psi), and the waterpotential of solutions may bedissectedintoindividualcomponents,



usually written as the following sum: Theterms *Y*s, *Y*p, and *Y*g denote the effects ofsolutes, pressure, and gravity, respectively,on the free energy of water. The referencestate used to define water potential is purewater at ambient pressure and temperature.The term *Y*s, called the **solute potential** orthe **osmotic potential**, represents the effectofdissolvedsolutesonwaterpotential.Solutesreducethefreeenergyofwaterby diluting thewater. Thisisprimarilyan entropyeffect;thatis,themixingofsolutesand waterincreasesthedisorderofthe systemandtherebylowersfreeenergy.

This means that the osmotic potential is independent of the specific nature of the solute. For dilutesolutions of non-disso-ciating substances, like sucrose, the osmotic potential maybe estimated by the**van'tHoffequation**:where*R*isthegasconstant(8.32Jmol–1K–1),*T*istheabsolutetemperature(in degrees Kelvin, or K), and *c*s is thesolute concentration of the solution, expressed as **osmolality**(molesoftotaldissolvedsolutesperliterofwater[molL–1]).Theminussignindicatesthatdissolved solutes reduce the water potential of a solution relative to the reference state of pure water.Table 2shows the values of *RT* at various temperaturesand the *Y*s values of solutions of different soluteconcentrations. For ionic solutes that dissociate into two or more particles, *c*s must be multiplied bythenumberofdissociated particlestoaccountfortheincreased numberofdissolved particles.

The term *Y*p is the **hydrostatic pressure** of the solution. Positive pressures raise the water potential;negativepressuresreduceit.Sometimes*Y*piscalled*pressurepotential*.The positivehydrostaticpressure within cells is the pressure referred to as *turgor pressure*. The value of *Y*p can also benegative, as is the case in the xylem and in the walls between cells, where a *tension*, or *negativehydrostatic pressure*, can develop. As we will see, negative pressures outside cells are very importantin moving water long distances through the plant. Hydrostatic pressure is measured as the deviationfrom ambient pressure. Remember that water in the reference state is at ambient pressure, so by thisdefinition *Y*p = 0 MPa for water in the standard state. Thus the value of *Y*p for pure water in an openbeakeris 0 MPa, even though its absolute.

Water can also leave the cell in response to a water potential gradient

Water can also leave the cell by osmosis. If, in the previous example, we remove our plant cell fromthe 0.1 *M* sucrose solution and place it in a 0.3 *M* sucrose solution, *Y*w (solution) (–0.732 MPa) ismore negative than *Y*w(cell) (–0.244 MPa), and water will move from the turgid cell to the solution.As water leaves the cell, the cell volume decreases. As the cell volume decreases, cell *Y*p and *Y*wdecreasealsountil*Y*w(cell)=*Y*w(solution)=–0.732MPa.Fromthewaterpotentialequation,wecan calculate that at equilibrium, *Y*p = 0 MPa. As before, we assume that the change in cell volume issmall, so we can ignore the change in *Y*s. If we then slowly squeeze the turgid cell by pressing itbetweentwoplates,weeffectivelyraisethecell*Y*p,consequentlyraisingthecell*Y*wandcreatinga <*Y*w such that water now flows *out* of the cell. If we continue squeezing until half the cell water isremoved and then hold the cell in this condition, the cell will reach a new equilibrium. However, thecomponents of the cell *Y*w will be quite different. Because half of the water was squeezed out of thecell while the solutes remained inside the cell (the plasma membrane is selectivelypermeable), thecell solution is concentrated twofold, and thus *Y*s is lower (–0.732= –1.464 MPa). Knowing the finalvalues for *Y*w and *Y*s, we can calculate the turgor pressure, using Equation 3.6, as *Y*p = *Y*w – *Y*s = (–0.244) – (–1.464) = 1.22 MPa. In our example we used an external force to change cell volumewithoutachangeinwaterpotential.Innature,itistypicallythewaterpotentialofthecell'senvironmentt hatchanges,andthecellgainsorloseswateruntilits*Y*wmatchesthatofitssurroundings. One point common to all these examples deserves emphasis: *Water flow is a passiveprocess. That is, water moves in response to physical forces, toward regions of low water potential orlowfree energy.*

Thewaterpotentialconcepthelps us evaluatethewater status ofaplant

The concept of water potential has two principal uses: First, water potential governs transport acrosscell membranes, as we have described. Second, water potential is often used as a measure of the *waterstatus* of a plant. Because of transpirational water loss to the atmosphere, plants are seldom fullyhydrated. They suffer from water deficits that lead to inhibition of plant growth and photosynthesis, aswell as to other detrimental effects. Figure 3 lists some of the physiological changes that plantsexperience as they become dry. The process that is most affected by water deficit is cell growth. Moreseverewaterstressleadstoinhibitionofcelldivision,inhibitionofwall andproteinsynthesis,accumulation of solutes, closing of stomata, and inhibition of photosynthesis. Water potential is onemeasure of how hydrated a plant is and thus provides a relative index of the *water stress* the plant. Inleaves of well-watered plants, *Y*w ranges from –0.2 to about –1.0 MPa, but thel eaves of plants in aridclimates can have much lower values, perhaps –2 to –5 MPa under extreme conditions. Because watertransportisa passiveprocess, plantscan takeup wateronlywhenthe plant*Y*w isless thanthe soil *Y*w. As the soil becomes drier, the plant similarly becomes less hydrated (attains a lower *Y*w). If thiswerenot the case, the soilwould beginto extract water fromthe plant.

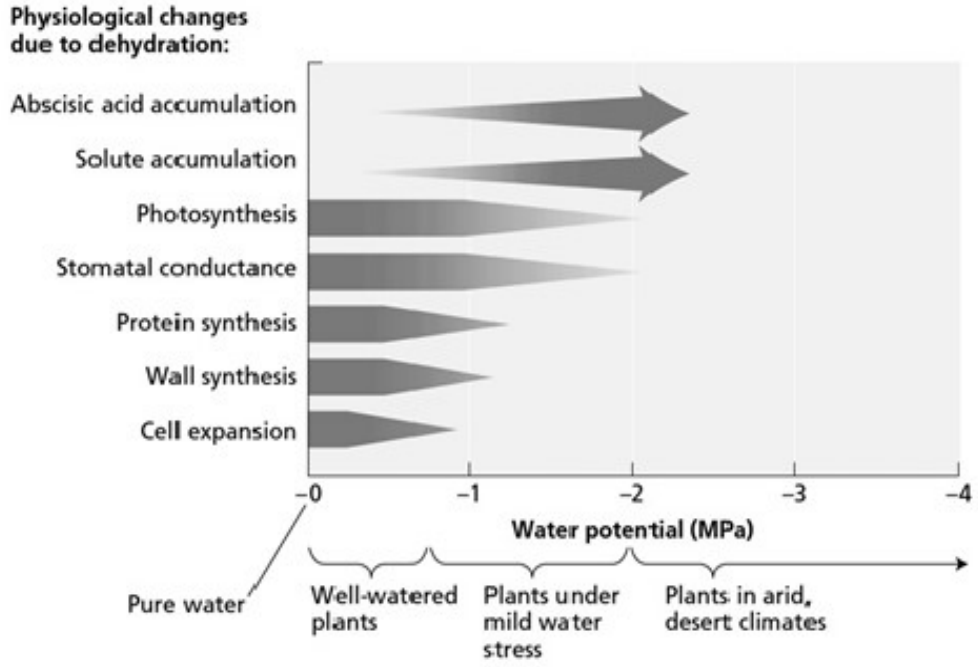


Figure3:Waterpotentialofplantsundervariousgrowingconditions,andsensitivityo fvariousphysiologicalprocessesto water potential.(AfterHsiao 1979.)

4.Water balance of Plants:Water in the Soil, Water absorption by roots, transport through Xylem, movement from leaf to atmosphere, Soil-Plant-atmosphere continuum.

Role of Water in Plant Physiology :

Of all the vital substances essential for plant growth and development , water is needed in enormous amounts . It is present throughout the plant body . Water constitutes more than 70 % of the fresh weight of a plant and in some of the growing cells its level may be up to 90 % . Water supply affects the growth rate of plants considerably.

(i)It is a major component of the plant body.

(ii)What is an essential solvent in which mineral nutrients are dissolved and translocated from the roots to the apex of the plant body . Minerals are also absorbed through water.

(iii)Large number of metabolic reactions take place in the water medium.

(iv)It maintains the structure of nucleic acids , proteins by supplying hydrogen bonding.

(v)Several processes like photosynthesis use water as a reactant of raw material . Thus formation of complex carbohydrates from the simple ones also involves the removal of water while the reverse reaction requires water as a reactant

(vi)This essential component is required to maintain the turgidity of the cell . Thus , it helps the cells to retain their tensile strength and provides proper shape to the cells . (vii)Water also acts as a temperature buffer since it has an exceptionally high heat capacity for specific heat.

(viii)Water molecules have the unique property of adhesion and cohesion and thus these processes keep the water molecules together . This property helps in upward movement of water in the plant body.

(ix)The elongation phase of cell growth is mostly dependent on water absorption

(x)Water is also a metabolic end product of respiration (xi)Plans absorb enormous quantities of water and simultaneously lose greater amounts of water through transpiration .

Water in soil

Though the soil particles often lie close to each other yet some angular space is always present there . All such spaces in the soil are collectively called pore space . The pore space comprises a fairly constant volume in the soil (40-60 % of total soil volume) which remains filled with water and gases in.

varying proportions. In a “dry” soil, water occupies a very small proportion of this space. In a “wet” soil, it is water that occupies most of this space. The soil, in fact, acts as a water reservoir for plants.

The water stored in the soil may be classified into the following four groups :

Different forms of water:

Water is mainly obtained through rain. Some of the water goes into the reservoirs. This is called run off or Run away water. Rest of the water enter into the land.

Water present in soil is following type:

(a) Gravitational water: Form of water, which reaches at the soil water table due to the gravitational force after the rainfall. This form is not available to plants but available by mechanical methods or by tube well irrigation.

(b) Hygroscopic water: Thin film of water is tightly held by the soil particles is called hygroscopic water. This water is also not available to the plants.

(c) Chemically combined water: The amount of water present in the chemical compounds, which are present in the particles of soil. This is not available to the plants.

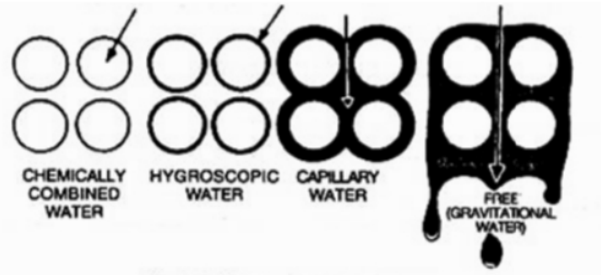


Fig. 2.1 Different types of soil water.

(d) Capillary water: Water exists between soil particles in small capillary pores is called Capillary water. It is the most common available form of water for absorption.

(e) Atmospheric humidity: This is water vapor present in air, which can be absorbed by hanging roots of the epiphytes due to presence of spongy velamen tissue and hygroscopic hairs.

Field Capacity or Water Holding Capacity of the Soil:

After heavy rainfall or irrigation of the soil, some water is drained off along the slopes while the rest percolates down in the soil. This amount of water retained by the soil after the drainage of gravita¬tional water has become very slow is called as field capacity or the water holding capacity of the soil. The field capacity is affected by soil profile, soil structure and temperature. For instance a fine textured soil overlying a coarse textured soil will have a higher field capacity than a uniformly fine textured soil. Similarly, the field capacity increases with decreasing temperature and vice versa.

Permanent Wilting Percentage or Wilting Coefficient:

The percentage of the soil water left after the plant growing in that soil has permanently wilted is called as permanent wilting percentage or the wilting coefficient. The permanent wilting percentage can be determined by growing the seedlings in small containers under conditions of adequate water supply till they develop several leaves. The soil surface is then covered and the water supply is cut until wilting occurs. The containers are now transferred to humid chamber.If the plants do not recover, they are considered to be permanently wilted. Otherwise, they are again transferred to normal atmospheric conditions. This process is repeated till they are permanently wilted. The percentage of the soil water is determined at this point after removing the plants from the containers and shaking off as much soil from their roots as possible. Thus the different plants if grown in the same soil wilt at different times depending upon their osmotic potential after the water supply to the soil is stopped.

Soil Texture in Relation to Water Absorption:

The texture of a soil depends upon the proportion of different sized soil particles in that soil and is a very important factor for the absorption of water in plants.

Depending upon their diameters the soil particles are classified as below:

Sandy Soils: Such soils are very rich in sand particles and though well aerated them have poor water holding capacity. Sandy soils are, therefore, not good for water absorption.

Clayey Soils: These are rich in clay particles and are poorly aerated. Such soils often become water-logged and are, therefore, neither good for water absorption nor for normal growth of the plants.

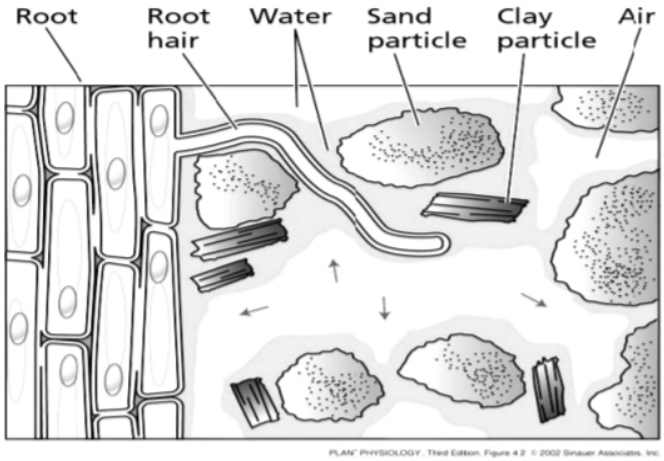
Loam: Such soils contain almost equal proportion of the different sized soil particles. They are sufficiently aerated and have good water holding capacity. Therefore, they are very good for water absorption and growth. The loam soil in which the proportion of sand is slightly higher is called as sandy loam while a loam soil in which clay particles predominate, is called as clayey loam.

Water absorption by roots:
Water is an important constituent of soil because all land plants depend upon it for their requirement of water. Deep in the soil and above the impermeable stratum, water occurs freely in the previous rocky matter. It is called ground water. Ground water bearing pervious straturn is known as aquifer.

The upper layer of ground water is called water table. In the region of water table soil is completely saturated with water. Air is excluded. The plants which do so, are called phreatophytes, e.g., *Populus deltoides*, *Alhagi pseudalhagi*, *Tamarix*, *Prosopis cineraria* etc. Phreatophytes have been used successfully to locate under-ground water since the period of Varahmihira. Soil water, important to most plants, is the one present in 1-2m of soil because their roots are generally restricted to this region.

Water absorption from soil:

- Water clings to the surface of soil particles.
- As soil dries out, water moves first from the center of the largest spaces between particles.
- Water then moves to smaller spaces between soil particles.
- Root hairs make intimate contact with soil particles – amplify the surface area for water absorption by the plant.



Water Moves through soil by bulk flow
Bulk flow:
Concerted movement of groups of molecules in mass, most often in response to a **pressure gradient**.

- Dependant on the radius of the tube that water is traveling in.
- This is the main method for water movement in **Xylem, Cell Walls** and in the **soil**.
- **It is Independent** of solute concentration gradients – to a point In addition, **diffusion** of water vapor accounts for some water movement.
- As water moves into root – less in soil near the root
- Results in a **pressure gradient** with respect to neighboring regions of soil.
- So there is a reduction in yp near the root and a **higher** yp in the neighboring regions of soil.
- Water filled pore spaces in soil are interconnected, water moves to root surface by bulk flow down the pressure gradient
- As water moves from soil into root the spaces fill with air. This **reduces** the flow of water

Mechanism of Absorption of Water:

In higher plants water is absorbed through root hairs which are in contact with soil water and form a root hair zone a little behind the root tips (Fig. 4.1). Root hairs are tubular hair like prolongations of the cells of the epidermal layer (when epidermis bears root hairs it is also known as piliferous layer) of the roots. The walls of root hairs are permeable and consist of pectic substances and cellulose which are strongly hydrophilic (water loving) in nature. Root hairs contain vacuoles filled with cell sap.

Mechanism of water absorption is of two types:

(1) Active Absorption of Water:

In this process the root cells play active role in the absorption of water and metabolic energy released through respiration is consumed.

Active absorption may be of two kinds:

(a) Osmotic absorption i.e., when water is absorbed from the soil into the xylem of the roots according to the osmotic gradient.

(b) Non-osmotic absorption i.e., when water is absorbed against the osmotic gradient.

(2) Passive Absorption of Water:

It is mainly due to transpiration, the root cells do not play active role and remain passive.

(1a) Active Osmotic Absorption of Water:

First step in the osmotic absorption of water is the imbibition of soil water by the hydrophilic cell walls of root hairs. Osmotic Pressure (O.P.) of the cell-sap of root hairs is usually higher than the O.P. of the soil water. Therefore, the Diffusion Pressure Deficit (D.P.D.) and the suction pressure in the root hairs become higher and water from the cell walls enters into them through plasma-membrane (semi-permeable) by osmotic diffusion. Now, the cortical cells adjacent to root hairs have higher O.P., suction pressure and D.P.D. in comparison to the root hairs. Therefore, water is drawn into the adjacent cortical cells from the root-hairs by osmotic diffusion.

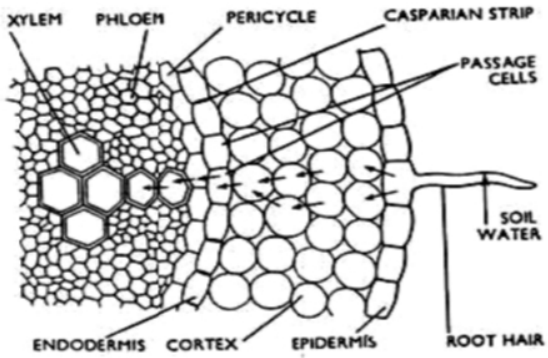


Fig. 4.2. A part of T.S. of typical dicot root. The arrows indicate the path of water.

Water from endodermal cells is drawn into the cells of pericycle by osmotic diffusion which now becomes turgid and their suction pressure is decreased. In the last step, water is drawn into xylem from turgid pericycle cells. (In roots the vascular bundles are radial and protoxylem elements are in contact with pericycle). It is because in absence of turgor pressure of the xylem vessels (which are

non-elastic), the suction pressure of xylem vessels become higher than the suction pressure of the cells of the pericycle. When water enters into xylem from pericycle, a pressure is developed in the xylem of roots which can raise the water to a certain height in the xylem. This pressure is called as root pressure.

(1b) Active Non-Osmotic Absorption of Water:

Sometimes, it has been observed that absorption of water takes place even when the O.P. of the soil water is higher than the O.P. of cell-sap. This type of absorption which is non- osmotic and against the osmotic gradient requires the expenditure of metabolic energy probably through respiration.

Following evidences support this view:

(i) The factors which inhibit respiration also decrease water absorption.
(ii) Poisons which retard metabolic activities of the root cells also retard water absorption.
(iii) Auxins which increase metabolic activities of the cells stimulate absorption of water.

(2) Passive Absorption of Water:

Passive absorption of water takes place when rate of transpiration is usually high. Rapid evaporation of water from the leaves during transpiration creates a tension in water in the xylem of the leaves. This tension is transmitted to water in xylem of roots through the xylem of stem and the water rises upward to reach the transpiring surfaces.

As a result, soil water enters into the cortical cells through root hairs to reach the xylem of roots to maintain the supply of water. The force for this entry of water is created in leaves due to rapid transpiration and hence, the root cells remain passive during this process.

During absorption of water by roots, the flow of water from epidermis to endodermis may take place through three different pathways:

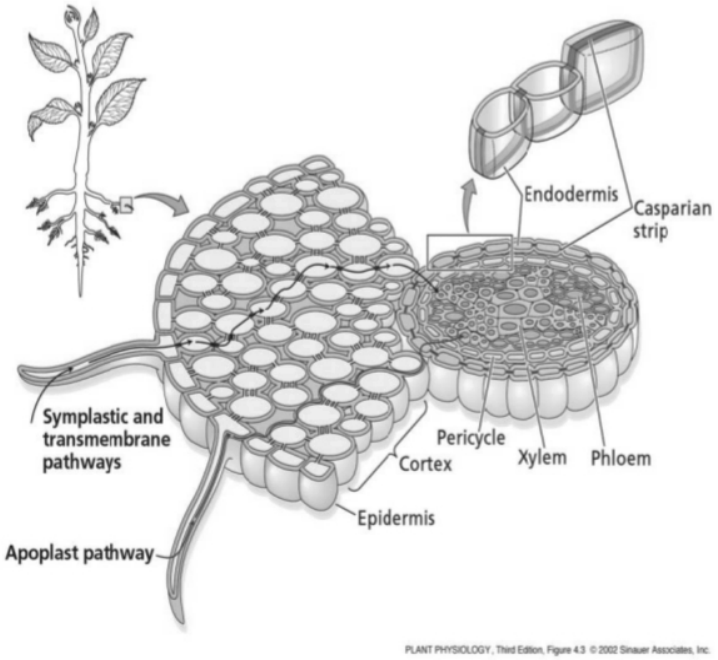
(i) Apoplastic pathway (cell walls and intercellular spaces),
(ii) Trans-membrane pathway (by crossing the plasma membranes) and
(iii) Symplast pathway (through plasmodesmata).

Combination of these three pathways is responsible for transport of water across the root.The mechanism of water absorption described earlier, in-fact belongs to the second category. The relative importance of these three pathways in water absorption by roots is not clearly established. However,

Movement of water and nutrients in the roots

Negative water potential continues to drive movement once water (and minerals) are inside the root; Ψ of the soil is much higher than Ψ or the root, and Ψ of the cortex (ground tissue) is much higher than Ψ of the stele (location of the root vascular tissue). Once water has been absorbed by a root hair, it moves through the ground tissue through one of three possible routes before entering the plant's xylem:

- **The symplast**: "sym" means "same" or "shared," so symplast is shared cytoplasm. In this pathway, water and minerals move from the cytoplasm of one cell in to the next, via plasmodesmata that physically join different plant cells, until eventually reaching the xylem.
- **The transmembrane pathway**: in this pathway, water moves through water channels present in the plant cell plasma membranes, from one cell to the next, until eventually reaching the xylem.
- **The apoplast**: "a" means "outside of," so apoplast is outside of the cell. In this pathway, water and dissolved minerals never move through a cell's plasma membrane but instead travel through the porous cell walls that surround plant cells.



Water and minerals that move into a cell through the plasma membrane has been "filtered" as they pass through water or other channels within the plasma membrane; however water and minerals that move via the apoplast do not encounter a filtering step until they reach a layer of cells known as the **endodermis** which separate the vascular tissue (called the stele in the root) from the ground tissue in the outer portion of the root. The endodermis is exclusive to roots, and serves as a checkpoint for materials entering the root's vascular system. A waxy substance called suberin is present on the walls of the endodermal cells. This waxy region, known as the **Casparian strip**, forces water and solutes to cross the plasma membranes of endodermal cells instead of slipping between the cells. This ensures that only materials required by the root pass through the endodermis, while toxic substances and pathogens are generally excluded.

Aquaporins:

In recent years some integral membrane proteins have been discovered which form water selective channels in cell membranes (lipid bilayers) and facilitate faster movement of water across the membranes into the plant cells. These channels have been called as aquaporins (Fig. 4.4). The direction of water transport across the membranes however, is not affected by aquaporins.

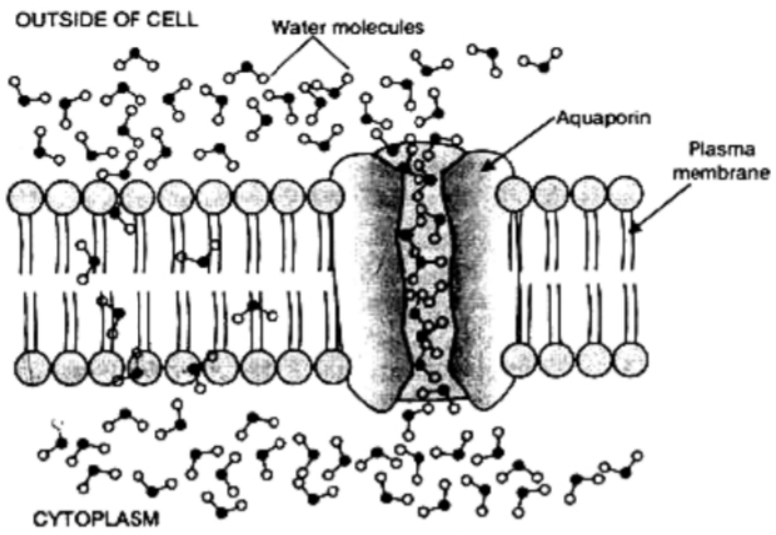


Fig. 4.4. Bulk flow of water across the cell membrane through aquaporin.

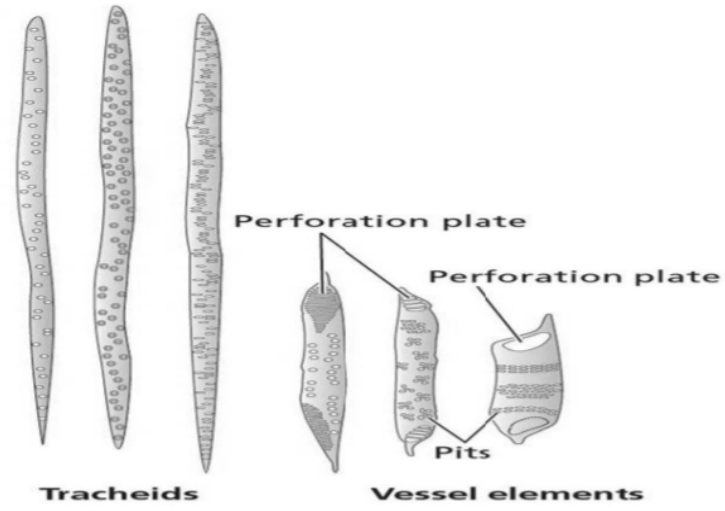
Aquaporin's are found in both plant and animal membranes but they are

relatively abundant in plants. The aquaporin's satisfactorily account for the observed rate of water movement across the membranes which could not be explained earlier simply by direct diffusion of water through lipid bilayer as the latter does not allow bulk flow of water across it. According to Tyerman et al (2002), expression and activity of aquaporin's appear to be regulated probably by protein phosphorylation in response to availability of water.

Water transport through xylem

Xylem:

- Tracheids: Elongated spindle-shaped cells –arranged in overlapping vertical files.
- Water flows between them via pits – areas with no secondary walls and thin porous primary walls
- Vessel elements: Shorter & wider. The open end walls provide an efficient low-resistance pathway for water movement.
- Perforation plate forms at each end – allow stacking end on to form a larger conduit called a vessel.



Among the reasons for this recognition of primary functions of xylem are the following:

(1) The anatomy of xylem obviously and clearly fits it for conducting purposes and it is also true that among the vascular tissues, xylem vessels have the right cross-sectional area to allow the upward transport of the large amounts of water required by plants;

(2) In the classical ringing experiments, it is observed that the removal of a ring of tissue external to xylem from the stem does not interfere significantly with upward movement of water to the organs situated above the ring whereas the removal of a cylinder of xylem from the stem certainly seriously disturbs or even completely stops conduction of water. The net result in many cases is almost complete wilting of leaves attached to the stem above the ring;

(3) Upward movement of water continues for some time even in shoots cut from plant, when placed in water. The rate of water movement through xylem vessels varies greatly from almost imperceptibly slow, to speeds as high as 75 cm per min.

Ringing Experiments:

Most land plants obtain the necessary water for normal growth and development from the soil. By far, the largest proportion of absorbed water is lost as vapour in the process of transpiration from the aerial parts.

Much smaller quantities are utilised for growth and for various other metabolic processes of plants. Water, therefore, must move continuously through the intervening tissues and organs from the absorbing regions of roots to the tissues in which it is utilised or from which, it escapes as vapour, the leaves.

In small herbs and shrubs, the distance to be traversed by water on its onward journey from the root tips to the leaves, is usually not more than a few feet. But even in such plants, they may

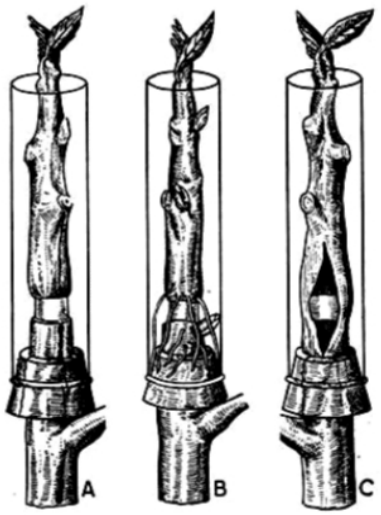


FIG. 686. Ringing experiments; A & B, all tissues external to xylem removed; C, only xylem removed.

have such deep-seated root system, that the ascending water has to cover distances as great as 6-10 metres (m) before even reaching the surface of the soil. It is in tall trees, however, in which the most striking illustrations of upward conduction of water occur. The tallest trees in record are a species of redwood in U.S.A., which attain immense heights of 90-120 m. The heights of our own eucalyptus and firs range between 60-90 m. Since in all trees, the root systems also penetrate at least a few feet into the ground, the actual vertical distance through which the absorbed water must be conducted in order to reach the topmost leaves, may easily be 120 m or even more!

A simple calculation reveals that the force required to lift water to the top of tallest trees must be enormous indeed. For example, in case of a 90 m tree, a pressure of 10 atm. is just sufficient to support the column of water and according to all available experimental evidence; another 10 atm. is needed to overcome resistance due to friction.

So altogether a minimum of 20 atm. is necessary if the water is actually moving up through the xylem vessels. The mechanism by which this feat is accomplished against the force of gravity has been the subject of much speculation and it has been one of the most fascinating problems of plant physiology which has intrigued generations of investigators, not to speak of laymen. This has been termed, ascent of sap.

We know that water enters the land plant entirely through the root hairs and after crossing the cells of the cortex, the peculiarly thick-walled endodermis and pericycle, finally reach the xylem .vessels or tracheids of the roots.

At this point, its upward movement begins. We know that xylem vessels form a continuous conducting system, just like a pipeline, from the root tips through the main tissues of the roots and through the stems, the petioles of the leaves and ultimately through veins and veinlets, ending up in the surrounding mesophyll tissue of the leaves. In the mesophyll cells, water moves onward from cell to cell and eventually the mesophyll cell walls, losing most of it by evaporation into the intercellular spaces, from where it escapes into the external air through stomata. It was generally agreed, that xylem vessels are the main pathway of the upward conduction of water. A cell-to-cell lateral movement of water in a radial direction undoubtedly occurs along the vascular rays in the stems of most species of plants.

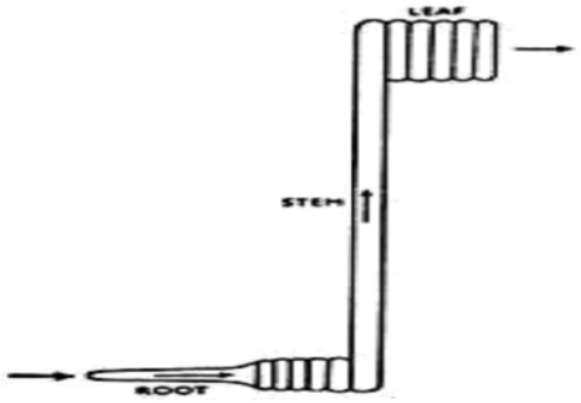


Fig. 685. Diagrammatic path of absorbed water from the root to the leaf and to external air.

Ascent of Sap:

The mechanism of water moving vertically in plants against the force of gravity is still a classical problem of plant physiology. Since diffusion is much too slow to account for the rates that commonly occur in plants, mass movement of water must be envisaged; the whole column must move simultaneously instead of molecular movement.

A number of different theories of the mechanism by which ascent of water are brought about in plants has been suggested and the present state of our knowledge justifies a discussion of only three possible mechanisms:

(1) That the ascent of water is caused by the activity of the living cells - vital theories;

(2) That upward movement occurs as a result of root pressure;

(3) That ascent of water has its explanations in a set of purely physical principles, supplemented by cohesion of water theory, proposed by Dixon.

How is water transported up a plant against gravity, when there is no “pump” to move water through a plant’s vascular tissue? There are three hypotheses that explain the movement of water up a plant

against gravity. These hypotheses are not mutually exclusive, and each contributes to movement of water in a plant, but only one can explain the height of tall trees:

1. **Root pressure** pushes water up
2. **Capillary action** draws water up within the xylem
3. **Cohesion-tension** pulls water up the xylem

We'll consider each of these in turn.

Vital Theories:

As living cells are more or less in intimate contact with the dead xylem elements through which upward conduction of water occurs; suggestions have often been made that the motive power of ascent of sap is provided by the vital activity of living cells. Our own J. C. Bose was the most ardent advocate of the vital theory of ascent of sap. Bose's idea was essentially an elaboration of the theory proposed by Godlewski in 1884. According to Godlewski, ascent of water resulted from periodic changes in the osmotic pressure of the living cells—wood-ray cells in the xylem. It visualised a repetitive process of alternate increase and decrease in the osmotic pressure of the wood-ray cells, resulting in driving water out and into the xylem vessel. Thus the movement of water was supposed to be due to an alternate contraction and expansion of wood-ray cells, which also supplied necessary energy for the mechanism, perhaps energy released in cellular respiration.

The concepts of Bose and Molisch have been destructively criticised by a number of investigators, including Smith, Benedict, MacDougal, and others.

Elaborate experiments carried out by these investigators, however, showed conclusively that water would continue to ascend for some time through the plant after all the living cells present in a woody stem were killed either by soaking the cut end into boiling water or by dipping it into a strong solution of picric acid.

Root Pressure:

Root pressure relies on positive pressure that forms in the roots as water moves into the roots from the soil. Water moves into the roots from the soil by osmosis, due to the low solute potential in the roots (lower Ψs in roots than in soil). This intake of water in the roots increases Ψp in the root xylem, driving water up. In extreme circumstances, root pressure results in **guttation**, or secretion of water droplets from stomata in the leaves. However, root pressure can only move water against gravity by a few meters, so it is not strong enough to move water up the height of a tall tree.

Capillary action:

Capillary action or capillarity is the tendency of a liquid to move up against gravity when confined within a narrow tube (capillary). Capillarity occurs due to three properties of water:

1. **Surface tension**, which occurs because hydrogen bonding between water molecules is stronger at the air-water interface than among molecules within the water.
2. **Adhesion**, which is molecular attraction between "unlike" molecules. In the case of xylem, adhesion occurs between water molecules and the molecules of the xylem cell walls.
3. **Cohesion**, which is molecular attraction between "like" molecules. In water, cohesion occurs due to hydrogen bonding between water molecules.

On its own, capillarity can work well within a vertical stem for up to approximately 1 meter, so it is not strong enough to move water up a tall tree.

Cohesion Tension Theory:

The **cohesion-tension** hypothesis is the most widely-accepted model for movement of water in vascular plants. Cohesion-tension essentially combines the process of capillary action with **transpiration**, or the evaporation of water from the plant stomata. Transpiration is ultimately the main driver of water movement in xylem. The cohesion-tension model works like this:

1. **Transpiration** (evaporation) occurs because stomata are open to allow gas exchange for photosynthesis. As transpiration occurs, it deepens the meniscus of water in the leaf, creating negative pressure (also called tension or suction).
2. The **tension** created by transpiration "pulls" water in the plant xylem, drawing the water upward in much the same way that you draw water upward when you suck on a straw.
3. **Cohesion** (water sticking to each other) causes more water molecules to fill the gap in the xylem as the top-most water is pulled toward the stomata.

In the application of physical principles for an explanation of the mechanism of ascent of sap in plants, atmospheric pressure naturally comes first to mind. But it is at once apparent that atmospheric pressure can only account for a rise of about 10 m. Suggestions were then put forward that water rose by imbibitional forces through the thick walls of the xylem vessels.

Physicists have shown that the forces of imbibition are very great, ranging from 100-1000 atm. and this at first sight would seem more than adequate for carrying water to any required height.

However, the rate of movement of water through imbibing colloids is extremely slow compared with known rates of water conduction in the xylem. Moreover, it was soon shown that water actually moves not through the cell walls, which would be required for an imbibitional transport, but through the lumen or cavities of the xylem vessels. We have seen that negative pressure (or tension) generally exists in the xylem during rapid transpiration from leaves.

This can be shown by the use of a very sensitive instrument known as dendrograph which measures diameters of the tree trunk and such measurements definitely show, at times at least, a contraction of trunk-diameter during the day (caused by tension or pull due to transpiration) and expansion at night (tension less, due to stopping of transpiration). Thus the ascent of sap is usually associated with a pull from above rather than a push from below (positive root pressure). Forces developing in aerial parts of the plants, especially in the leaves, cause the rise of water through the plant. It is easy to calculate mathematically whether this can be explained by a capillary pull in the vessels.

A vessel diameter of 0·1 mm would account for a rise of only 30 cm. But it must be understood clearly that it is not in the xylem vessels themselves that the main capillary pull occurs. Since this is clearly a surface tension phenomenon, the pull must occur at the water surface due to transpiration.

Thus the transpiration pull, it seems, is responsible not only for the movement of water within the plant, but also of absorption of water from the external root medium, i.e., soil solution.

The following three stages in the process then may be distinguished:

(1) Transpiration from the surface of microcapillaries,

(2) Capillary rise of fine threads of water due to the force of adhesion between water and cell wall, and

(3) The whole column of water moves all together and resists breaking because of the force of cohesion between the water molecules—similar particles always have tendency to stick to each other.

Of course, these three stages occur simultaneously. This concept is known as Dixon's transpiration-cohesion-tension theory of ascent of sap in plants.

Objections:

(i) The gases dissolved in sap shall form air bubbles under tension and high temperature. Air bubbles would break the continuity of water column and stop ascent of sap due to transpiration pull,

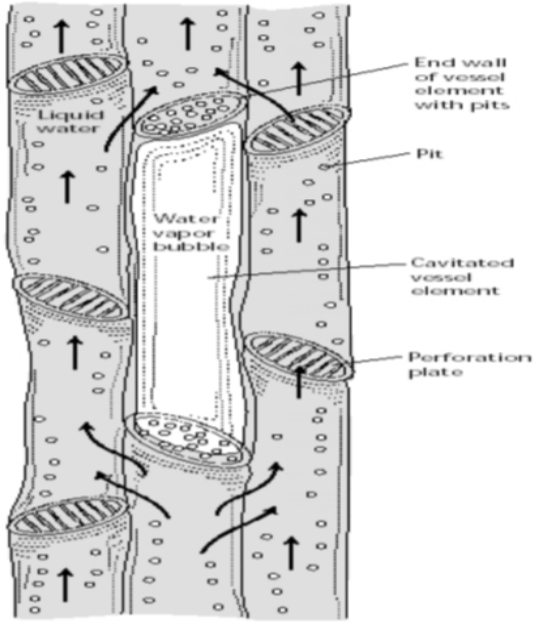
(ii) A tension of up to 100 atm has been reported in the xylem sap by Mac Dougal (1936) while the cohesive force of sap can be as low as 45 atm.

(iii) Overlapping cuts do not stop ascent of sap though they break the continuity of water column.The cohesion-tension theory of sap ascent is shown. Evaporation from the mesophyll cells produces a negative water potential gradient that causes water to move upwards from the roots through the xylem.

Transpiration Pull:

It is the pulling force responsible for lifting the water column. As water is lost in form of water vapour to atmosphere from the mesophyll cells by transpiration, a negative hydrostatic pressure is created in the mesophyll cells which in turn draw water from veins of the leaves.

The negative tension is then gradually transmitted downwards via xylem tissues of the leaf, stem and finally to the roots. Thus the transpiration pull acts as pull from above on the-whole of water column of the plant which pushes the water column of xylem vessels of roots lowers leaves i.e. in an upward direction. This is how ascent of sap is affected in plants. The whole process can be compared to a person (transpiration pull) pulling a bucket full of water with a steel rope (unbroken and continuous water column)



The Significance of Transpiration:

Although transpiration has been studied extensively, very little is known concerning the significance of transpiration in the life of the plant. There is even now some controversy whether transpiration is a necessary and unavoidable evil or whether it is in some way essential for the welfare of the plant.

In the first category, opinions prevail that transpiration brings nothing but harm to the plant, and the harmful effects ascribed to transpiration far outweigh its supposed beneficial effects, while in the second, there are opinions which consider transpiration as important as photosynthesis and respiration.

From the former point of view the primary function of stomata is to permit exchange of CO_2 and O_2 between the plant and the atmosphere in the process of photosynthesis and respiration and water is lost through the open stomata, because there is no stopping the passage of water vapour, while permitting CO_2 and O_2 exchange.

Thus the primary function of stomata is not to be thought of as one permitting water loss any more than the function of a main door in a busy office building is to let heat escape from the building in winter.

Just as loss of heat cannot be avoided if the door must be kept open to continuous streams of persons who enter and leave the building during business hours, similarly loss of water vapour from the leaf is inevitable when stomata are open to permit exchange of CO_2 and O_2between the plant and the atmosphere.

Regulation or Control of Water Loss by Stomata:

The general view came to be held that the stomata by opening and closing control or regulate the transpirational water loss from the plant.

The earlier idea was that stomata could close in anticipation of wilting and thus conserve the water supply of the leaf and prevent it from wilting. When later it was found that the stomata do not close until the leaf has wilted to some extent, it was then realised that the regulation, if any, was passive since under such conditions, the stomatal closure is not due to any special activity of the guard cells but to a decrease in their turgour caused by loss of water from the leaf cells.

The rate of transpiration is generally higher than the absorption of water by roots from soil during the day and the reverse is true at night. The water content of a leaf may vary over a considerable range during a 24-hour period without any visible signs of wilting of the aerial organs.

We have discussed before that no correlation generally exists between the number of stomata per unit area of the leaf surface, and the rate of transpiration. The general view now held by most investigations is that when the stomata are fully open or nearly so, the transpiration rate is primarily determined by the same physical factors which control evaporation from a free water surface.

Transpiration may actually increase when stomata begin to close and may decrease when they open wider. This is sometimes due to the fact, as we have seen before, that when the diameters of the stomatal pores are about 1 /10th to 1 /20th the distance between the two pores, no further opening of the pore can affect the rate of diffusion of water vapour through them. As the stomata gradually close, influence of physical factors of evaporation is lessened until at 50% opening of stomatal pore, variable atmospheric conditions become the primary factors in controlling transpiration, physiological regulation by the stomata, of the water loss, taking only a subsidiary role. Only when the pores are almost closed, the guard cells begin to exert a controlling influence on the water loss regardless of evaporation and climatic factors prevailing at the time.

Mechanism of Water Loss in Transpiration:

In order to form vapours, water present inside the exposed parts of the plant requires a source of heat energy. It is the radiant energy during the day and heat energy from the transpiring organ during the night. In both the cases the temperature of the transpiring organs comes to lie 2-5 °C below that of the atmosphere.

The atmosphere is rarely saturated with water vapours. The dry air of the atmosphere has a high DPD (or low water potential)-13.4 atm at 99% relative humidity or RH, 140 atm at 90% RH, 680 atm at 60% and 2055 atm at 20% RH.

Such a high DPD or low water potential can overcome various types of resistances water molecules have to meet in chang-ing from liquid phase to vapour phase and the movement of water vapours out of the transpiring organ.

The intercellular spaces of the transpiring organ is almost saturated with water vapours. When the stomata are open, the water vapours are drawn from the sub-stomatal cavities to the outside air due to high DPD of the latter. This increases the DPD of the sub-stomatal air which draws more water vapours from the intercellular spaces.

The latter in turn get water vapours from the wet walls of mesophyll cells. Stomatal transpiration will continue till the stomata are open. Mechanism of lenticular transpiration is similar to that of stomatal transpiration.

Cuticle is not much permeable to water. However, its molecules absorb water from the epidermal cells by imbibition. The imbibed water is slowly lost to the atmosphere which has a high DPD. Imbibition flow is reduced by the thickness of cuticle.

Therefore, a thick cuticle does not allow transpiration to occur through it. Cuticle is shrunken and thicker during the day but at night it expands and becomes loose. Therefore, cuticular transpiration can be more at night. Mechanism of bark transpiration is similar to that of cuticular transpiration.

Soil-plant-atmosphere continuum:

The soil-plant-atmosphere continuum (SPAC) is the pathway for water moving from soil through plants to the atmosphere. Continuum in the description highlights the continuous nature of water connection through the pathway. The low water potential of the atmosphere, and relatively higher (i.e. less negative) water potential inside leaves, leads to a diffusion gradient across the stomatal pores of leaves, drawing water out of the leaves as vapour. As water vapour transpires out of the leaf, further water molecules evaporate off the surface of mesophyll cells to replace the lost molecules since water in the air inside leaves is maintained at saturation vapour pressure. Water lost at the surface of cells is replaced by water from the xylem, which due to the cohesion-tension properties of water in the xylem of plants pulls additional water molecules through the xylem from the roots toward the leaf.

5. Cell wall structure, biogenesis; membrane transport processes; solute transport and photo assimilate translocation

Structure of Cell Wall:

A cell wall can have upto three parts— middle lamella, primary wall and secondary wall.

Middle Lamella It is a thin, amorphous and cementing layer between two adjacent cells. Middle lamella is the first layer which is depos-ited at the time of cytokinesis. It is just like brick work of the common wall be-tween two adjacent rooms.

Middle lamella is absent on the outer side of surface cells. It is made up of calcium and magnesium pectates. The softening of ripe fruits is caused by partial solubilisation of pectic compounds to produce jelly-like consistency.

Primary Wall:

It is the first formed wall of the cell which is produced inner to the middle lamella. The primary wall is com-monly thin (0.1-3.0 µm) and capable of exten-sion. It grows by intussusceptions or addition of materials within the existing wall. Some cells possess only primary wall, e.g., leaf cells, fruit cells, of cortex and pith.

Primary wall consists of a number of micro fibrils embedded in the amorphous gel like matrix or ground substance. In the majority of plants, the micro fibrils are formed of cellulose. They are synthesized at plasma membrane by particle rosettes (terminal com-plexes) having cellulose synthetize enzyme.

The wall is made of a polymer of P, 1-4 acetyl glucosamine or fungus cellulose in many fungi. Fungus cellulose is similar to chitin present in the exoskeleton of insects. Micro fibrils are oriented variously according to the shape and thickening of the wall. Usually they are arranged in a loose network due to incomplete cross-linking.

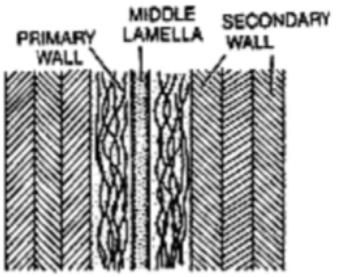


Fig. 8.14. Arrangement of microfibrils in the common wall between two adjacent cells as seen in L.S.

The matrix of the wall consists of water, pectin, hemicelluloses and glycoproteins. Pectin is the filler substance of the matrix. Proteins are structural and enzymatic. Protein expansin (Me Queen-Mason et al, 1992) is involved in loosening and expansion of cell wall through incorporation of more cellulose. Hemicellulose binds micro fibrils with matrix.

Secondary Wall:

It is produced in some mature cells when the latter have stopped growth, e.g., tracheids, vessel elements, fibres, collenchyma's. Secondary wall is laid inner to the primary wall by accretion or deposition of materials over the surface of existing structure. It is thick (3—10 µm) and made up of at least three layers, sometimes more (e.g., latex tube of Euphorbia milli). They are named as S1, S2, S3, Sx, etc.

The innermost layer of the secondary wall is sometimes distinct both chemically as well as in staining properties due to the presence of xylans. It is then called tertiary wall, e.g., tension wood in gymnosperms. Secondary wall may be absent, irregularly deposited or formed uniformly in the cells. This results in differentiation of cells— parenchyma, collenchyma, sclerenchyma, tracheids and vessels.

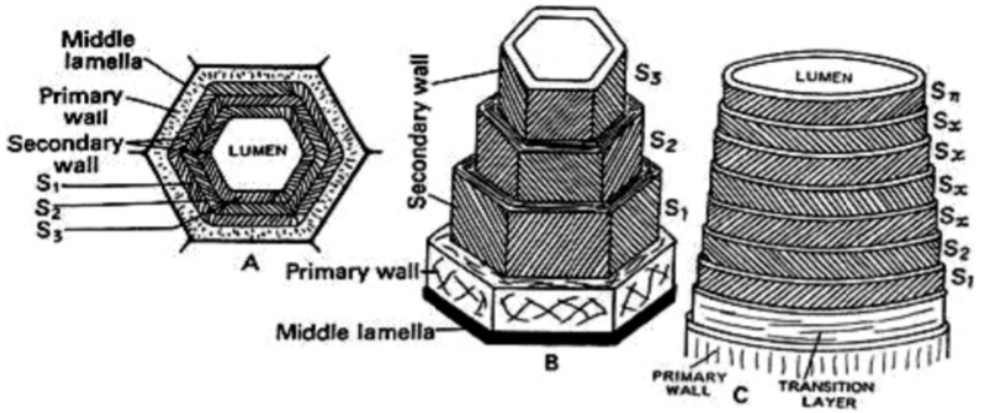


Fig. 8.15. Parts and layers of cell wall. A, a cell in T.S. showing parts of cell wall. B, typical wood fibre cut at various levels to show parts and layers of the wall. C, latex tube of *Euphorbia milli* (= *E. splendens*) cut at various levels to show parts.

The composition of secondary wall is basically similar to the primary wall in having cellulose micro-fibrils embedded in a matrix of pectin and hemicelluloses. Cellulose mi-cro-fibrils of the secondary wall lie close, parallel and at an angle to the longitudinal axis of the cell. Their orientation is different in the different layers of the secondary wall. A number of different materials may be deposited in the wall.

The important ones are:

(a) Lignin:

It reduces the water content of the wall matrix and increases its hardness. However, water permeability is not affected. The characteristic of lignification's (and cutinisation) has evolved with the evolution of land plants.

(b) Suberin:

The wall of cork and endodermal cells contains a special fatty substance called suberin. Suberin makes the walls impermeable.

(c) Cutin:

The epidermal cells possess another fatty substance called cutin. Cutin is also laid as a distinct layer on the outside of the epidermal cell walls. It is known as cuticle. Cutin reduces the rate of epidermal or surface transpiration. Other substances which can be deposited in the cell wall are silica (e.g., grasses), minerals, waxes, tannins, resins, gums, etc.

Plasmodesmata:

Plasmodesmata (singular- plasmodesma; Tangl, 1879; Strasburger, 1901) are cytoplasmic bridges between adjacent plant cells which develop in the minute pores of their walls. They form a protoplasmic continuum called symplast. Cell wall and intercellular spaces form a non-living component of the plant body called apoplasm. A plasmoderma is 40—50 nm in diameter. It may be simple or branched.

Plasmodesma is lined by plasma membrane. It encloses tubular extention of endoplasmic reticulum called desmotubule The space between desmotubule and plasma membrane contains 8-10 microchannels. Plasmodesmata

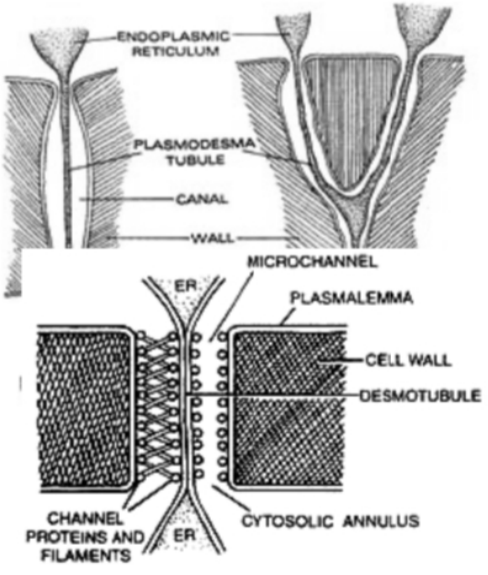


Fig. 8.17. Components of plasmodesmata.

form channels for controlled passage of small sized particles between adjacent cells as well as transfer of some specific signals.

Pits:

Pits are un-thickened areas in the secondary walls of plant cells. They, therefore, appear as depressions. Pits generally occur in pairs on the wall of two adjacent cells. A pit has a cavity or pit chamber and a pit membrane.

The pit membrane consists of primary wall and middle lamella. Pits are of two types, simple and bordered. Simple pit has uniform width of the pit chamber. In bordered pit, the pit chamber is flask-shaped because the secondary wall overarches its mouth.

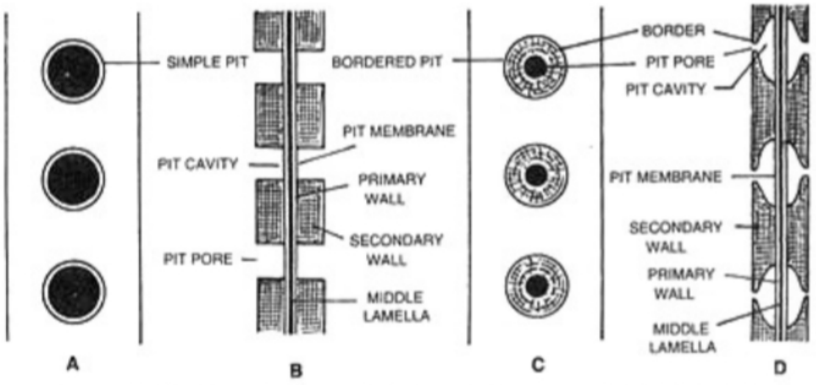


Fig. 8.18. Pits. A, surface view of simple pits. B, simple pit pairs in section. C, surface view of bordered pits. D, bordered pit pairs in section.

Pit membrane is permeable. It may have minute sub-microscopic pores. Therefore, pits help in rapid translocation between two adjacent cells.

Cell wall biogenesis:

The middle lamella is laid down first, formed from the cell plate during cytokinesis, and the primary cell wall is then deposited inside the middle lamella. The actual structure of the cell wall is not clearly defined and several models exist - the covalently linked cross model, the tether model, the diffuse layer model and the stratified layer model. However, the primary cell wall, can be defined as composed of cellulose microfibrils aligned at all angles. Cellulose microfibrils are produced at the plasma membrane by the cellulose synthase complex, which is proposed to be made of a hexameric rosette that contains three cellulose synthase catalytic subunits for each of the six units. Microfibrils are held together by hydrogen bonds to provide a high tensile strength. The cells are held together and share the gelatinous membrane called the middle lamella, which contains magnesium and calcium pectates (salts of pectic acid). Cells interact though plasmodesmata, which are inter-connecting channels of cytoplasm that connect to the protoplasts of adjacent cells across the cell wall.

In some plants and cell types, after a maximum size or point in development has been reached, a secondary wall is constructed between the plasma membrane and primary wall. Unlike the primary wall, the cellulose microfibrils are aligned parallel in layers, the orientation changing slightly with each additional layer so that the structure becomes helicoidal. Cells with secondary cell walls can be rigid, as in the gritty sclereid cells in pear and quince fruit. Cell to cell communication is possible through pits in the secondary cell wall that allow plasmodesmata to connect cells through the secondary cell walls.

Passive and active transport

According to Fick's first law, the movement of molecules by diffusion always proceeds spontaneously, down a gradient of concentration or chemical potential, until equilibrium is reached. The spontaneous "downhill" movement of molecules is termed **passive transport**. At equilibrium, no

further net movements of solute can occur without the applicationof a driving force. The movement of substances against or up a gradient of chemical potential (e.g., to a higher concentration) is termed **active transport**. It is not spontaneous, and itrequires that work be done on the system by the application of cellular energy. One way (but not the only way) of accomplishing this task is to couple transport to the hydrolysis of ATP. The driving force for diffusion, or, conversely, the energy input necessary to move substances against a gradient, by measuring the potential-energy gradient, which is often a simple function of the difference in concentration. Biological transport can be driven by four major forces: concentration, hydrostatic pressure, gravity, and electric fields. The **chemical potential** for any solute is defined as the sum of the concentration, electric, and hydrostatic potentials (and the chemical potential under standard conditions.

Transport of ions across a membrane barrier

If the two KCl solutions in the previous example are separated by a biological membrane, diffusion is complicated by the fact that the ions must move through the membrane as well as across the open solutions. The extent to which a membrane permits the movement of a substance is called **membrane permeability**. As will be discussed later, permeability depends on the composition of the membrane, as well as on the chemical nature of the solute. In a loosesense, permeability can be expressed in terms of a diffusion coefficient for the solute in the membrane. However, permeability is influenced by several additional factors, suchas the ability of a substance to enter the membrane, that are difficult to measure. Despite its theoretical complexity, we can readily measure permeability by determining the rate at which a solute passes through a membrane under a specific set of conditions.Generally the membrane will hinder diffusion and thus reduce the speed with which equilibrium is reached.The permeability or resistance of the membrane itself, however, cannot alter the final equilibrium conditions. Equilibrium occurs when $m\sim j = 0$.In the sections that follow we will discuss the factors that influence the passive distribution of ions across amembrane. These parameters can be used to predict there lationship between the electrical gradient and the concentration gradient of an ion.

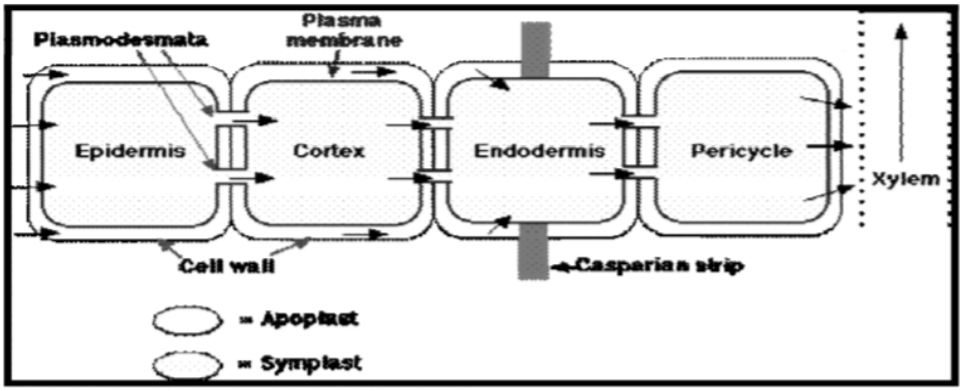


Figure 6: Three classes of membrane transport proteins: channels, carriers, and pumps. Channels and carriers can mediate the passive transport of solutes acrossmembranes

Carriers Bind and Transport Specific Substances

Unlike channels, **carrier** proteins do not have pores thatextend completely across the membrane. In transport mediated by a carrier, the substance being transported is the pore at the center. The pore-forming regions of the four subunits dip into the membrane, with a K+ selectivity finger region formed at the outer (near) part of the pore. (B) Side view of the inward rectifying K+ channel, showing a polypeptide chain of one subunit, with six membrane-spanning helices. The fourth helix contains positively-charged amino acids and acts as a voltage-sensor. The pore-forming region is a loop between helices 5 and 6. (A after Leng et al. 2002; B after Buchanan et al. 2000.) initially bound to a specific site on the carrier protein. This requirement for binding allows carriers to be highly selective for a particular substrate to be transported. Carriers therefore specialize in the transport of

specific organic metabolites. Binding causes a conformational change in the protein, which exposes the substance to the solution on the other side of the membrane. Transport is complete when the substance dissociates from the carrier's binding site.

Because a conformational change in the protein is required to transport individual molecules or ions, the rate of transport by a carrier is many orders of magnitude slower than through a channel. Typically, carriers may transport 100 to 1000 ions or molecules per second, which is about 106 times slower than transport through a channel. The binding and release of a molecule at a specific site on a protein that occur in carrier-mediated transport are similarto the binding and release of molecules from an enzyme in an enzyme-catalyzed reaction. Carrier-mediated transport (unlike transport through channels) can be either passive or active, and it can transport a much wider range of possible substrates. Passive transporton a carrier is sometimes called **facilitated diffusion**, although it resembles diffusion only in that it transports substances down their gradient of electrochemical potential, without an additional input of energy. (This term might seem more appropriately applied to transport through channels, but historically it has not been used in this way.)

Primary Active Transport Is Directly Coupled to Metabolic or Light Energy

To carry out active transport, a carrier must couple the uphill transport of the solute with another, energy-releasing, event so that the overall free-energy change is negative.**Primary active transport** is coupled directly to a source of energy other than *m*~j, such as ATP hydrolysis, an oxidation–reduction reaction (the electron transport chain of mitochondria and chloroplasts), or the absorption of light by the carrier protein (in halobacteria, bacteriorhodopsin).

The membrane proteins that carry out primary active transport are called **pumps**. Most pumpstransport ions, such as H^+ or $Ca2^+$. However, as we will see later in the chapter, pumps belonging to the "ATPbindingcassette" family of transporters can carry large organic molecules.Ion pumps can be further characterized as either electrogenicor electroneutral. In general, **electrogenic transport** refers to ion transport involving the net movement ofcharge across the membrane. In contrast, **electroneutral transport**, as the name implies, involves no net movementof charge. For example, the Na+/K+-ATPase of animal cells pumps three Na+ ions out for every two K+ ions in, resulting in a net outward movement of one positive charge. The Na+/K+-ATPase is therefore an electrogenic ion pump. In contrast, the H+/K+-ATPase of the animal gastric mucosa pumps one H+ out of the cell for every one K+ in, so thereis no net movement of charge across the membrane. Therefore, the H+/K+-ATPase is an electroneutral pump. In the plasma membranes of plants, fungi, and bacteria, as well as in plant tonoplasts and other plant and animal endomembranes, H+ is the principal ion that is electrogenically pumped across the membrane. The **plasma membrane H+-ATPase** generates the gradient of electrochemicalpotentials of H+ across the plasma membranes, while the **vacuolar H+-ATPase** and the **H+-pyrophosphatase** (**H+-PPase**) electrogenically pump prot.

In the plasma membranes of plants,fungi, and bacteria,as well as in plant tonoplasts and other plant and animal endomembranes, H+ is the principal ion that is electrogenicallypumped across the membrane. The **plasmamembrane**. **H+-ATPase** generatesthe gradient of electrochemical potentials of H+ across the plasmamembranes, while the **vacuolar H+-ATPase** and the (**H+-PPase**) electrogenically pump protons into the lumenof the vacuole and the Golgi cisternae.In plant plasma membranes, the most prominent pumpsare for H+ and Ca2+, and the

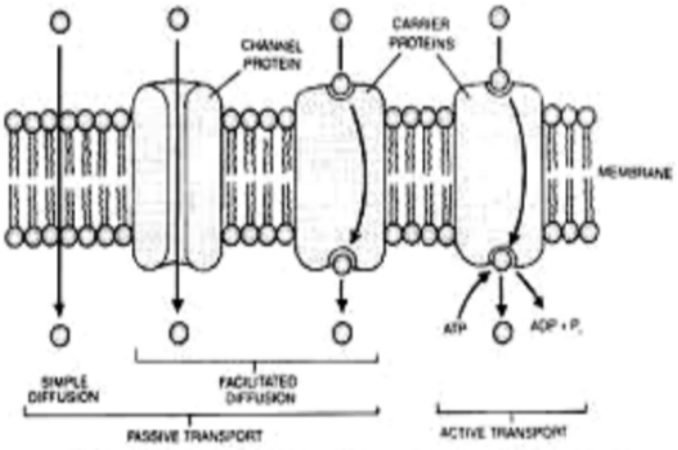


Fig. 11.1. Three ways of exchange of ions and solutes across membranes.

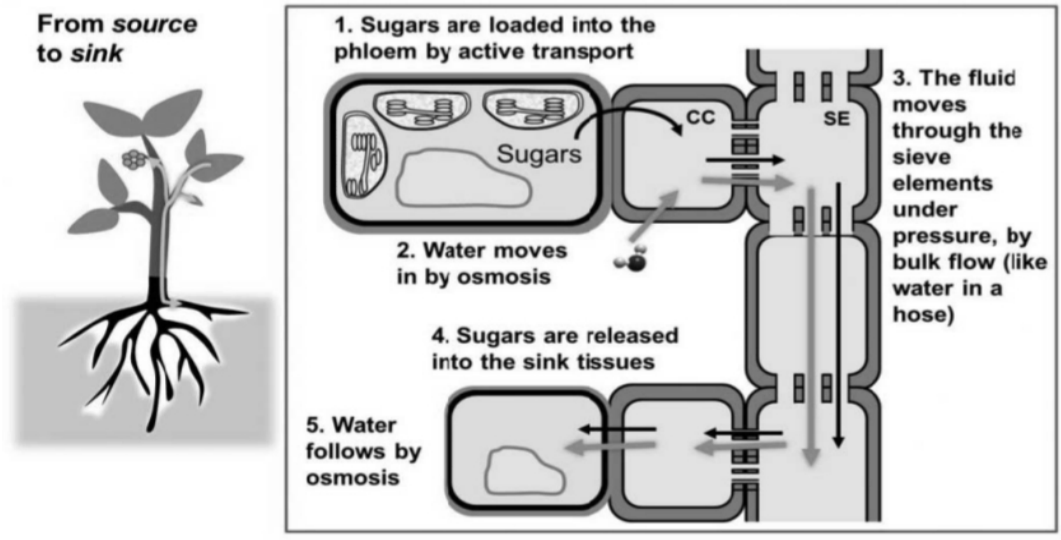
direction of pumping is outward. The other important way that solutes can be actively transported across a cmembrane against their gradient of electrochemical potential is by coupling of the uphill transport of one solute to the downhill transport of another. This type of carrier mediated co-transport is termed **secondary active transport**,

Secondary Active Transport Uses the Energy Stored in Electrochemical-Potential Gradients

Protons are extruded from the cytosol by electrogenic H+- ATPases operating in the plasma membrane and at the vacuole membrane. Consequently, a membrane potential and a pH gradient are created at the expense of ATP hydrolysis. This gradient of electrochemical potential for H+, $m_{\sim H^+}$,or (when expressed in other units) the **proton motive force** (**PMF**), or p, represents stored free energy in the form of the H+ gradient. The proton motive force generated by electrogenic H+ transport is used in secondary active transport to drive the transport of many other substances against their gradient of electrochemical potentials. Figure 6.9 shows how secondary transport may involve the binding of a substrate (S) and an ion (usually H+) to a carrier protein, and a conformational change in that protein. There are two types of secondary transport: symport and antiport. The **symport** (and the protein involved is called a *symporter*) because the two substances are moving in the same direction through the membrane. **Antiport** (facilitated by a protein called an *antiporter*) refers to coupled transport in which the downhill movement of protons drives the active (uphill) transport of a solute in the opposite direction.In both types of secondary transport, the ion or solutebeing transported simultaneously with the protons is moving against its gradient of electrochemical potential, so its transport is active. However, the energy driving this transport is provided by the proton motive force rather than

Solutes Move through Both Apoplast and Symplast

Thus far, our discussion of cellular ion transport has not included the cell wall. In terms of the transport of small molecules, the cell wall is an open lattice of polysaccharides through which mineral nutrients diffuse readily. Because all plant cells are separated by cell walls, ions can diffuse across a tissue (or be carried passively by water flow) entirely through the cell wall space without ever entering a living cell. This continuum of cell walls is called the *extracellular space*, or *apoplast*. We can determine the apoplastic volume of a slice of plant tissue by comparing the uptake of ^{3}H-labeled water and ^{14}C-labeled mannitol. Mannitol is a non-permeating sugar alcohol that diffuses within the extracellular space but cannot enter the cells. Water, on the other hand, freely penetrates both the cells and the cell walls. Measurements of this type usually show that 5 to 20% of the plant tissue volume is occupied by cell walls.



Ions Moving through the Root Cross Both Symplastic and Apoplastic Spaces

Ion absorption by the roots is more pronounced\in the root hair zone than in the meristem and elongation zones. Cells in the root hair zone have completed their elongation but have not yet begun secondary growth. The root hairs are simply extensions of specific epidermal cells that greatly increase the surface area available for ion absorption. An ion that enters a root may immediately enter the symplast by crossing the plasma membrane of an epidermal cell, or it may enter the apoplast and diffuse between the epidermal cells through the cell walls. From the apoplast of the cortex, an ion may either cross the plasma membrane of a cortical cell, thus entering the symplast, or diffuse radially all the way to the endodermis via the apoplast. In all cases, ions must enter the symplast before they can enter the stele, because of the presence of the Casparian strip. The apoplast forms a continuous phase from the root surface through the cortex. At the boundary between the vascular cylinder (the stele) and the cortex is a layer of specialized cells, the endodermis. A suberized cell layer in the endodermis, known as the Casparian strip, effectively blocks he entry of water and mineral ions into the stele via the apoplast. Once an ion has entered the stele through the symplastic connections across the endodermis, it continues to diffuse from cell to cell into the xylem. Finally, the ion reenters the apoplast as it diffuses into a xylem tracheid or vessel element. Again, the Casparian strip prevents the ion from diffusing back out of the root through the apoplast. The presence of the Casparian strip allows the plant to maintain a higher ionic concentration in the xylem than exists in the soil water surrounding the roots.

Xylem Parenchyma Cells Participate in Xylem

The movement of molecules and ions from one location to another is known as transport. Plants exchange solutes and water with their environment and among their tissues and organs. Both local and long-distance transport processes in plants are controlled largely by cellular membranes. Forces that drive biological transport, which include concentration gradients, electric-potential gradients, and hydrostatic pressures, are integrated by an expression called the electrochemical potential. Transport of solutes down a chemical gradient (e.g., by diffusion) is known as passive transport. Movement of solutes against a chemical potential gradient is known as active transport and requires energy input. The extent to which a membrane permits or restricts the movement of a substance is called membrane permeability. The permeability depends on the chemical properties of the particular solute and on the lipid composition of the membrane, as well as on the membrane proteins that facilitate the transport of specific substances. When cations and anions move passively across a membrane at different rates, the electric potential that developsis called the diffusion potential. For each ion, the relationship between the voltage difference across the membrane and the distribution of the ion at equilibrium is described by the Nernst equation. The Nernst equation shows that atequilibrium the difference in concentration of an ion between two compartments is balanced by the voltage difference between the compartments. That voltage difference, or membrane potential, is seen in all living cells because of the asymmetric ion distributions between the inside and outside of the cells. The electrical effects of different ions diffusing simultaneously across a cell membrane are summed by the Goldman equation. Electrogenic pumps, which carry out active transport and carry a net charge, change the membrane potential from the value created by diffusion. Membranes contain specialized proteins—channels, carriers, and pumps—that facilitate solute transport. Channels are transport proteins that span the membrane, forming pores through which solutes diffuse down their gradient of electrochemical potentials. Carriers bind a solute on one side of the membrane and release it on the other side. Transport specificity is determined largely by the properties of channels and carriers. A family of H+-pumping ATPases provides the primary driving force for transport across the plasma membrane of

plant cells. Two other kinds of electrogenic proton pumpsserve this purpose at the tonoplast. Plant cells also have calcium- pumping ATPases that participate in the regulation of intracellular calcium concentrations, as well as ATP binding cassette transporters that use the energy of ATP to transport large anionic molecules. The gradient of electrochemical potential generated by H+ pumping is used to drive the transport of other substances in a process called secondary transport. Genetic studies have revealed many genes, and their corresponding transport proteins, that account for the versatility of plant transport. Patch clamp electrophysiology provides unique information on ion channels, and it enables measurement of the permeability and gating of individual channel proteins .Solutes move between cells either through the extracellular spaces (the apoplast) or from cytoplasm to cytoplasm(via the symplast). Cytoplasms of neighboring cells are connected by plasmodesmata, which facilitate symplastic transport. When an ion enters the root, it may be taken up into the cytoplasm of an epidermal cell, or it may diffuse through the apoplast into the root cortex and enter the symplast through a cortical cell. From the symplast, the ion is loaded into the xylem and transported to the shoot.

6. Growth and developmental physiology: Embryogenesis and differentiation of plant organs Seed germination and seedling growth.

The vegetative phase of development begins with embryogenesis, but development continues throughout the life of a plant. Plant developmental biologists are concerned with questions such as, How does a zygote give rise to an embryo, an embryo to a seedling? How do new plant structures arise from preexisting structures? Organs are generated by cell division and expansion, but they are also composed of tissues in which groups of cells have acquired specialized functions, and these tissues are arranged in specific patterns. How do these tissues form in a particular pattern, and how do cells differentiate? What are the basic principles that govern the size increase (growth) that occurs throughout plant development?

Understanding how growth, cell differentiation, and pattern formation are regulated at the cellular, biochemical, and molecular levels is the ultimate goal of developmental biologists.

Embryogenesis

The developmental process known as **embryogenesis** initiates plant development. Although embryogenesis usually begins with the union of a sperm with an egg, forming a single-celled *zygote*, somatic cells also may undergo embryogenesis under special circumstances. Fertilization also initiates three other developmental programs: endosperm, seed, and fruit development. Here we will focus on embryogenesis because it provides the key to understanding plant development.

Embryogenesis transforms a single-celled zygote into a multicellular, microscopic, embryonic plant. A completed **embryo** has the basic body plan of the mature plant and many of the tissue types of the adult, although these are present in a rudimentary form.

In plants, as in all other eukaryotes, the union of one sperm with the egg forms a single-celled zygote. In angiosperms, however, this event is accompanied by a second fertilization event, in which another sperm unites with two polar nuclei to form the triploid endosperm nucleus, from which the ***endosperm*** (the tissue that supplies food for the growing embryo) will develop.

Embryogenesis occurs within the **embryo sac** of the ovule while the ovule and associated structures develop into the **seed**. Embryogenesis and endosperm development typically occur in parallel with seed development, and the embryo is part of the seed. Endosperm may also be part of the mature seed, but in some species the endosperm disappears before seed development is completed. Embryogenesis and seed development are highly ordered, integrated processes, both of which are initiated by double fertilization.

When completed, both the seed and the embryo within it become dormant and are able to survive long periods unfavorable for growth. The ability to form seeds is one of the keys to the evolutionary success of angiosperms as well as gymnosperms. the zygote is genetically programmed to develop in a particular way, and that cell division, cell expansion, and cell differentiation are tightly controlled during embryogenesis.

If these processes were to occur at random in the embryo, the result would be a clump of disorganized cells with no definable form or function. In this section we will examine these changes in greater detail. We will focus on molecular genetic studies that have been conducted with the model plant *Arabidopsis* that have provided insights into plant development.

Embryogenesis Establishes the Essential Features of the Mature Plant

Plants differ from most animals in that embryogenesis does not directly generate the tissues and organs of the adult. For example, angiosperm embryogenesis forms a rudimentary plant body, typically consisting of an embryonic axis and two cotyledons (if it is a dicot). Nevertheless, embryogenesis establishes the two basic developmental patterns that persist and can easily be seen in the adult plant:

1. The apical–basal axial developmental pattern.
2. The radial pattern of tissues found in stems and roots.

Embryogenesis also establishes the **primary meristems**. Most of the structures that make up the adult plant are generated after embryogenesis through the activity of merisstems. Although these primary meristems are established during embryogenesis, only upon germination will they become active and begin to generate the organs and tissues of the adult.

Axial patterning. Almost all plants exhibit an *axial polarity* in which the tissues and organs are arrayed in a precise order along a linear, or polarized, axis. The shoot apical meristem is at one end of the axis, the root apical meristem at the other. In the embryo and seedling, one or two cotyledons are attached just below the shoot apical meristem. Next in this linear array is the hypocotyl, followed by the root, the root apical meristem, and the root cap. This axial pattern is established during embryogenesis.

For example, whereas adventitious roots develop from the basal ends of stem cuttings, buds develop from the apical ends, even if they are inverted.

Radial patterning. Different tissues are organized in a precise pattern within plant organs. In stems and roots the tissues are arranged in a radial pattern extending from the outside of a stem or a root into its center. If we examine a root in cross section, for example, we see three concentric rings of tissues arrayed along a radial axis: An outermost layer of epidermal cells (the epidermis) covers a cylinder of cortical tissue (the cortex), which in turn overlies the vascular cylinder (the endodermis, pericycle, phloem, and xylem).

The **protoderm** is the meristem that gives rise to the epidermis, the **ground meristem** produces the future cortex and endodermis, and the **procambium** is the meristem that gives rise to the primary vascular tissue and vascular cambium.

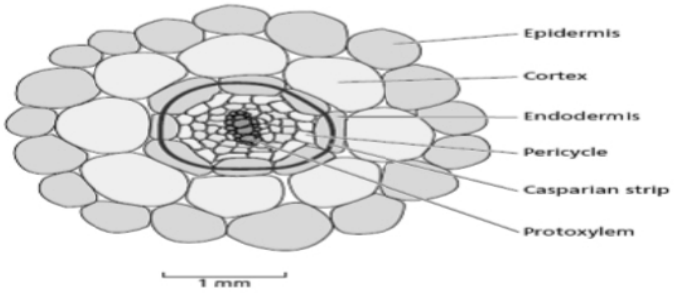


FIGURE 16.2 The radial pattern of tissues found in plant organs can be observed in a crosssection of the root. This crosssection of an *Arabidopsis* root was taken approximately 1 mm back from the root tip, a region in which the different tissues have formed.

***Arabidopsis* Embryos Pass through Four Distinct Stages of Development**

The *Arabidopsis* pattern of embryogenesis has been studied extensively and is the one we will present here, but keep in mind that angiosperms exhibit many different patterns of embryonic development, and this is only one type.

The most important stages of embryogenesis in *Arabidopsis*, and many other angiosperms, are these:

1. *The* ***globular stage*** *embryo*. After the first zygotic division, the apical cell undergoes a series of highly ordered divisions, generating an eight-cell (**octant**) globular embryo by 30 hours after fertilization. Additional precise cell divisions increase the number of cells in the sphere.

2. *The **heart stage** embryo*. This stage forms through rapid cell divisions in two regions on either side of
the future shoot apex. These two regions produce outgrowths that later will give rise to the cotyledons and give the embryo bilateral symmetry
3. *The **torpedo stage** embryo*. This stage forms as a result of cell elongation throughout the embryo axis and
further development of the cotyledons.
4. *The **maturation stage** embryo*. Toward the end of embryogenesis, the embryo and seed lose water and
become metabolically quiescent as they enter dormancy.

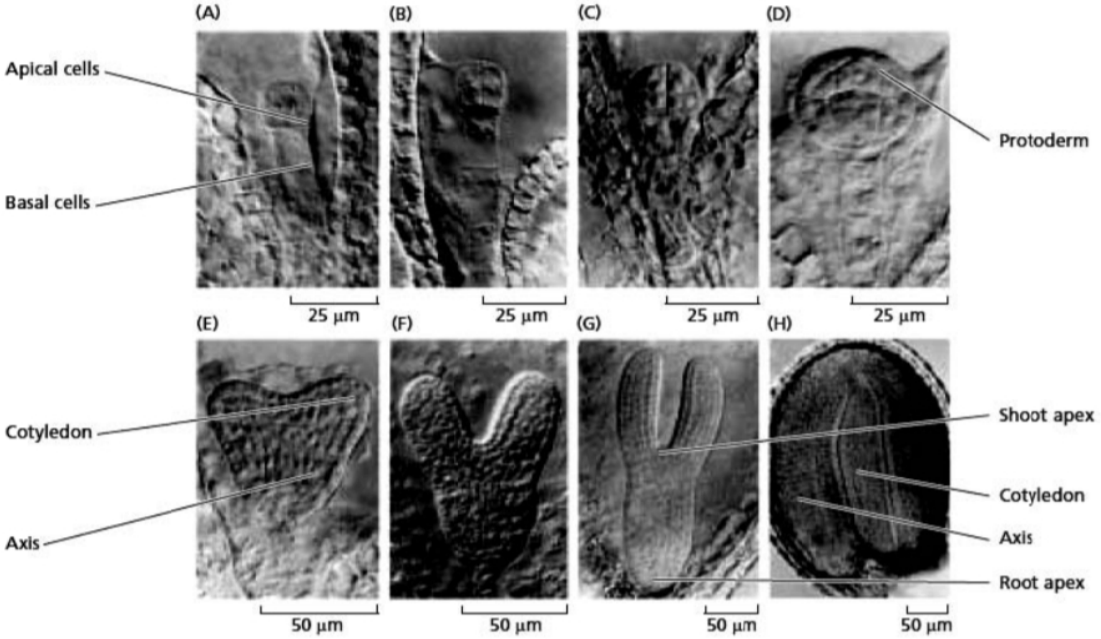


FIGURE 16.3 *Arabidopsis* embryogenesis is characterized by a precise pattern of cell division. Successive stages of embryogenesis are depicted here. (A) One-cell embryo after the first division of the zygote, which forms the apical and basal cells; (B) two-cell embryo; (C) eight-cell embryo; (D) early globular stage, which has developed a distinct protoderm (surface layer); (E) early heart stage; (F) late heart stage; (G) torpedo stage; (H) mature embryo. (From West and Harada 1993 photographs taken by K. Matsudaira Yee; courtesy of John Harada, © American Society of Plant Biologists, reprinted with permission.)

Cotyledons are food storage organs for many species, and during the cotyledon growth phase, proteins, starch, and lipids are synthesized and deposited in the cotyledons to be utilized by the seedling during the heterotrophic (nonphotosynthetic) growth that occurs after germination.
Although food reserves are stored in the *Arabidopsis* cotyledons, the growth of the cotyledons is not as extensive in this species as it is in many other dicots. In monocots, the food reserves are stored mainly in the endosperm. In *Arabidopsis* and many other dicots, the endosperm develops rapidly early in embryogenesis but then is reabsorbed, and the mature seed lacks endosperm tissue.

Embryogenesis Requires Specific Gene Expression

Analysis of *Arabidopsis* mutants that either fail to establish axial polarity or develop abnormally during embryogenesis has led to the identification of genes whose expression participates in tissue patterning during embryogenesis.

The* GNOM *gene: Axial patterning. Seedlings homozygous for mutations in the *GNOM* gene lack both roots and cotyledons. Defects in *gnom* embryos first appear during the initial division of the zygote, and they persist throughout embryogenesis. In the most extreme mutants, *gnom* embryos are

spherical and lack axial polarity entirely. We can conclude that *GNOM* gene expression is required for the establishment of axial polarity.1

The* MONOPTEROS *gene: Primary root and vascular tissue.

Mutations in the *MONOPTEROS* (*MP*) gene result in seedlings that lack both a hypocotyl and a root, although they do produce an apical region. The apical structures in the *mp* mutant embryos are not structurally normal, however, and the tissues of the cotyledons are disorganized (Berleth and Jürgens 1993). Embryos of *mp* mutants first show abnormalities at the octant stage, and they do not form a procambium in the lower part of the globular embryo, the part that should give rise to the hypocotyl and root. Although the *mp* mutant embryos lack a primary root when they germinate, they will form adventitious roots as the seedlings grow into adult plants.

The* SHORT ROOT *and* SCARECROW *genes: Ground tissue development.

Genes have been identified that function in the establishment of the radial tissue pattern in the root and hypocotyl during embryogenesis. These genes also are required for maintenance of the radial pattern during

postembryonic development. To identify these genes, investigators isolated *Arabidopsis* mutants that caused roots to grow slowly. Analysis of these mutants identified several that have defects in the radial tissue pattern. Two of the affected genes, *SHORT ROOT* (*SHR*) and *SCARECROW* (*SCR*), are necessary for tissue differentiation and cell differentiation not only in the embryo, but also in both primary and secondary roots and in the hypocotyl. Mutants of *SHR* and *SCR* both produce roots with a single- celled layer of ground tissue. Cells making up the single-celled layer of ground tissue have a mixed identity and show characteristics of both endodermal and cortical cells in plants with the *scr* mutation. These *scr* mutants also lack the cell layer called the **starch sheath**, a structure that is involved in the growth response to gravity.

The* HOBBIT *gene: The root meristem.

The primary root and shoot meristems are established during embryogenesis. Because in most cases they do not become active at this time, the term *promeristem* may be more appropriate to describe these structures. A **promeristem** may be defined as an embryonic structure that will become a meristem upon germination.

A molecular marker for the root promeristem has not yet been identified, but it appears to be determined early in embryogenesis. Root cap stem cells (the cells that divide to produce the root cap) are formed from the hypophysis at the heart stage of embryogenesis, indicating that the root promeristem is established at least by this stage of embryogenesis. The expression of the *HOBBIT* gene may be an early marker of root meristem identity (Willemsen et al. 1998).

The SHOOTMERISTEMLESS gene: The shoot promeristem.

The shoot promeristem can be recognized morphologically by the torpedo stage of embryogenesis in *Arabidopsis*. Oriented cell divisions of some of the cells between the cotyledons result in a layered appearance of this region that is characteristic of the shoot apical meristem (as described later in the chapter). However, the progenitors of these cells probably acquired the molecular identity of the shoot apical meristem cells much earlier, during the globular stage.

Embryo Maturation Requires Specific Gene Expression

The *Arabidopsis* embryo enters dormancy after it has generated about 20,000 cells. Dormancy is brought about by the loss of water and a general shutting down of gene transcription and protein

synthesis, not only in the embryo, but also throughout the seed. To adapt the cell to the special conditions of dormancy, specific gene expression is required.

For example, the *ABSCISIC ACID INSENSITIVE3* (*ABI3*) and *FUSCA3* genes are necessary for the initiation of dormancy and are sensitive to the hormone abscisic acid, which is the signaling molecule that initiates seed and embryo dormancy. *ABI3* also controls the expression of genes encoding the storage proteins that are deposited in the cotyledons during the maturation phase of embryogenesis.

The *LEAFY COTYLEDON1* (*LEC1*) gene also is active in late embryogenesis. Because *lec1* mutants cannot survive desiccation and do not enter dormancy, the embryos die unless they are rescued through isolation before desiccation occurs. The normal appearance and development of the mature *lec1* mutants indicates that the *LEC1* gene is required only during embryogenesis. Although the most obvious defects of the *lec1* mutants are seen only in the maturation phase embryo, mRNA from *LEC1* gene expression can be detected throughout embryogenesis.

Meristems in plant development

Meristems are populations of small, isodiametric (having equal dimensions on all sides) cells with embryonic characteristics. Vegetative meristems are self-perpetuating. Not only do they produce the tissues that will form the body of the root or stem, but they also continuously regenerate themselves. Ameristem can retain its embryonic character indefinitely, possibly even for thousands of years in the case of trees. The reason for this ability is that some meristematic cells do not become committed to a differentiation pathway, and they retain the capacity for cell division, as long as the meristem remains vegetative. Undifferentiated cells that retain the capacity for cell division indefinitely are said to be **stem cells**. Although historically called *initial cells* in plants, in function they are very similar, if not identical, to animal stem cells (Weigel and Jürgens 2002).

When stem cells divide, on average one of the daughter cells retains the identity of the stem cell, while the other is committed to a particular developmental pathway (Figure 16.12). Stem cells usually divide slowly. Their committed daughters, however, may enter a period of rapid cell division before they stop dividing and can be recognized as specific cell types. Stem cells represent the ultimate source of all the cells in the meristem and the entire rest of the plant— both roots, leaves, and other organs, as well as stems.

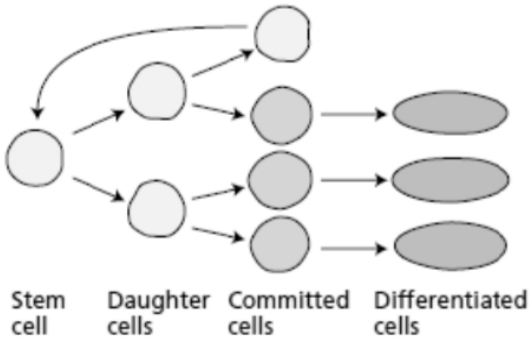


FIGURE 16.12 Stem cells generate daughter cells, some of which remain uncommitted and retain the property of stem cells, while others become committed to differentiate.

The Shoot Apical Meristem Contains Different Functional Zones and Layers

The shoot apical meristem consists of different functional regions that can be distinguished by the orientation of the cell division planes and by cell size and activity. The angiosperm vegetative shoot apical meristem usually has a highly stratified appearance, typically with *three distinct layers of cells*. These layers are designated **L1**, **L2**, and **L3**, where L1 is the outermost layer (Figure 16.13). Cell

divisions are anticlinal in the L1 and L2 layers; that is, the new cell wall separating the daughter cells is oriented at right angles to the meristem surface. Cell divisions tend to be less regularly oriented in the L3 layer. Each layer has its
own stem cells, and all three layers contribute to the formation of the stem and lateral organs.
Active apical meristems also have an organizational pattern called **cytohistological zonation**. Each zone is composed of cells that may be distinguished not only on the basis of their division planes, but also by differences in size and by degrees of vacuolation (see Figure 16.13B). These zones exhibit different patterns of gene expression, reflecting the different functions of each zone (Nishimura et al. 1999; Fletcher and Meyerowitz 2000).

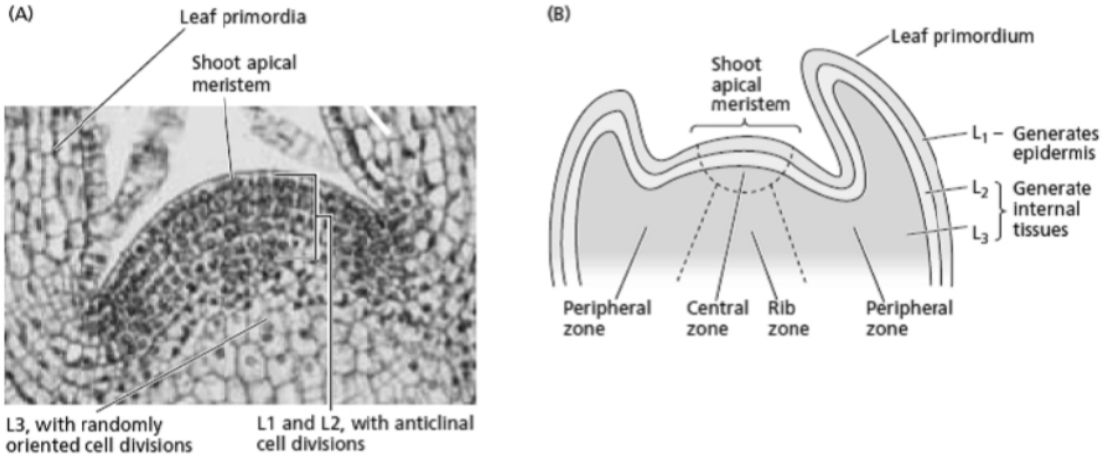


FIGURE 16.13 The shoot apical meristem generates the aerial organs of the plant. (A) This longitudinal section through the center of the shoot apex of *Coleus blumei* shows the layered appearance of the shoot apical meristem. Most cell divisions are anticlinal in the outer L1 and L2 layers, while the planes of cell divisions are more randomly oriented in the L3 layer. The outermost (L1) layer generates the shoot epidermis; the L2 and L3 layers generate internal tissues. (B) The shoot apical meristem also has cytohistological zones, which represent regions with different identities and functions. The central zone contains the stem cells, which divide slowly but are the ultimate source of the tissues that make up the plant body. The peripheral zone, in which cells divide rapidly, surrounds the central zone and produces the leaf primordia. A rib zone lies below the central zone and generates the central tissues of the stem. (A ©J. N. A. Lott/Biological Photo Service.)

The center of an active meristem contains a cluster of relatively large, highly vacuolate cells called the **central zone**. The central zone is somewhat comparable to the quiescent center of root meristems (which will be discussed later in the chapter). Adoughnut-shaped region of smaller cells, called the **peripheral zone**, flanks the central zone. A **rib zone** lies underneath the central cell zone and gives rise to the internal tissues of the stem.
These different zones most likely represent different developmental domains. The peripheral zone is the region in which the first cell divisions leading to the formation of leaf primordia will occur. The rib zone contributes cells that become the stem. The central zone contains the pool of stem cells, some fraction of which remains uncommitted, while others replenish the rib and peripheral zone populations (Bowman and Eshed 2000).

Leaf development

The leaves of most plants are the organs of photosynthesis. This is where light energy is captured and used to drive the chemical reactions that are vital to the life of the plant.

Although highly variable in size and shape from species to species, in general leaves are thin, flat structures with dorsiventral polarity. This pattern contrasts with that of the shoot apical meristem and stem, both of which have radial symmetry. Another important difference is that leaf primordial exhibit determinate growth, while the vegetative shoot apical meristem is indeterminate. As described in the

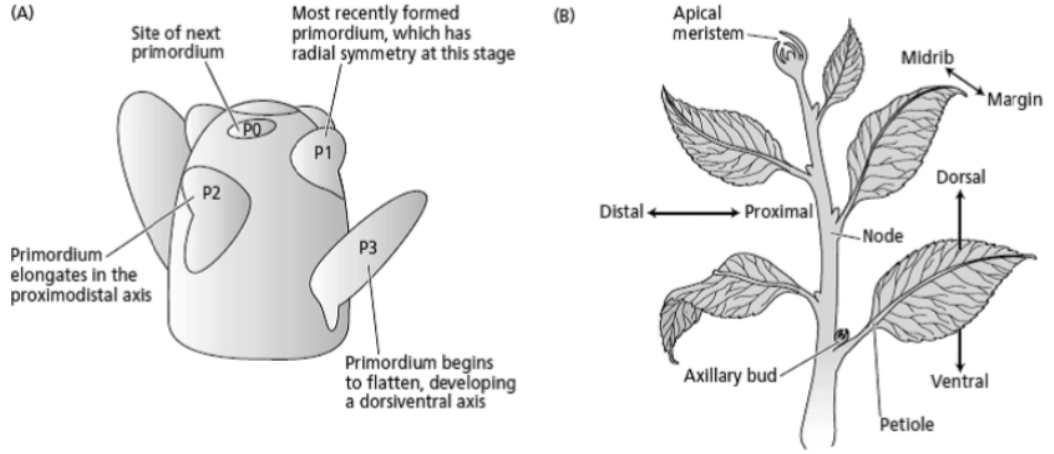


FIGURE 16.15 The origin of leaves at the shoot apex and their axes of symmetry on the stem (A) Leaf primordia in the flanks of the shoot apical meristem. (B) Diagram of a shoot showing the various axes along which development occurs. (After Christensen and Weigel 1998.)

sections that follow, several distinct stages can be recognized in leaf development (Sinha 1999).

Stage 1: Organogenesis. Asmall number of cells in the L1 and L2 layers in the flanks of the apical dome of the shoot apical meristem acquire the **leaf founder cell** identity. These cells divide more rapidly than surrounding cells and produce the outgrowth that represents the **leaf primordium** (plural *primordia*) (Figure 16.15A). These primordial subsequently grow and develop into leaves.

Stage 2: Development of suborgan domains. Different regions of the primordium acquire identity as specific parts of the leaf. This differentiation occurs along three axes: **dorsiventral** (abaxial–adaxial), **proximodistal** (apical–basal), and **lateral** (margin–blade–midrib) (Figure 16.15B). The upper (adaxial) side of the leaf is specialized for light absorption; the lower (abaxial) surface is specialized for gas exchange. Leaf structure and maturation rates also vary along the proximodistal and lateral axes.

Stage 3: Cell and tissue differentiation. As the developing leaf grows, tissues and cells differentiate. Cells derived from the L1 layer differentiate as epidermis (epidermal cells, trichomes, and guard cells), derivatives of the L2 layer differentiate as the photosynthetic mesophyll cells, and vascular elements and bundle sheath cells are derived from the L3 layer. These cells differentiate in a genetically determined pattern that is characteristic of the species but to some degree modified in response to the environment.

The Arrangement of Leaf Primordia Is Genetically Programmed

The timing and pattern with which the primordia form is genetically determined and usually is a characteristic of the species. The number and order in which leaf primordial form is reflected in the subsequent arrangement of leaves around the stem, known as **phyllotaxy** (Figure 16.16).

Root development

Roots are adapted for growing through soil and absorbing the water and mineral nutrients in the capillary spaces between soil particles. These functions have placed constraints on the evolution of root structure. For example, lateral appendages would interfere with their penetration through the soil. As a result, roots have a streamlined axis, and no lateral organs are produced by the apical meristem.

Branch roots arise internally and form only in mature, nongrowing regions. Absorption of water and minerals is enhanced by fragile root hairs, which also form behind the growth zone. These long, threadlike cells greatly increase the root's absorptive surface area.

The Root Tip Has Four Developmental Zones

Roots grow and develop from their distal ends. Although the boundaries are not sharp, four developmental zones can be distinguished in a root tip: the root cap, the meristematic zone, the elongation zone, and the maturation zone (Figure 16.17).

These four developmental zones occupy only a little more than a millimeter of the tip of the *Arabidopsis* root. The developing region is larger in other species, but growth is still confined to the tip. With the exception of the root cap, the boundaries of these zones overlap considerably:

The **root cap** protects the apical meristem from mechanical injury as the root pushes its way through the soil. Root cap cells form by specialized root cap stem cells. As the root cap stem cells produce new cells, older cells are progressively displaced toward the tip, where they are eventually sloughed

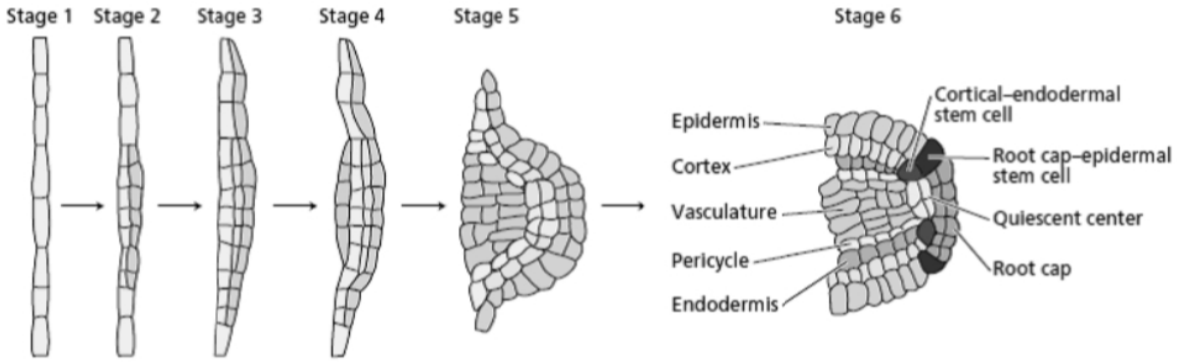


FIGURE 16.18 Model for lateral root formation in *Arabidopsis*. Six major stages are shown in the development of the primordium. The different tissue types are designated by colors. By stage 6, all tissues found in the primary root are present in the typical radial pattern of the branch root. (From Malamy and Benfey 1997.)

off. As root cap cells differentiate, they acquire the ability to perceive gravitational stimuli and secrete mucopolysaccharides (slime) that help the root penetrate the soil.

• The **meristematic zone** lies just under the root cap, and in *Arabidopsis* it is about a quarter of a millimeter

long. The root meristem generates only one organ, the primary root. It produces no lateral appendages.

• The **elongation zone**, as its name implies, is the site of rapid and extensive cell elongation. Although some cells may continue to divide while they elongate within this zone, the rate of division decreases progressively to zero with increasing distance from the meristem.

• The **maturation zone** is the region in which cells acquire their differentiated characteristics. Cells enter

the maturation zone after division and elongation have ceased. Differentiation may begin much earlier, but cells do not achieve the mature state until they reach this zone. The radial pattern of differentiated tissues becomes obvious in the maturation zone. Later in the chapter we will examine the differentiation and maturation of one of these cell types, the tracheary element.

As discussed earlier, lateral or branch roots arise from the pericycle in mature regions of the root. Cell divisions in the pericycle establish secondary meristems that grow out through the cortex and epidermis, establishing a new growth axis (Figure 16.18). The primary and the secondary root meristems behave similarly in that divisions of the cells in the meristem give rise to progenitors of all the cells of the root.

Cell differentiation

Differentiation is the process by which a cell acquires metabolic, structural, and functional properties that are distinct from those of its progenitor cell. In plants, unlike animals, cell differentiation is frequently reversible, particularly when differentiated cells are removed from the plant and placed in tissue culture. Under these conditions, cells dedifferentiate (i.e., lose their differentiated characteristics), reinitiate cell division, and in some cases, when provided with the appropriate nutrients and hormones, even regenerate whole plants.

This ability to dedifferentiate demonstrates that differentiated plant cells retain all the genetic information

required for the development of a complete plant, a property termed **totipotency**. The only exceptions to this rule are cells that lose their nuclei, such as sieve tube elements of phloem, and cells that are dead at maturity, such as vessel elements and tracheids (collectively referred to as tracheary elements) in xylem.

Initiation and regulation of Developmental pathways

Rapid progress has been made in identifying genes that play critical roles in regulating growth, cell differentiation, and pattern formation. This progress is largely a consequence of an intensive, international effort focused on *Arabidopsis*— first to sequence its genome, and subsequently to understand the function of all of its genes. However, many important discoveries have been made as a result of studies with other species, including *Antirrhinum*, maize, petunia, tomato, and tobacco.

However, many of these developmentally important genes have been found to encode either transcription factors (proteins with the ability to bind to specific DNA sequences and thus control the expression of other genes) or components of signaling pathways. The nature of these genes suggests some possible ways that development might be regulated.

Where these molecular genetic studies have been coupled with clonal analysis, cell biological, physiological,

and/or biochemical studies, it has been possible to identify important principles of plant development. Although we are far from a complete understanding, these insights include the following:

- The expression of genes that encode transcription factors determines cell, tissue, and organ identity.
- The fate of a cell is determined by its position and not its clonal history.
- Developmental pathways are controlled by networks of interacting genes.
- Development is regulated by cell-to-cell signaling.

Transcription Factor Genes Control Development

Transcription factors are proteins that have an affinity for DNA. They are able to turn the expression of genes on or off by binding to specific DNA sequences.

These 1500 transcription factor genes belong to numerous families. However, many members of two of

these families—the MADS box and homeobox genes— have been found to be particularly important in plant development.

MADS box genes are key regulators of important biological functions in plants, animals, and fungi.2 There are about 30 MADS box genes in the *Arabidopsis* genome, many of which control aspects of development. Specific MADS box genes are important for developmental events in the root, leaf, flower, ovule, and fruit (Riechmann and Meyerowitz 1997).

Homeobox genes encode homeodomain proteins that act as transcription factors. **Homeodomain proteins** play a major role in regulating developmental pathways in all eukaryotes. As with the MADS box genes, each homeobox gene participates in regulating a unique developmental event by controlling the expression of a unique set of target genes.

Many Plant Signaling Pathways Utilize Protein Kinases

Protein kinases are ATP-dependent enzymes that add phosphate groups to proteins. Protein phosphorylation is a key regulatory mechanism that is utilized extensively to regulate the activity of enzymes and transcription factors. Although widely utilized by all eukaryotes, plant genomes are especially rich in genes that encode these enzymes. The *Arabidopsis* genome contains over 1200

genes that encode protein kinases. Of these, more than 600 encode *receptor protein kinases* (Shiu and Bleecker 2001).

The functions of most of these receptor protein kinases are unknown, but recently some have been shown to play important signaling roles in plant development. *Arabidopsis* has two such genes: *BRI1*, which encodes a receptor kinase that functions in brassinosteroid signaling and *CLAVATA1* (*CLV1*), which encodes a

receptor kinase that participates in regulating the size of the uncommitted cell population in shoot apical meristem.

A Cell's Fate Is Determined by Its Position

In both the root and shoot meristem, a small number of stem cells are the ultimate source of any particular tissue, and most of the cells in a given tissue are clonal, having arisen. Inappropriate expression of the *KN1* gene during leaf development causes severe abnormalities around the leaf veins. The gain-of-function mutation *kn1*causes cell proliferation after normal cell division ceases; in addition, the division planes are abnormal, causing gross distortion of the blade surface.

However, most evidence supports the view that *cell fate does not depend on cell lineage, but instead is determined by positional information* (Scheres 2001). In the vast majority of cases, shoot epidermal cells are derived from a small number of stem cells in the L1 layer. However, the derivatives of the L1 layer are committed to become epidermal cells because they occupy the outermost layer and lie on top of the cortical cell layer, not because they were clonally derived from the stem cells in the L1 layer.

The plane in which a cell divides will determine the position of its daughter cells within a tissue, and this positioning in turn plays the most significant role in determining the fate of the daughter cells. The strongest evidence for the importance of position in determining a cell's ultimate fate comes from an examination of the fate of cells that are displaced from their usual position, such that they come to occupy a different layer.

Developmental Pathways Are Controlled by Networks of Interacting Genes

We have a great deal more to learn about the regulatory networks that control developmental pathways. However, several discoveries point to a model in which local and long-distance signaling events control the expression of genes that encode transcription factors. These transcription factors in turn determine the character or activities of a given tissue or cell. Often these mechanisms involve feedback loops in which two or more genes interact to regulate each other's expression. These interactions are seen most clearly in the case of the shoot apical meristem.

Expression of the *KNOX* gene *STM* (*SHOOTMERISTEMLESS*) is essential for the formation of the shoot apical meristem in the *Arabidopsis* embryo and for meristem function in the growing plant. *STM* is expressed throughout the apical dome of the vegetative meristem, except in the developing leaf primordia. Similarly, *STM* is expressed in the dome of the floral meristem, but it is silenced as floral organs appear. Two additional *KNOX* genes—*KNAT1* and *KNAT2*—also are expressed in the apical meristem of *Arabidopsis* and participate in maintaining the meristem cells in an undifferentiated state.

Development Is Regulated by Cell-to-Cell Signaling

How do cells know where they are? If a cell's fate is determined by its position and not by clonal lineage, then cells must be able to sense their position relative to other cells, tissues, and organs. Neighboring cells and distant tissues and organs provide positional information. Cells in multicellular plants usually are in close contact with others around them, and the behavior of each cell is carefully

coordinated with that of its neighbors throughout the life of the plant. Furthermore, each cell occupies a specific position within the tissue and organ to which it belongs.

Coordination of cellular activity requires cell–cell communication. That is, some developmentally important genes act *nonautonomously*. They do not have to be expressed in a given cell to affect the fate of that cell. Agiven gene or set of genes can exert an effect on development in neighboring cells or even cells in distant tissues through cell–cell communication, via at least three different mechanisms:

1. Ligand-induced signaling
2. Hormonal signaling
3. Signaling via trafficking of regulatory proteins and/or mRNAs

Other signaling mechanisms remain to be discovered.

The mechanism by which cells communicate has not been established in other cases, although it is clear that positional information is exchanged between cells in different tissues.

As presented earlier, the *SHR* and *SCR* genes are important for the establishment of the radial tissue patterns in roots. They encode rather similar transcription factors, but these two genes are expressed and function in different tissues. *SCR* is required for the asymmetric cell division that forms the epidermis and cortex, and it also determines the endodermis cell fate. *SCR* is expressed in the stem cell that will give rise to the ground tissue before it divides asymmetrically to form the precursors of endodermis and cortex.

The analysis of plant growth

How do plants grow? This deceptively simple question has challenged plant scientists for more than 150 years. New cells form continually in the apical meristems. Cells enlarge slowly in the apical meristem and more rapidly in the subapical regions. The resulting increase in cell volume can range from severalfold to 100-fold, depending on the species and environmental conditions. Classically, plant growth has been analyzed in terms of cell number or overall size (or mass).

However, these measures tell only part of the story. Tissue growth is neither uniform nor random. The derivatives of the apical meristems expand in predictable and site-specific ways, and the expansion patterns in these subapical regions largely determine the size and shape of the primary plant body. The total growth of the plant can be thought of as the sum of the local patterns of cell expansion.

The analysis of the motions of cells or "tissue elements" (and the related problem of cell expansion) is called *kinematics*. In this section we will discuss both the classical definitions of growth and the more modern, kinematic approach. As we will see, the advantage of the kinematic approach is that it allows one to describe the growth patterns of organs mathematically in terms of the expansion patterns of their component cells.

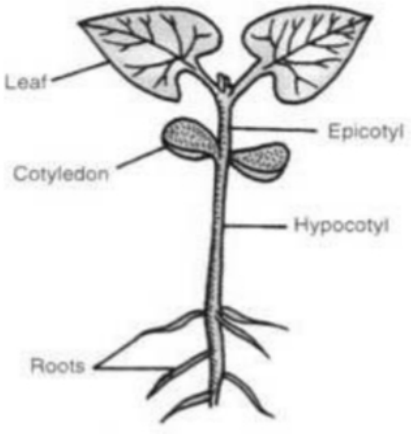


Fig. 3.3(*b*). Epicotyl and Hypocotyl.

Seed Germination:

Germination is a process by which the embryo in the seed becomes activated and begins to grow into a new seedling.

Types of Germination:

There are two types of germination:

1. Epigeal Germination:

In this type of germination, the hypocotyl elongates rapidly and arches upwards pulling the cotyledons which move above the soil. Bean, cotton, papaya, gourd, castor and onion have germination of this kind.

2. Hypogeal Germination:

In this type of germination, the epicotyl elongates and the cotyledons remain below the soil. Pea, mango, maize, rice, gram and groundnut have germination of this kind.

Epicotyl → The upper portion of the axis of the seedling above the cotyledons.

Hypocotyl → The portion of seedling between the cotyledons and the radicle.

3. Vivipary (Viviparous Germination):

Vivipary is the phenomenon of giving birth to young ones in advanced stage of development. It occurs in mammals (among animals) and mangrove plants. In mangrove plants (e.g., Rhizophora, Sonneratia, Heritiera) the seeds cannot germinate on the ground because of the excessive salt content and lack of oxygen in marshy habitat. In such plants seed dormancy is absent.

The embryo of the seed (present inside the fruit) continues growth while the latter is attached to the parent plant. Hypocotyl elongates and pushes the radicle out of the seed and the fruit. Growth continues till the hypocotyl and radicle become several centimetres long (more than 70 cm in Rhizophora). The seedling becomes heavy.

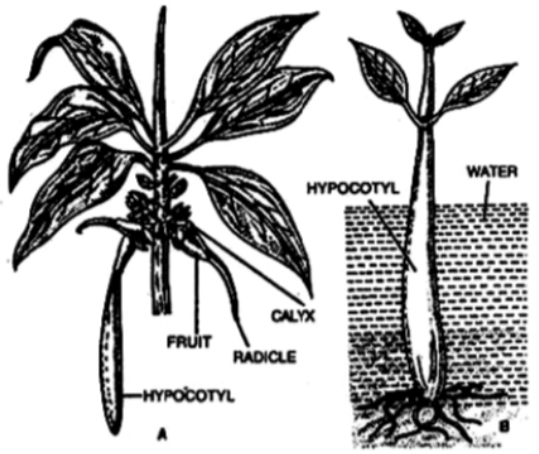


Fig. 4.9. Vivipary. A. twig of *Rhizophora* showing viviparous germination, B. A seedling has become established on tidal soil.

7. Photosynthesis: Light reactions, organization of light absorbing system, mechanism of electron and proton transport, Carbon concentrating mechanisms.

Photosynthesis literally means 'synthesis with the help of light'. It is the process that gives life to all living beings. The plants convert light energy into life energy. It is the only biological process that makes use of sun's light energy for driving the life machinery. Hence, photosynthesis is regarded as ' leader ' of all processes both biological and abiological. It is

the most fundamental of all biochemical reactions by which plants synthesize organic compounds in the chloroplast from carbon dioxide and water with the help of sunlight. It is an oxidation-reduction reaction between water and carbon dioxide.

Significance of photosynthesis:

Photosynthesis is a source of all our food and fuel. It is the only biological process that acts as the driving vital force for the whole animal kingdom and for the non-photosynthetic organism. It drives all other processes of biological and abiological world. It is responsible for the growth and sustenance of our biosphere. It provides organic substances, which are used in the production of fats, proteins, nucleoproteins, pigments, enzymes, vitamins, cellulose, organic acids, etc. Some of them become structural parts of the organisms. It makes use of simple raw materials such as CO_2, H_2O and inexhaustible light energy for the synthesis of energetic organic compounds. It is significant because it provides energy in terms of fossil fuels like coal and petrol obtained from plants, which lived millions and millions of years ago. Plants, from great trees to microscopic algae, are engaged in converting light energy into chemical energy, while man with all his knowledge in chemistry and physics cannot imitate them.

Site of photosynthesis:

Chloroplasts are the actual sites for photosynthesis. All green parts of a plant are involved in photosynthesis. Leaves are the most important organs of photosynthesis. In xerophytes like *Opuntia*, the stem is green and it performs photosynthesis. Over half a million chloroplasts are present in one square millimeter of a leaf. It measures about 4 to 6 micron. A typical chloroplast of higher plants is discoid shaped. It is a double membrane bound organelle containing chlorophyll, carotenoid, xanthophyll, cytochrome, DNA, RNA, manganese, etc. Chloroplasts are generally considerably larger than mitochondria. The space enclosed by the envelope is filled with matrix called stroma.

In the stroma, many grana are embedded. In each granum, several disc shaped lamellae are found. These disc shaped structures are called thylakoids. They resemble a stack of coins. This structure is known granum. Generally a chloroplast contains 40 to 60 grana. The photosynthetic pigments are found in grana. The stroma contains circular DNA, RNA and enzymes for starch synthesis.

Photochemical and biosynthetic phases:

The pigments involved in photosynthesis are called photosynthetic pigments. They are chlorophyll ‘a’, chlorophyll ‘b’, carotenoids, xanthophyll and phycobilins. Magnesium is an essential component for the formation of chlorophyll. Chlorophyll ‘a’ is a universal pigment present in the plants in which water is one of the raw materials for photosynthesis. Chlorophylls are highly efficient in absorbing solar energy and they are directly linked to photosynthetic electron transport. Photosynthetic pigments other than chlorophyll ‘a’ are generally called accessory pigments e.g. Chlorophyll ‘b’, carotenoids and xanthophyll, whereas chlorophyll ‘a’ is regarded as primary pigment. Photosynthetic pigments occur in the granum. They constitute the pigment system called photosystem. About 250 to 400 pigment molecules are present in a photosystem. Two types of photosystems are found in the granum. Photosystem I (PS I) has less accessory pigments and more

Chlorophyll ‘a’, while photosystem II (PS II) has more accessory pigments and less chlorophyll ‘a’. The primary function of photosystems is to trap light energy and converts it to chemical energy. The energy absorbed by accessory pigments is transferred to the chlorophyll ‘a’. The grana lamella where the photosynthetic pigments are aggregated to perform photosynthetic activities is called active center.

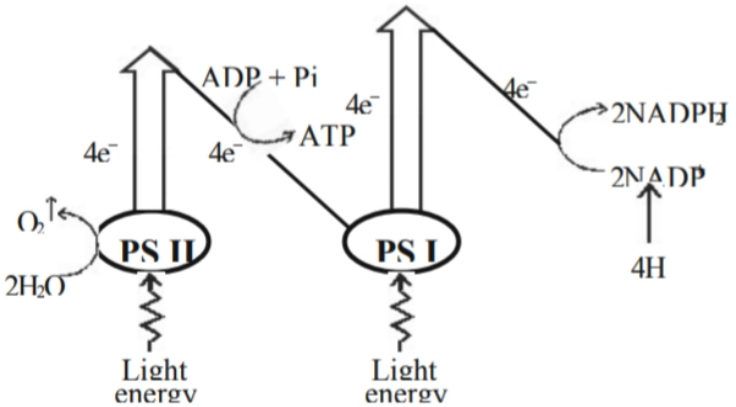
Mechanism of photosynthesis:

The overall reaction of photosynthesis can be written as follows.

$$CO_2 + 2H_2O \xrightarrow[Chlorophyll]{Solar\ energy} (CH_2O)_n + H_2O + O_2$$

The reactions of photosynthesis can be grouped into two – light reactions and dark reactions. The reactions involving pigments, solar energy and water that produce ATP and $NADPH_2$, are called light reactions. The photosynthetic reactions in which CO_2 is reduced to carbohydrates making use of ATP and $NADPH_2$ generated by light reactions are collectively called dark reactions.

The overall process of photosynthesis is illustrated in the following Fig.1 and Fig.2.



Electron transport system:

The light driven reactions of photosynthesis are referred to as electron transport chain. When PS II absorbs photons of light, it is excited and the electrons are transported through electron transport chain of plastoquinone, cytochrome b_6, cytochrome f and plastocyanin. The electrons released from PS II phosphorylate ADP to ATP. This process of ATP formation from ADP in the presence of light in chloroplast is called photophosphorylation.

Now, the PS II is in oxidised state. It creates a potential to split water molecules to protons, electrons and oxygen. This light dependent splitting of water molecules is called photolysis of water. Manganese, calcium and chloride ions play prominent roles in the photolysis of water. The electrons thus released are used in the reduction of PS II. Similar to PS II, PS I is excited by absorbing photons of light and gets oxidised. This oxidised state of the PS I draws electrons from PS II and gets reduced. The electrons released to PS I are transported through electron transport chain of ferredoxin reducing substrate, ferredoxin and ferredoxin NADP reductase to reduce $NADP^+$ to $NADPH_2$.

Cyclic and noncyclic photophosphorylation:

In chloroplasts, phosphorylation occurs in two ways – noncyclic photophosphorylation and cyclic photophosphorylation.

- **Noncyclic photophosphorylation**

When the molecules in the PS I are excited the electrons are released. So, an electron deficiency or a hole is made in the PS I. This electron is now transferred to ferredoxin to reduce $NADP^+$. When the molecules in the PS II get excited, electrons are released. They are transferred to fill the hole in PS I through plastoquinone, cytochrome b_6, cytochrome f and plastocyanin. When the electron is transported between plastoquinone and cytochrome f, ADP is phosphorylated to ATP.

The 'hole' in the PS I has been filled by the electron from PS II. Then the electrons are transferred from PS I to NADP for reduction. Therefore, this electron transport is called noncyclic electron transport and the accompanying phosphorylation as noncyclic photophosphorylation. The noncyclic electron transport takes place in the form of 'Z'. Hence, it is also called Z-scheme. ***Fig. 3.***

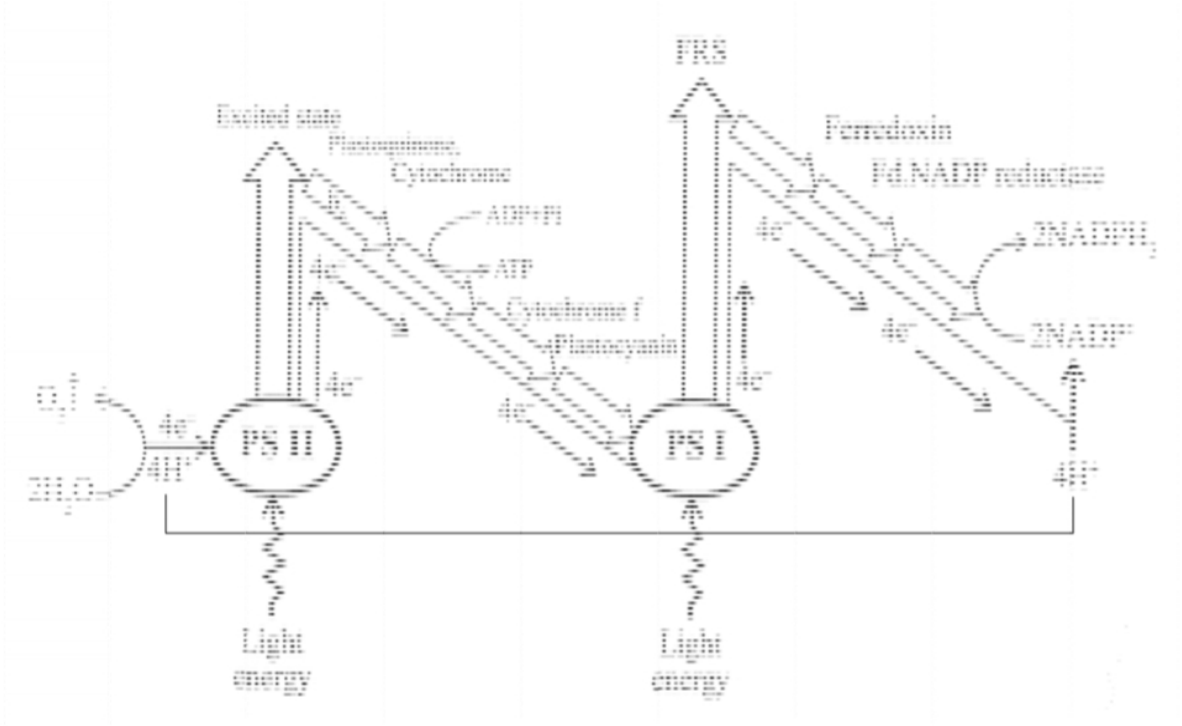
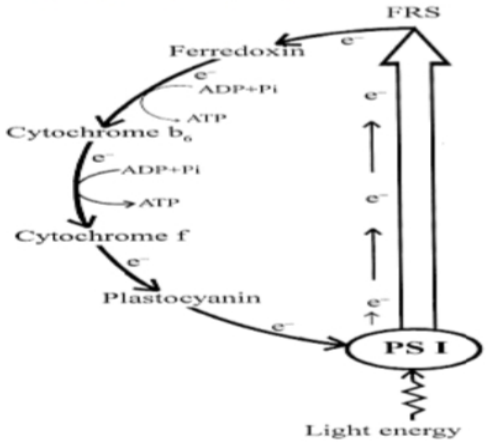


Fig. 3. Non-cyclic photophosphorylation

❖ **Cyclic photophosphorylation**



Under the conditions of (i) PS I only remains active (ii) photolysis of water does not take place (iii) requirement of ATP is more and (iv) no availability of NADP+ the cyclic photophosphorylation takes place. When the molecule in the PS I is excited, the electrons are released. The electrons are captured by ferredoxin through ferredoxin reducing substrate (FRS). Due to non-availability of NADP+, electrons from ferredoxin fall back to the molecules of PS I through the electron carrier's cytochrome b_6, cytochrome f and plastocyanin. These electron carriers facilitate the downhill transport of electrons from FRS to PS I.

Fig. 4. Cyclic photophosphorylation

Difference between cyclic and noncyclic electron transport and photophosphorylation

Cyclic photophosphorylation	Noncyclic photophosphorylation
1. It is associated with PS I	1. It is associated with both PS I and PS II.
2. The electron expelled from chlorophyll molecule is cycled back	2. The electrons are not cycled back but compensated by the electrons From photolysis of water.
3. Photolysis of water and evolution of oxygen do not take place.	3. Photolysis of water and evolution of oxygen take place.
4. Photophosphorylation takes place at two places.	4. Photophosphorylation takes place only at one place.
5. NADPis not reduced.	5. NADP+ is reduced to $NADPH_2$.

C_3 Cycle:

The enzymatic reduction of CO_2 by these reactions is also known as carbon fixation. These reactions that result in CO_2 fixation take place in a cyclic way and were discovered by Melvin Calvin. Hence, the cycle is called **Calvin cycle.**

Fixation of carbon dioxide in plants during photosynthesis occurs in three stages – fixation, reduction and regeneration of RuBP.

- **Fixation**

The acceptor molecule of CO_2 is a 5C compound called ribulose-1,5bisphosphate (RuBP). Fixation of a molecule of CO_2 to RuBP is catalyzed by the enzyme RuBP carboxylase. The resulting 6C compound is highly unstable and gets cleaved to form two molecules of 3C compounds called phosphoglyceric acid (PGA).

$$RuBP + CO_2 \xrightarrow{RuBP\,Carboxylase} 2\,moles\,of\,PGA$$

- **Reduction**

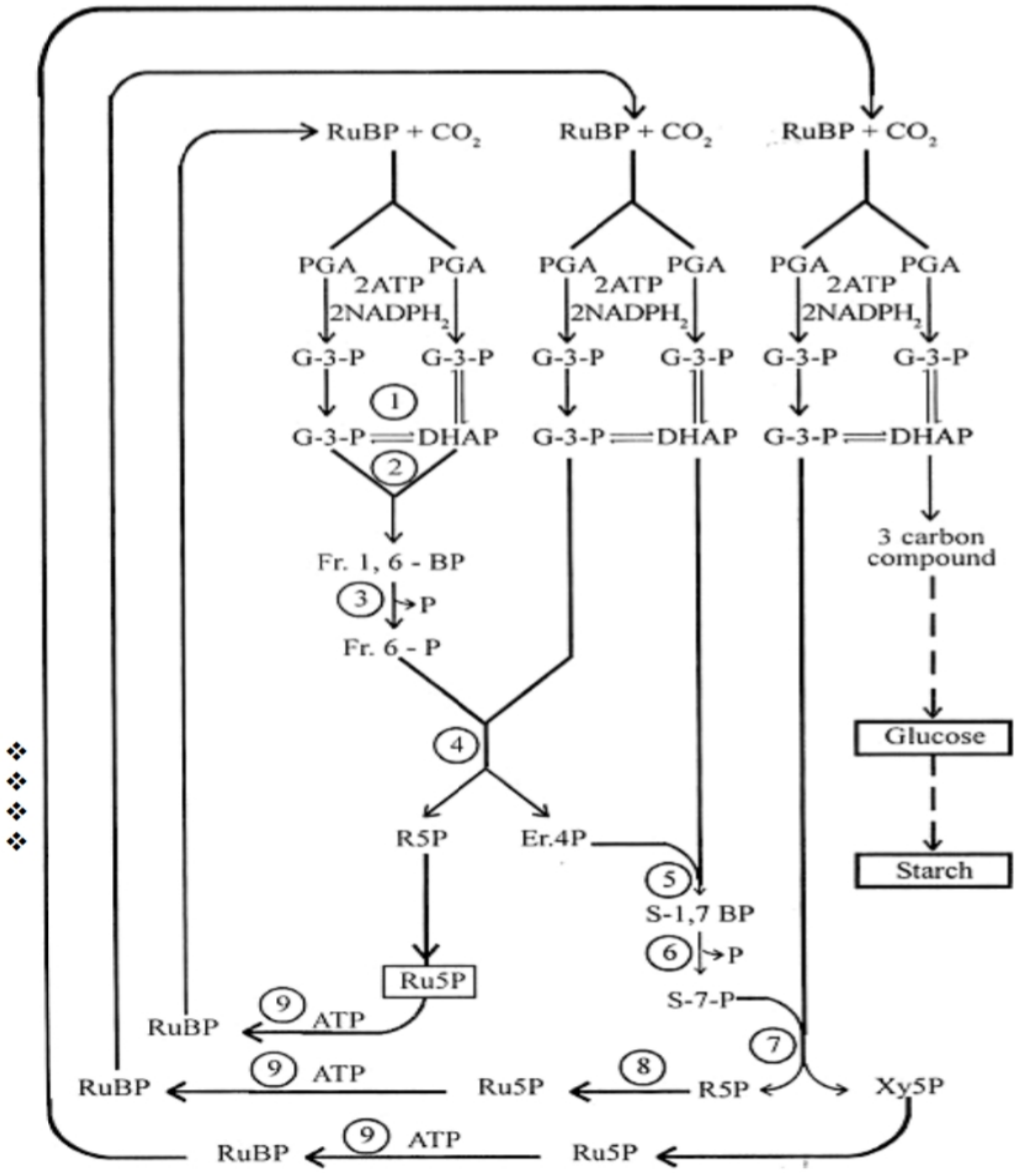
The two molecules of PGA are further reduced to glyceraldehyde-3phosphates in two steps. First, two PGA molecules are converted to 1, 3 -bisphosphoglyceric acids by the enzyme PGA kinase. This reaction consumes two molecules of ATP in the ratio of one ATP for each molecule of 1,3-bisphosphoglyceric acid formed.

In the second step, the two molecules of 1,3-bisphosphoglyceric acid are reduced to glyceraldehyde-3-phosphates by the enzyme glyceraldehyde3-phosphate dehydrogenase with the help of the light generated reducing

Fig. 5. Calvin cycle power $NADPH_2$. So, two molecules of $NADPH_2$ will be consumed during this reaction. To reduce one molecule of CO_2 up to reduction two ATP and two $NADPH_2$ are consumed.

$$2\,moles\,of\,PGA + 2\,ATP \xrightarrow{PGA\,kinase} 2\,moles\,1,3-bisphospho-glyceric\,acid$$

$$2\,moles\,1,3-bisphospho\,glyceric\,acid + 2NADPH_2 \xrightarrow{\substack{G-3-P\\ dehydrogenase}} 2\,moles\,G-3-P$$



❖ **Regeneration of RuBP**

The glyceraldehyde 3-phosphate molecules are converted to RuBP through a series of reactions, which generate 4C, 6C and 7C phosphorylated compounds as intermediates. For better and easy understanding of these reactions, a simplified scheme of Calvin cycle considering three CO_2 molecules fixation reactions is shown below.

The reactions of regeneration of RuBP are as follows.

1. Some of the Glyceraldehyde 3-phosphate molecules are converted to dihydroxyacetone phosphates.
2. Glyceraldehyde 3-phosphate combines with dihydroxyacetone phosphate to form fructose1,6-bisphosphate.
3. Fructose 1,6-bisphosphate undergoes dephosphorylation to form fructose 6-phosphate.
4. Fructose 6-phosphate combines with glyceraldehyde 3-phosphate obtained from the fixation of second molecule of CO_2 to form Ribose 5-phosphate (R5P) and Erythrose 4-phosphate (Er4P).
5. Erythrose 4-phosphate combines with DHAP obtained from the second CO_2 fixation, to form sedoheptulose 1,7-bisphosphate.
6. Sedoheptulose 1,7-bisphosphate undergoes dephosphorylation to form sedoheptulose 7-phosphate.
7. Sedoheptulose 7-phosphate combines with glyceraldehyde 3-phosphate obtained by the third CO_2 fixation, to form two molecules of 5C compounds – ribose 5-phosphate and xylulose 5-phosphate (Xy5P).
8. Ribose 5-phosphate and xylulose 5-phosphate molecules are transformed to ribulose 5-phosphate (Ru5P).
9. Ru5P molecules are then phosphorylated by ATP to form RuBP molecules, which again enter into the cycle of CO_2 fixation.

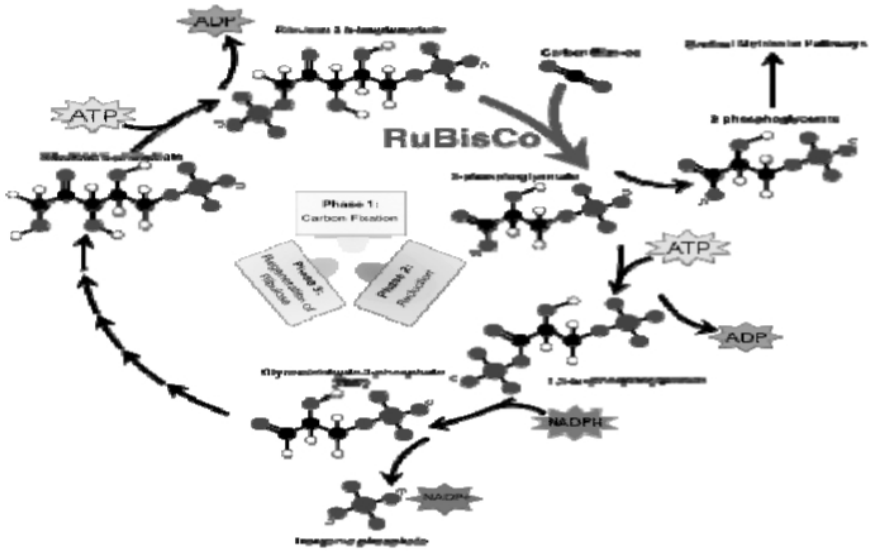


Fig.6. C_3 cycle

$$RuBP + CO_2 \xrightarrow{2ATP + 2NADPH_2} 2\,moles\,of\,G-3-P$$

$$Ru5P \xrightarrow{ATP} RuBP$$

In the above illustration, three CO_2 molecules are fixed and the net gain is a 3C called DHAP. These triose phosphate molecules combine to form hexose phosphates, which are used to form sucrose. For every carbon fixation 3ATP and 2 $NADPH_2$ are consumed.

C_4 Carbon Fixation

C_4 carbon fixation or **the Hatch–Slack pathway** is a photosynthetic process in some plants. It is the first step in extracting carbon from carbon dioxide to be able to use it in sugar and other biomolecules. It is one of three known processes for carbon fixation. "C_4" refers to the four-carbon molecule that is the first product of this type of carbon fixation.

C_4 fixation is an elaboration of the more common C_3 carbon fixation and is believed to have evolved more recently. C_4 overcomes the tendency of the enzyme RuBisCO to wastefully fix oxygen rather than carbon dioxide in the process of photorespiration. This is achieved by ensuring that RuBisCO works in an environment where there is a lot of carbon dioxide and very little oxygen. CO2 is shuttled via malate or aspartate from mesophyll cells to bundle-sheath cells. In these bundle-sheath cells CO2 is released by decarboxylation of the malate. C_4plants use PEP carboxylase to capture more CO2 in the mesophyll cells. PEP (phosphoenolpyruvate, three carbons) binds to CO2 to make oxaloacetic acid (OAA). OAA then makes malate (four carbons). Malate enters bundle sheath cells and releases the CO2. These additional steps, however, require more energy in the form of ATP. Using this extra energy, C_4 plants are able to more efficiently fix carbon in drought, high temperatures, and limitations of nitrogen or CO2. Since the more common C_3 pathway does not require this extra energy, it is more efficient in the other conditions.

The C_4 pathway was elucidated by Marshall Davidson Hatch and C. R. Slack, in Australia, in 1966; it is sometimes called the Hatch-Slack pathway.

In C_3 plants, the first step in the light-independent reactions of photosynthesis involves the fixation of CO2 by the enzyme RuBisCO into 3-phosphoglycerate. However, due to the dual carboxylase and oxygenase activity of RuBisCo, some part of the substrate is oxidized rather than carboxylated, resulting in loss of substrate and consumption of energy, in what is known as photorespiration.

In order to bypass the photorespiration pathway, C_4 plants have developed a mechanism to efficiently deliver CO2 to the RuBisCO enzyme. They use their specific leaf anatomy where chloroplasts exist not only in the mesophyll cells in the outer part of their leaves but in the bundle sheath cells as well. Instead of direct fixation to RuBisCO in the Calvin cycle, CO2 is incorporated into a four-carbon organic acid, which has the ability to regenerate CO2 in the chloroplasts of the bundle sheath cells. Bundle sheath cells can then use this CO2 to generate carbohydrates by the conventional C_3 pathway.

The first step in the pathway is the conversion of pyruvate to phosphoenolpyruvate (PEP), by the enzyme pyruvate orthophosphate dikinase. This reaction requires inorganic phosphate and ATP plus pyruvate, producing phosphoenolpyruvate, AMP, and inorganic pyrophosphate (PP_i).

The next step is the fixation of CO2 into oxaloacetate by the enzyme PEP carboxylase. Both of these steps occur in the mesophyll cells:

$$\text{pyruvate} + P_i + ATP \rightarrow PEP + AMP + PP_i$$

$$PEP + CO2 \rightarrow \text{oxaloacetate}$$

PEP carboxylase has a lower Km for HCO^-_3 — and, hence, higher affinity — than RuBisCO. Furthermore, O_2 is a very poor substrate for this enzyme. Thus, at relatively low concentrations of CO2, most CO2 will be fixed by this pathway.

The product is usually converted to malate, a simple organic compound, which is transported to the bundle-sheath cells surrounding a nearby vein. Here, it is decarboxylated to produce CO2 and pyruvate. The CO2 now enters the Calvin cycle and the pyruvate is transported back to the mesophyll cell.

Since every CO2 molecule has to be fixed twice, first by four-carbon organic acid and second by RuBisCO, the C_4 pathway uses more energy than the C_3 pathway. The C_3 pathway requires 18 molecules of ATP for the synthesis of one molecule of glucose, whereas the C_4 pathway requires 30 molecules of ATP. This energy debt is more than paid for by avoiding losing more than half of photosynthetic carbon in photorespiration as occurs in some tropical plant making it an adaptive mechanism for minimizing the loss.

There are several variants of this pathway:

1. The four-carbon acid transported from mesophyll cells may be malate, as above, or aspartate.
2. The three-carbon acid transported back from bundle-sheath cells may be pyruvate, as above, or alanine.
3. The enzyme that catalyses decarboxylation in bundle-sheath cells differs. In maize and sugarcane, the enzyme is NADP-malic enzyme; in millet, it is NAD-malic enzyme; and, in *Panicum maximum*, it is PEP carboxykinase.

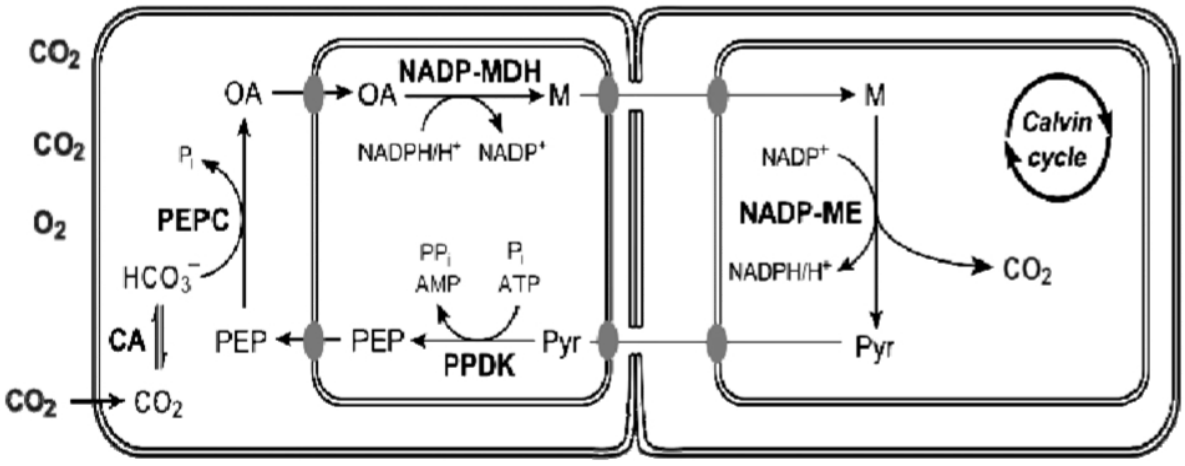


Fig 7. NADP-ME type C_4 pathway

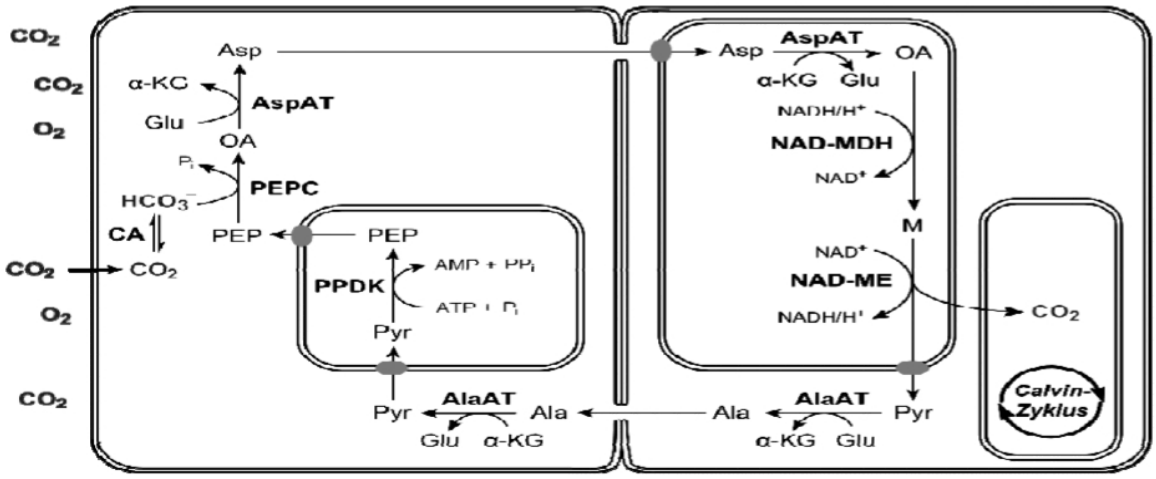


Fig 8. NAD-ME type C_4 pathway

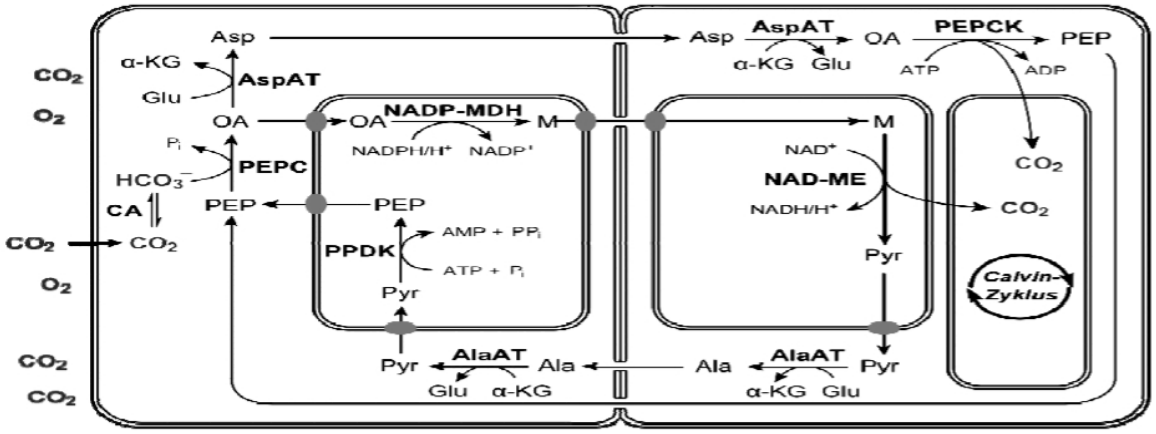


Fig 9. PEPCK type C4 pathway

Kranz anatomy:

The C_4 plants often possess a characteristic leaf anatomy called *kranz anatomy*, from the German word for wreath. Their vascular bundles are surrounded by two rings of cells; the inner ring, called bundle sheath cells, contains starch-rich chloroplasts lacking grana, which differ from those in mesophyll cells present as the outer ring. Hence, the chloroplasts are called dimorphic. The primary function of kranz anatomy is to provide a site in which CO_2 can be concentrated around RuBisCO, thereby avoiding photorespiration. In order to maintain

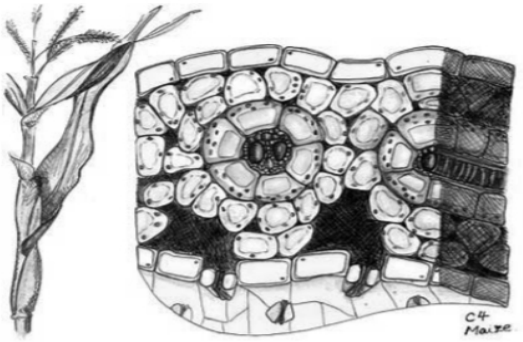


Fig 10. Cross section of a maize leaf, a C4 plant. Kranz anatomy

a significantly higher CO_2concentration in the bundle sheath compared to the mesophyll, the boundary layer of the kranz has a low conductance to CO_2, a property that may be enhanced by the presence of suberin. The carbon concentration mechanism in C_4 plants distinguishes their isotopic signature from other photosynthetic organisms.

Hatch-Slack pathway involves two carboxylation reactions. One takes place in chloroplasts of mesophyll cells and another in chloroplasts of bundle sheath cells.

1. The first step involves the carboxylation of phosphor-enol pyruvic acid in the chloroplasts of mesophyll cells to form a 4C compound, oxaloacetic acid. This reaction is catalyzed by the enzyme phosphor-enol pyruvate carboxylase

$$\underset{\text{pyruvic acid}}{\text{Phosphoenol}} + CO_2 + H_2O \xrightarrow{PEP\ Carboxylase} Oxaloacetic\ acid + \underset{\text{Phosphoric acid}}{H_3PO_4}$$

2. Oxaloacetic acid is converted into aspartic acid by the enzyme transaminase or it may be reduced to malic acid by $NADP^+$ specific malate dehydrogenase.

$$Oxaloacetic\ acid + NH_3 \xrightarrow{Transa\,min\,ase} Aspartic\ acid$$

$$Oxaloacetic\ acid + NADPH_2 \xrightarrow[malate\ dehydrogenase]{NADP^+\ specific} Malic\ acid + NADP^+$$

3. Malic acid or aspartic acid formed in chloroplast of mesophyll cells is transferred to the chloroplasts of bundle sheath where it is decarboxylated to form CO_2 and pyruvic acid in the presence of $NADP^+$ specific malic enzyme.

$$Malic\ acid + NADP^+ \xrightarrow[malic\ enzyme]{NADP^+\ specific} CO_2 + Pyruvic\ acid + NADPH_2$$

4. Now, second carboxylation occurs in chloroplasts of bundle sheath cells. Ribulose bisphosphate accepts CO_2 produced in step (3) in the presence of RuBP carboxylase and yields 3-phosphoglyceric acid. Some of the 3-phosphoglyceric acid molecules are utilized to produce sucrose and starch, while remaining PGA molecules are used for the regeneration of RuBP.

$$RuBP + CO_2 \xrightarrow{RuBP\ carboxylase} 2\,moles[3-phosphoglyceric\ acid]$$

5. The pyruvic acid produced in step (3) is transferred to the chloroplasts of mesophyll cells where it is phosphorylated to regenerate phosphor-enol pyruvic acid .This reaction is catalyzed by pyruvate kinase in the presence of Mg^{2+}.

$$\underset{acid}{Pyruvic} + ATP + Pi \xrightarrow[kinase]{Mg^{2+}\ /\ Pyruvic} \underset{pyruvic\ acid}{Phosphoenol} + AMP + \underset{pyro\ phosphate}{PPi}$$

$$AMP + ATP \overset{\text{Adenylate kinase}}{\rightleftharpoons} 2ADP$$

The AMP is phosphorylated by ATP in the presence of adenylate kinase to form 2 molecules of ADP. C_4 plants are photo synthetically more efficient than C_3 plants, because the net requirement of

ATP and $NADPH_2$ for the fixation of one molecule of CO2 is considerably lower in C4 plants than in C3 plants.

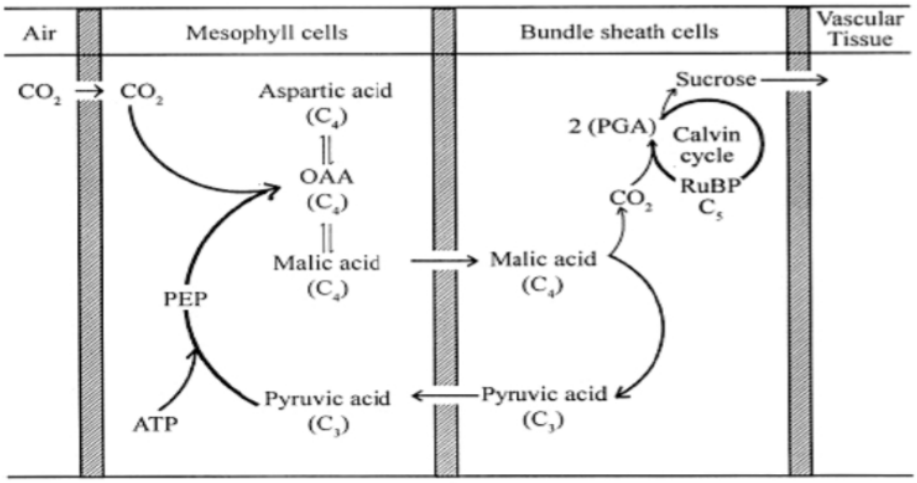


Fig 11. Hatch-Slack pathway of CO_2 fixation

Difference between C_3 and C_4 photosynthetic pathways

	C_3 pathway	C_4 pathway
1.	Photosynthesis occurs in meso - phyll cells.	Photosynthesis occurs in meso - phyll and bundle sheath cells.
2.	The CO_2 molecule acceptor is RuBP.	The CO_2 acceptor molecule is phosphoenol pyruvate.
3.	The first stable product is a 3C compound called 3 – PGA.	The first stable product is a 4C compound called OAA.
4.	Photorespiration rate is high and leads to loss of fixed CO_2. It decreases CO_2 fixation rate.	Photorespiration is negligible and it is almost absent. Hence, it increases CO_2 fixation rate.
5.	Optimum temperature is 20 to 25°C.	Optimum temperature is 30 to 45°C.
6.	Examples of C_3 plants are rice, wheat and potato.	Examples of C_4 plants are maize, sugarcane, Tribulus and Amaranthus

CAM Pathway:

Crassulacean acid metabolism, also known as CAM photosynthesis, is a carbon fixation pathway that evolved in some plants as an adaptation to arid conditions. In a plant using full CAM, the stomata in the leaves remain shut during the day to reduce evapotranspiration, but open at night to collect carbon dioxide (CO2). The CO2 is stored as the four-carbon acid malate in vacuoles at night, and then in the daytime, the malate is transported to chloroplasts where it is converted back to CO2, which is then

used during photosynthesis. The pre-collected CO2 is concentrated around the enzyme RuBisCO, increasing photosynthetic efficiency. The mechanism was first discovered in plants of the family Crassulaceae.

CAM was first suspected by de Saussure in 1804 in his Recherches Chimiques sur la Vegetation, confirmed and refined by Aubert, E. in 1892 in his Recherches physiologiques sur les plantes grasses and expounded upon by Richards, H. M. 1915 in Acidity and Gas Interchange in Cacti, Carnegie Institution. The term CAM may have been coined by Ranson and Thomas in 1940, but they were not the first to discover this cycle. It was observed by the botanists Ranson and Thomas, in the succulent family Crassulaceae (which includes jade plants and Sedum). Its name refers to acid metabolism in Crassulaceae, not the metabolism of "crassulacean acid"

Overview-A two part cycle:

During the night

During the night, a plant employing CAM has its stomata open, allowing CO2 to enter and be fixed as organic acids that are stored in vacuoles. During the day the stomata are closed (thus preventing water loss), and the carbon is released to the Calvin cycle so that photosynthesis may take place.

The carbon dioxide is fixed in the cytoplasm of mesophyll cells by a PEP reaction similar to that of C4 pathway. But, unlike the C4 mechanism, the resulting organic acids are stored in vacuoles for later use; that is, they are not immediately passed on to the Calvin cycle. The latter cannot operate during the night because the light reactions that provide it with ATP and NADPH cannot take place.

During the day

During the day, the CO2-storing organic acids are released from the vacuoles of the mesophyll cells and enter the stroma of the chloroplasts where an enzyme releases the CO2, which then enters into the Calvin cycle.

Benefits

The most important benefit of CAM to the plant is the ability to leave most leaf stomata closed during the day. Plants employing CAM are most common in arid environments, where water comes at a

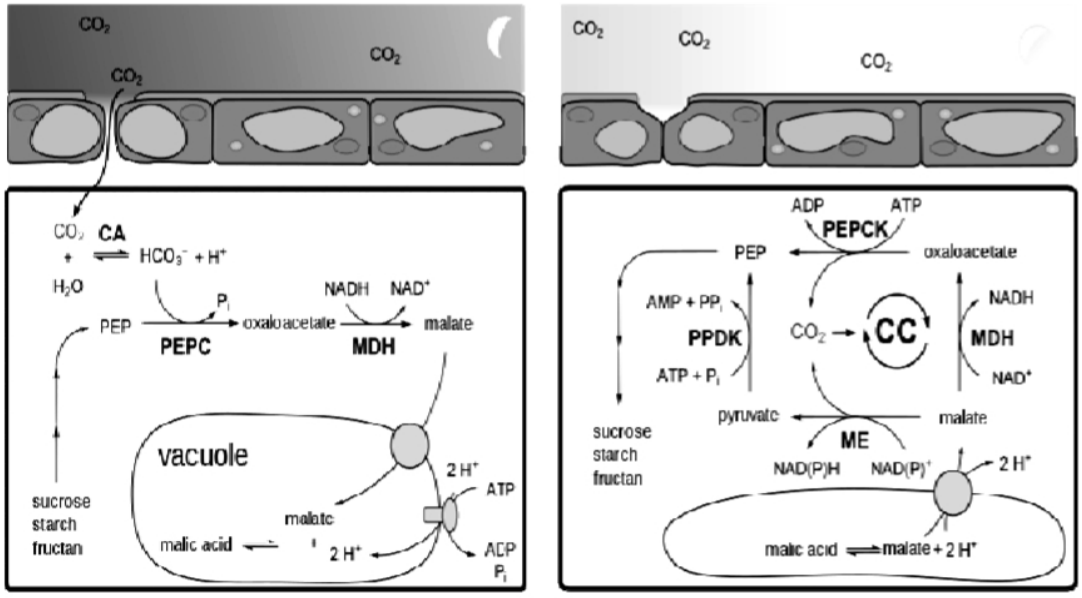


Fig 13. Overview of CAM

premium. Being able to keep stomata closed during the hottest and driest part of the day reduces the loss of water through evapotranspiration, allowing such plants to grow in environments that would otherwise be far too dry. Plants using only C3 carbon fixation, for example, lose 97% of the water they uptake through the roots to transpiration - a high cost avoided by plants able to employ CAM.

Comparison with C_4 metabolism

The C_4 pathway bears resemblance to CAM; both act to concentrate CO_2 around RuBisCO, thereby increasing its efficiency. CAM concentrates it temporally, providing CO_2 during the day, and not at night, when respiration is the dominant reaction. C_4 plants, in contrast, concentrate CO_2 spatially, with a RuBisCO reaction center in a "bundle sheath cell" being inundated with CO_2. Due to the inactivity required by the CAM mechanism, C_4 carbon fixation has a greater efficiency in terms of PGA synthesis.

Photorespiration or C_2 cycle:

In animals and bacteria, only one kind of respiration known as dark respiration occurs. This is not affected by the presence or absence of light. But in certain green plants, there are two distinct types of respiration – photorespiration and dark respiration. Respiration that occurs in photosynthetic tissues in the presence of light and results in increased rate of carbon dioxide evolution is called photorespiration or light respiration.

Photorespiration involves three organelles – chloroplasts, peroxisomes and mitochondria. Oxidation of RuBP in the presence of high oxygen is the first reaction of photorespiration. This reaction is catalyzed by Rubisco* enzyme called carboxylase. It leads to the formation of 2C compound – phosphoglycolic acid and 3C compound PGA. When PGA is used up in the Calvin cycle, the phosphoglycolic acid is dephosphorylated to form glycolic acid in the chloroplasts.

From the chloroplast, the glycolic acid diffuses into the peroxisome where it is oxidised to glyoxalic acid and hydrogen peroxide. In peroxisome from glyoxalic acid, glycine is formed.

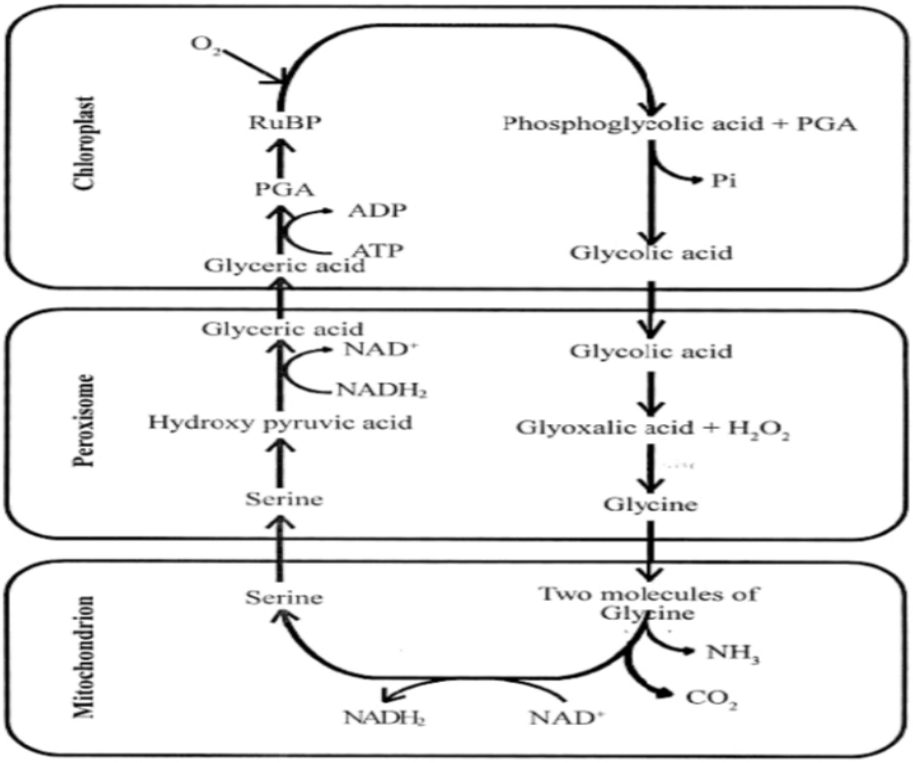


Fig.14. Photorespiratory pathway

Glycine molecules enter into mitochondria where two molecules of glycine combine to give a molecule of serine, NH_3 and CO_2. During this process, NAD^+ is reduced to $NADH_2$.

The amino acid serine is taken to peroxisome where, it is converted into hydroxyl pyruvic acid. Hydroxy pyruvic acid is reduced by $NADH_2$ to form glyceric acid.

The glyceric acid leaves peroxisome and enters chloroplast, where it is phosphorylated to PGA, which enters into Calvin cycle. During the photo respiratory pathway, one CO_2 molecule released in mitochondria is to be re-fixed.

Photorespiration is also known as photosynthetic carbon oxidation cycle or C_2 cycle. Under the conditions of high light and limited CO_2 supply, photorespiration protects the plants from photo oxidative damage. This means that, if enough CO_2 is not available to utilize light energy, excess energy causes damage to plant. However, photorespiration utilizes part of the light energy and saves the plant from photo oxidative damage. Increased O_2 level increases photorespiration whereas increased CO_2 level decreases photorespiration and increases photosynthesis.

Difference between photorespiration and dark respiration

	Photorespiration	Dark respiration
1.	It takes place only in photo synthetic cells in the presence of light.	It takes place in all living cells in the mitochondria.
2.	It is light dependent	It takes place in the presence and in the absence of light.
3.	It is the function of chloroplast, peroxisomes and mitochondria.	It is the function of mitochondria alone.

8. Cell wall structure, biogenesis; membrane transport processes; solute transport and photo assimilate translocation

Structure of Cell Wall:

A cell wall can have upto three parts— middle lamella, primary wall and secondary wall.

Middle Lamella It is a thin, amorphous and cementing layer between two adjacent cells. Middle lamella is the first layer which is depos-ited at the time of cytokinesis. It is just like brick work of the common wall be-tween two adjacent rooms.

Middle lamella is absent on the outer side of surface cells. It is made up of calcium and magnesium pectates. The softening of ripe fruits is caused by partial solubilisation of pectic compounds to produce jelly-like consistency.

Primary Wall:

It is the first formed wall of the cell which is produced inner to the middle lamella. The primary wall is com-monly thin (0.1-3.0 µm) and capable of exten-sion. It grows by intussusceptions or addition of materials within the existing wall. Some cells possess only primary wall, e.g., leaf cells, fruit cells, of cortex and pith.

Primary wall consists of a number of micro fibrils embedded in the amorphous gel like matrix or ground substance. In the majority of plants, the micro fibrils are formed of cellulose. They are synthesized at plasma membrane by particle rosettes (terminal com-plexes) having cellulose synthetize enzyme.

The wall is made of a polymer of P, 1-4 acetyl glucosamine or fungus cellulose in many fungi. Fungus cellulose is similar to chitin present in the exoskeleton of insects. Micro fibrils are oriented variously according to the shape and thickening of the wall. Usually they are arranged in a loose network due to incomplete cross-linking.

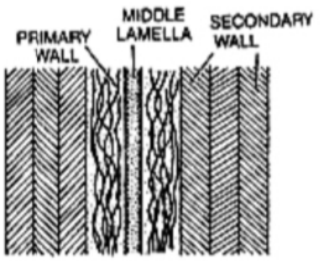


Fig. 8.14. Arrangement of microfibrils in the common wall between two adjacent cells as seen in L.S.

The matrix of the wall consists of water, pectin, hemicelluloses and glycoproteins. Pectin is the filler substance of the matrix. Proteins are structural and enzymatic. Protein expansin (Me Queen-Mason et al, 1992) is involved in loosening and expansion of cell wall through incorporation of more cellulose. Hemicellulose binds micro fibrils with matrix.

Secondary Wall:

It is produced in some mature cells when the latter have stopped growth, e.g., tracheids, vessel elements, fibres, collenchyma's. Secondary wall is laid inner to the primary wall by accretion or deposition of materials over the surface of existing structure. It is thick (3—10 µm) and made up of at least three layers, sometimes more (e.g., latex tube of Euphorbia milli). They are named as S1, S2, S3, Sx, etc.

The innermost layer of the secondary wall is sometimes distinct both chemically as well as in staining properties due to the presence of xylans. It is then called tertiary wall, e.g., tension wood in gymnosperms. Secondary wall may be absent, irregularly deposited or formed uniformly in the cells. This results in differentiation of cells— parenchyma, collenchyma, sclerenchyma, tracheids and vessels.

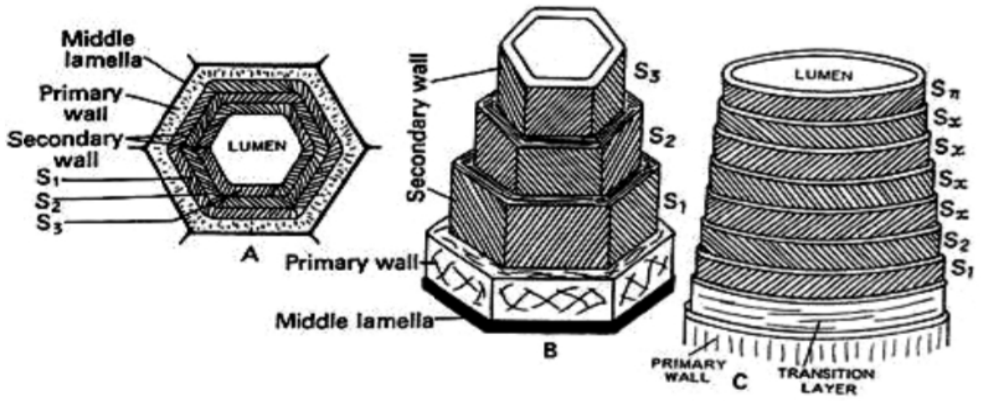


Fig. 8.15. Parts and layers of cell wall. A, a cell in T.S. showing parts of cell wall. B, typical wood fibre cut at various levels to show parts and layers of the wall. C, latex tube of *Euphorbia milli* (= *E. splendens*) cut at various levels to show parts.

The composition of secondary wall is basically similar to the primary wall in having cellulose micro-fibrils embedded in a matrix of pectin and hemicelluloses. Cellulose mi-cro-fibrils of the secondary wall lie close, parallel and at an angle to the longitudinal axis of the cell. Their orientation is different in the different layers of the secondary wall. A number of different materials may be deposited in the wall.

The important ones are:

(a) Lignin:

It reduces the water content of the wall matrix and increases its hardness. However, water permeability is not affected. The characteristic of lignification's (and cutinisation) has evolved with the evolution of land plants.

(b) Suberin:

The wall of cork and endodermal cells contains a special fatty substance called suberin. Suberin makes the walls impermeable.

(c) Cutin:

The epidermal cells possess another fatty substance called cutin. Cutin is also laid as a distinct layer on the outside of the epidermal cell walls. It is known as cuticle. Cutin reduces the rate of epidermal or surface transpiration. Other substances which can be deposited in the cell wall are silica (e.g., grasses), minerals, waxes, tannins, resins, gums, etc.

Plasmodesmata:

Plasmodesmata (singular- plasmodesma; Tangl, 1879; Strasburger, 1901) are cytoplasmic bridges between adjacent plant cells which develop in the minute pores of their walls. They form a protoplasmic continuum called symplast. Cell wall and intercellular spaces form a non-living component of the plant body called apoplasm. A plasmoderma is 40—50 nm in diameter. It may be simple or branched.

Plasmodesma is lined by plasma membrane. It encloses tubular extention of endoplasmic reticulum called desmotubule The space between desmotubule and plasma membrane contains 8-10 microchannels. Plasmodesmata

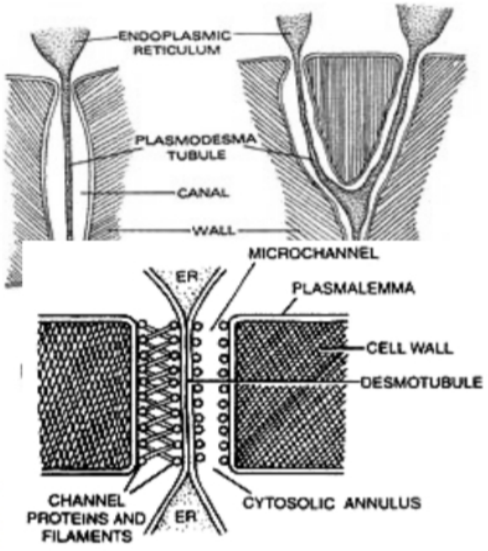


Fig. 8.17. Components of plasmodesmata.

form channels for controlled passage of small sized particles between adjacent cells as well as transfer of some specific signals.

Pits:

Pits are un-thickened areas in the secondary walls of plant cells. They, therefore, appear as depressions. Pits generally occur in pairs on the wall of two adjacent cells. A pit has a cavity or pit chamber and a pit membrane.

The pit membrane consists of primary wall and middle lamella. Pits are of two types, simple and bordered. Simple pit has uniform width of the pit chamber. In bordered pit, the pit chamber is flask-shaped because the secondary wall overarches its mouth.

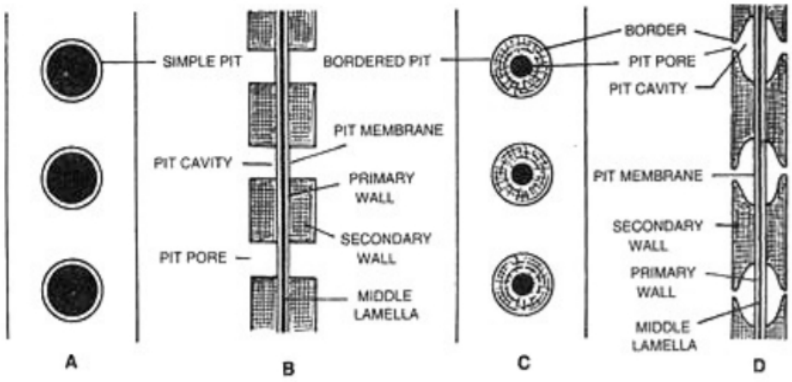


Fig. 8.18. Pits. A, surface view of simple pits. B, simple pit pairs in section. C, surface view of bordered pits. D, bordered pit pairs in section.

Pit membrane is permeable. It may have minute sub-microscopic pores. Therefore, pits help in rapid translocation between two adjacent cells.

Cell wall biogenesis:

The middle lamella is laid down first, formed from the cell plate during cytokinesis, and the primary cell wall is then deposited inside the middle lamella. The actual structure of the cell wall is not clearly defined and several models exist - the covalently linked cross model, the tether model, the diffuse layer model and the stratified layer model. However, the primary cell wall, can be defined as composed of cellulose microfibrils aligned at all angles. Cellulose microfibrils are produced at the plasma membrane by the cellulose synthase complex, which is proposed to be made of a hexameric rosette that contains three cellulose synthase catalytic subunits for each of the six units. Microfibrils are held together by hydrogen bonds to provide a high tensile strength. The cells are held together and share the gelatinous membrane called the middle lamella, which contains magnesium and calcium pectates (salts of pectic acid). Cells interact though plasmodesmata, which are inter-connecting channels of cytoplasm that connect to the protoplasts of adjacent cells across the cell wall.

In some plants and cell types, after a maximum size or point in development has been reached, a secondary wall is constructed between the plasma membrane and primary wall. Unlike the primary wall, the cellulose microfibrils are aligned parallel in layers, the orientation changing slightly with each additional layer so that the structure becomes helicoidal. Cells with secondary cell walls can be rigid, as in the gritty sclereid cells in pear and quince fruit. Cell to cell communication is possible through pits in the secondary cell wall that allow plasmodesmata to connect cells through the secondary cell walls.

Passive and active transport

According to Fick's first law, the movement of molecules by diffusion always proceeds spontaneously, down a gradient of concentration or chemical potential, until equilibrium is reached. The spontaneous "downhill" movement of molecules is termed **passive transport**. At equilibrium, no

further net movements of solute can occur without the applicationof a driving force. The movement of substances against or up a gradient of chemical potential (e.g., to a higher concentration) is termed **active transport**. It is not spontaneous, and itrequires that work be done on the system by the application of cellular energy. One way (but not the only way) of accomplishing this task is to couple transport to the hydrolysis of ATP. The driving force for diffusion, or, conversely, the energy input necessary to move substances against a gradient, by measuring the potential-energy gradient, which is often a simple function of the difference in concentration. Biological transport can be driven by four major forces: concentration, hydrostatic pressure, gravity, and electric fields. The **chemical potential** for any solute is defined as the sum of the concentration, electric, and hydrostatic potentials (and the chemical potential under standard conditions.

Transport of ions across a membrane barrier

If the two KCl solutions in the previous example are separated by a biological membrane, diffusion is complicated by the fact that the ions must move through the membrane as well as across the open solutions. The extent to which a membrane permits the movement of a substance is called **membrane permeability**. As will be discussed later, permeability depends on the composition of the membrane, as well as on the chemical nature of the solute. In a loosesense, permeability can be expressed in terms of a diffusion coefficient for the solute in the membrane. However, permeability is influenced by several additional factors, suchas the ability of a substance to enter the membrane, that are difficult to measure. Despite its theoretical complexity, we can readily measure permeability by determining the rate at which a solute passes through a membrane under a specific set of conditions.Generally the membrane will hinder diffusion and thus reduce the speed with which equilibrium is reached.The permeability or resistance of the membrane itself, however, cannot alter the final equilibrium conditions. Equilibrium occurs when $m\sim j = 0$.In the sections that follow we will discuss the factors that influence the passive distribution of ions across amembrane. These parameters can be used to predict there lationship between the electrical gradient and the concentration gradient of an ion.

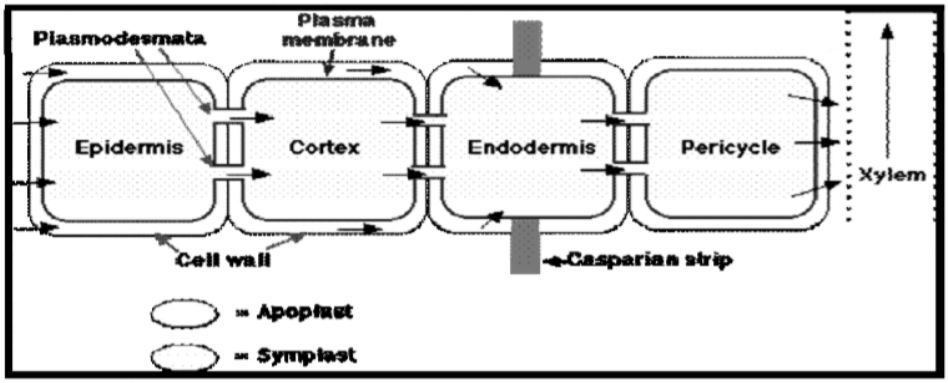


Figure 6: Three classes of membrane transport proteins: channels, carriers, and pumps. Channels and carriers can mediate the passive transport of solutes acrossmembranes

Carriers Bind and Transport Specific Substances

Unlike channels, **carrier** proteins do not have pores thatextend completely across the membrane. In transport mediated by a carrier, the substance being transported is the pore at the center. The pore-forming regions of the four subunits dip into the membrane, with a K+ selectivity finger region formed at the outer (near) part of the pore. (B) Side view of the inward rectifying K+ channel, showing a polypeptide chain of one subunit, with six membrane-spanning helices. The fourth helix contains positively-charged amino acids and acts as a voltage-sensor. The pore-forming region is a loop between helices 5 and 6. (A after Leng et al. 2002; B after Buchanan et al. 2000.) initially bound to a specific site on the carrier protein. This requirement for binding allows carriers to be highly selective for a particular substrate to be transported. Carriers therefore specialize in the transport of

specific organic metabolites. Binding causes a conformational change in the protein, which exposes the substance to the solution on the other side of the membrane. Transport is complete when the substance dissociates from the carrier's binding site.

Because a conformational change in the protein is required to transport individual molecules or ions, the rate of transport by a carrier is many orders of magnitude slower than through a channel. Typically, carriers may transport 100 to 1000 ions or molecules per second, which is about 106 times slower than transport through a channel. The binding and release of a molecule at a specific site on a protein that occur in carrier-mediated transport are similarto the binding and release of molecules from an enzyme in an enzyme-catalyzed reaction. Carrier-mediated transport (unlike transport through channels) can be either passive or active, and it can transport a much wider range of possible substrates. Passive transporton a carrier is sometimes called **facilitated diffusion**, although it resembles diffusion only in that it transports substances down their gradient of electrochemical potential, without an additional input of energy. (This term might seem more appropriately applied to transport through channels, but historically it has not been used in this way.)

Primary Active Transport Is Directly Coupled to Metabolic or Light Energy

To carry out active transport, a carrier must couple the uphill transport of the solute with another, energy-releasing, event so that the overall free-energy change is negative.**Primary active transport** is coupled directly to a source of energy other than *m*~j, such as ATP hydrolysis, an oxidation–reduction reaction (the electron transport chain of mitochondria and chloroplasts), or the absorption of light by the carrier protein (in halobacteria, bacteriorhodopsin).

The membrane proteins that carry out primary active transport are called **pumps**. Most pumpstransport ions, such as H^+ or $Ca2^+$. However, as we will see later in the chapter, pumps belonging to the "ATPbindingcassette" family of transporters can carry large organic molecules.Ion pumps can be further characterized as either electrogenicor electroneutral. In general, **electrogenic transport** refers to ion transport involving the net movement ofcharge across the membrane. In contrast, **electroneutral transport**, as the name implies, involves no net movementof charge. For example, the Na+/K+-ATPase of animal cells pumps three Na+ ions out for every two K+ ions in, resulting in a net outward movement of one positive charge. The Na+/K+-ATPase is therefore an electrogenic ion pump. In contrast, the H+/K+-ATPase of the animal gastric mucosa pumps one H+ out of the cell for every one K+ in, so thereis no net movement of charge across the membrane. Therefore, the H+/K+-ATPase is an electroneutral pump. In the plasma membranes of plants, fungi, and bacteria, as well as in plant tonoplasts and other plant and animal endomembranes, H+ is the principal ion that is electrogenically pumped across the membrane. The **plasma membrane H+-ATPase** generates the gradient of electrochemicalpotentials of H+ across the plasma membranes, while the **vacuolar H+-ATPase** and the **H+-pyrophosphatase** (**H+-PPase**) electrogenically pump prot.

In the plasma membranes of plants,fungi, and bacteria,as well as in plant tonoplasts and other plant and animal endomembranes, H+ is the principal ion that is electrogenicallypumped across the membrane. The **plasmamembrane**. **H+-ATPase** generatesthe gradient of electrochemical potentials of H+ across the plasmamembranes, while the **vacuolar H+-ATPase** and the (**H+-PPase**) electrogenically pump protons into the lumenof the vacuole and the Golgi cisternae.In plant plasma membranes, the most prominent pumpsare for H+ and Ca2+, and the

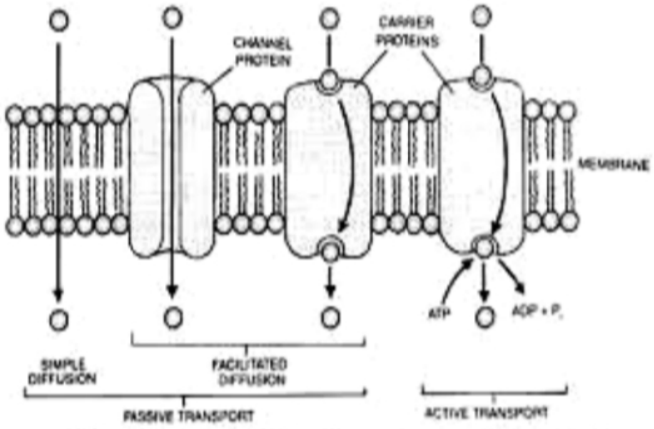


Fig. 11.1. Three ways of exchange of ions and solutes across membranes.

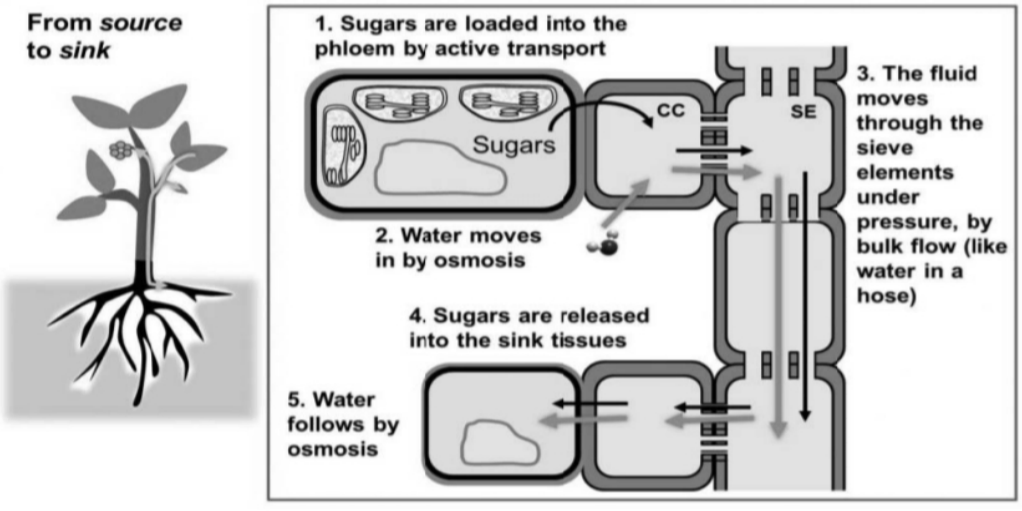
direction of pumping is outward. The other important way that solutes can be actively transported across a cmembrane against their gradient of electrochemical potential is by coupling of the uphill transport of one solute to the downhill transport of another. This type of carrier mediated co-transport is termed **secondary active transport**,

Secondary Active Transport Uses the Energy Stored in Electrochemical-Potential Gradients

Protons are extruded from the cytosol by electrogenic H+- ATPases operating in the plasma membrane and at the vacuole membrane. Consequently, a membrane potential and a pH gradient are created at the expense of ATP hydrolysis. This gradient of electrochemical potential for H+, m~H+,or (when expressed in other units) the **proton motive force** (**PMF**), or p, represents stored free energy in the form of the H+ gradient. The proton motive force generated by electrogenic H+ transport is used in secondary active transport to drive the transport of many other substances against their gradient of electrochemical potentials. Figure 6.9 shows how secondary transport may involve the binding of a substrate (S) and an ion (usually H+) to a carrier protein, and a conformational change in that protein. There are two types of secondary transport: symport and antiport. The **symport** (and the protein involved is called a *symporter*) because the two substances are moving in the same direction through the membrane. **Antiport** (facilitated by a protein called an *antiporter*) refers to coupled transport in which the downhill movement of protons drives the active (uphill) transport of a solute in the opposite direction.In both types of secondary transport, the ion or solutebeing transported simultaneously with the protons is moving against its gradient of electrochemical potential, so its transport is active. However, the energy driving this transport is provided by the proton motive force rather than

Solutes Move through Both Apoplast and Symplast

Thus far, our discussion of cellular ion transport has not included the cell wall. In terms of the transport of small molecules, the cell wall is an open lattice of polysaccharides through which mineral nutrients diffuse readily. Because all plant cells are separated by cell walls, ions can diffuse across a tissue (or be carried passively by water flow) entirely through the cell wall space without ever entering a living cell. This continuum of cell walls is called the *extracellular space*, or *apoplast*. We can determine the apoplastic volume of a slice of plant tissue by comparing the uptake of ^{3}H-labeled water and ^{14}C-labeled mannitol. Mannitol is a non-permeating sugar alcohol that diffuses within the extracellular space but cannot enter the cells. Water, on the other hand, freely penetrates both the cells and the cell walls. Measurements of this type usually show that 5 to 20% of the plant tissue volume is occupied by cell walls.



Ions Moving through the Root Cross Both Symplastic and Apoplastic Spaces

Ion absorption by the roots is more pronounced\in the root hair zone than in the meristem and elongation zones. Cells in the root hair zone have completed their elongation but have not yet begun secondary growth. The root hairs are simply extensions of specific epidermal cells that greatly increase the surface area available for ion absorption. An ion that enters a root may immediately enter the symplast by crossing the plasma membrane of an epidermal cell, or it may enter the apoplast and diffuse between the epidermal cells through the cell walls. From the apoplast of the cortex, an ion may either cross the plasma membrane of a cortical cell, thus entering the symplast, or diffuse radially all the way to the endodermis via the apoplast. In all cases, ions must enter the symplast before they can enter the stele, because of the presence of the Casparian strip. The apoplast forms a continuous phase from the root surface through the cortex. At the boundary between the vascular cylinder (the stele) and the cortex is a layer of specialized cells, the endodermis. A suberized cell layer in the endodermis, known as the Casparian strip, effectively blocks he entry of water and mineral ions into the stele via the apoplast. Once an ion has entered the stele through the symplastic connections across the endodermis, it continues to diffuse from cell to cell into the xylem. Finally, the ion reenters the apoplast as it diffuses into a xylem tracheid or vessel element. Again, the Casparian strip prevents the ion from diffusing back out of the root through the apoplast. The presence of the Casparian strip allows the plant to maintain a higher ionic concentration in the xylem than exists in the soil water surrounding the roots.

Xylem Parenchyma Cells Participate in Xylem

The movement of molecules and ions from one location to another is known as transport. Plants exchange solutes and water with their environment and among their tissues and organs. Both local and long-distance transport processes in plants are controlled largely by cellular membranes. Forces that drive biological transport, which include concentration gradients, electric-potential gradients, and hydrostatic pressures, are integrated by an expression called the electrochemical potential. Transport of solutes down a chemical gradient (e.g., by diffusion) is known as passive transport. Movement of solutes against a chemical potential gradient is known as active transport and requires energy input. The extent to which a membrane permits or restricts the movement of a substance is called membrane permeability. The permeability depends on the chemical properties of the particular solute and on the lipid composition of the membrane, as well as on the membrane proteins that facilitate the transport of specific substances. When cations and anions move passively across a membrane at different rates, the electric potential that developsis called the diffusion potential. For each ion, the relationship between the voltage difference across the membrane and the distribution of the ion at equilibrium is described by the Nernst equation. The Nernst equation shows that atequilibrium the difference in concentration of an ion between two compartments is balanced by the voltage difference between the compartments. That voltage difference, or membrane potential, is seen in all living cells because of the asymmetric ion distributions between the inside and outside of the cells. The electrical effects of different ions diffusing simultaneously across a cell membrane are summed by the Goldman equation. Electrogenic pumps, which carry out active transport and carry a net charge, change the membrane potential from the value created by diffusion. Membranes contain specialized proteins—channels, carriers, and pumps—that facilitate solute transport. Channels are transport proteins that span the membrane, forming pores through which solutes diffuse down their gradient of electrochemical potentials. Carriers bind a solute on one side of the membrane and release it on the other side. Transport specificity is determined largely by the properties of channels and carriers. A family of H+-pumping ATPases provides the primary driving force for transport across the plasma membrane of

plant cells. Two other kinds of electrogenic proton pumpsserve this purpose at the tonoplast. Plant cells also have calcium- pumping ATPases that participate in the regulation of intracellular calcium concentrations, as well as ATP binding cassette transporters that use the energy of ATP to transport large anionic molecules. The gradient of electrochemical potential generated by H+ pumping is used to drive the transport of other substances in a process called secondary transport. Genetic studies have revealed many genes, and their corresponding transport proteins, that account for the versatility of plant transport. Patch clamp electrophysiology provides unique information on ion channels, and it enables measurement of the permeability and gating of individual channel proteins .Solutes move between cells either through the extracellular spaces (the apoplast) or from cytoplasm to cytoplasm(via the symplast). Cytoplasms of neighboring cells are connected by plasmodesmata, which facilitate symplastic transport. When an ion enters the root, it may be taken up into the cytoplasm of an epidermal cell, or it may diffuse through the apoplast into the root cortex and enter the symplast through a cortical cell. From the symplast, the ion is loaded into the xylem and transported to the shoot.

9. Stress physiology: Response and adaptation to abiotic stress: water stress, temperature stress (heat and cold stress), stress induced gene expression.

Stress

The concept of plant stress is often used imprecisely, and stress terminology can be confusing, so it is useful to start our discussion with some definitions. **Stress** is usually defined as an external factor that exerts a disadvantageous influence on the plant. This chapter will concern itself with environmental or abiotic factors that produce stress in plants, although biotic factors such as weeds, pathogens, and insect predation can also produce stress. In most cases, stress is measured in relation to plant survival, crop yield, growth (biomass accumulation), or the primary assimilation processes (CO_2 and mineral uptake), which are related to overall growth. The concept of stress is intimately associated with that of **stress tolerance**, which is the plant's fitness to cope with an unfavorable environment. In the literature the term *stress resistance* is often used interchangeably with *stress tolerance*, although the latter term is preferred. Note that an environment that is stressful for one plant may not be stressful for another. For example, pea (*Pisum sativum*) and soybean (*Glycine max*) grow best at about 20°C and

30°C, respectively. As temperature increases, the pea shows signs of heat stress much sooner than the soybean. Thus the soybean has greater heat stress tolerance.

Water stress

The water-limited productivity of plants depends on the total amount of water available and on the water-use efficiency of the plant. A plant that is capable of acquiring more water or that has higher water-use efficiency will resist drought better. Some plants possess adaptations, such as the C_4 and CAM modes of photosynthesis that allow them to exploit more arid environments. In addition, plants possess acclimation mechanisms that are activated in response to water stress. **Water deficit** can be defined as any water content of a tissue or cell that is below the highest water content exhibited at the most hydrated state. When water deficit develops slowly enough to allow changes in developmental processes, water stress has several effects on growth, one of which is a limitation in leaf expansion. Leaf area is important because photosynthesis is usually proportional to it. However, rapid leaf expansion can adversely affect water availability.

Response and adaptation to water stress

1. Decreased Leaf Area Is an Early Adaptive Response to Water Deficit

Typically, as the water content of the plant decreases, its cells shrink and the cell walls relax. This decrease in cell volume results in lower turgor pressure and the subsequent concentration of solutes in the cells. The plasma membrane becomes thicker and more compressed because it covers a smaller area than before. Because turgor reduction is the earliest significant biophysical effect of water stress, turgor-dependent activities such as leaf expansion and root elongation are the most sensitive to water deficits.

2. Water Deficit Stimulates Leaf Abscission

The total leaf area of a plant (number of leaves × surface area of each leaf) does not remain constant after all the leaves have matured. If plants become water stressed after a substantial leaf area has developed, leaves will senesce and eventually fall off. Such a leaf area adjustment is an important long-term change that improves the plant's fitness in a water-limited environment. Indeed, many drought-deciduous, desert plants drop all their leaves during a drought and sprout new ones after a rain. This cycle can occur two or more times in a single season. Abscission during water stress results largely from enhanced synthesis of and responsiveness to the endogenous plant hormone ethylene.

3. Water Deficit Enhances Root Extension into Deeper, Moist Soil

Mild water deficits also affect the development of the root system. Root-to-shoot biomass ratio appears to be governed by a functional balance between water uptake by the root and photosynthesis by the shoot. Simply stated, *a shoot will grow until it is so large that water uptake by the roots becomes limiting to further growth*; conversely, *roots will grow until their demand for photosynthate from the shoot equals the supply*. This functional balance is shifted if the water supply decreases. All these factors lead to a preferential root growth into the soil zones that remain moist. As water deficits progress, the upper layers of the soil usually dry first. Thus, plants commonly show a mainly shallow root system when all soil layers are wetted, and a loss of shallow roots and proliferation of deep roots as water in top layers of the soil is depleted. Deeper root growth into wet soil can be considered a second line of defense against drought. Enhanced root growth into moist soil zones during stress requires allocation of assimilates to the growing root tips. During water deficit, assimilates are directed to the fruits and away from the roots. For this reason the enhanced water uptake resulting from root growth is less pronounced in reproductive plants than in vegetative plants. Competition for assimilates between roots and fruits is one explanation for the fact that plants are generally more sensitive to water stress during reproduction.

4. Stomata Close during Water Deficit in Response to Abscisic Acid

The preceding sections focused on changes in plant development during slow, long-term dehydration. When the onset of stress is more rapid or the plant has reached its full leaf area before initiation of stress, other responses protect the plant against immediate desiccation. Under these conditions, stomata closure reduces evaporation from the existing leaf area. Thus, stomatal closure can be considered a third line of defense against drought. Uptake and loss of water in guard cells changes

their turgor and modulates stomatal opening and closing. Because guard cells are located in the leaf epidermis, they can lose turgor as a result of a direct loss of water by evaporation to the atmosphere. The decrease in turgor causes stomatal closure by **hydropassive closure**. This closing mechanism is likely to operate in air of low humidity, when direct water loss from the guard cells is too rapid to be balanced by water movement into the guard cells from adjacent epidermal cells. A second mechanism, called **hydroactive closure**, closes the stomata when the whole leaf or the roots are dehydrated and depends on metabolic processes in the guard cells. A reduction in the solute content of the guard cells results in water loss and decreased turgor, causing the stomata to close; thus the hydraulic mechanism of hydroactive closure is a reversal of the mechanism of stomatal opening. However, the control of hydroactive closure differs in subtle but important ways from stomatal opening. Abscisic acid is synthesized continuously at a low rate in mesophyll cells and tends to accumulate in the chloroplasts. Besides ABA (Sauter et al. 2001), other si gnals, such as pH and inorganic ion redistribution, appear to play a role in long-distance signaling between the roots and the shoots.

5. Water Deficit Limits Photosynthesis within the Chloroplast

The photosynthetic rate of the leaf (expressed per unit leaf area) is seldom as responsive to mild water stress as leaf expansion is because photosynthesis is much less sensitive to turgor than is leaf expansion. However, mild water stress does usually affect both leaf photosynthesis and stomatal conductance. As stomata close during early stages of water stress, water-use efficiency may increase (i.e., more CO_2 may be taken up per unit of water transpired) because stomatal closure inhibits transpiration more than it decreases intercellular CO_2 concentrations. As stress becomes severe, however, the dehydration of mesophyll cells inhibits photosynthesis, mesophyll metabolism is impaired, and water-use efficiency usually decreases. Results from many studies have shown that the relative effect of water stress on stomatal conductance is significantly larger than that on photosynthesis. The response of photosynthesis and stomatal conductance to water stress can be partitioned by exposure of stressed leaves to air containing high concentrations of CO_2. Any effect of the stress on stomatal conductance is eliminated by the high CO_2 supply, and differences between photosynthetic rates of stressed and unstressed plants can be directly attributed to damage from the water stress to photosynthesis.

6. Water Deficit Increases Resistance to Liquid-Phase Water Flow

When a soil dries, its resistance to the flow of water increases very sharply, particularly near the *permanent wilting point*. At the permanent wilting point (usually about −1.5 MPa), plants cannot regain turgor pressure even if all transpiration stops. Because of the very large soil resistance to water flow, water delivery to the roots at the permanent wilting point is too slow to allow the overnight rehydration of plants that have wilted during the day. Rehydration is further hindered by the resistance within the plant, which has been found to be larger than the resistance within the soil over a wide range of water deficits (Blizzard and Boyer 1980). Several factors may contribute to the increased plant resistance to water flow during drying.

As plant cells lose water, they shrink. When roots shrink, the root surface can move away from the soil particles that hold the water, and the delicate root hairs may be damaged. In addition, as root extension slows during soil drying, the outer layer of the root cortex (the hypodermis) often becomes more extensively covered with suberin, a water-impermeable lipid, increasing the resistance to water flow.

Heat stress

Most tissues of higher plants are unable to survive extended exposure to temperatures above 45°C. Non-growing cells or dehydrated tissues (e.g., seeds and pollen) can survive much higher temperatures than hydrated, vegetative, growing cells. Actively growing tissues rarely survive temperatures above 45°C, but dry seeds can endure 120°C, and pollen grains of some species can endure 70°C. In general, only single-celled eukaryotes can complete their life cycle at temperatures

above 50°C, and only prokaryotes can divide and grow above 60°C. Periodic brief exposure to sublethal heat stresses often induces tolerance to otherwise lethal temperatures, a phenomenon referred to as **induced thermo tolerance**. The mechanisms mediating induced thermo tolerance will be discussed later in the chapter. As mentioned earlier, water and temperature stress are interrelated; shoots of most C_3 and C_4 plants with access to abundant water supply are maintained below 45°C by evaporative cooling; if water becomes limiting, evaporative cooling decreases and tissue temperatures increase. Emerging seedlings in moist soil may constitute an exception to this general rule. These seedlings may be exposed to greater heat stress than those in drier soils because wet, bare soil is typically darker and absorbs more solar radiation than drier soil.

Response and adaptation to heat stress

1. High Temperature Reduces Membrane Stability

The stability of various cellular membranes is important during high-temperature stress, just as it is during chilling and freezing. Excessive fluidity of membrane lipids at high temperatures is correlated with loss of physiological function. In oleander (*Nerium oleander*), acclimation to high temperatures is associated with a greater degree of saturation of fatty acids in membrane lipids, which makes the membranes less fluid (Raison et al. 1982). At high temperatures there is a decrease in the strength of hydrogen bonds and electrostatic interactions between polar groups of proteins within the aqueous phase of the membrane. High temperatures thus modify membrane composition and structure and can cause leakage of ions. Membrane disruption also causes the inhibition of processes such as photosynthesis and respiration that depend on the activity of membrane-associated electron carriers and enzymes. Photosynthesis is especially sensitive to high temperature. In their study of *Atriplex* and *Tidestromia*, O. Björkman and colleagues (1980) found that electron transport in photosystem II was more sensitive to high temperature in the cold-adapted *A. sabulosa* than in the heat-adapted *T. oblongifolia*. In these plants the enzymes ribulose-1,5-bisphosphate carboxylase, NADP:glyceraldehyde-3-phosphate dehydrogenase, and phosphoenolpyruvatecarboxylase were less stable at high temperatures in *A. sabulosa* than in *T. oblongifolia*. However, the temperatures at which these enzymes began to denature and lose activity were distinctly higher than the temperatures at which photosynthesis began to decline. These results suggest that early stages of heat injury to photosynthesis are more directly related to changes in membrane properties and to uncoupling of the energy transfer mechanisms in chloroplasts than to a general denaturation of proteins.

2. Several Adaptations Protect Leaves against Excessive Heating

In environments with intense solar radiation and high temperatures, plants avoid excessive heating of their leaves by decreasing their absorption of solar radiation. This adaptation is important in warm, sunny environments in which a transpiring leaf is near its upper limit of temperature tolerance. In these conditions, any further warming arising from decreased evaporation of water or increased energy absorption can damage the leaf. Both drought resistance and heat resistance depend on the same adaptations: reflective leaf hairs and leaf waxes; leaf rolling and vertical leaf orientation; and growth of small, highly dissected leaves to minimize the boundary layer thickness and thus maximize convective and conductive heat loss. Some desert shrubs—for example, white brittlebush (*Encelia farinosa*, family Compositae)—have dimorphic leaves to avoid excessive heating: Green, nearly hairless leaves found in the winter are replaced by white, pubescent leaves in the summer.

3. At Higher Temperatures, Plants Produce Heat Shock Proteins

In response to sudden, 5 to 10°C rises in temperature, plants produce a unique set of proteins referred to as **heat shock proteins** (**HSPs**). Most HSPs function to help cells withstand heat stress by acting as molecular chaperones. Heat stress causes many cell proteins that function as enzymes or structural components to become unfolded or misfolded, thereby leading to loss of proper enzyme structure and activity. Such misfolded proteins often aggregate and precipitate, creating serious problems within the cell. HSPs act as molecular chaperones and serve to attain a proper folding of misfolded, aggregated proteins and to prevent misfolding of proteins. This facilitates proper cell functioning at elevated, stressful temperatures. Heat shock proteins were discovered in the fruit fly (*Drosophila melanogaster*) and have since been identified in other animals, and in humans, as well as in plants, fungi, and

microorganisms. For example, when soybean seedlings are suddenly shifted from 25 to 40°C (just below the lethal temperature), synthesis of the set of mRNAs and proteins commonly found in the cell is suppressed, while transcription and translation of a set of 30 to 50 other proteins (HSPs) is enhanced. New mRNA transcripts for HSPs can be detected 3 to 5 minutes after heat shock (Sachs and Ho 1986). Although plant HSPs were first identified in response to sudden changes in temperature (25 to 40°C) that rarely occur in nature, HSPs are also induced by more gradual rises in temperature that are representative of the natural environment, and they occur in plants under field conditions. Some HSPs are found in normal, unstressed cells, and some essential cellular proteins are homologous to HSPs but do not increase in response to thermal stress (Vierling 1991). Plants and most other organisms make HSPs of different sizes in response to temperature increases. The molecular masses of the HSPs range from 15 to 104 kDa (kilodaltons), and they can be grouped into five classes based on size. Different HSPs are localized to the nucleus, mitochondria, chloroplasts, endoplasmic reticulum, and cytosol. Members of the HSP60, HSP70, HSP90, and HSP100 groups act as molecular chaperones, involving ATP-dependent stabilization and folding of proteins, and the assembly of oligomeric proteins. Some HSPs assist in polypeptide transport across membranes into cellular compartments. HSP90s are associated with hormone receptors in animal cells and may be required for their activation, but there is no comparable information for plants.

Low-molecular-weight (15–30 kDa) HSPs are more abundant in higher plants than in other organisms. Whereas plants contain five to six classes of low-molecular- weight HSPs, other eukaryotes show only one class (Buchanan et al. 2000). The different classes of 15–30 kDa molecular-weight HSPs (smHSPs) in plants are distributed in the cytosol, chloroplasts, ER and mitochondria. The function of these small HSPs is not understood. Cells that have been induced to synthesize HSPs show improved thermal tolerance and can tolerate exposure to temperatures that are otherwise lethal. Some of the HSPs are not unique to high-temperature stress. They are also induced by widely different environmental stresses or conditions, including water deficit, ABA treatment, wounding, low temperature, and salinity. Thus, cells previously exposed to one stress may gain cross-protection against another stress. Such is the case with tomato fruits, in which heat shock (48 hours at 38°C) has been observed to promote HSP accumulation and to protect cells for 21 days from chilling at 2°C.

4. Adaptation to Heat Stress Is Mediated by Cytosolic Calcium

Heat stress causes a reduction in cytosolic pH from the normal slightly alkaline value, probably by inhibiting proton-pumping ATPases and pyrophosphatases that pump protons across the plasma membrane or into the vacuole. Additionally, heat stress effects a change in calcium homeostasis inside the cell by affecting the influx of calcium into the cytosol through either plasma membrane or vacuolar calcium channels, or by action on efflux ATPases or proton cotransporters. This increase in cytosolic calcium leads to the activation of calmodulin (CaM), which binds to glutamate decarboxylase (GAD) converting it from the inactive to the active form. Glutamate conversion to γ− aminobutyric acid (GABA) is then accomplished consuming protons in the process and mediating an increase in cytosolic pH. CAX1 and CAX2 are transport proteins, ACA: Ca_2^+ ATPase.

Cold stress Chilling temperatures are too low for normal growth but not low enough for ice to form. Typically, tropical and subtropical species are susceptible to chilling injury. Among crops, maize, *Phaseolus* bean, rice, tomato, cucumber, sweet potato, and cotton are chilling sensitive. *Passiflora*, *Coleus*, and *Gloxinia* are examples of susceptible ornamentals. When plants growing at relatively warm temperatures (25 to 35°C) are cooled to 10 to 15°C, **chilling injury** occurs: Growth is slowed, discoloration or lesions appear on leaves, and the foliage looks soggy, as if soaked in water for a long time. If roots are chilled, the plants may wilt. Species that are generally sensitive to chilling can show appreciable variation in their response to chilling temperatures. Genetic adaptation to the colder temperatures associated with high altitude improves chilling resistance. In addition, resistance often increases if plants are first hardened (acclimated) by exposure to cool, but noninjurious, temperatures. Chilling damage thus can be minimized if exposure is slow and gradual. Sudden exposure to temperatures near 0°C, called *cold shock*, greatly increases the chances of injury.

Response and adaptation to cold stress

1. Membrane Properties Change in Response to Chilling Injury

Leaves from plants injured by chilling show inhibition of photosynthesis, slower carbohydrate translocation, lower respiration rates, inhibition of protein synthesis, and increased degradation of existing proteins. All of these responses appear to depend on a common primary mechanism involving loss of membrane function during chilling. For instance, solutes leak from the leaves of chillig sensitive *Passiflora maliformis* (conch apple) floated on water at 0°C, but not from those of chilling-resistant *Passiflora caerulea* (passionflower). Loss of solutes to the water reflects damage to the plasma membrane and possibly also to the tonoplast. In turn, inhibition of photosynthesis and of respiration reflects injury to chloroplast and mitochondrial membranes. Why are membranes affected by chilling? Plant membranes consist of a lipid bilayer interspersed with proteins and sterols. The physical properties of the lipids greatly influence the activities of the integral membrane proteins, including H+-ATPases, carriers, and channel-forming proteins that regulate the transport of ions and other solutes, as well as the transport of enzymes on which metabolism depends. As the membranes become less fluid, their protein components can no longer function normally. The result is inhibition of H+-ATPase activity, of solute transport into and out of cells, of energy transduction, and of enzyme-dependent metabolism. In addition, chilling- sensitive leaves exposed to high photon fluxes and chilling temperatures are photo-inhibited, causing acute damage to the photosynthetic machinery.

2. Ice Crystal Formation and Protoplast Dehydration Kill Cells

The ability to tolerate freezing temperatures under natural conditions varies greatly among tissues. Seeds, other partly dehydrated tissues, and fungal spores can be kept indefinitely at temperatures near absolute zero (0 K, or –273°C), indicating that these very low temperatures are not intrinsically harmful. Fully hydrated, vegetative cells can also retain viability if they are cooled very quickly to avoid the formation of large, slow-growing ice crystals that would puncture and destroy subcellular structures. Ice crystals that form during very rapid freezing are too small to cause mechanical damage. Conversely, rapid warming of frozen tissue is required to prevent the growth of small ice crystals into crystals of a damaging size, or to prevent loss of water vapor by sublimation, both of which take place at intermediate temperatures (–100 to –10°C).

3. ABA and Protein Synthesis Are Involved in Acclimation to Freezing

In seedlings of alfalfa (*Medicago sativa* L.), tolerance to freezing at –10°C is greatly improved by previous exposure toncold (4°C) or by treatment with exogenous ABA without exposure to cold. These treatments cause changes in them pattern of newly synthesized proteins that can be resolved on two-dimensional gels. Some of the changes are unique to the particular treatment (cold or ABA), but some of the newly synthesized proteins induced by cold appear to be the same as those induced by ABA or by mild water deficit. Protein synthesis is necessary for the development of freezing tolerance, and several distinct proteins accumulateduring acclimation to cold, as a result of changes in gene expression (Guy 1999). Isolation of the genes for these proteins reveals that several of the proteins that are induced by low temperature share homology with the RAB/LEA/DHN (responsive to ABA, late embryo abundant, and dehydrin, respectively) protein family. As described earlier in the section on gene regulation by osmotic stress, these proteins accumulate in tissues exposed to different stresses, such as osmotic stress. Their functions are under investigation.

4. Numerous Genes Are Induced during Cold Acclimation

Expression of certain genes and synthesis of specific proteins are common to both heat and cold stress, but some aspects of cold-inducible gene expression differ from that produced by heat stress (Thomashow 2001). Whereas during cold episodes the synthesis of "housekeeping" proteins (proteins made in the absence of stress) is not substantially down-regulated, during heat stress housekeeping-protein synthesis is essentially shut down. On the other hand, the synthesis of several heat shock proteins that can act as molecular chaperones is up-regulated under cold stress in the same way that it is during heat stress. This suggests that protein destabilization accompanies both heat and cold stress and that mechanism for stabilizing protein structure during both heat and cold episodes are important for survival.

Stress induced gene expression

Heat stress related gene expression

The five classes of heat shock proteins found in plants

HSP class	Size (kDa)	Examples (Arabidopsis / prokaryotic)	Cellular location
HSP100	100–114	AtHSP101 / ClpB, ClpA/C	Cytosol, mitochondria, chloroplasts
HSP90	80–94	AtHSP90 / HtpG	Cytosol, endoplasmic reticulum
HSP70	69–71	AtHSP70 / DnaK	Cytosol/nucleus, mitochondria, chloroplasts
HSP60	57–60	AtTCP-1 / GroEL, GroES	Mitochondria, chloroplasts
smHSP	15–30	Various AtHSP22, AtHSP20, AtHSP18.2, AtHSP17.6 / IBPA/B	Cytosol, mitochondria, chloroplasts, endoplasmic reticulum

Source: After Boston et al. 1996.

Cold stress related gene expression

More than 100 genes are up-regulated by cold stress. Because cold stress is clearly related to ABA responses and to osmotic stress, not all the genes up-regulated by cold stress necessarily need to be associated with cold tolerance, but most likely many of them are. Many cold stress–induced genes are activated by transcriptional activators called **C-repeat binding factors** (**CBF1**, **CBF2**, **CBF3**; also called DREB1b, DREB1c, and DREB1a, respectively) (Shinozaki and Yamaguchi-Shinozaki 2000). CBF/DREB1-type transcription factors bind to **CRT/DRE elements** (C-repeat/dehydration-responsive, ABA-independent sequence elements) in gene promoter sequences, which were discussed earlier in the chapter. CBF/DREB1 is involved in the coordinate transcriptional response of numerous cold and osmotic stress–regulated genes, all of which contain the CRT/DRE elements in their promoters. CBF1/DREB1b is unique in that it is specifically induced by cold stress and not by osmotic or salinity stress, whereas the DRE-binding elements of the DREB2 type (discussed earlier in the section on osmotic stresses) are induced only by osmotic and salinity stresses and not by cold. The expression of CBF1/DREB1b is controlled by a separate transcription factor, called ICE (*i*nducer of *C*BF *e*xpression). ICE transcription factors do not appear to be induced by cold, and it is presumed that ICE or an associated protein is post transcriptionally activated, permitting activation of CBF1/DRE1b, but the precise signaling pathway(s) of cold perception, calcium signaling, and the activation of ICE are presently under investigation. Transgenic plants constitutively expressing CBF1 have more cold–up-regulated gene transcripts than wild-type plants have, suggesting that numerous cold–up-regulated proteins that may be involved in cold acclimation are being produced in the absence of cold in these CBF1 transgenic plants. In addition, CBF1 tansgenic plants are more coldtolerant than control plants.

Transgenic strategy of stress in plants.

In Arabidopsis, overexpression of cDNA encoding DREB1 and DREB2 triggered expres-sion of many stress tolerance genes under normal growing conditions. Thus, transgenic strat-egy resulted in increased tolerance to drought, salinity and cold stress. Researchers from Japan International Research Center for Agricultural Science (JIRCAS) identified 12 stress-inducible genes which are targeted by DREBIA transcription factor protein.

In the transgene construct DREBIA) was driven under the control of Camv 35S promoter (35S:DREB1A). In the notable observation, all the transgenic plants carrying 35S:DREB1A transgene exhibited severe retar-dation under normal growth condition. This negative effect is probably due to different levels of expression of DREBIA transgenes.

Over production of transcription factor proteins resulted in the expression of all stress inducible target genes, rd29A, rdl7, cor 6.6, cor 15a, erd 10 and kinl. Under normal conditions, plants under this situation suffer heav-ily due to unnecessary drenching of energy for the production of several hundreds of proteins. This problem was effectively tackled by using stress inducible rd 29A promoter.

In an extended transgenic strategy DREBIA gene expressed under the control of stress inducible rd 29A pro-moter provides protection and shows minimal effect on plant growth. In addition, it provides high degree of tolerance to stress condition rather when it is expressed under 35S Camv pro-moter.

Since rd 29A promoter is stress inducible, it did not cause expression of the transcription factor gene (DREB).

Under unstressed conditions, instead, it rapidly enhances the expression of DREBIA transgene only under dehydration, high salt and low temperature conditions (Fig. 15.5). Overall rd 29A promoter may be useful to improve stress tolerance of agriculturally important crops by gene transfer. In the earlier transgenic work, Shinozaki and coworkers have amplified signalling pathway by overexpressing gene containing fusion of a DRE-containing promoter with a DREB gene.

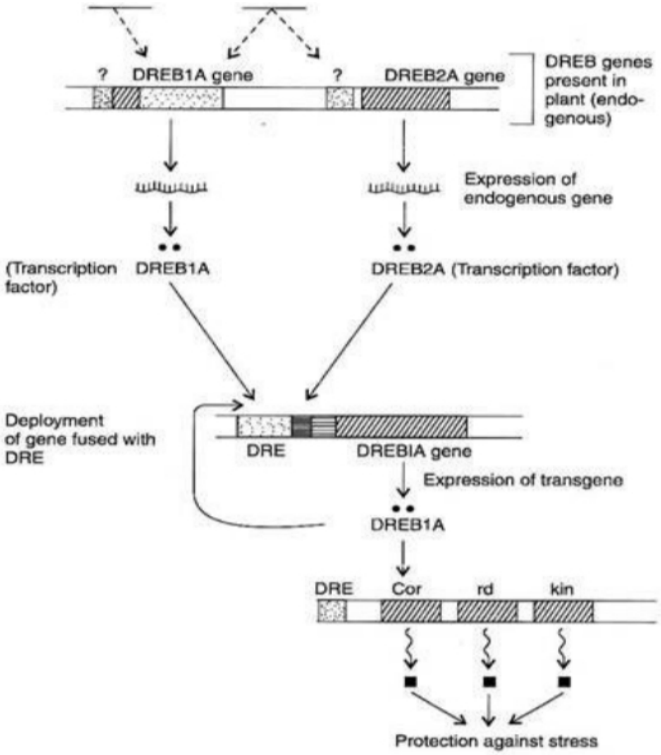


Fig. 15.5 Overexpression of stress inducible transcription factor protein and amplification of signalling pathway by overexpressing the introduced gene (DREB1) fused with DRE-*cis*-acting sequence (after Smirnoff and Bryant, 1999.)

This enhances further DREBIA expression in response to stress. This amplified strategy led to strong induction of DRE-containing target genes. Some contrasting experiments show that DREBIA cannot induce the expression of other drought-responsive genes (such as PSCS, erdl, rd22 and rd 29B) which contain no DRE element in their promoter region.

10. Principle of biophysical chemistry: pH, buffer, reaction kinetics, thermodynamics, law of mass action, acid base reactions, bond energy, energy rich compounds, redox potential, free energy.

A **buffer** solution (more precisely, pH buffer or hydrogen ion buffer) is an aqueous solutionconsisting of a mixture of a weak acid and its conjugate base, or vice versa. Its pH changes very little when a small amount of strong acid or base is added to it. Buffer solutions are used as a means of keeping pH at a nearly constant value in a wide variety of chemical applications. In nature, there are many systems that use buffering for pH regulation. For example, the bicarbonate buffering system is used to regulate the pH of blood.

Principles of buffering

Buffer solutions achieve their resistance to pH change because of the presence of an equilibrium between the acid HA and its conjugate base A−.

$HA \rightleftharpoons H+ + A-$

When some strong acid is added to an equilibrium mixture of the weak acid and its conjugate base, the equilibrium is shifted to the left, in accordance with Le Châtelier's principle. Because of this, the hydrogen ion concentration increases by less than the amount expected for the quantity of strong acid added. Similarly, if strong alkali is added to the mixture the hydrogen ion concentration decreases by less than the amount expected for the quantity of alkali added. The effect is illustrated by the simulated titration of a weak acid with pKa = 4.7. The relative concentration of undissociated acid is shown in blue and of its conjugate base in red. The pH changes relatively slowly in the buffer region, pH = pKa ± 1, centered at pH = 4.7 where [HA] = [A−]. The hydrogen ion concentration decreases by less than the amount expected because most of the added hydroxide ion is consumed in the reaction

$OH- + HA \rightarrow H2O + A-$ and only a little is consumed in the neutralization reaction which results in an increase in pH. $OH- + H+ \rightarrow H2O$

Once the acid is more than 95% deprotonated the pH rises rapidly because most of the added alkali is consumed in the neutralization reaction.

Applications

Buffer solutions are necessary to keep the correct pH for enzymes in many organisms to work. Many enzymes work only under very precise conditions; if the pH moves outside of a narrow range, the enzymes slow or stop working and can denature. In many cases denaturation can permanently disable their catalytic activity.[Industrially, buffer solutions are used in fermentation processes and in setting the correct conditions for dyes used in colouring fabrics. They are also used in chemical analysis and calibration of pH meters.

The majority of biological samples that are used in research are made in buffers, especially phosphate buffered saline (PBS) at pH 7.4. For buffers in acid regions, the pH may be adjusted to a desired value by adding a strong acid such as hydrochloric acid to the particular buffering agent. For alkaline buffers, a strong base such as sodium hydroxide may be added. Alternatively, a buffer mixture can be made from a mixture of an acid and its conjugate base. For example, an acetate buffer can be made from a mixture of acetic acid and sodium acetate. Similarly an alkaline buffer can be made from a mixture of the base and its conjugate acid.

Calculating buffer pH

First write down the equilibrium expression.

$HA \rightleftharpoons A^- + H^+$

This shows that when the acid dissociates equal amounts of hydrogen ion and anion are produced.

To find x, use the formula for the equilibrium constant in terms of concentrations:

$$K_a = \frac{[H^+][A^-]}{[HA]}$$

Substitute the concentrations with the values found in the last row of the ICE table:

$$K_a = \frac{x(x+y)}{C_0 - x}$$

Simplify to:

$$x^2 + (K_a + y)x - K_a C_0 = 0$$

With specific values for C_0, K_a and y this equation can be solved for x. Assuming that pH = $-\log_{10}[H^+]$ the pH can be calculated as pH = $-\log_{10}(x + y)$.

To find x, use the formula for the equilibrium constant in terms of concentrations: To find x, use the formula for the equilibrium constant in terms of concentrations:

Polyprotic acids

Polyprotic acids are acids that can lose more than one proton. The constant for dissociation of the first proton may be denoted as K_{a1} and the constants for dissociation of successive protons as K_{a2}, etc. Citric acid, H_3A, is an example of a polyprotic acid as it can lose three protons.

In chemistry, **pH** is a scale used to specify how acidic or basic a water-based solutionis. Acidic

Calculation of the pH with a polyprotic acid requires a speciation calculation to be performed. In the case of citric acid, this entails the solution of the two equations of mass balance

$$C_A = [A^{3-}] + \beta_1[A^{3-}][H^+] + \beta_2[A^{3-}][H^+]^2 + \beta_3[A^{3-}][H^+]^3$$
$$C_H = [H^+] + \beta_1[A^{3-}][H^+] + 2\beta_2[A^{3-}][H^+]^2 + 3\beta_3[A^{3-}][H^+]^3 - K_w[H^+]^{-1}$$

C_A is the analytical concentration of the acid, C_H is the analytical concentration of added hydrogen ions, β_q are the cumulative association constants

$$\log\beta_1 = pK_{a_3}, \quad \log\beta_2 = pK_{a_2} + pK_{a_3}, \quad \log\beta_3 = pK_{a_1} + pK_{a_2} + pK_{a_3}$$

K_w is the constant for self-ionization of water. There are two non-linear simultaneous equations in two unknown quantities $[A^{3-}]$ and $[H^+]$. Many computer programs are available to do this calculation. The speciation diagram for citric acid was produced with the program HySS.[8]

solutions have a lower pH, while basic solutions have a higher pH. At room temperature (25 °C), pure water is neither acidic nor basic and has a pH of 7.

The pH scale is logarithmic and approximates the negative of the base 10 logarithm of the molar concentration (measured in units of moles per liter) of hydrogen ions in a solution. More precisely it is the negative of the base 10 logarithm of the activity of the hydrogen ion. At 25 °C, solutions with a pH less than 7 are acidic and solutions with a pH greater than 7 are basic. The neutral value of the pH depends on the temperature, being lower than 7 if the temperature increases. Contrary to popular belief, the pH value can be less than 0 or greater than 14 for very strong acids and bases respectively.

pH

pH is defined as the decimal logarithm of the reciprocal of the hydrogen ion activity, a_{H^+}, in a solution.

$$\mathrm{pH} = -\log_{10}(a_{H^+}) = \log_{10}\left(\frac{1}{a_{H^+}}\right)$$

For example, for a solution with a hydrogen ion activity of 5×10^{-6} (at that level, this is essentially the number of moles of hydrogen ions per liter of solution) we get $1/(5\times10^{-6}) = 2\times10^{5}$, thus such a solution has a pH of $\log_{10}(2\times10^{5}) = 5.3$. For a commonplace example based on the facts that the masses of a mole of water, a mole of hydrogen ions, and a mole of hydroxide ions are respectively 18 g, 1 g, and 17 g, a quantity of 10^{7} moles of pure (pH 7) water, or 180 tonnes (18×10^{7} g), contains close to 1 g of dissociated hydrogen ions (or rather 19 g of H_3O^+ hydronium ions) and 17 g of hydroxide ions.

Note that pH depends on temperature. For instance at 0 °C the pH of pure water is 7.47. At 25 °C it's 7.00, and at 100 °C it's 6.14.

This definition was adopted because ion-selective electrodes, which are used to measure pH, respond to activity. Ideally, electrode potential, E, follows the Nernst equation, which, for the hydrogen ion can be written as

$$E = E^0 + \frac{RT}{F}\ln(a_{H^+}) = E^0 - \frac{2.303RT}{F}\mathrm{pH}$$

Reaction kinetics, thermodynamics, law of mass action:

Bioenergetics is the application of thermodynamic laws to the study of energy transformations in biological systems. The energetics of cellular processes can be related to chemical equilibrium and oxidation–reduction potentials of chemical reactions. Whether at the level of molecules, cells, or ecosystems, the flow of energy is central to the maintenance of life. A basic understanding of energy flow is therefore essential to grasping the true beauty, significance, and complexity of biology.

The field of study concerned with the flow of energy through living organisms is called bioenergetics. It is important to note that although the laws of bioenergetics provide critical insight into the driving forces that govern cellular processes, bioenergetics does not provide any insights into the biochemical reaction mechanisms underlying these processes. The purpose of this section is to facilitate an understanding of the laws that govern biological energy transformations by assembling, in the simplest form possible, some basic thermodynamic principles. Thus, a complete understanding of cellular physiology requires an integration of bioenergetics with biochemical reaction mechanisms.

The first law of thermodynamics refers to energy conservation

Biological energy transformations are based on two thermodynamic laws. The first law, commonly known as the **law of conservation of energy**, states that the energy of the universe is constant. This is not a difficult concept to comprehend—it means simply that there is a fixed amount of energy and, while it may be moved about or changed in form, it can all be accounted for somewhere. More to the point, energy is never ‘‘lost’’ in a reaction—an apparent decrease in one form of energy will be balanced by an increase in some other form of energy. In one of the examples mentioned earlier, some of the energy expended in displacing an object appears as work while some appears as heat generated due to friction. In the same way, some of the chemical energy released in the combustion of glucose

will also be found as heat in the environment, while some will be found as bond energy in the product molecules, CO_2 and water.

The second law of thermodynamics refers to entropy and disorder

As biologists we are concerned above all with how much work can be done. But, as suggested earlier, not all energy is available to do work. This brings us to the second law of thermodynamics and the concept of **entropy**. Because it involves the concept of entropy (S), the second law is a bit more difficult to comprehend. What is entropy? Entropy has been variously described as a measure of randomness, disorder, or chaos. However, since entropy is a thermodynamic concept, it is useful to describe it in terms of thermal energy. Temperature is defined as the mean molecular kinetic energy of matter. Thus, any molecular system not at absolute zero (−273◦C, or 0◦K) contains a certain amount of thermal energy—energy in the form of the vibration and rotation of its constituent molecules as well as their translation through space. This quantity of thermal energy and temperature go hand-in-hand: as the quantity of energy increases or decreases, so does temperature. Because temperature cannot be held constant when this energy is given up, it is said to be ‘‘isothermally unavailable’’ (Gr. *isos*, equal). Quantitatively, isothermally unavailable energy is given by the term TS, where T is the absolute temperature and S is entropy.

Since isothermally unavailable energy, and consequently, entropy, are related to the energy of molecular motion, it follows that the more molecules are free to move about, that is, the more random or less ordered or chaotic the system, the greater will be their entropy.

Free energy is related to chemical equilibria

Under appropriate conditions, all chemical reactions will achieve a state of equilibrium, at which there will be no further *net* change in the concentrations of reactants and products. There is a fairly straightforward relationship between free energy and chemical equilibria In a reaction where the reactant A is converted

to product B, K is the equilibrium mass-action ratio—the ratio of concentration of products to the concentration of reactants when the reaction has come to equilibrium. Thus

$$K_{eq} = [B]_{eq}/[A]_{eq} \qquad (5.6)$$

In E= mc^2 the slope of the line represents the change in free energy (_G) when a small amount of the reactant A is converted to the product B. Several useful points can be drawn from this diagram.

1. At equilibrium, the slope of the line is zero. Consequently, *when reactions are at equilibrium*, _G = 0

and no useful work can be accomplished.

2. The further the mass-action ratio is displaced from equilibrium (Keq), the greater the free energy change for conversion of the same small amount of A to B. The free energy change for a reaction is a function of its displacement from equilibrium. Therefore, *the further a reaction is poised away from equilibrium, the more free energy is available as the reaction proceeds toward equilibrium*.

3. As A approaches equilibrium, *Δ*G is negative and free energy is available to do work. However, as the

reaction proceeds past equilibrium toward B, *Δ*G is becomes positive and energy must be supplied. *A system can do work as it moves toward equilibrium*. Note that if the reaction were initiated with pure B, the direction of the arrows would be reversed and work could be done as B approached equilibrium. The relationship between the free energy change (*Δ*G) and the equilibrium constant (Keq) can be expressed

quantitatively as:

$$\Delta G = \Delta G^{\circ\prime} + RT \ln K_{eq} \qquad (5.7)$$

However, at equilibrium, *Δ*G = 0 and Equation 5.7 can be rearranged to give

$$\Delta G^{\circ\prime} = -RT \ln K_{eq} = -2.3RT \log K_{eq} \qquad (5.8)$$

Furthermore, the actual free energy change (*Δ*G) of a reaction *not* at equilibrium is given by:

$$\Delta G = \Delta G^{\circ\prime} + 2.3RT \log \Gamma \qquad (5.9)$$

where R is the universal gas constant, T is the absolute temperature, and *T*equals the observed (i.e., nonequilibrium) mass-action ratio. Equation 5.8 can then be substituted in Equation 5.9 and rearranged to give:

$$\Delta G = -2.3RT \log(K_{eq}/\Gamma) \qquad (5.10)$$

Equation 5.10 reinforces the observation that the value of ΔG is a function of the degree to which a reaction is displaced from equilibrium. When _*T*=Keq, the reaction is at equilibrium and $\Delta G = 0$ and no useful work can be done. When *T*is less than Keq, $\Delta G < 0$ and the reaction will occur spontaneously with the release of energy which can be used to perform useful work. However, when *T*is greater than Keq, $\Delta G > 0$ and the reaction will not occur spontaneously, but rather, requires an input of energy to proceed.

Acid–base reaction:

An acid–base reaction is a chemical reaction that occurs between an acid and a base, which can be used to determine pH. Several theoretical frameworks provide alternative conceptions of the reaction mechanisms and their application in solving related problems; these are called the acid–base theories, for example, Brønsted–Lowry acid–base theory.

Their importance becomes apparent in analyzing acid–base reactions for gaseous or liquid species, or when acid or base character may be somewhat less apparent. The first of these concepts was provided by the French chemist Antoine Lavoisier, around 1776.

Lewis definition

The hydrogen requirement of Arrhenius and Brønsted–Lowry was removed by the Lewis definition of acid–base reactions, devised by Gilbert N. Lewis in 1923, in the same year as Brønsted–Lowry, but it was not elaborated by him until 1938. Instead of defining acid–base reactions in terms of protons or other bonded substances, the Lewis definition defines a base (referred to as a Lewis base) to be a compound that can donate an electron pair, and an acid (a Lewis acid) to be a compound that can receive this electron pair.

For example, boron trifluoride, BF3 is a typical Lewis acid. It can accept a pair of electrons as it has a vacancy in its octet. The fluoride ion has a full octet and can donate a pair of electrons.

Acid–base equilibrium:

The reaction of a strong acid with a strong base is essentially a quantitative reaction. For example,

$$HCl_{(aq)} + Na(OH)_{(aq)} \rightarrow H_2O + NaCl_{(aq)}$$

In this reaction both the sodium and chloride ions are spectators as the neutralization reaction,

$$H^+ + OH^- \rightarrow H_2O$$

The equilibrium constant for this reaction can be derived from the acid dissociation constants of adenine and of the dihydrogen phosphate ion.

$$[A^-]\,[H^+] = K_{a1}[AH]$$
$$[HPO_4^{2-}]\,[H^+] = K_{a2}[H_2PO_4^-]$$

The notation [X] signifies "concentration of X". When these two equations are combined by eliminating the hydrogen ion concentration, an expression for the equilibrium constant, *K* is obtained.

$$[A^-]\,[H_2PO_4^-] = K[AH]\,[HPO_4^{2-}]; \qquad K = \frac{K_{a1}}{K_{a2}}$$

Acid–alkali reaction:

An acid–alkali reaction is a special case of an acid–base reaction, where the base used is also an alkali. When an acid reacts with an alkali salt (a metal hydroxide), the product is a metal salt and water. Acid–alkali reactions are also neutralization reactions.

In general, acid–alkali reactions can be simplified to

$$OH^-_{(aq)} + H^+_{(aq)} \rightarrow H_2O$$

by omitting spectator ions.

Acids are in general pure substances that contain hydrogen cations (H^+) or cause them to be produced in solutions. Hydrochloric acid (HCl) and sulfuric acid (H_2SO_4) are common examples. In water, these break apart into ions:

$$HCl \rightarrow H^+_{(aq)} + Cl^-_{(aq)}$$
$$H_2SO_4 \rightarrow H^+_{(aq)} + HSO^-_{4(aq)}$$

The alkali breaks apart in water, yielding dissolved hydroxide ions:

$$NaOH \rightarrow Na^+_{(aq)} + OH^-_{(aq)}$$

Redox potential, free energy:

Photosynthesis and respiration, are electrochemical phenomena. Each operates as a sequence of oxidation–reduction reactions in which electrons are transferred from one component to another. Thus, the oxidation of one component is linked or coupled to the reduction of the next component. Such coupled electron transfer reactions are known as *red*uction–*ox*idation or *redox* reactions. However, it is not clear how to treat the electrons, which have no independent existence. Moreover, our interest in redox couples is more in their tendency to accept electrons from or donate electrons to another couple, a tendency known as **redox potential**.

Redox potentials allow the feasibility and direction of electron transfers between components in a complex system to be predicted. Indeed, in order to understand electron flow in photosynthesis and respiration it is necessary to have a working understanding of redox potential and how it is applied.

The direction of electron transfer between redox couples can be predicted by comparing their midpoint potentials (E*m*). Thermodynamically spontaneous electron transfer will proceed from couples with the more negative (less positive) redox potential to those with the less negative (more positive) redox potential. The energy-transducing membranes of bacteria, mitochondria, and chloroplasts all contain electron-transport

systems involving a number of electron carriers with different midpoint redox potentials.

In addition to allowing us to predict the direction of electron transfer, redox potentials also permit the calculation of Gibbs free energy changes for electron-transfer reactions. This can be done using the following relationship:

$$\Delta G^{\circ\prime} = -n\mathbf{F}\Delta E_m \qquad (5.21)$$

where *n* is the number of electrons transferred and F is the Faraday constant (96 500 coulombs mol−1).

Biological electron transfers may involve either single electrons or pairs, but energy calculations are almost always based on *n*=2._Em is the redox interval through which the electrons are transferred and is determined as Thus, for a coupled transfer of electrons from water (the donor) to NADP+ (the

acceptor) as occurs in photosynthetic electron transport in chloroplasts, ΔEm = (−320) − (+820) = −1140mV = −1.14 V. Substituting this in Equation 5.21, the value of ΔG°_ for a two-electron transfer from H2O to NADP+ is +220 Kj mol−1. Note that the sign of ΔG° is positive, indicating that this electron transfer will not occur spontaneously.
In photosynthesis, light energy is used to drive this coupled endergonic reaction. In contrast, mitochondria transfer electrons from NADH to O_2. For this coupled electron-transfer reaction, the value of ΔEm is +1.14 V and consequentlyΔG°_ is−220 kJ mol−1, indicating that this electron transfer in mitochondria is exergonic and thus will occur spontaneously. It is important to note that the molecular mechanisms by which these electrons are transferred through complex processes such as photosynthesis, respiration, and nitrogen assimilation are not in the purview of bioenergetics.

11. Enzyme: Enzyme kinetics, catalytic reactions and regulatory properties, inhibitions, iso-enzymes, allosterism, ribozyme and abzymes.

Enzymes

Enzymes are proteins that act as catalysts within living cells. **Catalysts** increase the rate at which chemical reactions occur without being consumed or permanently altered themselves. A **chemical reaction** is a process that converts one or more substances (known as reagents, reactants, or substrates) to another type of substance (the product). As a catalyst, an enzyme can facilitate the same chemical reaction over and over again.

Examples

Lipases - a group of enzymes that help digest fats in the gut.

Amylase - helps change starches into sugars. Amylase is found in saliva.

Maltase - also found in saliva; breaks the sugar maltose into glucose. Maltose is found in foods such as potatoes, pasta, and beer.

Trypsin - found in the small intestine, breaks proteins down into amino acids.

Lactase - also found in the small intestine, breaks lactose, the sugar in milk, into glucose and galactose.

Acetylcholinesterase - breaks down the neurotransmitter acetylcholine in nerves and muscles.

Helicase - unravels DNA.

DNA polymerase - synthesize DNA from deoxyribonucleotides.

Properties of enzymes

(1) Enzymes are complex macromolecules with high molecular weight.
(2) They catalyze biochemical reactions in a cell. They help in the breakdown of large molecules into smaller molecules or bring together two smaller molecules to form a larger molecule.
(3) Enzymes do not start a reaction. However, they help in accelerating it.
(4) Enzymes affect the rate of biochemical reaction and not the direction of the reaction.
(5) Most of the enzymes have high turnover number. Turnover number of an enzyme is the number of molecules of a substance that is acted upon by an enzyme per minute under saturated substrate concentration. High turnover number of enzymes increases the efficiency of reaction.
(6) Enzymes are specific in action.

(7) Enzymatic activity decreases with increase in temperature and all enzymes show maximum activity at an optimum kmp of 30-40 0C.
(8) They show maximum activity at an optimum pH of 6 – 8.
(9) The velocity of enzyme increases with increase in substrate concentration and then, ultimately reaches maximum velocity.

Function of enzymes

Enzymes serve a wide variety of functions inside living organisms.

1. They are indispensable for signal transduction and cell regulation, often via kinases and phosphatases.

2. They also generate movement, with myosin hydrolyzing ATP to generate muscle contraction, and also transport cargo around the cell as part of the cytoskeleton. Other ATPases in the cell membrane are ion pumps involved in active transport.

3. Enzymes are also involved in more exotic functions, such as luciferase generating light in fireflies.

4. Viruses can also contain enzymes for infecting cells, such as the HIV integrase and reverse transcriptase, or for viral release from cells, like the influenza virus neuraminidase.

5. An important function of enzymes is in the digestive systems of animals. Enzymes such as amylases and proteases break down large molecules (starch or proteins, respectively) into smaller ones, so they can be absorbed by the intestines. Starch molecules, for example, are too large to be absorbed from the intestine, but enzymes hydrolyze the starch chains into smaller molecules such as maltose and eventually glucose, which can then be absorbed. Different enzymes digest different food substances. In ruminants, which have herbivorous diets, microorganisms in the gut produce another enzyme, cellulase, to break down the cellulose cell walls of plant fiber.

Enzyme kinetics

It is the study of the chemical reactions that are catalysed by enzymes. In enzyme kinetics, the reaction rate is measured and the effects of varying the conditions of the reaction are investigated. Studying an enzyme's kinetics in this way can reveal the catalytic mechanism of this enzyme, its role in metabolism, how its activity is controlled, and how a drug or an agonist might inhibit the enzyme.

Enzymes are usually protein molecules that manipulate other molecules—the enzymes' substrates. These target molecules bind to an enzyme's active site and are transformed into products through a series of steps known as the enzymatic mechanism

$$E + S \rightleftarrows ES \rightleftarrows ES^* \rightleftarrows EP \rightleftarrows E + P$$

Enzyme catalysis

Catalysis is a phenomenon in which the rate of the reaction is altered, and the substance used to accelerate remains unchanged regarding quantity and chemical properties. The substance used to change the speed of the reaction is called a catalyst. Enzymes are a type of catalysts which are

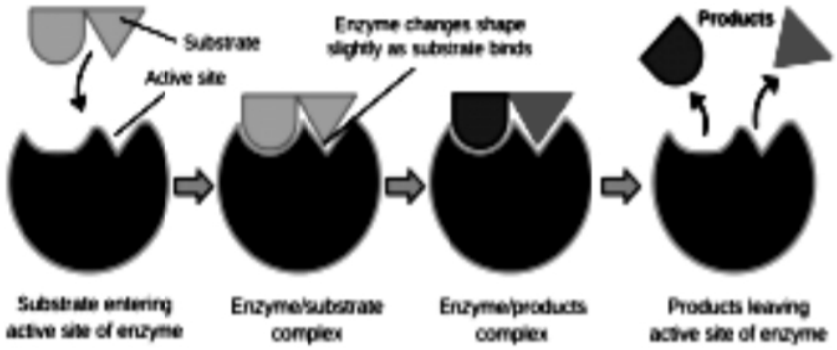
responsible for increasing the rate of reaction in plants and animals. The catalysis in which enzymes act as a catalyst is called enzyme catalysis.

Enzymes are complex compounds which are made up of nitrogen. Animals and plants produce these compounds. Enzymes are proteins which have high molecular mass and form a heterogeneous mixture when dissolved in water. These proteins act very efficiently and are responsible for various reactions which occur in the body of living beings. These proteins can also be called as biochemical catalysts and the catalysis as biochemical catalysis.

Characteristics of enzyme catalysis

- A single molecule of this catalysis can transform a million molecules of the reactant per second. Hence it is highly efficient.
- These biochemical catalysts are unique in nature i.e. the same catalyst cannot be used in more than one reaction.
- The effectiveness of a catalyst is maximum at its optimum temperature. The activity of the biochemical catalysts declines at either side of the optimum temperature.
- Biochemical catalysis is dependent upon the pH of the solution. A catalyst works best at an optimum pH which ranges between 5-7 Ph values.
- The activity of the enzymes usually increases in the presence of a coenzyme or an activator such as Na^{+}, Ca^{2+} .The rate of the reaction increases due to the presence of a weak bond which exists between the enzyme and a metal ion.

Mechanism of enzyme catalyst:



Catalysis with enzyme as a catalyst

The enzyme consists of a number of cavities which are present on the outer surface. These cavities possess groups such as -COOH, -SH, etc. These centres are called as the active centre of the biochemical particle. The substrate which has the opposite charge of the enzyme fits into the cavities just as a key fits into a lock. Due to the existence of the active groups, the complex formed decomposes to give the products.

Hence this happens in two steps:

Step1: Combining of enzyme and the reactant

E+R→ER

Step 2: Disintegration of the complex molecule to give the product

ER→E+P

Enzyme inhibition

Enzymes play central roles in life processes. It holds for most enzymes that their function is needed only in certain conditions. When those conditions do not apply, the activity of a given enzyme can be futile or even harmful. Accordingly, the activity of most enzymes is under strict control. Enzymes can be regulated at multiple levels, ranging from transcriptional regulation of the expression of the enzyme-encoding gene through the direct regulation of the activity of the enzyme molecule by effector molecules to the controlled proteolytic decomposition of the enzyme. In this chapter, only those inhibitors will be reviewed that reversibly and specifically bind to enzymes through non-covalent interactions and inhibit the substrate-binding and/or catalytic apparatus of the given enzyme. These inhibitors can be classified into three mechanistic groups based on their mechanism of action: competitive, uncompetitive and mixed inhibitors. The type of inhibition can be determined through enzyme kinetic measurements.

Competitive inhibition

Competitive inhibitors compete for the substrate-binding site of the enzyme with the substrate, because the substrate and the inhibitor bind to identical or overlapping sites. Due to the overlapping nature of the binding sites, a ternary complex—in which the substrate and the inhibitor would simultaneously bind to the enzyme—cannot form. Accordingly, in the enzyme-inhibitor complex, the enzyme is completely inactive.

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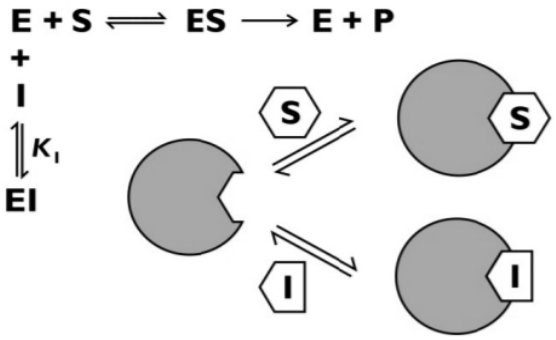


Figure. The scheme of competitive inhibition

Uncompetitive inhibition

Some inhibitors bind only to the ES complex without binding to the free enzyme. This interaction scheme is illustrated in Figure 9.8.

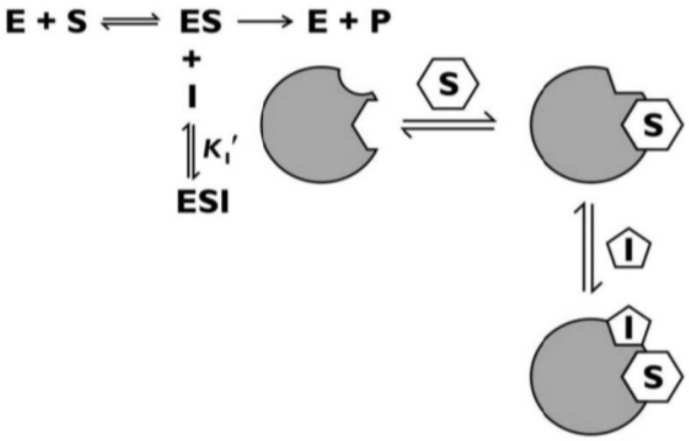


Figure. Uncompetitive inhibition

Mixed or non-competitive inhibition inhibition

There are inhibitors that can bind both to the free enzyme as well as to the ES complex.

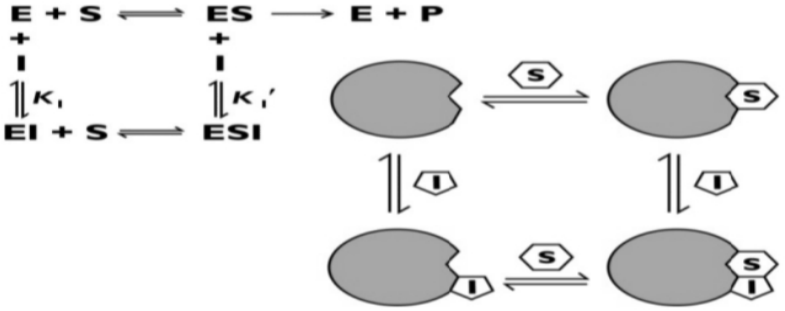


Figure. Mixed inhibition

Isozymes

These were first described by R. L. Hunter and Clement Markert (1957) who defined them as different variants of the same enzyme having identical functions and present in the same individual. This definition encompasses enzyme variants that are the product of different genes and thus represent different loci (described as isozymes) and enzymes that are the product of different alleles of the same gene (described as allozymes).

Isozymes are usually the result of gene duplication, but can also arise from polyploidisation or nucleic acid hybridization. Over evolutionary time, if the function of the new variant remains identical to the original, then it is likely that one or the other will be lost as mutation accumulate, resulting in a pseudogene. However, if the mutations do not immediately prevent the enzyme from functioning, but instead modify either its function, or its pattern of gene expression, then the two variants may both be favoured by natural selection and become specialised to different functions. For example, they may be expressed at different stages of development or in different tissues.

Allosterism

Allosteric regulation (allosterism) is the modulation of an enzyme's activity via the binding of an effector molecule (ligand) to a site *other* than the enzyme's active site (an allosteric site)

Allosteric binding causes a conformational change in the enzyme's structure which affects the enzyme's affinity for substrate

Allosteric regulation can be either positive (activation) or negative (non-competitive inhibition)

Positive Allosterism

An example of allosteric activation is seen in the binding of oxygen molecules to haemoglobin

Haemoglobin is composed of four distinct subunits and can bind up to four oxygen molecules (HbO8)

As each oxygen molecule binds, it changes the conformation of haemoglobin and increases its affinity for oxygen

This ensures that haemoglobin will transport the maximum amount of oxygen from oxygen-rich areas (i.e. the lungs)

Conversely, the release of an O2 molecule decreases haemoglobin's affinity for oxygen – promoting its release in the tissues

Negative Allosterism

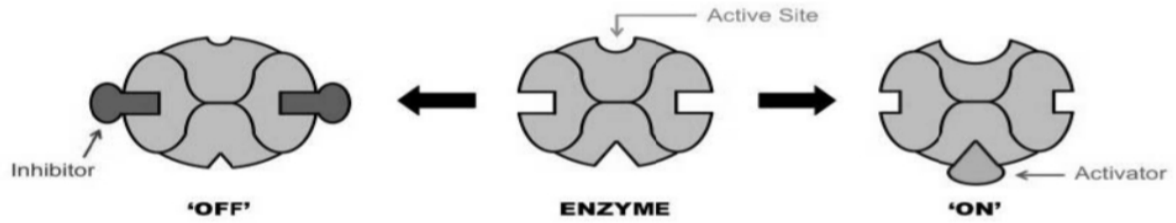
An example of allosteric inhibition can be seen in any example of non-competitive inhibition

Phosphofructokinase (PFK) is an enzyme involved in the breakdown of glucose during glycolysis (to make ATP)

ATP binds to an allosteric site on PFK and inhibits its activity – preventing glycolysis from occurring

Thus ATP prevents the further production of more ATP when energy stocks are high (end-product inhibition)

When energy stocks are low, there is insufficient ATP to inhibit PFK and glycolysis will be able to proceed



Allosteric Regulation

Ribozymes (ribonucleic acid enzymes)

They are RNA molecules that are capable of catalyzing specific biochemical reactions, similar to the action of protein enzymes. The 1982 discovery of ribozymes demonstrated that RNA can be both genetic material (like DNA) and a biological catalyst (like protein enzymes), and contributed to the

RNA world hypothesis, which suggests that RNA may have been important in the evolution of prebiotic self-replicating systems. The most common activities of natural or in vitro-evolved ribozymes are the cleavage or ligation of RNA and DNA and peptide bond formation. Within the ribosome, ribozymes function as part of the large subunit ribosomal RNA to link amino acids during protein synthesis. They also participate in a variety of RNA processingreactions, including RNA splicing, viral replication, and transfer RNA biosynthesis. Examples of ribozymes include the hammerhead ribozyme, the VS ribozyme, Leadzyme and the hairpin ribozyme.

Ribozymes have been proposed and developed for the treatment of disease through gene therapy. One major challenge of using RNA based enzymes as a therapeutic is the short half-life of the catalytic RNA molecules in the body. To combat this, the 2' position on the ribose is modified to improve RNA stability. One area of ribozyme gene therapy has been the inhibition of RNA-based viruses.

A type of synthetic ribozyme directed against HIV RNA called gene shears has been developed and has entered clinical testing for HIV infection. Similarly, a ribozyme has been designed to target the hepatitis C virus RNA. The ribozyme is able to cleave the conserved regions of the virus's genome which has been shown to reduce the virus in mammalian cell culture. Despite these efforts by researchers, these projects have remained in the preclinical stage.

Abzyme

Abzyme is a monoclonal antibody with catalytic activity. Abzymes are usually raised in lab animals immunized against synthetic haptens, but some natural abzymes can be found in normal humans (anti-vasoactive intestinal peptide autoantibodies) and in patients with autoimmune diseases such as systemic lupus erythematosus, where they can bind to and hydrolyze DNA. They are, however, subjects of considerable academic interest. Studying them has yielded important insights into reaction mechanisms, enzyme structure and function, catalysis, and the immune system itself.

Enzymes function by lowering the activation energy of the transition state of a chemical reaction, thereby enabling the formation of an otherwise less-favourable molecular intermediate between the reactant(s) and the product(s). If an antibody is developed to bind to a molecule that's structurally and electronically similar to the transition state of a given chemical reaction, the developed antibody will bind to, and stabilize, the transition state, just like a natural enzyme, lowering the activation energy of the reaction, and thus catalyzing the reaction. By raising an antibody to bind to a stable transition-state analog, a new and unique type of enzyme is produced.

So far, all catalytic antibodies produced have displayed only modest, weak catalytic activity. The reasons for low catalytic activity for these molecules have been widely discussed. Possibilities indicate that factors beyond the binding site may play an important, in particular through protein dynamics. Some abzymes have been engineered to use metal ions and other cofactors to improve their catalytic activity.

Vitamins co factors and coenzymes

Vitamins are organic compounds that are required in small amount (µg or mg/day) for normal growth metabolism and proper functioning of the body.

Vitamins are also known as accessory food factors or food hormones. Animal can't synthesize vitamins, and therefore depends upon plants and microbes for their supply.

Any deficiency of vitamins for a longer period or presence of anti-vitamins in the diet causes deficiency disease or avitaminosis.

However, treatment with antibiotics often results in deficiency of B-Complex vitamins because the intestinal bacteria that synthesize them get killed by such drugs.

For this reason, doctors recommend vitamin B-complex during antibiotic therapy. Excess intake of vitamins may cause some pathological conditions called hyper-vitaminosis. However, it is rare and occurs in case of fat soluble vitamins which can be stored in considerably high amounts.

Types of Vitamins:

On the basis of solubility, vitamins are of two types:

(a) Fat soluble vitamins: Vitamins A, D, E & K.

(b) Water soluble vitamins: Vitamins C and B-complex (B_1, B_2, B_6 etc.)

Vitaminoids:

These are the organic compounds having similar biological properties like vitamins but required in larger amounts.

They are of following types:

(a) Fat soluble vitaminoids:

Essential Fatty acids and ubiquinone's.

(b) Water soluble vitaminoids:

Biotin (Vit-B), Choline (Vit-B_4), Inositol (Vit-B_8), bioflavonoids, N- Lipoic acid etc.

History:

Lunin (1881) first discovered them and Funk (1912) coined the term 'vitamins'. Later, Drummond (1920) proposed the term vitamin by dropping the final 'e'

Pseudovitamin, Antivitamin and Provitamin:

Pseudovitamins (false vitamins) are certain organic compounds that are structurally similar to some vitamins but don't show the physiological actions of the vitamin. For example, methylcobalmine is a pseudovitamin of cyanocobalamine (Vit – B_{12}).

Antivitamin is a compound which resembles structurally to a vitamin but inhibits or antagonize the function of the vitamin. For example, pyrithiamine, galactoflavin and avidin are antivitamins of thiamine, riboflavin and biotin respectively. Provitamin is the precursor of a vitamin which when taken through diet is converted into the vitamin in the body, e.g. (3-carotene (provitamin (A), ergosterol (Provitamin D) etc.

Fat Soluble Vitamins:

Vitamin A:

Clinical name:

Axerophthol, antixeropthalmic or antinyctalopic vitamin.

Chemical name:

Chemically, vitamin A ($C_{20}H_{29}OH$) is an unsaturated alcohol called retinol. Vitamin A exists in two isomeric forms: vitamin A (retinol) is a trans-isomer and occurs naturally, whereas vitamin A_2(dehydroretinal or retional2) is a cisisomer and has 30 – 40% of vitamin A actively.

Sources:

Plant sources like green vegetables, fruits, and cereals supply pro vitamin A (β-carotene) in diet. Animal sources of vitamin A are liver, milk, butter, egg yolk etc. Liver of fresh water fish contain A_2.

Functions:

(i) Retinol (vitamin A aldehyde) combines with lysine residues of opsin protein to form rhodopsin pigments of rod cells of retina, so it is essential for night vision,

(ii) Retinoic acid (vitamin A acid) has some anticancer effects,

(iii) Vitamin A maintains the integrity of epithelial cells; permeability of cell membranes as well as the membranes of organelles,

(iv) In young animals vitamin A causes growth, formation of bones and teeth.

Deficiency Symptoms:

(i) Vitamin A deficiency causes the defective night vision called night blindness (nyctalopia or Henerolopia).

(ii) In children deficiency of vitamin A_2 causes xerophthalmia (drying of conjunctiva) and keratomalacia ulceration and softening of cornea) that may lead to complete blindness,

(iii) Toad's skin is another detectable early symptom of vitamin A where the skin becomes dry and rough particularly in the lateral part of forearms and sides of thigh.

Hypervitaminosis A:

In acute cases of hypervitaminosis A headache, nausea, vomiting and drowsiness noticed. But in chronic cases (prolonged intake) the symptoms develop are anorexia (loss of appetite), alopecia (loss of hair), cracking of lips, dry itchy skin, pain in bones and joints.

Vitamin D:

Clinical name:

Clinically, Vitamin D is called antirachitic vitamin because it possesses the property of curing or preventing ticket.

Chemical name:

Chemically Vitamin D is a steroid which is related to calcium metabolism. Hence it is called calciferol. Vitamin D exists in 2 forms i.e. Vitamin D_2 (ergocalciferol) and Vitamin D_3 (cholecalciferol). They are synthesized from the provitamins by the action of UV-rays of sunlight. Because of it, vitamin D is called sunshine vitamin. The provitamin D_2 (ergosterol) occurs in plants and the provitamin D_3 (7-dehydrocholesterol) occurs in animals. The term vitamin D_1 is no longer used.

Sources:

Vit. D_3 obtained from fish liver oil, egg, milk, butter, ghee etc.

Functions:

(i) Vitamin D_3 is also considered as a prohormone which gives rise to a hormone colcitriol (1, 2, 5 Dihydroxy cholecalciferol) by various metabolic changes. Calcitriol has a role in calcium and phosphate metabolism,

(ii) Vitamin D activates the transcription of mRNA for calcium binding protein,

(iii) It helps in the growth and development of bone and teeth,
(iv) Increase the excretion of phosphate.

Deficiency symptoms:

Vitamin D deficiency leads to rickets in children and osteomalacia in adult. Rickets is characterized by bowlegs, knock knees, bending of ribs leading to pigeon's breast, enlargement of ankles, knees, wrists, elbow etc. In osteomalacia the bones become weak and fragile instead of being soft due to decalcification of bones.

Hypervitaminosis D:

Excess intake of vitamin D cause increase level of Ca and P. As a result, kidney, arteries, muscles, etc. become calcified. The early symptoms cause anorexia (loss of appetite), thrist, constipation and polyuria followed by nausea, vomiting and diarrhea.

Vitamin E:

Clinical name:

It is called antisterility vitamin or fertility vitamin because of its requirement in proper functioning of reproductive system.

Chemical name:

Tocopherol (tokes = child birth; phero = to bear, ol = alcohol)

Source:

Vegetable oil, leafy vegetables, milk, cheese, egg, meat etc.

Function:

(i) It acts as an antioxidant and prevents oxidation of vitamin A, K, essential fatty acids.
(ii) It keeps the skin glowing by reducing keratinization. Hence, it is also called as beauty vitamin,
(iii) It helps in the normal functioning of skeletal muscles, gonads and renal tubules.

Deficiency symptoms:

(i) Causes sterility and miscarriage,
(ii) Causes muscular weakness and dystrophy (degeneration.)

Vitamin K:

Clinical name:

It is called antihemorrhagic vitamin or vitamin for blood clotting or coagulation vitamin.

Chemical name:

Chemically vitamin K is a naphthoquinone derivative. Naturally it occurs in two forms, i.e. vitamin K_1 (phylloquinone) and vitamin K_2(menaquinone or farnoquinone). The vitamin K_3 (mendione) is a synthetic product.

Source:

Green leafy vegetables, soybean, carrots, potatoes, milk, fish and meats etc.

Dietary requirement:

140 – 200 mg.

Function:

(i) Act as co-enzyme Q and participate in oxidative phosphorylation in ETC.,
(ii) Acts as a co-factor of carboxylase,
(iii) Required for fat absorption.

Deficiency symptoms:
(i) Delay in blood clotting,
(ii) Cause hemorrhagic disease of newborn,
(iii) Defective functioning of liver.
Hypervitaminosis K:
Develop hyperbilirubinemia.
Water Soluble Vitamin
Vitamin C:
Clinical name:
It is called antiscorbutic acid because it prevents scurvy. It is also known as anti- rabies or and cancer vitamin.
Chemical name:
Chemically it exists in L-ascorbic acid and L-dehydroascorbic acid (ascorbone). The L-ascorbic acid is ay-lactone (with internal esterification) that is synthesized in plants and most animals except primates and guinea pigs.
Source:
Citrus fruits (lemons, oranges), grapes, apple, papaya, guava, vegetables etc.
Daily requirement:
40 – 50 mg.
Functions:
(i) It is required for absorption of iron,
(ii) It keeps gums and capillary walls healthy.
(iii) It gives resistance against cold and viruses. Hence, it is often called as anti-viral vitamin,
(iv)It is necessary for wound healing,
(v) It acts as co-enzyme for hydroxylation and oxidation -reduction reactions. Thus, it helps in metabolism of amino acids, collagen synthesis etc.
Deficiency symptoms: It causes scurvy (Sailor's disease) characterized by bleeding gums, loosening of teeth, fragile capillaries, failure in wound healing, anemia, general weakness etc.
Vitamin B-Complex:
It includes at least 10-11 different water soluble vitamins. They are grouped together as B-complex because all the members' acts as coenzymes and their actions are closely related.
Thousands of chemical reactions proceed very rapidly at any given instant within all living cells of an organism. Virtually all of these reactions are mediated by remarkable molecular devices called enzymes. That is, the enzymes are central to every biochemical reaction and are called the catalysts of biological systems (biocatalysts).
They in organized sequences and catalyse the hundreds of stepwise reactions by which nutrient molecules are degraded, chemical energy is conserved and transformed, and biological macromolecules are made from simple precursors. Through the action of regulatory enzymes, metabolic pathways are highly coordinated to yield a harmonious interplay among the many different activities necessary to sustain life.
Enzymes catalyse an enormous diversity of biochemical reactions due to their capacity to specifically bind a very wide range of molecules. By utilizing the full repertoire of intermolecular forces, enzymes

bring substrates together in an optimal orientation, the prelude to making and breaking chemical bonds.

They catalyse reactions by stabilizing transition states, the highest energy-species in reaction pathways. By selectively stabilizing a transition state, an enzyme determines which one of several potential biochemical reactions actually takes place.

Until 1980s, all enzymes were believed to be proteins. Then, Tom Cech and Sidney Altman independently discovered that certain RNA molecules may function as enzymes may be effective biocatalysts. These RNA biocatalysts have come to be known as ribozymes.

An enzyme is a protein that is synthesised in a living cell and catalyses or speeds up a thermodynamically possible reaction so that the rate of the reaction is compatible with the biochemical process essential for the maintenance of the cell. It is sometimes called as organic catalyst or biocatalyst. Over 90% of enzymes are simple globular proteins (Fig. 8.14). The remainder is conjugated proteins, which have a non-protein fraction called the prosthetic group. Many enzymes have relative molecular mass of between 10,000 and 50,000da.

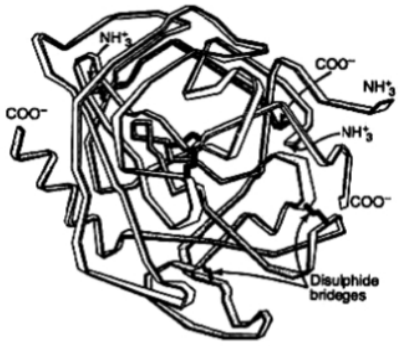


Fig. 8.14 : Three-dimensional globular structure of chymotrypsinogen. It is an inactive precursor of pancreatic chymotrypisn

The first enzyme discovered was amylase, which catalyses the conversion of starch to maltose, in 1833 by two French chemists Payen and Persoz. However, it was not well-known until 1876 when Wilhelm Kuhne, the distinguished German biochemist, proposed the term enzyme.

Unit of Enzyme:

The actual molar amount of the enzyme in an enzyme-catalyzed reaction is not known in many situations. In such cases the amount of enzyme can be expressed in terms of the enzyme activity observed.

The International Commission on Enzymes established by International Union of Biochemistry defines One International Unit of enzyme as the amount of enzyme that catalyzes the formation of one micromole of product in one minute.

In determining the One International Unit the conditions of assay must be specified because enzymes are very sensitive to factors such as pH, temperature, and ionic strength. Another definition for units of enzyme is the 'katal'. One katal is defined as the amount of enzyme that catalyses the conversion of one mole of substrate to product in one second. Thus, one katal equals 6 x 107 international units.

Chemical Nature of Enzymes:

All enzymes are globular proteins with the exception of recently discovered RNA enzymes. Some enzymes may additionally contain a non-protein group. Accordingly there are two types of enzymes, simple and conjugate.

Simple Enzyme:
It is an enzyme which is wholly made up of protein. Active site is formed by specific grouping of its own amino acids. Additional substance or group is absent, e.g., pepsin, trypsin, urease.

Conjugate Enzyme:
It is an enzyme which is formed of two parts— a protein part called apoenzyme (e.g., flavoprotein) and a nonprotein part named cofactor. The complete conjugate enzyme, consisting of an apoenzyme and a cofactor, is called holoenzyme. Active site is formed jointly by apoenzyme and cofactor.
Cofactor is small, heat stable and dialysable part of conjugate enzyme. It may be inorganic or organic in nature. Organic cofactors are of two types, coenzymes and prosthetic groups.
Coenzymes are easily separable non-protein organic cofactors. Prosthetic groups are non-protein organic cofactors firmly attached to apoenzymes, e.g., heme (=haem), biotin, pyridoxal phosphate. Heme (= haem) is iron containing prosthetic group in cytochromes, haemoglobin, myoglobin, catalase and peroxidase.
The last two cause breakdown of hydrogen peroxide to water and oxygen. FMN and FAD are considered prosthetic groups by some workers while others consider them to be coenzymes.
Both coenzyme and prosthetic group take part in group transfer reactions. Prosthetic group requires a single apoenzyme for picking up the group and transferring the same. Coenzyme requires two Apo enzymes, one for picking up the group and the second for transferring the group, e.g., NAD^+, $NADP^+$, CoA.

Active Site or Active Spot:
The whole of enzyme molecule is not active in catalysing a chemical reaction. Only a small portion of it is active. It is called active site or active spot. An enzyme may have one to several active sites. An active site or spot is an area of the enzyme which is capable of attracting and holding particular substrate molecules by its specific charge, size and shape so as to allow the chemical change.
It fails to recognise other molecules. Active site consists of a few amino acids and their side groups which are brought together in a particular fashion due to secondary and tertiary folding of a protein molecule (Fig. 9.27) and its association with the cofactor, if any.
For example, the active site for aldolase is glycine-histidine-alanine while that of pyruvic oxidase is aspartic acid-cysteine-alanine. The remaining amino acids help maintain the shape of the enzyme molecule.

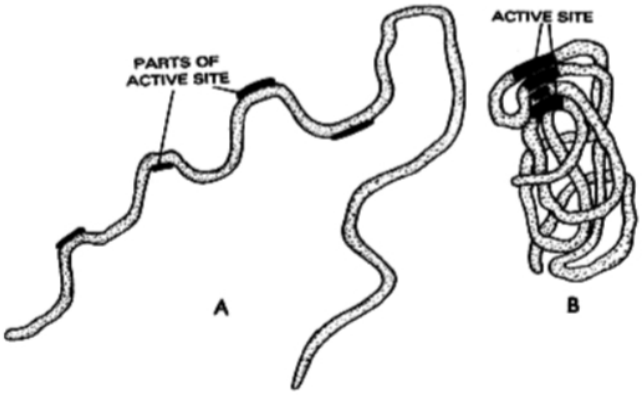


Fig. 9.27. Grouping of amino acids of a polypetide during the formation of tertiary structure to produce an active site.

Characteristics of Enzymes:

All enzymes are proteins, but a functional enzyme has different components and these components are named differently, viz.,

Holoenzyme:
A conjugated protein and functional enzyme.

Apoenzyme:
Polypeptide segment of the enzyme, which is catalytically inactive.
Coenzyme:
The non-protein organic moiety, which can frequently be separated from the apoenzyme.
Prosthetic group:
If a substance is firmly (covalently) attached to the protein part of the enzyme, it is referred to as a prosthetic group. It is the non-protein portion of any conjugated protein. So coenzyme is a specific example of prosthetic group.
Activator:
There are many metalloprotein enzymes in which the metal ion (e.g. Mg^{++}, Mn^{++}, and Zn^{++}) is bonded either to the apoenzyme or to the coenzyme. The metal is usually designated as activator. They form a co-ordination complex between the enzyme and the substrate, and activate the substrate by prompting electronic shifts.
Pro-enzyme or Zymogens:
They are simple protein enzymes, which are secreted, in an inactive form.

Modes of Enzyme Action:

There are two view points by which enzymes are supposed to bring about chemical reaction.
i. Lock and Key Hypothesis:
It was put forward by Emil Fischer in 1894. According to this hypothesis, both enzyme and substrate molecules have specific geometrical shapes. 'In the region of active sites the surface configuration of the enzyme is such as to allow the particular substrate molecules to be held over it. The active sites also contain special groups having —NH2, —COOH, —SH for establishing contact with the substrate molecules.
The contact is such that the substrate molecules or reactants come together causing the chemical change. It is similar to the system or lock and key. Just as a lock can be opened by its specific key, a substrate molecule can be acted upon by a particular enzyme. This also explains the specificity of enzyme action.
After coming in contact with the active site of the enzyme, the substrate molecules or reactants form a complex called enzyme-substrate complex. In the complexed state the molecules of the substrate undergo chemical change.
The products remain attached to the enzyme for some time so that an enzyme-product complex is also formed. However, the products are soon released (Fig. 9.34) and the freed enzyme is able to bind more substrate molecules.

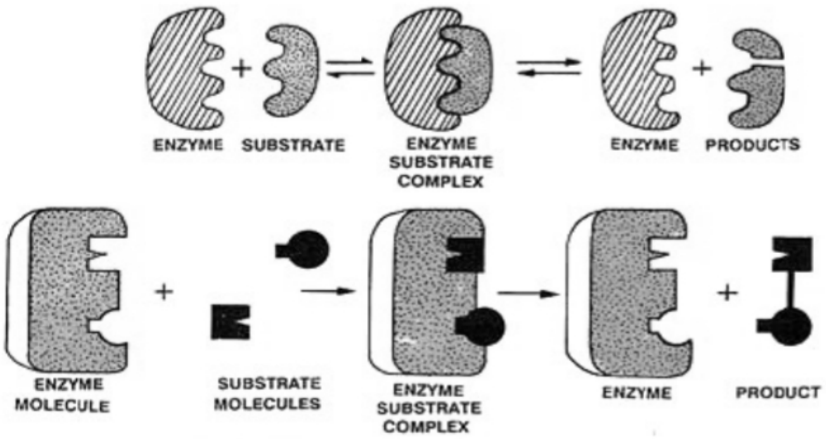


Fig. 9.33. Lock and key theory of enzyme action.
Upper Series – Breakdown Reaction Lower Series – Biosynthetic Reaction

Enzyme + Substrate ⇋ Enzyme – Substrate Complex
Enzyme – Substrate Complex ⇋ Enzyme – Products Complex
Enzyme – Products Complex ⇋ Enzyme + Products
Thus we see that the chemical reactants do not cause any alteration in the composition or physiology of the enzyme.

The same enzyme molecule can be used again and again (Fig. 9.35). Hence, enzymes are required in very small concentrations.

Evidences:

1. Blow and Steitz (1970) have found the formation of complex between the enzyme chymotrypsin and its substrate.
2. Keilen and Maun have observed that the absorption spectra of the same enzyme are different in the free state and in the presence of the substrate.
3. The theory explains how a small concentration of enzyme can act upon a large amount of the substrate.
4. Lock and key theory explains how the enzyme remains unaffected at the end of chemical reaction.
5. It is able to predict the increase in the rate of chemical reaction on the addition of more enzyme or substrate.
6. The theory explains how a substance having a structure similar to the substrate can work as competitive inhibitor.

ii. Induced-Fit Theory (Fig. 9.35):

It is modification of lock and key hypothesis which was proposed by Koshland in 1959. According to this theory the active site of the enzyme contains two groups, buttressing and catalytic. The buttressing group is meant for supporting the substrate. The catalytic group is able to weaken the bonds of reactants by electrophilic and nucleophilic forces.

The two groups are normally at a distance. As soon as the substrate comes in contact with the buttressing group, the active site of the enzyme undergoes conformational changes so as to bring the catalytic group opposite the substrate bonds to be broken.

Catalytic group helps in bringing about chemical reaction. The substrate is converted into product. The product is unable to hold on the buttressing site due to change in its structure and bonds. Buttressing group reverts to its original position. The product is released.

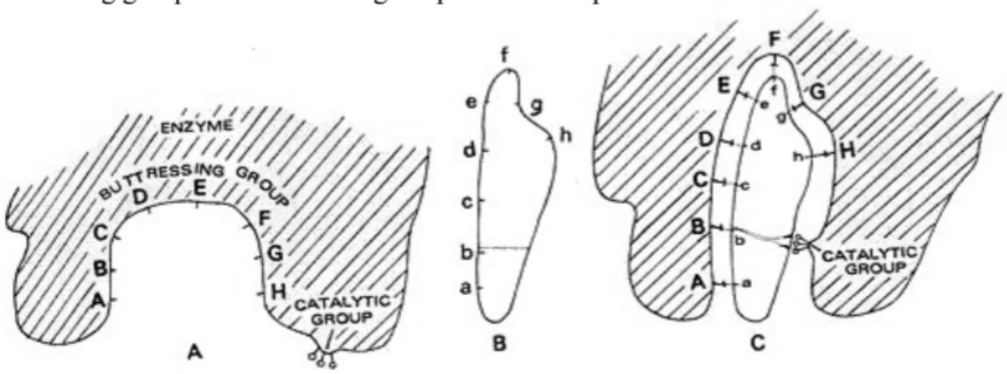


Fig. 9.35. Induced-fit theory of enzyme action. A, active site of enzyme. B, substrate molecule. C, enzyme-substrate complex with conformational changes so as to bring the catalytic group against the substrate bonds to be broken.

Inhibition of Enzyme Action:

Four common types of enzymes inhibition are as follows:

i. Protein Denaturation:

Enzyme activity is dependent upon the maintenance of tertiary structure of the protein moiety. The latter is destroyed by several factors like heat, high energy radiations and salts of heavy metals.

ii. Competitive inhibition:

It is the inhibition of enzyme activity by the presence of a chemical that competes with the substrate for binding to the active site of the enzyme. The inhibitor chemical is also called substrate analogue or competitive inhibitor.

It resembles the substrate in structure and gets bound up to the active site of the enzyme without getting transformed by the latter (Fig. 9.37). As a result, the enzyme cannot participate in catalytic change of the substrate. This is similar to the jamming of a lock by a key similar to original one.

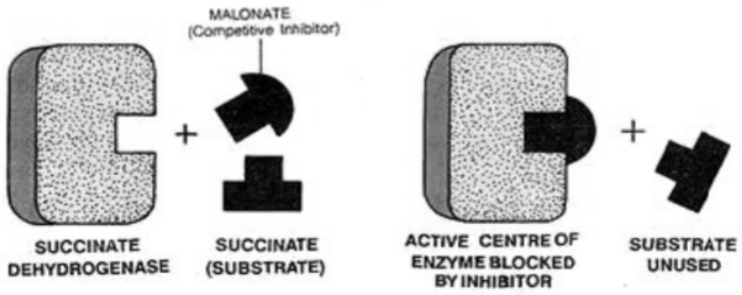


Fig. 9.37. Competitive inhibition of enzyme action.

Equilibrium constant for inhibitor binding is called K_i. A high $K_i$reduces enzyme activity while a low K_i allows enzyme activity to continue though at a reduced rate. Classical example of competitive inhibition is reduction of activity of succinate dehydrogenase by malonate, oxaloacetate and other anions which resemble succinate in their structure.

Competitive inhibition is usually reversible since the addition of more substrate tends to reduce the effect of the inhibitor.

The inhibition is important in that:

(i) It gives evidence for lock and key hypothesis of enzyme action,
(ii) Substrate analogues are not metabolized by enzymes,
(iii) Control of bacterial pathogens has been effected through competitive inhibition.
Sulpha drugs (e.g., sulphanilamide) inhibit the synthesis of folic acid in bacteria by competing with p-amino benzoic acid (PABA) for the active site of enzyme. Preformed folic acid is obtained by animal cells. Therefore, sulpha drugs do not harm them.

iii. Non-competitive Inhibition:

It is an irreversible inhibition of enzyme activity by the presence of a substance that has no structural similarity with the substrate. It is of two types, reversible and irreversible.

The irreversible non-competitive inhibitor destroys or combines irreversibility with a functional group of enzyme that is essential for its catalytic function. Cyanide inhibits the activity of cytochrome oxidase by combining with its metallic ions.

It has no structural similarity with the substrate of the enzyme, namely cytochrome c. Cytochrome oxidase is a respiratory enzyme. In its inhibition, the animal is unable to perform the respiration properly and gets killed. Di-isopropyl fluorophosphates (DFP, a nerve gas) prevents impulse transfer by combining irreversibly with amino acid serine of acetylcholine esterase.

It also poisons a number of other enzymes like trypsin, chymotrypsin, phosphoglucomutase, elastase, etc. lodoacetamide inhibits enzymes having sulphahydryl (—SH) or imidazole group.

iv. Allosteric Modulation or Feed Back Inhibition:

It is a type of reversible inhibition found in allosteric enzymes. The inhibitor is non-competitive and is usually a low molecular intermediate or product of a metabolic pathway having a chain of reactions involving a number of enzymes. It is, therefore, also called end product or feedback inhibition.

The inhibitor is also called modulator. Modulator is a substance that attaches with an allosteric enzyme at a site other than catalytic one but influences the latter, either inhibiting or activating the same. An example of feed back or allosteric inhibition is stoppage of activity of enzyme hexokinase (glucokinase) by glucose-6-phosphate, the product of reaction catalysed by it (Fig. 9.38).

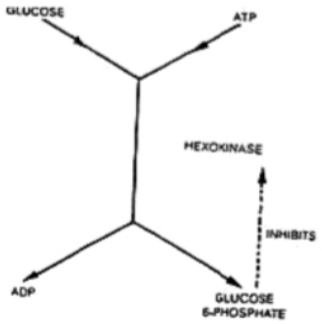


Fig. 9.38. Feedback or allosteric inhibition of hexokinase.

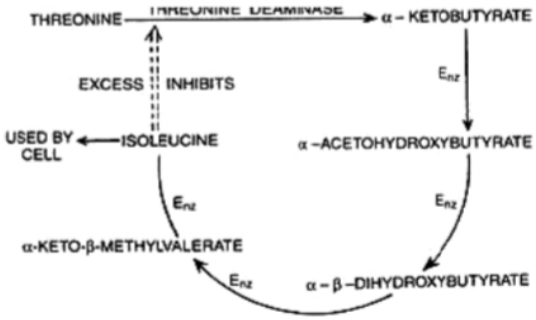


Fig. 9.39. Feedback or end product in case of isoleucine.

Another example is inhibition of threonine deaminase by isoleucine (Fig. 9.3). Amino acid isoleucine is formed in bacterium Escherichia coli in a 5-step reaction from threonine. Each step requires a separate enzyme. When isoleucine accumulates beyond a threshold value, its further production stops. Isoleucine added to the medium of bacterium also stops its internal production showing that its excess prevents some step of the reaction. The latter was found out to be enzyme threonine deaminase which is involved in the first step of the reaction (threonine to a-ketobutyrate).

Importance:

(i) It has a regulatory role on enzyme activity,
(ii) Enzyme inhibitors have been used in the study of metabolic pathways,
(iii) Some inhibitors are used in controlling pathogenic activity, e.g., sulpha drugs,
(iv) Use of inhibitors have shown the mechanism of enzyme action.

Feedback Inhibition of Enzymes:

Feedback inhibition (also called end-product inhibition or allosteric modulation) is one in which the end- product of the reaction acts as inhibitor and inhibits the activity of regulatory enzyme, usually, enzyme of the first step of a biosynthetic pathways. In multi-enzyme system synthesis of a product is completed in a number of steps, each step being catalyzed by a specific enzyme.

In some of such systems, the regulatory enzyme is specifically inhibited by the end-product of the pathway whenever the concentration of the end-product exceeds the cell's requirement. When the reaction catalyzed by the regulatory enzyme is slowed, all subsequent enzymes act at reduced rates due to the depletion of their substrates.

The rate of production of the pathway's end-product is thereby brought into balance as per the requirement of the cell. Feedback inhibition is beautifully illustrated by biosynthesis of L-isoleucine from L-threonine (Fig. 10-12).

In this system, the first enzyme, threonine dehydratase, is inhibited by L-isoleucine. No other intermediate in the sequence inhibits threonine dehydratase, nor is any other enzyme of the system inhibited by L-isoleucine.

L-isoleucine binds not to the active site, but to regulatory site on the enzyme molecule; this is called allosteric modulation. However, when the concentration of the end-product drops sufficiently, the enzyme reactivates and the end-product is resynthesized.

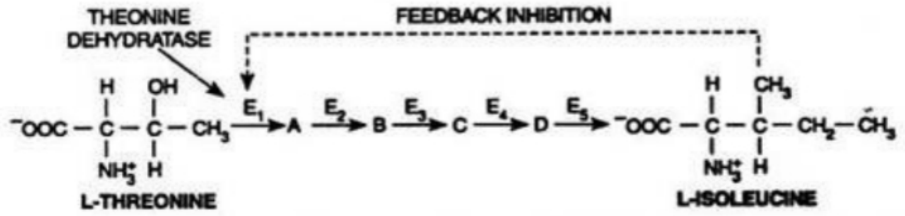


FIG. 27.12. Feedback inhibition. The conversion of L-threonine to L-isoleucine is a multistep system catalyzed by a sequence of five enzymes (E_1 to E_5). Threonine dehydratase (E_1) is specifically inhibited by L-isoleucine, the end-product of the pathway.

Regulation of Enzyme:

Biochemical reaction studies have shown that the pace of a chemical reaction in a biological system is maintained by the activities of the enzymes. Enzymes are rather unstable molecules and are synthesized and degraded simultaneously. Their activities may be regulated either through their synthesis or by modifying the existing enzyme molecules.

The activities of enzyme molecules are regulated by several ways which are the following:

I. Allosteric Regulation:

Allosteric regulation is a fine mechanism of controlling a reaction through the enzyme activity. Some enzymes (called allosteric enzymes), show sigmoidal curve between the substrate concentration and the activity. The activity of these enzymes is modified by several metabolites. The effect of different concentrations of 'activator' and 'inhibitor' on these enzymes is also sigmoid.

These effector molecules have a structure different from the substrate molecules. In most of the cases, allosteric inhibitors are the end products of the reaction; inhibiting the first enzyme in the series.

Thus, this kind of inhibition is called feedback inhibition, end product inhibition or retro-inhibition. The allosteric activators arc normally one of the substrates or cofactors of the enzyme. The effect of the allosteric 'inhibitor' or 'activator' on the enzyme is reversible.

When they are withdrawn, the enzyme resumes the original activity:

i. Allosteric Inhibition:

Inhibition of threonine deaminase by isoleucine is an example of allosteric inhibition. Threonine deaminase deaminates threonine to α-ketrobutyrate. The final product of the reaction is isoleucine.

Whenever the accumulation of isoleucine occurs, conversion of threonine to α-ketobutyrate and consequently formation of other intermediaries in the biosynthesis of isoleucine is stopped. When isolcucine is used up, threonine deaminase is reactivated and reactions for the biosynthesis of isoleucine start again.

ii. Allosteric Activation:

Activation of glycogen synthetase by glucose-6-phosphate is an example of allosteric activation. Another example of allosteric regulation (of both inhibitory and activating type) is observed during Pasteur effect. Pasteur effect is the inhibition of glycolysis and fermentation by oxygen. The molecular basis of this effect is the allosteric inhibition of enzyme phosphofructokinase by ATP and citrate and its activation by AMP.

Like many others, this kind of regulation is also of adaptive significance. As the level of AMP increases due to increased use of ATP in the cell, glycolysis is increased by the activation of phosphofructokinase with the result of more formation of ATP. When ATP level exceeds normal requirement of the cell, inhibition of glycolysis occurs through the same enzyme phosphofructokinase and ATP synthesis is stopped.

iii. Mechanism of Allosteric Regulation:

Regarding the mechanism of allosteric regulation, it is proposed that allosteric enzymes have two active centers; one for the substrate and the other for effector. These two sites lie either on same or on two different subunits. Binding of a effector molecule to one type of subunit changes the structure of the enzyme molecule in such a way that binding of the substrate to the other subunit is affected.

To explain the mechanism, an example of allosteric regulation of aspartate transcarbamylase may be cited. Asparate transcarbamylase contains two types of subunits. These two types of subuntis may be split apart by treatment with mercurials; with one type retaining the ability to bind with the substrate, whereas the other to recognize the inhibitor.

When these two species of subunits are together (active enzyme molecule), binding of the inhibitor to one type of subunit changes the structure of other subunits in such a way that the binding of the substrate is inhibited. When the subunits containing binding sites for the inhibitor are removed enzyme is not affected by the inhibitor.

Further, it gives a typical Michaelis-Menten curve with the substrate concentration. Similarly, the binding of activator may change the molecular structure in such a way that the binding of substrate is facilitated.

12. Let's sum up

- Water potential is the chemical potential of water divided by the partial molal volume of water. The major factors influencing the water potential in plants are concentration, pressure,and gravity.
- In higher plants water is absorbed through root hairs which are in contact with soil water and form a root hair zone a little behind the root tips. During absorption of water by roots, the flow of water from epidermis to endodermis may take place through three different pathways:(i) Apoplastic pathway, (ii) Trans-membrane pathway and (iii) Symplast pathway.
- Three different theories of the mechanism by which ascent of water are brought about in plants. These hypotheses are (i) Root pressure (ii) Capillary action (iii) Cohesion-tension.
- A cell wall can have up to three parts— middle lamella, primary wall and secondary wall. The middle lamella is laid down first, formed from the cell plate during primary cell wall is then deposited inside the middle lamella.
- Signal transduction is the process by which a chemical or physical signal is transmitted through a cell as a series of molecular events, most commonly protein phosphorylation catalyzed by protein kinases, which ultimately results in a cellular response. Proteins responsible for detecting stimuli are generally termed receptors, although in some cases the term sensor is used.
- In plants, red and blue light are especially effective in inducing a photo-morphogenetic response. Phytochrome is a blue protein pigment responsible for the perception of light in photo-physiological processes. It is possibly the only photoreceptor in photoperiodism and the flowering process.

- Plants produce signaling molecules, called hormones that have profound effects on development at vanishingly low concentrations. Until quite recently, plant development was thought to be regulated by only five types of hormones: auxins, gibberellins, cytokinins, ethylene, and abscisic acid.

- Embryogenesis transforms a single-celled zygote into a multicellular, microscopic, embryonic plant. Differentiation is the process by which a cell acquires metabolic, structural, and functional properties that are distinct from those of its progenitor cell. Germination is a process by which the embryo in the seed becomes activated and begins to grow into a new seedling.

- Senescence is the process of aging in plants. Plants have both stress-induced and age-related developmental aging. Chlorophyll degradation during leaf senescence reveals the carotenoids, and is the cause of autumn leaf color in deciduous trees. Leaf senescence has the important function of recycling nutrients, mostly nitrogen, to growing and storage organs of the plant.

- Flower initiation takes place by the transformation of vegetative apex into a reproductive structure. It signifies a transition from vegetative to the floral state. The shoot meristem is reduced and is also induced to develop sepals, petals, stamens and carpels in place of leaves.The ABC model of flower development in angiosperm demonstrates the presence of three classes of genes that regulate the development of floral organs. The genes are referred to as class A genes, class B genes and class C gene.
- Stress is usually defined as an external factor that exerts a disadvantageous influence

on the plant. In most cases, stress is measured in relation to plant survival, crop yield, growth (biomass accumulation), or the primary assimilation processes which are related to overall growth.

- A buffer solution is an aqueous solutionconsisting of a mixture of a weak acid and its conjugate base, or vice versa. pH is defined as the decimal logarithm of the reciprocal of the hydrogen ion activity, aH+, n a solution.

- Enzymes are proteins that act as catalysts within living cells. Catalysts increase the rate at which chemical reactions occur without being consumed or permanently altered themselves.
- A chemical reaction is a process that converts one or more substances to another type of substance.

- Carbohydrates are a group of organic compounds consisting of C, H, O usually in the ratio of 1: 2: 1. Lipids are heterogeneous group of water insoluble compounds which are oily or greasy in consistency but soluble in non-polar solvents. Proteins are organic nitrogenous compounds in which a large number of amino acids are joined together by peptide linkages to form long polypeptide chains.

- Photosynthesis is the process that gives life to all living beings. The plants convert light energy into life energy. It is the only biological process that makes use of sun's light energy for driving the life machinery.

- In respiration carbohydrate is broken down, as a result the potential energy is transformed into kinetic form. Though it is a destructive or katabolic process, yet respiration is extremely beneficial, because it releases the necessary energy for performing the life functions.

13. Suggested Reading

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5. Mathews, C.K., Van Holder, K.E. & Ahren, K.G. Bio-Chemistry (3rd ed.), 2000, Pearson Education.
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10. Dainty, J. (1976) Water relations of plant cells. In Transport in Plants,Vol. 2, Part A: Cells (Encyclopedia of Plant Physiology, New
11. Series, Vol. 2.), U. Lüttge and M. G. Pitman, eds., Springer, Berlin, pp. 12–35.

14. Assignment

1. What will be the value of DPD in a fully turgid cell?
2. Where does photorespiration take place?
3. Discuss physiology of flowering with special reference to photoperiodism
4. Describe the mechanisms of enzyme actions.
5. Describe how plants use light and hormones to influence the germination of the seed.
6. How does phytochrome mediate the photomorphogenetic response?
7. Differentiate between cyclic and non-cyclic photophosphorylation.
8. Define buffer. Give an example.
9. Give an account of the secondary structure of protein.
10. What is Leghaemoglobin?
11. Write a short note on mode of action of auxin.
12. What do you mean by turgor pressure?
13. Explain the mechanism of water Absorption in plant.
14. What do you mean by Ascent of Sap?
15. What is the meaning of CAM? State the significance of it.
16. How does gibberellin induce α-amylase synthesis in aleurone layer of cereals?
17. Differentiate between apoplastic and symplastic pathway.

SYLLABUS
COURSE–BOTCOR T206
(Plant Physiology and Biochemistry) (Full Marks–75)
Group B (Plant Metabolism & Development)

Course	Group	Details Contents Structure		Study hour
BOTCOR T206	Group B (Plant Metabolism & Development)	Phytohormones and Growth Regulators in Plant Development:	**Unit 8.** Chemistry, biosynthesis, physiological effects, and signal transduction pathways of auxins, gibberellins,	1
			Unit 9. Signal transduction pathways of cytokinin, ethylene, abscisic acid, brassinosteroids, polyamines, jasmonates.	1
		Signal Transduction and Sensory Photobiology:	**Unit 10.** Signal transduction in higher plants and Light control of plant development; phytochrome: properties, phytochrome induced response, phytochrome signaling pathways, blue light responses.	1
		Control of Flowering:	**Unit 11.** Floral meristem and floral organ development, floral evocation.	1
		Senescence and Programmed Cell Death:	**Unit 12.** Types of senescence, metabolic changes associated with senescence. Senescence and its regulation, influence of hormones.	1
		Plant Products in Metabolism:	**Unit 13.** Structure and properties of carbohydrates, lipids, amino acids, proteins, nucleic acids;	1
		Energy Yielding Metabolisms:	**Unit 14.** Paths of energy synthesis through Glycolysis, Citric acid cycle, plant mitochondrial electron transport chain, alternative oxidase, PPP cycle, regulation of respiratory pathways, Lipid metabolism: fatty acid biosynthesis and oxidation.	1
		Nitrogen Metabolism:	**Unit 15.** Biological and non-biological nitrogen fixation, nitrate and ammonium assimilation.	1

Content

COURSE – BOTCOR T206

GROUP-B (Plant Growth & Biochemical Processes)

Content Structure

1. Introduction
2. Course Objective
3. Phytohormones and Growth Regulators in Plant Development: Chemistry, biosynthesis, physiological effects, and signal transduction pathways of auxins, gibberellins, cytokinin, ethylene, abscisic acid, brassinosteroids, polyamines, jasmonates. (6)
4. Signal Transduction: Signal transduction in higher plants. (2)
5. Sensory Photobiology: Light control of plant development; phytochrome: properties, phytochrome induced response, phytochrome signaling pathways, blue light responses. (3)
6. Control of Flowering: Floral meristem and floral organ development, floral evocation. (3)
7. Senescence and Programmed Cell Death: Types of senescence, metabolic changes associated with senescence and its regulation, influence of hormones. (2)
8. Plant Products in Metabolism: Structure and properties of carbohydrates, lipids, amino acids, proteins, nucleic acids; secondary metabolites. (5)
9. Energy Yielding Metabolisms: Paths of energy synthesis through Glycolysis, Citric acid cycle, plant mitochondrial electron transport chain, alternative oxidase, PPP cycle, regulation of respiratory pathways, Lipid metabolism: fatty acid biosynthesis and oxidation.
10. Nitrogen Metabolism: Biological and non-biological nitrogen fixation, nitrate and ammonium assimilation.
11. Let us sum up
12. Suggested Reading
13. Assignment

1.Introduction

Plant physiology is a subdiscipline of botany concerned with the functioning, or physiology, of plants. Closely related fields include plant morphology (structure of plants), plant ecology (interactions with the environment), phytochemistry (biochemistry of plants), cell biology, genetics, biophysics and molecular biology. Fundamental processes such as photosynthesis, respiration, plant nutrition, plant hormonefunctions, tropisms, nastic movements, photoperiodism, photomorphogenesis, circadian rhythms, environmental stress physiology, seed germination, dormancy and stomata function and transpiration, both parts of plant water relations, are studied by plant physiologists. The field of plant physiology includes the study of all the internal activities of plants-those chemical and physical processes associated with life as they occur in plants. This includes study at many levels of scale of size and time. At the smallest scale are molecularinteractions of photosynthesis and internal diffusion of water, minerals, and nutrients. At the largest scale are the processes of plant development, seasonality, dormancy, and reproductive control. Major subdisciplines of plant physiology include phytochemistry (the study of the biochemistry of plants) and phytopathology (the study of disease in plants). The scope of plant physiology as a discipline may be divided into several major areas of research. Finally, plant physiology includes the study of plant response to environmental conditions and their variation, a field known as environmental physiology. Stress from water loss, changes in air chemistry, or crowding by other plants can lead to changes in the way a plant functions. These changes may be affected by genetic, chemical, and physical factors.

2. Course Objectives

- The objective of this module is not only to develop a better aptitude towards the knowledge of physio-biochemical metabolism of plant system, but also to underline the need for better management with knowledge and skills to handle the task.
- On completion of this study material will be better knowledge –
  - Water potential activity and its significance in plant system
  - Different metabolic analysis of plants
  - Adaptation of plants and regulation of hormonal system
  - Energy conservation, signaling system

3. Phytohormones and Growth Regulators in Plant Development: Chemistry, biosynthesis, physiological effects, and signal transduction pathways of auxins, gibberellins, cytokinin, ethylene, abscisic acid, brassinosteroids, polyamines, jasmonates.

In higher plants, regulation and coordination of metabolism, growth, and morphogenesis often depend on chemical signals from one part of the plant to another. This idea originated in the nineteenth century with the German botanist Julius von Sachs (1832–1897).

Sachs proposed that chemical messengers are responsible for the formation and growth of different plant organs. He also suggested that external factors such as gravity could affect the distribution of these substances within a plant. Although Sachs did not know the identity of these chemical messengers, his ideas led to their eventual discovery.

Plants produce signaling molecules, called *hormones* that have profound effects on development at vanishingly low concentrations. Until quite recently, plant development was thought to be regulated by only five types of hormones: auxins, gibberellins, cytokinins, ethylene, and abscisic acid. However, there is now compelling evidence for the existence of plant steroid hormones, the brassinosteroids, that have a wide range of morphological effects on plant development.

Biosynthesis and metabolism of auxin:

Went's studies with agar blocks demonstrated unequivocally that the growth-promoting "influence" diffusing from the coleoptile tip was a chemical substance. The fact that it was produced at one location and transported in minute amounts to its site of action qualified it as an authentic plant hormone.

In the years that followed, the chemical identity of the "growth substance" was determined, and because of its potential agricultural uses, many related chemical analogs were tested. This testing led to generalizations about the chemical requirements for auxin activity. In parallel with these studies, the agar block diffusion technique was being applied to the problem of auxin transport. Technological advances, especially the use of isotopes as tracers, enabled plant biochemists to unravel the pathways of auxin biosynthesis and breakdown.

Multiple Pathways Exist for the Biosynthesis of IAA

IAA is structurally related to the amino acid tryptophan, and early studies on auxin biosynthesis focused on tryptophan as the probable precursor. However, the incorporation of exogenous labeled tryptophan (e.g., [3H]tryptophan) into IAA by plant tissues has proved difficult to demonstrate.

Nevertheless, an enormous body of evidence has now accumulated showing that plants convert tryptophan to IAA by several pathways, which are described in the paragraphs that follow.

The IPA pathway. The **indole-3-pyruvic acid** (**IPA**) pathway (see Figure 19.6C), is probably the most common of the tryptophan-dependent pathways. It involves a deamination reaction to form IPA, followed by a decarboxylation reaction to form indole-3-acetaldehyde (IAld). Indole-3- acetaldehyde is then oxidized to IAA by a specific dehydrogenase.

The TAM pathway. The **tryptamine** (**TAM**) **pathway** (see Figure 19.6D) is similar to the IPApathway, except that the order of the deamination and decarboxylation reactions is reversed, and different enzymes are involved. Species that do not utilize the IPApathway possess the TAM pathway. In at least one case (tomato), there is evidence for both the IPAand the TAM pathways (Nonhebel et al. 1993).

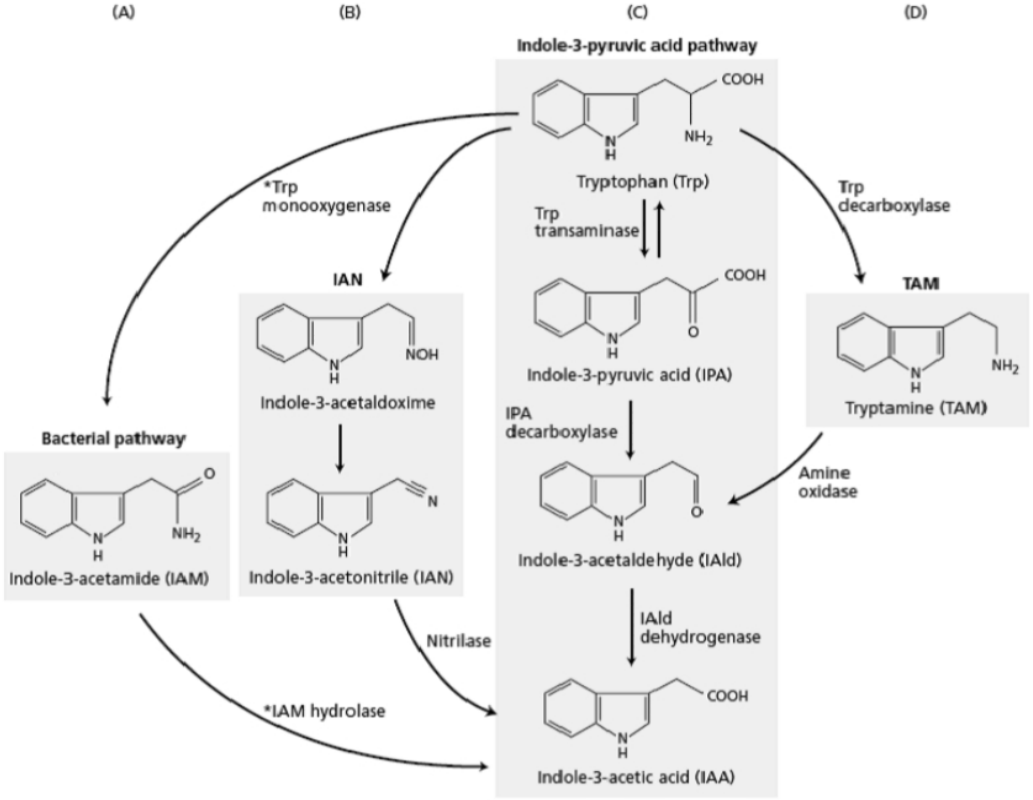


FIGURE 19.6 Tryptophan-dependent pathways of IAA biosynthesis in plants and bacteria. The enzymes that are present only in bacteria are marked with an asterisk. (After Bartel 1997.)

The IAN pathway. In the **indole-3-acetonitrile** (**IAN**) pathway (see Figure 19.6B), tryptophan is first converted to indole-3-acetaldoxime and then to indole-3-acetonitrile. The enzyme that converts IAN to IAA is called *nitrilase*. The IAN pathway may be important in only three plant families: the Brassicaceae (mustard family), Poaceae (grass family), and Musaceae (banana family). Nevertheless, nitrilase- like genes or activities have recently been identified in the Cucurbitaceae (squash family), Solanaceae (tobacco family), Fabaceae (legumes), and Rosaceae (rose family).

Four genes (*NIT1* through *NIT4*) that encode nitrilase enzymes have now been cloned from *Arabidopsis*. When *NIT2* was expressed in transgenic tobacco, the resultant plants acquired the ability to respond to IAN as an auxin by hydrolyzing it to IAA (Schmidt et al. 1996).

Another tryptophan-dependent biosynthetic pathway— one that uses **indole-3-acetamide** (**IAM**) as an intermediate (see Figure19.6A)—is used by various pathogenic bacteria, such as *Pseudomonas savastanoi* and *Agrobacterium tumefaciens*. This pathway involves the two enzymes tryptophan monooxygenase and IAM hydrolase. The auxins produced by these bacteria often elicit morphological changes in their plant hosts.

IAA Is Degraded by Multiple Pathways

Like IAA biosynthesis, the enzymatic breakdown (oxidation) of IAA may involve more than one pathway. For some time it has been thought that peroxidative enzymes are chiefly responsible for IAA oxidation, primarily because these enzymes are ubiquitous in higher plants and their ability to degrade IAA can be demonstrated in vitro (Figure 19.10A). However, the physiological significance of the peroxidase pathway is unclear. For example, no change in the IAA levels of transgenic plants was observed with either a tenfold increase in peroxidase expression or a tenfold repression of peroxidase activity (Normanly et al. 1995).

On the basis of isotopic labeling and metabolite identification, two other oxidative pathways are more likely to be involved in the controlled degradation of IAA. The end product of this pathway is oxindole- 3-acetic acid (OxIAA), a naturally occurring compound in the endosperm and shoot tissues of *Zea mays*. In one pathway, IAA is oxidized without decarboxylation to OxIAA.

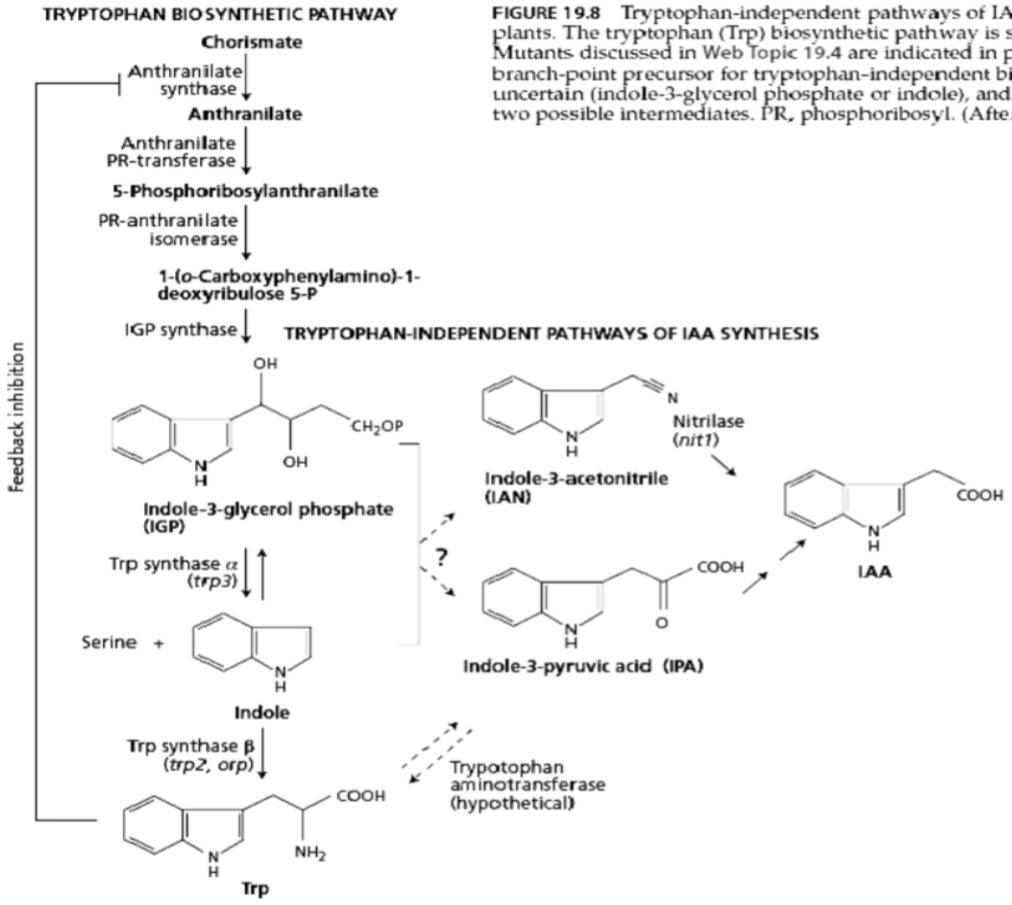


FIGURE 19.8 Tryptophan-independent pathways of IAA biosynthesis in plants. The tryptophan (Trp) biosynthetic pathway is shown on the left. Mutants discussed in Web Topic 19.4 are indicated in parentheses. The branch-point precursor for tryptophan-independent biosynthesis is uncertain (indole-3-glycerol phosphate or indole), and IAN and IPA are two possible intermediates. PR, phosphoribosyl. (After Bartel 1997.)

Physiological Effects of Auxin in Plants:

The following points highlight the eight physiological effects of auxin in plants:

1. Cell Elongation:

The primary physiological effect of auxin in plants is to stimulate the elongation of cells in shoot. A very common example of this can be observed in phototropic curvatures where the unilateral light unequally distributes the auxin in the stem tip (i.e., more auxin on shaded side than on illuminated side).

The higher concentration of auxin on the shaded side causes the cells on that side to elongate more rapidly resulting in bending of the stem tip towards the unilateral light. Many theories have been proposed to explain the mechanism of cell elongation due to auxin.

(i) By increasing the osmotic solutes of the cells,

(ii) By reducing the wall pressure,

(iii) By increasing the permeability of cells to water,

(iv) By an increase in the wall synthesis and

(v) By inducing the synthesis of specific DNA dependent new m-RNA and specific enzymic proteins. The latter bringing about an increase in cell plasticity and extension resulting ultimately in cell enlargement.

2. Apical Dominance:

It has been a common observation in many vascular plants especially the tall and sparsely branched ones that if the terminal bud is intact and growing, the growth of the lateral buds just below it remained suppressed. Removal of the apical bud results in the rapid growth of the lateral buds. This phenomenon in which the apical bud dominates over the lateral buds and does not allow the latter to grow is called as apical dominance.

In recent years, experiment with transgenic plants by plant physiologists and plant molecular biologists have made it quite clear that by removing the apical bud (or decapitating) the shoot), the concentration of auxin in lateral buds situated below was not decreased but there was in-fact a manifold increase in auxin conc., a few hours after decapitation. For instance, Gocal et al (1991) have observed fivefold increase in auxin conc. in the axillary buds of Phaseolus vulgaris 4 hours after decapitation.

It is now generally held that inhibitory effect of auxin from shoot apex on lateral buds is not direct but is indirect possibly through the involvement of other growth hormones such as cytokinins and abscisic acid (ABA). (In many plant species it has been observed that application of cytokinins to lateral buds stimulates bud growth. The auxin in shoot apex makes it a sink for cytokinins which are synthesized in roots. By decapitating the shoot, the supply of cytokinins is diverted to lateral buds, thereby relieving the buds from apical dominance.

Application of auxin to cut apical stump retards accumulation of cytokinins in lateral buds resulting in inhibition of bud growth. The ratio of auxin to cytokinins appears to be one of the key factors involved in apical dominance. Higher auxin to cytokinins ratio suppresses the growth of lateral buds.

3. Root Initiation:

In contrast to the stem, the higher concentration of auxin inhibits the elongation of root but the number of lateral branch roots is considerably increased i.e., the higher conc. of auxin initiates more lateral branch roots. Application of IAA in lanolin paste to the cut end of a young stem results in an early and extensive rooting. This fact is of great practical importance and has been widely utilised to promote root formation in economically useful plants which are propagated by cuttings.

4. Prevention of Abscission:

Natural auxins have controlling influence on the abscission of leaves, fruits etc.

5. Parthenocarpy:

Auxin can induce the formation of parthenocarpic fruits. In nature also, this phenomenon is not uncommon and in such cases the concentration of auxins in the ovaries has been found to be higher

than in the ovaries of plants which produce fruits only after fertilization. In the latter cases, the concentration of the auxin in ovaries increases after pollination and fertilization.

6. Respiration:

It has been established that the auxin stimulates respiration and there is a correlation between auxin induced growth and an increased respiration rate. According to French and Beevers (1953), the auxin may increase the rate of respiration indirectly through increased supply of ADP (Adenosine diphosphate) by rapidly utilizing the ATP in the expanding cells.

7. Callus Formation:

Besides cell elongation, the auxin may also be active in cell division. In fact, in many tissue cultures where the callus growth is quite normal, the continued growth of such callus takes place only after the addition of auxin.

8. Vascular Differentiation:

Auxin induces vascular differentiation in plants. This has also been confirmed in tissue culture experiments and from studies with transgenic plants. Cytokinins are also known to participate in differentiation of vascular tissues and it is believed that vascular differentiation in plants is probably under the control of both auxin and cytokinins.

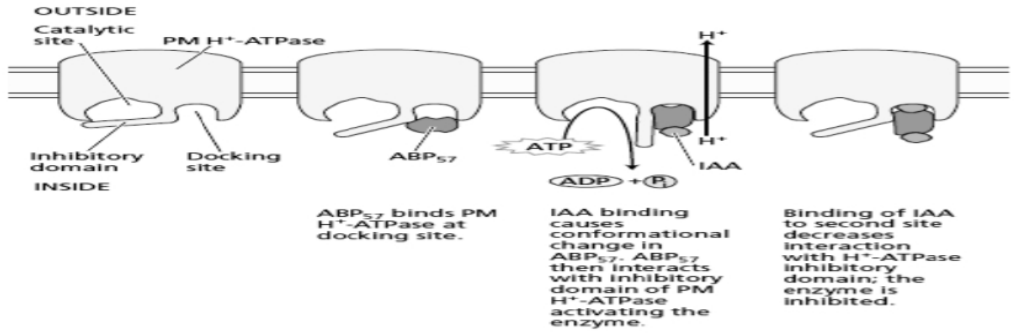
Auxin signal transduction pathways

The ultimate goal of research on the molecular mechanism of hormone action is to reconstruct each step in the signal transduction pathway, from receptor binding to the physiological response. The various signaling pathways that have been implicated in auxin action. Finally we will turn our attention to auxinregulated
gene expression.

ABP1 Functions as an Auxin Receptor

In addition to its possible direct role in plasma membrane H+-ATPase activation (discussed earlier), the auxin-binding protein ABP1 appears to function as an auxin receptor in other signal transduction pathways. ABP1 homologs have been identified in a variety of monocot and dicot species (Venis and Napier 1997). Knockouts of the *ABP1*gene in *Arabidopsis* are lethal, and less severe mutations result in altered development (Chen et al. 2001). Recent studies indicate that, despite being localized primarily on the endoplasmic reticulum (ER), a small amount of ABP1 is secreted to the plasma membrane outer surface where it interacts with auxin to cause protoplast swelling and H+- pumping (Venis et al. 1996; Steffens et al. 2001).

However, it is unlikely that ABP1 mediates all auxin response pathways because expression of a number of auxin-responsive genes is not affected when protoplasts are incubated with anti-ABP1 antibodies. It is also unclear what role the ABP1 in the ER plays in auxin-responsive signal



transduction. Finally, it remains to be determined whether ABP57, the soluble and unrelated ABP from rice that activates the H+-ATPase (see Figure 19.24), is involved in a signal transduction pathway.

Calcium and Intracellular pH Are Possible Signaling Intermediates

Calcium plays an important role in signal transduction in animals and is thought to be involved in the action of certain plant hormones as well. The role of calcium in auxin action seems very complex and, at this point in time, very uncertain. Nevertheless, some experimental evidence shows that auxin increases the level of free calcium in the cell. Changes in cytoplasmic pH can also serve as a second messenger in animals and plants. In plants, auxin induces a decrease in cytosolic pH of about 0.2 units within 4 minutes of application. The cause of this pH drop is not known. Since the cytosolic pH is normally around 7.4, and the pH optimum of the plasma membrane H+-ATPase is 6.5, a decrease in the cytosolic pH of 0.2 units could cause a marked increase in the activity of the plasma membrane H+-ATPase. The decrease in cytosolic pH might also account for the auxin-induced increase in free intracellular calcium, by promoting the dissociation of bound forms. MAP kinases that play a role in signal transduction by phosphorylating proteins in a cascade that ultimately activates transcription factors have also been implicated in auxin responses. When tobacco cells are deprived of auxin, they arrest at the end of either the G1 or the G2 phase and cease dividing; if auxin
is added back into the culture medium, the cell cycle resumes (Koens et al. 1995). Auxin appears to exert its effect on the cell cycle primarily by stimulating the synthesis of the major cyclin-dependent protein kinase (CDK): Cdc2 (*c*ell *d*ivision *c*ycle *2*).

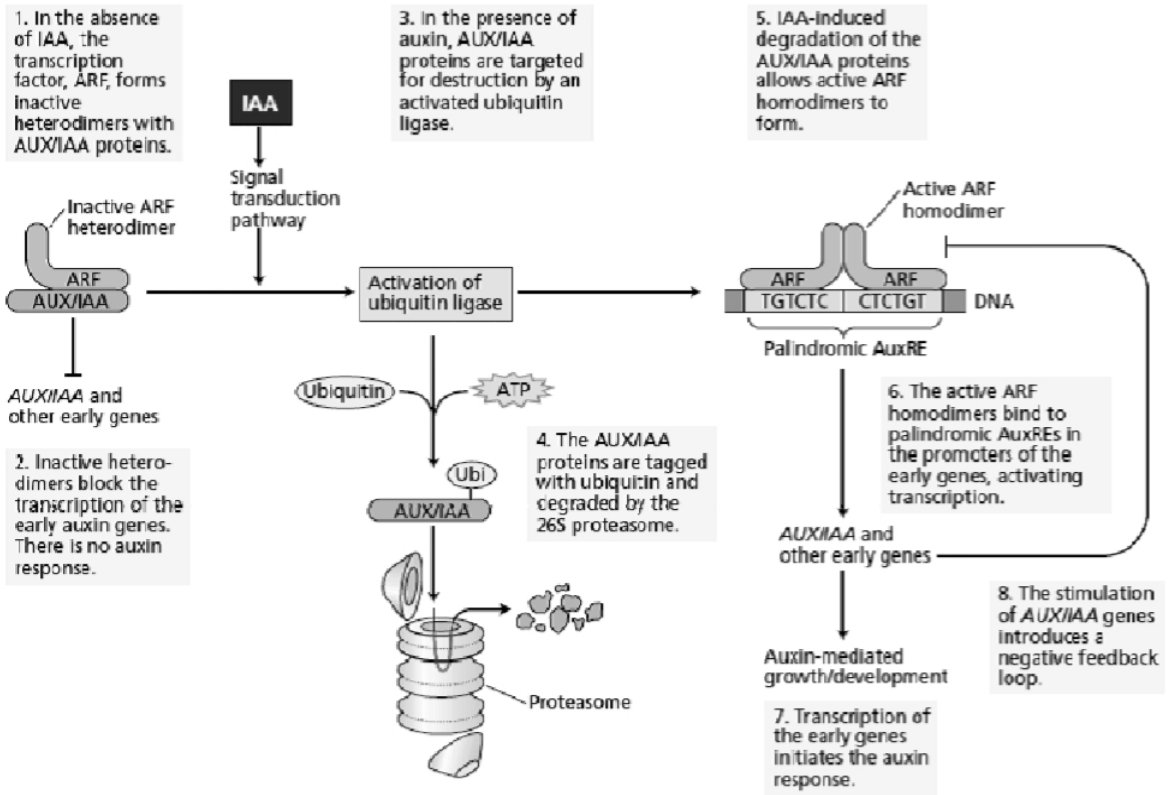


FIGURE 19.41 A model for auxin regulation of transcriptional activation of early response genes by auxin. (After Gray et al. 2001.)

Gibberellins:

In the 1950s the second group of hormones, the gibberellins (GAs), was characterized. The gibberellins are a large group of related compounds (more than 125 are known) that, unlike the auxins, are defined by their chemical structure rather than by their biological activity. Gibberellins are most often associated with the promotion of stem growth, and the application of gibberellin to intact plants can induce large increases in plant height. As we will see, however, gibberellins play important roles in a variety of physiological phenomena.

The biosynthesis of gibberellins is under strict genetic, developmental, and environmental control, and numerous gibberellin-deficient mutants have been isolated. Mendel's tall/dwarf alleles in peas are a famous example. Such mutants have been useful in elucidating the complex pathways of gibberellin biosynthesis.

Biosynthesis and metabolism of gibberellin

Gibberellins Are Synthesized via the Terpenoid Pathway in Three Stages

Gibberellins are tetracyclic diterpenoids made up of four isoprenoid units. Terpenoids are compounds made up of five-carbon (isoprene) building blocks: joined head to tail. Researchers have determined the entire

gibberellin biosynthetic pathway in seed and vegetative tissues of several species by feeding various radioactive precursors and intermediates and examining the production of the other compounds of the pathway (Kobayashi et al. 1996).

The gibberellin biosynthetic pathway can be divided into three stages, each residing in a different cellular compartment (Figure 20.6) (Hedden and Phillips 2000).

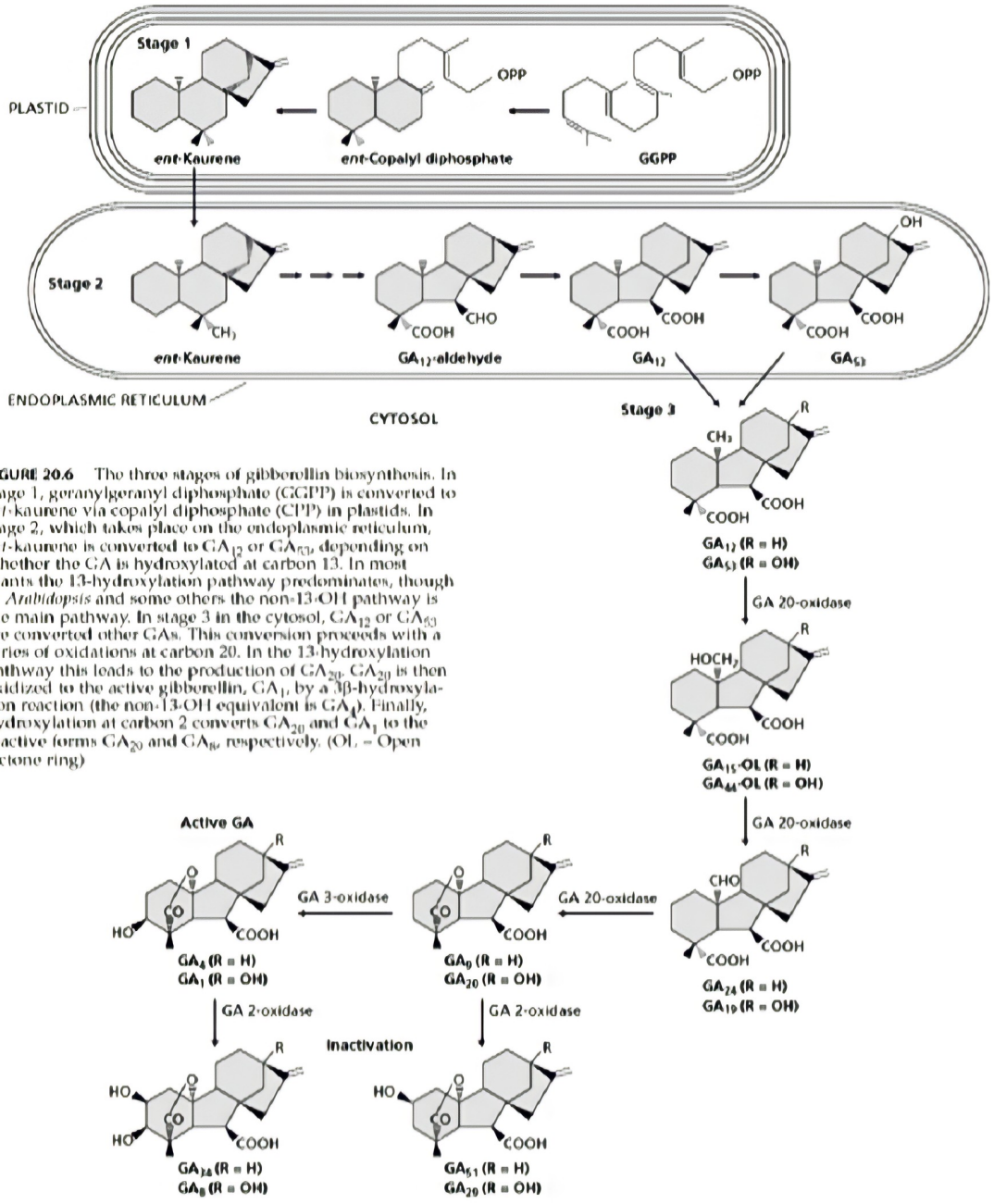


FIGURE 20.6 The three stages of gibberellin biosynthesis. In stage 1, geranylgeranyl diphosphate (GGPP) is converted to *ent*-kaurene via copalyl diphosphate (CPP) in plastids. In stage 2, which takes place on the endoplasmic reticulum, *ent*-kaurene is converted to GA_{12} or GA_{53}, depending on whether the GA is hydroxylated at carbon 13. In most plants the 13-hydroxylation pathway predominates, though in *Arabidopsis* and some others the non-13-OH pathway is the main pathway. In stage 3 in the cytosol, GA_{12} or GA_{53} are converted other GAs. This conversion proceeds with a series of oxidations at carbon 20. In the 13-hydroxylation pathway this leads to the production of GA_{20}. GA_{20} is then oxidized to the active gibberellin, GA_1, by a 3β-hydroxylation reaction (the non-13-OH equivalent is GA_4). Finally, hydroxylation at carbon 2 converts GA_{20} and GA_1 to the inactive forms GA_{29} and GA_8, respectively. (OL = Open lactone ring)

Stage 1: Production of terpenoid precursors and ent-kaurene in plastids.

The basic biological isoprene unit is isopentenyl diphosphate (IPP).2 IPP used in gibberellin biosynthesis in green tissues is synthesized in plastids from glyceraldehyde-3-phosphate and pyruvate (Lichtenthaler et al. 1997). However, in the endosperm of pumpkin seeds, which are very rich in gibberellin, IPP is formed in the cytosol from mevalonic acid, which is itself derived from acetyl-CoA. Thus the IPP used to make gibberellins may arise from different cellular compartments in different tissues. Once synthesized, the IPP isoprene units are added successively to produce intermediates of 10 carbons (geranyl diphosphate), 15 carbons (farnesyl diphosphate), and 20 carbons (geranylgeranyl diphosphate, GGPP). GGPP is a precursor of many terpenoid compounds, including carotenoids and many essential oils, and it is only after GGPP that the pathway becomes specific for gibberellins.

The cyclization reactions that convert GGPP to *ent*-kaurene represent the first step that is specific for the gibberellins (Figure 20.6). The two enzymes that catalyze the reactions are localized in the proplastids of meristematic shoot tissues, and they are not present in mature chloroplasts (Aach et al. 1997). Thus, leaves lose their ability to synthesize gibberellins from IPP once their chloroplasts mature.

Compounds such as AMO-1618, Cycocel, and Phosphon D are specific inhibitors of the first stage of gibberellin biosynthesis, and they are used as growth height reducers.

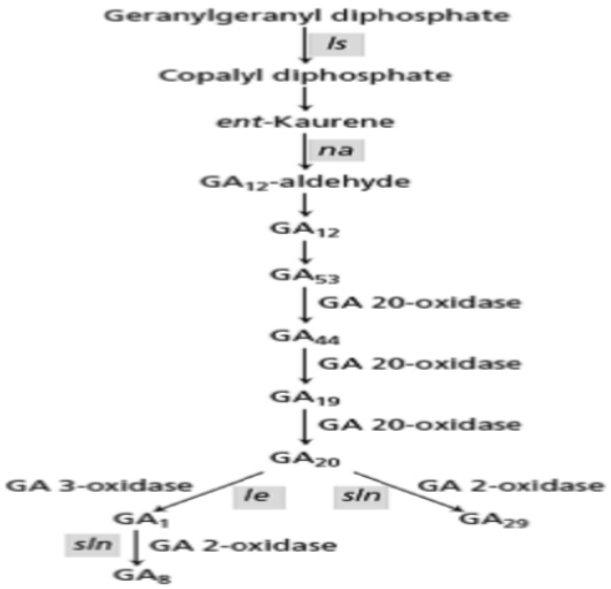


FIGURE 20.7 A portion of the gibberellin biosynthetic pathway showing the abbreviations and location of the mutant genes that block the pathway in pea and the enzymes involved in the metabolic steps after GA_{53}.

Stage 2: Oxidation reactions on the ER form GA12 and GA53.

In the second stage of gibberellin biosynthesis, a methyl group on *ent*-kaurene is oxidized to a carboxylic acid, followed by contraction of the B ring from a six- to a five-carbon ring to give GA12-aldehyde. GA12-aldehyde is then oxidized to **GA12**, the first gibberellin in the pathway in all plants and thus the precursor of all the other gibberellins (see Figure 20.6).

Many gibberellins in plants are also hydroxylated on carbon 13. The hydroxylation of carbon 13 occurs next, forming GA53 from GA12. All the enzymes involved are monooxygenases that utilize cytochrome P450 in their reactions. These P450 monooxygenases are localized on the endoplasmic

reticulum. Kaurene is transported from the plastid to the endoplasmic reticulum, and is oxidized *enroute* to kaurenoic acid by kaurene oxidase, which is associated with the plastid envelope (Helliwell et al. 2001).

Further conversions to GA12 take place on the endoplasmic reticulum. Paclobutrazol and other inhibitors of P450 monooxygenases specifically inhibit this stage of gibberellin biosynthesis before GA12-aldehyde, and they are also growth retardants.

Stage 3: Formation in the cytosol of all other gibberellins from GA12 or GA53.

All subsequent steps in the pathway (see Figure 20.6) are carried out by a group of soluble dioxygenases in the cytosol. These enzymes require 2-oxoglutarate and molecular oxygen as cosubstrates, and they use Fe2+ and ascorbate as cofactors.

The specific steps in the modification of GA12 vary from species to species, and between organs of the same species.

Two basic chemical changes occur in most plants:

1. Hydroxylation at carbon 13 (on the endoplasmic reticulum) or carbon 3, or both.
2. A successive oxidation at carbon 20 ($CH_2 \rightarrow CH_2OH \rightarrow CHO$). The final step of this oxidation is the loss of carbon 20 as CO_2 (see Figure 20.6).

When these reactions involve gibberellins initially hydroxylated at C-13, the resulting gibberellin is GA20.

GA20 is then converted to the biologically active form, GA1, by hydroxylation of carbon 3. (Because this is in the beta configuration [drawn as if the bond to the hydroxyl group were toward the viewer], it is referred to as 3β-hydroxylation.)

Finally, GA1 is inactivated by its conversion to GA8 by a hydroxylation on carbon 2. This hydroxylation can also remove GA20 from the biosynthetic pathway by converting it to GA29.

Inhibitors of the third stage of the gibberellin biosynthetic pathway interfere with enzymes that utilize 2-oxoglutarate as cosubstrates. Among these, the compound prohexadione (BX-112), is especially useful because it specifically inhibits GA 3-oxidase, the enzyme that converts inactive GA20 to growth-active GA1.

Gibberellin signal transduction:

Cereal aleurone layers

Genetic analyses of gibberellin-regulated growth, such as the studies described in the previous section, have identified some of the genes and their gene products, but not the biochemical pathways involved in gibberellin signal transduction.

The biochemical and molecular mechanisms, which are probably common to all gibberellin responses, have been studied most extensively in relation to the gibberellin- stimulated synthesis and secretion of α-amylase in cereal aleurone layers (Jacobsen et al. 1995).

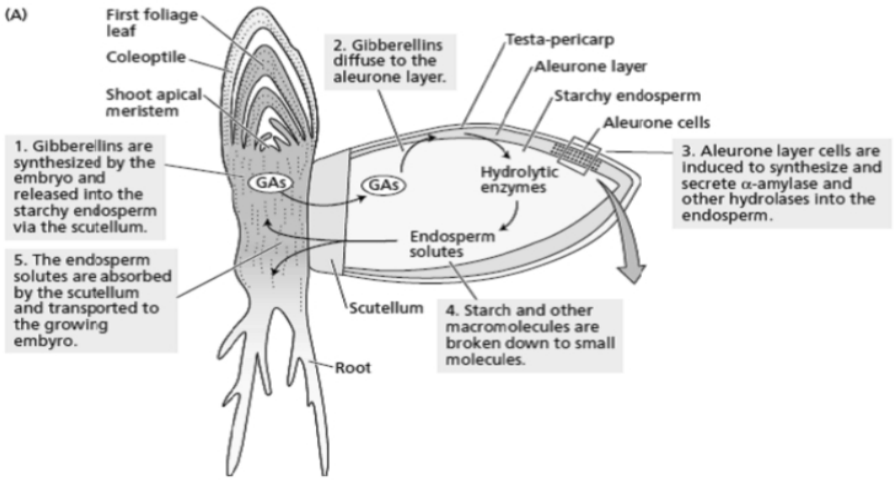
The gibberellin receptor, the transcriptional regulation of the genes for α-amylase and other proteins, and the possible signal transduction pathways involved in the control of α-amylase synthesis and secretion by gibberellin.

Gibberellin from the Embryo Induces α-Amylase

Production by Aleurone Layers

Cereal grains (*caryopses*; singular *caryopsis*) can be divided into three parts: the diploid embryo, the triploid endosperm, and the fused testa–pericarp (seed coat–fruit wall). The embryo part consists of the plant embryo proper, along with its specialized absorptive organ, the *scutellum* (plural *scutella*),

which functions in absorbing the solubilised food reserves from the endosperm and transmitting them to the growing embryo. The endosperm is composed of two tissues: the centrally located starchy endosperm and the aleurone layer (Figure 20.33A).



The starchy endosperm, typically nonliving at maturity, consists of thin-walled cells filled with starch grains. The aleurone layer surrounds the starchy endosperm and is cytologically and biochemically distinct from it. Aleurone cells are enclosed in thick primary cell walls and contain large numbers of protein-storing vacuoles called *protein bodies* (Figures 20.33B–D), enclosed by a single membrane. The protein bodies also contain phytin, a mixed cation salt (mainly Mg^{2+} and K^{+}) of *myo*-inositolhexaphosphoric acid (phytic acid). During germination and early seedling growth, the stored food reserves of the endosperm—chiefly starch and protein—are broken down by a variety of hydrolytic enzymes, and the solubilized sugars, amino acids, and other products are transported to the growing embryo. The two enzymes responsible for starch degradation are α- and β-amylase. α-Amylase hydrolyzes starch chains internally to produce oligosaccharides consisting of α-1,4-linked glucose residues. β-Amylase degrades these oligosaccharides
from the ends to produce maltose, a disaccharide. Maltase then converts maltose to glucose.
α-Amylase is secreted into the starchy endosperm of cereal seeds by both the scutellum and the aleurone layer (see Figure 20.33A). The sole function of the aleurone layer of the seeds of graminaceous monocots (e.g., barley, wheat, rice, rye, and oats) appears to be the synthesis and release of hydrolytic enzymes. After completing this function, aleurone cells undergo programmed cell death.

Gibberellic Acid Enhances the Transcription of α-Amylase mRNA

Before molecular biological approaches were developed, there was already physiological and biochemical evidence that gibberellic acid might enhance α-amylase production at the level of gene transcription (Jacobsen et al. 1995). The two main lines of evidence were as follows:
1. GA3-stimulated α-amylase production was shown to be blocked by inhibitors of transcription and translation.
2. Heavy-isotope- and radioactive-isotope-labeling studies demonstrated that the stimulation of α-amylase activity by gibberellin involved de novo synthesis of the enzyme from amino acids, rather than activation of preexisting enzyme.

Gibberellin Receptors May Interact with GProteins on the Plasma Membrane

Acell surface localization of the gibberellin receptor is suggested from the fact that gibberellin that has been bound to microbeads that are unable to cross the plasma membrane is still active in inducing

α-amylase production in aleurone protoplasts (Hooley et al. 1991). In addition, microinjection of GA3 into aleurone protoplasts had no effect, but when the protoplasts were immersed in GA3 solution, they produced α-amylase (Gilroy and Jones 1994). These results suggest that gibberellin acts on the outer face of the plasma membrane.

Two gibberellin-binding plasma membrane proteins have been isolated through the use of purified plasma membrane and a radioactively labeled gibberellin that was chemically modified to permanently attach to protein to which it was weakly bound. Because the presence of excess gibberellin reduces binding, and these proteins from a semidwarf, gibberellin-insensitive sweet pea bind gibberellin less strongly, they may represent the gibberellin receptors (Lovegrove et al. 1998).

Cyclic GMP, Ca2+, and Protein Kinases Are Possible Signaling Intermediates

In animal cells, G-proteins can activate the enzyme guanylyl cyclase, the enzyme that synthesizes cGMP from GTP, leading to an increase in cGMP concetration. Cyclic GMP, in turn, can regulate ion channels, Ca2+ levels, protein kinase activity, and gene transcription. Gibberellin has been reported to cause a transient rise in cGMP levels in barley aleurone layers, suggesting a possible role for cGMP in α-amylase production (Pensen et al. 1996).

In conclusion, gibberellin signal transduction in aleurone cells seems to involve G-proteins as well as cyclic

GMP, leading to production of the transcription factor GAMYB, which induces α-amylase gene transcription. α-Amylase secretion has similar initial components but also involves an increase in cytoplasmic calcium and protein phosphorylation. The detailed signaling pathways remain to be worked out.

Physiological Effects of Gibberellins in Plants:

The following points highlight the eight physiological effects of gibberellins: 1. Seed Germination 2. Dormancy of Buds 3. Root Growth 4. Elongation of the Internodes 5. Bolting and Flowering 6. Parthenocarpy 7. Light Inhibited Stem Growth and 8. De novo Synthesis of the Enzyme-α-Amylase.

1. Seed Germination:

Certain light sensitive seeds e.g., lettuce and tobacco show poor germination in dark. Germination starts vigorously if these seeds are exposed to light or red light. This requirement of light is overcome if the seeds are treated with gibberellic acid in dark.

2. Dormancy of Buds:

In temperate regions the buds formed in autumn remain dormant until next spring due to severe colds. This dormancy of buds can be broken by gibberellin treatment. In potatoes also, there is a dormant period after harvest, but the application of gibberellin sprouts the eyes vigorously.

3. Root Growth:

Gibberellins have little or no effect on root growth. At higher concentration in some plants, however, some inhibition of root growth may occur. The initiation of roots is markedly inhibited by gibberellins in isolated cuttings.

4. Elongation of the Internodes:

Most pronounced effect of gibberellins on the plant growth is the elongation of the internodes, so much so that in many plants such as dwarf pea, dwarf maize etc., they overcome the genetic dwarfism. For instance, the light grown dwarf pea plants have short internodes and expanded leaves. But, when treated with gibberellin the internodes elongate markedly and they look like tall plants.

It is considered that in such dwarf plants (i) the gene for producing gibberellin is missing, or (ii) the concentration of the natural inhibitors is higher. When external gibberellin is applied the deficiency of the endogenous gibberellins is made good or the external gibberellin overcomes the effect of natural inhibitors which fall short.

Deepwater rice (*Oryza sativa*) is another notable example of pronounced effect of gibberellins on elongation of internodes so that its foliage may remain above water in the field. Striking growth rates of as much as 25 cms. per day have been observed in rice plants under field conditions.

(Partial submergence of rice plants is believed to reduce partial pressure of O_2 which triggers ethylene biosynthesis in submerged tissues. Ethylene in turn reduces the level of ABA (abscisic acid) which acts as antagonist of GA. Submerged rice tissues thus become more responsive to endogenous GA resulting in marked elongation of internodes).

5. Bolting and Flowering:

In many herbaceous plants the early period of growth shows rosette-habit with short stem and cauline leaves. Under short days the rosette habit is retained while under long days bolting occurs i.e., the stem elongates rapidly and is converted into floral axis bearing flower primordia. This bolting can also be induced in such plants e.g. Rudbeckia speciosa (It is a Long Day Plant) by the application of gibberellin even under non-inductive short days.

In Hyoscyamus niger (also a Long Day Plant) gibberellin treatment causes bolting and flowering under non-inductive short days. While in Long Day Plants the gibberellin treatment usually results in early flowering, its effects are quite variable in Short Day Plants. It may either have no effect, or inhibit, or may activate flowering.

6. Parthenocarpy:

Germination of the pollen grains is stimulated by gibberellins, likewise the growth of the fruit and the formation of parthenocarpic fruits can be induced by gibberellin treatment. In many cases e.g., pome and stone fruits where auxins have failed to induced parthenocarpy the gibberellins have proven to be successful. Seedless and fleshy tomatoes and large sized grapes are produced by gibberellin treatment on commercial scale.

7. Light Inhibited Stem Growth:

It is common observation that the dark grown plants become etiolated and have taller, thinner and pale stems while the light grown plants have shorter, thicker and green stems, and it may be concluded that light has inhibitory effect on stem elongation. Treatment of light grown plants with gibberellin also stimulates the stem growth and they appear to be dark brown. In such cases the protein content of the stem falls while soluble nitrogen content increases probably due to more breakdowns of proteins than their synthesis.

It is considered that the light in some way lowers the level of endogenous gibberellins and inhibits the stem growth.

8. De novo Synthesis of the Enzyme-α-Amylase:

One of the important functions of gibberellins is to cause de novo (i.e., a new) synthesis of the enzyme a- amylase in the aleurone layer surrounding the endosperm of cereal grains during germination. This enzyme brings about hydrolysis of starch to form simple sugars which are then translocated to growing embryo to provide energy source.

Cytokinins:

THE CYTOKININS WERE DISCOVERED in the search for factors that stimulate plant cells to divide (i.e., undergo cytokinesis). Since their discovery, cytokinins have been shown to have effects on many other physiological and developmental processes, including leaf senescence, nutrient mobilization, apical dominance, the formation and activity of shoot apical meristems, floral development, the breaking of bud dormancy, and seed germination. Cytokinins also appear to mediate many aspects of light-regulated development, including chloroplast differentiation, the development of autotrophic metabolism, and leaf and cotyledon expansion.

Although cytokinins regulate many cellular processes, the control of cell division is central in plant growth and development and is considered diagnostic for this class of plant growth regulators. For these reasons

we will preface our discussion of cytokinin function with a brief consideration of the roles of cell division in normal development, wounding, gall formation, and tissue culture.

The regulation of plant cell proliferation by cytokinins. Then turn to cytokinin functions not directly related to cell division: chloroplast differentiation, the prevention of leaf senescence, and nutrient mobilization. Finally, we will consider the molecular mechanisms underlying cytokinin perception and signaling.

Biosynthesis, metabolism of cytokinins:

The side chains of naturally occurring cytokinins are chemically related to rubber, carotenoid pigments, the

plant hormones gibberellin and abscisic acid, and some of the plant defense compounds known as phytoalexins. All of these compounds are constructed, at least in part, from isoprene units.

Isoprene is similar in structure to the side chains of zeatin and iP. These cytokinin side chains are synthesized from an isoprene derivative. Large molecules of rubber and the carotenoids are constructed by the polymerization of many isoprene units; cytokinins contain just one of these units. The precursor(s) for the formation of these isoprene structures are mevalonic acid or pyruvate plus 3-phosphoglycerate, depending on which pathway is involved. These precursors are converted to the biological isoprene unit dimethylallyl diphosphate (DMAPP).

IPT Catalyzes the First Step in Cytokinin Biosynthesis

The first committed step in cytokinin biosynthesis is the transfer of the isopentenyl group of dimethylallyl diphos-phate (DMAPP) to an adenosine moiety. An enzyme that catalyzes such an activity was first identified in the cellular slime mold *Dictyostelium discoideum*, and subsequently the *ipt* gene from *Agrobacterium* was found to encode such an enzyme. In both cases, DMAPP and AMP are converted to isopentenyladenosine-5′-monophosphate (iPMP).

As noted earlier, cytokinins are also present in the tRNAs of most cells, including plant and animal cells. The tRNA cytokinins are synthesized by modification of specific adenine residues within the fully transcribed tRNA. As with the free cytokinins, isopentenyl groups are transferred to the adenine molecules from DMAPP by an enzyme call tRNA-IPT.

The genes for tRNA-IPT have been cloned from many species. The possibility that free cytokinins are derived from tRNA has been explored extensively. Although the tRNAbound cytokinins can act as hormonal signals for plant cells if the tRNA is degraded and fed back to the cells, it is unlikely that any significant amount of the free hormonal cytokinin in plants is derived from the turnover of tRNA.

An enzyme with IPT activity was identified from crude extracts of various plant tissues, but researchers were unable to purify the protein to homogeneity. Recently, plant *IPT* genes were cloned after the *Arabidopsis* genome was analyzed for potential *ipt*-like sequences (Kakimoto 2001; Takei et al. 2001). Nine different *IPT* genes were identified in *Arabidopsis*—many more than are present in

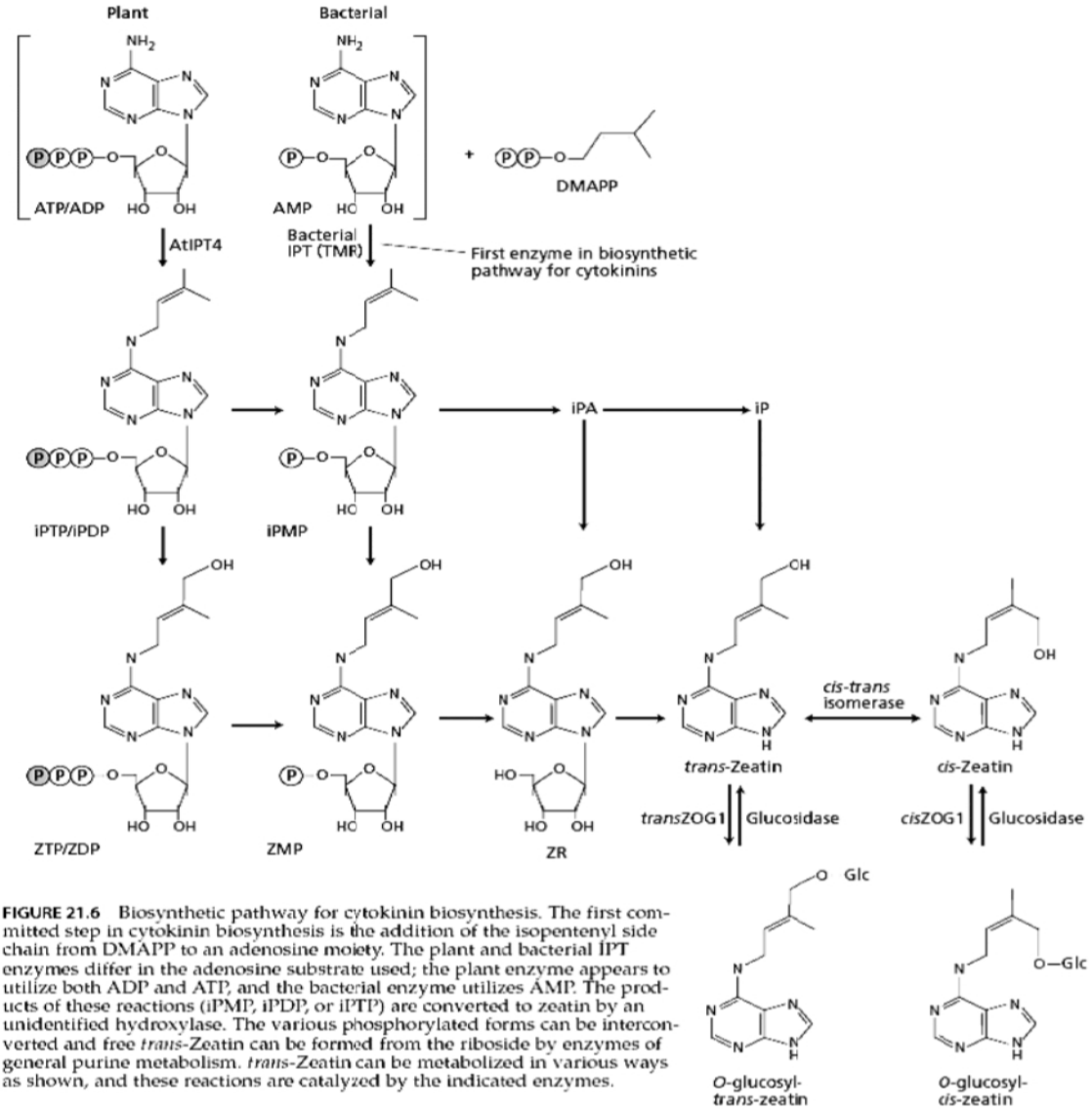


FIGURE 21.6 Biosynthetic pathway for cytokinin biosynthesis. The first committed step in cytokinin biosynthesis is the addition of the isopentenyl side chain from DMAPP to an adenosine moiety. The plant and bacterial IPT enzymes differ in the adenosine substrate used; the plant enzyme appears to utilize both ADP and ATP, and the bacterial enzyme utilizes AMP. The products of these reactions (iPMP, iPDP, or iPTP) are converted to zeatin by an unidentified hydroxylase. The various phosphorylated forms can be interconverted and free *trans*-Zeatin can be formed from the riboside by enzymes of general purine metabolism. *trans*-Zeatin can be metabolized in various ways as shown, and these reactions are catalyzed by the indicated enzymes.

animal genomes, which generally contain only one or two such genes used in tRNA modification. Phylogenetic analysis revealed that one of the *Arabidopsis IPT* genes resembles bacterial tRNA-*ipt*, another resembles eukaryotic tRNA-*IPT*, and the other seven form a distinct group or clade together with other plant sequences. The grouping of the seven *Arabidopsis IPT* genes in this unique plant clade provided a clue that these genes may encode the cytokinin biosynthetic enzyme.

The proteins encoded by these genes were expressed in *E. coli* and analyzed. It was found that, with the exception of the gene most closely related to the animal tRNA-*IPT* genes, these genes encoded

proteins capable of synthesizing free cytokinins. Unlike their bacterial counterparts, however, the *Arabidopsis* enzymes that have been analyzed utilize ATP and ADP preferentially over AMP.

The biological roles of cytokinins

Although discovered as a cell division factor, cytokinins can stimulate or inhibit a variety of physiological, metabolic, biochemical, and developmental processes when they are applied to higher plants, and it is increasingly clear that endogenous cytokinins play an important role in the regulation of these events in the intact plant.

Cytokinins Regulate Cell Division in Shoots and Roots

As discussed earlier, cytokinins are generally required for cell division of plant cells in vitro. Several lines of evidence suggest that cytokinins also play key roles in the regulation of cell division in vivo.

Much of the cell division in an adult plant occurs in the meristems. Localized expression of the *ipt* gene of *Agrobacterium* in somatic sectors of tobacco leaves causes the formation of ectopic (abnormally located) meristems, indicating that elevated levels of cytokinin are sufficient to initiate cell divisions in these leaves (Estruch et al. 1991). Elevation of endogenous cytokinin levels in transgenic *Arabidopsis* results in overexpression of the KNOTTED homeobox transcription factor homologs *KNAT1* and *STM*—genes that are important in the regulation of meristem function. Interestingly, overexpression of *KNAT1* also appears to elevate cytokinin levels in transgenic tobacco, suggesting an interdependent relationship between *KNAT* and the level of cytokinins.

Cytokinins Regulate Specific Components of the Cell Cycle

Cytokinins regulate cell division by affecting the controls that govern the passage of the cell through the cell division cycle. Zeatin levels were found to peak in synchronized culture tobacco cells at the end of S phase, mitosis, and G1 phase.

Cytokinins were discovered in relation to their ability to stimulate cell division in tissues supplied with an optimal level of auxin. Evidence suggests that both auxin and cytokinins participate in regulation of the cell cycle and that they do so by controlling the activity of cyclin-dependent kinases.

cyclin-dependent protein

kinases (*CDKs*), in concert with their regulatory subunits, the *cyclins*, are enzymes that regulate the eukaryotic cell cycle.

The Auxin: Cytokinin Ratio Regulates Morphogenesis in Cultured Tissues

Shortly after the discovery of kinetin, it was observed that the differentiation of cultured callus tissue derived from tobacco pith segments into either roots or shoots depends on the ratio of auxin to cytokinin in the culture medium. Whereas high auxin:cytokinin ratios stimulated the formation of roots, low auxin:cytokinin ratios led to the formation of shoots. At intermediate levels the tissue grew as an undifferentiated callus (Figure 21.13) (Skoog and Miller 1965).

Cytokinins Modify Apical Dominance and Promote Lateral Bud Growth

One of the primary determinants of plant form is the degree of apical dominance. Plants with strong apical dominance, such as maize, have a single growing axis with few lateral branches. In contrast, many lateral buds initiate growth in shrubby plants. Although apical dominance may be determined primarily by auxin, physiological studies indicate that cytokinins play a role in initiating the growth of lateral buds. For example, direct applications of cytokinins to the axillary buds of many species stimulate cell division activity and growth of the buds.

Cytokinins Induce Bud Formation in a Moss

Thus far we have restricted our discussion of plant hormones to the angiosperms. However, many plant hormones are present and developmentally active in representative species throughout the plant kingdom. The moss *Funaria hygrometrica* is a well-studied example. The germination of moss spores gives rise to a filament of cells called a *protonema* (plural *protonemata*). The protonema elongates and undergoes cell divisions at the tip, and it forms branches some distance back from the tip.

Cytokinin Overproduction Has Been Implicated in Genetic Tumors

Many species in the genus *Nicotiana* can be crossed to generate interspecific hybrids. More than 300 such interspecific hybrids have been produced; 90% of these hybrids are normal, exhibiting phenotypic characteristics intermediate between those of both parents. The plant used for cigarette

tobacco, *Nicotiana tabacum*, for example, is an interspecific hybrid. However, about 10% of these interspecific crosses result in progeny that tend to form spontaneous tumors levels in tumor-prone hybrids are five to six times higher
than those found in either parent.

Cytokinins Delay Leaf Senescence

Leaves detached from the plant slowly lose chlorophyll, RNA, lipids, and protein, even if they are kept moist and provided with minerals. This programmed aging process leading to death is termed **senescence**. Leaf senescence is more rapid in the dark than in the light. Treating isolated leaves of many species with
cytokinins will delay their senescence. Although applied cytokinins do not prevent senescence completely, their effects can be dramatic, particularly when the cytokinin is sprayed directly on the intact plant. If only
one leaf is treated, it remains green after other leaves of similar developmental age have yellowed and dropped off the plant. Even a small spot on a leaf will remain green if treated with a cytokinin, after the surrounding tissues on the same leaf begin to senesce.

Cytokinins Promote Movement of Nutrients

Cytokinins influence the movement of nutrients into leaves from other parts of the plant, a phenomenon known as *cytokinin-induced nutrient mobilization*. This process is revealed when nutrients (sugars, amino acids, and so on) radiolabeled with 14C or 3H are fed to plants after one leaf or part of a leaf is treated with a cytokinin. Later the whole plant is subjected to autoradiography to reveal the pattern of movement and the sites at which the labeled nutrients accumulate.

Cytokinins Promote Chloroplast Development

Although seeds can germinate in the dark, the morphology of dark-grown seedlings is very different from that of lightgrown seedlings: Dark-grown seedlings are said to be **etiolated**. The hypocotyl and internodes of
etiolated seedlings are more elongated, cotyledons and leaves do not expand, and chloroplasts do not mature.

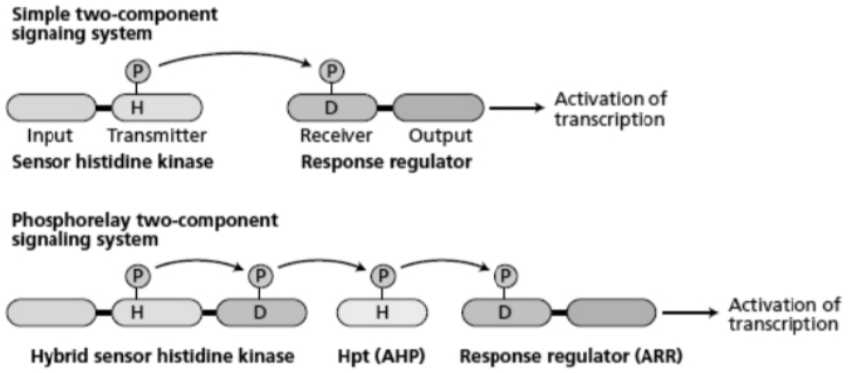


FIGURE 21.22 Simple versus phosphorelay types of two-component signaling systems. (A) In simple two-component systems, the input domain is the site where the signal is sensed. This regulates the activity of the histidine kinase domain, which when activated autophosphorylates on a conserved His residue. The phosphate is then transferred to an Asp residue that resides within the receiver domain of a response regulator. Phosphorylation of this Asp regulates the activity of the output domain of the response regulator, which in many cases is a transcription factor. (B) In the phosphorelay-type two-component signaling system, an extra set of phosphotransfers is mediated by a histidine phosphotransfer protein (Hpt), called AHP in *Arabidopsis*. The *Arabidopsis* response regulators are called ARRs. H = histidine, D = aspartate.

Cytokinins Promote Cell Expansion in Leaves and Cotyledons

The promotion of cell enlargement by cytokinins is most clearly demonstrated in the cotyledons of dicots with leafy cotyledons, such as mustard, cucumber, and sunflower.

The cotyledons of these species expand as a result of cell enlargement during seedling growth. Cytokinin treatment promotes additional cell expansion, with no increase in the dry weight of the treated cotyledons.

Cytokinins Regulate Growth of Stems and Roots

Although endogenous cytokinins are clearly required for normal cell proliferation in the apical meristem, and therefore normal shoot growth (see Figure 21.9), applied cytokinins typically inhibit the process of cell elongation in both stems and roots. For example, exogenous cytokinin inhibits hypocotyl elongation at concentrations that promote leaf and cotyledon expansion in the dark-grown seedlings.

Cellular and molecular modes of cytokinin action

The diversity of the effects of cytokinin on plant growth and development is consistent with the involvement of signal transduction pathways with branches leading to specific responses. Although our knowledge of how cytokinin works at the cellular and molecular levels is still quite fragmentary, significant progress has been achieved.

A Cytokinin Receptor Related to Bacterial Two-Component Receptors Has Been Identified

The first clue to the nature of the cytokinin receptor came from the discovery of the *CKI1* gene. *CKI1* was identified in a screen for genes that, when overexpressed, conferred cytokinin-independent growth on *Arabidopsis* cells in culture.

As discussed already, plant cells generally require cytokinin in order to divide in culture. However, a cell line that overexpresses *CKI1* is capable of growing in culture in the absence of added cytokinin.

Cytokinins Cause a Rapid Increase in the Expression of Response Regulator Genes

One of the primary effects of cytokinin is to alter the expression of various genes. The first set of genes to be upregulated in response to cytokinin are the ***ARR*** (A*rabidopsis r*esponse *r*egulator) genes. These genes are homologous to the receiver domain of bacterial two-component response regulators, the downstream target of sensor histidine kinases.

In *Arabidopsis*, response regulators are encoded by a multigene family. They fall into two basic classes: the **type-A *ARR*** genes, which are made up solely of a receiver domain, and the **type-B *ARR*** genes, which contain a transcription factor domain in addition to the receiver domain (Figure 21.23).

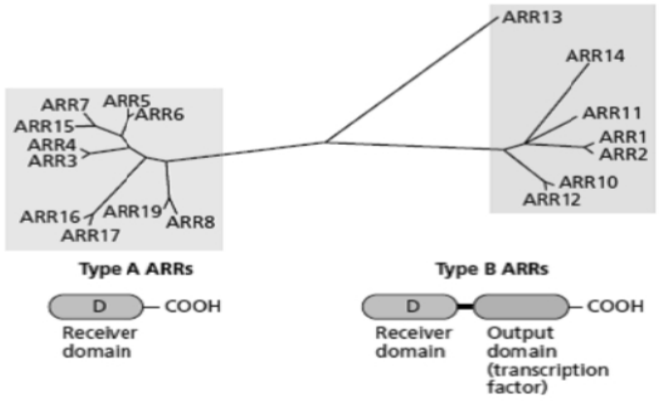


FIGURE 21.23 Phylogenetic tree of *Arabidopsis* response regulators. The top part of the figure shows a phylogenetic tree that represents the degree of relatedness of the receiver domains present in the *Arabidopsis* genome. The closer two proteins are on the tree, the more similar are their amino acid sequences. Note that these proteins fall into two distinct groups, or clades, called the type-A ARRs (blue) and the type-B ARRs (red). These differences in sequence are also reflected in a distinct domain structure, as depicted below the tree. The type-A ARRs consist solely of a receiver domain, but the type-A proteins also contain a fused output domain at the carboxy-terminus.

Histidine Phosphotransferases May Mediate the Cytokinin Signaling Cascade

From the preceding discussions we have seen that cytokinin binds to the CRE1 receptors to initiate a response that culminates in the elevation of transcription of the type-A *ARR*s. The type-A ARR proteins, in turn, may regulate the expression of numerous other genes, as well as the activities of various target proteins that ultimately alter cellular function. How is the signal propagated from CRE1 (which is at the plasma membrane) to the nucleus to alter type-A *ARR* transcription?

Cytokinin-Induced Phosphorylation Activates Transcription Factors

The question now becomes, How do the activated AHPs, once in the nucleus, act to regulate gene transcription? Genetic studies in intact *Arabidopsis* plants and overexpression studies in isolated *Arabidopsis* protoplasts using a cytokinin responsive reporter have provided a likely answer (Hwangand Sheen 2001; Sakai et al. 2001).

The mechanism of action of cytokinin is just beginning to emerge. Acytokinin receptor has been identified in *Arabidopsis*. This transmembrane protein is related to the bacterial two-component sensor histidine kinases. Cytokinins increase the abundance of several specific mRNAs. Some of these are primary response genes that are similar to bacterial two-component response regulators. The signal transduction mechanism from CRE1 to transcriptional activation of the type-A *ARR*s involves other homologs of two-component elements.

Ethylene:

In 1901, Dimitry Neljubov, a graduate student at the Botanical Institute of St. Petersburg in Russia, observed that dark-grown pea seedlings growing in the laboratory exhibited symptoms that were later termed the *triple response*: reduced stem elongation, increased lateral growth (swelling), and abnormal, horizontal growth. When the plants were allowed to grow in fresh air, they regained their normal morphology and rate of growth. Neljubov identified ethylene, which was present in the laboratory air from coal gas, as the molecule causing the response.

The first indication that ethylene is a natural product of plant tissues was published by H. H. Cousins in 1910. Cousins reported that "emanations" from oranges stored in a chamber caused the premature ripening

of bananas when these gases were passed through a chamber containing the fruit. However, given that oranges synthesize relatively little ethylene compared to other fruits, such as apples, it is likely that the oranges used by Cousins were infected with the fungus *Penicillium*, which produces copious amounts of ethylene. In 1934, R. Gane and others identified ethylene chemically as a natural product of plant metabolism, and because of its dramatic effects on the plant it was classified as a hormone.

Structure, biosynthesis, and measurement of ethylene

Ethylene can be produced by almost all parts of higher plants, although the rate of production depends on the type of tissue and the stage of development. In general, meristematic regions and nodal regions are the most active in ethylene biosynthesis. However, ethylene production also increases during leaf abscission and flower senescence, as well as during fruit ripening. Any type of wounding can induce ethylene biosynthesis, as can physiological stresses such as flooding, chilling, disease, and temperature or drought stress.

The amino acid methionine is the precursor of ethylene, and ACC (1-aminocyclopropane-1-carboxylic acid) serves as an intermediate in the conversion of methionine to ethylene. The complete pathway is a cycle, taking its place among the many metabolic cycles that operate in plant cells.

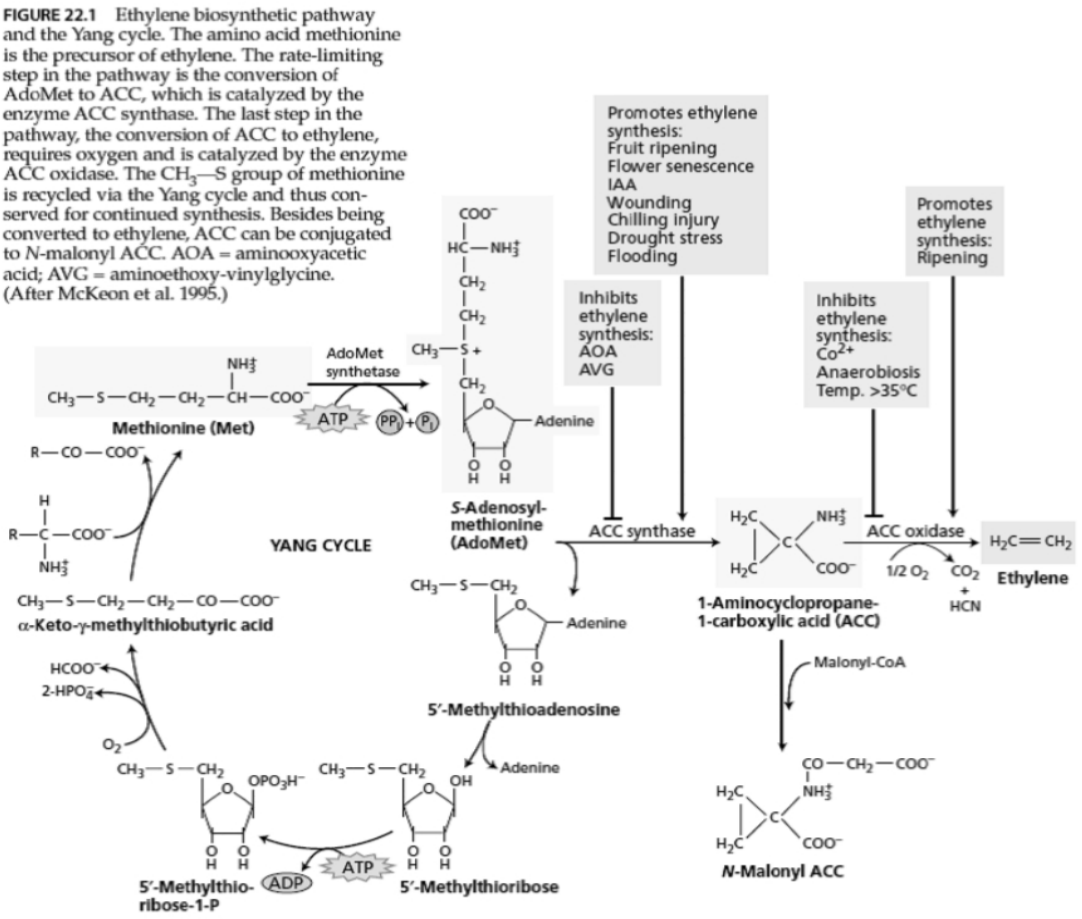


FIGURE 22.1 Ethylene biosynthetic pathway and the Yang cycle. The amino acid methionine is the precursor of ethylene. The rate-limiting step in the pathway is the conversion of AdoMet to ACC, which is catalyzed by the enzyme ACC synthase. The last step in the pathway, the conversion of ACC to ethylene, requires oxygen and is catalyzed by the enzyme ACC oxidase. The CH_3—S group of methionine is recycled via the Yang cycle and thus conserved for continued synthesis. Besides being converted to ethylene, ACC can be conjugated to *N*-malonyl ACC. AOA = aminooxyacetic acid; AVG = aminoethoxy-vinylglycine. (After McKeon et al. 1995.)

Developmental and physiological effects of ethylene:

Ethylene was discovered in connection with its effects on seedling growth and fruit ripening. It has since been shown to regulate a wide range of responses in plants, including seed germination, cell expansion, cell differentiation, flowering, senescence, and abscission.

Ethylene Promotes the Ripening of Some Fruits

In everyday usage, the term *fruit ripening* refers to the changes in fruit that make it ready to eat. Such changes typically include softening due to the enzymatic breakdown of the cell walls, starch hydrolysis, sugar accumulation, and the disappearance of organic acids and phenolic compounds, including tannins. From the perspective of the plant, fruit ripening means that the seeds are ready for dispersal.

For seeds whose dispersal depends on animal ingestion, *ripeness* and *edibility* are synonymous. Brightly colored anthocyanins and carotenoids often accumulate in the epidermis of such fruits, enhancing their visibility. However, for seeds that rely on mechanical or other means for dispersal, *fruit ripening* may mean drying followed by splitting.

Ethylene Induces Lateral Cell Expansion

At concentrations above 0.1 µL L–1, ethylene changes the growth pattern of seedlings by reducing the rate of elongation and increasing lateral expansion, leading to swelling of the region below the hook.

These effects of ethylene are common to growing shoots of most dicots, forming part of the **triple response**. In *Arabidopsis*, the triple response consists of inhibition and swelling of the hypocotyl, inhibition of root elongation, and exaggeration of the apical hook.

Ethylene Breaks Seed and Bud Dormancy in Some Species

Seeds that fail to germinate under normal conditions (water, oxygen, temperature suitable for growth) are said to be dormant. Ethylene has the ability to break dormancy and initiate germination in certain seeds, such as cereals. In addition to its effect on dormancy, ethylene increases the rate of seed germination of several species. In peanuts (*Arachis hypogaea*), ethylene production and seed germination are closely correlated. Ethylene can also break bud dormancy, and ethylene treatment is sometimes used to promote bud sprouting in potato and other tubers.

Ethylene Promotes the Elongation Growth of Submerged Aquatic Species

Although usually thought of as an inhibitor of stem elongation, ethylene is able to promote stem and petiole elongation in various submerged or partially submerged aquatic plants. These include the dicots *Ranunculus sceleratus*, *Nymphoides peltata*, and *Callitriche platycarpa*, and the fern *Regnellidium diphyllum*. Another agriculturally important example is the cereal deepwater rice.

Ethylene Induces the Formation of Roots and Root Hairs

Ethylene is capable of inducing adventitious root formation in leaves, stems, flower stems, and even other roots. Ethylene has also been shown to act as a positive regulator of root hair formation in several species.

This relationship has been best studied in *Arabidopsis*, in which root hairs normally are located in the epidermal cells that overlie a junction between the underlying cortical cells (Dolan et al. 1994).

In ethylene-treated roots, extra hairs form in abnormal locations in the epidermis; that is, cells not overlying a cortical cell junction differentiate into hair cells (Tanimoto et al. 1995). Seedlings grown in the presence of ethylene inhibitors (such as Ag+), as well as ethylene-insensitive mutants, display a reduction in root hair formation in response to ethylene.

These observations suggest that ethylene acts as a positive regulator in the differentiation of root hairs.

Ethylene Induces Flowering in the Pineapple Family

Although ethylene inhibits flowering in many species, it induces flowering in pineapple and its relatives, and it is used commercially in pineapple for synchronization of fruit set. Flowering of other species, such as mango, is also initiated by ethylene. On plants that have separate male and female flowers (monoecious species), ethylene may change the sex of developing flowers. The promotion of female flower formation in cucumber is one example of this effect.

Ethylene Enhances the Rate of Leaf Senescence

As, senescence is a genetically programmed developmental process that affects all tissues of the plant. Several lines of physiological evidence support roles for ethylene and cytokinins in the control of leaf senescence:

The Role of Ethylene in Defense Responses Is Complex

Pathogen infection and disease will occur only if the interactions between host and pathogen are genetically compatible. However, ethylene production generally increases in response to pathogen attack in both compatible (i.e., pathogenic) and noncompatible (nonpathogenic) interactions.

Ethylene Has Important Commercial Uses

Because ethylene regulates so many physiological processes in plant development, it is one of the most

widely used plant hormones in agriculture. Auxins and ACC can trigger the natural biosynthesis of ethylene and in several cases are used in agricultural practice. Because of its high diffusion rate, ethylene is very difficult to apply in the field as a gas, but this limitation can be overcome if an ethylene-releasing compound is used. The most widely used such compound is ethephon, or 2-chloroethylphosphonic acid, which was discovered in the 1960s and is known by various trade names, such as Ethrel.

CELLULAR AND MOLECULAR MODES OF ETHYLENE ACTION

Despite the broad range of ethylene's effects on development, the primary steps in ethylene action are assumed to be similar in all cases: They all involve binding to a receptor, followed by activation of one or more signal transduction pathways leading to the cellular response. Ultimately, ethylene exerts its effects

primarily by altering the pattern of gene expression. In recent years, remarkable progress has been made in our understanding of ethylene perception, as the result of molecular genetic studies of *Arabidopsis thaliana*.

One key to the elucidation of ethylene signaling components has been the use of the triple-response morphology of etiolated *Arabidopsis* seedlings to isolate mutants affected in their response to ethylene

Ethylene Receptors Are Related to Bacterial Two- Component System Histidine Kinases

The first ethylene-insensitive mutant isolated was *etr1*(*e*thylene-*r*esistant *1*). The *etr1* mutant was identified in a screen for mutations that block the response of *Arabidopsis* seedlings to ethylene. The amino acid sequence of the carboxy-terminal half of *ETR1* is similar to bacterial two-component histidine kinases—receptors used by bacteria to perceive various environmental cues, such as chemo-sensory stimuli, phosphate availability, and osmolarity. Bacterial two-component systems consist of a sensor histidine kinase and a response regulator, which often acts as a transcription factor.

High-Affinity Binding of Ethylene to Its Receptor Requires a Copper Cofactor

Even prior to the identification of its receptor, scientists had predicted that ethylene would bind to its receptor via a transition metal cofactor, most likely copper or zinc. This prediction was based on the high affinity of olefins, such as ethylene, for these transition metals. Recent genetic and biochemical studies have borne out these predictions. Analysis of the ETR1 ethylene receptor expressed in yeast demonstrated that a copper ion was coordinated to the protein and that this copper was necessary for highaffinity ethylene binding (Rodriguez et al. 1999). Silver ion could substitute for copper to yield high-affinity binding, which indicates that silver blocks the action of ethylene not by interfering with ethylene binding, but by preventing the changes in the protein that normally occur when ethylene binds to the receptor.

Unbound Ethylene Receptors Are Negative Regulators of the Response Pathway

In *Arabidopsis*, tomato, and probably most other plant species, the ethylene receptors are encoded by multigene families. Targeted disruption (complete inactivation) of the five *Arabidopsis* ethylene receptors (ETR1, ETR2, ERS1, ERS2, and EIN4) has revealed that they are functionally redundant (Hua and Meyerowitz 1998). That is, disruption of any single gene encoding one of these proteins has no effect, but a plant with disruptions in all five receptor genes exhibits a constitutive ethylene response phenotype.

A Serine/Threonine Protein Kinase Is Also Involved in Ethylene Signaling

The recessive *ctr1* (*c*onstitutive *t*riple *r*esponse *1* = triple response in the absence of ethylene) mutation was identified in screens for mutations that constitutively activated ethylene responses. The fact that the mutation

caused an *activation* of the ethylene response suggests that the wild-type protein also acts as a *negative regulator* of the response pathway (Kieber et al. 1993), similar to the ethylene receptors. CTR1 appears to be related to RAF-1, a MAPKKK serine/threonine protein kinase (*m*itogen-*a*ctivated *p*rotein *k*inase *k*inase *k*inase) that is involved in the transduction of various external regulatory signals and developmental signaling pathways in organisms ranging from yeast to humans

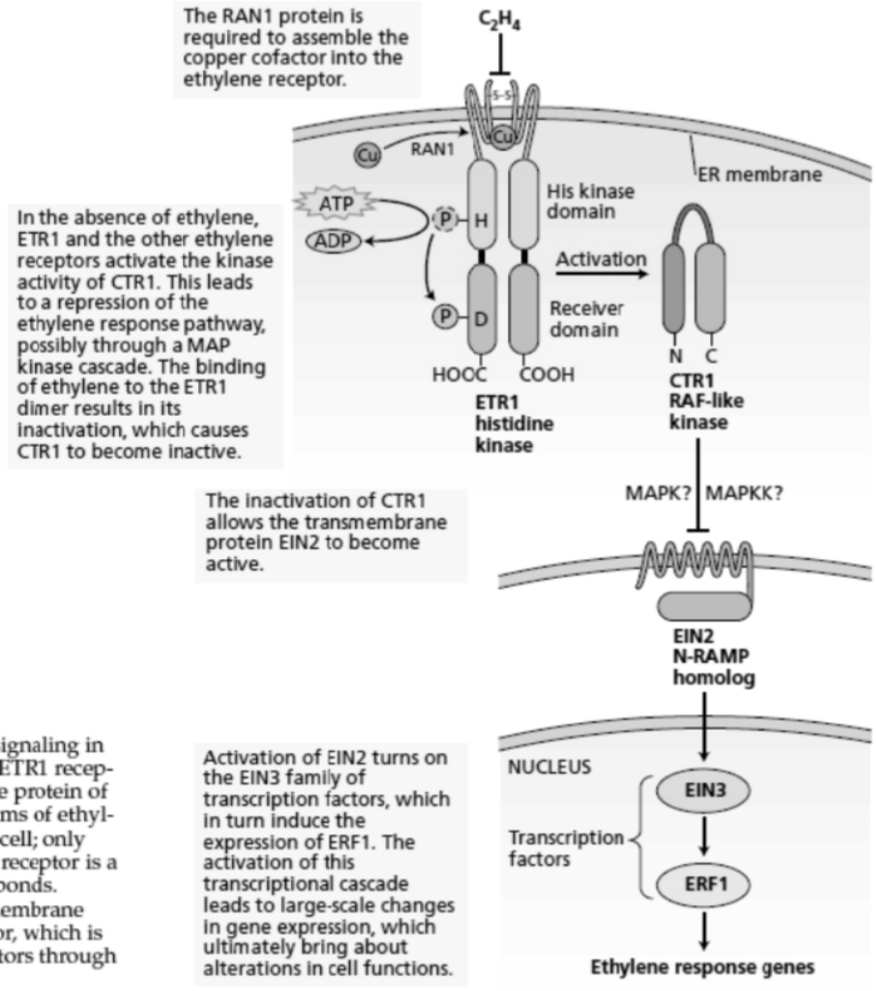


FIGURE 22.16 Model of ethylene signaling in *Arabidopsis*. Ethylene binds to the ETR1 receptor, which is an integral membrane protein of the ER membrane. Multiple isoforms of ethylene receptors may be present in a cell; only ETR1 is shown for simplicity. The receptor is a dimer, held together by disulfide bonds. Ethylene binds within the trans-membrane domain, through a copper co-factor, which is assembled into the ethylene receptors through the RAN1 protein.

Ethylene Regulates Gene Expression

One of the primary effects of ethylene signaling is an alteration in the expression of various target genes. Ethylene affects the mRNA transcript levels of numerous genes, including the genes that encode cellulase, as well as ripening-related genes and ethylene biosynthesis genes. Regulatory sequences called **ethylene response elements**, or **EREs**, have been identified from the ethylene-regulated genes. Key components mediating ethylene's effects on gene expression are the EIN3 family of transcription factors.

Genetic Epistasis Reveals the Order of the Ethylene Signaling Components

The order of action of the genes *ETR1*, *EIN2*, *EIN3*, and *CTR1* has been determined by the analysis of how the mutations interact with each other (i.e., their epistatic order). Two mutants with opposite phenotypes are
crossed, and a line harboring both mutations (the double mutant) is identified in the F2 generation. In the case of the ethylene response mutants, researchers constructed a line doubly mutant for *ctr1*, a constitutive ethylene response mutant, and one of the ethylene-insensitive mutations.

Ethylene regulates fruit ripening and other processes associated with leaf and flower senescence, leaf and fruit abscission, root hair development, seedling growth, and hook opening. Ethylene also regulates the expression of various genes, including ripening-related genes and pathogenesis-related genes.

The ethylene receptor is encoded by a family of genes that encode proteins similar to bacterial two-component histidine kinases. Ethylene binds to these receptors in a transmembrane domain through a copper cofactor. Downstream signal transduction components include CTR1, a member of the RAF family of protein kinases; and EIN2, a channel-like transmembrane protein. The pathway activates a cascade of transcription factors, including the EIN3 and EREBP families, which then modulate gene expression.

Abscisic Acid:

THE EXTENT AND TIMING OF PLANT GROWTH are controlled by the coordinated actions of positive and negative regulators. Some of the most obvious examples of regulated nongrowth are seed and bud dormancy, adaptive features that delay growth until environmental conditions are favorable. For many years, plant physiologists suspected that the phenomena of seed and bud dormancy were caused by inhibitory compounds, and they attempted to extract and isolate such compounds from a variety of plant tissues, especially dormant buds. Early experiments used paper chromatography for the separation of plant extracts, as well as bioassays based on oat coleoptile growth. These early experiments led to the identification of a group of growth-inhibiting compounds, including a substance known as *dormin* purified from sycamore leaves collected in early autumn, when the trees were entering dormancy. Upon discovery that dormin was chemically identical to a substance that promotes the abscission of cotton fruits, *abscisin II*, the compound was renamed **abscisic acid** (**ABA**), to reflect its supposed involvement in the abscission process.

It is now known that ethylene is the hormone that triggers abscission and that ABA-induced abscission of cotton fruits is due to ABA's ability to stimulate ethylene production.

Biosynthesis, metabolism, and transport of ABA

As with the other hormones, the response to ABA depends on its concentration within the tissue and on the sensitivity of the tissue to the hormone. The processes of biosynthesis, catabolism, compartmentation, and transport all contribute to the concentration of active hormone in the tissue at any given stage of development. The complete biosynthetic pathway of ABA was elucidated with the aid of ABA-deficient mutants blocked at specific steps in the pathway.

ABA Is Synthesized from a Carotenoid Intermediate

ABA biosynthesis takes place in chloroplasts and other plastids via the pathway depicted in Figure 23.2. Several ABA-deficient mutants have been identified with lesions at specific steps of the pathway. These mutants exhibit abnormal phenotypes that can be corrected by the application of exogenous ABA. For example, *flacca* (*flc*) and *sitiens* (*sit*) are "wilty mutants" of tomato in which the tendency of the leaves to wilt (due to an inability to close their stomata) can be prevented by the application of exogenous ABA. The *aba* mutants of *Arabidopsis* also exhibit a wilty phenotype. These and other mutants have been useful in

elucidating the details of the pathway (Milborrow 2001). The pathway begins with isopentenyl diphosphate (IPP), the biological isoprene unit, and leads to the synthesis of the C40 xanthophyll (i.e., oxygenated carotenoid) **violaxanthin** (see Figure 23.2). Synthesis of violaxanthin is catalyzed by zeaxanthin epoxidase (ZEP), the enzyme encoded by the *ABA1* locus of *Arabidopsis*. This discovery provided conclusive evidence that ABA synthesis occurs via the "indirect" or carotenoid pathway, rather than as a small molecule.

Maize mutants (*vp*) that are blocked at other steps in the carotenoid pathway also have reduced levels of ABA and exhibit **vivipary**—the precocious germination of seeds in the fruit while still attached to the plant (Figure 23.3). Vivipary is a feature of many ABA-deficient seeds. Violaxanthin is converted to the C40 compound **9′-*cis*neoxanthin**, which is then cleaved to form the C15 com-pound **xanthoxal**, previously called *xanthoxin*, a neutral growth inhibitor that has physiological properties

similar to those of ABA. The cleavage is catalyzed by **9-*cis*-epoxycarotenoid dioxygenase** (**NCED**), so named because it can cleave both 9-*cis*-violaxanthin and 9′-*cis*-neoxanthin.

Developmental and physiological effects of ABA

Abscisic acid plays primary regulatory roles in the initiation and maintenance of seed and bud dormancy and in the plant's response to stress, particularly water stress. In addition, ABA influences many other aspects of plant development by interacting, usually as an antagonist, with auxin, cytokinin, gibberellin, ethylene, and brassinosteroids. In this section we will explore the diverse physiological effects of ABA, beginning with its role in seed development.

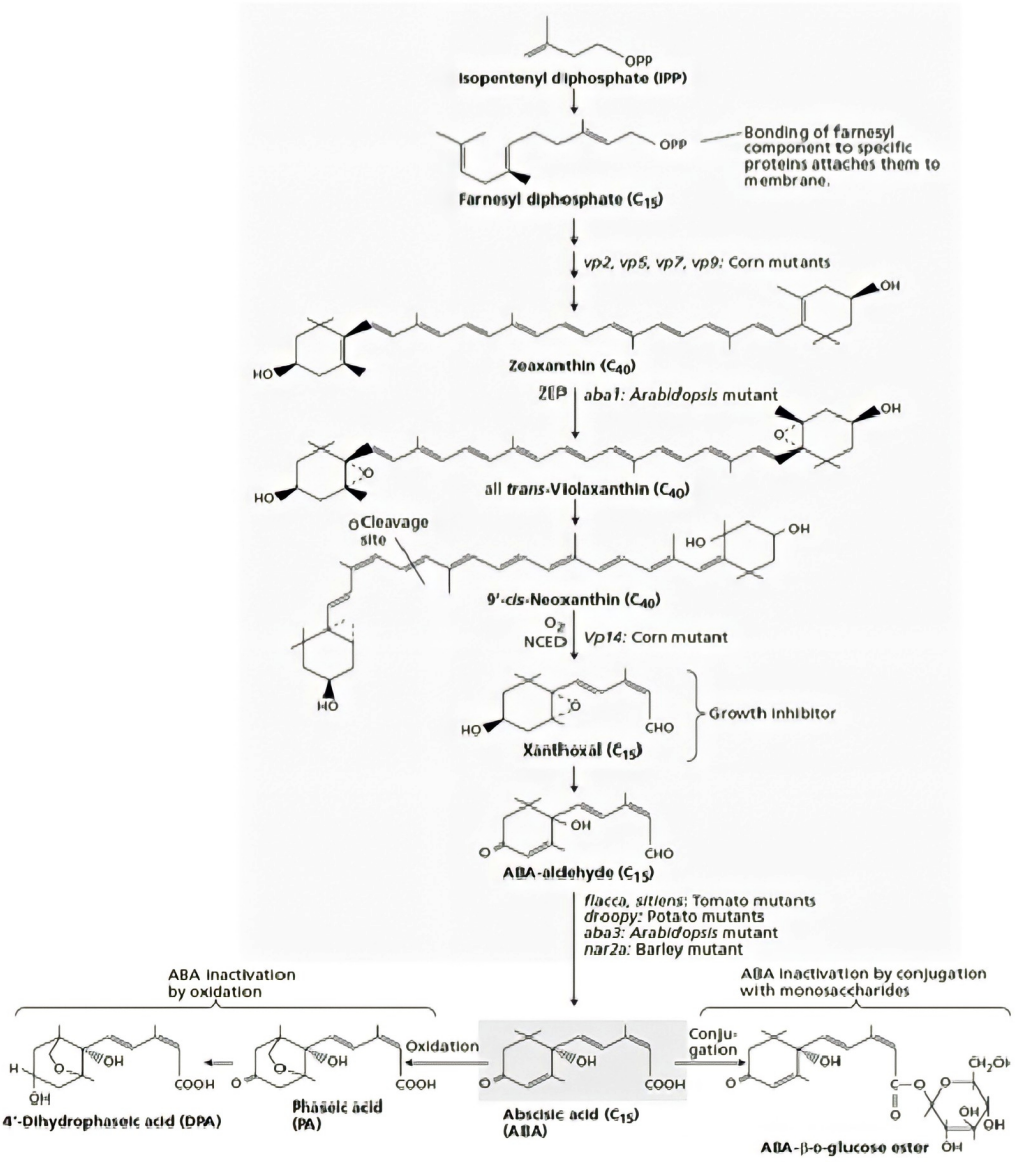


FIGURE 23.2 ABA biosynthesis and metabolism. In higher plants, ABA is synthesized via the terpenoid pathway (see Chapter 13). Some ABA-deficient mutants that have been helpful in elucidating the pathway are shown at the steps at which they are blocked. The pathways for ABA catabolism include conjugation to form ABA-β-D-glucosyl ester or oxidation to form phaseic acid and then dihydrophaseic acid. ZEP = zeaxanthin epoxidase; NCED = 9-cis-epoxycarotenoids dioxygenase.

ABA levels in seeds peak during embryogenesis

Seed development can be divided into three phases of approximately equal duration:

1. During the first phase, which is characterized by cell divisions and tissue differentiation, the zygote undergoes embryogenesis and the endosperm tissue proliferates.
2. During the second phase, cell divisions cease and storage compounds accumulate.
3. In the final phase, the embryo becomes tolerant to desiccation, and the seed dehydrates, losing up to 90% of its water. As a consequence of dehydration, metabolism comes to a halt and the seed enters a **quiescent** ("resting") state. In contrast to dormant seeds, quiescent seeds will germinate upon rehydration.

The latter two phases result in the production of viable seeds with adequate resources to support germination and the capacity to wait weeks to years before resuming growth. Typically, the ABA content of seeds is very low early in embryogenesis, reaches a maximum at about the halfway point, and then gradually falls to low levels as the seed reaches maturity. Thus there is a broad peak of ABA accumulation in the seed corresponding to mid- to late embryogenesis.

The hormonal balance of seeds is complicated by the fact that not all the tissues have the same genotype. The seed coat is derived from maternal tissues the zygote and endosperm are derived from both parents.

Genetic studies with ABA-deficient mutants of *Arabidopsis* have shown that the zygotic genotype controls

ABAsynthesis in the embryo and endosperm and is essential to dormancy induction, whereas the maternal genotype controls the major, early peak of ABA accumulation and helps suppress vivipary in midembryogenesis (Raz et al. 2001).

ABA Promotes Desiccation Tolerance in the Embryo

An important function of ABAin the developing seed is to promote the acquisition of desiccation tolerance. As desiccation can severely damage membranes and other cellular constituents. During the mid- to late stages of seed development, specific mRNAs accumulate in embryos at the time of high levels of endogenous ABA. These mRNAs encode so-called **late-embryogenesis-abundant (LEA)** proteins thought to be involved in desiccation tolerance.

Synthesis of many LEA proteins, or related family members, can be induced by ABA treatment of either young embryos or vegetative tissues. Thus the synthesis of most LEA proteins is under ABA control.

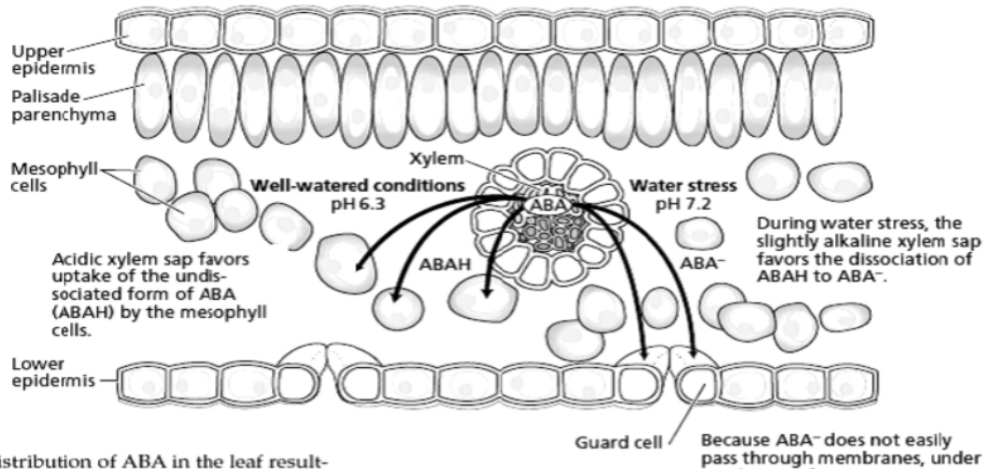


FIGURE 23.4 Redistribution of ABA in the leaf resulting from alkalinization of the xylem sap during water stress.

ABA Promotes the Accumulation of Seed Storage Protein during Embryogenesis

Storage compounds accumulate during mid- to late embryogenesis. Because ABA levels are still high, ABA
could be affecting the translocation of sugars and amino acids, the synthesis of the reserve materials, or both.

Studies in mutants impaired in both ABAsynthesis and response showed no effect of ABA on sugar translocation.

In contrast, ABA has been shown to affect the amounts and composition of storage proteins. For example, exogenous ABA promotes accumulation of storage proteins in cultured embryos of many species, and some ABA-deficient or -insensitive mutants have reduced storage protein accumulation. However, storage protein synthesis is also reduced in other seed developmental mutants with normal ABAlevels and responses, indicating that ABAis only one of several signals controlling the expression of storage protein genes during embryogenesis.

Seed Dormancy May Be Imposed by the Coat or the Embryo

During seed maturation, the embryo enters a quiescent phase in response to desiccation. Seed germination can be defined as the resumption of growth of the embryo of the mature seed; it depends on the same environmental conditions as vegetative growth does. Water and oxygen must be available, the temperature must be suitable, and there must be no inhibitory substances present.

In many cases a viable (living) seed will not germinate even if all the necessary environmental conditions for growth are satisfied. This phenomenon is termed **seed dormancy**. Seed dormancy introduces a temporal delay in the germination process that provides additional time for seed dispersal over greater geographic distances. It also maximizes seedling survival by preventing germination under unfavorable conditions. Two types of seed dormancy have been recognized: coat-imposed dormancy and embryo dormancy.

ABA Inhibits Precocious Germination and Vivipary

When immature embryos are removed from their seeds and placed in culture midway through development before the onset of dormancy, they germinate precociously—that is, without passing through the normal quiescent and/or dormant stage of development. ABA added to the culture medium inhibits precocious germination. This result, in combination with the fact that the level of endogenous ABA is high during mid- to late seed development, suggests that ABA is the natural constraint that keeps developing embryos in their embryogenic state.

ABA Inhibits GA-Induced Enzyme Production

ABA inhibits the synthesis of hydrolytic enzymes that are essential for the breakdown of storage reserves in seeds. For example, GA stimulates the aleurone layer of cereal grains to produce α-amylase and other hydrolytic enzymes that break down stored resources in the endosperm during germination. ABAinhibits this GA-dependent enzyme synthesis by inhibiting the transcription of α-amylase mRNA.

ABA Closes Stomata in Response to Water Stress

Elucidation of the roles of ABA in freezing, salt, and water stress led to the characterization of ABA as a stress hormone. As noted earlier, ABA concentrations in leaves can increase up to 50 times under drought conditions— the most dramatic change in concentration reported for any hormone in response to an environmental signal. Redistribution or biosynthesis of ABA is very effective in causing stomatal closure, and its accumulation in stressed leaves plays an important role in the reduction of water loss by transpiration under water stress conditions.

ABA Promotes Root Growth and Inhibits Shoot Growth at Low Water Potentials

ABAhas different effects on the growth of roots and shoots, and the effects are strongly dependent on the water status of the plant. The growth of shoots and roots of maize seedlings grown under either abundant water conditions (high water potential) or dehydrating conditions (low water potential). Two types of seedlings were used: (1) wild-type seedlings with normal ABA levels and (2) an ABA-deficient, viviparous mutant.

When the water supply is ample (high water potential), shoot growth is greater in the wild-type plant (normal endogenous ABAlevels) than in the ABA-deficient mutant. The reduced shoot growth in the ABA-deficient mutant could be due in part to excessive water loss from the leaves. In maize and tomato, however, the stunted shoot growth of ABA-deficient plants at high water potentials seems to be due to the overproduction of ethylene, which is normally inhibited by endogenous ABA (Sharp et al. 2000). This finding suggests that endogenous ABApromotes shoot growth in well-watered plants by suppressing ethylene production.

ABA Promotes Leaf Senescence Independently of Ethylene

Abscisic acid was originally isolated as an abscission-causing factor. However, it has since become evident that ABA stimulates abscission of organs in only a few species and that the primary hormone causing abscission is ethylene. On the other hand, ABA is clearly involved in leaf senescence, and through its promotion of senescence it might indirectly increase ethylene formation and stimulate abscission.

Cellular and molecular modes of ABA action

ABA is involved in short-term physiological effects (e.g., stomatal closure), as well as long-term developmental processes (e.g., seed maturation). Rapid physiological responses frequently involve alterations in the fluxes of ions across membranes and may involve some gene regulation as well, and long-term processes inevitably involve major changes in the pattern of gene expression.

Genetic studies have shown that many conserved signaling components regulate both short- and long-term responses, indicating that they share common signaling mechanisms.

ABA Is Perceived Both Extracellularly and Intracellularly

Although ABA has been shown to interact directly with phospholipids, it is widely assumed that the ABA receptor is a protein. To date, however, the protein receptor for ABA has not been identified. Experiments have been performed to determine whether the hormone must enter the cell to be effective, or whether it can act externally by binding to a receptor located on the outer surface of the plasma membrane.

The results so far suggest multiple sites of perception. Some experiments point to a receptor on the outer surface of the cell. For example, microinjected ABA fails to alter stomatal opening in the spiderwort *Commelina*, or to inhibit GA-induced α-amylase synthesis in barley aleurone protoplasts (Anderson et al. 1994; Gilroy and Jones 1994). Furthermore, impermeant ABA–protein conjugates have been shown to activate both ion channel activity and gene expression (Schultz and Quatrano 1997; Jeannette et al. 1999).

ABA Increases Cytosolic Ca2+, Raises Cytosolic pH, and Depolarizes the Membrane

As stomatal closure is driven by a reduction in guard cell turgor pressure caused by a massive long-term efflux of K+ and anions from the cell. During the subsequent shrinkage of the cell due to water loss, the surface area of the plasma membrane may contract by as much as 50%. Where does the extra membrane go? The answer seems to be that it is taken up as small vesicles by endocytosis—a process that also involves reorganization of the actin cytoskeleton. However, the first changes detected after exposure of guard cells to ABA are transient membrane depolarization caused by the net influx of positive charge, and transient increases in the cytosolic calcium concentration.

ABAstimulates elevations in the concentration of cytosolic Ca2+ by inducing both influx through plasma membrane channels and release of calcium into the cytosol from internal compartments, such as the central vacuole (Schroeder et al. 2001). Stimulation of influx occurs via a pathway that uses **reactive oxygen species (ROS)**, such as hydrogen peroxide (H_2O_2) or superoxide (O_2), as secondary messengers leading

to plasma membrane channel activation (Pei et al. 2000).

ABA Activation of Slow Anion Channels Causes Long-Term Membrane Depolarization

The rapid, transient depolarizations induced by ABA are insufficient to open the K+ efflux channels, which require long-term membrane depolarization in order to open. However, long-term depolarizations in response to ABA have been demonstrated. According to a widely accepted model,

long-term membrane depolarization is triggered by two factors: (1) an ABA-induced transient depolarization of the plasma membrane, coupled with (2) an increase in cytosolic calcium.

ABA Stimulates Phospholipid Metabolism

As discussed previously, much evidence supports a role for calcium both in the promotion of stomatal closing and in the inhibition of stomatal opening. According to the classic calcium-dependent signal transduction pathway of animal cells, IP3 is released, along with diacylglycerol (DAG), when phospholipase C is activated by a G-protein in the plasma membrane. Does ABAuse the same pathway when it induces stomatal closure?

1. ABA binds to its receptors.

2. ABA-binding induces the formation of reactive oxygen species, which activate plasma membrane Ca^{2+} channels.

3. ABA increases the levels of cyclic ADP-ribose and IP_3, which activate additional calcium channels on the tonoplast.

4. The influx of calcium initiates intracellular calcium oscillations and promotes the further release of calcium from vacuoles.

5. The rise in intracellular calcium blocks K^+_{in} channels.

6. The rise in intracellular calcium promotes the opening if Cl^-_{out} (anion) channels on the plasma membrane, causing membrane depolarization.

7. The plasma membrane proton pump is inhibited by the ABA-induced increase in cytostolic calcium and a rise in intracellular pH, further depolarizing the membrane.

8. Membrane depolarization activates K^+_{out} channels.

9. K^+ and anions to be released across the plasma membrane are first released from vacuoles into the cytosol.

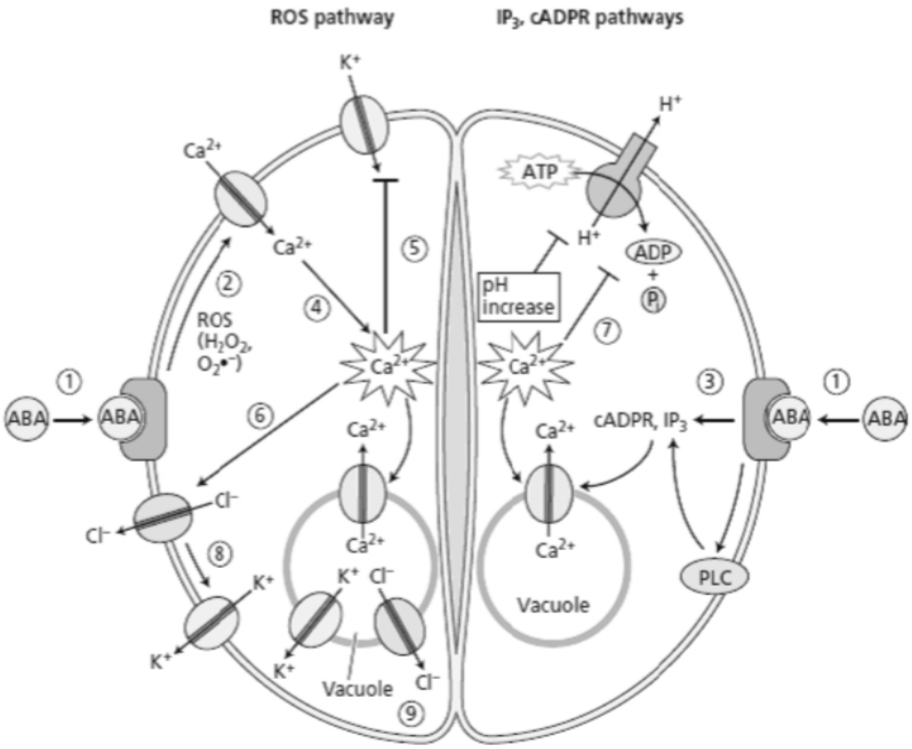


FIGURE 23.12 Simplified model for ABA signaling in stomatal guard cells. The net effect is the loss of potassium and its anion (Cl^- or $malate^{2-}$) from the cell. (R = receptor; ROS = reactive oxygen species; cADPR = cyclic ADP-ribose; G-protein = GTP-binding protein; PLC = phospholipiase C.)

Protein Kinases and Phosphatases Participate in ABA Action

Nearly all biological signaling systems involve protein phosphorylation and dephosphorylation reactions at some step in the pathway. Thus we can expect that signal transduction in guard cells, with their multiple sensory inputs, involves protein kinases and phosphatases. Artificially raising the ATP concentration inside guard cells by allowing the cytoplasm to equilibrate with the solution inside a patch pipette strongly activates the slow anion channels.

This activation of the slow anion channels by ATP is abolished by the inclusion of protein kinase inhibitors in the patch pipette solution (Schmidt et al. 1995). Protein kinase inhibitors also block ABA-induced stomatal closing.

ABA Regulation of Gene Expression Is Mediated by Transcription Factors

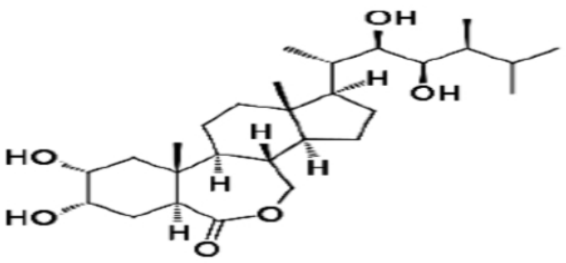
Downstream of the early ABA signal transduction processes already discussed, ABA causes changes in gene

expression. ABAhas been shown to regulate the expression of numerous genes during seed maturation and under certain stress conditions, such as heat shock, adaptation to low temperatures, and salt tolerance (Rock 2000). The ABAand stress-induced genes are presumed to contribute to adaptive aspects of induced tolerance. They include genes encoding proteases, chaperonins, proteins similar to LEA proteins, enzymes of sugar or other compatible solute metabolism, ion and water channel proteins, enzymes that detoxify active oxygen species, and regulatory proteins such as transcription factors and protein kinases.

In general, the ABAresponse appears to be regulated by more than one signal transduction pathway, even within a single cell type. This redundancy is consistent with the ability of plant cells to respond to multiple sensory inputs. There is genetic evidence for cross-talk between ABA signalling and the signaling of all other major classes of phytohormones, as well as sugars.

Brassinosteroids:

Brassinosteroids (BRs) are a class of polyhydroxysteroids that have been recognized as a sixth class of plant hormones and may have utility as an anticancer drug for endocrine-responsive cancers to induce apoptosis and inhibit growth. These brassinosteroids were first explored during the 70s, when Mitchell et al. reported promotion in stem elongation and cell division by the treatment of organic extracts of rapeseed (*Brassica napus*) pollen. Brassinolide was the first isolated brassinosteroid in 1979, when pollen from *Brassica napus* was shown to promote stem elongation and cell divisions, and the biologically active molecule was isolated. The yield of brassinosteroids from 230 kg of *Brassica napus* pollen was only 10 mg. Since their discovery, over 70 BR compounds have been isolated from plants.



Brassinolide, the first brassinosteroid isolated and shown to have biological activity

Biosynthesis

The BR is biosynthesised from campesterol. The biosynthetic pathway was elucidated by Japanese researchers and later shown to be correct through the analysis of BR biosynthesis mutants in *Arabidopsis thaliana*, tomatoes, and peas. The sites for BR synthesis in plants have not been experimentally demonstrated. One well-supported hypothesis is that all tissues produce BRs, since BR biosynthetic and signal transduction genes are expressed in a wide range of plant organs, and short distance activity of the hormones also supports this. Experiments have shown that long distance transport is possible and that the flow is from the base to the tips (acropetal), but it is not known if this movement is biologically relevant.

Hormonal activity

BRs have been shown to be involved in numerous plant processes: Promotion of cell expansion and cell elongation; works with auxin to do so. It has an unclear role in cell division and cell wall regeneration.

Promotion of vascular differentiation; BR signal transduction has been studied during vascular differentiation. Is necessary for pollen elongation for pollen tube formation. Acceleration of senescence in dying tissue cultured cells; delayed senescence in BR mutants supports that this action may be biologically relevant.

Can provide some protection to plants during chilling and drought stress. Extract from the plant *Lychnis viscaria* contains a relatively high amount of Brassinosteroids. Lychnis viscaria increases the disease resistance of surrounding plants. 24-Epibrassinolide (EBL), a brassinosteroid isolated from *Aegle marmelos* Correa (Rutaceae), was further evaluated for the antigenotoxicity against maleic hydrazide (MH)-induced genotoxicity in *Allium cepa* chromosomal aberration assay. It was shown that the percentage of chromosomal aberrations induced by maleic hydrazide (0.01%) declined significantly with 24-epibrassinolide treatment. BRs have been reported to counteract both abiotic and biotic stress in plants. Application of brassinosteroids to cucumbers was demonstrated to increase the metabolism and removal of pesticides, which could be beneficial for reducing the human ingestion of residual pesticides from non-organically grown vegetables. In all Type of brassinosteroids 28-homoBL is the most effective type of brassinosteroids. Brassinosteroids increased tolerance to high temperature in Brassica juncea L. The ability of 28-homobrassinolide to confer resistance to stress in *Brassica juncea* L. has also established. Application of 24-epiBL have any protective role on shoot, root length, soluble protein, proline content and peroxidases along with proline content PPO and IAA in seedlings of *B. juncea* L. under seasonal stress BRs have also been reported to have a variety of effects when applied to rice seeds (*Oryza sativa* L.). Seeds treated with BRs were shown to reduce the growth inhibitory effect of salt stress. When the developed plants fresh weight was analyzed the treated seeds outperformed plants grown on saline and non-saline medium however when the dry weight was analyzed BR treated seeds only outperformed untreated plants that were grown on saline medium. When dealing with tomatoes (*Lycopersicon esculentum*) under salt stress the concentration of cholophyll a and cholophyll b were decreased and thus pigmentation was decreased as well. BR treated rice seeds considerably restored the pigment level in plants that were grown on saline medium when compared to non-treated plants under the same conditions.

Signalling mechanism

BRs are perceived at the cell membrane by a co-receptor complex, comprising BRASSINOSTEROID INSENSITIVE 1(BRI1) and BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1). BRI1 acts as a kinase, but in the absence of BR its action is inhibited by another protein, BRI1 KINASE INHIBITOR 1 (BKI1). When BR binds to the BRI1:BAK1 complex, BKI1 is released, and a phosphorylation cascade is triggered which results in the de-activation of another kinase, BRASSINOSTEROID INSENSITIVE 2 (BIN2). BIN2 and its close homologues inhibit several transcription factors.

The inhibition of BIN2 by BR releases these transcription factors to bind to DNA and to enact certain developmental pathways.

Agricultural uses

BR might reveal to have a prominent interest in the role of horticultural crops. Based on extensive research BR has the ability to improve the quantity and quality of horticultural crops and protect plants against many stresses that can be present in the local environment. With the many advances in technology dealing with the synthesis of more stable synthetic analogues and the genetic manipulation of cellular BR activity, using BR in the production of horticultural crops has become a more practical and hopeful strategy for improving crop yields and success. BR could also help bridge the gap of the consumers' health concerns and the producers need for growth. A major benefit of using BR is that it does not interfere with the environment because they act in natural doses in a natural way. Since it is a “plant strengthening substance” and it is natural, BR application would be more favorable than pesticides and does not contribute to the co-evolution of pests.

In Germany, extract from the plant is allowed for use as a "plant strengthening substance."

Polyamines:

A **polyamine** is an organic compound having more than two amino groups. Alkyl polyamines occur naturally but are also synthetic. Alkylpolyamines are colorless, hygroscopic, and water soluble. Near neutral pH, they exist as the ammonium derivatives. Most aromatic polyamines are crystalline solids at room temperature.

Natural polyamines

Low-molecular-weight linear polyamines are found in all forms of life. The principal examples are the triamine and tetraamine spermidine and spermine. They are structurally and biosynthetically related to the diamines putrescine and cadaverine. Polyamine metabolism is regulated by the activity of the enzyme ornithine decarboxylase (ODC). Polyamines are found in high concentrations in the mammalian brain.

Synthetic polyamines

Several synthetic polyamines are used in chemical industry and the research laboratory. They are mainly of interest as additives to motor oil and as co-reactants (hardeners) with epoxy resins. Many synthetic polyamines feature NCH2CH2N linkages:

Diethylenetriamine, abbreviated dien or DETA, The related permethylated derivative pentamethyldiethylenetriamine is used as a chelating agent in organolithium chemistry. Triethylenetetramine tetraethylenepentamine, pentaethylenehexamine.

Biological function

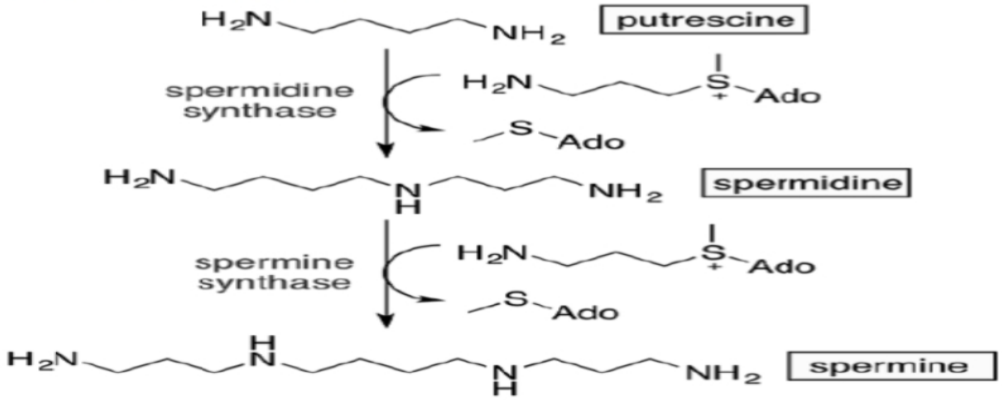
Although it is known that the biosynthesis of polyamines is highly regulated, the biological function of polyamines is only partly elucidated. In their cationic ammonium form, they bind to DNA, and, in structure, they represent compounds with cations that are found at *regularly spaced intervals* (unlike, say, Mg^{2+} or Ca^{2+} which are point charges). They have also been found to act as promoters of programmed ribosomal frameshifting during translation.

Inhibition of polyamine biosynthesis, retards or stops cell growth. The provision of exogenous polyamines restores the growth of these cells. Most eukaryotic cells have a polyamine transporter system on their cell membrane that facilitates the internalization of exogenous polyamines. This system is highly active in rapidly proliferating cells and is the target of some chemotherapeutics currently under development.

Polyamines are also modulators of a variety of ion channels, including NMDA receptors and AMPA receptors. They block inward-rectifier potassium channels so that the currents of the channels are inwardly rectified, thereby the cellular energy, i.e. K+ ion gradient across the cell membrane, is conserved. In addition, polyamine participate in initiating the expression of SOS response of Colicin E7 operon and down-regulate proteins that are essential for colicin E7 uptake, thus conferring a survival advantage on colicin-producing *E. coli* under stress conditions. Polyamines can enhance the permeability of the blood–brain barrier. They are involved in modulating senescence of organs in plants and are therefore considered as a plant hormone. In addition, they are directly involved in regulation of programmed cell death.

Biosynthesis of spermidine, spermine, thermospermine

Spermidine is synthesized from putrescine, using an aminopropyl group from decarboxylated *S*-adenosyl-L-methionine (SAM).



Biosynthesis of spermidine and spermine from putrescine. Ado = 5'-adenosyl.

The reaction is catalyzed by spermidine synthase. Spermine is synthesized from the reaction of spermidine with SAM in the presence of the enzyme spermine synthase. Thermospermine (NH_2-$(CH2)_3$-NH-$(CH_2)_3$-NH-$(CH_2)_4$-NH_2) is a structural isomer of spermine and a novel type of plant growth regulator. It is produced from spermidine by the action of thermospermine synthase, which is encoded by a gene named ACAULIS5 (ACL5).

Polyamine analogues

The critical role of polyamines in cell growth has led to the development of a number of agents that interfere with polyamine metabolism. These agents are used in cancer therapy. Polyamine analogues upregulate p53 in a cell leading to restriction of proliferation and apoptosis. It also decreases the expression of estrogen receptor alpha in ER positive breast cancer.

Jasmonates:

Jasmonate (JA) and its derivatives are lipid-based plant hormones that regulate a wide range of processes in plants, ranging from growth and photosynthesis to reproductive development. In particular, JAs are critical

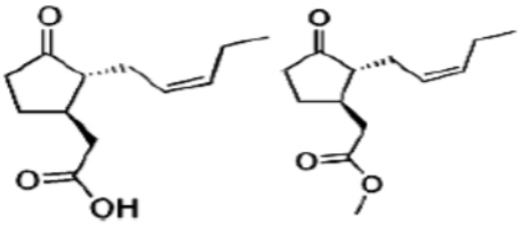
for plant defense against herbivory and plant responses to poor environmental conditions and other kinds of abiotic and biotic challenges. Some JAs can also be released as volatile organic compounds (VOCs) to permit communication between plants in anticipation of mutual dangers.

The isolation of methyl jasmonate from jasmine oil derived from *Jasminum grandiflorum* led to the discovery of the molecular structure of jasmonates and their name.

Chemical structure

Jasmonates (JA) are an oxylipin, i.e. a derivative of oxygenated fatty acid. It is biosynthesized from linolenic acid in chloroplast membranes. Synthesis is initiated with the conversion of linolenic acid to 12-oxo-phytodienoic acid (OPDA), which then undergoes a reduction and three rounds of oxidation to form (+)-7-iso-JA, jasmonic acid. Only the conversion of linolenic acid to OPDA occurs in the chloroplast; all subsequent reactions occur in the peroxisome. JA itself can be further metabolized into active or inactive derivatives. Methyl JA (MeJA) is a volatile compound that is potentially responsible for interplant communication. JA conjugated with amino acid isoleucine (Ile) results in JA-Ile, which is currently the only known JA derivative needed for JA signaling. JA undergoes decarboxylation to give cis-jasmone.

Mechanism of signalling



Structures of active jasmonate derivatives

In general, the steps in jasmonate (JA) signaling mirror that of auxin signaling: the first step comprises E3 ubiquitin ligase complexes, which tag substrates with ubiquitin to mark them for degradation by proteasomes. The second step utilizes transcription factors to effect physiological changes. One of the key molecules in this pathway is JAZ, which serves as the on-off switch for JA signaling. In the absence of JA, JAZ proteins bind to downstream transcription factors and limit their activity. However, in the presence of JA or its bioactive derivatives, JAZ proteins are degraded, freeing transcription factors for expression of genes needed in stress responses. Because JAZ did not disappear in null *coi1* mutant plant backgrounds, protein COI1 was shown to mediate JAZ degradation. COI1 belongs to the family of highly conserved

F-box proteins, and it recruits substrates for the E3 ubiquitin ligase SCFCOI1. The complexes that ultimately form are known as SCF complexes. These complexes bind JAZ and target it for proteasomal degradation. However, given the large spectrum of JA molecules, not all JA derivatives activate this pathway for signaling, and the range of those participating in this pathway is unknown. Thus far, only JA-Ile has been shown to be necessary for COI1-mediated degradation of JAZ11. JA-Ile and structurally related derivatives can bind to COI1-JAZ complexes and promote ubiquitination and thus degradation of the latter.

This mechanistic model raises the possibility that COI1 serves as an intracellular receptor for JA signals. Recent research has confirmed this hypothesis by demonstrating that the COI1-JAZ complex acts as a co-receptor for JA perception.

Specifically, JA-Ile binds both to a ligand-binding pocket in COI1 and to a 20 aminoacid stretch of the conserved Jas motif in JAZ. This JAZ residue acts as a plug for the pocket in COI1, keeping JA-Ile bound in the pocket. Additionally, co-purification and subsequent removal of inositol pentakisphosphate (InsP5) from COI1 suggest InsP5 is a necessary component of the co-receptor and plays a role in potentiating the co-receptor complex. Once freed from JAZ, transcription factors can activate genes needed for a specific JA response. The best-studied transcription factors acting in this pathway belong to the MYC family of transcription factors, which are characterized by a basic helix-loop-helix (bHLH) DNA binding motif. These factors (of which there are three, MYC2, 3, and 4) tend to act additively. For example, a plant that has only lost one myc becomes more susceptible to insect herbivory than a normal plant. A plant that has lost all three will be as susceptible to damage as coi1 mutants, which are completely unresponsive to JA and cannot mount a defense against herbivory. However, while all these MYC molecules share functions, they vary greatly in expression patterns and transcription functions. For instance, MYC2 has a greater effect

on root growth compared to MYC3 or MYC4.

Additionally, MYC2 will loop back and regulate JAZ expression levels, leading to a negative feedback loop. These transcription factors all have different impacts on JAZ levels after JA signaling. JAZ levels in turn affect transcription factor and gene expression levels. In other words, on top of activating different response genes, the transcription factors can vary JAZ levels to achieve specificity in response to JA signals.

Function

Although jasmonate (JA) regulates many different processes in the plant, its role in wound response is best understood. Following mechanical wounding or herbivory, JA biosynthesis is rapidly activated, leading to expression of the appropriate response genes. For example, in the tomato, wounding produces defense molecules that inhibit leaf digestion in the insect's gut. Another indirect result of JA signaling is the volatile emission of JA-derived compounds. MeJA on leaves can travel airborne to nearby plants and elevate levels of transcripts related to wound response. In general, this emission can further upregulate JA synthesis and signaling and induce nearby plants to prime their defenses in case of herbivory.

Following its role in defense, JAs have also been implicated in cell death and leaf senescence. JA can interact with many kinases and transcription factors associated with senescence. JA can also induce mitochondrial death by inducing the accumulation of reactive oxygen species (ROSs). These compounds disrupt mitochondria membranes and compromise the cell by causing apoptosis, or programmed cell death. JAs' roles in these processes are suggestive of methods by which the plant defends itself against biotic challenges and limits the spread of infections. JA and its derivatives have also been implicated in plant development, symbiosis, and a host of other processes included in the list below.

By studying mutants overexpressing JA, one of the earliest discoveries made was that JA inhibits root growth. The mechanism behind this event is still not understood, but mutants in the COI1-dependent signaling pathway tend to show reduced inhibition, demonstrating that the COI1 pathway is somehow necessary for inhibiting root growth. JA plays many roles in flower development. Mutants in JA synthesis or in JA signaling in Arabidopsis present with male sterility, typically due to delayed development. The same genes promoting male fertility in Arabidopsis promote female fertility in tomatoes. Overexpression of 12-OH-JA can also delay flowering. JA and MeJA inhibit the germination of nondormant seeds and stimulate the germination of dormant seeds. High levels of JA encourage the accumulation of storage proteins; genes encoding vegetative storage proteins are JA responsive. Specifically, tuberonic acid, a JA derivative, induces the formation of tubers. JAs also play a role in symbiosis between plants and microorganisms; however, its precise role is still unclear. JA currently appears to regulate signal exchange and nodulation regulation between legumes and rhizobium. On the other hand, elevated JA levels appear to regulate carbohydrate partitioning and stress tolerance in mycorrhizal plants.

Role in pathogenesis

Pseudomonas syringae causes bacterial speck disease in tomatoes by hijacking the plant's jasmonate (JA) signalling pathway. This bacteria utilizes a type III secretion system to inject a cocktail of viral effector proteins into host cells.

One of the molecules included in this mixture is the phytotoxin coronatine (COR). JA-insensitive plants are highly resistant to *P. syringae* and unresponsive to COR; additionally, applying MeJA was sufficient to rescue virulence in COR mutant bacteria. Infected plants also expressed downstream JA and wound response genes but repressed levels of pathogenesis-related (PR) genes. All these data suggest COR acts through the JA pathway to invade host plants.

Activation of a wound response is hypothesized to come at the expense of pathogen defense. By activating the JA wound response pathway, *P. syringae* could divert resources from its host's immune system and infect more effectively. Plants produce N-acylamides that confer resistance to necrotrophic pathogens by activating JA biosynthesis and signalling. Arachidonic acid (AA), the counterpart of the JA precursor a-LeA occurring in metazoan species but not in plants, is perceived by plants and acts through an increase in JA levels concomitantly with resistance to necrotrophic pathogens. AA is an evolutionarily conserved signalling molecule that acts in plants in response to stress similar to that in animal systems.

Cross talk with other defense pathways

While the jasmonate (JA) pathway is critical for wound response, it is not the only signaling pathway mediating defense in plants. To build an optimal yet efficient defense, the different defense pathways must be capable of cross talk to fine-tune and specify responses to abiotic and biotic challenges.

One of the best studied examples of JA cross talk occurs with salicylic acid (SA). SA, a hormone, mediates defense against pathogens by inducing both the expression of pathogenesis-related genes and systemic acquired resistance (SAR), in which the whole plant gains resistance to a pathogen after localized exposure to it. Wound and pathogen response appear to be interact negatively. For example, silencing phenylalanine ammonia lyase (PAL), an enzyme synthesizing precursors to SA, reducesSAR but enhances herbivory resistance against insects.

Similarly, overexpression of PAL enhances SAR but reduces wound response after insect herbivory. Generally, it has been found that pathogens living in live plant cells are more sensitive to SA-induced defenses, while herbivorous insects and pathogens that derive benefit from cell death are more susceptible to JA defenses. Thus, this trade-off in pathways optimizes defense and

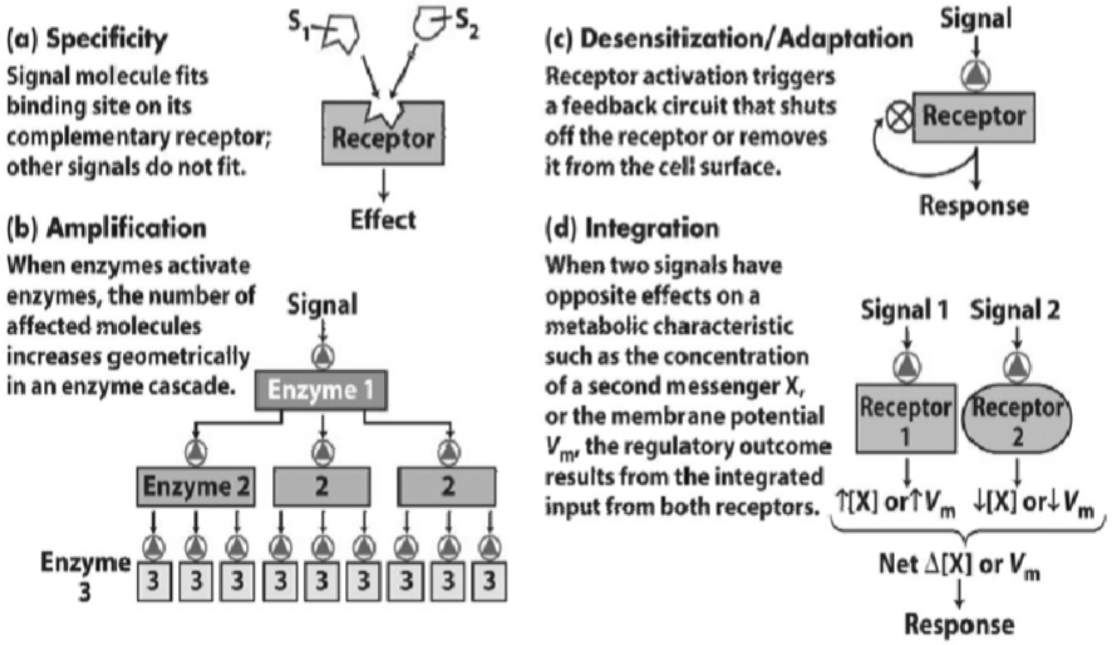
saves plant resources.
Cross talk also occurs between JA and other plant hormone pathways, such as those of abscisic acid (ABA) and ethylene (ET). These interactions similarly optimize defense against pathogens an herbivores of different lifestyles. For example, MYC2 activity can be stimulated by both JA and ABA pathways, allowing it to integrate signals from both pathways. Other transcription factors such as ERF1 arise as a result of JA and ET signaling. All these molecules can act in combination to activate specific wound response genes.
Finally, cross talk is not restricted for defense: JA and ET interactions are critical in development as well, and a balance between the two compounds is necessary for proper apical hook development in *Arabidopsis* seedlings. Still, further research is needed to elucidate the molecules regulating such cross talk.

6. Signal transduction: Signal transduction in eukaryotes.

Signal transduction is the process by which a chemical or physical signal is transmitted through a cell as a series of molecular events, most commonly protein phosphorylation catalyzed by protein kinases, which ultimately results in a cellular response. Proteins responsible for detecting stimuli are generally termed receptors, although in some cases the term sensor is used. The changes elicited by ligand binding (or signal sensing) in a receptor give rise to a biochemical cascade, which is a chain of biochemical events as a signaling pathway.

When signaling pathways interact with one another they form networks, which allow cellular responses to be coordinated, often by combinatorial signaling events. At the molecular level, such responses include changes in the transcription or translation of genes, and post-translational and conformational changes in proteins, as well as changes in their location. These molecular events are the basic mechanisms controlling cell growth, proliferation, metabolism and many other processes. In multicellular organisms, signal transduction pathways have evolved to regulate cell communication in a wide variety of ways.

Characteristics of Signal Transduction:



1. RECEPTORS:

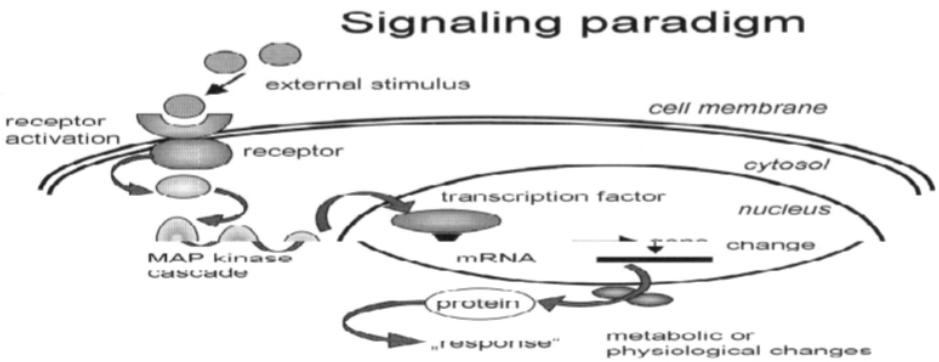


Fig. 6.1 Visualization of the signaling paradigm (for description, see text). The receptor is stimulated by a ligand or another kind of signal, and it changes its own state from susceptible to active. The active receptor initiates the internal signaling cascade, including a series of protein phosphorylation state changes. Subsequently, transcription factors are activated or deactivated The transcription factors regulate the transc tion rate of a set of genes. The absolute amo or the relative changes in protein concentrat alter the state of the cell and trigger the actu response to the signal.

Simple receptor activation

• The simplest mechanism is the reversible binding of the ligand L to the receptor R to form the complex RL $R + L = \leftrightarrow RL$

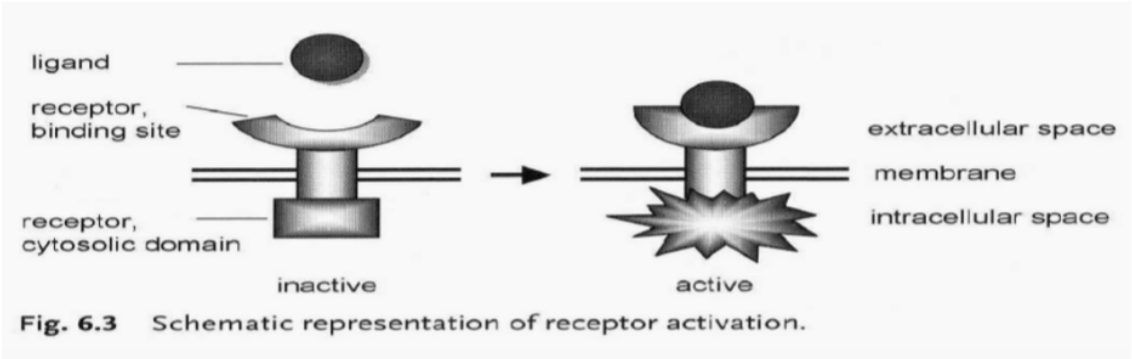


Fig. 6.3 Schematic representation of receptor activation.

The Signalling System:

Signalling system is very complex which may be compared to electronic circuits. You know that electronic system is such that can integrate, modulate and amplify inputs and generate output signals when switched on or switched off after getting suitable signals.

The signalling systems include few basic type of modules. There are four main processes, but the signalling system uses the one or more processes.

The types of signalling modules used in intracellular signalling are:

(a) Receptor kinases (e.g. tyrosine kinase, serine kinase, histidine kinase),
(b) Receptor non-kinases (e.g. serpentine, cytokine, His-Asp phosphorelay),
(c) Protein kinase (intracellular enzymes e.g. cycline families, Asp kinase),
(d) Lipid modifying intracellular enzymes (e.g. p13K, p15K, PLC),
(e) Cyclic nucleotides (e.g. cAMP, cGMP), and
(f) Metal ions (e.g. Ca^{++}).

The four main processes are
These are:
(a) Protein phosphorylation by kinases,
(b) Small molecule and protein interaction, often involving phosphates,
(c) Protein-protein interaction, often mediated by common motifs (specific protein sequences) which frequently results in membrane recruitment when one component is tethered to a membrane, and
(d) Protein and DNA interaction that promotes gene expression or gene inhibition.
When many signals are interconnected in a series or parallel, the intracellular signalling becomes complex. General principles of these modules, the way of processing and onward transmission of signals in prokaryotes and eukaryotes are the main topics of discussion.

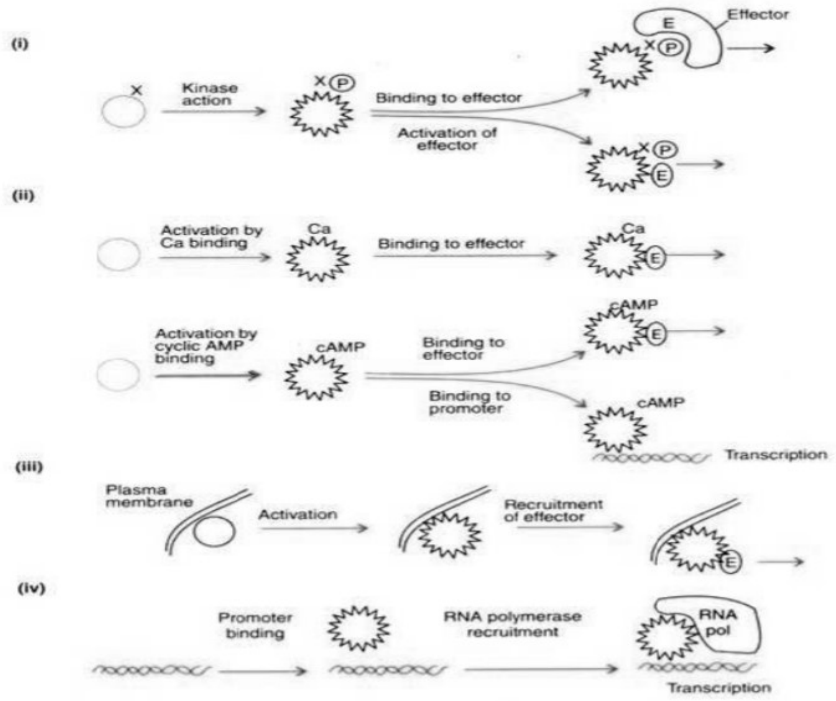


Fig. 27.8: Signalling molecules that use different types of interactions. *(i)* protein phosphorylation, where X=Tyr, Ser, Thr, His or Asp, *(ii)* interaction between small molecules and proteins, e.g. Ca^{++}, or cAMP, *(iii)* interaction between protein and protein, and *(iv)* interaction between protein and DNA which regulates transcription.

The Basic Building Blocks used in Signalling:
(a) Protein Phosphorylation:
Protein phosphorylation is closely linked to cellular signaling. It exits in all signalling modules. The terminal y-phosphate is directly transferred from ARP (in some cases) to an acceptor protein by a protein kinase. The activity of the acceptor is modified example mitogen-activated protein (MAP) kinases in eukaryotes and histidyl-aspartyl phosphorelay in bacteria.
In some cases, indirect phosphorylation of protein also occurs (e.g. in G protein when binding of GTP activates their function, while GDP binding inactivates). There are secondary messengers which are used in intracellular signalling such as phosphorylated inositol's or cyclic nucleotides (cAMP, cGMP).
Kinases are regulated by any of a number of mechanisms: threonine and/or tyrosine phosphorylation, legend occupancy resulting in autophosphorylation or interaction with small molecules (e.g. cAMP or Ca^{++}).

i. Histidine Kinases:

These are found in bacteria, lower eukaryotes and plants as transmembrane protein. They are stimulated to undergo self-phosphorylation by ligand occupancy.

ii. Protein Phosphatases:

Proteins which remove phosphate groups from proteins are called protein phosphatases. Protein kinases add phosphate group to proteins and play a key role in activation of signals.

Specific phosphatases e.g. dephosphorylate phosphotyrosine and phosphoserine/phosphothreonme play a key role in control of proliferation, differentiation and cell cycle Phosphoproteins take part in signalling. They moderate the phosphorylation status by regulating the balance of phosphatases and kinases.

(b) Nucleotide-Binding Proteins:

The three nucleotides (GTP, cGMP and cAMP) play a major role in the intracellular signalling.

iii. GTP-Binding Proteins:

There is a set of eukaryotic proteins (G proteins) that show GTPase activity. They bind to GTP and remove the terminal phosphate of GTP and produce GDP bound to G protein. This cycle operates similar to ATP and ADP cycles. When GDP dissociates from the G protein and GTP binds again, the cycle is repeated. G proteins are of two type: the heterotrimeric G proteins (the dominating proteins), and the small G proteins or membrane of Ras super family (the intermediate member of the signalling pathways).

The heterotrimeric G proteins consist of three different subunits- α, β and y subunits The a-subunit has GTP-binding domain; hence Gβ has a role in signal transduction.

The βy subunits transmit signals by non-covalent interaction with effector molecules. Activation of G proteins and Association of α-subunit from βy subunits are given in Fig. 27.9. GTPase activity results in after binding the Gα subunit with GDP and subsequent association with Gβy and down regulation.

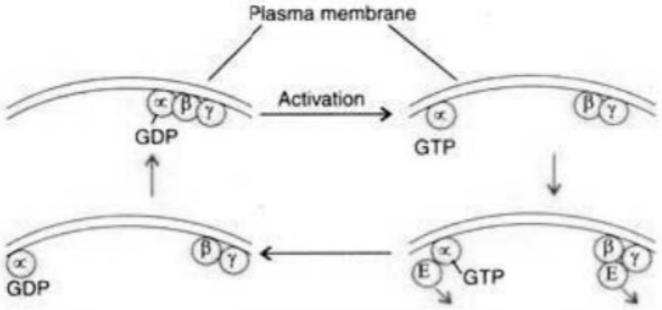


Fig. 27.9 : The function of membrane-bound heterotrimeric G proteins having α, β and γ subunits.

The small G proteins (Ras super family or p21 family) play a key role in many cellular functions such as proliferation and differentiation (Ras family), cytoskeletal organisation (Rho) and nuclear membrane transport (Ran). The activity of small G proteins is modulated after interaction with several classes of proteins. GDP-dissociation inhibitors (GDI) inhibit the loss of bound GDP and keep the G proteins in an inactive form to attenuate signalling from the activated G proteins. GTPase activity IS stimulated by GTPase-activating proteins (GAP). The removal of the bound GDP IS helped by guanine nucleotide exchange factors (GEF) which enable the GTP to bind and activate G proteins. Some of these factors have shown to be proto-oncogenes.

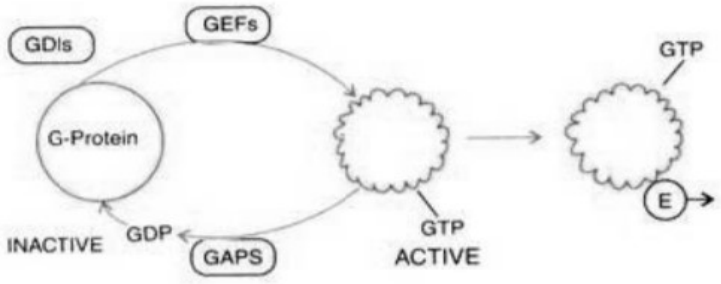


Fig. 27.10 : Functioning of small G proteins.

iv. Cycline Nucleotide-Binding Proteins:

In 1950s, cAMP was identified as the first intracellular signalling molecules. It mediates hormone action and acts as molecules transmitting the primary signal that has been received at the cell membrane). The cAMP mediates the response to chemo-attractants. The adenylate cyclase and guanylate cyclase regulate the concentration of cAMP and cGMP, respectively. The soluble bacterial adenylate cyclases produce cAMP which binds to c AMP receptor protein (CRP) and activate them. CRP is a transcription factor. The cAMP influences the expression of many of genes. Consequently bacteria become able to express metabolic enzymes which are required during growth. The cAMP also regulates the expression of the other genes which can cause pathogenesis. In eukaryotes heterotrimeric G proteins regulate the membrane-bound adenylate cyclases which produce cAMP. G proteins are coupled to transmembrane receptors. The cAMP-dependent protein kinases (protein kinase A) are the main effects of the cAMP signals. While in the inactive form, protein kinase A consists of a dimer of regulatory (A) subunits and two catalytic (C) sub- units. The molecules of cAMP binds to reach R subunits and induce conformational changes. Consequently activated C subunits are released. This activated protein kinase A phosphorylates many substrates on serine or threonine (Fig. 27.11).

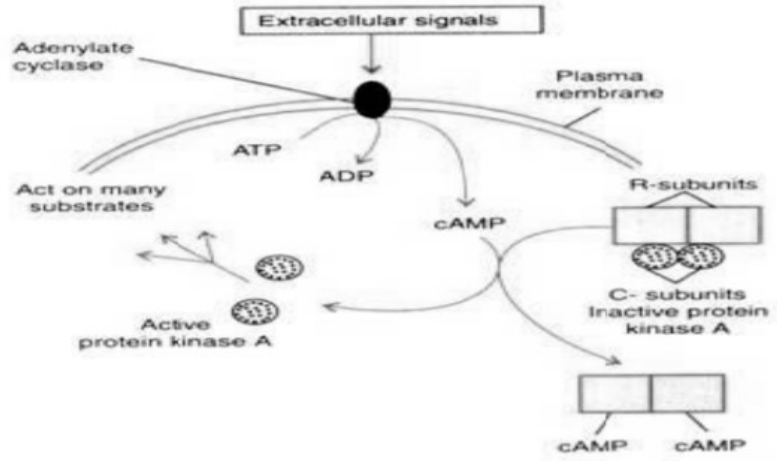


Fig. 27.11 : Production of cAMP and activation of protein kinase A.

Both the cycles work in eukaryotes by direct binding to proteins which form cation channels. Binding events result in opening of the channel. The G-protein-linked cell surface receptor generates small intracellular mediators thought cAMP pathways (Fig. 27.12).

(c) Role of Intracellular Concentration of Ca^{++} in Cell Signalling:

Calcium is found in Cytoplasm and maintained in a very low concentration (10-100 nM). But its concentration varies with cell cycle, exogenous source or release from the stores. It gets complexes in membrane bound vesicles acting as stores. A highly specific protein calmodulin (CaM) binds to Ca^{++} and transmit the signal. Ca-binding to CaM brings about changes in conformation of CaM.

Consequently CaM interacts with many effectors including CaM-modulated kinase. The most extensively studied CaM is the phosphatase calcineurin which is associated with several cellular activities such as NO synthesis, apoptosis, and induction of T lymphocytes. In eukaryotic cells Ca^{++} acts as a second messenger. Fig. 27.12 shows the two major pathways by which G-protein-linked cell surface receptors generate small intracellular mediators.

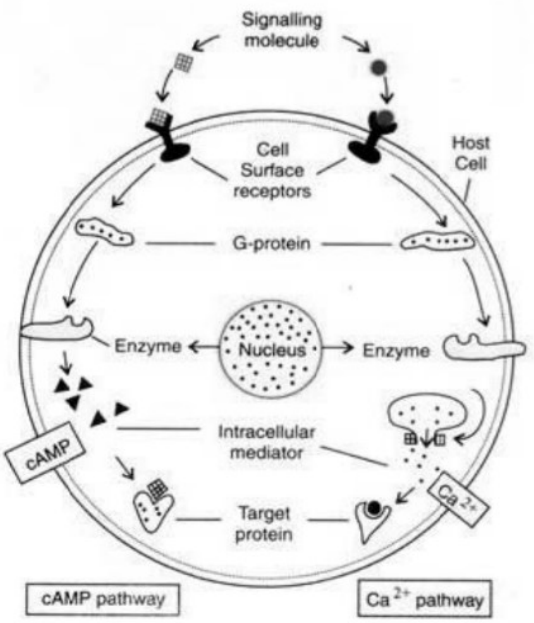


Fig. 27.12 : Generation of small intracellular mediators by G-protein-linked cell.

(d) Role of Phosphorylated Lipids in Cell Signalling:

In eukaryotes lipids are involved in signalling process. Cellular phospholipases attack the lipid moieties of the membrane to produce different types of signalling molecules. For example, phosphatidylinositol lipids play a role in cellular stimulation. They have inositol as head, the six-membered carbon ring with a -OH group on each carbon. On the basis of phosphorylation status of inositol head group, several phosphatidylinositols are found in the cells. The activity of three enzymes triggers their signalling role. These are: phosphoinositide 5′-kinase (P15p, phosphoinositide 3′-kinase (P13K), and phospholipase C (PLC). Extracellular signals regulate all these enzymes.

(e) Regulation of Transcription:
Both types of cells are able to respond to any signal by changing their gene expression. In a signalling pathway the end point acts as signal. Regulators causing changes in expression of many genes in bacteria are called 'global regulators'. In prokaryotes post-transcriptional events regulate expression of many of the transcriptional factors for example cAMP-mediated CRP-DNA interactions. In prokaryotes, phosphorylation or protein-protein interactions regulate the control of transcriptional factors and also select the other factors to the promotors. Besides, some other factors also get translocated from cytoplasm to the nucleus and regulate transcription.

(f) Role of Cell Membrane in Signalling:
Cell membrane acts as boundary of the cell through which extracellular signal has to enter. In bacteria histidine kinases act as receptor and directs signals across the membrane. Besides, there are many signal molecules which are associated with cell membrane because the end effect is membrane-associated. The components can be well organised in three-dimensional way in cell membrane. The signalling components recruit the other molecules to the membrane where they interact with other factors. For example, GTP-bound Ras activates Raf kinase to recruit Raf to the membrane where the membrane-bound kinase activates it through phosphorylation.

3. Prokaryotic Signalling Mechanisms:
Intracellular signalling is very complex like electronic circuit. Genome size of different bacteria varies and those organisms work according to genes present in them.

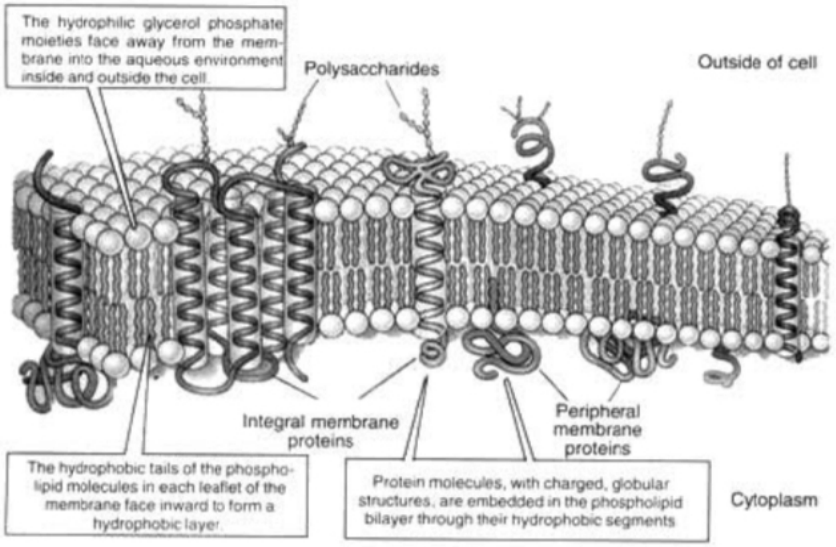
In bacteria the generic mechanism of regulation is called signalling systems which include:
(a) The histidyl-aspartae phosphorelay systems (the main module of bacteria used to receive and process incoming signals such as chemotaxis, response to osmolarity, oxygen and phosphate, and virulence system),
(b) The cAMP and CRP (involved in regulation of hundreds of genes. The cAMP is controlled at transcriptional and post-transcriptional levels. Binding of CRP- cAMP complex induces gene expression).

4. Eukaryotic Signalling Pathway:
Earlier it was thought that signalling process in eukaryotes was very complex to understand in molecular terms. Fragmented understanding about individual components could be known. The knowledge of signalling expanded with the development of new techniques such as genome sequencing, increasing number of reagents (isolated components, specific probes e.g. antibodies for individual components and selective inhibitors). In spite of all these, no pathway has been fully elucidated. The best characterised pathway is the Ras activation and MAP kinases of which several details are unclarified. They are interconnected and cannot work without reference to others.

(a) The Ras/MAP Kinase Pathway:
In this pathway the ligand occupancy leads to receptor transphosphorylation, membrane recruitment of GrB2, Sos (son-of-sevenless) where sevenless is the Drosophila (gene), Ras activation and activation of the Ras kinase, and stimulation by phosphorylation of the MAP kinase pathway resulting in various sequels in the cells.

(b) The Phospholipase C/Inositol Triphosphate Pathway:
The phospholipase C, beta or gamma is activated by membrane signalling events and cleaves PIP2 to produce diacylyglycerol (DAG) and inositol triphosphate (IP3). These activates the release of Ca++ions.

and results in activation of protein kinase C (PKC), which phosphorylates many additional protein substrates.



Bacterial cell membrane structure : Extracellular signal has to enter via cell membrane which acts as boundary of the cell.

(c) The Adenylate Cyclase, cAMP and Protein Kinase A Pathway:

Adenylate cyclase is activated at the membrane by interaction with the activated heterotrimeric G protein G_5. The cAMP is generated and binds to and activates protein kinase A (PKA), which phosphorylates many substrates.

(d) Integrin's, the Rho Family and Organisation of Cytoskeletal:

The integrins are the signalling molecules that interact with the extracellular matrix on the outside of the cell and various proteins-linked to actin on the cells interior. The proteins involved include α-actin, lalin, tensin, vinculin and pavilin.

A local adhesion is formed upon activation that includes focal adhesion kinase (FAK). The Src kinase is recruited and several proteins in the complex are activated by phosphorylation by Src and FAC. These signals lead to the Ras/Raf, Rho signalling pathway and to cytoskeletal rearrangement.

In eukaryotes, the central role of signalling pathway of a cell is to define its phenotype and function. The increasing novel knowledge about the components of signalling pathways and the types of genes which they interact are already being applied in new strategy to combat the cancer. For example, genetically engineered viruses are attempted to grow in such cells that lack functional p53 and kill these cells.

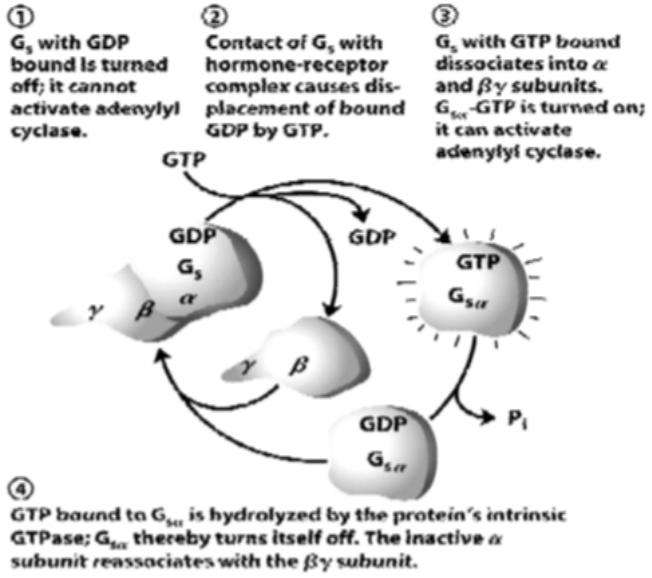
There are about 2000-5000 signal transduction proteins in mammalian cells. Bacteria have capacity to utilise eukaryotic signalling pathway during the process of infection. These findings make a line between the signalling pathways involved in infection and the other responsible for the pathology in diseases such as cancer and inflammation.

STRUCTURAL COMPONENTS OF SIGNALING:

- **G protein cycle**
- **MAP kinase cascade**
- **G-Protein mediated Signaling:**

Guanosine nucleotide-binding protein (G protein)

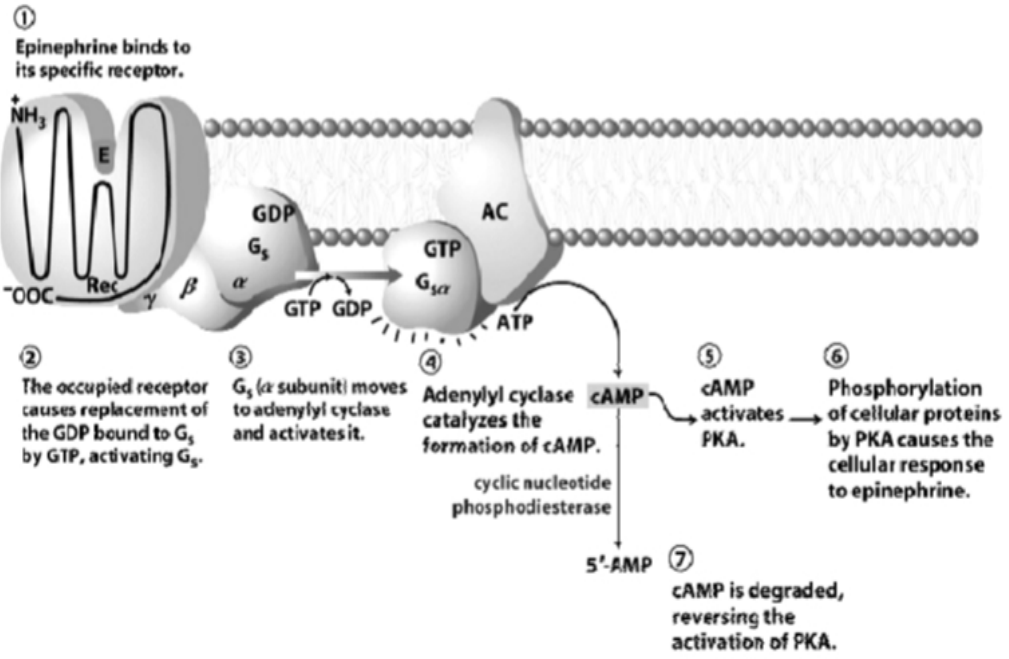
• The **G protein** is an heterotrimer, consisting of 3 different subunits (alpha, beta and gamma)
• The alpha subunit bound to GDP (guanosine di-[two] phosphate) has high affinity for the beta and gamma subunits
• When it is stimulated by the activated receptor, the alpha subunit exchanges bound GDP for GTP (guanosine tri-[three] phosphate)
• The GTP-bound alpha subunit dissociates from the beta/gamma components, and it binds to a nearby enzyme, altering its activity
• GTP is then hydrolysed to GDP (looses a phosphate group) by the intrinsic GTPase activity of the alpha subunit, that regains its affinity for the beta/gamma subunits



The G-Protein cycle

G protein Coupled Receptors

• The human genome encodes more than 1000 Gprotein Coupled Receptors (GPCR), that transduce messages as diverse as light, smells, taste, and hormones
• An example is the beta-adrenergic receptor, that mediates the effects of epinephrine on many Tissues



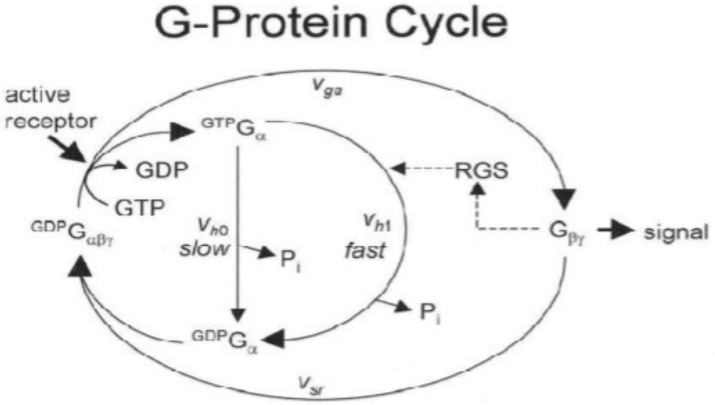


Fig. 6.6 Activation cycle of G protein. Without activation, the heterotrimeric G protein is bound to GPD. Upon activation by the activated receptor, an exchange of GDP with GTP occurs and the G protein is divided into GTP-bound Gα and the heterodimer Gβγ. Gα-bound GTP is hydrolyzed, either slowly in reaction v_{h0} or fast in reaction v_{h1}, supported by the RGS protein. GDP-bound Gα can re-associate with Gβγ (reaction v_{sr}).

Ras Protein and MAPK Pathway

The gene family ras encodes small GTPases that are involved in cellular signal transduction. Ras the super-family of proteins regulates diverse cell behaviors such as cell growth, differentiation and survival. Since Ras communicates signals from outside the cell to the nucleus, mutations in ras genes can permanently activate it and cause inappropriate transmission inside the cell even in the absence of extracellular signals. Because these signals result in cell growth and division, disregulated Ras signaling can ultimately lead to oncogenesis and cancer.

Ras proteins function as binary molecular switches that control intracellular signaling networks. Ras-regulated signal pathways control processes such as actin- cytoskeletal integrity, proliferation, differentiation, cell adhesion, apoptosis, and cell migration. Ras and Ras-related proteins are often deregulated in cancers, leading to increased invasion and metastasis, and decreased apoptosis. Activated Ras activates the protein kinase activity of RAF kinase. RAF kinase phosphorylates and activates MEK. MEK phosphorylates and activates a mitogen-activated protein kinase (MAPK).

Mitogen-activated protein (MAP) kinases are serine/threonine-specific protein kinases that respond to extracellular stimuli (mitogens, osmotic stress, heat shock and pro-inflammatory cytokines) and regulate various cellular activities, such as gene expression, mitosis, differentiation, proliferation, and

Tab. 6.1 Names of the components of MAP kinase pathways in different organisms and different pathways.

Organism	*Budding yeast*		*Xensopus oocytes*	*Human, cell cycle regulation*		
	HOG pathway	Pheromone pathway			p38 pathway	JNK pathway
MAPKKK	Ssk2/Ssk22	Ste11	Mos	Rafs (c-, A- and B-),	Tak1	MEKKs
MAPKK	Pbs2	Ste7	MEK1	MEK1/2	MKK3/6	MKK4/7
MAPK	Hog1	Fus3	p42 MAPK	ERK1/2	p38	JNK1/2

cell survival/apoptosis. MAPK pathways are activated within the protein kinase cascades called "MAPK cascade". Each one consists of three enzymes, MAP kinase, MAP kinase kinase (MKK, MEKK, or MAP2K) and MAP kinase kinase kinase (MKKK or MAP3K) that are activated in series. A MAP3K that is activated by extracellular stimuli, which phosphorylates a MAP2K on its serine and threonine residues and this MAP2K activates a MAP kinase through phosphorylation on its serine and

tyrosine residues. This MAP kinase signaling cascade has been evolutionary well-conserved from yeast to mammals. Cascades convey information to effectors, coordinates incoming information from other signaling pathways, amplify signals, and allow for a variety of response patterns. Down-regulation of MAP kinase pathways may occur through dephosphorylation by serine/threonine phosphatases, tyrosine phosphatases, or dual-specificity phosphatases and through feedback inhibitory mechanisms that involve the phosphorylation of upstream kinases. Drugs that selectively down-regulate MAP kinase cascades could prove to be valuable as therapeutic agents in the control of malignant disease. Mitogen-activated protein kinases (MAPKs)

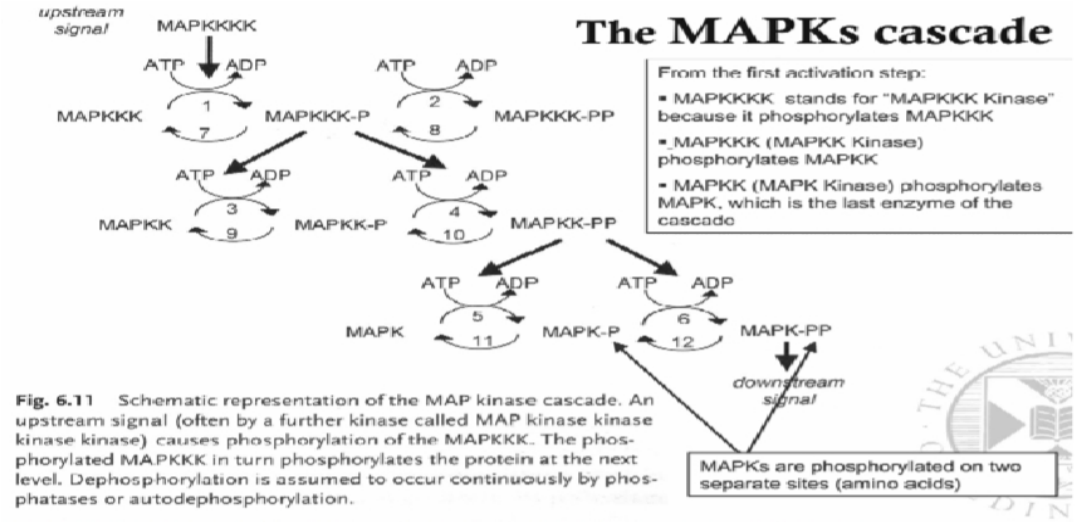


Fig. 6.11 Schematic representation of the MAP kinase cascade. An upstream signal (often by a further kinase called MAP kinase kinase kinase kinase) causes phosphorylation of the MAPKKK. The phosphorylated MAPKKK in turn phosphorylates the protein at the next level. Dephosphorylation is assumed to occur continuously by phosphatases or autodephosphorylation.

•MAPKs belong to a family of serine/threonine kinases (kinases add phosphate groups to/phosphorylate other proteins)

•They participate in cell growth, differentiation, transformation, apoptosis (controlled cell death) and others

•They are conserved from yeast to mammal, but their names differ in different species:

Single Transduction Pathways in Plants:

Plant cells, due to their sessile nature, are able to interact with its surrounding environment. Plants use various environmental signals to alter their mode of developmental morphology. Throughout their life cycle, plant and plant cells respond to both internal and external signals, such as nutrients, organic metabolites, water availability, light, temperature, germination, growth and flowering. Sometimes plants respond to harsh environmental stresses at cellular and molecular level, as well as at physiological levels to confer tolerance of the stress and ensure better survivality. The genome sequences of Arabidopsis and rice have now been determined and have revealed the presence of complex gene families that encode signalling molecules and transcription factors (TFs). There are as many as 1800 genes that encode transcription factor, more than 600 genes that encode protein kinases and major junk of 600 genes that encode F-box proteins particularly in Arabidopsis genome. The participation and stability of signalling factors and TFs is indispensable for the regulation of signal pathways. In addition, post transcriptional regulation at RNA level also leads to various other signalling pathways (Fig. 4.1).

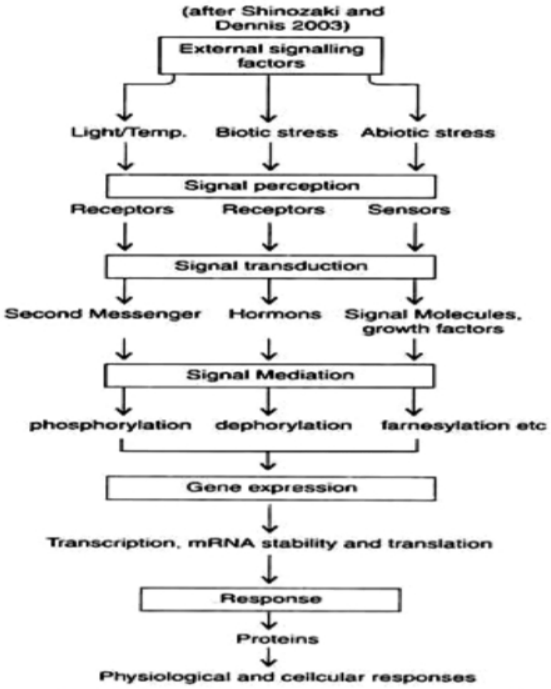


Fig. 4.1 Signal transduction pathways in plants

Mechanism of Signal Transduction:

Generally, signal transduction is initiated by sensing of signal by a receptor. These receptors are either located in the plasma membrane or in the cytoplasm or restricted to cellular compartments. The receptors happen to be a protein. The plasma membrane by virtue of its membrane potential can act as receptor by employing proteinaceous pores, called channels, to control in and out flux of ions through the cell. As a consequence of alteration in membrane potential opens a group of voltage gated channel that allow Ca^{2+} to enter and initiate transduction sequence. Several signals such as light wavelength (red < blue), fungal elicitors or growth regulators can modify membrane potential. Several unique receptors have been characterized in the cells. ome transmembrane protein receptors are phosphorylated by protein kinases. In plants, receptor-like protein consists of a large extra cytoplasmic domain with active site of a protein kinase involves in signal transduction process. Binding of ligand leading to dimerization of the receptor and brings protein domains into very close proximity of the cytoplasm. This receptor complex is then activated by phosphorylation. The active RLK complex interacts with membrane bound or soluble transduction proteins to initiate the signal transduction in a different direction. Several RLKs have been characterised in plant cells including protein kinases which are implicated in incompatibility process and precludes fertilization.

Calcium Signalling in Plants:

The Ca^4 ion is being established as a signalling molecule in plants. Several plant signal transduction processes have been shown to employ Ca^{2+} as an integral signalling molecule. In plants, Ca^{+} ion acts as second messenger, a term often used to describe readily diffusible molecules, conveys information from outside to the largest enzymes within the cell.

The cytosolic level of Ca^{2+} plays a significant role in understanding signalling. Several Ca^{2+} signalling mediated responses have been observed in plants. In plants, Ca^{2+} ions in cytosol are maintained at many orders of magnitude lower than in the cell wall. During signalling, elevation of Ca^{2+} level takes place which is associated with initiation of responses. The calcium act on several proteins involved in signalling of which protein kinasesis prominent. Among Ca^{2+} mediated signal responses, prominent ones are initiation of Stomatal aperture closure in guard cell, direction of growth in tubes and wall thickening in seedling in response to wind.

In stomatal guard cell, abscisic acid induces elevation in $[Ca^{2+}]$ and cytochromes were found to be unevenly distributed. The Ca^{2+}signalling in plants is in the form of a Ca^{2+} waves. Increase in Ca^{2+}elevation after exposure to hypo-osmotic stress leads to clustering of Ca^{2+} and is thaliana guard cells showed that SIP modules guard cell turgor by affecting the activities of the plasma membrane K^+ channels and slow anion channels. Sphingosine-1-phosphate mediated changes in guard cell turgor can be transduced via the second messenger Ca^{2+} and through a protein a subunit (GPA1) (Fig. 4.5).

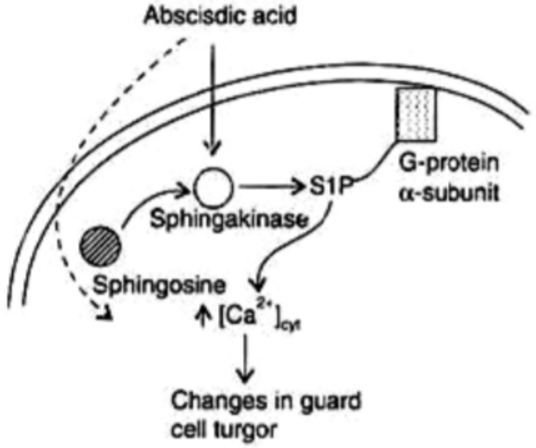


Fig. 4.5 Sphingolipid-mediated signalling in plants

Other Plant Signaling Molecules:

Jasmonate:

Jasmonates are biologically, active signalling molecules controlling metabolic, development and defence response in plants. Jasmonic acid (JA) is synthesised at its terminal end product in octadocanoid pathways, and several intermediates in this pathway for JA synthesis, also act as signalling molecule which affect a variety of plant processes including fruit ripening, production of viable pollen, root growth and biotic as well as abiotic stress, particularly in defence response against insect and pathogen attack.

In addition to JA, its precursor 12- oxophytodienoic acid (OPDA) and other oxylipins act as signal molecule for defence suggests that plant response to pathogen attack may be regulated by a complex mix of signals, otherwise termed as oxylipin signature.

The production of JA leads to the induction of many genes such as vegetative storage protein and a plant defence in. It also facilitates transcription of genes that regulate JA synthesis. Microarray analysis confirmed that at least five out of 41 Jasmonate responsive genes are involved in JA biosynthesis.

In tomato plant, systemic induction of JA response occurs through system in signal pathway. System in, an 18-aminoacid polypeptide acts as primary signal for the activation of defence genes in leaves of wounded tomato plants. System in causes a cascade of intracellular signalling events leading to the release of linolenic acid from plasma membrane. Conversion of linolenic acid into oxylipin signals the expression of defense genes.

Plant Hormone Signaling:

1. Cytokinin Signalling:

Cytokininis have been registered as one of the key plant hormones involved in differential development and other physiological process in plants, such as cell division, root and shoot growth, chlorophyll development, leaf senescence and including biotic and abiotic stress response.

Comprehensive progress has been made in analysing cytokinin perception and signalling. Several painful efforts resulted in the identification of three cytokinin receptor proteins such as GK11, a receptor histidine kinase, CRE_1/AHK_4 histidine kinase and AHK_2/AHK_3 histidine kinase receptor in Arabidopsis due to the availability of its genome sequence.

In addition cytokinis down-stream transmitters like AHPs (Arabidopsis Histidine Phosphotransfer protein) and ARR (Arabidopsis Responsive Regulator) have been characterized. Expression of cytokinin receptors takes place mainly in the roots whereas remaining two is expressed in all other major organs.

Three cytokinin receptors contain two to three trans membrane domains at the N-tranasminal part, transmitter (histidin Kinase) and two receiver domains. The extracellular ligand-binding regions of 210 amino acid long, have drawn special attention as they are the main recognition sites for cytokinins.

These domains are present in variety of functionally diverse membrane receptor proteins that recognise cytokinin like adenine derivations or peptide ligands. This domain has been coined as the CHASE domain (cyclases/histidine-kinase-associated sensory extracellular domain). This domain is specific for AHK_2, AHK_3 and CRE_1/AHK_4 receptors in Arabidopsis.

Cytokinin signal-transduction pathway consists of Arabidopsis five histidine phospho-transmitters (AHPs) and 22 responsive regulators (ARRs). Five AHPs genes encodes proteins of 12 kDa, transmit the signal from the receptor, which is localised in the plasma membrane to ARRs, which are probably present in the nucleus. The ARRs are divided into two major classes, such as A and B-type.

There are 22 predicted responsive regulator (ARR) genes. Presence of receiver domain is the characteristic feature of type A-ARR genes. By contrast, the type B-ARR contains a C-terminal output domain in addition to the receiver domain. Transcription of A- type ARR increases rapidly within 10 min in response to cytokinin. When cytokinin binds to the receptor, it undergoes dimerization and autophosphorylation. Following activation of receptor complex, phosphoryl group is transferred to AHPs which transport the signal from the cytoplasm to type B ARRs in the nucleus.

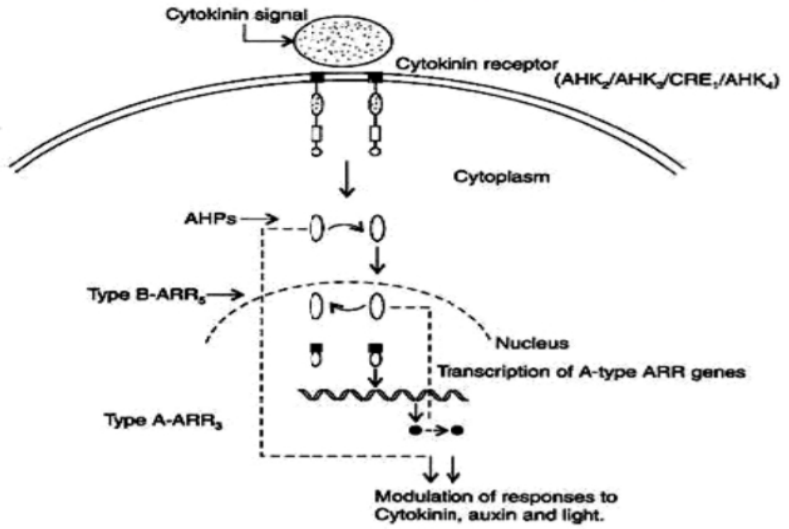


Fig. 4.2 Hypothetical depiction for cytokinin signal transduction. (Reproduced with permission from Elsevier. Copyright 2003. Current Opinion in Plant Biology.)

Following transmission of signal inside the nucleus type-B response regulators transcribe target genes, such as type-A ARR genes. In this signal process, type-A response regulations may down regulate the primary cytokinin signal response via a negative feedback process (Fig. 4.2).

2. Auxin Signalling:

Indole 3 acetic acid (IAA) is a naturally occurring auxin known to involve in cell division elongation or expansion, besides participation in other morphogenesis. During past several decades intensive research have been done in the identification of receptors for the auxin signal. The possible auxin receptor known as auxin-binding protein (ABP_1), has recently been characterized.

In addition, several other auxin binding proteins such as glutathione S-transferase, β-glucanase, and a cytokinin glucosidase are known to express in stressed plants. ABP_1 is a small family of 23 kDa protein that bind to auxins like Indole-3-acetic acid (IAA) and napthalene-1-acetic acid (NAA). ABP_1 contains a C-terminal HDEL endoplasmic reticulum retention domain.

Over expression of ABP_1 in transgenic tobacco evidenced its role of controlling cell expansion. Recent studies have established that AtP_1N_1, a 67 kDa protein is a trans membrane component involved in polar auxin transport. Similarly another member of the auxin efflux carrier family AtP_1N_2 is probably involved in controlling movement of auxin in elongation zone.

3. Gibberellin Signalling:

Gibberellins (GA) are associated with plant growth, seed germination, stem elongation, flowering and fruit development. Besides, it is also involved in the regulation of gene expression in the cereal aleurone layer.

Recent findings on the GA signalling pathways shows GAMYB is a GA-regulated MYB transcription factor, which is involved in the activation of a-amylase expression in barley aleurone cells and anther development. In addition, GAMYB (GA regulated MYB transcription factor) interacts with KGM (KINASE-associated with GAMYB). KGM is a member of protein kinase sub group, represses GAMYB functions in barley aleurone.

Recent studies on GA signalling shows that PHORI (photoperiod-Responsive 1) acts as positive regulator in GA signalling particularly in the expression of ent-Kaurene oxidase gene. When GA binds to an unidentified GA receptor (s), activates G proteins (D_1) that enhance the GA signal.

Enhanced GA signal then mediates PHORI transport into the nucleus, where it acts as a positive regulation by GA signalling. Meanwhile nucleus located protein kinases and GID_1 (GA insensitive dwarf 1) are also activated by GA signal, which in turn trigger GID_2/SLY_1-mediated degradation of DELLA proteins (SLR/RGA). These DELLA proteins directly or indirectly inhibit the expression of

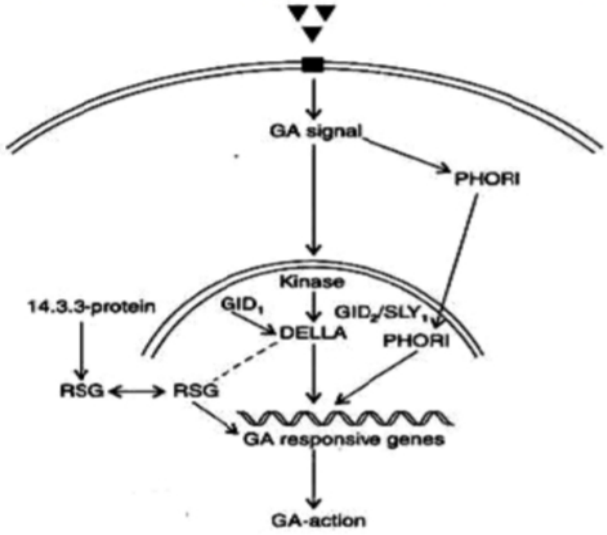


Fig. 4.3 GA signalling shows positive and negative regulation of GA responsive Genes. (After Gomi and Matsuoka, 2003 with permission from Elsevier Copyright 2003.)

GA-induced genes in the absence of GA in cells. Therefore in presence of GA, PHORI regulates expression of GA-responsive genes. Similarly 14.3.3 proteins regulates the sub-cellular localisation of RSG (Repression of Shoot Growth), which in turn controls the expression of the GA-response genes and GA mediated action take
lecules are known to be involved in signalling pathway. These specialised photoreceptors are involved in bundantly synthesised in the dark as inactive form and are degraded upon exposure to light.
Phytochrome undergoes inter convertable form from inactive red (R) light absorbing Pfr form into active far red (FR) absorbing Pfr form after absorption of a light photon. Phytochrome in this active status in turn can be transformed back into the Pr form after absorption of FR. There are five distinct phytochromes in Arabidopsis designated phy A to phy E and play a key role in different photomorphog PIF_3(Phytochrome Interacting Factor). In this process, phy B can bind specifically to PIF_3 that is already bound to light responsive G-box DNA sequence site (CACGTG) and facilitates transcriptional activation of specific gene (Fig. 4.4).

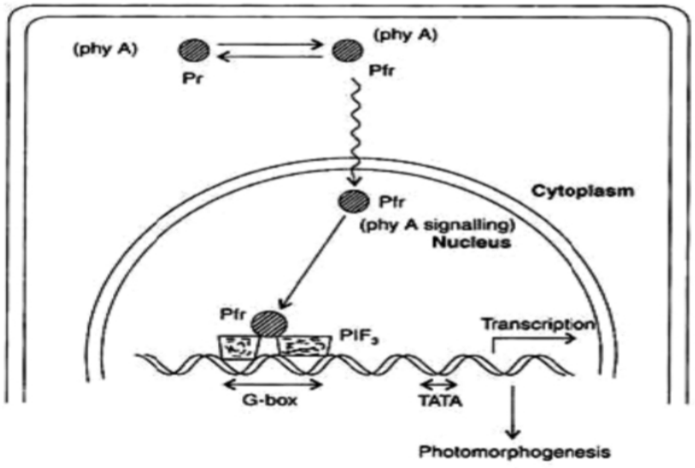


Fig. 4.4 Phytochrome signalling and control of gene expression. (After Wang and Deng, 2003. With permission from Elsevier Copyright 2003. Trends in Plant Science.)

5.<u>Sensory photobiology: Light control of plant development; phytochrome: properties, phytochromeinduced response, phytochrome signaling pathways, blue light responses.</u>

Growth and development of plants are influenced by several environmental factors including light. However, light causes several responses other than photosynthesis. These responses greatly influence the course of plant development and the final plant appearance. They are photomorphogenetic responses. For example, the seeds of many plants do not germinate unless they are exposed to light. Besides photosynthesis in which light is harvested by green plants and is converted- into chemical energy, there are numerous other plant responses to light such as phototropism, germination of some light sensitive seeds e.g. lettuce, de-etiolation of monocot and dicot seedlings etc., which are quite independent of photosynthesis and in which light just acts as environmental signal to bring about the particular photo response.

Most of these photo-responses control genetically defined structural development or morphogenesis (i.e., origin of form) of plants. The role of light in regulating morphogenesis is known as photo-morphogenesis. In plants, red and blue light are especially effective in inducing a photo-morphogenetic response. The effect of light in controlling morphogenesis can best be demonstrated by comparing a monocot (maize) or dicot (bean) seedling grown in light with one grown in darkness both of which have been reared from genetically identical seeds. It can easily be noticed that dark grown seedling has become etiolated (i.e., pale and weak) while the one grown in light has stockier and green appearance with short stem and large leaf area (Fig. 25.1). Since both etiolated and light grown seedlings were reared from genetically identical seeds, light must have altered the gene expression during germination so that the appearance or form of etiolated and light grown seedlings looks different.

De-etiolation of light grown seedling can be done in very short period (hours) by placing it even in dim light. During de-etiolation, marked reduction in the rate of stem elongation, straightening of apical hook and development of green pigments can easily be noticed. The etiolated form of the seedling is thus gradually transformed to stockier green appearance and is the result of photo-morphogenesis. The development of seedling in darkness is called as skoto-morphogenesis (from Greek word Skotos = darkness).

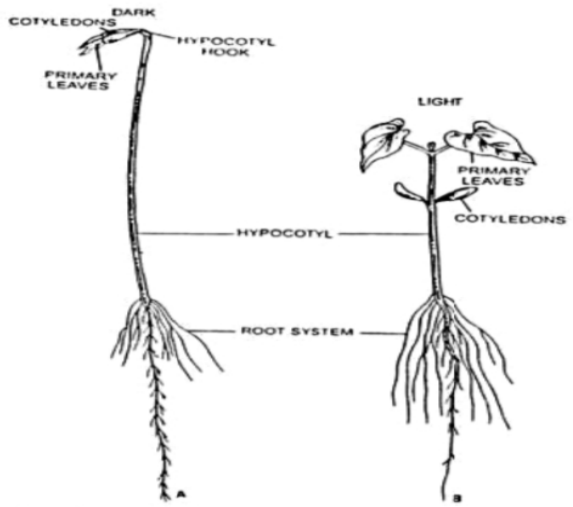


Fig. 25.1. Effect of light on seedling development in bush bean (*Phaseolus vulgaris*)

According to Hans Mohr (1983), there are two important stages of photo-morphogenesis:
(i) Pattern specification, in which cells and tissues develop specific ability or competence to respond to light during certain developmental stage and
(ii) Pattern realization, during which time the photo-response occurs

Photoperiodism:
The discovery of photoperiodism, i.e., the growth response to the length of light and dark periods by Garner and Allard, completely changed the concept of flower initiation. It was demonstrated by experiments that the length of the dark period rather than the light period is the critical factor in the photoperiod response.

As per the result of investigations, It became apparent that a majority of plants fell into one of three categories:

(i) **Short day** (ii) **Long day** (iii) **Day-neutral plants**.

Short-day plants require a dark period exceeding some critical length to flower, and cannot flower under continuous illumination (light). Long-day plants are inhibited from flowering when the dark period exceeds some critical length, and they can flower under continuous illumination.

Day-neutral plants can flower under any night length. In some plants, the leaves need only to be exposed to one light-dark cycle of the proper day length to cause flower initiation, whereas most plants require several or many such cycles. It has been shown that photoperiodic responses may be altered by brief exposure to low light intensity. For example, interruption of the dark period in short-day plants has shown that it is red light (wave length 660 mμ) which is effective. Similarly it has been proved experimentally that, far-red light (wave length 730 mμ) reverses the red light effect, i.e., if exposure to red light is followed by exposure to far-red, the result is as if there had been no exposure at all. If repeated alternating exposures to red and far-red are given, the final exposure determines the response.

Light and Plant Development

Light provides the signal for photomorphogenesis.

Plants detect parts of the light spectrum that are relevant for photosynthesis.

Classes of major plant photoreceptors:

1) Phytochromes: detect red light
2) Cryptochromes: detect blue light
3) Phototropins: detect blue light

Phytochromes

Discovery of Phytochrome:

Phytochrome is a blue protein pigment responsible for the perception of light in photo-physiological processes. It is possibly the only photoreceptor in photoperiodism and the flowering process. The discovery of phytochrome is closely associated with studies on flowering. However, many other light controlled plant responses other than photosynthesis, collectively called photo-morphogenesis, are the effects of phytochrome action. In 1932, Beltsville research group of the USDA headed by Borthwick and Hendricks showed that red light (630 to 680 nm) elicits the germination of lettuce seeds, whereas far-red light (710 to 740 nm) inhibits the process.

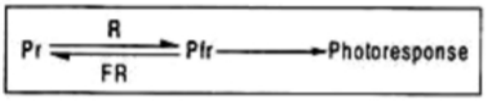
The phytochrome involvement in the flowering process was envisioned when in 1952. Borthwick. Hendricks and Parker demonstrated that red light inhibition of flowering in Xanthium could be reversed by a subsequent far-red light treatment. The action spectra for inhibition and promotion of flowering shows that the red light near 660 nm and far-red light near 730 nm respectively, are maximally effective.

From these observations, Borthwick and his associates concluded that phytochrome, the photoreceptor, exists as two inter-convertible forms, one absorbing red radiation (Pr or P_{660}), the other with an absorption maximum in the far-red region of the spectrum (Pfr or P_{730}).

The Pr form of pigment is converted by red light (R) to the Pfr form, conversely, far-red light (PR) changes the Pfr form of pigment to Pr. The effect of natural white light is like red light (R). Pfr form

of phytochrome, produced by exposure to R, is thought to be the physiologically active form since relatively small amount of Pfr brings about a response.

A simple model of phytochrome action can be represented as follows:



In 1959, Butler and his associates first extracted phytochrome from etiolated oat coleoptiles. It occurs as a chromo-protein in which the chromophore is a linear tetrapyrrole similar to C-phycocyanin.

Phytochrome is widely distributed in the plant kingdom. Although green leaves are the organs, which perceive day light most effectively, their phytochromecontent is very low. For this reason, most studies with phytochrome have used etiolated seedlings from which phytochrome has been obtained in highly purified form. The absorption spectra of the two forms of phytochrome, i.e., P660 and P730 overlap considerably (Fig. 14.1). The overlap is the reason why total photochemical conversion is not possible when irradiated with either red or far-red.

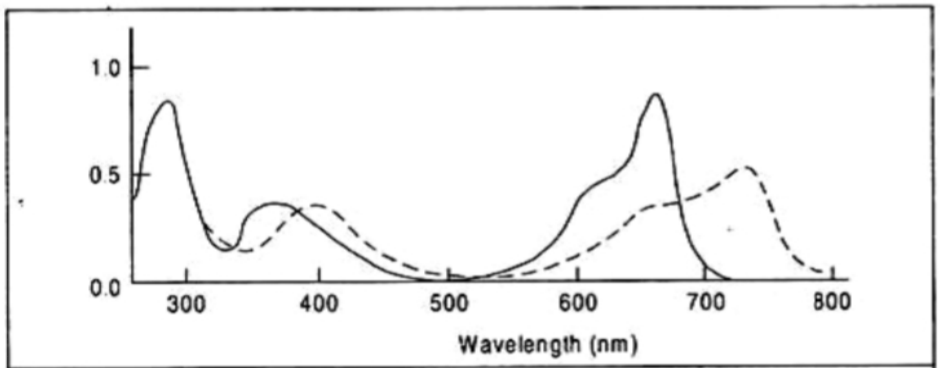
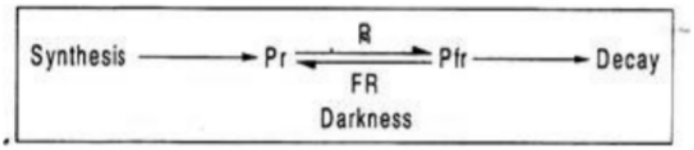


Fig. 14.1 : Absorption spectra of Pr (—) and Pfr (---) froms of isolated phytochrome

Besides photochemical conversions, non-photochemical reactions also occur in vivo. Thus, Pfr may undergo dark reversion to Pr. Since natural white light acts like R, phytochrome will remain mainly in the Pfr form at the end of the day. It has been observed that after several hours of .darkness, plants become sensitive to R indicating that Pfr is present in a large amount. Thus, it is inferred that Pfr is

converted spontaneously to Pr in darkness. Phytochrome decay or destruction is also a dominant irreversible process in a seedling, which is the thermo-chemical transformation of Pfr to an inactive form.

Thus, the model of phytochrome action including synthesis, dark reversion and decay can be presented as:



Structure and Biosynthesis of Phytochrome:

Phytochrome is a soluble chromo-protein with a molecular mass of 250 kDa it occurs as a dimer made up of two subunits, each of 125 kDa. Each subunit consists of two components - light- absorbing pigment molecule, chromophore and a polypeptide chain, Apo protein. The Apo protein monomer has a molecular mass of 125 kDa. Apo protein and chromophore together make up the holochrome. The chromophore is a linear tetrapyrrole similar to phycocyanin termed phytochromobilin and it is a ring attached to the protein through thioether-linkage to a cysteine residue.

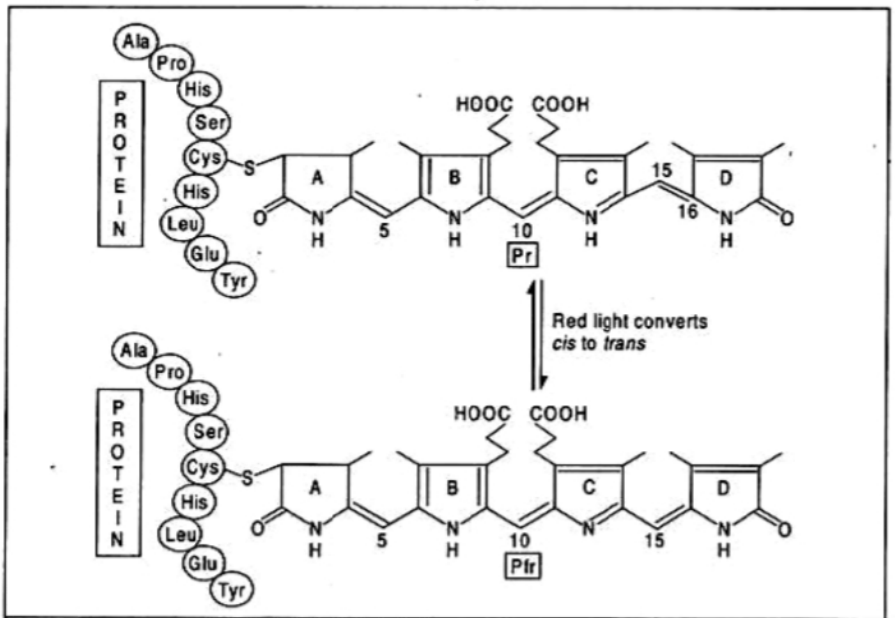


Fig. 14.2 : Structure of the phytochrome chromophore and its binding to the apoprotein. The chromophore is covalently linked to the protein at cysteine residue via a thioether bond. The chromophore undergoes *cis-trans* isomerization at carbon 15 in response to red and far-red light

The principal difference between the Pr chromophore and the Pfr chromophore appears to be a cistrans isomerization of the methane-bridge between rings C and D. The absorption of red light provides the energy required to overcome a high activation energy for rotation around the double bond. There is further evidence that the protein also undergoes photo chemically induced conformational changes.

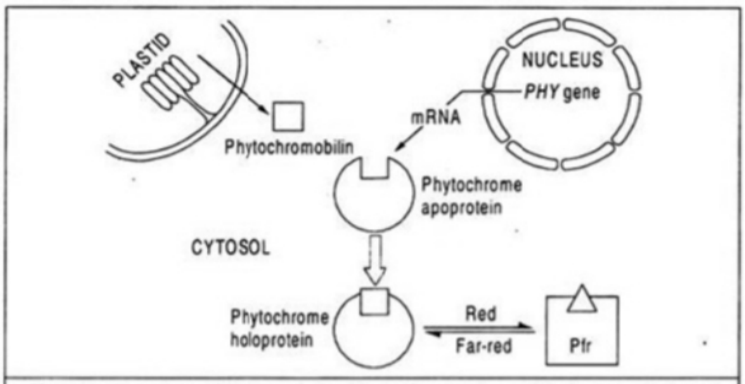


Fig. 14.3 : Phytochromobilin is synthesized in plastid and released into the cytosol, where it assembles with apoprotein to form phytochrome holoprotein. Both chromophore and protein undergo conformational changes (After Kendrick *et. al.*, 1997)

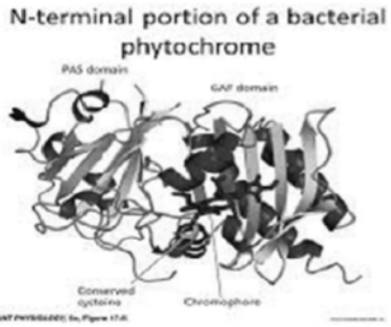
Phytochromobilin is Synthesized in Plastids:

Phytochrome Apo protein alone cannot absorb red or far-red light. Light can be absorbed only when the polypeptide is covalently linked with phytochromobilin to form the holoprotein. Phytochromobilin is synthesized within plastids. After synthesis, it leaks out of the plastid into the cytosol. Assembly of Apo protein with chromophore is autocatalytic, that is, it occurs spontaneously when purified Apo protein is mixed with purified chromophore in test tube, for which no cofactors are necessary. Assembly in vivo of these two components is also autocatalytic (Fig. 14.3).

(a) Phytochrome is encoded by a Multi-gene Family:

Complementary DNA (cDNA) copies of mRNAs were isolated from oat and zucchini (*Cucurbita pepo*) seedlings. Using these clones as probe, five structurally related phytochrome genes were identified in Arabidopsis. This gene family is known as PHY, and its 5 individual members are PHYA, PHYB, PHYC, PHYD and PHYE. The Apo protein without chromophore is also called PHY, and the holoprotein with chromophore is called phy. Phytochrome sequences from other higher plants are named according to their homology with Arabidopsis PHY genes.

- The phytochrome apoprotein alone cannot absorb red or far-red light
- Light can be absorbed only when the polypeptide is covalently linked with phytochromobilin to form the holoprotein.



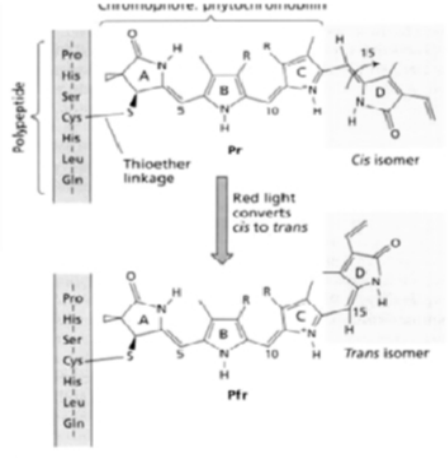
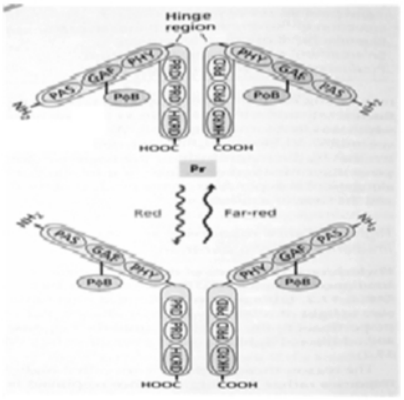


Figure: Structure of Pr and Pfr forms of chromophore (Phytochromobilin) and the peptide region bound to the chromophore through a thioether linkage. The chromophore undergoes a cis-trans isomerization at carbon 15 in response to red and far-red light

Phytochrome structure and function



- Phytochrome functions as a dimer. Each subunit consist of a light-absorbing chromophore covalently linked to a polypeptide chain, the apoprotein. Only the holoprotein can absorb red and far-red light.

There are several conserved domains within the phytochromes:

- **PAS:**(N-terminal domain) The term PAS comes from the first letter of each of the three founding member proteins of the family-PER,ARNT and SIM
- **GAF** (contains bilin-lyase domain): is necessary for autocatalytic assembly of the chromophore with the peptide
- **PHY:** stabilizes phytochrome in the Pfr form.
- A hinge region separates the N-terminal and C-terminal halves and plays a critical role in conversion of PR to Pfr
- **PRD** (PAS-related domain): these domains mediate phytochrome dimerisation.
- **HKRD** (HIS kinase-related domain): This C-terminal domain is essential for autophosphorylation .
- PφB, phytochromobilin.

(b) Phytochrome Controlled Responses:

Phytochrome is the photoreceptor involved in many developmental responses of plants to light. It is involved as a light detector and also in the measurement of light duration. The regulatory effects of light on plant growth and development are visualized most prominently at two stages in the life cycle of the plant — firstly, at the stage of seed germination and seedling development, and secondly, at the stage of transition from the vegetative to the flowering phase.

Type I: Fast Responses:

The type I responses include those processes in which the quantum energy absorbed by the plant is transduced to another form of energy. Examples of this type of essentially energy-transducing responses include leaf movement of Mimosa and chloroplast movement in Mougeotia, Other

examples are surface potential changes, membrane potential changes and ion fluxes. These phenomena are relatively rapid, occurring on a time scale of seconds and minutes.

Type II: Slow Responses:

The rates and activation of certain aspects of growth and development are switched on or modulated under the influence of the quality of light (red or far-red). Examples of type II responses include stem elongation, seed germination, hook opening, leaf expansion, flower initiation and pigment biosynthesis.

Type II responses are relatively slow responses occurring on a time scale of hours and days. The phytochrome molecule is thought to act as a photo chrome sensor that controls the photo-morphogenetic machinery of plants.

(c) Variation in Lag Time, Escape Time and Light Quanta for Phytochrome Responses:

Morphological responses to the photo-activation of phytochrome may be visually observed after a lag time ranging from a few minutes (chloroplast rotation in green alga Mougeotia) to as long as a few weeks (flower initiation). It has further been established that red-light induced effects are reversible by far-red light only for a limited period of time after which the response escapes from the photo-reversible control.

Not only the lag and escape times differ in diverse phytochrome responses, but different amounts of light (fluence) are required to induce them. The amount of light is termed **"fluence"** which is defined as the number of photons per unit surface area. Each phytochrome response is characterized by a specific range of light fluences over which the magnitude of response is proportional to the fluence.

These responses can be categorized into three major groups based on their sensitivities to fluence, viz., (a) very low fluence response (VLFR), (b) low fluence response (LFR) and (c) high irradiation response.

(i) Very Low Fluence Responses (VLFRs):

Examples can be provided by Arabidopsis seeds, which can germinate with very low red light. The reciprocal relationship between fluence and time, known as the Bunsen-Roscoe law of reciprocity is valid in case of VLFRs, which, however, fail to show reversal control by light.

This means that a response can be induced either by brief pulse of red light that is quite bright or by a very dim light for a longer duration. Another point of interest is that far-red cannot reverse VLFRs. The reason is that about 3% of the total phytochrome remaining after far-red exposure is sufficient to induce VLFRs.

(ii) Low Fluence Responses (LFRs):

They exhibit characteristic induction with red light and reversion with far-red light. The law of reciprocity, i.e., light-induced response is a function of total fluence (fluence rate x irradiation time) and independent of the fluence rate or irradiation time holds for LFRs. Such responses include classic red/far-red photo-reversible responses, such as promotion of lettuce seed germination and leaf movement.

(iii) High Irradiation Responses (HIRs):

Some photo-morphogenetic responses require prolonged or continuous exposure to light of high irradiance and are proportional to irradiance, but the reciprocity law is not followed here.

Examples of HIRs are:

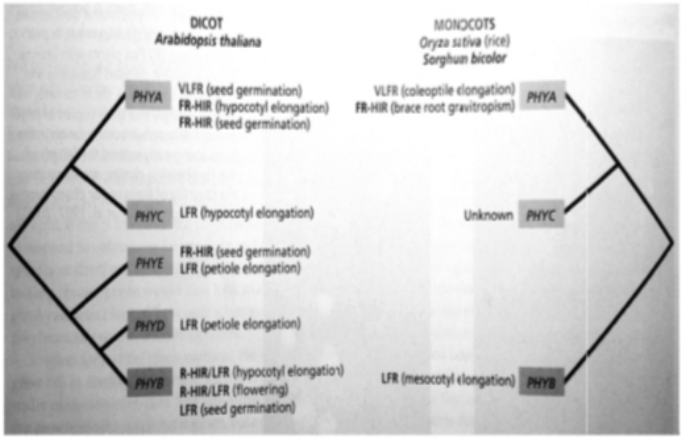
(i) Anthocyanin synthesis in dicot seedlings and apple skin

(ii) Inhibition of seedling elongation (hypocotyl)

(iii) Flower induction
(iv) Plumular hook opening
(v) Cotyledon expansion in mustard
(vi) Ethylene production to Sorghum
(vii) De-etiolation of seedlings. However, the effect is not photo-reversible. The reason that these responses are called high irradiance responses (HIR) rather than high fluence responses (HFR) is that they are proportional to irradiance (i.e., brightness of source) rather than to fluence.

Genetic Analyis of Phytochrome Function

- **Phytochrome A mediates responces to continuous far-red light but also mediated red-light responses. It also controls flowering in Arabidopsis and rice**
- **Phytochrome B mediates responses to continuous red or white light, and regulates responses to shade such as accelerated flowering and increased elongation.**
- **Phytochromes C, D, and E also have specific developmental roles that may be partially redundant with those of phytochrome A and B.**
- **The signaling pathway components downstream from phytochromes vary and can lead to different responses in different organs or taxa.**



Mode of Action in Phytochrome:
Pfr is regarded as the physiologically active form of phytochrome. Conversion of Pr to Pfr by light will produce a particular response depending on the localization of phytochromeand the state of differentiation of the responding cells. It is also possible that the photo stationary state ratio Pfr/P_{total} acts as a signal perceived by the plant under certain conditions. For example, the HIR response for the inhibition of hypocotyl growth in lettuce can be explained in term of the ratio $Pfr/P_{total} = 0.03$, i.e., 3% Pfr level is necessary for the HIR response. First step is the absorption of light by the pigment. Then the absorbed light alters the molecular properties of phytochrome, which induces a sequence of cellular events ultimately leading to a change in growth, development or position of an organ. Generally, two consistent lines of evidences are sought to explain the effects of phytochrome. One is concerned with the Pfr effect on changes in the properties of cellular membranes. The second theme is that Pfr regulates gene expression.

Cryptochromes

Apart from phytochrome mediated photo-responses, large number of photo-responses in plants are known which are controlled by blue light and are believed to be mediated through a group of yet unidentified pigments called crypto chrome (crypto from cryptogams), the latter acting as photoreceptor in such responses. Blue light responses have been reported in algae, fungi, ferns and higher plants.

Some of the typical and most commonly known blue-light responses in plants are:

(i) Phototropism
(ii) Stomatal opening
(iii) Inhibition of hypocotyl elongation
(iv) Sun tracking by leaves
(v) Phototaxis
(vi) Movements of chloroplasts within the cells and
(vii) Stimulation of synthesis of carotenoids and chlorophylls etc.

Crypto chrome absorbs light rays mostly in violet-blue region of the spectrum (400 – 500 nm). It also absorbs long wave ultraviolet rays in UV-A region (320 to 400 nm). However, most photo-responses of plants caused by crypto chrome result from absorption in violet-blue region of the spectrum but they are simply called as blue-light responses. Although phytochrome and some other photoreceptors also absorb blue light, but the typical blue-light morphogenetic responses differ from photo-responses mediated by them in being insensitive to red light and there is no red/far- red reversibility.

i. The action spectra of many blue-light responses in higher plants such as phototropism, stomatal movement, inhibition of hypocotyl elongation etc. are similar and characteristic. They show three peaks in blue region (400 – 500 nm) of the spectrum of visible light. This three peaked, action spectrum is also known as three fingers action spectrum (because of its resemblance in shape with three fingers) and is typical of most blue light responses (Fig. 25.3). Three fingers action spectrum is not observed in phytochrome mediated photo-responses or photo-responses mediated by other photoreceptors other than crypto-chrome.

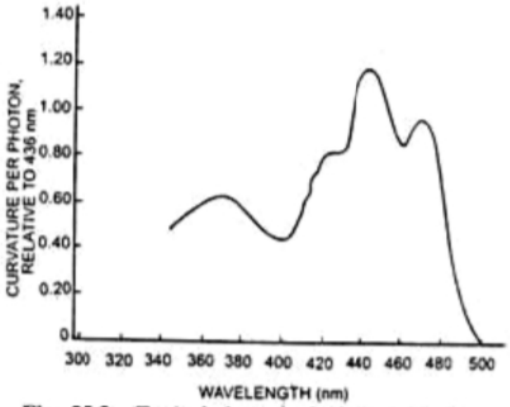


Fig. 25.3. Typical three peaked or three fingers action spectrum for blue light stimulated phototropism in ***Avena*** coleoptile. (After Thimann and Curry, 1960).

ii. Scientists have implicated roles of yellow pigment carotenoids or flavins as photoreceptors in blue-light responses of plants for a long time. However, the spectroscopy of blue-light responses is complex and it is not easy to distinguish between these two types of pigments by comparing available action and absorption spectra.

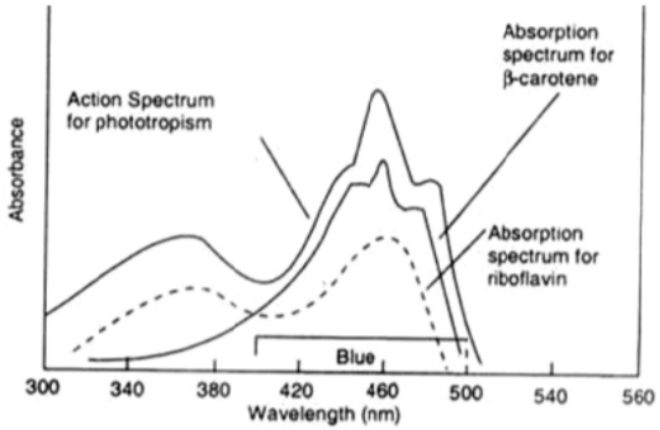


Fig. 25.4. Action spectrum for phototropism compared with the absorption spectra of riboflavin and carotene.

Schmidt (1984) has summarised arguments in favour of flavins or carotenoids as photoreceptor pigments in blue light responses of plants as follows:

(a) Arguments in Favour of Flavins:

(i) Action spectra show UV maximum between 350-400 nm.

(ii) Primary steps of the blue light response are dependent on presence of O_2.

(iii) Flavin reactions are often redox reactions.

(iv) Light can be substituted by oxidants while reductants suppress the blue light reaction.

(v) Blue light reaction is inhibited by flavin inhibitors such as KI.

(vi) Blue light action spectra resemble low temp, spectra of flavins.

(vii) Neurospora mutant which is free of carotenoids shows blue light response.

(viii) Half life of carotenoids in first excited singlet state is very short (10^{-13} seconds)

(b) Arguments in Favour of Carotenoids:

(i) Three peaked (three fingers) action spectra resemble absorption spectra of carotenoids.

(ii) Small or no UV maximum in some action spectra.

(iii) Energy transfer from UV absorbing pigment to carotenoids is feasible.

(iv) Carotenoids from diatom mutant do not show blue light response.

Earlier evidences suggested crypto chrome to be one or both of the yellow pigments, carotenoids (such as β-carotene, zeaxanthin) and/or flavins (such as riboflavin, FAD) which mediate blue-light responses in plants.

However, with extensive researches done with mutants and transgenic plants and over expression studies beginning in early 1990s, the vexed problem of identification of blue-light receptors in plants has gradually been resolved now.

The term crypto chrome is now applied specifically to flavoprotein photoreceptor that mediates inhibition of hypocotyl (stem) elongation caused by blue-light. Blue-light photoreceptor in phototropism and chloroplasts movements in plants is phototropin which is also a flavoprotein. The carotenoid zeaxanthin is blue-light photoreceptor involved in stomatal opening.

Cryptochrome Structure:

The first protein with characteristics of blue-light receptor was isolated in 1993 from Arabidopsis. It was found that hy4 mutant of Arabidopsis had lost the capacity to respond specifically to blue-light in that it showed an elongated hypocotyl even on irradiation with blue-light (In the wild type, blue-light causes inhibition of hypocotyl elongation).

Isolation of the hy4 gene (later named as cryl) showed that it encoded a 75 kDa protein called crypto-chrome 1 (CRY1) with remarkable sequence similarity (homology) to DNA photolyase in having two chromophores: a flavin adenine dinucleotide (FAD) and a pterin attached to the apoprotein (Fig. 25.5). This led to the establishment of cryptochrome to be a flavoprotein that was involved in inhibition of hypocotyl elongation in response to blue-light. (The structure of pterin is given in figure 25.6. For structure of FAD).

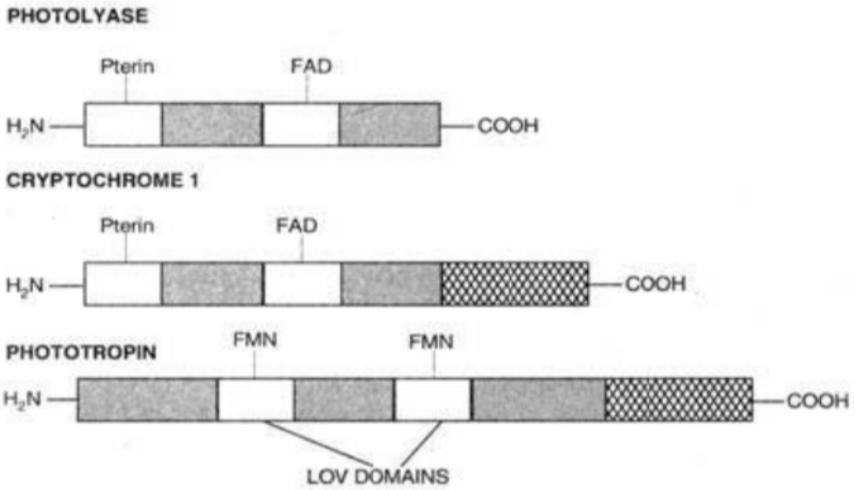


Fig. 25.5. Diagrammatic representation of the chromophore binding domains of microbial photolyase, cryptochrome 1 (cry1) and phototropin. Cross-hatched blocks represent carboxy-terminal domains with kinase activity.

(DNA photolyase is a blue-light activated flavoenzyme which repairs UV-induced damage to microbial DNA. Cryptochrome differs from photolyase mainly in two respects. Firstly, the cryptochrome does not show photolyase activity and secondly, unlike photolyase it has an extended carboxy-terminal domain (Fig. 25.5) with kinase activity).

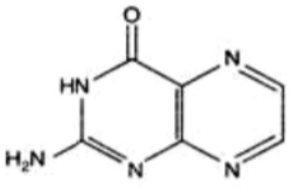


Fig. 25.6. Structure of pterin (fully oxidised)

A second cryptochrome 2 (CRY2) also with two chromophores like CRY1, has also been isolated from Arabidopsis (Lin 2000). CRY2, mediates blue-light stimulated inhibition of hypocotyl elongation, increase in cotyledon expansion and anthocyanin production. It also has a role in determining flowering time. Both CRY1 and CRY2 appear to be ubiquitous in plant kingdom, but while CRY1 is stable in light grown seedling, CRY2 is rapidly degraded in light.

Mechanism of action of cryptochrome:

The mechanism of action of crypto chrome remains elusive so far. The flavins are known to participate in oxidation-reduction reactions and photolyases repair damaged DNA (as a result of UV-radiations) by transferring electrons to pyrimidine dimers. Crypto chromes may act probably in a similar way through some electron transfer mechanism.

Phototropins:

Phototropins are blue-light receptors that mediate phototropism and chloroplasts movements in plants. In late 1980s, it was found that blue-light stimulated phosphorylation of a 120 kDa protein located on plasmamembrane of actively growing regions of etiolated seedlings. These regions were also most responsive to phototropic stimulus. Extensive biochemical and physiological studies showed this protein to be a kinase autophosphorylating in blue-light and which could be the photoreceptor for phototropism.

Later on, a mutant nph1 (won phototropic hypocotyl 1) was isolated from Arabidopsis which lacked phototropic response in the hypocotyl and also the 120 kDa membrane protein. It was genetically independent of the hy4 mutant as it showed blue-light induced inhibition of hypocotyl elongation.

The nph1 gene was cloned and it was found (as postulated) to encode a 120 kDa protein nph1. The nph1 gene was renamed as phot1 and the protein encoded by it was named phototropin (Briggs and Christie, 2002).

Phototropin is also a flavoprotein with two flavin mononucleotide (FMN) chromophores. The protein has a carboxy-terminal domain with a serine/threonine kinase activity. In the amino-terminal half, there are two domains called LOV domains (of about 100 amino acids each) to which are attached the chromophores (Fig. 25.5). (LOV domains are so called because they are characteristics of microbial proteins which regulate response to light, oxygen and voltage).

Recent spectroscopic studies done by Swartz et al, 2000) have shown that in dark, FMN molecules remain non covalently bound to LOV domains, but on irradiations with blue-light they become covalently bound to cysteine residues of the apoprotein through a sulphur atom forming a cysteine-flavin covalent adduct. The reaction is reversed in dark. A second gene called phot 2 has also been isolated from Arabidopsis which is related to phot 1. It is believed that phototropic response involves both phot 1 and phot 2.

Mechanism of action of phototropins:

The mechanism of action of phototropins is not clear. It has been observed that blue-light causes a transient increase in cytosolic calcium concentration and there are indications that phototropin signalling chain may partly involve regulation of cytoplasmic calcium concentration.

Zeaxanthin:

The carotenoid zeaxanthin has been shown to be blue-light receptor in guard cells that plays central role in blue-light stimulated stomatal opening. (See chapter 17 for structure of zeaxanthin).

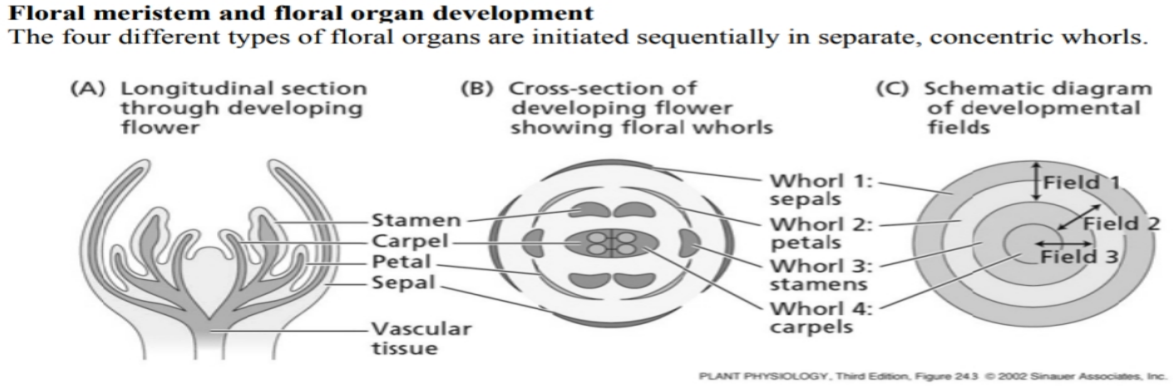
Following evidences strongly support role of zeaxanthin in stomatal opening:
(i) The absorption spectrum of zeaxanthin closely resembles the action spectrum of blue- light stimulated stomatal opening.
(ii) During stomatal opening in intact leaves, the incident radiation, zeaxanthin concentration in guard cell, and stomatal apertures have been found to be directly correlated.
(iii) Blue-light sensitivity of guard cells increases with an increased concentration of zeaxanthin in guard cells.
(iv) There is complete inhibition of blue-light stimulates stomatal opening by 3mM conc. of dithiothreitol (DTT) which is a potent inhibitor of the enzyme that converts violaxanthin to zeaxanthin.
(v) In facultative CAM plant species such as MeSembryanthemum crystallinum, there is a shift from C3 to CAM mode of carbon metabolism in response to accumulation of salts. In C3 mode, the guard cells accumulate zeaxanthin and exhibit blue-light response. But, in CAM mode, neither there is accumulation of zeaxanthin in guard cells nor they respond to blue- light. (In CAM plants, stomata remain closed during the day).

6.Control of flowering: Floral meristem and floral organ development, floral evocation.

Growth and development of plants are influenced by several environmental factors including light. However, light causes several responses other than photosynthesis. These responses greatly influence the course of plant development and the final plant appearance. They are photomorphogenetic responses. For example, the seeds of many plants do not germinate unless they are exposed to light. Germination of seeds in light shows that the seedlings require light to grow. Phototropic responses of seedlings and of leaves of mature plants are also beneficial photomorphogenetic processes. Photomorphogenetic responses are also important to older plants. Many such responses respond to the relative lengths of day and night by forming reproductive structures or by forming dormant buds that can resist a cold winter (i.e., phenomenon of photoperiodism and vernalization.) Flower formation is a transitional phase in the life cycle of a plant. It is of immense importance for perpetuation and origin of variability in the next generations. Flower initiation takes place by the transformation of vegetative apex into a reproductive structure.It signifies a transition from vegetative to the floral state. The shoot meristem is reduced and is also induced to develop sepals, petals, stamens and carpels in place of leaves. The pattern and timing of flower initiation vary from species to species. Thus, the flowers may be terminal, axillary, single or grouped into an inflorescence. A plant must attain a specific state of 'ripeness to respond' before it flowers.Once this stage is reached, then it can be induced to flower. Thus by and large, two phases are recognized at this point. These are flower induction and flower differentiation. Both the processes are subjected to variable and diverse controls. The induction of flowering implies whereby all the cells of the shoot meristem instead of giving rise to

leaves are turned towards the formation of floral organs.Once such a switch over has been accomplished then the differentiation steps leading to flower formation follow. Of the two phases, the former has attracted the attention of several plant physiologists for a long time. One simple reason could be that once the induction of flowering has been accomplished, the differentiation would follow automatically.With experience it is now widely accepted that during flower induction a plant must be subjected, for specific period of time, to an external condition e.g., light, temperature or even some chemicals, etc.This time interval is generally referred to as induction period and the external conditions as inductive-conditions. Similarly, the external conditions under which a given plant continues to grow vegetatively is called non-inductive conditions.The two main inductive conditions (i.e., light and temperature), which induce flowering. The flowering response to day length is called photoperiodism while low temperature treatment is called vernalization.A large number of plant species undergo a state of vigorous vegetative growth and during this period, they cannot be induced to flower. This is called a juvenile phase. It is as if such species must attain 'ripeness-to-flower' even though suitable conditions are provided. On the contrary, several grass species like Loliumremotum, L. temulentum can be made to flower at any stage of growth.The duration for ripeness-to-flower varies considerably. It may be a few days (Xanthium), a few weeks (Lunaria) or even several years (Mains, Citrus). In such instances, the plant must develop specific number of nodes and also minimal number of leaves before flowering is induced. It is this difference in the number of leaves which is critical for different species to flower at variable times.In a longitudinal section of the vegetative shoot apex, a central dome or corpus is seen along with one or several layered tunica. Corpus is differentiated into central mother cell zone, flank meristem and rib meristem. During the transition of vegetative apex into reproductive primordium, several histological and biochemical changes occur.One of these is an increase in the mitotic activity between rib meristem and central mother zone. Cells derived from this zone become central core of floral primordium. The reproductive shoot is an enlarged structure with several cells. Such a transformation also induces synthesis of nucleic acids.Through the usage of antimetabolites i.e., inhibitors of nucleic acids synthesis, the suppression of such transformation is clearly made out. In Chenopodium there is a rapid increase in RNA content following photoperiodic induction. Similarly, with radio-active precursors of RNA an enormous amount of incorporation was observed in Lolium following inductive long-day conditions. Murret (1977) has reviewed on the environmental interaction and the genetics of

flowering. In brief, it may be said that before the initiation of flowering, there is reprogramming of genetic activity in the shoot apices. It is generally assumed that stimulus induces some of the passive genes blocks, The latter are activated and these undertake synthesis of new messenger RNA; such genes are activated floral genes. The RNA thus synthesized controls flowering through the synthesis of new enzyme systems.lt is also believed that the flowering stimulus is a transmissible molecule which has the potentiality to combine with and inactivate the repressor i.e., floral gene repressors. Moreover, DNA may also be exerting a significant regulatory role through nucleotides. Genes may influence flowering through enzyme synthesis, membrane and transport phenomenon, energy supply. switching and regulator) mechanism, etc.In tomato, flower differentiation is solely determined by genotype of the plant. In several plant species in addition to genetic make-up, day length, intensity and quality of light, temperature and mineral nutrition also influence flower formation enormously. In fact, the response of plants to the factors mentioned above is an adaptive property for their perpetuation during unfavourable conditions.



Two major types of genes regulate floral development

1. Meristem identity genes : Positive regulators of floral organ identity in the developing floral meristem. Encode transcription factors that are necessary for initial induction of organ identity genes.

• FLORICAULA (FLO) : Identified from snapdragon *(Antirrhinum*)

• *flo* mutants develop an inflorescence that does not produce flowers i.e. develop additional inflorescence meristems in the bract axils.

• The wild type FLO gene controls the determining step in which floral meristem identity is established.

• LEAFY (LFY) and SUPRESSOR OF CONSTANS 1 (SOC1):

• LFY is *Arabidopsis* version of FLO gene.

• Both LFY and SOC1 serve as master regulators for the initiation of floral development.

- APETALA1 (AP1) : another floral meristem identity gene identified from *Arabidopsis*

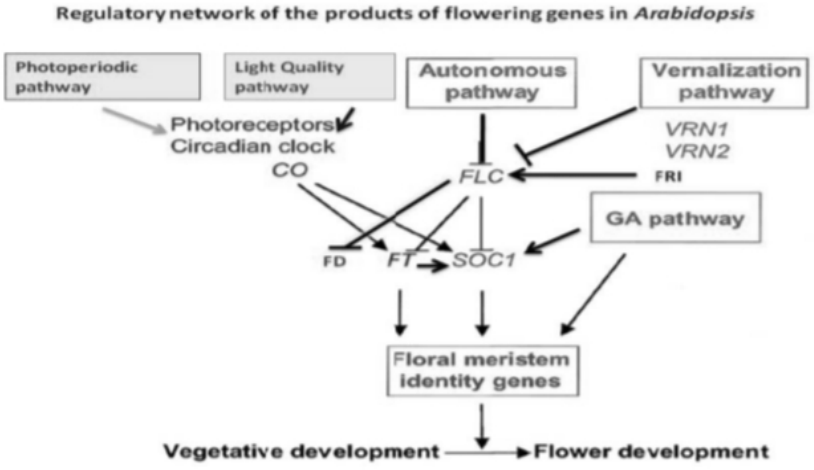
2. **Floral organ identity genes :** directly control floral identity. The proteins encoded by these genes are transcription factors that likely control the expression of other genes whose products are involved in the formation and/ or function of floral organs.

- The genes that determine floral organ identity are known as **floral homeotic genes**.
- Most of plant homeotic genes belong to a class of relate sequences known as **MADS box genes**.
- The **MADS box genes** have a charateristic conserved nucleotide sequence known as **MADS box**, which encodes a protein known as **MADS domain**.
- The MADS domain enables these transcription factors to bind DNA at specific site.
- Some floral homeotic genes: **DEFICIENS** gene of snapdragon and the **AGAMOUS (AG)**, **PISTILLATA (PI)** and **APETALA3 (AP3)** genes of *Arabidopsis*.

Mutations in homeotic floral identity genes alter the types of organs produced in each of whorls.

Control of Flower Primordium Specification by LEAFY and APETALAI/CAULI FLOWER:

The



knowledge regarding the mechanism by which flowers are specified on the flanks of the shoot apex is well understood though same cannot be said regarding mechanisms involved in the production of floral stimulus in the leaves.

The available information is:

i. Replacement of flowers with indeterminate shoots in lfy and apcal double mutants indicates that LFY and API/CAL are crucial for flower primordia specifications.

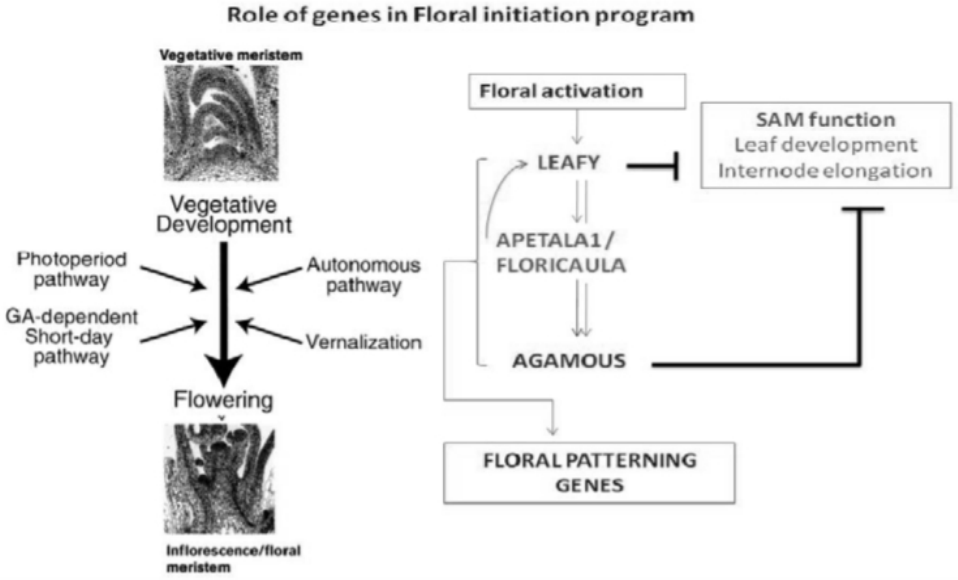
ii. In ecotypically expressed LFY and API these genes are sufficient to specify flowers when expressed in shoot primordia.

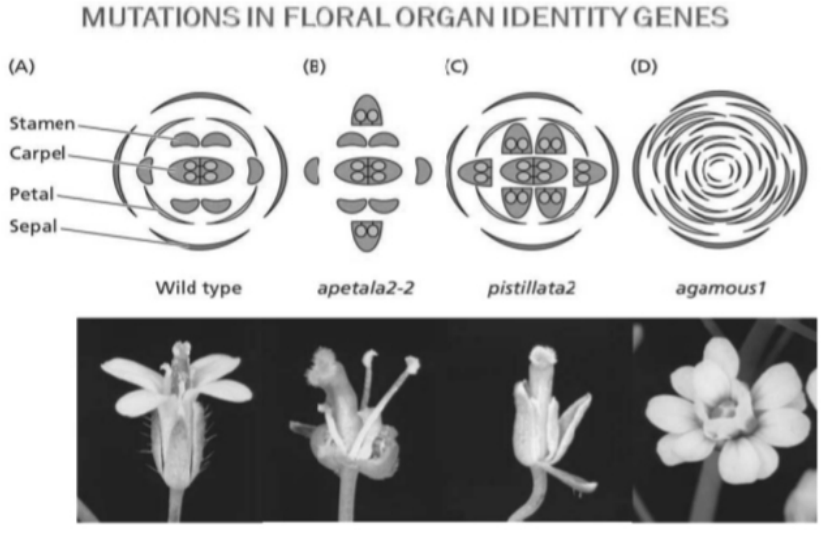
iii. Many other genes may be involved in the specifications of flowers along with LFY and API/CAL.

Hempel (2000) have discussed molecular interactions between LFY and MADS-BOX genes, API/CAL and AGAMOUS (AG). In wild type LFY is expressed throughout flower primordia early in their ontogeny. API and CAL expression also takes place throughout flower primordia, though the expression of these two genes occurs in primordia only after they have become distinct from the meristem.

The upregulation of API during floral induction treatments does not occur until several hours after LFY has been upregulated. In lfy mutants, API expression is weak and delayed whereas ecotypic expression of LFY induces the ecotypic expression of LFY induces the ecotypic expression of API in leaf primordia and in axillary flower primordia.

These data show that LFY is a formal regulator of API. The recent data also show that the API promoter is a direct target of LFY, and it has a LFY-responsive enhancer that is needed for its activity. Clearly LFY has direct and distinct roles in the specification of flowers and in the patterning of floral organs.





Interactions between LFY, API and TERMINAL FLOWERI (TFLI) during the Transition to Flowering: It is the interactions between these genes (LFY and API/CAL) and TFLI that inflorescence

is regulated. TFLI prevents the expression of floral meristem identity genes in the shoot meristem and promotes indeterminate growth.

ABC model of flowering:

The ABC model of flower development in angiosperm demonstrates the presence of three classes of genes that regulate the development of floral organs. The genes are referred to as class A genes, class B genes and class C gene. These genes and the interaction between them induce the development of floral organs.Many literatures on molecular genetics and Internet Websites provide articles on ABC model. In the following essay the basic concept of ABC model will be discussed in brief. The analysis of ABC model is based on the use of molecular genetics and formulated on the observation that mutants induce right floral organs to develop in wrong whorls.

In the flower of angiosperms there are usually four concentric whorls of organs, i.e. sepal, petal, stamen and carpel that are formed in whorl 1, whorl 2, whorl 3 and whorl 4 respectively, the whorl 1 being on the peripheral side. In the whorl 1 class A genes when expressed induce the development of sepals. The interaction between class A and class B genes induce the development of petals in the whorl 2. Stamens are formed in the whorl 3 as a result of interaction between class B and class C genes. In the whorl 4 class C gene induces the formation of carpel. So the summary of ABC model is: class A genes together and class C gene alone are responsible for the development of sepals and carpel respectively. The class B genes and class A genes function cooperatively to determine the development of petals. The class B genes and class C gene act together to induce the development of stamens.

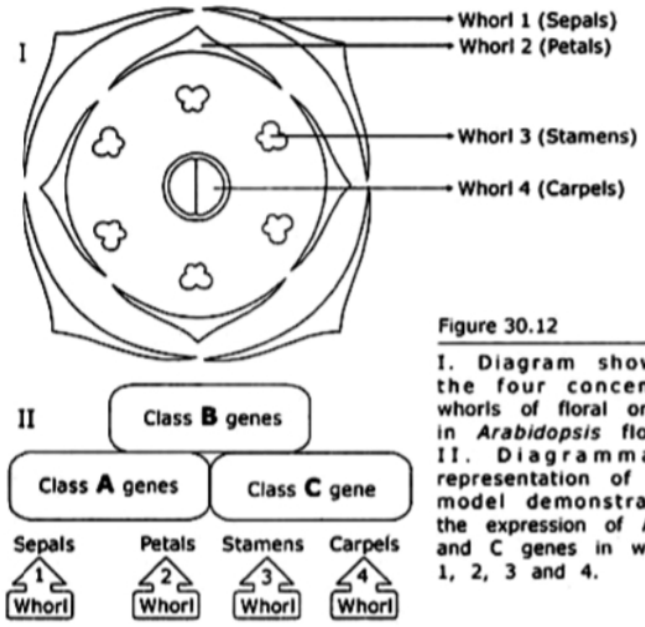


Figure 30.12

I. Diagram showing the four concentric whorls of floral organs in *Arabidopsis* flower. II. Diagrammatic representation of ABC model demonstrating the expression of A, B and C genes in whorls 1, 2, 3 and 4.

Coen et al. (1991) formulated the ABC model. While analyzing the mutations affecting flower structure Coen et al. identified the class ABC genes that direct flower development. They also formulated the molecular models of how floral meristem and organ identity may be specified. They

have shown that the distantly related angiosperm plants use homologous mechanisms in pattern formation of floral organs. Ex. *Arabidopsis thaliana* and *Antirrhinum majus*.

The following two have led to formulate ABC model:

(1)The discovery of homeotic mutants (homeotic genes identify specific floral organs and help the organ to develop in respective whorl. The homeotic mutant has inappropriate expression—that is, it induces right organ to develop in wrong whorl. As for example - petals emerge in the whorl where normally stamens develop).

(2) The observation that each of the genes that induce the formation of an organ in a flower has an effect on two groups of floral organs, i.e. sepal and petals or petals and stamens. Class A, B and C genes are homeotic genes. They determine the identity of different floral organs and induce the organs to develop in their respective whorls. The homeotic mutants have defects in floral organ development and induce the right organs to develop in wrong whorls/place, i.e. one floral organ develops in the whorl, which is the normal position of another floral organs. Petals, for example, develop in the whorl where stamens are normally to be formed.In each whorl of a flower there is one or more homeotic genes and their cooperative functions determine the organ to be formed in that whorl. For example, the activity of class A genes is restricted to whorls 1 and 2. The class B genes have function in whorls 2 and 3. The class C gene functions in whorls 3 and 4.Another way of describing the function of class A, B and C genes is that —in whorl 1, the class A gene-function alone determines the formation of sepals; in whorl 2, class A and B gene-functions both determine the formation of petals; in whorl 3, class B and C gene-functions both determine the emergence of stamens and in whorl 4, class C gene-function alone determines the carpel formation.

In *Arabidopsis* there are two genes in class A, two genes in class B and one gene in class C. The most characteristic feature of these homeotic genes is in the identification of floral organs and in the determinacy of position / whorl of their emergence in a floral meristem. The two genes of class A and the two genes of class B act cooperatively.

The function of class A genes is confined to whorls 1 and 2. Similarly the function of class C gene is restricted in whorls 3 and 4. This can be interpreted in another way. In the whorls 1 and 2 the function of class A genes prevents class C gene from functioning in the same whorls. Similarly the function of class C gene prevents class A genes from functioning in the whorls 3 and 4.

Any mutation in class A genes with defects in floral organ development will invite class C gene to express in whorls 1 and 2. The class C gene, in class A mutants, will express in whorls 1 and 2 in addition to the normal whorls 3 and 4.

Similarly any mutation in class C gene with defects in floral organ development will lead to the encroachment of the function of class A genes. The class A genes will express in the whorls 3 and 4 in addition to the normal whorls 1 and 2.

Class	*Arabidopsis*
Class A	*APETALA 1 (AP1), APETALA 2 (AP2)*
Class B	*APETALA 3 (AP3), PISTILLATA (P1)*
Class C	*AGAMOUS (AG)*

Table 30.1
Illustrating Homeotic genes in *Arabidopsis*.

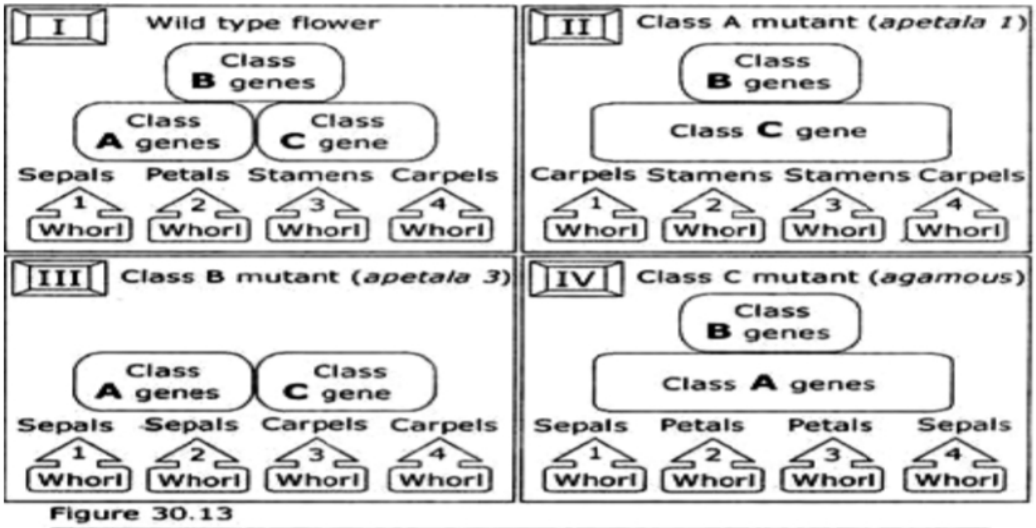


Figure 30.13

Diagrammatic illustrations of the floral organs formed in wild type and homeotic mutants of *Arabidopsis* flower. I. Wild type flower. The individual and cooperative expressions of class A, B and C genes are in the formation of sepals, petals, stamens and carpels in whorls of 1, 2, 3 and 4 respectively. II. Class A mutant, *apetala 1*. This homeotic mutant contains loss-of-function A genes. Class C gene expresses alone in whorl 1 in addition to whorl 4. Class C gene and class B genes together express in whorls 2 and 3. As a result stamens are formed in both whorls 2 and 3. III. Class B mutant, *apetala 3*. This homeotic mutant contains loss-of-function B genes. Class A genes express in whorls 1 and 2. As a result sepals are formed in both whorls 1 and 2. Class C gene expresses in whorls 3 and 4. So carpels are formed in both whorls 3 and 4. IV. Class C mutant, *agamous*. This homeotic mutant contains loss-of-function C gene. Class A genes express in whorls 3 and 4 in addition to whorls 1 and 2. As a result petals and sepals are formed in whorls 3 and 4 respectively. In whorl 3 class A and class B genes together form petals. In whorls 1 and 4 the sole group of class A genes expresses and so sepals are formed.

The following three examples of homeotic mutant genes will illustrate the above discussion:

(1) The flower of Arabidopsis with class A mutants, such as apetala 1(ap 1) shows the following pattern of floral organs (Fig. 3.13.II): whorl 1 shows bract-like structure with carpelloid characteristics; whorl 2 shows stamens; whorl 3 shows stamens and whorl 4 shows carpel.

The pattern of floral organ formation in whorls 1 and 2 is changed. In ap 1 mutants the activity of two genes of class A is lost. So the class C gene expressed in whorls 1 and 2 in addition to whorls 3 and 4. As a result carpelloid organ developed in whorl 1 and stamens formed in whorl 2. In the whorls 3 and 4 stamens and carpel respectively are formed similar to wild type (Fig. 30.13.I).

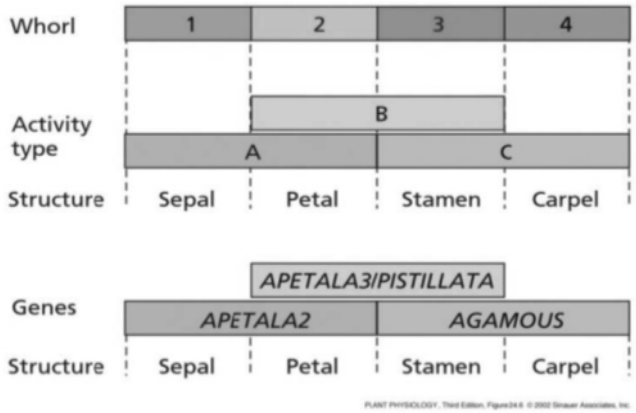
(2) Example: Flower of Arabidopsis with class B mutant, such as apetala 3 (ap 3): The flower shows sepals only both in whorls 1 and 2, while the whorls 3 and 4 show carpel only. Class B mutant

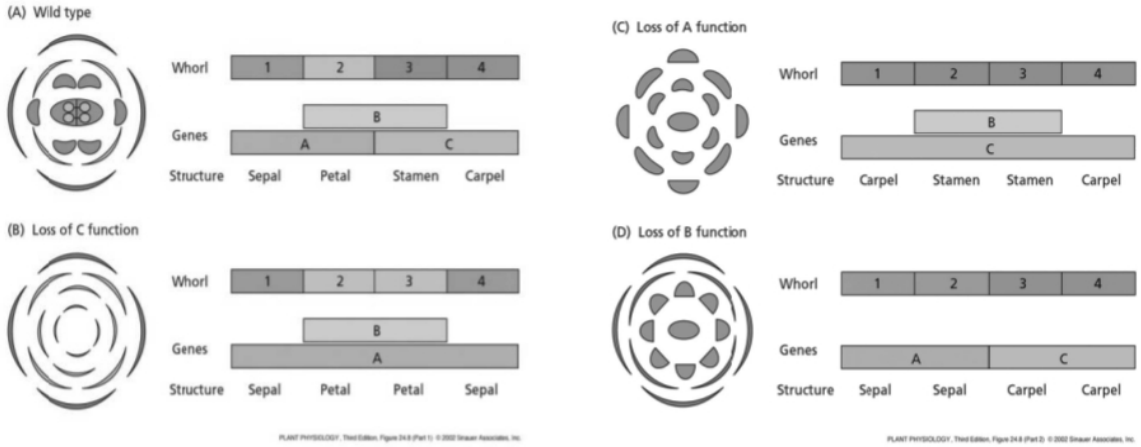
contains loss-of-function genes and as a result class A genes express in whorls 1 and 2; and class C gene alone expresses in whorls 3 and 4. In ap 3 mutants in whorl 2, sepals are formed instead of petals and in whorl 3, carpel is formed instead of stamens.

(3) In Arabidopsis the class C gene contains the sole gene agamous (ag). Arabidopsis flower with agamous (ag) mutant consists of many sepals and petals. The reproductive organs – stamens and carpel are not formed in the whorls 3 and 4. Class C gene with ag mutant contains loss-of-function gene. As a result class A genes express in whorls 3 and 4 in addition to 1 and 2. In ag mutant sepals and petals are formed in whorls 3 and 4 instead of stamens and carpel. The literature of Howell provides the scan electron micrograph of flower phenotypes of the floral homeotic mutants of class A, B and C genes.

In Arabidopsis it was observed that in all the mutants one homeotic gene remains functional in each whorl. The flower with class ABC triple mutant shows sepals in each whorl. In ABC triple mutant, the genes required for floral organ formation become nonfunctional. As a result sepals or leaves are formed in each whorl, as homeotic mutants specify no floral organs. This observation led Botanists to regard 'flowers as modified leaves' on the basis of molecular genetics.

The important feature of ABC model is that it can predict the type of floral organ to be induced to develop in any whorl. Krizek et al. (1996) was successful to induce any one of the four different floral parts in whorl 1 of Arabidopsis flower. This became possible by genetic manipulations of right combination of homeotic selector genes.The ABC model appears to be simple, but a completely different picture is obtained when it is analyzed on the basis of molecular genetics and in molecular terms.The analysis includes the structure of different classes of homeotic genes, the homeotic mutants, the co-operative function between homeotic genes, mutual exclusion in the expression of class A and C genes in the same whorl, the identification of floral homeotic genes and their isolation by cloning, the production of MADS box protein by homeotic mutants, the study of genes that mediate the interaction between floral meristem and floral organ development, presence or absence of different classes of transcription factors etc., the details of which can be obtained in the literatures on molecular genetics.





Arabidopsis thaliana belongs to the family Brassicaceae and has become the model organism for understanding the genetics and molecular biology of flowering plants like mice and Drosophila in animal researches due to following reasons:

(i) It has five chromosomes (n = 5) and so this small-size-genome is advantageous in gene mapping and sequencing.
(ii) The size of plant is small and so can be cultivated in a small space and requires modest indoor facilities.
(iii) It has rapid life cycle and takes about six weeks from germination to mature seeds.
(iv) An individual plant produces several thousand seeds.
(v) 'The Arabidopsis genome is among the smallest in higher plants, with a haploid size of about 100 megabases (mb) of DNA. With a small genome size it was expected that there would be fewer problems with gene duplication'— Howell.
(vi) It is easily transformable with T-DNA mediated transformation.

In 2004 ABCE model has been formulated. The characterization of sepallata 1, 2, 3 triple mutants in Arabidopsis has led to the above formulation. It is regarded that the class E genes have important role in the development of floral organs.

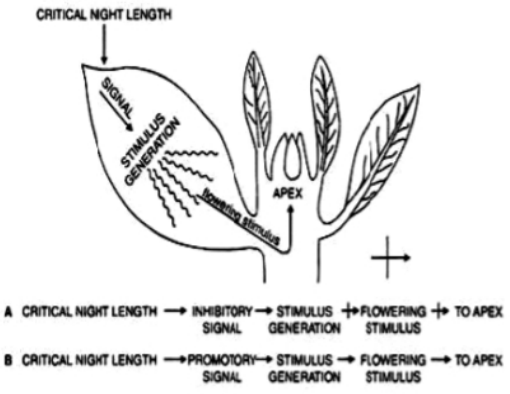


Fig. 22-12. Regulation of flowering in long day (A) and short day plants (B).

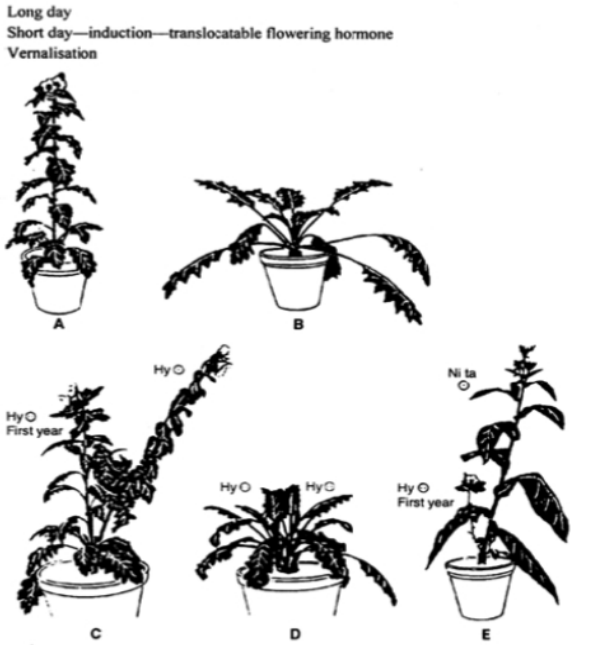


Fig. 22-12 A. Grafting experiments on *Hyoscyamus*. (A) Annual, (B) biennial in the first year, (C) annual (right) grafted on biennial in the first year, (D) control biennial graft on biennial in first year ; (E) *Nicotiana tabacum* graft on biennial in the first year (from Kuhn, 1965)

Floral Induction:
Floral induction is not a rapid process and the transformation of vegetative apex to flowering depends upon the intensity of stimulus. Different plant species require different number of inductive photoperiods for complete flowering. The inductive stimulus has to be of specific intensity and the induction can be reversed.

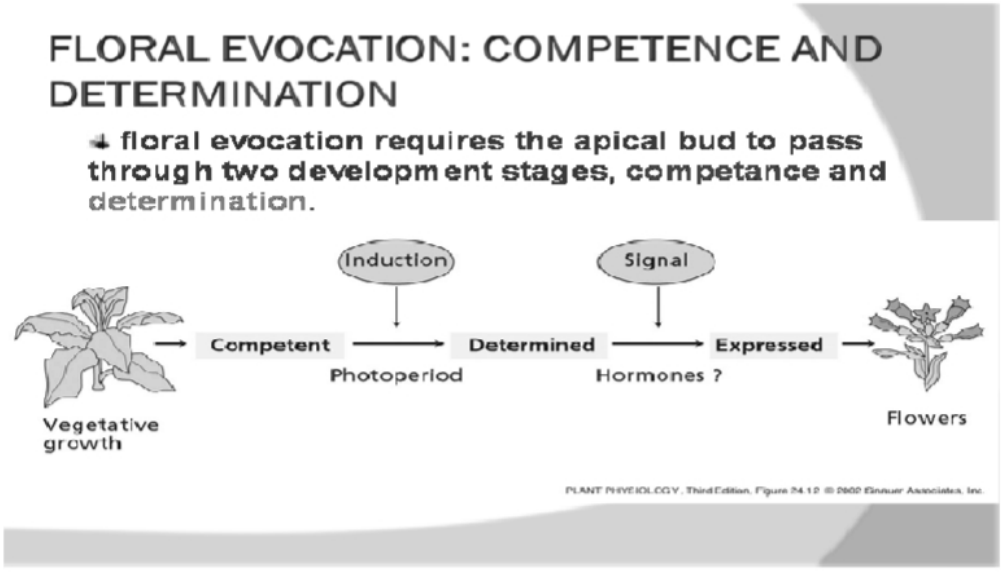
We have already shown that leaf is the real site of perception of the light signals that induce or stop flowering. Obviously something must be translocated from the leaf to the stem tip. According to the Russian plant physiologist C. Kh. Chailakhyan, flowering response needs atleast four steps and these are perception of stumulus; the transformation of the perceiving organ; the translocation of stimulus; the translocation of a stimulator and finally a specific response in the growing tip and thus the formation of flower.
It has come to be realized that perception is through phytochrome. The stimulus takes several hours to move out of the leaf and then initiate flowering. L.T. Evans (1971) has also shown that rate of translocation of the flowering stimulus varies in the LD and SD plants. He also showed that the role of stimulus was different from photosynthate. Flowering inducing stimulus are possibly translocated through phloem. .
Thus differences in LD and SD plants could be due to the concentrations of flowering hormone or else secondary reactions may be induced in the two types as follows:
In general it is believed that florigens are the floral stimulating substances. Florigens are in fact a concept. Some experiments have indicated the occurrence of flowering inhibitors. This inhibitor effect is opposite to that of florigen.

Floral evocation : the events occurring in the shoot apex that specifically commit the apical meristem to produce flowers.

Internal (autonomous) and external (environment-sensing) control systems enable plant to precisely regulate and time flowering for reproductive success.

Floral development can be response to:

- PHOTOPERIODISM: changes in day length.
- VERNALISATION: prolonged cold temperature.



Circadian Rhythms:

Like all other living organisms, the plants are and have always been exposed to strong and rhythmic environmental changes caused by planetary movements. It is but natural, that these environmental rhythmicities or periodicities find their counterparts in biological rhythms which control many behavioural and physiological activities of living organisms including plants. When the period of biological rhythmicities matches with those of the cycles of day and night, such rhythms are called as circadian rhythms (Circa = about; diem = a day).

Rhythmic behaviour in plants under natural conditions in known to scientist for over two thousand years. The so-called sleep movements (or up and down movements) of leaves in certain plants such as bean (Phaseolus multiflorus) is one such example and amongst the earliest observations on circadian phenomena.

In 1729, the French astronomer De Mairan carried out a very important experiment in which he transferred the plants to the continuous darkness of a cellar (a room under a house) and observed that the leaves showed rhythmic up and down movements for a number of days. He concluded that these were not dependent on the daily cycle of light and darkness even though they were normally synchronized with the latter. According to him these movements were controlled by an internal mechanism and were, therefore endogenous.

All circadian rhythms oscillate with a period length close to, but seldom equal to 24 hrs. when organisms are kept under constant conditions of light, temperature and other possible geophysical factors. The pattern of phase response curves may vary from organism to organism.

The persistence of rhythm varies with quality of light and also duration of exposure. In many animals, circadian rhythms in locomotory activity persist in continuous light (LL) as well as in constant darkness (DD) but with their 't' altered.

Circadian rhythms are endogenous. They are both an organismal and a cellular phenomenon. Light/dark cycle works as one of the strongest entrainers of these rhythms, although other periodic cycles can also entrain circadian rhythms. They free-run in the absence of a light/dark cycle.

This suggests that there is a pacemaker(s) which generates circadian rhythms in various physiological, biochemical and behavioural variables. Thus a basic circadian system has necessarily three important components such as photoreceptors, pacemaker(s), and observable rhythmic outputs.

The entraining pathways transduce information between the photo receptive elements and the pacemaker(s). The coupling pathways are also necessary as a link (the efferent pathways) between the pacemaker(s) and the multiple effector systems. The effector outputs are the overt rhythms that allow us to study the properties of the central clockwork (pacemaker) (Figs. 11.1 and 11.2).

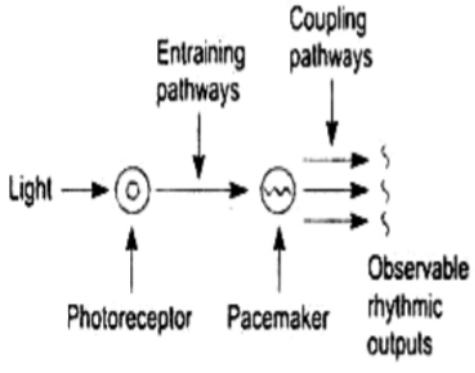


Fig. 11.1: A model showing a simple unidirectional pathway consisting of basic components of circadian systems.

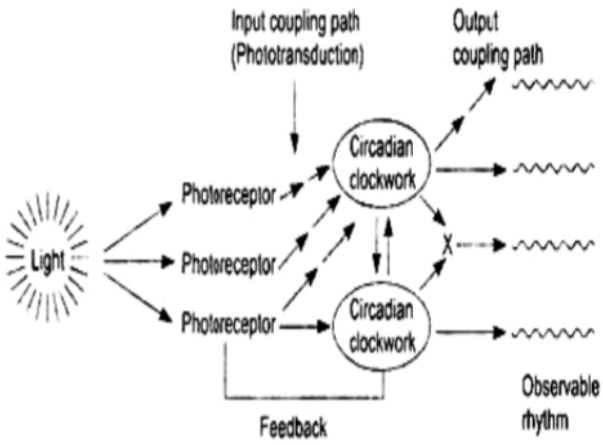


Fig. 11.2: A hypothetical model for the organisation of the major components of circadian systems

Occurrence of Circadian Rhythms in Plants:

Circadian rhythms have been found in some members of almost all major groups of plants except Bryophyta and Gymnosperms. Some of the examples of circadian rhythms in plants are given in Table 22.1.

Table 22.1. Some Examples of Circadian Rhythms in Plants.

Plant Group	Name of Organisms	Rhythmic Process
Algae	*Gonyaulax polyedra* (Unicellular dinoflagellate)	Bioluminescence, Photosynthetic capacity, Growth.
	Euglena gracilis (Unicellular)	Phototaxis
	Oedogonium cardiacum	Sporulation
	Acetabularia major (Unicellular)	Photosynthetic capacity
Fungi	*Pilobolus sphaerosporous*	Spore discharge
	Neurospora crassa	Mycelial growth
Pteridophyta	*Selaginella serpens*	Plastid Shape
Angiosperms	*Phaseolus multiflorus*	Leaf movements.
	Avena sativa	Growth rate of coleoptile
	Bryophyllum fedtschenkoi	Dark CO_2 fixation
	Kalanchoe blossfeidiana	Petal movements

Biological Clock:

The plants showing endogenous circadian rhythms have time measuring system or 'biological clock' inside their cells which measures the passage of time in much the same way as a pendulum. The nature and functioning of biological clock is not yet clearly understood.

There are, however, several evidences for the possible participation of:

(i) Membrane physiology, cytoplasmic organelles or

(ii) Nucleus and protein synthesis or

(iii) Higher frequency biochemical oscillations in the cells.

Accordingly, different models have been given by scientists to explain the working of circadian oscillator. For example, in Chronon model, the 24 hrs. period is ascribed to the time taken for the transcription of a hypothetical linear sequence of DNA, cistron by cistron. According to a membrane model, this period is attributed to the slower processes involved in the lateral fusion of proteins with the lipid bilayer. According to another model, the circadian period is believed to be the result of interactions between higher frequency biochemical oscillations within the cell.

The location of the 'biological clock' too is not clear in the cells. Because circadian rhythms are not observed in cell-free extracts or isolated cell-organelles, it is likely that the biological clock does not lie within the cell but the whole cell itself probably acts as the biological clock.

Photoperiodism:

The plants in order to flower require a certain day length i.e., the relative length of day and night which is called as photoperiod. The response of plants to the photoperiod expressed in the form of flowering is called as photoperiodism.

The phenomenon of photoperiodism was first discovered by Garner and Allard (1920, 22) who observed that the Biloxi variety of Soybeans (*Glycine max*) and 'Maryland Mammoth' variety of tobacco (*Nicotiana tabacum*) could be made to flower only when the daily exposure to the light was reduced below a certain critical duration and after many complex experiments concluded that 'the relative length of the day is a factor of the first importance in the growth and development of plants'. Depending upon the duration of the photoperiod, they classified plants into three categories.

(1) Short Day Plants (SDP):

These plants require a relatively short day light period (usually 8-10 hours) and a continuous dark period of about 14-16 hours for subsequent flowering (Fig. 18.1A). Some examples of these plants which are also known as long-night-plants are Maryland Mammoth variety of tobacco (*Nicotiana tabacum*) Biloxi variety of Soybeans (*Glycine max*), Cocklebur (*Xanthium pennsylvanicum*).

i. In short day plants the dark period is critical and must be continuous. If this dark period is interrupted even with a brief exposure of red light (660-665 mμ wavelength), the short day plant will not flower.

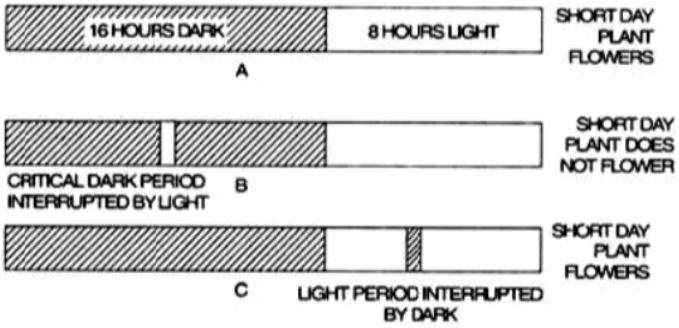


Fig. 18.1. Effect of a brief exposure of red light during dark and interruption of light period by dark on flowering in a short day plant.

ii. Maximum inhibition of flowering with red light occurs at about the middle of critical dark period.

iii. However, the inhibitory effect of red light can be overcome by a subsequent exposure with far-red light (730-735 mu wavelengths).

iv. Interruption of the light period by dark does not have inhibitory effect on flowering in short day plants.

v. Prolongation of the continuous dark period initiates early flowering in short day plants.

(2) Long Day Plants (LDP):

These plants require a longer day light period (usually 14-16 hours) in a 24 hours cycle for subsequent flowering. Some examples of these plants which are also called as short night plants are *Hyoscyamus niger* (Henbane) *Spinacea* (spinach) *Beta vulgaris* (Sugar beet).

i. In long day plants the light period is critical.

ii. A brief exposure in the dark period or the prolongation of the light period stimulates flowering in long day plants.

(3) Day Neutral Plants:

These plants flower in all photoperiods ranging from 5 hours to 24 hours continuous exposure. Some of the examples of these plants are tomato, cotton, sunflower, cucumber and certain varieties of peas and tobacco. During recent years certain intermediate categories of plants have also been recognised. They are,

Long Short Day Plants:

These are short day plants but must be exposed to long days during early periods of growth for subsequent flowering. Some of the examples of these plants are certain species of Bryophyllum.

Short-Long Day Plants:

These are long day plants but must be exposed to short days during early periods of growth for subsequent flowering. Some of the examples of these plants are certain varieties of wheat (*Triticum*) and rye (*Secale*).

Photoperiodic Induction:

Plants may require one or more inductive cycles for flowering. An appropriate photoperiod in 24 hours cycle constitutes one inductive cycle. If a plant which has received sufficient inductive cycles is subsequently placed under un-favourable photoperiods, it will still flower. Flowering will also occur if

a plant receives inductive cycles after intervals of un-favourable photoperiods (i.e., discontinuous inductive cycles). This persistence of photoperiodic after effect is called as photoperiodic induction.

i. An increase in the number of inductive cycles results in early flowering of the plant. For instance Xanthium (a short day plant) requires only one inductive cycle and normally flowers after about 64 days. It can be made to flower even after 13 days if it has received 4-8 inductive cycles. In such cases the number of flowers is also increased.

ii. Continuous inductive cycles promote early flowering than discontinuous inductive cycles.

Some of the example of plants which require more than one inductive cycles for subsequent flowering are Biloxi soybean (SDP) —2 inductive cycles; *Salvia occidentalis* (SDP) — 17 inductive cycles; *Plantago lanceolata* (LDP)—25 inductive cycles.

Perception of the Photoperiodic Stimulus and Presence of a Floral Hormone:

It is now well established that the photoperiodic stimulus is perceived by the leaves. As a result, a floral hormone is produced in the leaves which is then translocated to the apical tip, subsequently causing the initiation of floral primordia.

That the photoperiodic stimulus is perceived by the leaves can be shown by simple experiments on cocklebur (*Xanthium pennsylvanicum*), a short day plant. Cocklebur plant will flower if it has previously been kept under short-day conditions (Fig. 18.2 A). If the plant is defoliated and then kept under short day condition, it will not flower (Fig. 18.2 B). Flowering will also occur even if all the leaves of the plant except one leaf have been removed (Fig. 18.2 C).

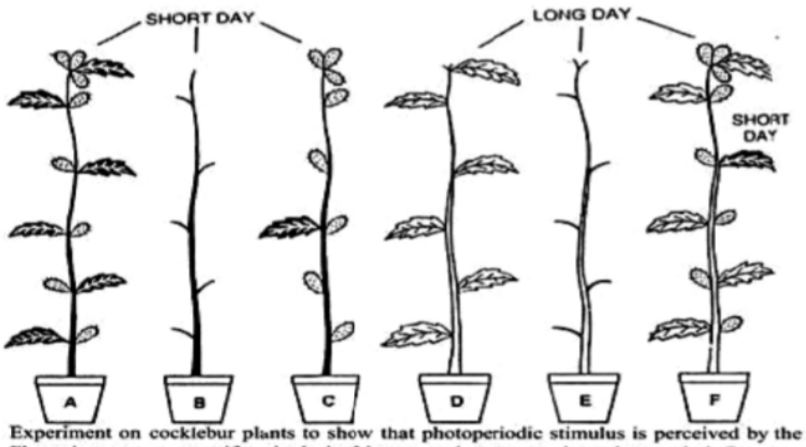


Fig. 18.2. Experiment on cocklebur plants to show that photoperiodic stimulus is perceived by the leaves. Flowering occurs even if a single leaf is exposed to appropriate photoperiod. See text

If a cocklebur plant whether intact of defoliated, is kept under long day conditions it will not flower (Fig. 18.2 D, E). But, if even one of its leaves is exposed to short day condition and the rest are under long day photoperiods, flowering will occur (Fig. 18.2 F).

The photoperiodic stimulus can be transmitted from one branch of the plant to another branch. For example, if in a two branched cocklebur plant one branch is exposed to short day and other to long day photo period, flowering occurs on both the branches (Fig. 18.3 A).

Flowering also occurs if one branch is kept under long day conditions and other branch from which all the leaves except one have been removed is exposed to short day condition (Fig. 18.3 B). However, if one branch is exposed to long photoperiod and the other has been defoliated under short day condition, flowering will not occur in any of the branches (Fig. 18.3 C).

Both the species will flower under a photoperiod of 13 hours. Subjecting LD and SD plants to light and darkness, other than 24 hours, shows that flowering in plants is more of a response to the dark period than to the light.

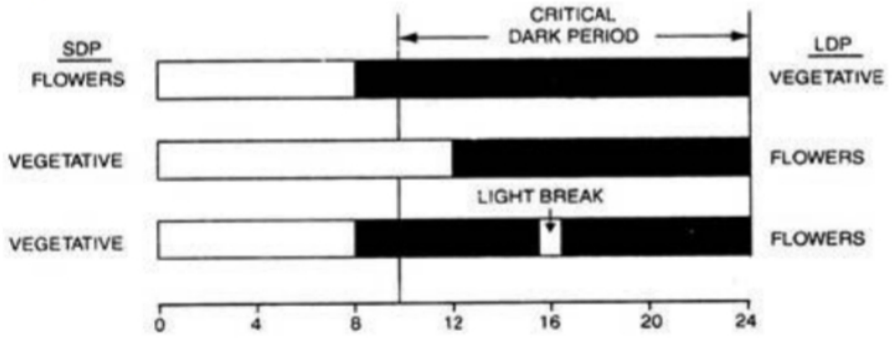


Fig. 22-2. Effect of breaks of light during the dark period on flowering in LDP (long day plant) and SDP (short day plant). Critical dark period is also marked out.

The critical night is that period of darkness, which must be exceeded before SD plants would flower. In LD plants, the dark period must be shorter than the critical night if flowering is to occur. Thus Xanthium, a SD plant, will not flower if the long dark period is interrupted by a single brief flash of light (Fig. 22-3).

On the other hand, interrupting the light period for a short period of darkness had no effect. It may be interpreted to mean that the long dark period which is necessary for flowering in Xanthium, consists of two short dark periods. Most LD plants will flower when so treated.

Alternatively, the SD plants may be called long night plants and LD plants as short night plants. While the length of dark period determines initiation of floral primordia, the length of photoperiod determines their number. In Glycine, the optimum Response is obtained with a photo-cycle comprising 16 hours of darkness and 11 hours of light, respectively.

Table 22-1 gives selective list of some important short day (SD) and long day plants (LD):

Table 22-1. A list of some important SD and LD plants.

Short day plants (SD)	Day Neutral plants	Long day plants (LD)
Cannabis sativus	*Cucumis sativus*	*Lactuca sativa*
Nicotiana tabacum		*Allium cepa*
Xanthium strumarium		*Hyoscyamus niger*
Helianthus tuberosus		*Daucus caryota*
Gossypium hirsutum		*Beta vugaris*
Cosmos bipinnatus		*Spinacea oleracea*
Coffea arabica		*Hordeum vulgare*
Glycine max		*Triticum aestivum*
Oryza sativa		*Raphanus sativus*
Zea mays		*Trifolium species*
Saccharum officinarum		*Pisum sativum*
		Petunia hybrida

Perception of Photoperiodic Stimulus:

In spinach, a LD plant, it was shown that the leaves are the receptors of the photoperiodic stimulus. It was assumed that something was produced in the leaves in response to photo-inductive cycle and then it was translocated to the apical bud where it initiated flower primordia. The experiments with flowering clearly illustrate this point. If a single leaf of Xanthium is exposed to short photoperiod (16 HD + 8 HL) while the rest of the plant is subjected to a long photoperiod there is formation of flowers. Grafting of photo-induced leaves from one plant to the one with non-inductive cycle also promotes flowering on the receptor plant. The receptor plant is defoliated before the initiation of the experiment to eliminate the antagonistic effect of the natural leaves.

Phytochrome:

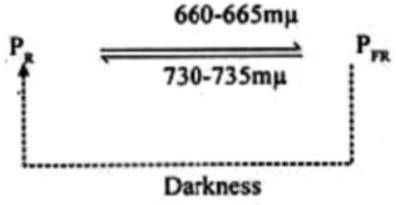
It has already been seen that a brief exposure with red light during critical dark period inhibits flowering in short-day plants and this inhibitory effect can be reversed by a subsequent exposure with far-red light. Similarly, the prolongation of the critical light period or the interruption of the dark period stimulates flowering in long-day plants. This inhibition of flowering in short-day plants and the stimulation of flowering in long-day plants involves the operation of a proteinaceous pigment called as phytochrome.

i. The pigment phytochrome exists in two different forms:
(i) Red light absorbing from which is designated as P_R and
(ii) Far-red light absorbing form which is designated as P_{FR}.

ii. These two forms of the pigment are photo chemically inter convertible.

iii. When P_R form of the pigment absorbs red light (660-665mp), it is converted into P_{FR} form.

iv. When P_{FR} form of the pigment absorbs far-red light (730-735mp), it is converted into P_R form.



v. The P_{FR} form of the pigment gradually changes into P_R form in dark.

It is considered that during the day the P_{FR} form of the pigments is accumulated in the plant which is inhibitory to flowering in short-day plants but is stimulatory in long–day plants. During critical dark period in short-day plants, this form gradually changes into P_R form resulting in flowering.

Light Quality and Phytochrome:

If a long light of a photo-inductive cycle for Xanthium is interrupted by a brief flash of light the plant does not flower. The most effective radiation in light break reactions is the red light. Its effect is reversed by far-red light suggesting participation of the phytochrome pigment. The phytochrome exists in two forms, one red absorbing form (Pr) and the other far-red absorbing (Pfr).

According to Borthwick, the far-red absorbing form of phytochrome (Pfr) accumulates in the plant during the day (white light). It is stimulatory to flowering in LD plants and inhibitory to flowering in SD plants.

At the onset of a dark period the Pfr is converted to Pr form which is stimulatory to flowering in SD plants and inhibitory to flowering in LD plants. The interruption of the dark period with red light will return the accumulated Pr form to Pfr form, thus inhibiting flowering in SD plants. If the red light break is followed by a far-red break the red light influence is reversed, since Pfr is converted to pr form, and flowering occurs (Fig 22-5).

The role of red and far-red light on the initiation of flowering in the LD and SD plants is shown in Fig. 22-6. It may be said that the precise role of phytochrome in flower initiation is not clear.

However, it may be added that this pigment is not the flowering stimulus but possibly aids the stimulation of flowering stimulus.

The effect of phytochrome on the flowering of SD plants may be explained as follows. At the end of light period Pfr is in high concentration and Pfr and Pr ratio is such that it prevents formation of flowering stimulus. If the dark period is prolonged, then either Pfr is destroyed or it is reverted to Pr state. At this stage the ratio of Pfr to Pr is such which triggers processes leading to formation of flowering stimulus. Under the situation where dark period is briefly interrupted by red light, there is formation of Pfr from Pr and the ratio of two is altered and therefore formation of flowering stimulus is prevented.

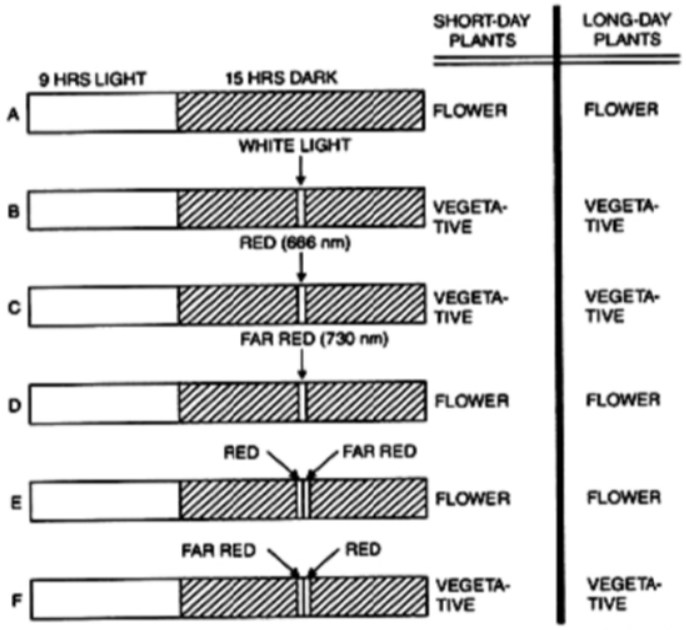


Fig. 22-6. The effect of red and far-red light during the night break on the flowering of LD and SD plants. The light is obtained either from sunlight or high-intensity radiation. Observe contrasting effects of two spectral qualities of light on SD and LD plants.

In the LD plant the role of Phytochrome can be explained as follows long day plants need high ratio of Pfr to Pr to trigger flowering stimulus. This ratio is attained at the end of a long day. Once the night is long then Pfr is reversed back to Pr and the formation of flowering stimulus is prevented.

Under the conditions where night is interrupted by a red light, there Pr is changed to Pfr. Here the ratio of the two pigments is high and flowering stimulus is produced. These explanations, however, fail to provide explanation for the initiation of flowering stimulus as mediated by phytochrome.

Figure 22-7 shows a possible hypothesis regarding photoinduction of a short day plant. According to Hess (1975), day light has more red than far-red light and therefore, concentration of P_{730} in the leaf is high and causes 'chemical inhibition' of flowering hormone. In the absence of light, P_{730} is converted into P_{660}.

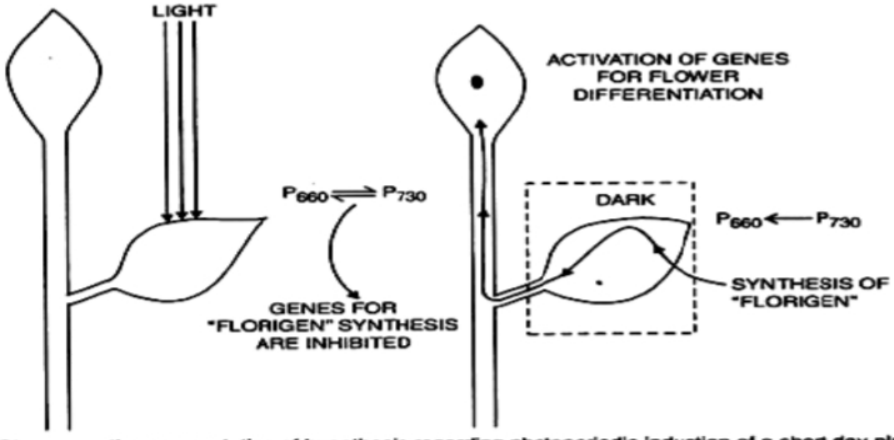


Fig 22.7. Diagrammatic representation of hypothesis regarding photoperiodic induction of a short day plant. Notice synthesis of florigen in the dak and its translocation to the apex for the activation of genes for flower differentiation.

The continued absence of light leaves very little of P_{730} and consequently synthesis of flowering hormone begins. Since antimetabolites inhibit flowering and GA promotes it, a working explanation has been proposed. It is believed that in plants exposed to inductive dark period more of "florigen" is synthesized in the leaf and this is translocate through phloem to the shoot apical flower.

GA and the flowering response. The application of GA to most LD plants will cause them to flower when on a non-inductive cycle but it has no effect on SD plants on a non-insuctive cycle.

Brian has suggested the role of GA in a scheme explaining photoperiodic reactions. According to him, a "GA-like" hormone is produced during the photoperiod as follows:

Red light promotes conversion of the precursor to the GA-like-hormone. During the dark the hormone is reconverted to the precursor. This back reaction is accelerated by far-red light. The GA-like hormone is then converted to florigen.

The steps of conversion are different in LD and SD plants. In LD plant a high level of GA-like hormone is required for the production of florigen. In SD plants, a low level of the GA- like hormone is optimum for a flowering response. However, if enough florigen is produced flowering will occur in both the LD and SD plants.

Genetic Approach to Photoperiod:

The basic approach is to identify the mutant that influences timing at any level, then the wild type gene is isolated and its gene product is analysed for clues to its role in the timing mechanism. In fact in several plant breeding programmes, flowering genes were also sought after. In crop species it is highly desirable to select early flowering photoperiod insensitive genes.

In pea and wheat, genetics of photoperiodic processes have received much attention. Incidentally, both the species are qualitative long-day plants. In pea several genes which affect photoperiodic timing and the onset of flowering have been identified, fsd (flowering short day) is a recessive mutant and makes the plant behave as a qualitative SD plant. When mutant is grafted to a wild-type stock under LD, the mutant will flower.

Recently approach in flowering genes has been made in Arabidopsis where several genes have been identified. This plant is a quantitative LD plant with a critical photoperiod of 8 to 10 hours. Under LD, it flowers with 4 to 7 leaves in the rosette. Under SD, flowering is delayed until 20 leaves are formed.

Flowering is also enhanced when the plant is exposed to blue or FR light suggesting the role of phytochrome in photoperiod phenomenon. Search is made to locate and isolate early and late flowering mutants. Here the flowering time refers to the number of rosette leaves produced before the flowering stem appears.

Incidentally, the mutants that affect phytochrome also affect flowering, hyl mutant is defective in the synthesis of the phytochromechromophore. Hence such mutants show an elongated hypocotyl but they flower earlier than the wild type under both LD and SD. Since the mutants flower under both conditions, they still show a response to photoperiod.

Several mutants which are insensitive to photoperiod and flower either earlier or later than the wild type plants have been identified, elf-3(early flower (-ing 3)—This is an early flowing type. On the contrary co-constants and gi-gigantea are later flowering, day-length insensitive mutants. They flower under LD conditions but SD has no effect.

Grafting experiments have shown that wild type genes, GI, operates earlier than CO gene in the same pathway and the floral promotion under LD depends on the amount of COmRNA transcribed. This

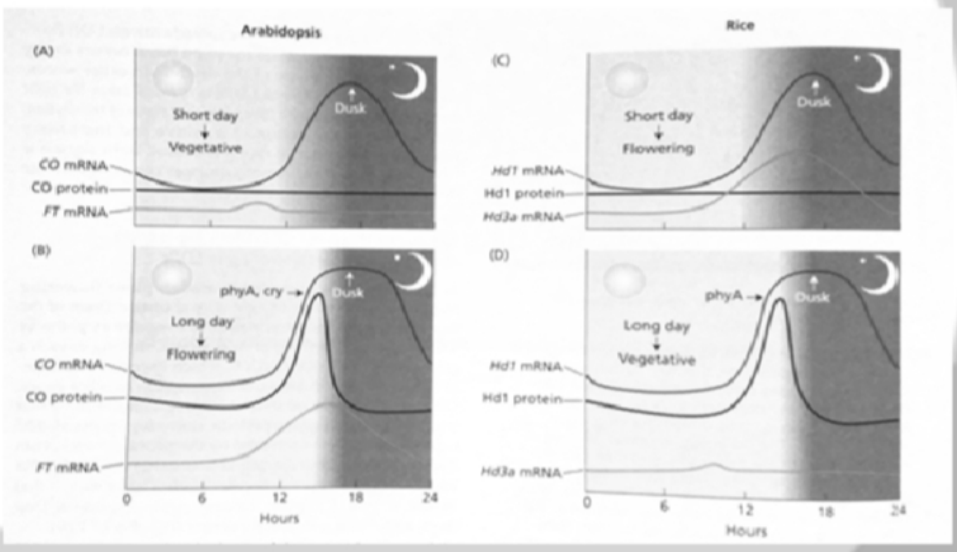
mutant is also of special attention since it appears to interact with endogenous clock. elf3 gene affects leaf movement, CAB gene expression besides advancing flowering.

Through traditional plant physiology techniques photoperiodic signal transduction chain and endogenous clock proved difficult to investigate, the molecular genetic approach has provided insight into mechanisms that have eluded researchers for more than seven decades. There are many other instances where modern approaches have solved several of the plant physiology enigmas.

COINCIDENCE MODEL

❖ **In the coincidence model, flowering is induced in both SDPs and LDPs when light exposure is coincident with the appropriate phase of the oscillator.**

❖ **CO (in Arabidopsis) and Hd1 (in Rice) regulate flowering by controlling the transcription of floral stimulus genes.**

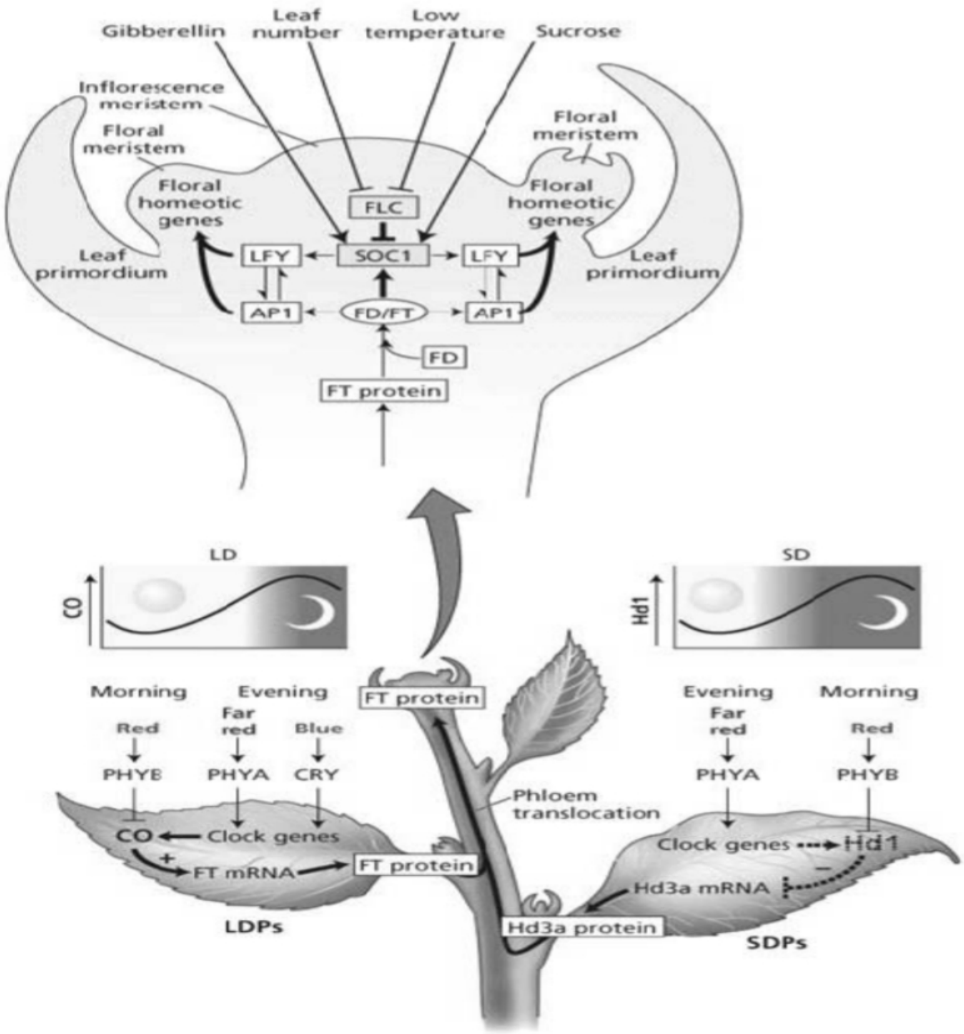


Long-distance signaling involved in flowering

In photoperiodic plants, **a long-range signal** is **transmitted in the phloem** from **leaves** to the **apex** , permitting **floral evocation**.

The discovery of florigen

- **FT** is a small, globular protein that exhibits the **properties** that would be **expected of florigen.**
- **Ft protein moves via the phloem** from the leaves to the shoot apical meristem under inductive photoperiods. In the meristem, **FT forms a complex with the transcription factor FD** forms a complex with the transcription factor **FD** to **activate floral identity genes**.
- **The four distinct pathways** that control flowering coverge to increase the expression of key regulators: **FT** in the vasculature and **SOC1,LFY, and AP1** in the meristem.



Gibberellins and the Flowering Response:

It is now well known that the gibberellins can induce flowering in long-day plants even under non-inductive short days. It is also definite that the gibberellins alone do not constitute the 'florigen', but it is usually held that the gibberellins are in some way connected with the overall process of flowering.

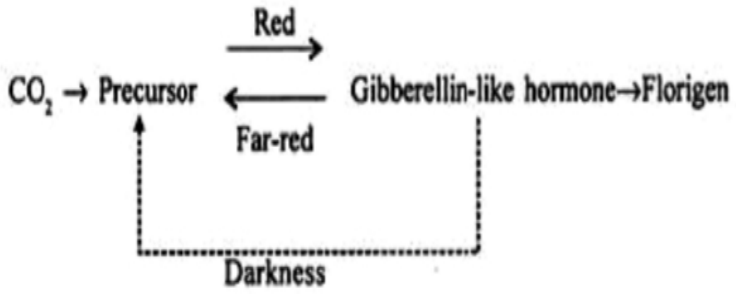
According to a scheme proposed by Brian (1958), a gibberellin like hormone is produced in the leaves during the photoperiod somewhat as follows:

CO_2 → Precursor (P) → Gibberellin-like hormone

The precursor may be slightly stimulatory or inactive or antagonistic to the gibberellin-like hormone. Red irradiations promote the conversion of the precursor to the gibberellin-like hormone. In the dark there is a slow reconversion of the gibberellin-like hormone to the precursor.

This reconversion is accelerated by far-red irradiations. It is further presumed that high concentration of the gibberellin-like hormone leads to the synthesis of florigen in long-day plants. In short-day plants the synthesis of florigen takes place when the level of gibberellin-like hormone is low. But, flowering eventually follows once the florigen synthesis has taken place in both the cases.

The whole scheme is diagrammatically shown below:



Importance of Photoperiodism:

(i) The knowledge of the phenomenon of photoperiodism has been of great practical importance in hybridisation experiments.

(ii) Although the floral hormone 'florigen' has not yet been isolated, the isolation and characterization of this hormone will be of utmost economic importance.

(iii) The phenomenon of photoperiodism is an excellent example of physiological preconditioning (or after-effect) where an external factor (i.e., the photoperiodic stimulus) induces some physiological changes in the plant the effect of which is not immediately visible. It lingers on in the plant and prepares the latter for a certain process (i.e., flowering) which takes place at a considerably later stage during the life history of the plant.

Vernalization:

Many plants do not come to flower before they experience a low temperature. These plants remain vegetative during the warm season, receive low temperature during winter, grow further and then bear flowers and fruits. Requirement of low temperature prevents precocious reproductive development in autumn.

It allows the plant to reach vegetative maturity before reproduction can occur. The condition occurs in winter varieties of some annual food plants (e.g., Wheat, Barley, and Rye), some biennial (e.g., Cabbage, Sugar beet, Carrot) and perennial plants (e.g., Chrysanthemum). The annual winter plants also possess spring varieties. The spring varieties are planted in spring. They come to flower and bear fruits prior to end of growing season. If the winter varieties are sown similarly, they fail to flower and produce fruits before the end of growing season. They are planted in autumn, form seedlings in which form they cover winter. The seedlings resume growth in spring. They bear flowers and fruits in summer.

7. Senescence and programmed cell death: Types of senescence, metabolic changes associated with senescence and its regulation, influence of hormones.

Senescence is the process of aging in plants. Plants have both stress-induced and age-related developmental aging. Chlorophyll degradation during leaf senescence reveals the carotenoids, and is the cause of autumn leaf color in deciduous trees. Leaf senescence has the important function of recycling nutrients, mostly nitrogen, to growing and storage organs of the plant. Unlike animals, plants continually form new organs and older organs undergo a highly regulated senescence program to maximize nutrient export. The plants or their organs like all other living organisms have a certain span of life during which they develop, grow, attain maturity and after some time at the end die. But prior to death, distinctive but natural deteriorative processes occur in them in growth and synthetic activities. These deteriorative processes that naturally terminate their functional life are collectively called as senescence and the plants or plant organs at this stage are called as senescent. These deteriorative processes may terminate in death either gradually or abruptly depending upon the plant. Senescence is a normal energy dependent developmental process which is controlled by plants own genetic programme and the death of the plant or plant part consequent to senescence is called as programmed cell death (PCD). Senescence is not confined only to whole plant. It may be limited to a particular plant organ such as leaf and flowers or cells such as phloem and xylem or cell-organelles such as chloroplasts and mitochondria etc. Senescence is closely associated with the phenomenon of aging and both are sometimes considered as the same by many workers. But according to Medawar (1957), the term senescence should be used to refer to natural changes towards termination of life while 'aging' to refer to changes in time without reference to the natural development of death.

Types of senescence:

Leopold (1961) has recognised 4 types of senescence patterns in whole plant (Fig17.41) which are as follows:

1. Overall Senescence:

This type of senescence occurs in annuals where whole of the plant is affected and dies.

2. Top Senescence:

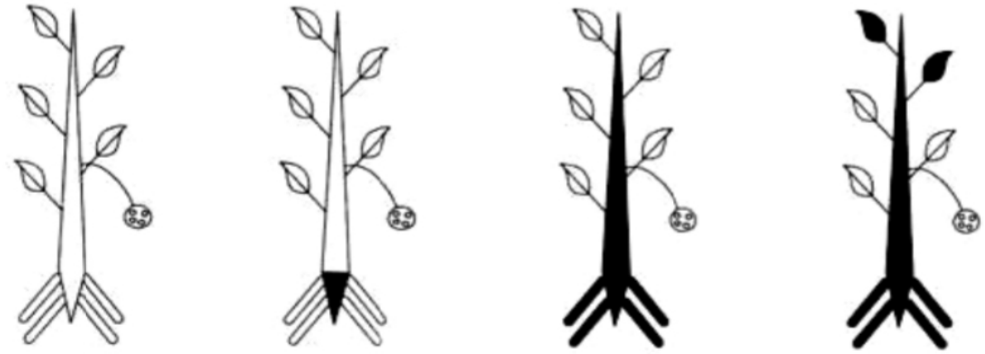
This is represented by perennial herbs where senescence occurs only in the above ground parts, the root system and underground system remaining viable.

3. Deciduous Senescence:

This type of senescence is less drastic and takes place in woody deciduous plants. Here senescence occurs in all the leaves simultaneously but the bulk of the stem and root system remains alive.

4. Progressive Senescence:

This is characterized by gradual progression of senescence and death of leaves from the base upwards as the plant grows. (The senescence of the entire plant after a single reproductive cycle is also known as monocarpic senescence) Senescence can best be studied in leaves or similar other organs of plants e.g., cotyledons, sepals, petals etc. or cell organelles like isolated chloroplast.



Some of the metabolic changes recognized so far are given briefly here:

i. Senescing cells and tissues are metabolically very active and an ordered series of cytological and biochemical events occur during senescence.

ii. Senescence is characterized by increased respiration, declining photosynthesis and an orderly disintegration of macromolecules.

iii. At the cellular level, chloroplasts are the first organelles to be disintegrated. Nuclei remain structurally and functionally intact until the last stage of senescence. Meanwhile, other cell organelles and bio-membranes also gradually deteriorate.

iv. Expression of senescence down-regulated genes (SDGs) decreases. Such genes encode proteins in photosynthesis and other biosynthetic processes. Concentration of growth promoting hormones especially cytokinins decline.

v. Expression of senescence associated genes (SAGs) increases. Such genes encode hydrolytic enzymes such as proteases, ribonucleases and lipases as well as enzymes involved in biosynthesis of deteriorative hormones such as abscisic acid (ABA) and ethylene.

vi. Some of the SAGs have secondary functions in senescence that are useful to plant. These genes encode enzymes that are involved in conversion and remobilization of nutrients and substrates from senescing tissues and their reallocation to other parts of the plant that survive (i.e., not senescing).

vii. Brilliant Colors are developed in leaves of many plants during senescence.

viii. This is due to degradation of chlorophylls, resulting in unmasking of more stable carotenoid pigments.

ix. Towards the end of senescence, the cells and tissues also lose respiratory control.
x. It is marked by general decline in ATP synthesis and chloroplast activities.
xi. Decrease in DNA and RNA
xii. Semi permeability of cytoplasmic membranes in the cells starts declining.
xiii. Impairment of the process of formation of new cells to replace the old and dead cells.
xiv. Due to anomalies in protein synthesis caused by senescence chromosomal aberrations and gene mutations may occur in the body cells which may be fatal.

Several environmental factors especially those which suppress normal plant growth also tend to enhance the rate of senescence. These are deficiency of soil nutrients, high temperatures, water deficit, darkness etc. In many plants on the other hand, removal of flowers, fruits and vegetative growing points can markedly delay the senescence of leaves. The role of externally supplied cytokinins in delaying senescence especially in detached plant parts is also well established.

Regulation of Senescence Process in Plants:

Senesce processes are tightly controlled processes in which the sequence of events is usually highly ordered until the terminal stages (death) are underway. Various factors which control the processes of senescence in different organs during life cycle of a flowering plant are given below.

It is evident from these details that at all stages a wide range of growth substances appears to be involved:

(a) Senescence of root: which also includes senescence of vascular tissues, root cap and hairs, is regulated by auxins and cytokinins.
(b) Senescence of cotyledons: is regulated by axis, shoot, light fruit, root and all hormones.
(c) Senescence of leaf: which also includes senescence of abscission zone, is regulated by fruit, root, light, all hormones and ethylene.
(d) Senescence of stem: which also includes senescence of vascular tissues, is regulated by auxins, cytokinins and sugars.
(e) Senescence of fruit: which also includes senescence of seeds is controlled by all hormones and ethylene.
(f) Senescence of flower: is regulated by pollination, ABA (Abscisic acid), cytokinins and ethylene.
(g) Senescence of apex: is regulated by fruits, day-length and gibberellins.
(h) Senescence of whole plant (monocarpism): is regulated by fruits, roots, day-length, auxins, ABA (Abscisic acid) and cytokinins.

Hormonal influence of senescence:

Programmed senescence seems to be heavily influenced by plant hormones. The hormones abscisic acid, ethylene, jasmonic acid and salicylic acid are accepted by most scientists as promoters of senescence, but at least one source lists gibberellins, brassinosteroids and strigolactone as also being involved. Cytokinins help to maintain the plant cell and expression of cytokinin biosynthesis genes late in development prevents leaf senescence.A withdrawal of or inability of the cell to perceive cytokinin may cause it to undergo apoptosis or senescence. In addition, mutants that cannot perceive ethylene show delayed senescence. Genome-wide comparison of mRNAs expressed during dark-induced senescence versus those expressed during age-related developmental senescence demonstrate that jasmonic acid and ethylene are more important for dark-induced (stress-related) senescence while salicylic acid is more important for developmental senescence.

Annual versus perennial benefits

Some plants have evolved into annuals which die off at the end of each season and leave seeds for the next, whereas closely related plants in the same family have evolved to live as perennials. This may be a programmed "strategy for the plants.

The benefit of an annual strategy may be genetic diversity, as one set of genes does continue year after year, but a new mix is produced each year. Secondly, being annual may allow the plants a better survival strategy, since the plant can put most of its accumulated energy and resources into seed production rather than saving some for the plant to overwinter, which would limit seed production. Conversely, the perennial strategy may sometimes be the more effective survival strategy, because the plant has a head start every spring with growing points, roots, and stored energy that have survived through the winter. In trees for example, the structure can be built on year after year so that the tree and root structure can become larger, stronger, and capable of producing more fruit and seed than the year before, out-competing other plants for light, water, nutrients, and space. This strategy will fail when environmental conditions change rapidly. If a certain bug quickly takes advantage and kills all of the nearly identical perennials, then there will be a far lesser chance that a random mutation will slow the bug compared to more diverse annuals.

Plant self-pruning

There is a speculative hypothesis on how and why a plant induces part of itself to die off. The theory holds that leaves and roots are routinely pruned off during the growing season whether they are annual or perennial. This is done mainly to mature leaves and roots and is for one of two reasons; either both the leaves and roots that are pruned are no longer efficient enough nutrient acquisition-wise or that energy and resources are needed in another part of the plant because that part of the plant is faltering in its resource acquisition.

•Poor productivity reasons for plant self pruning – the plant rarely prunes young dividing meristematic cells, but if a fully grown mature cell is no longer acquiring nutrients that it should acquire, then it is pruned.

oShoot efficiency self pruning reasons – for instance, presumably a mature shoot cell must on average produce enough sugar, and acquire enough oxygen and carbon dioxide to support both it and a similar sized root cell. Actually, since plants are obviously interested in growing it is arguable, that the "directive" of the average shoot cell, is to "show a profit" and produce or acquire more than enough sugar and gases than is necessary to support both it and a similar sized root cell. If this "profit" isn't shown, the shoot cell is killed off and resources are redistributed to "promising" other young shoots or leaves in the hope that they will be more productive.

oRoot efficiency self pruning reasons – similarly a mature root cell must acquire on average, more than enough minerals and water needed to support both it and a similar sized shoot cell that does not acquire water and minerals. If this does not happen, the root is killed off and resources sent to new young root candidates.

•Shortage/need-based reason for plant self pruning – this is the other side of efficiency problems.

oShoot shortages – if a shoot is not getting enough root derived minerals and water, the idea is that it will kill part of itself off, and send the resources to the root to make more roots.

oRoot shortages – the idea here is that if the root is not getting enough shoot derived sugar and gases it will kill part of itself off and send resources to the shoot, to allow more shoot growth.

This is an oversimplification, in that it is arguable that some shoot and root cells serve other functions than to acquire nutrients. In these cases, whether they are pruned or not would be "calculated" by the plant using some other criteria. It is also arguable that, for example, mature nutrient-acquiring shoot cells would have to acquire more than enough shoot nutrients to support both it and its share of both shoot and root cells that do not acquire sugar and gases whether they are of a structural, reproductive, immature, or just plain, root nature.

The idea that a plant does not impose efficiency demands on immature cells is that most immature cells are part of so-called dormant buds in plants. These are kept small and non-dividing until the plant needs them. They are found in buds, for instance in the base of every lateral stem.

Theory of hormonal induction of senescence

There is little theory on how plants induce themselves to senesce, although it is reasonably widely accepted that some of it is done hormonally. Plant scientists generally concentrate on ethylene and abscisic acid as culprits in senescence, but neglect gibberellin and brassinosteroid which inhibits root

growth if not causing actual root pruning. This is perhaps because roots are below the ground and thus harder to study.

1. Shoot pruning – it is now known that ethylene induces the shedding of leaves much more than abscisic acid. ABA originally received its name because it was discovered to have a role in leaf abscission. Its role is now seen to be minor and only occurring in special cases.

oHormonal shoot pruning theory – a new simple theory says that even though ethylene may be responsible for the final act of leaf shedding, it is ABA and strigolactones that induces senescence in leaves due to a run away positive feedback mechanism. What supposedly happens is that ABA and strigolactones are released by mostly mature leaves under water and or mineral shortages. The ABA and strigolactones act in mature leaf cells however, by pushing out minerals, water, sugar, gases and even the growth hormones auxin and cytokinin (and possibly jasmonic and salicylic acid in addition). This causes even more ABA and strigolactones to be made until the leaf is drained of all nutrients. When conditions get particularly bad in the emptying mature leaf cell, it will experience sugar and oxygen deficiencies and so lead to gibberellin and finally ethylene emanation. When the leaf senses ethylene it knows its time to excise.

2. **Root pruning** – the concept that plants prune the roots in the same kind of way as they abscise leaves, is not a well discussed topic among plant scientists, although the phenomena undoubtedly exists. If gibberellin, brassinosteroid and ethylene are known to inhibit root growth it takes just a little imagination to assume they perform the same role as ethylene does in the shoot,that is to prune the roots too.

o**Hormonal root pruning theory** – in the new theory just like ethylene, GA, BA and Eth are seen both to be induced by sugar (GA/BA) and oxygen (ETH) shortages (as well as maybe excess levels of carbon dioxide for Eth) in the roots, and to push sugar and oxygen, as well as minerals, water and the growth hormones out of the root cell causing a positive feedback loop resulting the emptying and death of the root cell. The final death knell for a root might be strigolactone or most probably ABA as these are indicators of substances that should be abundant in the root and if they cannot even support themselves with these nutrients then they should be senesced.

3**. Parallels to cell division** – the theory, perhaps even more controversially, asserts that just as both auxin and cytokinin seem to be needed before a plant cell divides, in the same way perhaps ethylene and GA/BA (and ABA and strigolactones) are needed before a cell would senesce.

Senescence is the process of aging in plants. Plants have both stress-induced and age-related developmental aging. Chlorophyll degradation during leaf senescence reveals the carotenoids, and is the cause of autumn leaf color in deciduous trees. Leaf senescence has the important function of recycling nutrients, mostly nitrogen, to growing and storage organs of the plant. Unlike animals, plants continually form new organs and older organs undergo a highly regulated senescence program to maximize nutrient export.

The plants or their organs like all other living organisms have a certain span of life during which they develop, grow, attain maturity and after some time at the end die. But prior to death, distinctive but natural deteriorative processes occur in them in growth and synthetic activities.

These deteriorative processes that naturally terminate their functional life are collec¬tively called as senescence and the plants or plant organs at this stage are called as senes¬cent. These deteriorative processes may terminate in death either gradually or abruptly depending upon the plant. Senescence is a normal energy dependent developmental process which is controlled by plants own genetic programme and the death of the plant or plant part con¬sequent to senescence is called as programmed cell death (PCD).

Senescence is not confined only to whole plant. It may be limited to a particular plant organ such as leaf and flowers or cells such as phloem and xylem or cell-organelles such as chloroplasts and mitochondria etc. Senescence is closely associated with the phenomenon of aging and both are sometimes considered as the same by many workers. But according to Medawar (1957), the term senes¬cence should be used to refer to natural changes towards termination of life while ‘aging’ to refer to changes in time without reference to the natural development of death**.**

8. plant products in metabolism: structure and properties of carbohydrates,.lipids, amino acids, proteins, nucleic acids, secondary metabolites.

Carbohydrates are a group of organic compounds consisting of C, H, O usually in the ratio of 1: 2: 1 and include such well known compounds as sugars, starch, cellulose etc. Previously, the carbohydrates were regarded as hydrates of carbon and corresponded to general formula ($C.H_2O$)

But the group name 'carbohydrates' was sometimes found misleading because:

(i) Some organic compounds e.g., formaldehyde (HCHO), acetic acid (CH_3COOH), lactic acid ($C_3H_6O_1$) inositol ($C_6H_{12}O_6$) etc. correspond to the general formula but are not carbohydrates.

(ii) Some carbohydrates e.g., rhamnose ($C_6H_{12}O_5$), rhamnohexose ($C_7H_{14}O_6$), digitoxose ($C_6H_{12}O_4$) do not correspond to the above general formula.

(iii) Besides containing C, H and O, some carbohydrates also contain nitrogen and sulphur.

Therefore, the carbohydrates are more appropriately referred to as polyhydroxyaldehydes or polyhydroxyketones and their derivatives or the substances which yield these on hydrolysis. But, the group name 'carbohydrates' is still retained traditionally.

The metabolism of carbohydrates is of utmost importance to organisms individually and collectively. Fundamentally, all organic foodstuffs are ultimately derived from the synthesis of carbohydrates through photosynthesis. The catabolism of carbohydrates provides the major share of the energy requirement for maintenance of life and preformation of work. Moreover carbohydrates act as energy reservoirs and serve architectural functions and are important constituents of nucleic acids.

Classification of the Carbohydrates:

Depending upon their complexity and behaviour on hydrolysis, the carbohydrates are classified into following 3 categories (Fig. 13.1).

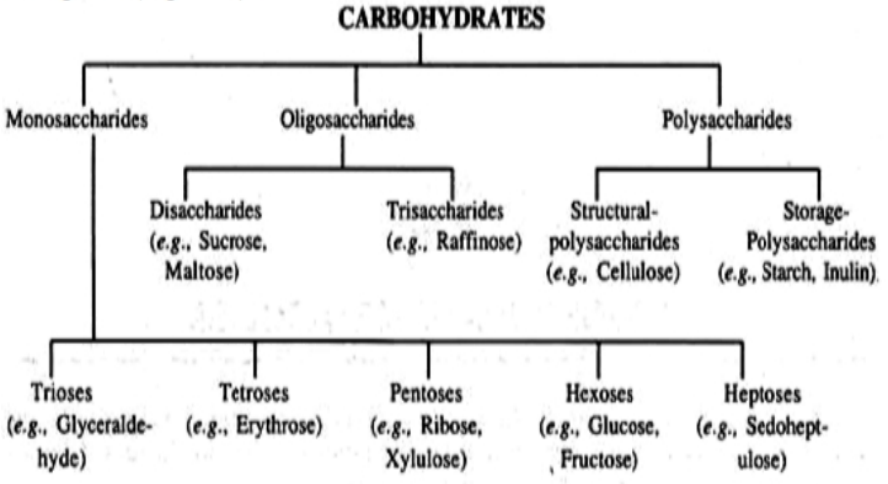


Fig. 13.1. Classification of the Carbohydrates

1. **Monosaccharide's (Simple sugars):**
2. i. These are simplest of carbohydrates and are known as sugars.
3. ii. These are the building units of complex carbohydrates.
4. iii. These cannot be hydrolysed.
5. iv. These are sweet-tasting, crystalline and soluble in water.
6. v. They have a potential aldehyde or keto group and hence, are reducing in nature.
7. vi. Aldehyde group or the reducing centre always lies at C No. 1 of the monosaccharide molecule. Such sugars are known as aldoses or aldose sugars.
8. vii. Monosaccharide's having keto group are known as ketoses or ketose sugars. In such sugars the keto group or the reducing centre always lies at C No. 2.

   Monosaccharides are polyhydroxyaldehydes or polyhydroxyketones containing 3-7 carbons. Monosaccharide's are the basic units, of carbohydrate which cannot be further hydrolyzed into simpler saccharides.

   On the basis of carbonyl group, monosaccharides are of two types i.e. aldoses (contain terminal aldehyde, —CHO) and ketoses (contain subterminal ketone, > C= O). Sugars with 3,4,5,6 and 7 carbon atoms are called trioses, tetroses, pentoses, hexoses and heptoses respectively.

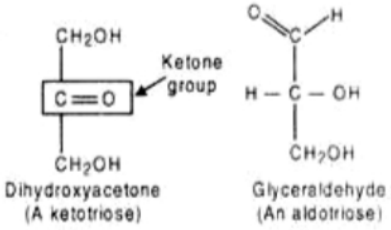
   Depending upon the number of the C atoms, the monosaccharide's are further classified as follows:

   (i) Triose Sugars, $C_3H_6O_3$ (e.g., glyceraldehyde, dihydroxyaeetone)

   (ii) Tetrose Sugars, $C_4H_8O_4$ (e.g., erythrose)

   (iii) Pentose Sugars, $C_5H_{10}O_5$ (e.g., ribose, ribulose, xylose, xylulose, arabinose).

Carbon atoms	Aldoses	Ketoses
Trioses, $(CH_2O)_3$ –	Glyceraldehyde	Dyhydroxyacetone
Tetroses, $(CH_2O)_4$ –	Erythrose, Threose	Erythrulose
Pentoses, $(CH_2O)_5$ –	Ribose, Arabinose, Xylose, Lyxose	Ribulose, Xylulose
Hexoses, $(CH_2O)_6$ –	Glucose, Mannose, Galactose	Talose, Fructose
Heptoses, $(CH_2O)_7$ –	Glucoheptose, Galactoheptose	Sedoheptulose



   (iv) Hexose Sugars, $C_6H_{12}O_6$ (e.g., glucose, fructose, galactose mannose).

   (v) Heptose Sugars, $C_7H_{14}O_7$ (e.g., sedoheptulose).

Properties of monosaccharides:

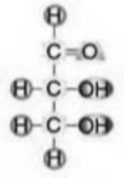
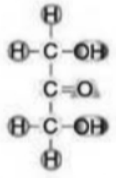
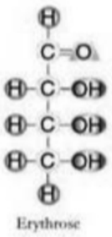
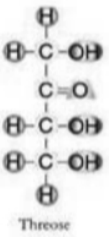
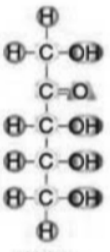
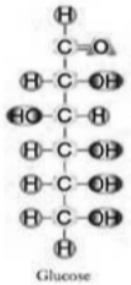
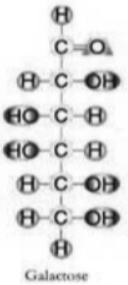
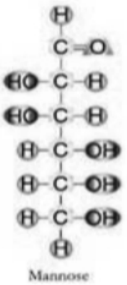
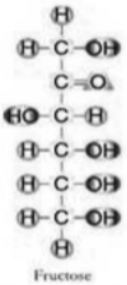
Physical properties:

All monosaccharides are colourless, crystalline, water soluble, sweet in taste and low MW organic compounds.

1. Isomerism:

Except dihydroxyacetone, all monosaccharides have asymmetric or chiral carbon atoms (carbon atoms attached to four different atoms). This allows the formation of stereoisomers or in short, isomers. Stereoisomers have the same structural formula but differ in spatial configuration. The number of possible isomers depends on the number of asymmetric carbon atoms (n) and is equal to 2^n. For glucose n = 4, so there are 16 isomers. Ketoses have one less asymmetric carbon atom than do aldoses with same number of carbon atoms.

Depending upon the number of carbon atoms, aldoses and ketoses are further classified as:

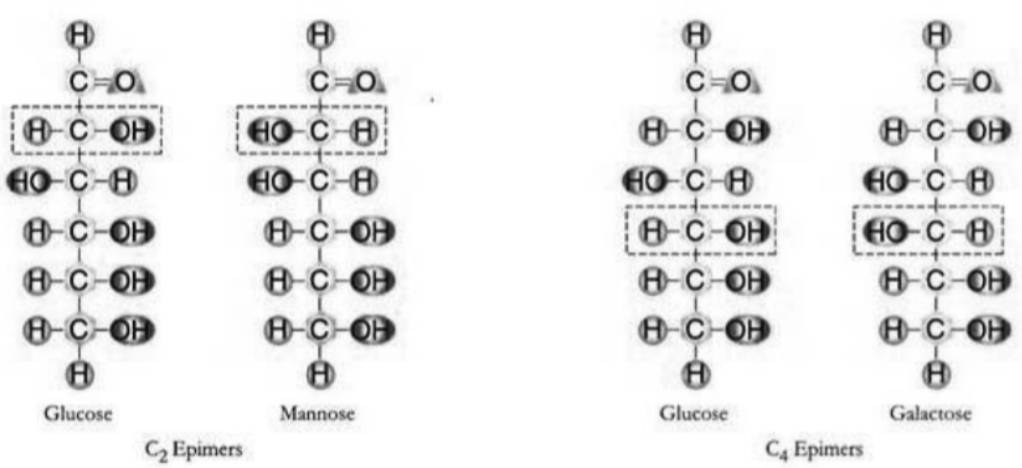
ALDOSE SUGAR	KETOSE SUGAR
(a) Trioses: Sugars containing three carbon atoms	
 Glyceraldehyde	 Dihydroxyacetone
(b) Tetroses: Sugars containing four carbon atoms	
 Erythrose	 Threose
(c) Pentoses: Sugars containing five carbon atoms	
 Ribose	Others 2-Deoxyribose Arabinose Xylose Lyxose
	 Ribulose
(d) Hexoses: Sugars containing six carbon atoms	
 Glucose  Galactose  Mannose	 Fructose

(e) Heptoses: Sugars containing seven carbon atoms: An aldo-heptose is sedoheptose and a keto-heptose is sedoheptulose.

The important isomers of monosaccharide's are as follows:

(i) Epimers:

When sugars differ only in the configuration around one specific carbon atom they are called epimers. e.g., Glucose and mannose are epimers at C_2 whereas glucose and galactose are epimers at C_4.



(ii) D- and L-isomers:

D-sugars and L-sugars are optical isomers. In biological systems all sugars are found in D-forms. The optical activity is due to the asymmetric carbon atoms which can be able to rotate the plan of polarized light to either left (levorotatory) or right (dextrorotatory). Hence the mono saccharides are two types i.e. L-sugars and D-sugars. The D-sugars can be represented by the orientation of-OH group of asymmetric carbon farthest from aldehyde or ketone group is on right. When it is on left, it is a member of L-sugars. As the D-sugars are mirror images of L-sugars, they are called enantiomers. For example, D- and L-glycerol, D- and L- glucose, etc.

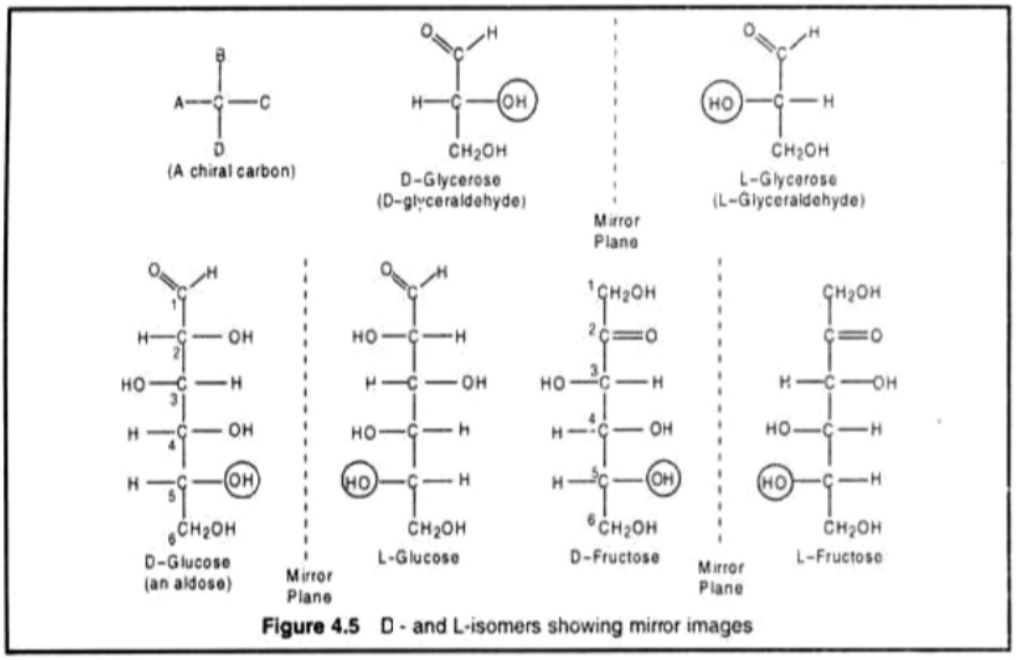


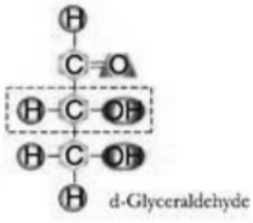
Figure 4.5 D - and L-isomers showing mirror images

(iii) Enantiomers:

Non super-imposable mirror images are known as enantiomers. e.g., D and L sugars.

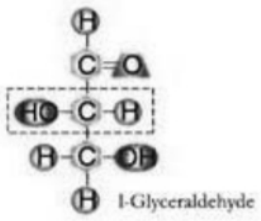
Explanation:

When white light (which is a mixture of different wavelengths) is passed through a Nichol prism, then the emerging light will be of a single wavelength and this is known as plane polarized light. When this plane polarized light is passed through a solution containing carbohydrate, the light is deflected either towards the right or left, which depends upon the configuration of atoms around the chiral centre.



When the solution containing glyceraldehyde with the configuration in the figure given below around the chiral centre is taken, wherein the —OH group on the asymmetric carbon atom is towards right, when written on paper in the straight line projection form, then the light is deflected towards right. Hence this glyceraldehyde is known as dextrorotatory sugar or compound and is designated as d-sugar (+ sugar).

When the solution containing glyceraldehyde with the following configuration around the chiral centre is taken, wherein the —OH group on the asymmetric carbon atom is towards left, when written on paper in the straight line projection form, then the plane polarized light is rotated towards left. Hence this glyceraldehyde is known as levorotatory sugar or compound and is designated as l-sugar (— sugar).

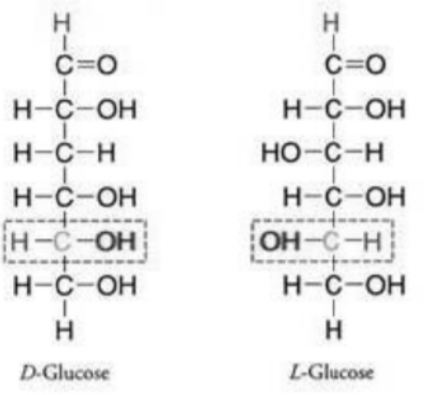


Glyceraldehyde has only one chiral centre or asymmetric carbon atom, whereas tetroses, pentose's and hexoses have more than one chiral centre. In such cases the rotation of the plane polarized light is dependent upon many factors, viz., the configuration around each of the chiral centre, the solvent in which it is present, temperature, etc.

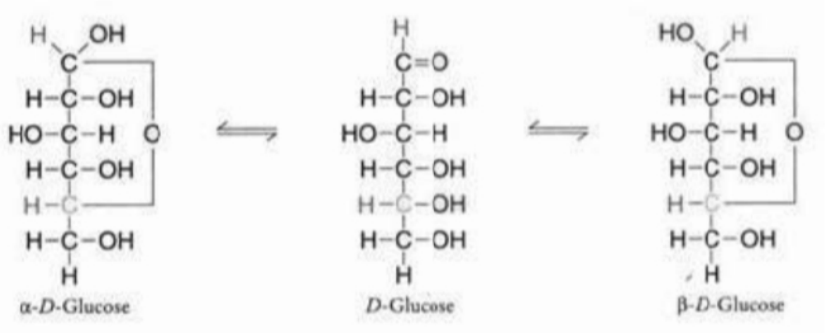
Under such circumstances there is no relation between the configurations of the sugar to the rotation of the plane polarized light. Hence other compounds (sugars, amino acids, etc.) are grouped into two categories namely D series compounds and L series compounds.

D series compounds are those compounds that contain the reference group on the right side of the last chiral centre from the functional group. If glucose is taken, the functional group is the aldehydic group (—CHO) and the chiral centre furthermost from it is the 5th carbon atom and the reference group -OH is present on the right side of the straight chain.

Hence it is known as D-glucose. This glucose may or may not be dextrorotatory. It may also be levorotatory. If this glucose is dextrorotatory then it is designated as D-(+)-glucose and if this glucose is levorotatory then it is designated as D-(-)-glucose.



L series compounds are those compounds that contain the reference group on the left side of the last chiral centre from the functional group. If the -OH group is present at the left side on 5th carbon of the straight chain form of glucose then it is known as L-glucose. This glucose may or may not be levorotatory. It may also be dextrorotatory. If this glucose is dextrorotatory, then it is designated as L-(+)-glucose and if this glucose is levorotatory then it is designated as L-(-)-glucose.



Racemic mixture:

A solution containing equal number of d (+) & l (-) forms of a sugar is known as a racemic mixture.

(iv) Anomers:

Sugars differing at the anomeric carbon atom are known as anomers.

Explanation:

When an aldehydic group (or carbonyl carbon) reacts with an alcoholic group, then it results in the formation of a hemiacetal. Carbohydrates contain both aldehydic (carbonyl) and alcoholic groups within the molecule. Hence it is possible that the aldehydic group present at the 1st carbon atom of the sugars can react with any of the alcoholic groups present on the other carbon atoms, thus resulting in the creation of an additional chiral centre at the 1st carbon atom and this chiral centre is now known as the anomeric carbon atom. Sugars differing at this anomeric carbon atom are known as anomers.

Two anomers for each of the sugars are possible. If the -OH group on the anomeric carbon atom is towards the right then it is known as alpha (α) anomer. If the —OH group on the anomeric carbon atom is towards the left, then it is known as beta (β) anomer or β-sugar.

(v) Pyranose and Furanose rings:

All sugars exist in linear o open-chain form. But in solution pentoses and nexoses cyclize into ring-forms. Ring-forms are two types, pyranose and furanose. Pyranose is 6-membered sugar ring similar to pyran with five carbon atom and one oxygen atom. While furanose is 5-membered sugar ring similar to furan with four carbon atom and one oxygen atom. The sugar rings are represented by Haworth projections. In Haworth projection (Haworth, 1928) the plane of sugar ring can be imagined as perpendicular to the plane of the paper, with the heavy line on the ring closest to the reader.

(iii) α-and β-anomers:

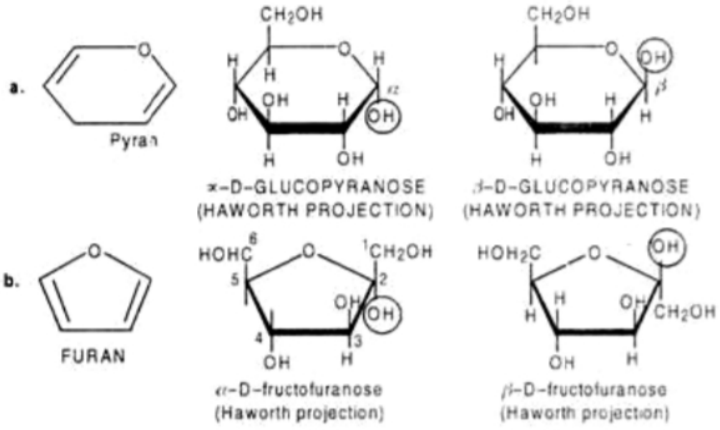
In a sugar ring an additional asymmetric carbon atom is created called anomeric carbon. For example, in glucose (aldose) C-l becomes anomeric and in fructose (ketose) C-2 becomes anomeric. The sugar isomers on anomeric carbon are called anomers. In α-anomer, the -OH group of anomeric carbon is written below and in β -anomer it is written above. The interconvertion of α- and β-anomers in solution is called mutarotation. In solution, glucose exist in 3 inter convertible forms- open-chain form (0.02%), α-D-fructofuranose (36%) and β- D-glucopyranose (67%).

Mutarotation:

Change in the specific rotation of an optically active compound without any change in its other properties is knows as mutarotation.

Explanation:

Glucose crystallized from cold water is α-D-glucose and it shows a specific rotation of $\{\alpha\}^{20}_{D}$ =



+112.2°. If it is dissolved in water, the specific rotation gradually changes with time and reaches a stable value of 52.7°.

This change in specific rotation is because α-D-glucose isomerizes to β-D-glucose via a straight chain intermediate and finally an equilibrium mixture of about 1/3rd of α-D-glucose, 2/3rds of β-D- glucose and a little of straight chain form is formed. This change in specific rotation is known as mutarotation.

Similarly, β-D-glucose, which can be obtained on crystallization from pyridine shows a specific rotation of $\{\alpha\}^{20}_{D}$ = +19°. When this is dissolved in water its rotation gradually changes and finals to 52.1°. This is again due to mutarotation and formation of α, β and straight chain forms of glucose in an equilibrium of 1/3: 2/3: 0/1 (n).

Chemical properties:

1.Reducing nature:

All monosaccharides are reducing. Disaccharides like lactose and galactose are also reducing, except sucrose. The reducing nature is due to presence of free aldehyde or ketone group which can reduce cupric ion (Cu^{2+}) to cuprous form (Cu^{+}).This is the basis of Benedict's test & Fehling's test for sugar detection in urine.

$C_6H_{12}O_6 + 2Cu(OH)_2$—» $C_6H_{12}O_7 + Cu_2O + H_2O$

Glucose Blue Gluconic acid red

In Toliens reagent (an ammonia solution), a reducing sugar reduce Ag^{+} of silver oxide (Ag_2OT txr a metallic silver (Ag) lining in the reaction vessel.

$C_6H_{12}O_6 + Ag_2O$—> $C_6H_{12}O_7 + 2Ag$

Silver oxide Silver

In alkaline medium, the aldehydic or ketonic group of sugars can reduce a number of substances (metals) like copper, silver, mercury and bismuth. Copper salts are reduced to cuprous hydroxide or oxide in solution. The sugars are identified in the urine and blood based upon this principle. Benedict's reagent is commonly used for the detection of sugars in urine.

(a) Reducing sugars:

Sugars having a free aldehydic or ketonic group are known as reducing sugars, e.g., glucose, fructose, galactose and all other monosaccharide's. Among disaccharides maltose and lactose are reducing sugars.

Benedict's test:

This is a semi quantitative test most commonly used for the detection of the percentage of sugar in urine. Benedict's test is carried out in a mild alkaline media. Hence weak reducing agents like uric acid and creatinine in urine cannot reduce Benedict's reagent. Therefore, this test is very specific for glucose or other reducing sugars in urine.

(b) Non-reducing sugars:

Sugars that do not have a free aldehydic or ketonic group are called as non-reducing sugars, e.g., sucrose and trehalose. Note: Though polysaccharides have at least one free aldehydic or ketonic group, but still they are non- reducing sugars owing to their larger molecular size and complexity of the structure. Hence the aldehydic or ketonic group is not available for the reducing action.

2.Glycosidic bond:

Glycosidic bond is a covalent bond formed by the condensation reaction between a sugar and the -OH group of a second compound which may or may not be the sugar. Glycosidic bonds are of two types i.e. O- glycosidic bond (-C-0- C) and N-Glycosidic bond (-C-N-C). Sugar can be joined to each other by O-glycosidic bonds to form disaccharides, oligosaccharides and polysaccharides. N-glycosidic bond formed when the anomeric carbon of a sugar liked to the nitrogen atom of an amine, as in nucleosides.

3. Formation of osazones:

Phenyl hydrazine reacts with reducing sugars to form osazones. It involves carbonyl carbon and the adjacent carbon. Osazone is a crystalline compound and is used as an identification test for sugars.

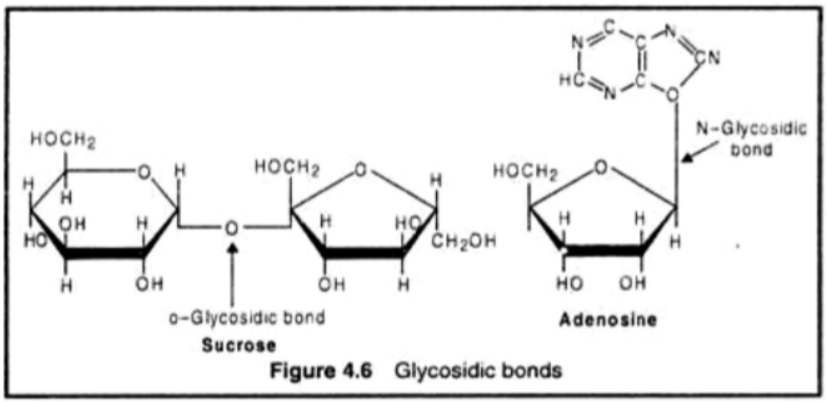
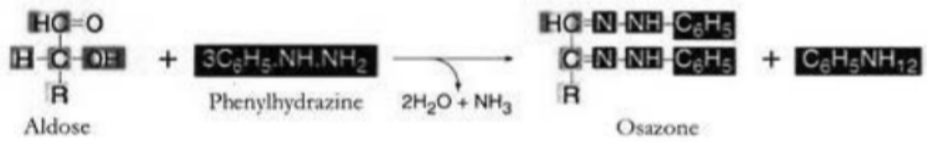


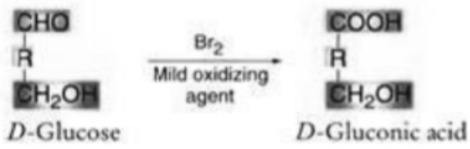
Figure 4.6 Glycosidic bonds



Fructose and glucose forms a broom stick shaped crystal in 3 and 5 minutes respectively. Maltose forms star shaped crystals in 20 minutes whereas lactose forms puff shaped crystals in 30 minutes time.

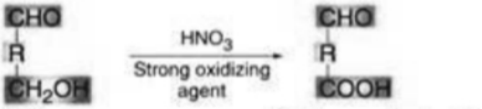
4. Oxidation of sugars:

(a) Mild oxidizing agent like bromine oxidizes the aldehydic group of carbohydrates converting it to an acid group.



D-Glucose *D*-Gluconic acid

(b) Strong oxidizing agent like nitric acid oxidizes the primary alcohol of the carbohydrates forming saccharic acids.

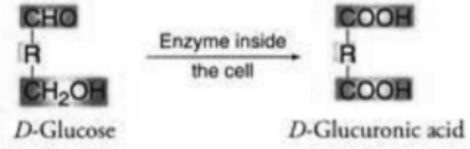


D-Glucose *D*-Glucosaccharic acid

Galactose forms mucic acid, which in insoluble in water. This forms an identification test for galactose known as mucic acid test.

(c) Enzymes:

Inside the cell, the enzymes oxidize both the aldehydic and primary alcoholic groups of the carbohydrates forming uronic acids.



D-Glucose *D*-Glucuronic acid

D-glucuronic acid is a component of structural materials like chondroitin sulphate, mucoitin sulphate and glycoproteins (proteoglycons). It plays an important role in detoxification of bile pigments. L-glucose forms ioduronic acid.

5. Dehydration with strong acids:

Concentrated H_2SO_4 removes the adjacent —OH groups as water (H_2O) forming furfural form pentose's and hydroxymethyl furfural from hexoses.

Furfural Hydroxymethyl Furfural

Furfural condenses with α-naphthol in presence of alcohol forming a purple violet coloured complex. This is the principle of Molisch's test which is a common identification test for all carbohydrates.

6. Derived sugars:

Substances formed from sugars on oxidation, reduction or addition/replacement of any group are called derived sugars.

(a) Amino sugars:

The hydroxyl group at the second carbon of a sugar is replaced by an amino group to form an amino sugar, e.g., glucosamine, galactosamine.

(b) Deoxy sugars:

These sugars are formed due to removal of one of the oxygen from the alcoholic group, e.g., 2-Deoxy-ribose, here the 'O' of the 2nd alcoholic group is removed. It is present in DNA. L-fucose is 6-Deoxy L-galactose, L-rhamnose is 6-Deoxy L-mannose.

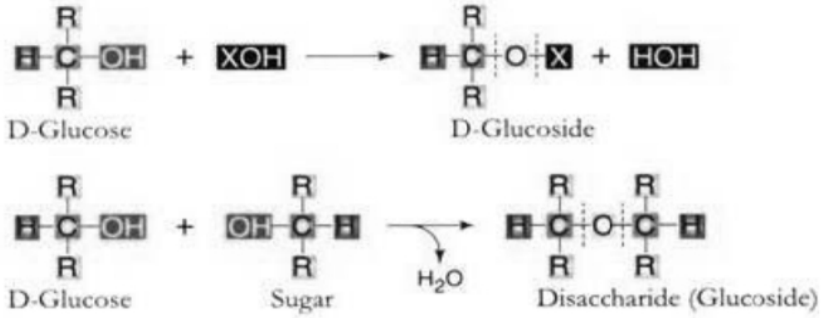
(c) Oxidation products of carbohydrates:

Uronic acids and saccharic acids are also derived sugars.

6. Formation of glycosides with alcohol:

When two alcoholic groups react with each other, a glycoside is formed. Carbohydrates contain many alcoholic groups. Hence two carbohydrates can react with alcoholic groups of one another sugars,

forming glycosides. Union of two carbohydrates is known as a disaccharide, three is trisaccharide and many is a polysaccharide.



Monosaccharide derivatives:

Some other important modified monosaccharide's are as follows-

(a) Deoxyribose:

The deoxygenation of ribose at C-2 position produces deoxyribose. It is important component of deoxyribonucleotides of DNA.

(b) Phosphorylated sugars:

The transfer of phosphorylated group from ATP to -OH group of a sugar produces phosphorylated sugar, e.g. glucose-6-phosphate. The phosphorylated sugars are anionic which prevent their escape from cell and also acts as reactive intermediates.

(c) Sugar alcohol:

It is formed by reduction of aldehyde and ketone group of sugar, e.g., mannitol, glycerol, sorbitol etc. Mannitol is a sugar alcohol in brown algae, some fruits and honey. It is used as a medicine for patients with brain edema. Glycerol required in fat synthesis. Sorbitol, a sugar alcohol commercially obtained from reduction of glucose and fructose, is used in diets of diabetics because it is absorbed very slowly and metabolized after conversion to glucose, but it does not raise blood sugar.

(d) Sugar acids:

Ascorbic acid or vitamin-C is a sugar acid required for collagen synthesis. Glucouronic acid and iduronic acid are components of mucopolysaccharides.

(e) Amino sugars (hexosamines):

Glucosamine forms chitin and hyaluronic acid.

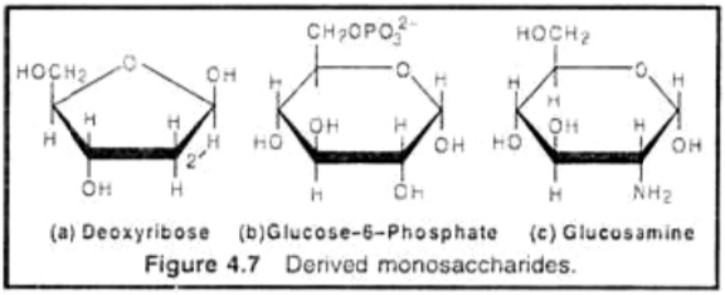


Figure 4.7 Derived monosaccharides.

2. Oligosaccharides:

i. These consist of more than one but fewer number of monosaccharide molecules joined together by glycosidic bonds.

ii. On hydrolysis, they yield the monosaccharide units which may be similar or dissimilar.

iii. These are also sweet tasting, crystalline, soluble sugars.

iv. These may or may not have a free -OH group at the reducing centre and accordingly may or may not be reducing.

Depending upon the number of the monosaccharide molecules which constitute them, the oligosaccharides are grouped in following categories:

(i) Disaccharides. $C_{12}H_{22}O_{11}$ (e.g., sucrose, maltose, lactose etc.)

(ii) Trisaccharides, $C_{18}H_{32}O_{16}$ (e.g., raffinose, gentianose etc.)

- **Disaccharides (Double sugars):**

Disaccharides are composed of 2 monosaccharide's linked by O-glycosidic bond.

The physiologically important disaccharides are:

Maltose (malt sugar) = Glucose + Glucose

Lactose (milk sugar) = Galactose + Glucose

Sucrose (cane sugar) = Glucose + Fructose

Maltose is a reducing disaccharide composed of two glucose units linked by α1,4-glycosidic bond. The reducing property of maltose is due to free aldehyde group of C-l of second glucose. Maltose is an enzymatic hydrolysis product of starch. It is formed in the germinating cereals by enzyme diastase (an amylase) and also formed during starch digestion by α-amylases of salivary and pancreatic juices.

Lactose is also a reducing disaccharide consists of galactose joined to glucose by a β 1,4 glycosidic bond. Its reducing nature is due to free aldehyde of C-l of glucose residue. Lactose present naturally in milk of mammals where its concentrations vary from 0-7%, depending on species.

Souring of milk occurs when bacteria convert lactose into lactic acid. Lactose is nutritionally important because it provides galactose which is essential for development of nervous tissue in the young ones.

Sucrose is a non-reducing disaccharide as it lacks any free reducing group in contrast to other sugars. It is commercially extracted from sugarcane and beet roots. It is the principal form of carbohydrate transported from source (leaves) to sink (site of utilization) in higher plants. Sucrose is dextrorotatory but on hydrolysis by sucrase produces a mixture of equal amount of glucose and fructose which is laevorotatory.

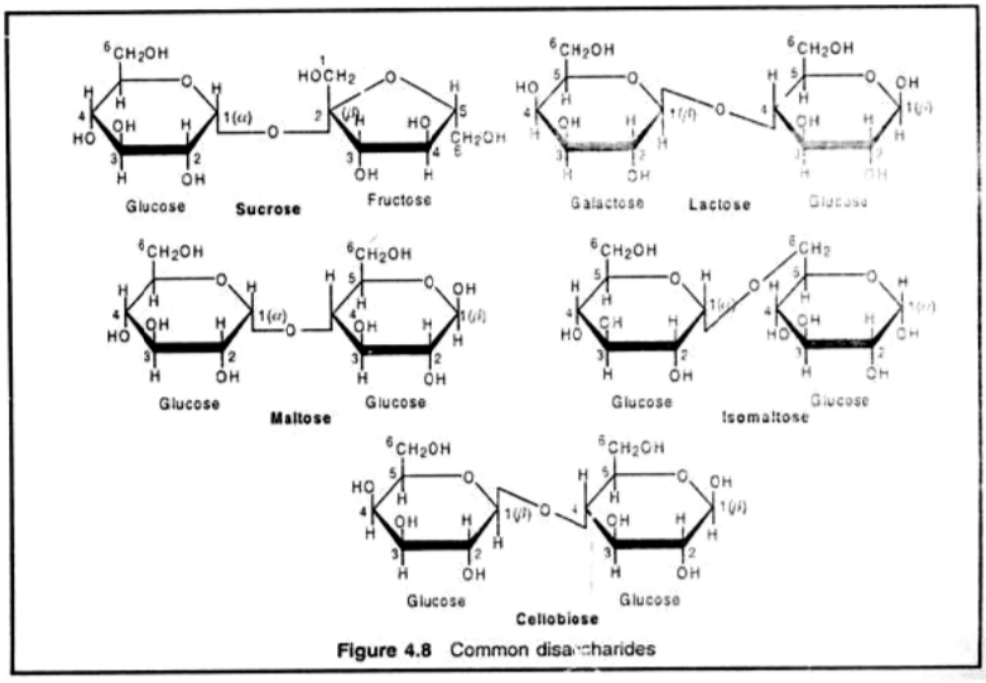


Figure 4.8 Common disaccharides

Cellobiose is the repeating disaccharide of cellulose in which β-glucosidic bond present.

Oligosaccharides may be composed of 3-9 monosaccharide units linked by O-glycosidic bonds. They may be referred as trisaccharides, tetrasaccharides etc. When linked with proteins and lipids they form glycoproteins and glycolipids respectively.

Gentianose (glucose-glucose-glucose), raffinose (galactose-glucose-fructose) are found in some plants. Webb and Burley (1964) found that stachyose (galactose-galactose-glucose) is a transport carbohydrate in plants like Frcixinusaыеnсапа, Cucurbitci pepo and Verbascum thapus.

Functions of oligosaccharides:

i. Transport form of Sugar:

Although glucose is the common transport sugar for many animals, sugars are transported in plants and other organisms as disaccharides.

ii. Storage Food:

Sucrose is the reserve storage product in sugarcane and sugar beet.

iii. Recognition Point and other Functions in Cells:

Oligosaccharides attached to cell membranes form cell coat of glycocalyx.

They are important for:

(a) Cell recognition,
(b) Cell attachment,
(c) Receptor molecules (for receiving and responding to external stimuli),
(d) Antigen specificity for human blood groups (A, B, Rh),
(e) Components of antibodies which are large molecules with attached carbohydrates,
(f) Glycoproteins of some viral coats for attaching to and invading host cell.
(g) Lactose (a disaccharide) is the natural sugar of milk.

3. Polysaccharides (Glycans):

Polysaccharides are macromolecules polysaccharides consist of at least more than ten monosaccarbohydrates. They may be linear or branched chains. Chemically, polysaccharides are of 2 types, homopolysaccharides and heteropolysaccharides. Homopolysacccharides are polymers of similar type of monosaccharides residue.

They may be glucosan or glucan (polymer of glucose) fructosan or fructan (polymer of fructose), galactosan or galactan (polymer of galactose), pentosan (polymer of pentose), hexosan (Polymer of hexoses), araban (polymer of arabinose, a pentose), xylan (polymer of xylose, a pentose) etc. Heteropolysaccharides are polymers of 2 or more type of monosaccharides residues, e.g., glycosamionoglycans (also called mucopolysaccharides), pectin, hemicelluloses etc. Heteropolysaccharide and structural polysaccharides secreted outside the cell for functioning.

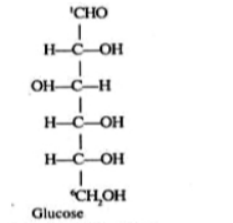
Structural Features:

Open Chain and Ring Forms of Monosaccharides:

Many monosaccharide's e.g ribose, glucose, fructose etc. exist both in open straight chain and ring form. If the ring is 5-membered it is called as furanose sugar. Sugar with a 6-membered ring is called as pyranose sugar e.g.,

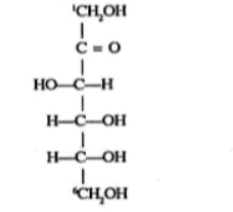
(The names pyranose and furanose are derived from six and five membered cyclic ethers called pyran and furan respectively with which these sugars bear a formal resemblance).

If the highest numbered asymmetric carbon atom of the sugar molecules contains—OH group on right-hand side in open chain structure, the sugar is known as D-Sugar and if on left side it is known as L-Sugar. For example, in open chain structure of glucose carbon number 2, 3, 4 and 5 are asymmetric. The highest

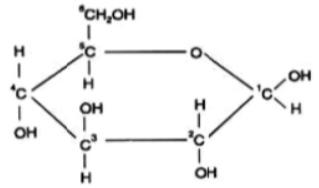


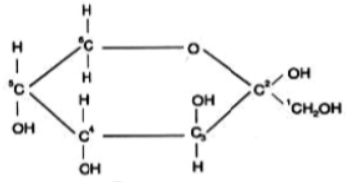
Glucose
(Open Chain Structure)
or Fischer's projection formula)*

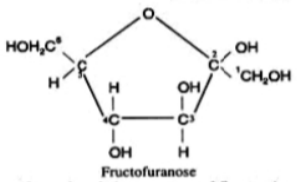
Similarly,



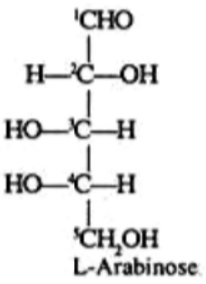
Fructose
(Open Chain Structure)

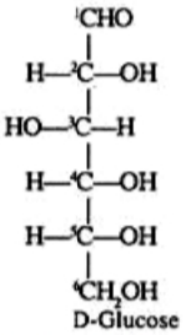

Glucopyranose
(Six membered ring structure of Glucose)


Fructopyranose
(6-membered ring structure of Fructose)


Fructofuranose
(5-membered ring-structure of Fructose)

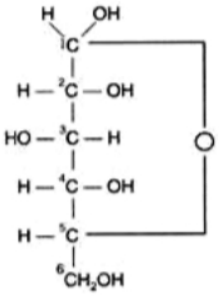
numbered asymmetric C-atom is therefore, 5. As shown below, it bears -OH group on right-hand side and hence, glucose is called a D-sugar and written as D-Glucose or D-Glucopyranose (in case of 6-membered ring structure).
In arabinose (a pentose sugar), the highest numbered asymmetric carbon atom is 4 which bears—OH group on left-hand side, therefore, this is a L-Sugar and called as L-Arabinose.
(The carbon atom whose 4 valancies are satisfied by four different groups or atoms is called as asymmetric carbon atom).
i. Almost all the sugars in plants are D-Sugars.

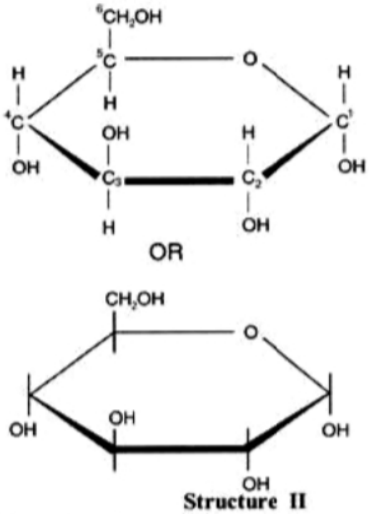

L-Arabinose


D-Glucose

ii. Symbols D and L should not be confused with the optical activity. If the compound is optically active and rotates the plane polarised light to right, it is called as dextrorotary and is denoted by small italic letter d or + sign. But, if it rotates the plane to left side it is called as laevorotatory and is denoted by I or – sign. For example, D-Glucose is dextrorotatory and D- Fructose leavorotatory and are written as:-
D (+) Glucose or D (d) Glucose
D (-) Fructose or D (l) Fructose
iii. The ring form of sugars is in fact a cyclic hemi-acetal or hemiketal structure formed by combination of the carbonyl group (i.e., aldehyde group at C_1 in aldose sugars and keto group at C_2 in ketose sugars) and one of the hydroxyl groups (usually at the highest numbered asymmetric C atom) of the sugar molecule in open chain structure.
The hemi acetal or hemiketal bond formation creates a new asymmetric centre at C_1 in aldose sugars and C_2 in ketose sugars which is now called as anomeric carbon atom. Taking for example a-D-Glucopyranose, the ring form of the sugars is usually depicted by following two structural formulas:

Although structure I indicates the basic features of a-D-Glucopyranose, it provides little insight as to the actual shape of the molecule and the spatial relationship of the various functional groups to one another. The latter features are clearly represented by structure II which is also called as Haworth's formula.
iv. In Haworth's perspective formula, the plane of the pyranose ring is considered to be perpendicular to that of the page on which it is written with the substituents (-H, -OH and -CH_2OH groups) either above or below the plane of the ring. The thin bonds of the ring are behind the plane of the paper while the thick bonds in front of it.


Structure I

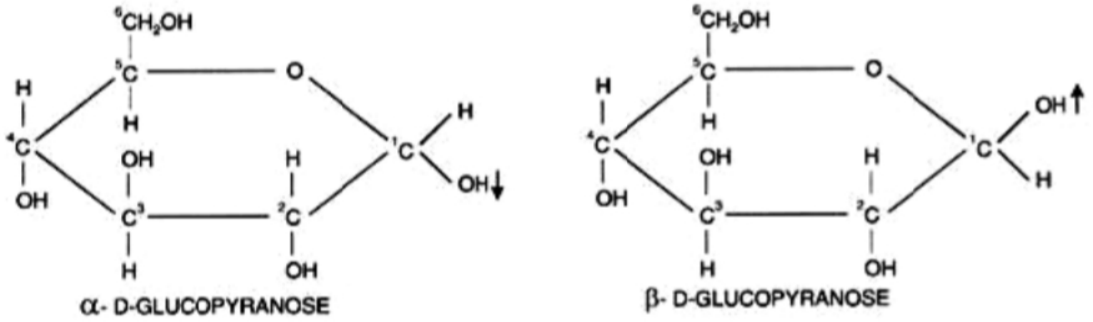

Structure II

α-D-Glucopyranose

(As a rule, the substituents written to the right side of the C chain in structure I are shown below the plane of the ring in Haworth's representation while those on left side above the plane of ring. However, opposite rule applies to that C atom whose—OH group is involved in the formation of cyclic hemiacetal. Thus, the CH_2OH group is written in an above position and the H atom on the same carbon written below despite the fact that it is on the left side in structure I.

In practice, however, the carbon atoms of the ring and the H atoms are omitted and often the thick bonds of the ring are not shown. The formula may also be inverted or twisted in oligo and polysaccharides to show the linkages between various monosaccharide molecules more clearly.)

If in the Haworth representation of the sugars the—OH group at the reducing centre (i.e., C No. 1 in aldose sugars and C No. 2 in ketose sugars) is present below the plane of the ring, the sugar is said to be in a-form. And if it is present above the plane of the ring, the sugar is said to be β-form. For example,



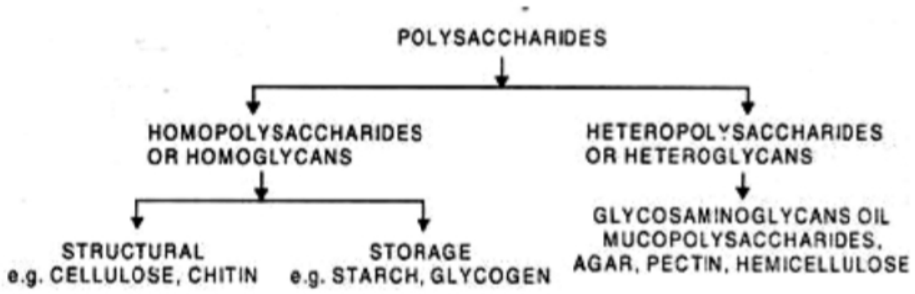
When α and β-forms change into one another the phenomenon is called as mutarotation.

There is an equilibrium between these two forms of ring compound. For example, the equilibrium in the mutarotation of a synthetic sample of D-Glucopyranose corresponds to a mixture of 36% of the a-form and 64% of the β-form.

α and β-forms of a given sugar are anomers of each other.

(The proportion of the open chain and ring forms of sugars in solution differs with different sugars. For most of the aldohexoses and aldopentoses the ring form is predominant one. Fructose (a ketose sugar) and most of the aldopentoses exist predominantly in the furnose form while glucose and other aldohexoses exist mainly in pyranose form. Ketopentoses such as ribulose usually prefer open chain form).

Functionally, polysaccharides are of 3 types – storage, structural and mucosubstances.



(A) Structural Polysaccharides:

They are the macromolecules that perform architectural functions in plant cell wall and skeleton of animals.

Cellulose:

Cellulose is the most abundant organic compound in the biosphere. It is estimated that about 10^{15} kg of cellulose are synthesized and degraded annually. Cellulose is the structural polysaccharide of plant cell walls, some protists and tunic of urochordates.

Each cellulose chain is a linear glucan of about 2000-25,000 D-glucose residues joined by β1→4 glycosidic linkages. The adjacent monomers lie at an angle of 180° and the straight chain is stabilized by hydrogen bonds. The individual chain is about 1-5 mm (micrometer) long.

Nearly 100 individual cellulose chains lie side by side and bonded to each other to make a crystalline domain or micelle. About 2.0-40 micelles embedded in an amorphous matrix to form a cellulose micro fibril. Micro-fibrils are synthesized on the plasma membrane by protein complexes called particle rosettes. About 250 micro-fibrils arranged in a bigger bundle called macro-fibrils.

Vertebrates don't have celluloses (P-amylase /β1 → 4) and therefore, can't digest cellulose. However, the rumen (1st stomach chamber) of herbivores, gut of termites contains symbiotic microorganisms that secretes cellulase and thus can digest cellulose.

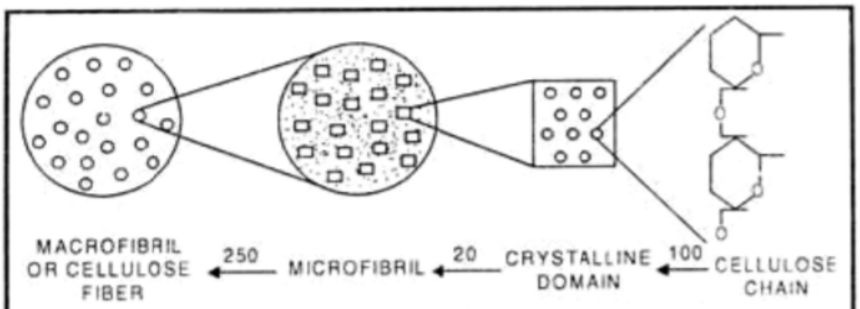


Figure 4.9 Association of cellulose chains in cross section, diagrammatic.

Chitin (fungal cellulose):

Chitin is the main structural polysaccharides of the exoskeletons of arthropods and also in the cell wall of most fungi and algae. Chitin is a linear polymer of N-acetyl-glucosamine (NAM) residues in/β→4 linkage. Structurally chitin is similar to cellulose. It becomes hard when impregnated with $CaCO_3$ and proteins. Chitin is soft leathery and provides strength and elasticity.

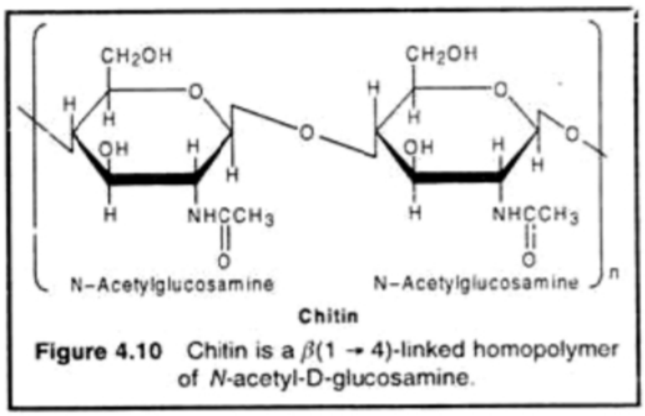


Figure 4.10 Chitin is a β(1 → 4)-linked homopolymer of *N*-acetyl-D-glucosamine.

(B) Storage Polysaccharides:

These polysaccharides serve as reserve food. At the time of need, they are hydrolyzed to release sugars available to the living cells for respiration and biosynthesis.

Followings are some important food storage

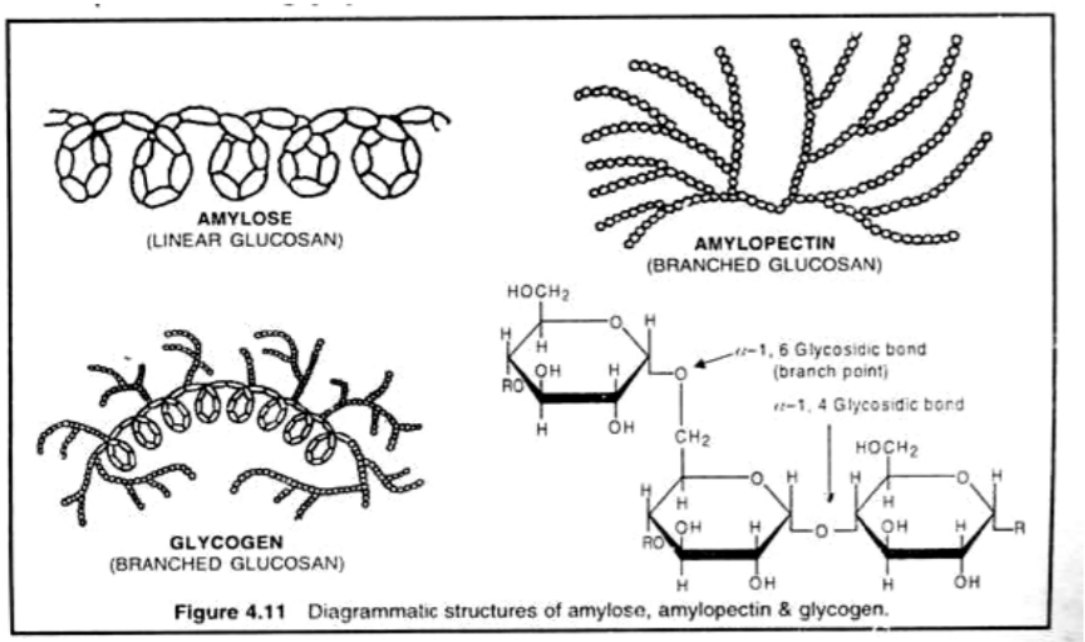


Figure 4.11 Diagrammatic structures of amylose, amylopectin & glycogen.

polysaccharides:

(i) Dextran:

It is the storage polymer in bacteria and yeasts. Dextran is a glucosan where the glucose resides are linked by αl-6 linkage with occasional branches occur by αl-4 linkages. Dextrans are non-toxic hence used chemically as substitute of plasma proteins.

(ii) Levan:

It is the storage polysaccharides in bacteria. Levan is a fructosan composed of fructose residue joined by 2-6 linkages.

(iii) Starch (=amylum):

Starch is a mixture of glucans that synthesized as end product of photosynthesis. It is stored in form of insoluble granules (starch grains) in chloroplasts, amyloplasts and cytoplasm of plant cells. Starch grain occur singly (simple) or in groups (compound). Starch grains have characteristic shape which differs in plant species. Each starch grain has a common point called hilum surrounded layer of starch ring.

Each starch grain consists of a mixture of two type of glucans i.e. amylose (20-30%) and amylopectin (70-80%). Amylose generally found in core while amylopectin in outer part of starch grain. Amylase is a linear but coiled polymer of about 200-1000 D-glucose residues linked by α1 →4 linkages.

Amylopectin is a branched structure of at least 80 chains each with 24-30 glucose residues, inter linked byal →6 linkages. Each branch found at an interval of 24-30 glucose residues. Thus amylopectin is like a glycogen except for its lower degree of branching. Amylose gives blue-black colour with iodine solution while amylopectin gives red-violet colour.

Cooking or boiling causes swelling of amylopectin component of starch grain which ruptures the cell wall and facilitates digestion. But amylase dissolves in water and separates. Partial hydrolysis of starch produce oligosaccharides called dextrins which are water soluble. Initially larger dextrins formed called erythrodextrin which give red colour to iodine and later smaller dextrins produce called achrodextrins which give no colour with iodine.

Dextrins also produce when flour in browned or bread is toasted. However, dextrins may be found naturally in honey and plant leaves.

(iv) Inulin (Dahlia starch):

Inulin is a water soluble fructosan consists of 25-35 /β-D fructose residues joined by/β1-2 linkages. It is a storage homoglycan deposited in the form of fan-shaped crystals in root tubers of Dahlia, Dandelion, stem tubers of Chicory, bulbs of onion and garlic etc. It gives no colour in iodine. Insulin is not metabolized in human body and therefore, used for the determination of rate of glomerular filtration (GFR) in kidney. Insulin is smallest polysaccharide.

(v) Glycogen (animal starch):

Glycogen in the storage polysaccharide of animals that present in all cells but most prevalent in liver and muscles where it occurs as cytoplasmic granules usually associated with SER. It is also found in yeasts and fungi but not in green plants. In liver and muscles, excess glucose convert into glycogen by glycogenesis and during starvation glycogen hydrolyzed to release glucose by glycogenosis.

Glycogen is water soluble and gives red colour to iodine. Structurally, a glycogen molecule resembles amylopectin of starch but glycogen is more branched with branch points occur every 8-12 glucose residues. Each glycogen consists of 5000-30,000 glucose residues linked by αl-4 glycosidic bands with αl, 6-linkage of branch point.

(C) Mucosubstances:

Mucopolysaccharides (glucosaminoglycans), mucoproteins (glycoproteins) are mucosubstances forming slime, mucus or mucilaginous compounds in both plant and animal body. They have structural and protective functions. Mucopolysaccharides are heteropolysaccharides present in the matrix of cell wall and the ground substances of connective tissues in animals. They are characterized by the presence of amino sugars and uronic acids.

Some mucopolysaccharides are as follows:

(i) Hemicelluloses and Pectins:

These are heterogeneous group of polysaccharides that found in the amorphous matrix of plant cell wall. Hemicelluloses are flexible cellulose-linking glycans. They are not the modified cellulose as the name indicates. Some common hemicelluloses are xyloglucan, xylan and glucomannan.

Pectins are linear or highly branched polysaccharides that form a hydrophilic gel in which hemicellulose-cellulose network is embedded. The most common pectins are homoglactouronan (pectic acid), arabinans, arabinogalactan, rhamoglactouronan etc. Pectins of cell wall determine the hydration and orientation of cellulose microfibrils. In wood, the matrix contains lignin, a phenolic polymer.

(ii) Agar (Agar -agar):

It is a cell wall mucopolysaccharide of some red algae (Gelidium, Gracilaria). It is used in culture medium, Cosmetics, leather, laxative, baked goods, meat industry and an emulsifier in dairy products.

(iii) Carrageenin:

It is related to agar and found in red algae Chordrus crispus. It is used as a component of tooth pastes, deodorants, cosmetics etc.

(iv) Hyaluronic acid:

It is a linear polymer of 250 to 25,000 disaccharide units that composed of D- glucuronic acid and N-acetylglucosamine joined by β l → 4 linkages. It occurs in the ground substance of connective tissue, synovial fluid and vitreous humor of eye.

The other glycosaminoglycans of ground substance of connective tissue are chondroitin sulphate, dermatan sulphate and keratin sulphate. But heparin is a sulfated glycosaminoglycan present in most cells of connective tissue and acts as anti-coagulant.

(v) Peptidoglycan (Murein, Mucopeptide):

It is made of heteropolysaccharide chains cross- linked by short peptides (generally tetrapeptide). Heteropolysaccharide chains are formed of two alternate amino-sugar molecules, N-acetylglucosamine (NAG) and N-acetylmurainic acid (NAM).

(vi) Lipopolysaccharides:

It is a complex of lipid and polysaccharides which forms the outer membrane of Gram negative bacteria. Lipopolysaccharide induces fever, shock and other toxic effects.

Functions of Polysaccharides:

Some of the important functions of Polysaccharides are listed below:

1. Starch and glycogen are the major storage foods of organic world.
2. On hydrolysis storage carbohydrates provide both energy and carbon chains.
3. Chitin is the structural carbohydrate of fungal walls and exoskeleton of arthropods.
4. Cellulose is the structural substance of cell walls in most of the plants.
5. Cellulose is economically important in the production of furniture, shelter, fuel, paper, textiles, ropes, rayon, cellophane, plastics, shatter proof glass, propellant explosives, emulsifier and raw material for several fermentation products.
6. For human beings cellulose has a roughage value but it is a food for ruminants, snails and termites.
7. Mucilage present as a protective coating around aquatic plants, bacteria, blue-green algae and some animals is derived from polysaccharides.
8. Mucopolysaccharides in human body have several functions— lubrication of ligaments and tendons, formation of some types of body fluids like cerebrospinal fluid, synovial fluid, vitreous humor, providing strength and flexibility to skin, connective tissue and cartilage, binding of proteins in cell walls and holding water in interstitial spaces.
9. Heparin prevents blood clotting inside blood vessels of animals.
10. Some mucopolysaccharides have medicinal and other commercial importance, e.g., husk of Plantago ovata, mucilage of Aloe, and alginic acid, agar, carragenin of marine algae.
11. Pectin's form matrix of cell wall. As calcium pectate they produce middle lamella or cementing layer between adjacent plant cells. Pectin's are commercial jellying agents.

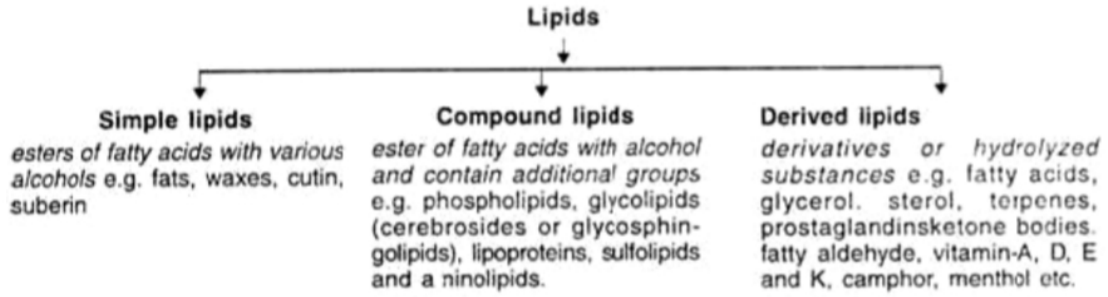
Lipids

Lipids are heterogeneous group of water insoluble compounds which are oily or greasy in consistency but soluble in non-polar solvents like ether, chloroform, benzene etc.

For examples, fatty acids, fats, oils, waxes, certain vitamins and hormones are considered as lipids. Lipids are composed of C, H, O, like carbohydrates but poor in oxygen and therefore require more oxygen for oxidation to release energy.

Classification:

Bloor (1943) coined the term 'lipid' and classified them into three types: Simple, Compound and Derived.



1. SIMPLE LIPIDS

(A) Fatty acids:

Fatty acids are monocarboxylic acids (R-COOH) with long hydrocarbon chains. Fatty acids don't occur free in nature; rather occur as esters in natural fats and oils. The fatty acid is called an acyl group when it is a part of ester. In biological systems, fatty acids usually contain an even number of carbon atoms, typically between 14 and 24. The most common fatty acids have 16-18 carbon and 0-3 double bonds. For example, plamitic (C16), stearic (C18), oleic (C18), linoleic (C18) etc.

Fatty acids are amphipathic molecules because their carboxylic groups (-COOH) are hydrophilic or polar and the hydrocarbon chains are hydrophobic or non-polar. In physiological conditions, fatty acids found in ionized forms. For example, palmitic acid exist in form of palmitate and so on.

The carbon atoms of fatty acids are numbered from the carboxyl carbon (C-l). The C-2 and C3 are called α-carbon and/β-carbon respectively. The methyl carbon at the end is called ω-carbon. Fatty acids are of 2 types, saturated and unsaturated. Saturated fatty acids contain no double bonds (saturated in hydrocarbon chain, e.g., $CH_3(CH_2)_{14}COOH$ (palmitic acid), $CH_3(CH_2)_{16}COOH$ (stearic acid), $CH_3(CH_2)_{18}$ carboxylic COOH (arachidonic acid) etc. They have higher group melting points and are solid at room temp. Unsaturated fatty acids contain one or more double bonds (unsaturated) in hydrocarbon chain. A double bond in an unsaturated fatty acid has two possible configurations, cis or Trans. The double bonds in most unsaturated fatty acids have the cis orientation that introduces a bend or kink in the hydrocarbon side chain.

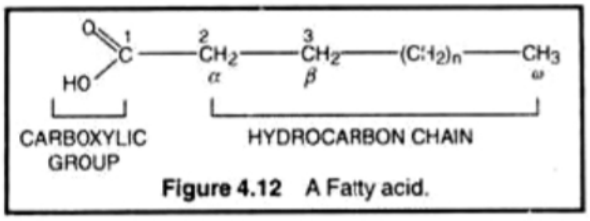
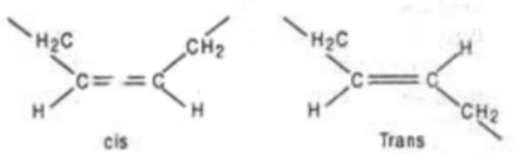


Figure 4.12 A Fatty acid.



Essential Fatty acids (EFA; Durr and Burr, 1930):

Bacterial and plants can synthesize all the required fatty acids. But animals can't synthesize 3 polyunsaturated fatty acids – linoleic, linolenic and arachidonic acids – that are required for growth and synthesis of prostaglandins. If linoleic acids are sufficiently available in the diet then other two EFA can be synthesized from it. EFAs are popularly known as Vitamin F.

Importance of Fatty acids:

1. Fatty acids are fuel molecules, stored as triglycerides in the fat-cells (adipose cells). Under the influence of hormone adrenaline adipose cells hydrolyze the triglycerides into free fatty acids that are

released into the blood. The complete oxidations of 3 fatty acids of a triglyceride release 9 Kcal/g, in contrast to carbohydrate and protein that release 4 Kcal/g.

2. They are the building blocks of phospholipids, glycolipids and lipoproteins that found in the biological membranes.

3. Eicosanoids (prostaglandins, thromboxane's and leukotriene's) are the polyunsaturated fatty acids, which serve as hormones.

4. Fatty acid derivatives act as intracellular messengers, e.g. IP_3(inositol 1, 4, 5-triphosphate) and DAG (diacylglycerol).

5. Deficiency of EFA (essential fatty acids) in human diet causes sterility, kidney failure, skin lesions like phrenoderma (hard skin), eczema etc.

6. Provide the insight to many diseases like obesity atherosclerosis etc.

7. Hydrogenation of unsaturated fatty acids converts oil to solid fat at room temperature. This is the basis of manufacturing edible vanaspati ghee or margarine from inedible and cheap cotton seed oil.

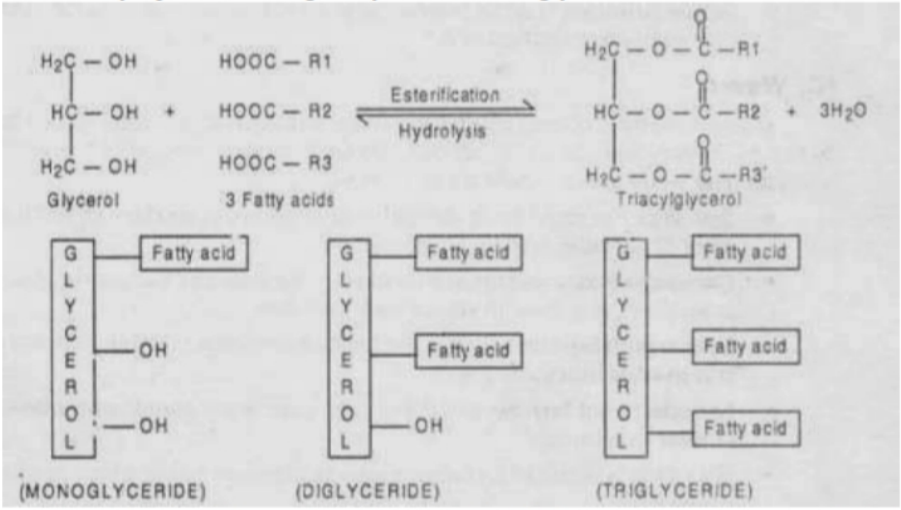
8. Physicians recommend taking PUFA to those having high blood cholesterol or cardiovascular disease.

9. The cleaning action of soap is due to reduction of surface tension of water by fatty acids

(B) Fats (= True fats, neutral fats, triglycerides or triacylglycerol)

They are the unchanged ester of 3 fatty acids and a gtycerol. When 2 or 1 fatty acids esterified with glycerol, then they are called mono-glycerides respects. In pure fats all the 3 fatty acids are similar e.g tripalmitin, tristearin. In mixed fat (triglyceride) all the three or at least one or 2 fatty adds are dissimilar e.g. butter.

Triglycerides are high efficiency storage fuel. In mammals, triglycerides are deposited in the form of large fat globules in the cytoplasm. In migratory birds, the triglycerides are stored under the skin, in



muscle, in abdominal cavity & in liver. About two-thirds of this stored fat is consumed in the long flight over water. Triglycerides store six time as much energy as does glycogen because they are deposited in highly reduced and anhydrous form.

Hence, in evolutionary process triglycerides were selected over glycogen as the major energy reservoir. In a typical 70kg man, triglycerides constitute about 11kg of total body weight with a fuel

reserve of 10^5kcal. If this amount of energy is stored in the form of glycogen, his total body weight would be 55kg greater.

- **Saturated Fatty Acids:**

Their general formula is: CH_3 — $(CH_2)_n$ — COOH. The most frequent are palmitic acid (C_{10}) and stearic acid (C_{18}). In lower concentration are found the fatty acids with 14 or 20 carbon atoms. Longer fatty acids (up to 36 carbon atoms) are present in numerous cells (bacteria, unicellular eucaryotes, plants, vertebrates).

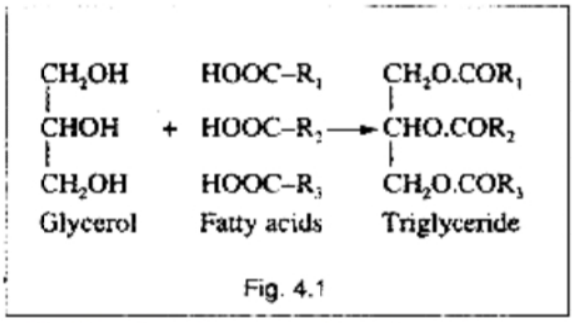


Fig. 4.1

They are generally present in some types of lipids. Milk on the contrary, is rich in short-chain fatty acids. Besides the even-carbon fatty acids, are generally found small quantities of fatty acids having 15, 17 or 19 carbon atoms.

- **Unsaturated Fatty Acids:**

Fatty acids are numbered from the terminal carboxyl (carbon 1) to the CH_3 group (carbon n). The double bond is indicated by the sign Δ, accompanied by the number corresponding to the first carbon atom participating in the double bond. The sign: is being increasingly used; it is followed by the number of double bonds, the position of the latter being indicated within brackets.

There is also a biochemical nomenclature. In this case carbon 1 is the terminal methyl. The place of the last double bond is indicated by ω followed by the number of atoms of the carbon existing up to this double bond. In practically all biological unsaturated fatty acids, the double bond, has a cis isomerism.

The principal unsaturated fatty acids are:

A. Monounsaturated Fatty Acids (1 Double Bond):

Oleic acid (C_{18}), double bond between carbon atoms C_9 and C_{10} , abbreviated as: (C_{18}, Δ^9 or 18 :1(9) or 18 ω 9).

CH_3-$(CH_2)_7$-CH = CH-$(CH_2)_7$-COOH

B. Polyunsaturated Fatty Acids (Several Double Bonds):

In the most common of such acids, the non-conjugated double bonds are separated by a methylene group. Plants can however contain fatty acids with conjugated double bonds, for example, eleostearic acid.

Linoleic acid (C_{18}, $\Delta^{9,12}$ or 18:2 (9,12) or 18 ω 6)

CH_3-$(CH_2)_4$-CH = CH-CH_2-CH = CH-$(CH_2)_7$-COOH

Linolenic acid (C_{18}, $\Delta^{9,12,15}$ or 18 : 3 (9,12,15) or 18 ω 3)

CH_3—CH_2—CH = CH—CH_2—CH = CH—CH_2—CH = CH—$(CH_2)_7$—COOH

Arachidonic acid (C_{20}, $\Delta^{5,8,11,14}$).

Docosahexaenoic acid (C_{22} $\Delta^{4,7,10,13,16,19}$).

Eleostearic acid (C_{18}, $\Delta^{9,11,3}$).

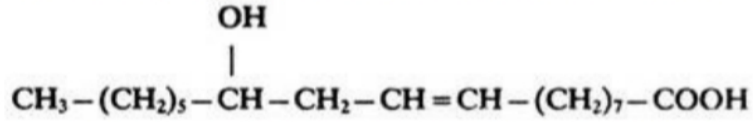
In mammals, polyunsaturated fatty acids can have up to 22 carbon atoms and 6 double bonds, but in plants these acids do not exceed 18 carbon atoms and 4 double bonds.

An important physical property of fatty acids is their melting point; it decreases with increasing number of double bonds. For example, the melting point of stearic acid (18: 0) is 70°C, whereas that of oleic acid (18 :1) is 13°C, that of linoleic acid (18: 2), -5.8°C and that of arachidonic acid (20: 4), –49.5°C.

- **Hydroxylated Fatty Acids:**

Plants can synthesize a series of hydroxylated fatty acids like ricinoleic acid for example:

Some of these hydroxylated fatty acids lead to the formation of cutin.
Other types of hydroxylated fatty acids are found in mammals. Some glycolipids contain large



quantities of α-hydroxylated acids (OH on carbon 2) with 22, 23, 24 and 25 carbon atoms. Moreover, cells of the epiderm have lipids containing very long-chain ω hydroxylated acids which play a role in the structure of this particular tissue.

- **Branched Fatty Acids:**

Example: 15 methylhexadecaenoic acid
$(CH_3)_2 - CH - (CH_2)_{13} - COOH$
The above type of fatty acid is particularly abundant in $Gram^+$bacteria.

Properties of Fats:

1. The fats of animal and plant origin are mixtures of different triglycerides. Plant fats have more unsaturated fatty acids and animal fats have saturated fatty acids. For commercially used fats are different into hard fats (animal fats) and oils (vegetable fats). Fats are solid at room temperature (20°C) but when liquid at 20°C called oils.
2. The property of fat depends upon chain length and their degree of saturation. Short chain length and un-saturation of fatty acids lower the melting point and increase the fluidity.
3. Hydrolysis: Lipases hydrolase the ester bonds so that a triglyceride forms 3 fatty acids and 1 glycerol. It is the basis of fat digestion.
4. Oxidation: On the outer mitochondrial membrane fatty acids first linked to coenzyme A by the enzyme fatty acid thiokinase. This is called fatty acid activation. Subsequently the activated fatty acids enter into the mitochondrial matrix where /β-oxidation (oxidation at /β-carbon) takes place to liberate energy.
5. Saponification: It is the process of formation of soap (metallic salts of fatty acids) by boiling a fat or oil with alkali like KOH, NaOH etc.
6. Saponification number: It is the number of milligrams of KOH required to saponify 1 gm of a given fat or oil. Saponification gives an idea about the molecular weight or chain length of the fatty acids of a fat. A fat having smaller fatty acids have higher saponification.
7. Rancidity: It is the development of unpleasant odour and taste of a fat or oil when kept for a long time, or exposed to air, moisture, heat etc. Rancidity is caused by microbial degradation of fatty acids, hydrolysis of a fat and formation of peroxides at the double bonds. Vegetable fats can be preserved for longer period than animal fat. This is because vegetable fats (oils) contain antioxidants like Vitamin E, Phenols etc. which prevent rancidity.
8. Acid Number: It is the number of milligrams of KOH required to neutralize the free fatty acids present in 1 gm of fat. The acid number indicates the degree of rancidity of a given fat. Higher the acid number, greater is the rancidity or fat.
9. Iodine Number: It is the number of grams of iodine absorbed by 100 gm. of fat. It indicates the degree of un-saturation of fat.

Identification of Fats and Oils:

(a) Hydrolysis:

1. Hydrolysis of triacylglycerol takes place by lipases producing fatty acids and glycerol.
2. Phospholipases attack the ester linkage of phospholipids.

(b) Saponification:

1. Boiling with an alcoholic solution of strong metallic alkali hydrolyses triglycerides into glycerol and fatty acids —this is called saponification.

2. The products are glycerol and the alkali salts of the fatty acids which are called soaps.
3. Fats, phospholipids, glycolipids and waxes are called saponifiable lipids.
4. Steroids, polyisoprenoids and higher alcohols are grouped as un-saponifiable lipids because they cannot give rise to soap.

(c) Saponification number:

1. The number of milligrams of KOH required to saponify 1 gram of fat or oil.
2. The amount of alkali needed to saponify a given quantity of fat will depend upon the number of-COOH group present. It is inversely proportional to the average molecular weight of the fatty acids in the fat i.e. the fats containing short chain fatty acids will have more -COOH groups per gram than long chain fatty acids—this will take up more alkali and, hence, will have higher saponification number.

Example:

Butter—containing a larger proportion of short chain fatty acids such as butyric and caproic acids, has relatively high saponification number 220 to 230.

(d) Acid number:

1. The number of milligrams of KOH required to neutralize the free fatty acids of 1 gram of fat.
2. Significance: The acid number indicates the degree of rancidity of the given fat.

(e) Iodine number:

1. This is the amount (in grams) of iodine absorbed by 100 grams of fat.
2. This is the measure of the degree of unsaturation of a fat.
3. Significance: If the fat contains higher number of unsaturated fatty acids, it becomes essential for the protection of heart disease. These unsaturated fatty acids, combined with the cholesterol, are oxidized in the liver—producing bile acids, bile salts, vit., D, gonadotrophin hormones. They prevent atherosclerosis.

(f) Acetyl number:

1. The number of milligrams of KOH required to neutralize the acetic acid obtained by saponification of 1 gram of fat after it has been acetylated.
2. This is a measure of the number of hydroxy acid groups in the fat.

(g) Polenske number:

1. The number of milliliters of 0.1 (N) KOH required to neutralize the insoluble fatty acids from 5 grams of fat.

(h) Reichert-Miessl number:

1. This is same as the Polenske number except that the soluble fatty acids are measured by titration of the distillate obtained by steam distillation of the saponification mixture.

Significance: It measures the amount of volatile soluble fatty acids.

(i) Halogenation:

1. Chlorine, bromine and iodine atoms may be added to the double bonds of unsaturated fatty acids containing fats.

(j) Rancidity:

1. Nearly all natural fats are oxidized when exposed to air, light, moisture, particularly, if warm, it develops an unpleasant odour and taste. The enzyme lipase—in the presence of moisture and warm temperature— bring about hydrolysis rapidly.
2. This happens due to the formation of peroxides at the double bonds of unsaturated fatty acids.
3. Vitamin E is an important natural antioxidant and prevents development of rancidity.

(B) Soaps:

1. Soaps are metallic salts of fatty acids.
2. Soaps are formed by adding alkalis to fatty acids.
3. Soaps of unsaturated fatty acids are softer and more water soluble than those of saturated fatty acids.
4. Potassium soap of an acid is more water-soluble and softer than the sodium soap; calcium and magnesium soaps are far less soluble.

(B) Waxes:
1. They are esters of fatty acids with higher alcohols other than glycerol.
2. In the human body, the commonest waxes are esters of cholesterol.
3. They are mainly three types:
(a) True waxes are esters of higher fatty acids with acetyl alcohol or other higher straight chain alcohols.
(b) Cholesterol esters are esters of fatty acid with cholesterol.
(c) Vitamin A and vitamin D esters are palmitic or stearic acid esters of vitamin A (Retinol) or vitamin D, respectively.

The well known waxes are as follows:

i. Bee-Wax:
Secreted from the abdominal glands of worker bees to build honeycombs. It is the ester of palmitic acid and hexacosonol.

ii. Carnauba wax:
It occurs as a coating on the leaves of Carnauba palm tree of Brazil (Copernicia prunifera). It is used in automobile polishes.

iii. Spermaceti (Sperm oil):
It is the hardest known wax obtained from the head of sperm whale. It is used as lubricating wax.

iv. Lanolin (wool fat):
Secreted from the cutaneous glands of fur bearing animals. It is closing similar to sebum.

v. Wax-D:
It is secreted by tuberculosis and leprosy causing bacteria. It is a measure cause of the diseases.

vi. Cerumen (ear wax):
Secreted from the ceruminous glands of external auditory canal.

vii. Paraffin wax:
It is obtained from petroleum. Candles are prepared from paraffin wax and stearic acid.

(C) Cutin:
It is formed by cross-esterification and polymerization of hydroxyl fatty acids. Along with wax (cuticular wax), cut in form cuticle on leaf epidermis which check transpiration. The cuticle is frequently covered by a layer of epicuticular wax.

(D) Suberin:
It is composed of glycerol and phellonic acid. It is found in the walls of cork cells and from Casparian strip of root endodermis. It is water impermeable and checks infection in plants.

2. Compound Lipids:

A. Phospholipids (phosphatides):
(i) They are esters of fatty acids with glycerol containing an esterified phosphoric acid and a nitrogen base.
(ii) They are present in large amounts in nerve tissue, brain, liver, kidney, pancreas and heart.

Biological functions of phospholipids:
(i) They increase the rate of fatty acid oxidation.
(ii) They act as carriers of inorganic ions across the membranes.
(iii) They help blood-clotting.
(iv) They act as prosthetic group to certain enzymes.
(v) They form the structures of membranes, matrix of cell wall, myelin sheath, microsomes and mitochondria.

Classification:
It is based on the type of alcohol present in the phospholipid.

There are three types:
1. Glycerophosphatides — In this, glycerol is the alcohol group.

Example:
(i) Phosphatidyl ethanolamine (cephalin).
(ii) Phosphatidyl choline (Lecithin).
(iii) Phosphatidyl serine.
(iv) Plasmalogens.
(v) Phosphatidic acid.
2. Phosphoinositides — In this, inositol is the alcohol.
Example:
Phosphatidyl inositol (Lipositol).
3. Phosphosphingosides — In this, sphingosine is an amino alcohol.
Example:
Sphingomyelin.
The phospholipids include the following groups:
1. Phosphatidic acid and phosphatidyl glycerol's:
Phosphatidic acid is important as an intermediate in the synthesis of triacylglycerol's and phospholipids.
Cardiolipin:
(a) It is formed from phosphatidyl glycerol.
(b) Chemically, it is di-phosphatidyl glycerol.
(c) It is found in inner membrane of mitochondria and bacterial wall.

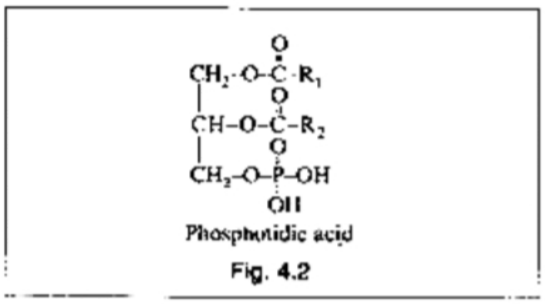


Fig. 4.2

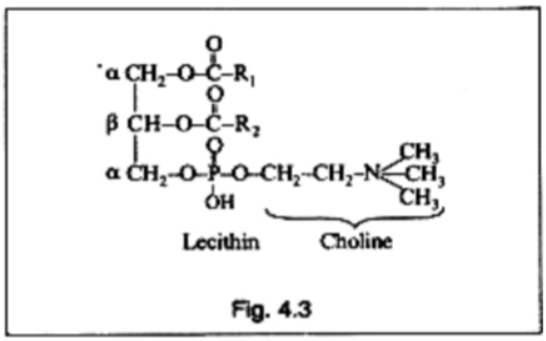


Fig. 4.3

2. Lecithin (Phosphatidylcholine):
The lecithin's contain glycerol and fatty acids, phosphoric acid and choline (nitrogenous base). Lecithin's generally contain a saturated fatty acid at α position and an unsaturated fatty acid at β position. They can exist in α or β forms.
Physical Properties:
(i) Lecithin's are waxy, white substances but become brown soon when exposed to air
(ii) They are soluble in ordinary fat solvents except acetone.
(iii) They decompose when heated.
(iv) They constitute valuable agents for the emulsifications of fats and oils.
Chemical Properties of Lecithin:
(i) When aqueous solution of lecithin's are shaken with H_2SO_4, choline is split off, forming phosphatidic acid.
(ii) When lecithin's are boiled with alkalis or mineral acids, not only choline is split off, but phosphatidic acid is further hydrolyzed to glycerophosphoric acid and 2 molecules of fatty acids:

Lecithin $\xrightarrow{H_2SO_4}$ Phosphatidic acid + choline.

Lecithin → H_2SO_4 Phosphatidic acid + choline.
Phosphatidic acid → Glycerophosphoric acid + fatty acids (2 mol)

Physiological Functions of Lecithin:

(i) It facilitate the combinations with proteins to from lipoproteins of plasma and cells.

(ii) Acetylcholine formed from choline has an important role in the transmission of nervous impulses across synapses.

(iii) Choline is the most important lipotropic agent as it can prevent formation of fatty liver.

(iv) Lecithin lowers the surface tension of lung alveoli. Dipalmityl lecithin is a major constituent of "lung surfactant" which prevents the adherence of the inner surface of the alveoli of the lungs (preventing the collapse of the alveoli) by its surface tension lowering effect. The absence of this in the alveolar membrane of some premature infants causes the respiratory distress syndrome in them.

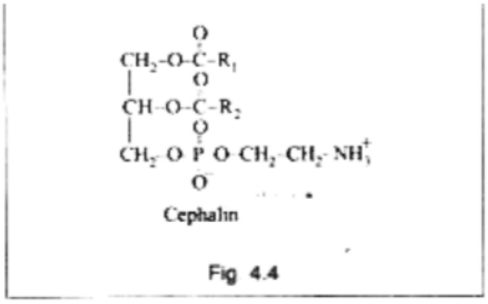
(v) It lowers the surface tension of water molecule and helps in the emulsification of fat.

Difference of Lecithin and Cephalin:

Cadmium chloride compound of cephalin is soluble but cadmium chloride compound of lecithin is insoluble.

3. Cephalitis (Phosphatidyl ethanolamine):

They always occur in the tissues in association with lecithin's and are very similar in properties. The only difference is the nitrogenous base.

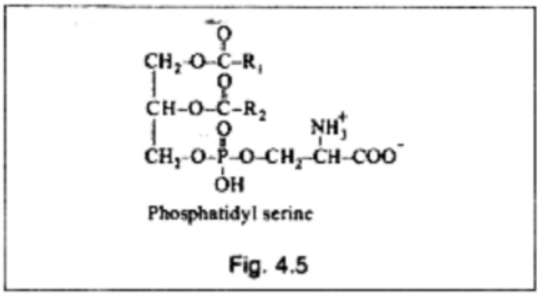


Cephalin

Fig 4.4

4. Phosphatidyl Serine:

A cephaline like phospholipid is found in tissues.



Phosphatidyl serine

Fig. 4.5

5. Phosphatidyl inositol (Lipositol or Phosphoinositides):

(i) It acts as second messenger in Ca^{++} dependent hormone action.

(ii) Some signals must provide communication between the hormone receptor on the plasma membrane and intracellular Ca^{++} reservoirs.

(iii) They are more acidic than the other phospholipids.

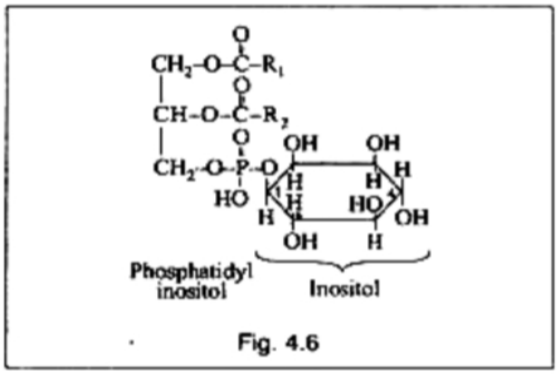


Fig. 4.6

6. Lysophospholipids:

(i) These are phosphoacylglycerols containing only one acyl radical in a position e.g., Lysolecithin.

(ii) Formation:

(a) By the action of phospholipase.

(b) By interaction of lecithin and cholesterol in presence of the enzyme lecithin cholesterol acyl transferase, so lysolecithin and cholesterol ester are formed

Lecithin + cholesterol

↓ LCAT

Lysolecithin + cholesterol ester.

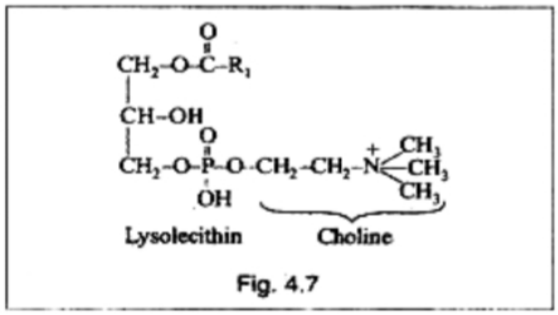


Fig. 4.7

7. Plasmalogens:

(i) These are the contents of brain and muscle.

(ii) Structurally, these resemble lecithin's and cephalins but give a positive reaction when tested for aldehydes with Schiff s reagent (fuchsin-sulphurous acid) after pretreatment of the phospholipid with mercuric chloride.

(iii) They possess an ether link in a position instead of ester link. The alkyl radical is an unsaturated alcohol.

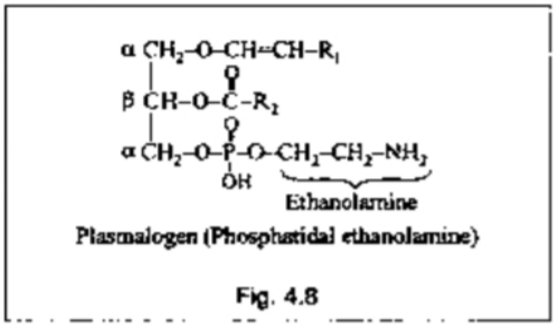


Fig. 4.8

8. Sphingomyelins:

(i) These are found in large quantities in brain and nerve tissue.

(ii) The concentrations of these phospholipids are increased in Niemann-Pick disease in the liver and spleen.

(iii) These contain sphingosine (18 carbon) (amino alcohol) fatty acid, phosphoric acid and choline. No glycerol is present.

(iv) In sphingosine molecule -NH_2 group binds a fatty acid by an amide linkage to produce ceramide. When phosphate group is attached to ceramide it is called ceramide phosphate.

(v) When choline is split off from sphingomyelin, ceramide phosphate is left.

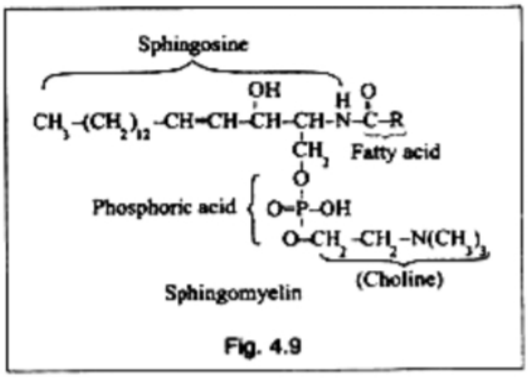


Fig. 4.9

CLINICAL ASPECT

- In Niemann-Pick disease excess amount of sphingomyelin are deposited in brain, liver, spleen.
- It is a lipid storage disease (lipidoses) and hereditary. It is caused by the deficiency of enzyme sphingomyelinase.
- The clinical findings are :
  - (a) Enlarged liver and spleen.
  - (b) Mental retardation.
  - (c) Nervous system is affected.
  - (d) Anemia and leukocytosis.

Action of Phospholipase:

(a) Phospholipase A_1 attacks the ester bond in position 1 of phospholipid.

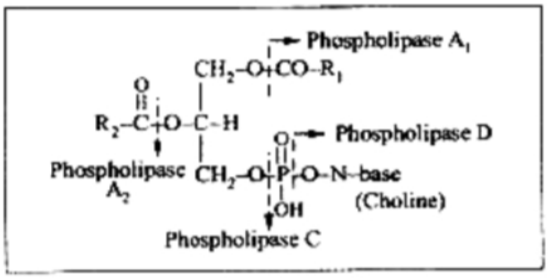
(b) Phospholipase A_2 attacks β position and form

Lysolecithin + one mol. fatty acid.

(c) Phospholipase B (lysophospholipase) attacks lysolecithin and hydrolyzes ester bond in α position and forms glyceryl phosphoryl choline + 1 mol. fatty acid.

(d) Phospholipase C hydrolyzes phosphate ester bond and produces α, β di-acyl glycerol + phosphoryl choline.

(e) Phospholipase D-splits off choline and phosphatidic acid is formed



B. Glycolipids:

These contain an amino alcohol (sphingosine or iso-sphingosine) attached with an amide linkage to a fatty acid and glycosidically to a carbohydrate moiety (sugars, amino sugar, sialic acid).

These are further classified into:

(i) Cerebrosides.

(ii) Gangliosides.

(i) Cerebrosides:

(a) Cerebrosides contain galactose, a high molecular weight fatty acid and sphingosine. Therefore, they may also be classified as sphingolipids.

(b) They are the chief constituent of myelin sheath.

(c) They may be differentiated by the type of fatty acid in the molecule.

These are:

Kerasin—Containing lignoceric acid [$CH_3 — (CH_2)_{22} — COOH$].

Cerebron—Containing a hydroxylignoceric acid (cerebronic acid).

[$CH_3—(CH_2)_2, — CH(OH)—COOH$].

Nervon—Containing an unsaturated homologue of lignoceric acid called nervonic acid. [$CH_3 — (CH_2)_7 — CH = CH — (CH_2)_{13} — COOH$].

Oxynervon—Containing hydroxy-nervonic acid [$CH_3 — (CH_2)_7 — CH = CH — (CH_2)_{12}— CH(OH) — COOH$].

(d) Stearic acid is a major component of the fatty acids of rat brain cerebrosides.

(e) Cerebrosides, specially cerebronic acid, increases in Gaucher's disease and the Kerasin characterized by glucose replacing galactose.

(f) The cerebrosides are in much higher concentration in medullated than in non-medullated nerve fibers.

CLINICAL ASPECT OF CEREBROSIDE

Gaucher's disease

- The cerebroside content of the reticuloendothelial cell (spleen) is very high.
- In cerebroside molecule, the kerasin is characterised by glucose replacing galactose.
- The disease is caused by the deficiency of enzyme glucocerebrosidase.

Symptoms

- Spleen is increased, signs of leucopenia.
- Liver is enlarged.
- Eyes show a yellow-brown wedge shaped elevation.

(ii) Gangliosides:

(a) These are glycolipids occurring in the brain.

(b) Gangliosides contain ceramide (sphingosine + fatty acids), glucose, galactose, N-acetylgalactosamine and sialic acid.

(c) Some gangliosides also contain di-hydro-sphingosine or gangliosine in place of sphingosine.

(d) Most of the gangliosides contain a glucose, two molecules of galactose, one N-acetylgalactosamine and up to three molecules of sialic acid.

Types of Ganglioside:

Tay Sach's disease (GM_2 gangliosidosis)

- This disease is characterised by increased accumulation of GM_2 ganglioside in brain and spleen.
- It is caused by deficiency of enzyme hexosaminidase A.

Symptom

- Mental retardation, blindness, muscular weakness.
- Red spot appears in the muscular region of eye in first year of life.

GM_1 ganglioside :

Gal → Gal NAC → Gal → Glucose → Ceramide
↓
NANA

GM_2 ganglioside :

Gal NAC → Gal → Glucose → Ceramide
↓
NANA

GM_3 ganglioside :

Gal → Glucose → Ceramide
↓
NANA

G = Ganglioside, M = Monosialo variety.

1, 2, 3 = Position in chromatography.

GM_1 gangliosidosis : This is due to deficiency of enzyme β-galactosidase.

C. Other compound lipids:

1. Lipoproteins:

(i) Triacylglycerol (45%), phospholipids (35%), cholesterol and cholesteryl esters (15%), free fatty acids (less than 5%) and also protein combine to form a hydrophilic lipoprotein complex.

(ii) Since pure fat is less dense than water, the proportion of lipid to protein in lipoproteins in plasma is by ultracentrifugation.

(iii) The density of lipoproteins increases as the protein content rises and the lipid content falls and the size of the particle becomes smaller.

(iv) Lipoproteins may be separated on the basis of their electrophoretic properties and may be identified more accurately by means of immuno-electrophoresis.

(v) Four major groups of lipoproteins have been identified which are important physiologically and in clinical diagnosis in some metabolic disorders of fat metabolism.

These are:

(a) Chylomicrons.

(b) Very low density lipoproteins (VLDL or pre-β-lipoproteins).

(c) Low density lipoproteins (LDL or β-lipoproteins).

(d) High density lipoproteins (HDL or α-lipoproteins).

(vi) Chylomicrons and VLDL: Predominant lipid is triacylglycerol (50%) and cholesterol (23%). The concentrations of these are increased in atherosclerosis and coronary thrombosis etc.

LDL:

Predominant lipid is cholesterol (46%) and phospholipids (23%). Increase in atherosclerosis and coronary thrombosis, etc.

HDL:

Predominant lipid is phospholipid (27%) and proteins (45%).

(vii) The protein moiety lipoprotein is known as an apoprotein which constitute nearly 60% of some HDL and 1% of chylomicrons. Many lipoproteins contain more than one type of apoprotein polypeptide.

(viii) The larger lipoproteins (such as chylomicrons and VLDL) consist of a lipid core of nonpolar triacylglycerol and cholesteryl ester surrounded by more polar phospholipid, cholesterol and Apo proteins.

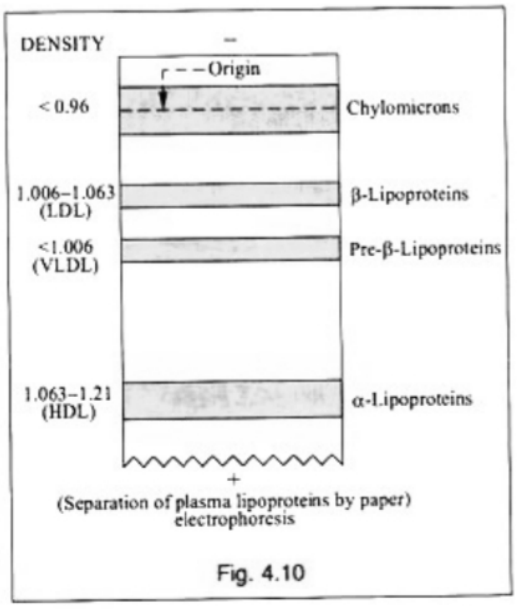


Fig. 4.10

Importance:

(i) To transport and deliver the lipids to tissues.

(ii) To maintain structural integrity of cell surface and subcellular particles like mitochondria and microsomes.

(iii) The β-lipoprotein fraction increases in severe diabetes mellitus, atherosclerosis etc. Hence determination of the relative concentrations of α-and β-lipoproteins and pre-β- lipoproteins are of diagnostic importance.

2. Amino lipids:

Phosphatidyl ethanolamine and serines are amino lipids and sphingomyelins and gangliosides contain substituted amino groups.

3. Sulpholipids (Sulphatides):

(i) These have been isolated from brain and other animal tissues.

(ii) These are sulphate derivatives of the galactosyl residue in cerebrosides.

3. Derived Lipids:

Prostaglandins (PG):

(a) They virtually exist in every mammalian tissue and act as local hormones.

(b) They have important physiologic and pharmacologic activities.

(c) They are synthesized in vivo by cyclization of the center of the carbon chain of 20-C polyunsaturated fatty acids (e.g., arachidonic acid) to form a cyclopentane ring.

(d) Three different eicosanoic fatty acids give rise to three groups of eicosanoids characterized by the number of double bonds in the side chains, e.g., PG_1, PG_2, PG_3. Variations in the substituent groups attached to the rings give rise to different types in each series of prostaglandins, as for example, "E" type of Prostaglandin has a keto group in position 9, whereas the "F" type has a hydroxyl group in this position.

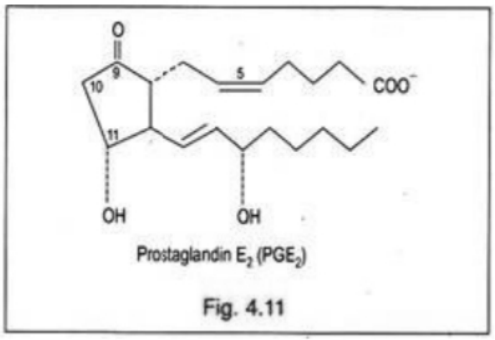


Fig. 4.11

Prostacyclin's (PGI):

(a) They are formed in vascular endothelium and continually formed in heart. They are also formed in kidneys.

(b) They are formed from cyclic endo-peroxide PGH_2 by the action of microsomal Prostacyclin synthetase.

(c) They inhibit platelet aggregation and gastric secretion from the pyloric mucosa.

(d) They decrease blood pressure and protect coronary arteries.

(e) They increase renal blood flow and stimulate renin production.

(f) They are inhibited by hyperlipemia, vit. E deficiency and radiation.

Thromboxane's:

(a) They contract smooth muscles on blood vessels, GI Tract, uterus, bronchioles.

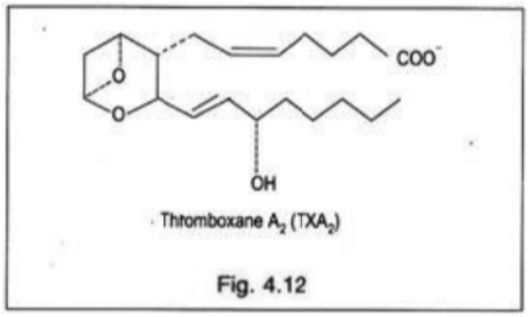
(b) They are discovered in platelets, and have the cyclopentane ring interrupted with an oxygen atom (Oxane ring).

(c) The substituent groups attached to the rings being varied give rise to different types in each series of thromboxane's labelled A, B, etc.

(d) They produce vasoconstriction and increase blood pressure.

(e) They cause release of serotonin and calcium ion (Ca^{++}) from platelet granules.

(f) Imidazole's inhibit their synthesis.



Thromboxane A_2 (TXA_2)

Fig. 4.12

Leukotriene's:

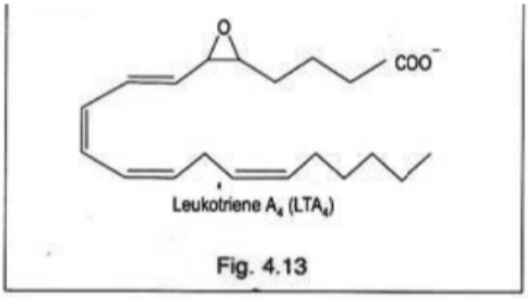
(a) They are the third group of eicosanoid derivatives formed via the lipoxygenase pathway rather than cyclization of the fatty acid chain.

(b) They are first described in leukocytes.

(c) They are characterized by the presence of three conjugated double bonds.

(d) They are stimulators of mucus secretion and are responsible for vasoconstriction of bronchial muscles.

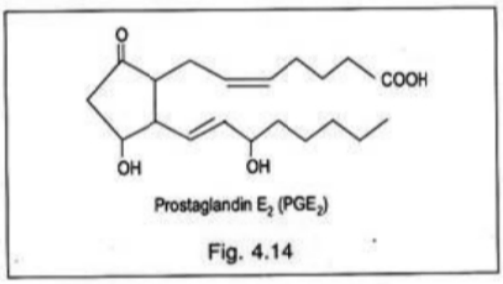
(e) They are inhibited by prolonged use of aspirin.



Leukotriene A_4 (LTA_4)

Fig. 4.13

The group of compounds known as prostaglandins are synthesized from arachidonic acid in the body. They have pharmacologic and biochemical activity.



Prostaglandin E_2 (PGE_2)

Fig. 4.14

Example : Prostaglandin E_2(PGE_2)

C. Many Other Fatty Acids:

(i) These have been detected in biologic material.

Example:

Fish oil contain 5 and 6 unsaturated fatty acids having carbon atoms 22.

(ii) Various other structures with hydroxy groups (ricinoleic acid) or cyclic groups have been found in nature.

Example of cyclic groups is chaulmoogric acid which was used many years ago in the treatment of leprosy.

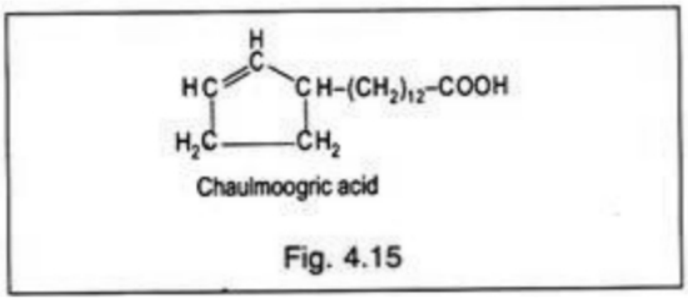


Fig. 4.15

AMINO ACIDS

Amino acids are the building blocks of proteins. Among the thousands of amino acids available in nature, proteins contain only 20 different kinds of amino acids, all of them are L-alpha-amino acids. The same 20 standard amino acids make proteins in all the living cells, may it either be a virus, bacteria, yeast, plant or human cell. These 20 amino acids combine in different sequences and numbers to form various kinds of proteins.

The number of proteins that can be had from these 20 amino acids can be calculated from 20 factorial, i.e., 20 x 19 x 18 x 17 x 16 x ..... x 2 x 1 = 2.4 x 10^{18}. In human beings alone there are more than 100 000 different types of proteins.

The general formulae for an amino acid can be written as 'R-CH-NH_2—COOH'. Depending upon the 'R' group present in the amino acid it is named accordingly. The 20 amino acids found in the proteins are known as primary or standard amino acids. In addition to these, some other amino acids are also found in proteins like 4-hydroxyproline, 5-hydroxylysine, 6-IV-methyllysine, gamma carboxyglutamic acid and desmosine, all of these are derivatives of standard amino acids.

Classification of Amino Acids:

I. Depending upon the Charge:

Amino acids can be broadly classified into three major groups:

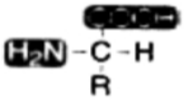
(1) Neutral

(2) Acidic and

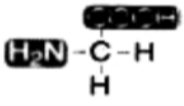
(3) Basic.

1. Neutral amino acids:

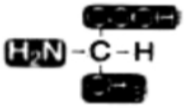
Those amino acids that do not contain any charge on the 'R' group.



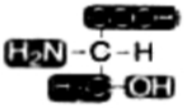
GENERAL FORMULA



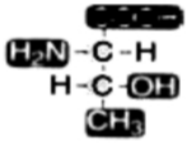
Glycine (Gly)
Mw = 75.08



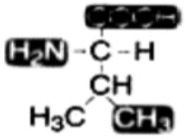
Alanine (Ala)



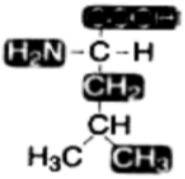
Serine (Ser)



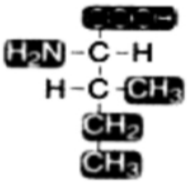
Threonine (Thr)



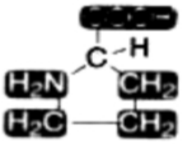
Valine (Val)



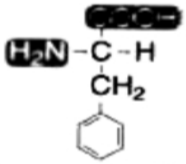
Leucine (Leu)



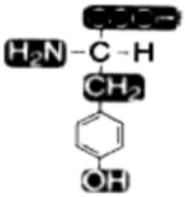
Isoleucine (Ile)



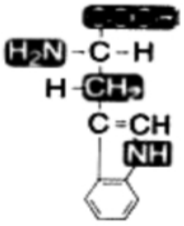
Proline (Pro)



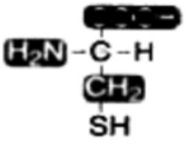
Phenylalanine (Phe)



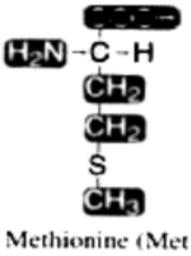
Tyrosine (Tyr)



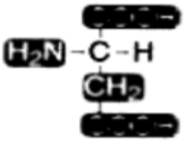
Tryptophan (Trp)
Mw = 206.27



Cystine (Cys)

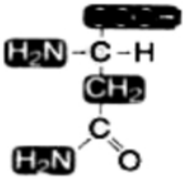


Methionine (Met)



Aspartic acid (Asp)

Glutamic acid (Glu)



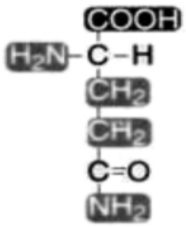
Asparagine (Asn)

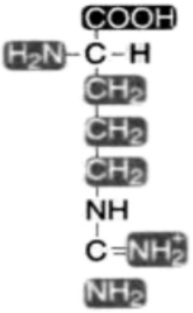
They are further classified into the following categories:

(a) Aliphatic:

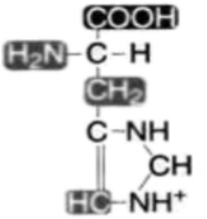
Those amino acids whose 'R' group contains a chain of carbon atoms—Gly, Ala, Ser, Thr, Val, Leu, lie, Asn, Gin.

(b) Aromatic:
Those amino acids whose 'R' group has a benzene ring—Phe, Tyr, Trp.
(c) Heterocyclic:
The "R" group has a heterocylic ring, i.e., any of the ring structures which contain different atoms—Pro, His.
(d) Sulphur containing:
Those amino acids which contain a sulphur atom-Cys, Met.
2. Acidic amino acids:
Those amino acids that contain a negative charge or an acidic group-Asp, Glu.
3. Basic amino acids:
Those amino acids that contain a positive charge or a basic group-Arg, Lys and His.


Glutamine (Gln)


Arginine (Arg)
Mw = 175.25


Lysine (Lys)


Histidine (His)

II. Depending upon the Solubility in Water:

The amino acids can also be grouped into two different categories, depending upon their solubility in water. They are—
1. Hydrophobic amino acids:
Amino acids insoluble in water are known as hydrophobic amino acids. They are—Ala, Val, Leu, lie, Pro, Met, Phe, Trp.
2. Hydrophilic amino acids:
Amino acids soluble in water are known as hydrophilic amino acids. They are—Gly, Ser, Thr, Cys, Tyr, Asp, Asn, Glu, Gin, Lys, Arg, His.
III. Depending upon their Nutritional Requirements:

The amino acids are classified into two groups.
They are:
1. Essential amino acids:
Are those which cannot be synthesized by the human body and hence they should be taken through the diet. There are 10 essential amino acids. Among these amino acids, arginine and histidine are known as semi-essential amino acids.

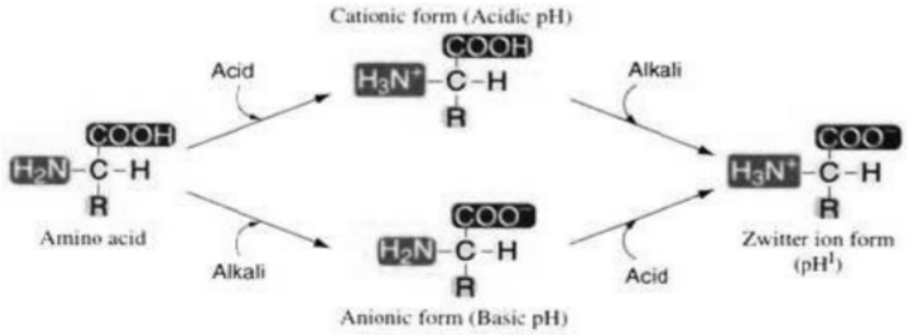
M – Methionine
A – Arginine
T – Threonine
T – Tryptophan
V – Valine
I – Isoleucine
L – Leucine
P – Phenylalanine
H – Histidine
Ly – Lysine

2. Non-essential amino acids:
These acids are those that can be synthesized in the human body and are not required in the diet. These include gly, ala, ser, pro, tyr, cys, asp, asn, glu, gin.

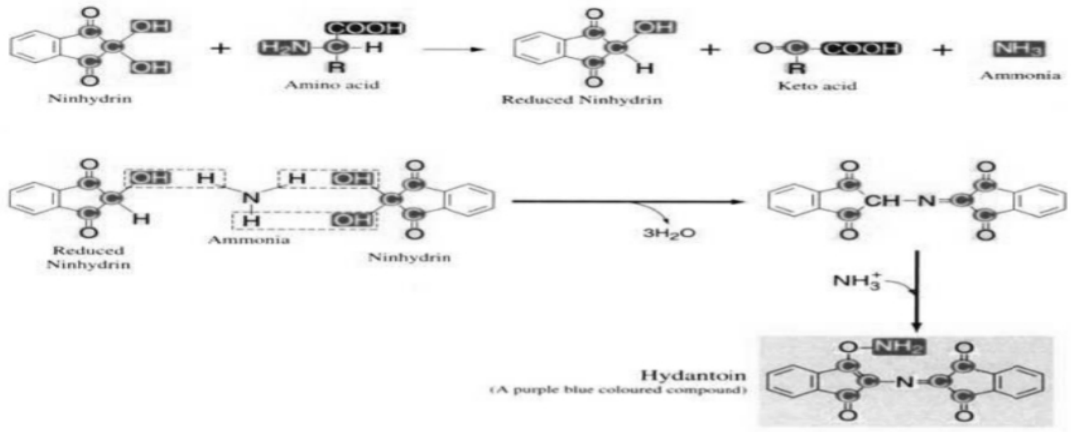
Reactions of Amino Acids:
Physical Characters of Amino Acids:

1. Zwitter ions:
Amino acids have an acidic group (—COOH group), i.e., a proton, donor. They also have a basic group (—NH_2 group), i.e., a proton, acceptor. A compound capable of both donating and accepting protons and thus able to act either as an acid or a base is known as amphoteric molecule. Amino acids have both anions and cations in solution and such compounds are called zwitter ions.

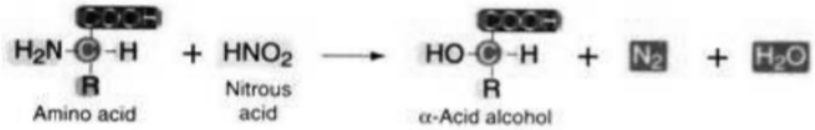
2. Isoelectric pH (pH^1):
The pH at which the positive charge on the amino acid (or any other molecule) is equal to the negative charges, is known as isoelectric pH. At this pH the net charge will be zero and hence it does not move either to positive (anode) or to negative (cathode) electrode, when subjected to an electric field. At pH^1 all the molecules exists in zwitter ion form.



Chemical Properties:
1. Reactions due to amino group:
(a) Ninhydrin test:
This test identifies or detects amino acids. If amino acids are heated with ninhydrin, they form a purple blue coloured compound, which is measured colorimetrically.

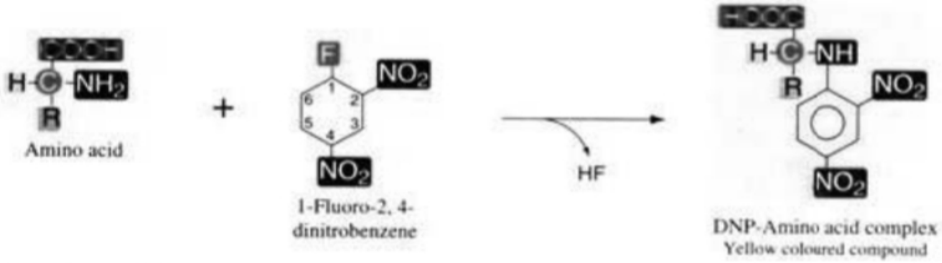


(b)Reaction with nitrous acid:
It is a method by which amino acids are measured depending upon the amount of nitrogen released.

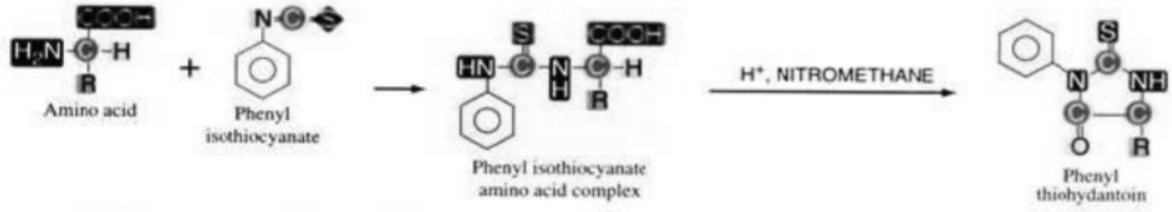


(c) Reaction with carbonyl compounds (RCHO):
The amino group in the amino acids reacts with carbonyl compounds forming a Schiff's base.

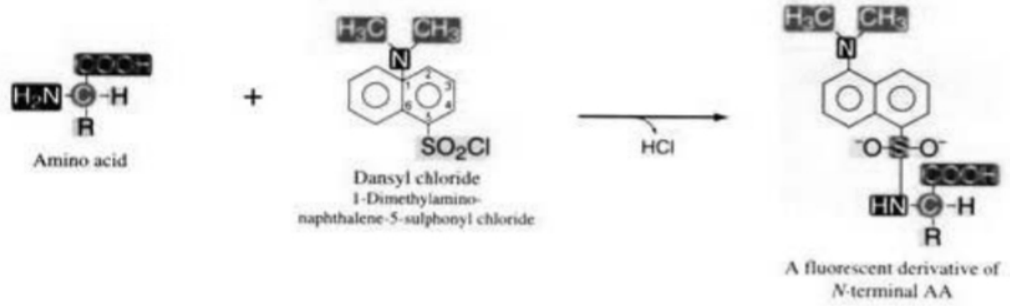
(d) Reaction with Sanger's reagent:
Amino acids react with Sanger's reagent, i.e., 1-fluoro-2, 4-dinitrobenzene, forming a yellow coloured complex. This reagent is used to detect the IV-terminal amino acid in the proteins.



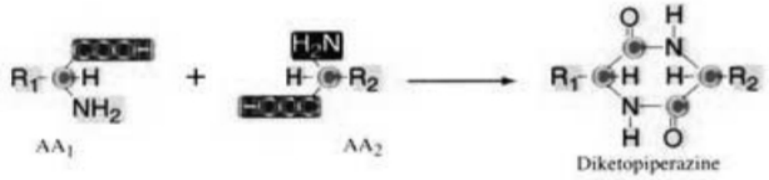
(e) Edmann's reaction:
Edmann's reagent is phenyl isothiocyanate, which is also used to detect the N-terminal amino acid in a protein. It forms a purple coloured derivative.



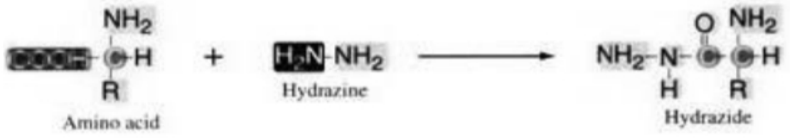
(f) Reaction with dansyl chloride:
Dansyl chloride, i.e., 1-dimethyl-amino-naphthalene-5-sulphonyl chloride forms a fluorescent derivate of the N-terminal amino acid of proteins. This is yet another reagent available for the detection of N-terminal amino acid.



(g) Condensation of two amino acids to form diketopiperazine:
Two amino acids react with amino groups of each amino acid and the carboxylic groups of the other amino acid forming a diketopiperazine.

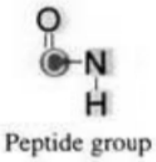


2. Reaction due to carboxylic:
(a) Reaction with hydrazine:
Hydrazine is used to detect the C-terminal amino acid in proteins. It forms a complex with the amino acid by reacting with the carboxylic group.



3. Reaction due to both amino and carboxylic group:
Due to the presence of both amino (basic) and carboxylic (acid) groups in amino acids, the amino group of one amino acid reacts with the carboxylic group of another amino acid to form a peptide bond.

Polymerization of amino acids in a similar manner gives a polypeptide chain.
Peptide bond:
The bond linking two amino acids is known as a peptide bond. It is formed due to reaction between an amino group of one amino acid and carboxylic group of another amino acid.
Peptide group:
The group forming the peptide bond is known as peptide group. It has a double bond character and hence is very rigid in nature.

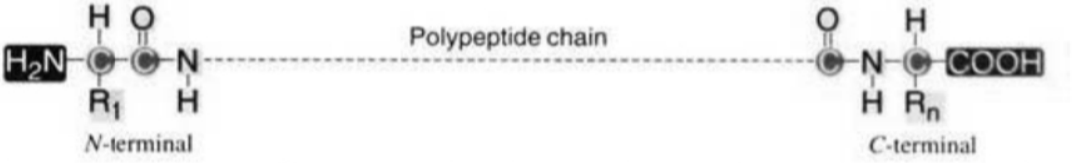


Polypeptide or peptide:
A chain made up of two or more amino acids, linked by a peptide bond is known as a polypeptide or just a peptide.
Difference between a peptide and a protein:
A peptide is that which has less than 50 amino acids or whose molecular weight is less than 5000 Daltons. A protein is that which has more than 50 amino acids or whose molecular weight is more

than 5000 Daltons. This differentiation is based upon the immunological property of the two units. Peptides are non-immunogenic, whereas proteins are immunogenic.

N-terminal and C-terminal of a protein:

The end of a protein or polypeptide where the amino group is free in known as N-terminal end and that amino acid whose amino group is free is known as N-terminal amino acid. Sanger's, Edmann's and Dansyl chloride are the reagents used to determine the N-terminal amino acids.



The end of the protein or polypeptide whose carboxylic group is free is known as C-terminal end and that amino acid whose carboxylic group is free in the protein is known as C-terminal amino acid. Hydrazine is used to detect the C-terminal amino acid. While representing a protein on paper, the N-terminal amino acid is written first (on the left) and the C-terminal amino acid is the last one (written at the right side of the paper).

Peptides of physiological importance:

(a) Glutathione:

It is a tripeptide made up of Glu, Cys and Gly. It is found in RBC and other tissues and functions to prevent oxidation of —SH groups of many enzymes.

(b) Bradykinin and kallidin:

These are small polypeptides containing 9 and 10 amino acids respectively. They are formed by partial hydrolysis of plasma protein due to snake poisoning (venom). They are powerful vasodepressors and inhibitors of heart function. Others are tyrocidin, gramicidin, glucagon, insulin, oxytocin, etc.

Derivatives of standard amino acids:

Some polypeptides chain unusual or rare amino acid residues which are derived by the post transcriptional modification from one of the 20 amino acids. The rare amino acids are not coded by DNA.

Some of them are as follows:

1. N-formylmethionine. It is the 1st amino acid of prokaryotic protein.
2. 4-hydroxyproline and 5-hydroxylysine found in collagen fibers in connective tissue.
3. carboxyglutamic acid found as a constituent of blood clotting factor prothrombin.
4. Cystine. The sulfhydryl groups (- SH) of two cysteine resides oxidized to form a cystine residue having disulfide bond (-S -S-). Cystine residues mostly found in extracellular proteins.
5. Acetylation: The N- termini when acetylated, makes the proteins more resistance to degradation.
6. Phosphorylation: Phsophoserine, phosphothreonine and phosphotyrosine are formed by the phosphorylation of their – OH group.

Non protein amino acids:

Over 150 modified amino acids are known that are not components of proteins.

A few examples are as follows:

(a) D-amino acids present in the peptides of bacterial cell wall are defensive in nature as they are not hydrolyzed by peptidases.

(b) Ornithine and citrulline, participate in Urea cycle.

(c) λ-aminobutyric acid (GABA) is a neurotransmitter derived from decarboxylation of glutamate.

{d) Dopamine is also a neurotransmitter derived from tyrosine.

(e) Thyroxin, a thyroid hormone, is a product of tyrosine.

(f) Histamine is a decarboxylation product of histidine. It is a vasodilator, causes allergic response, powerful stimulant of gastric secretion and constrict bronchial smooth muscles.

(g) Azaserine, valinomycin, atinomycin D, gramicidin S and tyrocidine are the useful components of antibiotics.

Properties:

Some of the major properties of amino acids are as follows:

1. Amino acids are colourless crystalline water soluble but largely insoluble in organic solvents. Most α-amino acid have melting points near 300°C whereas their non-ionic derivative usually melt around 100°C.

2. Glutamic acid was the first amino acid formed during the amino acid synthesis. From this all other amino acids are formed by reductive deamination and transamination.

3. Stereoisomerism:

Glycine is the simplest AA where the R-group is a hydrogen atom. Except glycine, all amino acids are asymmetrical (optically active) and exist in two stereo-isomeric forms i.e. L- isomer and D-isomer. Only the a-amino acids are found in proteins, while D-amino acids are rare in nature. Recently 2 free D-amino acids found in mammals, i.e. D-serine in the forebrain and D-aspertate in brain and in PNS.

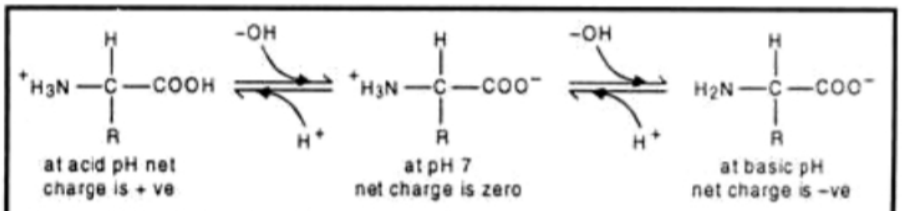


Figure 4.16 Ionization of an amino acid at different pH. At acid pH, amino acid is in cationic form and in neutral pH amino acid is in zwitter ionic form and at basic pH amino acid is in anionic form.

4. Ionization:

Amino acids have two ionizable groups i.e. a-amino and a-carboxyl groups. In addition to these, some amino acids have additional ionizable side-chain group (Asp, Glu, Arg, Lys,). In neutral solution (pH=7.0), amino acids are predominantly zwitterions or dipolar ions, where the α- amino group is protonated ($-NH_3^+$) and the a-carboxyl group is dissociated ($-COO^-$).

Therefore, a- amino acids are ampholytes (amphoteric electrolytes) i.e. can act as both acid (protein donor) and base (proton acceptor). In acid solution (e.g., pH1), only the a-amino group is ionized. In alkaline solution (e.g., pH1), only the a-carboxyl group is ionized. The pH at which an amino acid bears no net electric charge i.e. exist in form of Zwitter ion is called pi or isoelectric pH. pI can be calculated as the midpoint between the pK_1 and pK_2 values.

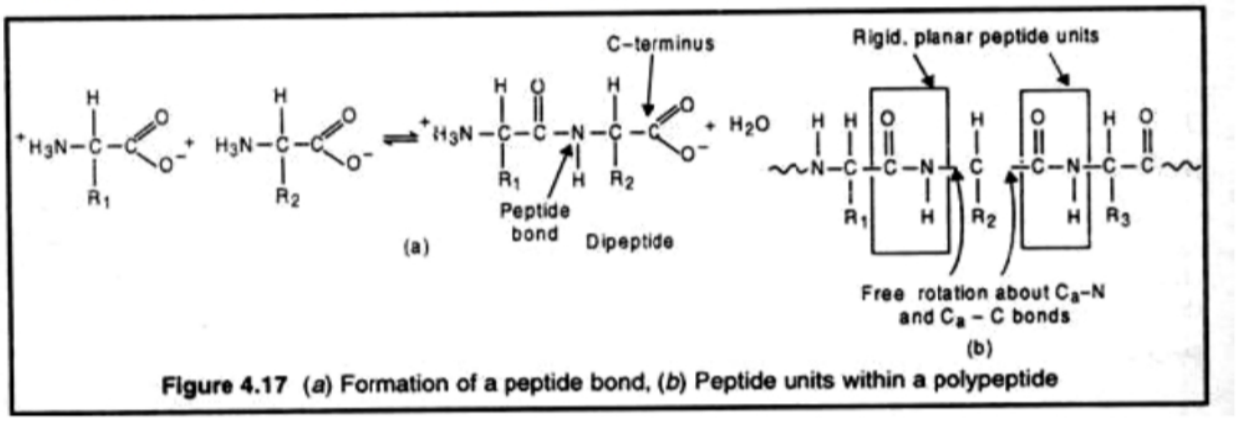


Figure 4.17 (*a*) Formation of a peptide bond, (*b*) Peptide units within a polypeptide

5. pK value :

All amino acids possess at least two weakly acidic functional groups. However, – COOH is several thousand times stronger acid than – NH^{3+}. The pK value is the pH at which these acidic groups are half dissociated. The pK ot- COOH group and – NH^{3+} group are respectively called pK_1 and pK_2. In all the 20 standard amino acids, the value of pK_1 varies from 1.8-2.9 and the pK_2 value varies from 8,

84 -10.78. Aromatic amino acids like tryptophan, tyrosine, histidine and phenylalanine absorb UV-light.

6. Peptide bond formation:

The peptide or amide bond (CO-NH) is a covalent linkage formed by removal of one water molecule between the a-amino group of one amino acid and the a-carboxyl group of another amino acid. After the formation of peptide bond, each amino acid is known as amino acid residue.

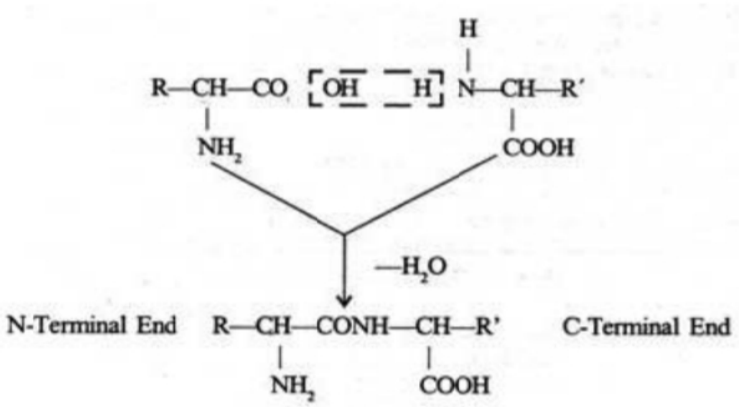
The peptide group is rigid and planer, while the peptide bond has partial double bond character with no rotational freedom. A peptide is a molecule that consists of two or more amino acid residues, e.g., dipeptide (2 amino acid residues), tripeptide (3 amino acid residue) etc. A polypeptide usually contains 20-4000 residues.

The backbone of the polypeptide includes the repeating sequence of carbon atoms and peptide bonds while the side chains of different amino acids responsible for functioning of the protein. A polypeptide with more than 100 residues is generally called a protein. A protein may consist of one or more polypeptide. A peptide is a polar molecule starting with the amino terminus (N-terminus) and ends with the carboxy terminus (C-terminus).

Proteins

Proteins are organic nitrogenous compounds in which a large number of amino acids are joined together by peptide linkages to form long polypeptide chains. Peptide-linkage (—CONH—) is formed when amino group (—NH_2) of one amino acid condenses with carboxylic group (—COOH) of another amino acid eliminating one molecule of water.

That end of the polypeptide chain where the —COOH group of the amino acid is not involved in peptide linkage is called as C-terminal end. The other end of the polypeptide chain with amino acid having free —NH_2 group is called as N-terminal end.



Although there may be hundreds of amino acids in a single polypeptide chain but fundamentally there are only about 20 different types of amino acids that constitute proteins in plants (there may be repetition of amino acids continuously or at intervals in the polypeptide chain).

Because of their very large size the proteins are often called as gigantic molecules or macromolecules of the cells. Their molecular weight may range from few thousands to over a million (10 lakhs.)

Structure of the Proteins:

The structure of the proteins can be studied under the following heads:

(1) Primary Structure of the Proteins:

Specific sequence or the arrangement of amino acids in the polypeptide, chain constitutes the primary structure of the proteins (Fig. 9.25).

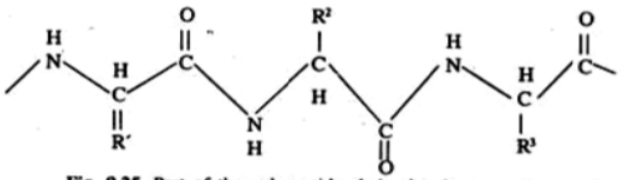


Fig. 9.25. Part of the polypeptide chain showing arrangement of amino acids.

(2) Secondary Structure of the Proteins :

Three types of helical structures are found:

(a) Alpha helix

(b) Beta pleated and

(c) Reverse turn.

1. Alpha helix:

α means the first and the structure described below was the first among the helical structures to be discovered, hence known as alpha (α) helix.

Polypeptide chain of the protein molecule is held in a coiled or helical shape by hydrogen bonds which are established in between the peptide linkages. The coiled or helical shape of polypeptide chain constitutes the α-helix or secondary structure of the protein (Fig. 9.26).

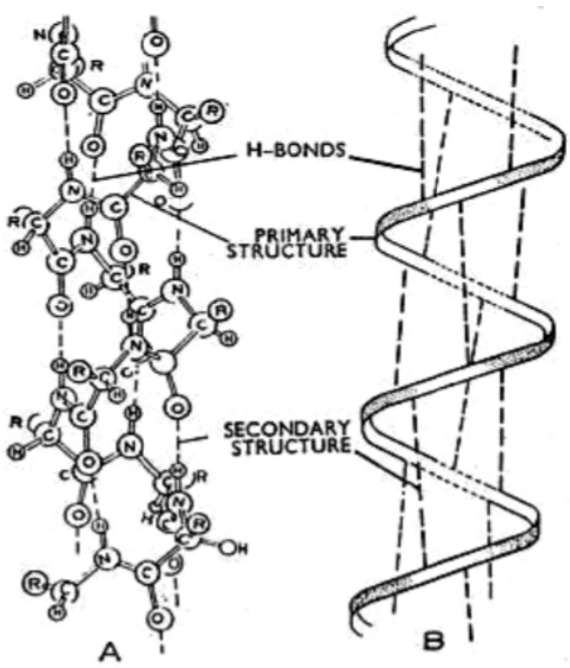


Fig. 9.26. α-helix structure of a protein (A) Molecular (B) Diagrammatic.

Although hydrogen bonds are very weak but when they are present in very large number all along the backbone of the polypeptide chain, they reinforce one another to stabilize the helical structure.

In a typical helical protein,

i. Each NH group (of peptide bond) is connected to a C=O group (of another peptide bond) by a H-bond at a distance equivalent to 3 amino acid residues.

ii. An α-helix or complete turn of a coil contains about 3.67 amino acid residues.

iii. The pitch of the helix is 5.4 Å (vertical distance along the axis from any point on the helix to a corresponding point directly above it is called as the pitch of the helix).

iv. Therefore, each amino acid is about 1.5 Å (5.4/3.6) distant from the next amino acid residue.

Besides a-helix structure, other types of secondary structures of proteins also occur. Among these, β-conformations (P-pleated sheets) are most common that are found in fibrous proteins called β-keratins. In β-pleated sheets, a number of polypeptide chains (which do not form helices) are cross linked with H-bonds in which amino acid to carboxyl terminal orientation may be in the same direction (parallel) or inverse (antiparallel).

Linus Pauling and Robert Corey are credited with doing pioneer work on peptide bond and organization of proteins. They even predicted the existence of secondary structures of proteins many years before the first complete structure of protein was elucidated.

The salient features of this structure are as under:

i. Here the polypeptide is twisted or coiled to form a right handed helical structure.

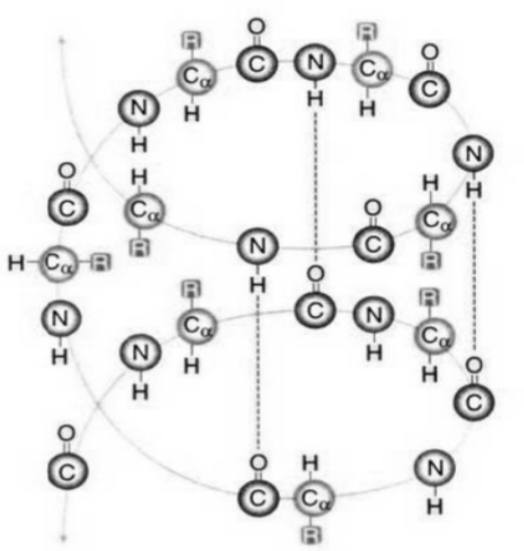
ii. The distance between each turn of the coil is 5.4 Å.

iii. There are 3.6 amino acids per turn.

iv. The 'R' groups are seen protruding out of the helix.

v. There are intra chain hydrogen bonding, wherein the hydrogen of —NH group combines with oxygen of -CO group of the 4th amino acid behind it. So every peptide group participates in hydrogen bonding.

vi. This type of structure is found in many proteins in combination with other structures. Pure a-helix structure is seen in hair protein, i.e., keratin.



2. Beta pleated:

β means the second and the structure described below was the second discovery after α helix.

The salient features of this structure are:

i. Here the chain is not helical but zigzag.

ii. The distance between each turn is 7 Å.

iii. Polypeptide chains are arranged side by side in the form of pleats.

iv. There is inter-chain hydrogen bonding between the chains and each peptide group participates in hydrogen bonding.

The chains are anti-parallel to each other.

3. Reverse turn:

Folds back on itself in reverse direction of the chain.

(3) Tertiary Structure of the Proteins:

The coiled (α-helix) polypeptide chain is further folded in various ways. This folding which is very specific for a particular protein constitutes its tertiary structure and is determined by its primary structure (Fig. 9.27).

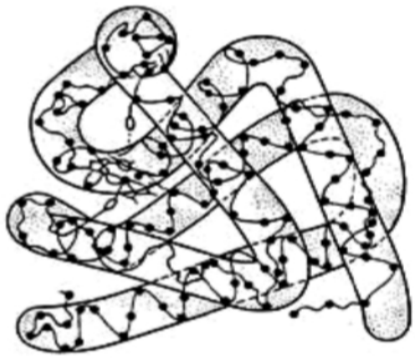


Fig. 9.27. Diagrammatic representation of the tertiary structure of a protein (sperm whale myoglobin).

(Tertiary structure of the proteins is essential for biologically active proteins i.e., the enzymes. They are rendered useless (or are denatured) if their tertiary structure is lost).

The tertiary structure of the protein is stabilized by the following forces which have also been shown in Fig. 9.28.

i. H-bonds (other than those established between the peptide linkages).

ii. Di-sulphide (S-S) bonds.

iii. Ionic bonds or salt linkages.

iv. Steric Effects i.e., the interaction of non-polar side chains caused by the mutual repulsion of the solvent.

v. Van der Waals forces.

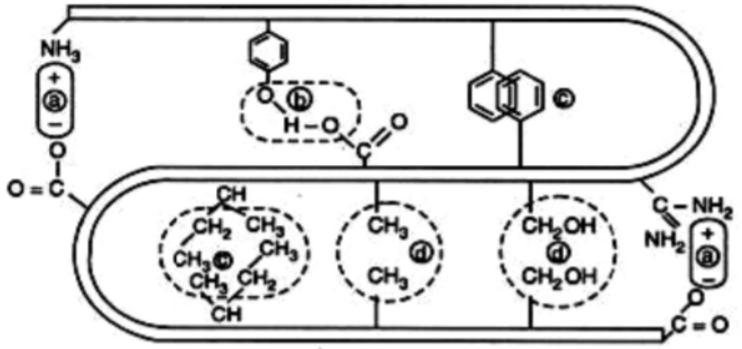


Fig. 9.28. Some types of non-covalent bonds which stabilize protein structure (*a*) electrostatic interaction (*b*) hydrogen bonding between tyrosine residues and carboxylate groups on side chains (*c*) interaction of non-polar side chains caused by the mutual repulsion of solvent (*d*) Van der Waals interactions.

1. Hydrogen bonds:

Formed between hydrogen and an electronegative atom like oxygen or nitrogen in the 'R' group of amino acids.

2. Ionic interactions:

Formed between acidic (glutamic and aspartic) and basic (arginine, lysine or histidine) amino acids.

3. Disulphide bonds:

This is a strong bond formed between the sulphahydryl groups of two cysteine amino acids. The resultant dimer structure formed is known as cystine (an amino acid found in proteins only and not in free form).

4. Hydrophobic interactions:

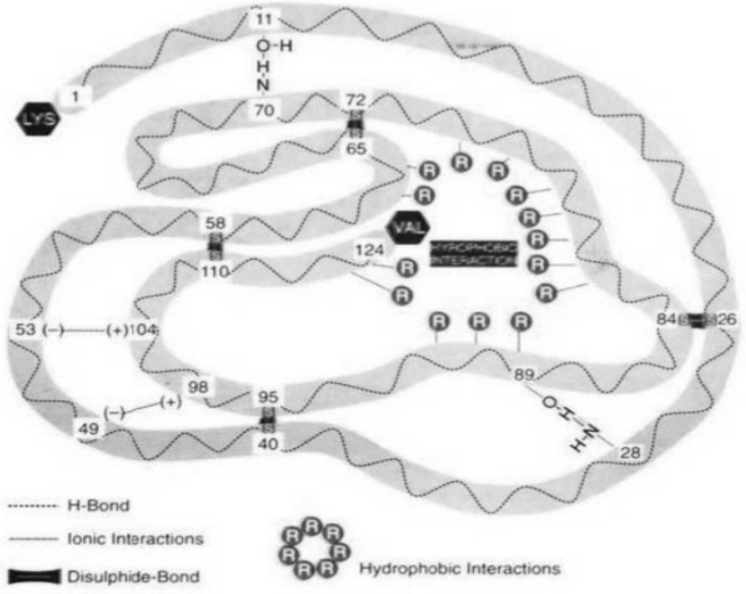
The 'R' groups of the hydrophobic amino acids aggregate together in the centre away from water, thereby developing a force of attraction between each "R" group and a force of repulsion from the water and these interactions are known as hydrophobic interactions.

(All the molecules exert a week force of attraction upon one another due to mutual interaction of their electrons and nuclei. There is a electrostatic attraction between the electrons of one molecule and the nuclei of the other while on other hand, there is electrostatic repulsion of nuclei and electrons of the molecule by the nuclei and electrons of the other molecule respectively. The resultant week force of attraction between the two molecules is known as the Van der Waals attraction or Van der Waals force. The energy of such forces is about 1 k.cal/mole).

(4) Quaternary Structure of the Proteins:

Sometimes more than one polypeptide chains are associated together to form a relatively more stable super molecule of protein. This constitutes the quaternary structure of the protein.

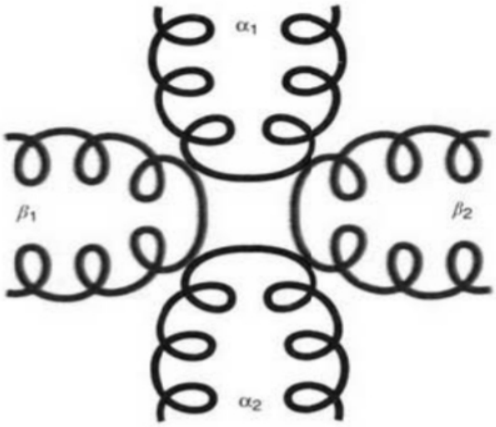
For example, in blood haemoglobin there are four polypeptide chains or subunits that constitute the protein.



Quaternary structure is maintained by various forces like di-sulphide-linkages, H-bonds etc., between the different polypeptide chains of the protein.

Oligomeric proteins:

Are those which have two or more polypeptide chains.



Quaternary structure refers to the type of arrangement of the polypeptides in an oligomeric protein. These polypeptides are held together by either hydrogen bonds, ionic bonds or Vander Waals' forces, e.g., Hemoglobin has four polypeptide chains which are arranged in a particular fashion that is referred to the quaternary structure of hemoglobin.

The Quaternary structure of hemoglobin describes that it is made up of four polypeptide chains; two of which are α (α_1 & α_2) and the other two are β (β_1 & β_2). The two alpha chains are opposite to each other and adjacent to each β-chain. The α chains and the β chains are linked together by salt bridges.

Structure function relationship in proteins:

Hemoglobin plays a vital role in transport of oxygen from the lungs to the peripheral tissues and transport of carbon dioxide from the tissue to the lungs.

There are three types of normal hemoglobin with the following polypeptides:

(1) Adult hemoglobin (Hb A) has 2α2β chains.
(2) Foetal hemoglobin (Hb F) has 2α2γ chains.
(3) Minor adult hemoglobin (Hb A_1) has 2α2δ chains.

The number of amino acids in α chains is 141 amino acids and the other chains, i.e., β, γ & δ chains have 146 amino acids. These chains are differentiated based upon the difference in the sequence of arrangement of the amino acids in the chains. The quaternary structure of hemoglobin creates a cavity in between the tetramer in which 2, 3, diphosphoglycerate (DPG or BPG) is present forming a salt bridge with the amino terminal of β-chain that stabilizes the hemoglobin thereby lowering the affinity to oxygen.

In the lungs, the partial pressure of oxygen is high which results in binding of O_2 to one of the chains of Hb thereby rupturing the salt bridges between the four subunits. Subsequent oxygen binding (sigmoid curve of Hb-O_9 association) is facilitated by rupture of the salt bridges altering secondary, tertiary and quaternary structures thus allowing rotation of one α/β subunit with respect to another α/β chain thereby compressing the tetramer and release of DPG. This results in increasing its affinity towards oxygen (the R state of Hb).

In the peripheral tissues, CO_2 binds with the a-amino group of the amino terminal with its conversion from positive to negative charge which favours salt bridge formation between the polypeptide chains with return to the deoxy state (T-state), i.e., release of oxygen from Hb. Release of O_2 from the Hb is also facilitated by binding of DPG to the tetramer.

When a person takes off on a flight, the aero plane slowly rises in altitude resulting in lowering of the O_2 tension due to which oxygenation of Hb is not possible. Thus the person feels hypoxic, but the physiological mechanism of the body starts decreasing the production of DPG, due to which Hb can bind the oxygen even at lower pressure of oxygen.

Therefore, when the aero plane reaches the maximum altitude and stays stable, the person feels comfortable. When Hb reaches the tissues DPG level increases enhancing release of oxygen. Similarly, the above process reverses, when a person dives deep into the sea. The high O_2 pressure results in increased production of DPG facilitating oxygenation of Hb in the lungs and deoxygenating in the peripheral tissues.

Classification of Proteins:

Proteins are classified based upon:

(1) Their solubility and

(2) Their structural complexity.

A. Classification Based upon Solubility:

On the basis of their solubility in water, proteins are classified into:

1. Fibrous proteins:

These are insoluble in water. They include the structural proteins. They have supportive function (e.g., collagen) and/or protective function (e.g., hair keratin and fibrin).

2. Globular proteins:

They are soluble in water. They include the functional proteins, e.g., enzymes, hemoglobin, etc.

B. Classification Based upon Structural Complexity:

On the basis of their structural complexity they are further divided into:

(1) Simple

(2) Conjugated and

(3) Derived proteins.

1. Simple proteins:

Proteins which are made up of amino acids only are known as simple proteins.

They are further sub-divided into:

(a) Albumins:

They are water soluble, heat coagulable and are precipitated on full saturation with ammonium sulphate, e.g., serum albumin, lactalbumin and ovalbumin.

(b) Globulins:

They are insoluble in water, but soluble in dilute salt solutions. They are heat coagulable and precipitate on half-saturation with ammonium sulphate, e.g., serum globulin and ovo-globulin.

(c) Glutelins:

They are insoluble in water and neutral solvents. Soluble in dilute acids and alkalies. They are coagulated by heat, e.g., glutelin of wheat.

(d) Prolamines:

Water insoluble but soluble in 70% alcohol, e.g., gliadin of wheat, proteins of corn, barley, etc.

(e) Histories:

Water soluble, basic in nature due to the presence of arginine and lysine, found in nucleus. They help in DNA packaging in the cell. They form the protein moiety of nucleoprotein.

(f) Protamine's:

Water soluble, basic in nature, not-heat coagulable. Found in sperm cells, hence component of sperm nucleoprotein.

(g) Globin's:

They are water soluble, non-heat coagulable. e.g., globin of haemoglobin.

(h) Albuminoids or scleroproteins:

Insoluble in all neutral solvents, dilute acids or alkalies, e.g., keratin of hair and proteins of bone and cartilage.

2. Conjugated proteins:

Proteins which are made up of amino acids and a non-amino acid/protein substance called the prosthetic group are known as conjugated proteins.

The various types of conjugated proteins are:

(a) Chromo proteins:

Here the non-protein part is a coloured compound in addition to the protein part. Ex. Haemoglobin has heme as the prosthetic group and cytochromes also have heme.

(b) Nucleoproteins:

These proteins are bound to nucleic acids, e.g., chromatin (histones + nucleic acids).

(c) Glycoproteins:

When a small amount of carbohydrate is attached to a protein it is known as glycoproteins, e.g., mucin of saliva. (Note: Glycoproteins have major amounts of protein and some amount of carbohydrates and proteoglycans contain major amounts of carbohydrates and little amount of proteins).

(d) Pbosphoprotein:

Phosphoric acid is present with the protein. Ex. Milk casein and egg yolk (vitellin).

(e) Lipoproteins:

Proteins in combination with lipids, e.g., LDL, HDL.

(f) Metalloproteins:

They contain metal ion in addition to the amino acids, e.g., hemoglobin (iron), ceruloplasmin (copper).

3. Derived proteins:

They are the proteins of low molecular weight produced from large molecular weight proteins by the action of heat, enzymes or chemical agents.

Proteins → Proteans → Proteoses → Peptones → Peptides → Amino acids

Depending upon the nature of the prosthetic group associated with them, conjugate proteins are classified as follows:-

1. Nucleoproteins (or Histones):

These are associated with nucleic acids.

2. Glycoproteins:

These are associated with some carbohydrate.

3. Chromoproteins:

These proteins are associated with some colouring matter e.g., chlorophylls, carotenoids, phycobillins etc.

4. Lipo-proteins:

These are associated with some lipid or fatty substances. (They are chiefly found in cy- to membranes).

5. Iron-porphyrin Proteins:

These are associated with iron-porphyrin compounds e.g., the cytochromes.

6. Simple Metal Containing Proteins:

These proteins are associated with some metal directly e.g., ferredoxin where Fe atoms are directly attached to the protein molecule.

7. Flavoproteins:

These are associated with some flavin compound e.g., FAD (Flavin Adenine-Dinucleotide).

Properties of Proteins:

1. Denaturation:

Partial or complete unfolding of the native (natural) conformation of the polypeptide chain is known as denaturation. This is caused by heat, acids, alkalies, alcohol, acetone, urea, beta- mercaptoethanol.

2. Coagulation:

When proteins are denatured by heat, they form insoluble aggregates known as coagulum. All the proteins are not heat coagulable, only a few like the albumins, globulins are heat coagulable.

3. Isoelectric pH (pH^I):

The pH at which a protein has equal number of positive and negative charges is known as isoelectric pH. When subjected to an electric field the proteins do not move either towards anode or cathode, hence this property is used to isolate proteins. The proteins become least soluble at pH^I and get precipitated. The pH^I of casein is 4.5 and at this pH the casein in milk curdles producing the curd.

4. Molecular Weights of Proteins:

The average molecular weight of an amino acid is taken to be 110. The total number of amino acids in a protein multiplied by 110 gives the approximate molecular weight of that protein. Different proteins have different amino acid composition and hence their molecular weights differ. The molecular weights of proteins range from 5000 to 10^9Daltons. Experimentally the molecular weight can be determined by methods like gel filtration, PAGE, ultra centrifugation or viscosity measurements.

Nucleic acids

In 1868 Meischer isolated a substance from the nucleus of pus cells. By digesting pus cells in HC1, he obtained a pure material and named it nuclein. Nuclein had strong acidic properties and contained considerable amount of phosphorus.

In 1889 Altmann coined the term nucleic acid. Later existence of two types of nucleic acid, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) was established.

The present knowledge about the structure of DNA is the result of research by great scientists like Chargaff, Stendel, Levene, Todd, Wilkins, Rosalind. Franklin, Astbury, Watson, Crick, Hargobind Khorana and many others.

All living organisms possess, nucleic acids. Nucleic acids are macromolecules of utmost biological importance. Nucleic acids possess all the information needed for an organism's cell structure, function, development and reproduction.

DNA:

DNA is mainly present in the chromosomes in the nucleus. A small amount of DNA is present in the mitochondria and chloroplasts. Mitochondria and chloroplasts are self- replicating bodies.
DNA molecule occupies central position among biological macromolecules. It is a storehouse of genetic information. Nucleotides sequences of DNA encode proteins and enzymes, which directly or indirectly control the synthesis of all cellular components.
DNA has an excellent mechanism for stable storage of genetic information. The enzymes that synthesize DNA, faithfully copy DNA molecules containing millions of bases. They perform this function with high degree of accuracy and speed. DNA is the only macromolecule for which repair mechanism exists if any damage is caused to it.

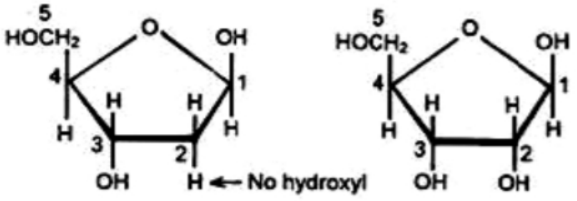
Structure of DNA:

DNA molecules are largest molecules of the cell, much larger than those of proteins. DNA is found in all animal and plant cells, prokaryotes and most of the viruses. In addition, it is also found in mitochondria and chloroplasts.

Chemical Composition of DNA:

DNA is made up of numerous monomer units called deoxyribonucleotides. Each deoxyribonucleotide consists of three components.
1. Pentose sugar – deoxyribose
2. A nitrogenous base – Purine or Pyrimidine
3. Phosphorus molecule.

Pentose sugar:
In DNA, the pentose sugar is deoxyribose. A similar pentose sugar ribose is present in RNA. The only difference between the two is the absence of hydroxyl group at position number two of the sugar ring in DNA. Therefore, it is called 2′-deoxyribose. This makes DNA more stable. Deoxyribose can be stained with Feulgen stain.



Deoxyribose $C_5H_{10}O_4$ Ribose $C_5H_{10}O_5$

Nitrogen Bases:

Nitrogen bases in DNA are of two kinds, Purine and Pyrimidine.

Purine:
Purines are dicyclic and have fused five and six member rings. Purines are of two kinds, Adenine (A) and Guanine (G).

Pyrimidines:
Pyrimidines are monocyclic and have a six member ring. Pyrimidines are of two kinds – Thymine (T) and Cytosine (C).
In RNA, thymine is replaced by uracil (U). The only difference between uracil and thymine is the presence of a methyl subunit at position C-5 in thymine.

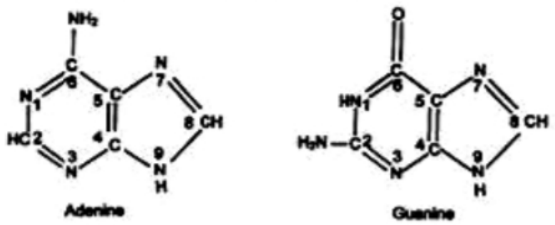


Fig. 3.2

In this way DNA consists of four bases A, G, C, T and RNA has A, G, C, U.

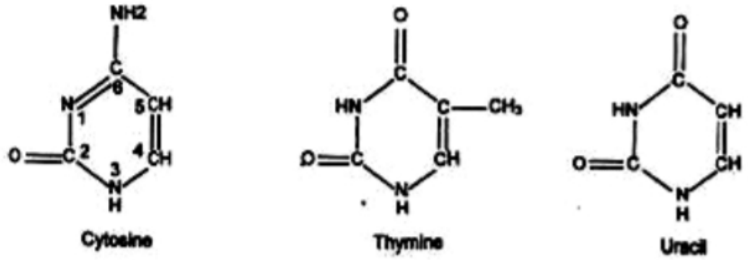


Fig. 3.3

The nitrogen base is linked to the sugar molecule by a glycosidic bond. The bond is formed between the first carbon atom of the sugar and nitrogen at position 1 in the case of pyrimidine and at position 9 in the case of purine.

Phosphate Group:

Phosphate group is attached to the 5′-carbon of deoxyribose sugar of one nucleotide and 3′-carbon of the deoxyribose sugar of next nucleotide. Phosphate group provides strong negative charge to the nucleic acid. The bond between phosphate and deoxyribose sugar is phosphodiester bond.

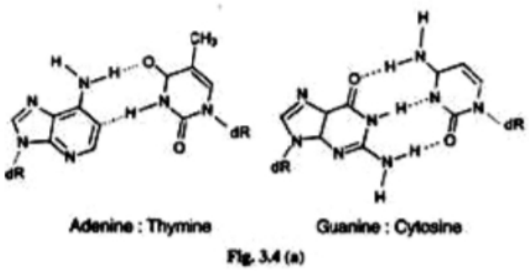


Fig. 3.4 (a)

Nucleosides and Nucleotides:

Within the structure of the nucleic acid, a nitrogen base is linked to sugar to form nucleoside. Nucleosides are called deoxyribouncleosides. Four kinds of nucleosides are formed in DNA.

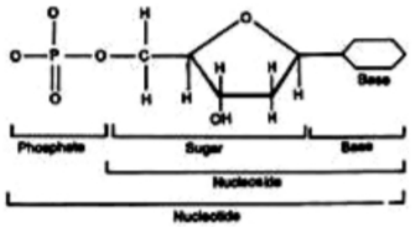


Fig. 3.4. (b)

Deoxyribose + Adenine → Deoxyadenosine

Deoxyribose + Guanine → Deoxyguanosine

Deoxyribose + Cytosine → Deoxycytidine

Deoxyribose + Thymine → Deoxythymidine

Purine nucleosides are suffixed by sine while pyramidine nucleosides are suffixed by dine.

Nucleotides:

Nucleotides are phosphoric acid esters of nucleosides with phosphorous at position C-5 of the sugar of one nucleoside and at C-3 of the next nucleoside. Nucleoside is joined to phosphorus by phosphodiester bands.

Base	Ribonucleoside	Deoxyribonucleoside
Adenine	Adenosine	Deoxyadenosine
Guanine	Guanosine	Deoxyguanosine
Cytosine	Cytidine	Deoxycitidine
Uracil	Uridine	—
Thymine	—	Thymidine (since only the deoxyriboside of thymine occurs naturally)

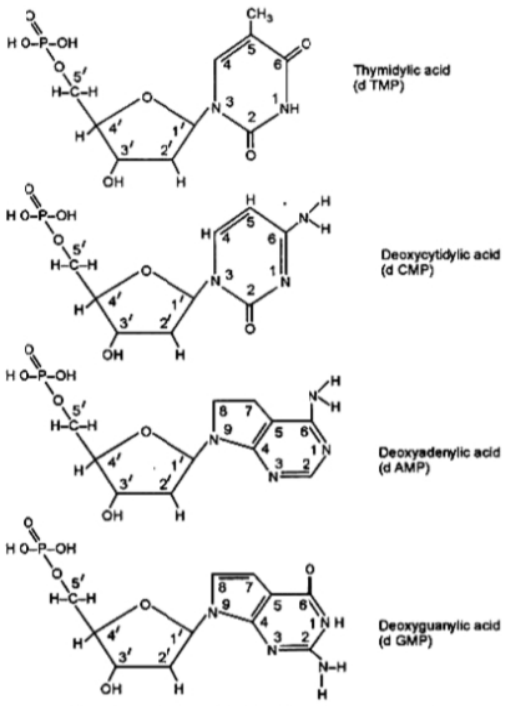


Fig. 3.5. The four deoxyribonucleotides found in DNA. The ring positions of bases are designated by plain numbers.

TABLE 3.2. The bases and nucleotides usually found in RNA and DNA

Base	RNA nucleotide	DNA nucleotide
Adenine (A)	Adenylic acid or Adenosine monophosphate (AMP)	Deoxyadenylic acid or deoxyadenosine monophosphate (dAMP)
Guanine (G)	Guanylic acid or guanosine monophosphate (GMP)	Deoxyguanylic acid or deoxyguanosine monophosphate (dGMP)
Cytosine (C)	Cytidylic acid of cytidine monophosphate (CMP)	Deoxycytidylic acid or deoxycytidine monophosphate (dCMP)
Thymine (T)	–	Thymidylic acid or Thymidine monophosphate
Uracil (U)	Uridylic acid or uridine monophosphate (UMP)	–

Polymerization:

Both strands of DNA are polynucleotide strands. Nucleotides undergo polymerization to form long chain of nucleotides. Nucleotides are repeating units. They are joined end to end to form chain or strands. Adjacent nucleotides are linked to each other – phosphodiester bonds between 5′-C of one nucleotide and 3′-C of the next nucleotide.

Each strand may possess thousands to millions of nucleotides within a single macromolecule. Each – DNA strand has free 5′-phosphate at one end and free 3′-hydroxyl at the other end.

Phosphodiester linkages provide polarity to DNA. Each strand has a polarity. One polynucleotide chain is written 3′ → 5′ direction upward or 5′ → 3′ direction downward. The other strand is antiparallel By convention the sequence of nucleotides is written in 5′ → 3′ direction from the left.

Nucleotides are building blocks of nucleic acids. Alternating series of sugar-phosphate- sugar molecules form the backbone of the each strand linked by phosphodiester bonds. During nucleic acid synthesis, the chain elongation takes place by addition of nucleotides one by one.

The 5′-end of the triphosphate of one nucleotide reacts with 3′-OH group of sugar of the next nucleotide. A phosphodiester bond is formed between first phosphate of incoming nucleotide with 3′-OH of the sugar of next nucleotide. The other two phosphate groups are released as the single pyrophosphate molecule.

Arthur Korenberg demonstrated that nucleotide building blocks for DNA are energy rich d ATP, *d*GTP, *d*CTP and *d*TTP.

Order of bases along polynucleotide chain is irregular. This unique irregularity along with long length is the basis for the enormous information stored in DNA. Thus arrangement of bases is specific for every gene.

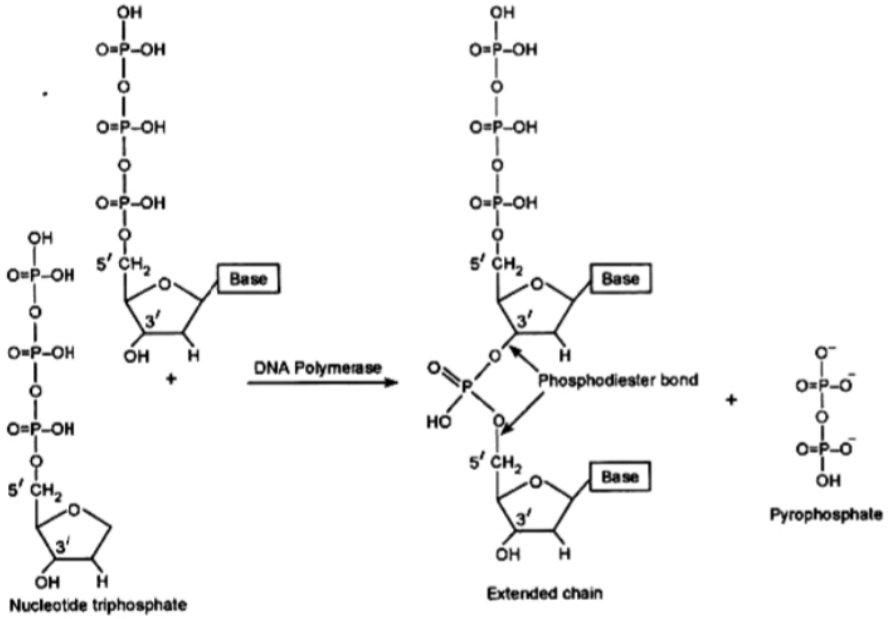


Fig. 3.5. Polymerisation reaction catalysed by DNA polymerase. Nucleotides are added to the 3′ end of the DNA chain since the DNA polymerase has the absolute requirement of a free 3′ OH in the chain to which it adds new bases.

Genetic Information in DNA:

The genetic information in DNA is conveyed by the sequence of four nucleotides. The genetic specificity exists in the linear sequence of four nucleotide building blocks. The four bases A, G, T and C can carry infinite number of genetic messages (4^N).

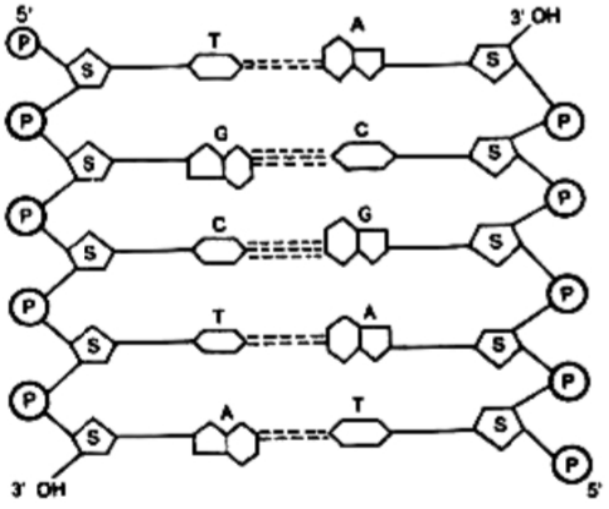


Fig. 3.6. Double polynucleotide strands of DNA.

Chargaff Equivalence Rule:

In 1950 Erwin Chargaff analysed and measured the base composition of DNA from different organisms by using paper chromatography. Exact ratios of of the four nucleotides vary from species to species. He discovered that in all DNAs the amount of purine was equal to the amount of pyrimidine. Thus A + G = C + T. Further, the amount of Adenine (A) was equal to the amount of thymine (T) and amount of cytosine (C) was equal to the amount of guanine (G).

However, A + T/G + C ratio varies from species to species. A + T/G + C ratio in human sperm is 1.62, in yeast it is 1.79 and in bacteriophages T_2 it is 1.86. In higher plants and animals generally A – T composition is high whereas in lower plants and animals G – C composition is high.

X-Ray crystallographic studies of DNA:

By X-ray crystallographic studies of DNA, Astbury gave three dimensional structure of DNA. Based on X-ray diffraction data provided by Wilkins, Rosalind Franklin and others, now famous pair of American scientist James Watson and English scientist Francis Crick proposed a model for DNA structure in 1953 in the journal Nature.

Watson and Crick were awarded Nobel Prize for this in 1962. They shared the prize with Wilkins who investigated X-ray diffraction photographs of DNA. Rosalind Franklin missed the Nobel prize because of her death in 1958.

This DNA model called double helix DNA model was a landmark research in the field of biology. It provided explanation for base composition, DNA replication and its biological properties.

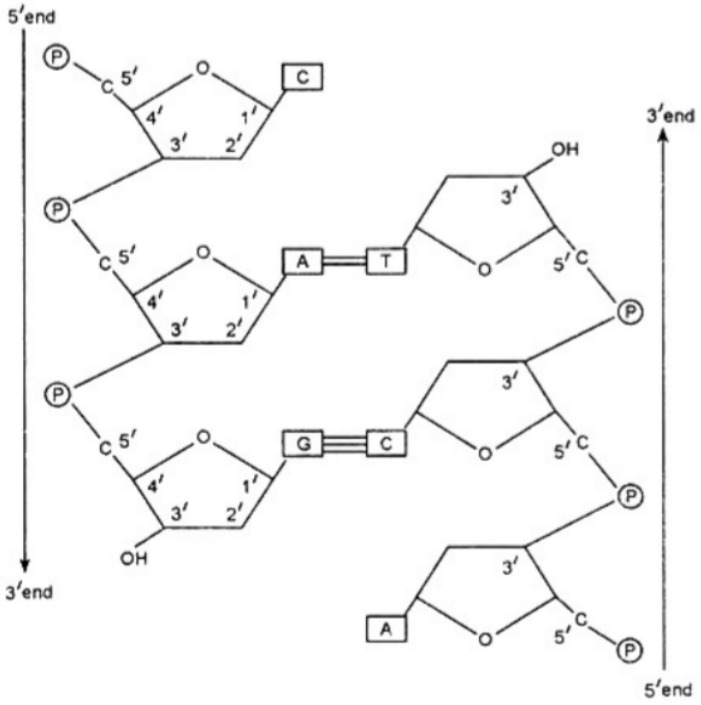


Fig. 3.7. Representation of the DNA double helix showing opposite polarities of the sugar-phosphate linkages in two strands.

Double helix structure of DNA:

Watson and Crick model of DNA has the following characteristic features:

DNA molecule consists of two polynucleotide strands twisted around each other to form a double helix. The two polynucleotide chains are held together by weak, non-covalent bonds between pairs of bases.

Each nucleotide of a polynucleotide chain consists of phosphate-sugar-base components. Each strand is made up of alternating sugar and phosphate molecules. Bases lie in pairs perpendicular to the axis of the helix. Base pairs are stacked above each other like steps of the staircase.

In double helix molecule of DNA, adenine always pairs with thymine and cytosine with guanine. According to Watson and Crick model hydrogen bonds between nitrogen bases bind

the two polynucleotide strands. Adenine is bonded to thymine by two hydrogen bonds A = T and guanine is bonded to cytosine by triple hydrogen bonds C = G.
These hydrogen bonds are weak and can easily break. This enables the two strands to separate easily. Hydrogen bonds between complementary bases contribute to thermodynamic stability of the helix and specificity of base pairing.

(1) A DNA molecule is made up of two complementary polynucleotide chains that are oriented in opposite polarity $\begin{matrix} 5' \rightarrow 3' \\ 3' \leftarrow 5' \end{matrix}$ to each other (Fig. 3.7, 3.8).

(2) Adenine (A) of one chain pairs with thymine (T) of the other, while guanine (G) of one chain pairs with cytosine (C) of the other. Thus both polynucleotide chains are complementary to each other. Thus DNA double-helix gives a quantitative relationship in which (A + G) = (C + T) or A + G/C + T = 1.

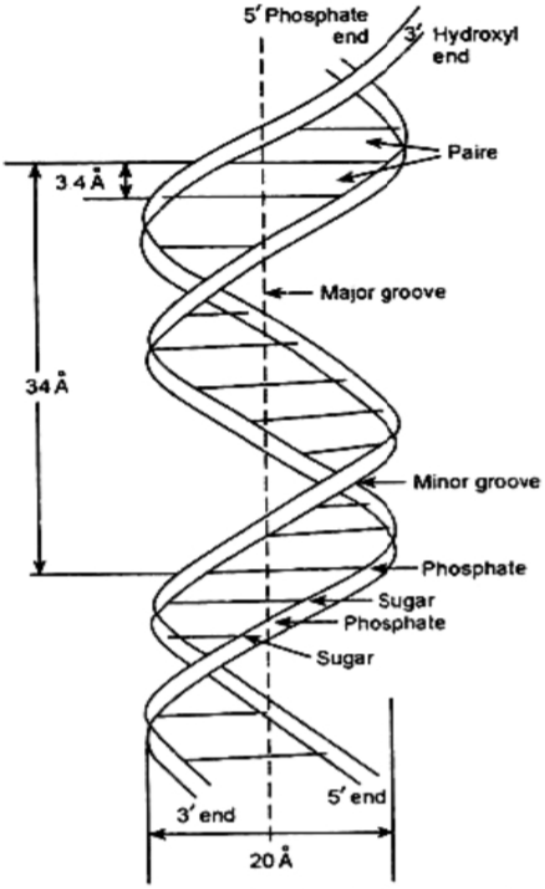


Fig. 3.8. The Watson Crick model of the DNA molecule. The phosphate sugar backbone is shown as long ribbon. Distance between two nucleotides is 3.4 Å and a complete turn of the double helix (pitch) takes 34 Å. The dashed line indicates the imaginary axis.

Two strands of double helix have complementary sequences:
As the adenine of one strand always pairs with thymine of the other strand and cytosine of one strand always pairs with guanine of the other strand, bases of one strand are complementary to the bases of the other strand. The sequence of bases of one strand dictates the base sequence of the other strand. This is known as Watson-Crick base pairing rule.

To have a constant diameter of DNA double helix, the adenine-thymine and cytosine- guanine pairs are the only ones that can fit the physical dimensions of the helical model. Because purine-purine pair (dicyclic) will be too thick and pyrimidine-pyrimidine pair (monocyclic) too thin.

```
5′   A T C T G A G A A G C 3′ one strand
     ‖ ‖ ||| ‖ ||| ‖ ||| ‖ ‖ ||| |||
3′   T A G A C T C T T C G 5′ complementary strand
```

Following are the other important properties revealed by X-ray diffraction data:

1. The diameter of the helix is 20A or 2 nm. It means the distance between two polynucleotide strands is 20A (1 nm = 10 Angstrom units).
2. The distance between two adjacent base pairs is 3.4 A or 0.34 nm.
3. One complete turn of the helix i.e., 360° takes 34 A or 3.4 nm length of DNA. In this way there are 10 base pairs in one complete turn.
4. Two strands or polynucleotide chains of a helix run in opposite directions, therefore they are antiparallel. One strand has 5′ → 3′ polarity while the other has 3′ → 5′ polarity.

In this way 10 base pairs make one complete turn of 360°. Helical coiling of two strands around the common axis is right handed. Such a DNA is called B-DNA.

The twisting or coiling makes alternating minor and major grooves. The major groove is rich in chemical information and allows regulatory proteins to bind to specific sequences on DNA.

Types of DNA:

DNA is not a rigid molecule. It can exist in different forms depending upon different conditions.

B-Form DNA (B-DNA):

Most of the double helix DNA in the cell has right handed coiling (clockwise) and is called B-form DNA. It has 10 base pairs per turn. Base pairs lie perpendicular to the axis of the helix. It is most stable configuration. When humidity is high (92%) and concentration of ions is low, DNA exists in B-Form. The B-Form of DNA is stable but can change to A, C and D form depending upon the concentration of excess salts and sequence of nucleotides.

A-Form DNA (A-DNA):

A form DNA is also right handed helix. It exists at 75% humidity in the presence of Na^+, K^+ ions. There are eleven base pairs per turn, which tilt from the axis of helix by 20.2°. It can quickly change to D-form.

C-Form DNA (C-DNA):

C-form of DNA is found at 66% relative humidity in the presence of lithium (Li^+) ions. Number of base pairs is 9.33 per turn. The base pairs show a negative tilt of 7.8°.

D-Form DNA (D-DNA):

It is rarely found. There are eight bases per turn. Base pairs show a negative tilt of 16.7°.

Z-Form DNA (Z-DNA):

It is a left handed (sinistral) coiling double helix. The sugar- phosphate backbones are zigzag instead of regular helix. That is why it is called Z-Form. It has 12 base pairs per turn.

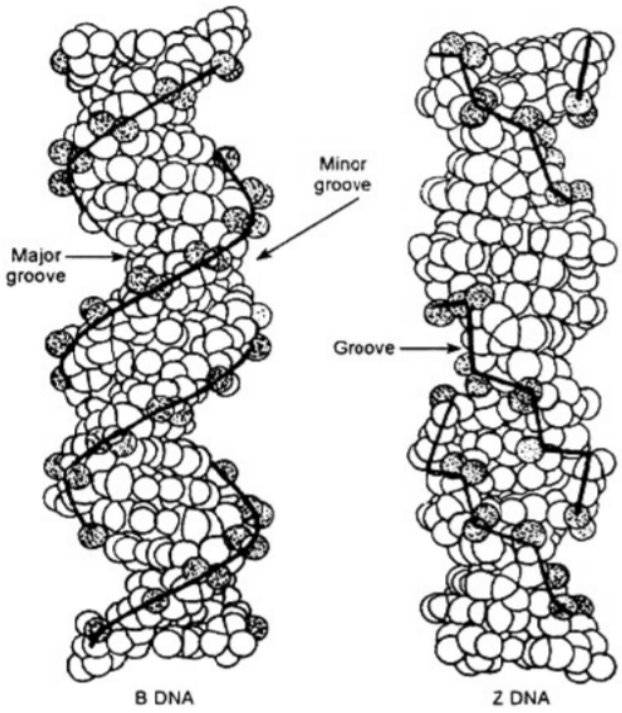


Fig. 3.10. B and Z forms of DNA double helices

The Z-form DNA makes one complete turn at 45A as compared to 34 A in B-DNA.

It has 18 A diameter therefore it is thin. Z-form DNA is assumed to play a role in gene regulation. Z-form is found in large number of living organisms including mammals, protozoans and plants.

TABLE 3.4. Chief distinguishing Characteristics of B-DNA and Z-DNA

Characteristic	B-DNA	Z-DNA
1. Helix	Right-handed	Left-handed
2. Sugar-phosphate backbone	Regular	Zig-Zag
3. Repeating unit	Mononucleotide	Dinucleotide
4. Helix pitch (turn)	34 Å	45 Å
5. Base pairs per turn of helix	10.4	12 (6 dinucleotides)
6. Axis rises per base pair	3.4 Å	3.7 Å
7. Diameter of helix	20 Å	18 Å
8. Angle of turn per base pair	36°	60°

Single Stranded DNA (ssDNA):

All organisms contain double stranded DNA (ds DNA) except a few viruses such as bacteriophage φ x 174, Provirus phage fd which contains single strand DNA (ds DNA). This single strand DNA is a circular molecule. It becomes double stranded only at the time of replication.

Denaturation and Renaturation:

Because the structure of DNA double helix is kept intact by weak hydrogen bonds, it is possible to separate the two strands. The separation of two strands is called melting or denaturation of DNA. The two hydrogen bonds of A = T can be broken more easily than the three bonds of C = G which require higher temperature. Therefore the temperature at which the two DNA strands become separate depends upon AT/CG ratio. Therefore G : C base pairs contribute more to the stability of DNA than A: T pair.

Similarly, at higher salt concentration of the solution, DNA denatures at higher temperature. This is because two DNA strands contain phosphate groups, which carry a negative charge. These negative charges of two strands repel each other causing their separation. At high salt concentration, the negative charges are shielded by cations, thus providing stability to the helix. Therefore, at low ionic strength, the helix is less stable.

When the temperature is brought down, the complementary strands again form hydrogen bonds to restore original double helix. This process is called renaturation or annealing. It helps to estimate the size of DNA molecule. Time taken to anneal depends upon the length of DNA molecule.

The size of a genome can be estimated by denaturation of DNA and allowing it to re-aneal. The speed of reassociation can be measured in cot units which is concentration x time.

Functions of DNA:

DNA is the genetic material. DNA molecules possess all the information needed for an organism's cell structure, function, development and reproduction.

It performs the following very important functions:

1. Inheritance:

DNA which is the genetic material carries genetic information from cell to cell and to the next generation. This function is performed by DNA replication. It forms exact copies of itself. During DNA replication the two strands separate and each strand functions as a template for the new strand.
In this way one molecule of DNA gives rise to two daughter molecules of DNA both identical to the parent molecule. Each daughter molecule has two strands, one parental (template) and the other new complementary strand.

2. Synthesis of proteins and enzymes:

DNA is transcribed into mRNA and other RNAs. The information contained in the sequence of bases (triplet codons) is translated into proteins and enzymes.

3. DNA molecule is capable of variation:

Variations are caused by mutation and recombination. These variations lead to change in structure and functions of the organisms and lead to evolution of new species.

RNA:

RNA is a polymer of ribo-nucleoside-phosphates. Dr. Severo Ochoa discovered the RNA and got 1959 Nobel Prize for Medicine. The sequence of the 77 nucleotides of yeast RNA was found by Robert W. Holley in 1964, winning Holley the 1968 Nobel Prize for Medicine. In 1976, Walter Fiers and his team at the University of Ghent determined the complete nucleotide sequence of bacteriophage MS2-RNA.
Its backbone is comprised of alternating ribose and phosphate groups. Ribose is a five carbon sugar with carbons numbered 1' through 5′. A base is attached to the Y position, generally adenine (A), cytosine (C), guanine (G) or uracil (U). Adenine and guanine are purines, cytosine and uracil are pyrimidine's. A phosphate group is attached to the 3′ position of one ribose and the 5′ position of the next. Most cellular RNA is single stranded, although some viruses have double stranded RNA (Fig. 5.1).

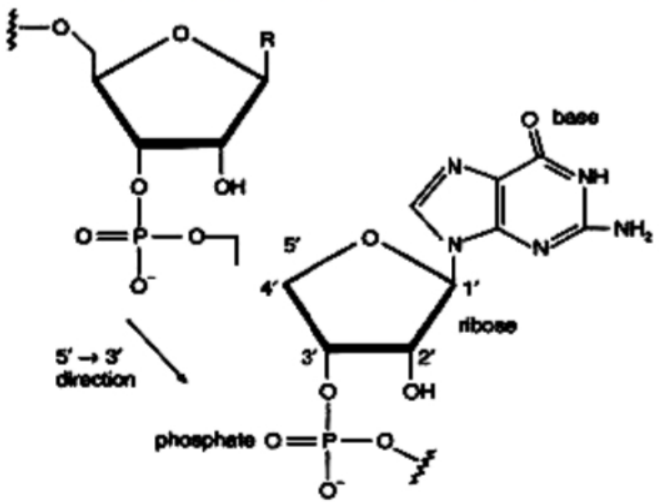


Fig. 5.1. Chemical structure of RNA.

Several other bases are occasionally found in RNAs including: thymine, pseudouridine and methylated cytosine and guanine. However, there are also numerous modified bases and sugars found in RNA that serve many different roles. Pseudouridine (Ψ), in which the linkage between uracil and ribose is changed from a C-N bond to a C-C bond, and ribothymidine (T), are found in various places (most notably in the TΨC loop of tRNA). Thus, it is not technically correct to say that uracil is found in RNA in place of thymine. Another notable modified base is hypoxanthine (a deaminated Guanine base whose nucleoside is called Inosine).
Inosine plays a key role in the Wobble Hypothesis of the Genetic Code. There are nearly 100 other naturally occurring modified nucleosides, of which pseudouridine and nucleosides with 2′-0-

methylribose are by far the most common. The specific roles of many of these modifications in RNA are not fully understood. However, it is notable that in ribosomal RNA, many of the post-translational modifications occur in highly functional regions, such as the peptidyl transferase center and the subunit interface, implying that they are important for normal function. The single RNA strand is folded upon itself, either entirely or in certain regions.

In the folded region a majority of the bases are complementary and are joined by hydrogen bonds. This helps in the stability of the molecule. In the unfolded region the bases have no complements. Because of this RNA does not have the purine pyrimidine equality that is found in DNA.

Inside of cells, there are three major types of RNA: messenger RNA (mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA). There are a number of other types of RNA present in smaller quantities as well, including small nuclear RNA (snRNA), small nucleolar RNA (snoRNA) and the 4.5S signal recognition particle (SRP) RNA. Novel species of RNA continue to be identified. RNA serves a multitude of roles in living cells.

These include: serving as a temporary copy of genes that is used as a template for protein synthesis (mRNA), functioning as adaptor molecules that decode the genetic code (tRNA) and catalyzing the synthesis of proteins (rRNA). There is much evidence implicating RNA structure in biological regulation and catalysis. Interestingly, RNA is the only biological polymer that serves as both a catalyst (like proteins) and as information storage (like DNA).

For this reason, it has be postulated RNA, or an RNA-like molecule, was the basis of life early in evolution. RNA is a nucleic acid polymer consisting of nucleotide monomers that plays several important roles in the processes that translate genetic information from deoxyribonucleic acid (DNA) into protein products; RNA acts as a messenger between DNA and the protein synthesis complexes known as ribosomes, forms vital portions of ribosomes, and acts as an essential carrier molecule for amino acids to be used in protein synthesis.

Synthesis of RNA:

There are three types of RNA; messenger RNA (mRNA) or template RNA, ribosomal RNA (rRNA) and soluble RNA (sRNA) or transfer RNA (tRNA). Ribosomal and transfer RNA comprise about 98% of all RNA. All three forms of RNA are made on a DNA template. Transfer RNA and messenger RNA are synthesized on DNA templates of the chromosomes, while ribosomal RNA is derived from nucleolar DNA.

The three types of RNA are synthesized during different stages in early development. Most of the RNA synthesized during cleavage is mRNA. Synthesis of tRNA occurs at the end of cleavage, and rRNA synthesis begins during gastrulation.

Synthesis of RNA is usually catalyzed by an enzyme RNA polymerase using DNA as a template, a process known as transcription. Initiation of transcription begins with the binding of the enzyme to a promoter sequence in the DNA (usually found “upstream” of a gene).

The DNA double helix is unwound by the helicase activity of the enzyme. The enzyme then progresses along the template strand in the 3′ to 5′ direction, synthesizing a complementary RNA molecule with elongation occurring in the 5′ to 3′ direction.

The DNA sequence also dictates where termination of RNA synthesis will occur. RNAs are often modified by enzymes after transcription. For example, a poly (A) tail and a 5′ cap are added to eukaryotic pre-mRNA and introns are removed by the splice some.

There are also a number of RNA-dependent RNA polymerases that use RNA as their template for synthesis of a new strand of RNA. For instance, a number of RNA viruses (such as poliovirus) use this type of enzyme to replicate their genetic material.

TABLE 3.5 Differences between DNA and RNA

Characteristic	DNA	RNA
1. Pentose sugar	2′-deoxyribose	Ribose
2. Bases	Adenine (A), Guanine (G), Thymine (T) and Cytosine (C)	Adenine (A), Guanine (G) Uracil (U) and Cytosine (C)
3. Strands	Generally double stranded ; single-stranded DNA is found in some viruses, *e.g.*, ϕX174 viruses,	Generally single-stranded; double-stranded RNA is found in some viruses, *e.g.*, reovirus
4. Native from	Double-stranded DNA usually in B-form	Double stranded RNA usually in A-form
5. Origin	Replication of pre-existing DNA	Usually synthesized on a DNA template; only genetic RNA may replicate to yield RNA
6. Function	As genetic material	(i) Functions as genetic material in some viruses (ii) Generally, nongenetic function, as tRNA, mRNA, rRNA, chromosomal RNA, primer RNA.

Types of RNA:

A. Messenger RNA (mRNA):

Messenger RNA (mRNA) is only 5-10% of total RNA present in the cell. In 1961, Francis Jacob and Jacques Monod for the first time proposed the name mRNA. This RNA carries genetic information from DNA to the ribosome. Since mRNA is transcribed on DNA (genes), its base sequence is complementary to that of the segment of DNA on which it is transcribed. Usually each gene transcribes its own mRNA. Therefore, there are approximately as many types of mRNA molecules as there are genes.

The structure of prokaryotic and eukaryotic mRNA is as follows:

1. 5′ Cap:

At the 5′ end of the mRNA molecule in most eukaryote cells and animal virus molecules is found a 'cap'. This cap is formed by the methylation of any of the four nucleotides. The cap helps mRNA to bind to the ribosomes. Without the cap mRNA molecules bind very poorly to the ribosomes. The bacterial mRNA does not have 5'cap. But they have specific ribosome binding site about six nucleotide long, which occurs at several places in the mRNA molecules. These are located at four nucleotides upstream from AUC.

2. Non-coding Regions:

As the name indicates these regions do not code for protein. There are two non-coding regions. First non-coding region (NCI) is followed by a 5'cap and is 10 to 100 nucleotides in length. This region is rich in A and U residues. The second non- coding region (NC2) is followed by termination codon and is 50-150 nucleotides long and contains an AAUAAA residues.

3. Initiation Codon:

Initiation codon is AUG in both prokaryotes and eukaryotes. Bacterial ribosomes bind directly to the AUG region of the mRNA to start the protein synthesis, whereas this is not there in the case of eukaryotes.

4. The Coding Region:
It consists of about 1,500 nucleotides. This region is responsible for coding protein with several ribosomes. The combination of mRNA strand with several ribosomes is called polyribosomes.

5. The Termination Codon:
The termination codon is required to give the signal to stop protein synthesis.

6. The poly (A) Sequence:
The non-coding region II is followed by poly (A) sequence in the eukaryotic mRNA. The prokaryotic mRNAs lack poly (A). The poly (A) sequences of 200-250 nucleotides are present at 3'OH end of mRNA. Poly (A) sequences are added when mRNA is present inside the nucleus. The function of poly (A) sequence in translation is unknown (Fig. 5.3).

Difference between prokaryotic and eukaryotic mRNA:

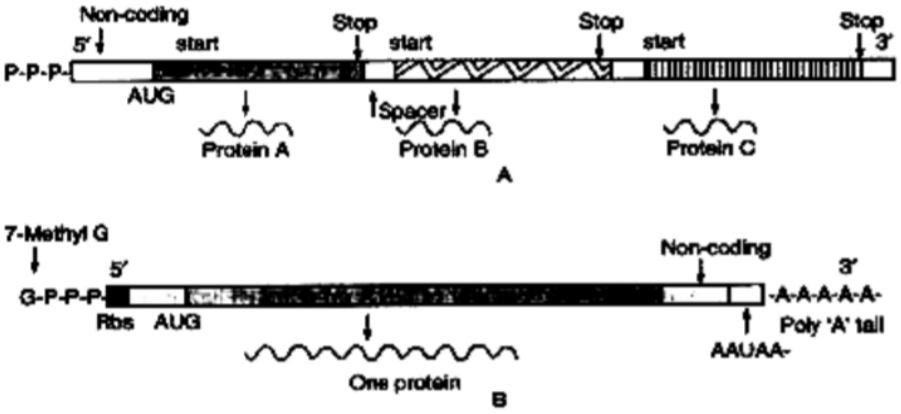


Fig. 5.3. Structure of prokaryotic (A) and eukaryotic (B) mRNA showing gene product. This requires processing of mRNA in eukaryotes before translation.

Prokaryotic mRNA:

1. Translation begins when the mRNA is still being transcribed on DNA.

2. Prokaryote mRNA are very short lived. It constantly under goes breakdown to its constituent ribonucleotides by ribonucleases.

3. In Prokaryotic mRNA are polycistronic.

4. The mRNA undergo very little processing after being transcribed.

5. Prokaryotic mRNA do not have poly (A) tail.

Eukaryotic mRNA:

1. Translation begins when the transcription is Completed.

2. Eukaryotic nRNAs are long lived. Thus are metabolically stable.

3. In Eukaryotic mRna are monocistronic .

4. The mRNA undergoes several processing after being transcribed such as polyadenylation capping and methylation.

5. Eukaryotic mRNA have poly (A) tail.

B. Ribosomal RNA (rRNA):

Ribosomal RNA is extremely abundant and makes up to 80% of RNA found in a typical eukaryotic cytoplasm. Ribosomal RNA consists of a single strand twisted upon itself in some regions. It has

helical regions connected by intervening single strand regions. The helical regions may show presence or absence of positive interaction. In the helical region most of the base pairs are complementary, and are joined by hydrogen bonds. In the unfolded single strand regions the bases have no complements.
In the cytoplasm, ribosomal RNA and protein combine to form a nucleoprotein called a ribosome. Ribosomal RNA (rRNA) is a component of the ribosomes. The base sequence of rRNA is complementary to that of the region of DNA where it is synthesized. Ribosomal RNA is formed from only a small section of the DNA molecule, and hence there is no definite base relationship between rRNA and DNA as a whole.

Primarily there are two types of ribosomes, one is 70s (prokaryotes) and the other is 80s (eukaryotes). The 70S ribosome of prokaryotes consists of a 30S subunit and a 50S subunit. The 30S subunit contains 16S rRNA, while the 50S subunit contains 23S and 5S rRNA. The 80S eukaryote ribosome consists of a 40S and a 60S subunit.

In vertebrates the 40S subunit contains 18S rRNA, while the 60S subunit contains 28-29S, 5.8S and 5S rRNA. In plants and invertebrates, the 40S subunit contains 16-18S RNA, while the 60S subunit contains 25S and 58 and 5.8S rRNA.

C. Transfer or tRNA:

The tRNA molecules are key to the translation process of the mRNA sequence into the amino acid sequence of proteins (at least one type of tRNA for every amino acid). To be precise, the amino-acyl-tRNA-synthase proteins are the 'true' translators of the genetic code into an amino acid sequence. These synthetases acetylate tRNA molecules with the proper amino acid that corresponds to the anti-codon in the structure of the tRNA molecule.

The anti-codon later recognizes the codon, the triple base sequence which 'codes' for the amino acid along the mRNA strand. A failure of properly acetylating the tRNA with the right amino acid results in an amino acid mutation even though the DNA sequence has not been changed. tRNA molecules are small nucleic acids of 60- 95 nucleotides, mostly 76, with a molecular weight 18-20kD, with the secondary structure resembling a clover leaf. Here are a few common features shared by all tRNA molecules found in various organisms.

(1) 5′ terminus is always phosphorylated.

(2) 7 bp stem, may have non-Watson & Crick pairing (like GU) acceptor or amino acid stem at 3′ terminus in which last three nucleotides are CCA-3′-OH. These are added after transcription and amino acylation occurs at 3′-OH group of last base 'A'.

(3) 3-4 bp stem and loop contains the base dihydrouridine (D) [D- arm.]

(4) 5 bp stem and loop containing anti-codon triplet [anti-codon arm].

(5) 5 bp stem and loop contains sequence T C, standing for [T- arm] pseudouridine.

(6) Variable arm (between anti-codon and T-arm) length, 3-21 nucleotides.

(7) Contains numerous modified bases (up to 25%) which are all post-transcriptionally modified.

The three dimensional structure of tRNA resembles an L-shaped molecule with the D-arm and anti-codon loop building one stretch and the T-arm and acceptor stem building the other stretch being deposed by ~90 to one another (interstem angle of 82 by X-ray refinement and 92 in an electron microscopy study). The molecule is about 6 nm in each direction with the anti-codon to acceptor 3′-term ends being 7.6 nm apart. The diameter of both arms is about 2.0 to 2.5 nm.

The structural complexity of tRNA is reminiscent of that of a protein with 71 out of 76 bases participating in stacking interaction (of which 42 in double helical stem structures). 9 bp interactions are cross linking the tertiary structure, i.e., they interact with bases from a different stem and loop

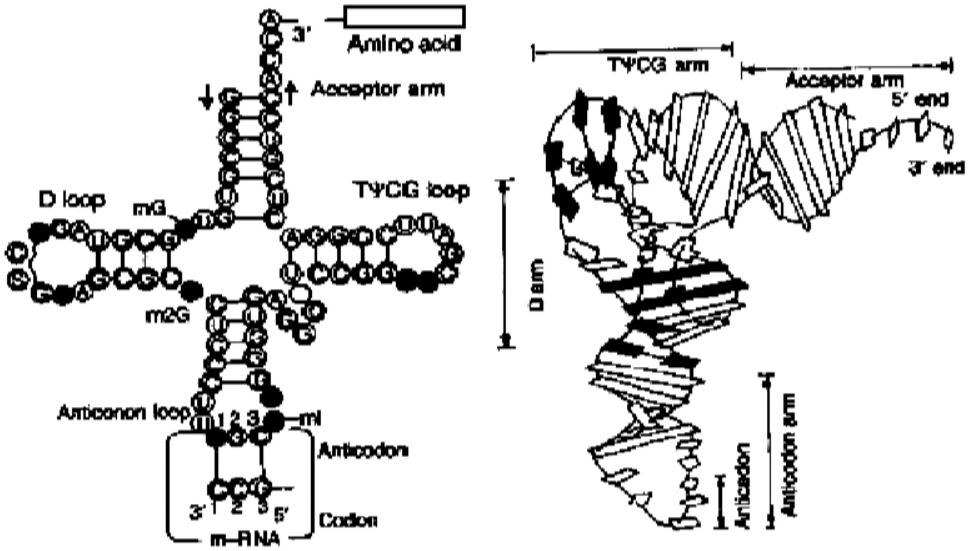


Fig. 5.4. A 3D structure of tRNA: Clover leaf model

Table 5.3. Difference between the three types of RNAs

	Ribosomal RNA (rRNA)	Messenger RNA (mRNA)	Transfer RNA (tRNA)
Percentage of total RNA of cell	~ 80%	5 to 10%	10 to 15%
Sedimentation coefficient	28S, 18S, 5.8S, 5S 23S, 16S, 5S	8S	3.8S
Number of nucleotides	5S RNA: 120 nucleotides. 16-18S RNA: 1,600 to 2,500 nucleotides. 23-28S RNA : 3,200 to 5,500 nucleotides	*E. coli:* 900 to 1,500 nucleotides	73 to 93 nucleotides
Unusual bases	Small amount of methylated bases (*E. coli*: 1 per 100-150 nucleotides).	Small amount of unusual bases.	High content of unusual bases. (*E. coli*: 1 per 3040 nucleotides).
Site of synthesis	Derived from nucleolar DNA.	Synthesized in nucleus on DNA template.	Synthesized in nucleus on DNA template.
Beginning of synthesis	Synthesis begins at gastrulation, and increases as development proceeds.	Some mRNAs are found in the ovum. New mRNA is synthesized during early cleavage.	tRNA synthesis occurs at the end of cleavage stages.
Base relationship with DNA	No obvious base relationship to DNA. rRNA is formed from only small sections of of DNA.	mRNA shows base relationship with DNA. It is formed from all sections of DNA.	rRNA also shows base relationship with DNA. It is formed from all sections of DNA.
Function	Unpaired bases may bind mRNA and tRNA to ribosomes.	Conveys genetic information from DNA to the ribosomes, where it takes part in protein synthesis.	Adaptor for attaching amino acids to mRNA template.

region. All of these 9 bp are non-Watson-Crick associations and are highly conserved which makes it likely to predict similar structures for all tRNA molecules (in fact, only few tRNA molecules have been crystallized and their structure determined) (Fig. 5.4).

New Types of RNA:

Besides mRNA, tRNA and rRNA, the three classically known RNA molecules, new types of RNA molecules are discovered in recent years, which are involved in various activities directly or they modulate the activity.

Some of the new types of RNA which are involved in various activities directly are as follows:

1. Non-Coding RNA:

RNA genes (sometimes referred to as non-coding RNA or small RNA) are genes that encode RNA that is not translated into a protein. The most prominent examples of RNA genes are transfer RNA (tRNA) and ribosomal RNA (rRNA), both of which are involved in the process of translation.

2. Small Nuclear RNA (snRNA):

About a dozen genes for snRNAs have been described, each present in multiple copies in the genome. Small nuclear RNAs combine with certain U-proteins to form snRNPs. These executive molecules have roles in editing other classes of RNA. The "U" designation was given to the snRNAs because they were found to be rich in uridylic acid.

An important example is the small ribonuclear proteins (snRNPs) that are components of the spliceosomes.

3. Small Nucleolar RNA (snoRNA):

In eukaryotic cells, rRNA and snRNA are extensively modified and processed in the nucleolus. Much of this activity is affected by snoRNAs. It appears that the coding for snoRNAs lies in introns and other intergenic regions (non-protein coding). Small nucleolar RNA can be considered to be a subgroup of the snRNAs but should not be confused with the snRNAs mediating mRNA splicing, i.e. the spliceosomal RNAs.

4. Short Interfering RNAs (siRNA):

Short interfering RNAs and miRNAs were discovered in different works, but their biogenesis and assembly into RNA-protein complexes and their function in down regulating gene expression are closely related. Short interfering RNAs and mi RNAs share common RNAse III processing enzyme,

5. Micro-RNAs (miRNAs):

Micro-RNAs are a class of small, non-coding RNAs that regulate gene expression in a sequence specific manner as required in embryonic development. Micro-RNAs have been found throughout diverse eukaryotes genomes including plants. They can inhibit protein expression by shutting off translation or by targeting mRNA for degradation. Micro-RNAs were first discovered in 2001 in the widely studied worm Caenorhabdtis elegans.

6. Ribozymes:

Ribozymes are catalytic RNA enzymes that act to alter covalent structure in other classes of RNAs and certain molecules. They occur in ribosomes, nucleus and chloroplasts of eukaryotic organisms. Some viruses including several bacteriophages also have ribozymes. An optimum concentration of metal ions such as Mg^{++} and K^{+} is associated with their effective functioning. Ribozymes generally act as molecular scissors cutting precursor RNA molecules at specific sites. Surprisingly, they also serve as molecular staplers, which ligate or join two RNA molecules together.

7. XISTRNA:

XIST stands for X-inactive-specific transcript. It is a large (~ 17kb) RNA coded by a gene on the X chromosome (8 exons are involved with human xist). xistRNA accumulates in female somatic cells

along the X chromosome containing the active xist gene and proceeds to inactivate nearly all the 100s of genes on that X chromosome..

8. Double-Stranded RNA (dsRNA):

Double-stranded RNA (dsRNA) is RNA with two complementary strands, similar to the DNA found in all 'higher' cells. dsRNA forms the genetic material of some viruses. In eukaryotes, it acts as a trigger to initiate the process of RNA interference and is present as an intermediate step in the formation of siRNAs (small interfering RNAs). siRNAs are often confused with miRNAs; siRNAs are double-stranded, whereas miRNAs are single-stranded.

Although initially single stranded, there are regions of intra-molecular association causing hairpin structures in pre-miRNAs. Very recently, dsRNA has been found to induce gene expression at transcriptional level, a phenomenon named "small RNA induced gene activation (RNAa)". Such dsRNA is called "small activating RNA (saRNA)".

9. RNA Secondary Structures:

The functional form of single stranded RNA molecules (like proteins) frequently requires a specific tertiary structure. The scaffold for this structure is provided by secondary structural elements which are hydrogen bonds within the molecule. This leads to several recognizable "domains" of secondary structure like hairpin loops, bulges and internal loops.

The secondary structure of RNA molecules can be predicted computationally by calculating the minimum free energies (MFE) structure for all different combinations of hydrogen bonding's and domains. There has been a significant amount of research directed at the RNA structure prediction problem.

Significance of nucleic acids:

Deoxyribonucleic acids and ribonucleic acids are the key centres which control all the metabolic activities of cell and in turn the whole organism.

(1) If there occurs any deficiency in the DNA amount, nucleus loses its capacity to support adenosine triphosphate (ATP) synthesis.

(2) Nucleus also becomes inefficient to incorporate amino acids into proteins.

(3) Besides, DNA is the main genetic material constituting genes and chromosomes which carry hereditary information from generation to generation. DNA helps in the RNA synthesis in the cell. If the loops of amphibian oocytic chromosome (lamp brush) are exposed to actinomycin (which has the property to fuse with DNA and thereby causing decrease in DNA amount), RNA synthesis is inhibited.

(4) Recently, McConnell and Cameron (1968) have produced the evidence that RNA amount increases the intelligence and learning capacity of men.

9. **Energy yielding metabolisms: Paths of energy synthesis through Glycolysis, Citric acid cycle, plant mitochondrial electron transport chain, alternative oxidase, PPP cycle, regulation of respiratory pathways, Lipid metabolism: fatty acid biosynthesis and oxidation.**

In respiration carbohydrate is broken down, as a result the potential energy is transformed into kinetic form. Though it is a destructive or katabolic process, yet respiration is extremely beneficial, because it releases the necessary energy for performing the life functions. Plants take in free oxygen from the air which enters through the stomata and lenticels and diffuses through the continuous intercellular space system to reach the living cells. It oxidises sugar, particularly glucose, into carbon dioxide and water vapour, thereby liberating considerable energy.

The reaction may be represented thus:

$C_6H_{12}O_6 + 6O_6 = 6CO_2 + 6H_2O$ + energy (674 cal.)

Though sugar is the main oxidisable material used during the process, other materials and in extreme cases, even protoplasm may be broken down for the liberation of energy. Carbon dioxide and water vapour go out through the stomata and lenticels. At night when stomata remain closed gaseous exchange takes place through the lenticels. Thus respiration is essentially a process of biological oxidation in which oxygen absorbed from the air breaks down the food (sugar) into carbon dioxide and water, thereby releasing the stored energy.

During, day time when the rate of photosynthesis is higher than the rate of respiration and when both the processes are going on simultaneously, all the oxygen liberated in photosynthesis is not consumed in respiration, whereas all the CO_2 liberated in respiration is completely consumed in photosynthesis and some more CO_2 must enter the plant for the higher rate of photosynthesis.

As a result, during day time, the exchange of gases between the plant and the atmosphere is that O_2 is liberated and CO_2 is consumed. Thus the effects of respiration are completely masked during day time. During night, however, there is undoubtedly no photosynthesis, and only exchange of gases between the plant and the atmosphere is the effect of the process of respiration—O_2 consumed and CO_2 liberated.

$$\text{Pyruvate} + \text{NAD+} + \text{Co-A} \xrightarrow[\text{dehydrogenase}]{\text{pyruvate}} \text{Acetyl Co-A} + \text{NADH} + H^+ + CO_2$$

Acetyl Co-A can enter into mitochondria while pyruvic acid cannot.

Definition:

The term respiration (L. respirare = to breath) was first used to describe the breathing i.e. exchange of gases between the organism and the environment.

Subsequently the term respiration was used in a wider sense including breathing, transport of gases as well as the oxidation of food leading to liberation of CO_2 and energy.

It is now agreed that respiration has two fundamental processes-external respiration and internal respiration. The external respiration or breathing is a physical process of exchange of respiratory gases ($O_2$and CO_2) between the organisms and the surroundings. Lavoisier (1789) first studied external respiration in animals. He observed that animals take in oxygen and give out $CO_2 + H_2O$. Higher animal possesses special organs for gas exchange but plants have stomata and lenticels for this purpose. This is also facilitated by air spaces present in the parenchyma.

Internal respiration, on the other hand, is a chemical process of food oxidation that occurs within the cell to liberate free energy, CO_2 and H_2O. Internal respiration takes place in the cytosol and mitochondria of eukaryotic cells. Hence, it is also known as cellular or tissue respiration.

Types of Respiration:

Depending on the availability of oxygen, Sachs (1890) classified cellular respiration into two major types: aerobic and anaerobic.

(i) Aerobic Respiration:

It uses oxygen and completely oxidizes the organic food to carbon dioxide and water. It, therefore, releases the entire energy available in glucose. It occurs in most plants and animals. The organisms which carry on this type of respiration are called aerobes.

$$C_6H_{12}O_6 + 6O_2 \xrightarrow{\text{Enzymes}} 6CO_2 + 6H_2O + 686 \text{ kcal/2870 kJ}$$

686 kcal or 2870 kJ of energy is liberated per mole of glucose. The value was previously calculated to be 673 Kcal. One Kcal is equal to 1000 calories. It is that amount of energy (as heat) which can raise the temperature of one litre of water 1° C.

(ii) Anaerobic Respiration:

It does not use molecular oxygen and incompletely oxidizes the organic food with or without production of carbon dioxide. It, therefore, releases a small amount of energy. It occurs in yeasts, certain bacteria and some parasitic worms (e.g. Ascaris, Taenia). The organisms which carry on anaerobic respiration are termed anaerobes. Anaerobes may be either facultative or obligate.

(a) Facultative anaerobes:

Organisms which normally require oxygen but which can live anaerobically when grown on suitable media e.g., Butyric acid bacteria, Lactic acid bacteria, Bacillus phosphoresces.

(b) Obligate anaerobes:

They live in the absence of oxygen or in the presence of negligible concentration of this gas. The common products of anaerobic respiration are CO_2 ethyl alcohol and lactic acid.

$$C_6H_{12}O_6 \xrightarrow{\text{Enzymes}} \underset{\text{ethyl alcohol}}{2CO_2 + 2C_2H_5OH} + 59\ \text{kcal} / 247\ \text{kJ}$$

$$C_6H_{12}O_6 \xrightarrow{\text{Enzymes}} \underset{\text{lactic acid}}{2C_3H_6O_3} + 36\ \text{kcal} / 150\ \text{kJ}$$

Anaerobic respiration is the only mode of respiration in some micro-organisms. In higher organisms it occurs as a temporary measure.

Anaerobic respiration cannot continue for long in higher organisms because:

(i) It yields little energy;

(ii) More substrate is decomposed so that little is left for growth and repair;

(iii) Some of the end products and intermediates of anaerobic respiration are toxic in higher concentration.

Protoplasmic and Floating Respiration:

Respiration which utilizes proteins as substrate is called protoplasmic respiration, whereas that uses carbohydrates or fats is termed floating respiration. Protoplasmic respiration cannot be continued for long as it depletes protoplasm of structural and functional proteins as well as liberates toxic ammonia.

Gaseous Exchange:

Lavosier observed that in respiration of animals, oxygen is taken in from the air. In return they give out carbon dioxide and water. There is a regular exchange of $O_2 \rightarrow CO_2$ in most of the organisms. Some organisms, especially micro-organisms, do not require oxygen for their respiration. Some of them give out CO_2 while a few do not do so. In these organisms there is no gaseous exchange.

Gaseous exchange occurs not only between the organisms and its environment but also between every cell and its surrounding environment. An animal cell exchanges gases with the extracellular fluid while a plant cell does so with the air present in intercellular spaces. An organism shows exchange of gases in a liquid or gaseous environment depending upon the habitat.

Mechanism of Respiration:

There are two major phases of respiration:

(i) Glycolysis, and

(ii) Krebs cycle.

During process of respiration, carbohydrates are converted into pyruvic acid through a series of enzymatic reactions. This series of reactions is known as glycolysis which takes place in cytosol.

Now, pyruvic acid enters mitochondria, where several enzymes catalyse the reactions, and pyruvic acid finally converts into CO_2 and water. This series of enzymatic reactions is known as Krebs cycle (after name of its discoverer Sir Hans Adolf Krebs (1900-1981), awarded Nobel Prize in 1953), or tricarboxylic acid (TCA) or citric acid cycle.

1.Glycolysis:

Glycolysis is a term used to describe the sequential series of reactions present in a wide variety of tissues that starts with a hexose sugar (usually glucose) and ends with pyruvic acid. This term has originated from Greek words, glycos = sugar and lysis = splitting.

The scheme of glycolysis was discovered by three German Scientists, Gustav Embden, Otto Meyerhof and J. Parnas, and therefore, referred as EMP pathway, after the abbreviation of their last names.

Glycolysis is the first stage in the breakdown of glucose and is common to all organisms. This means, glycolysis is common to both aerobic and anaerobic modes of respiration. In anaerobic organisms, this is only process in respiration. Glycolysis occurs in cytoplasm of cells. During this process, glucose undergoes partial oxidation to form two molecules of pyruvic acid.

In plants, glucose is derived from sucrose, which is the end product of photosynthetic carbon reactions (also known as dark reactions) or from storage carbohydrates.

Sucrose is converted into glucose and fructose by the enzyme invertase. Now, these two monosaccharides (i.e., glucose and fructose) enter glycolysis or EMP pathway.

The main steps of glycolytic pathway are as follows:

Glycolysis is carried out in following different steps:

a. Phosphorylation of Sugar (i.e., First Phosphorylation):

Glucose and fructose are phosphorylated to give rise to glucose-6-phosphate and fructose-6-phosphate, respectively, by the activity of enzyme hexokinase, in presence of ATR The phosphorylated form of glucose then isomerises to produce fructose-6-phosphate. Isomerisation takes place with the help of enzyme phosphohexose isomerase.

Further steps of metabolism of glucose and fructose are quite similar.

Equations are as follows:

$$\text{Glucose (6C)} + \text{ATP} \xrightarrow[Mg^{2+}]{\text{hexokinase}} \text{Glucose-6-phosphate} + \text{ADP}$$

Now isomerisation occurs :

$$\text{Glucose-6-phosphate} \underset{\text{isomerase } Mg^{2+}}{\overset{\text{phosphohexose}}{\rightleftharpoons}} \text{Fructose-6-phosphate}$$

b. Phosphorylation of Fructose-6-Phosphate (i.e., Second Phosphorylation):

Now, fructose-6-phosphate is phosphorylated and fructose-1, 6-bisphosphate produced by the action of enzyme phosphofructokinase in presence of ATP.

$$\text{Fructose-6-phosphate} + \text{ATP} \xrightarrow[Mg^{2+}]{\text{phosphofructokinase}} \text{Fructose-1, 6-bisphosphate} + \text{ADP}$$

c. Splitting:

Now, fructose- 1, 6-bisphosphate splits into two molecules of triose phosphate, i.e., 3-phosphoglyceraldehyde (PGAL) and dihydroxyacetone phosphate (Di HAP), which are interconvertible.

$$\text{Fructose-1, 6-bisphosphate} \overset{\text{aldolase}}{\rightleftharpoons} \text{3-phosphoglyceraldehyde (PGAL)} + \text{dihydroxyacetone phosphate (Di HAP).}$$

d. Oxidative Dehydrogenation:

After formation of 3-phosphoglycerldehyde (PGAL), the glycolytic pathway enters the energy conserving phase. Here, it is oxidized to a carboxylic acid, i.e., 1,3-bisphosphoglycerate, and NAD is reduced to NADH.

$$\text{3-phosphoglyceraldehyde} + \text{NAD} \underset{\text{dehydrogenase}}{\overset{\text{glyceraldehyde phosphate}}{\rightleftharpoons}} \text{1, 3-bisphosphoglycerate} + \text{NADH} + \text{H}^{+}$$

e. Formation of ATP:

In next step of glycolysis, 3-phosphoglycerate is formed from 1, 3-bisphosphoglycerate by enzymatic activity of phosphoglycerate kinase, and ATP is generated during this process. Direct synthesis of ATP from intermediate metabolites is called substrate level phosphorylsation.

$$\text{1, 3-bisphosphoglycerate} + \text{ADP} \underset{\text{kinase} - Mg^{2+}}{\overset{\text{phosphoglycerate}}{\rightleftharpoons}} \text{3-phosphoglycerate} + \text{ATP}$$

This type of formation of ATP, where a phosphate group is directly transferred from a substrate to ADP to form ATP, is different from the ATP produced by ATP synthesis during oxidative phosphorylation in mitochondria or in chloroplasts (During photophosphorylation in photosynthesis).

f. Isomerisation:

In next step 3-phosphoglycerate converts into its isomer 2-phosphoglycerate by catalytic activity of enzyme phosphoglyceromutase.

$$\text{3-phosphoglycerate} \xrightleftharpoons[\text{glyceromutase} - Mg^{2+}]{\text{phospho-}} \text{2-phosphoglycerate}$$

g. Dehydration:

In subsequent step 2-phosphoglycerate converts into phosphoenol pyruvate (PEP) in the presence of enzyme pyruvate kinase and liberates ATP.

$$\text{2-phosphoglycerate} \xrightleftharpoons{\text{pyruvate kinase}} \text{phosphoenol pyruvate} + \text{ATP}$$

$$\text{2-phosphoenol pyruvate} + 2\ \text{ADP} \rightleftharpoons \text{pyruvic acid} + 2\ \text{ATP}$$

h. Generation and Utilisation of ATP during Glycolysis:

During glycolytic pathway, the molecules of ATP are produced as follows:

(i) Direct transfer of phosphate to ATP.

(ii) Oxidation of NADH produced during glycolytic pathway to NAD^+.

i. In the end of glycolysis net gain of ATP:

(i) During glycolysis two triose phosphate molecules are formed from one glucose molecule, and 4 ATP molecules are produced.

(ii) Out of 4 ATP molecules, 2 ATP molecules are utilised in first few steps in converting glucose to fructose-1, 6 bisphosphate.

(iii) Moreover, three ATP molecules are produced from oxidation of each of two molecules of NADH produced during catabolism of glucose.

(iv) In all, a net gain of 8 molecules occurs during process of glycolysis.

(v) However, in anaerobic respiration, NADH + H^ is not converted to ATP, and therefore, only 2 ATP molecules are produced.

$$\text{Glycolysis} \longrightarrow 8\ \text{ATP}$$

$$\text{Pyruvic acid} \longrightarrow \text{Acetyl Co-A} \longrightarrow 6\ \text{ATP}$$

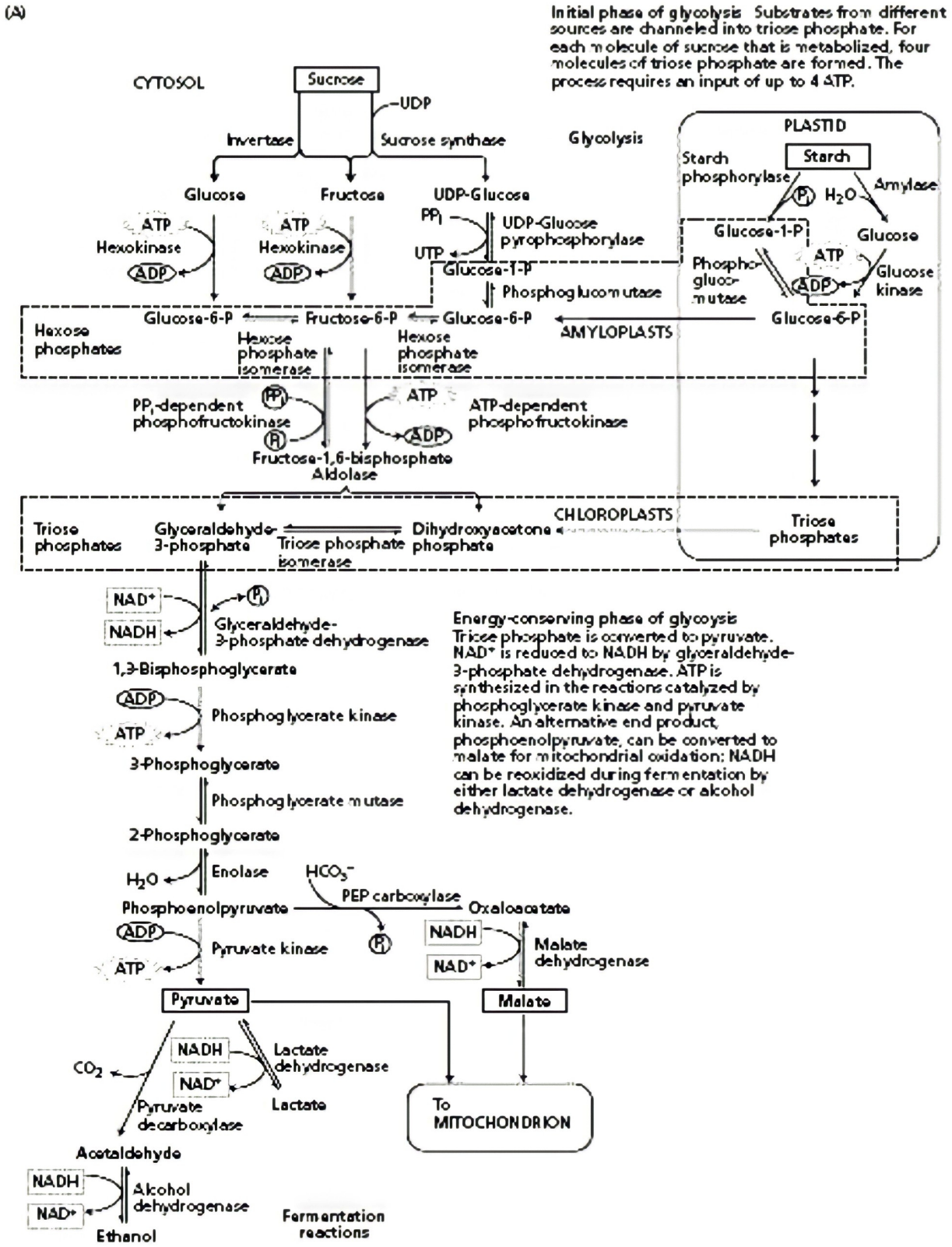
$$\text{Krebs cycle} \longrightarrow 24\ \text{ATP}$$

$$\text{Total} \longrightarrow 38\ \text{ATP}$$

$$C_6H_{12}O_6 + 6O_2 + 8H_2O + 38\ (ADP + H_3PO_4) \longrightarrow 6CO_2 + 14H_2O + 38\ (ATP + H_2O).$$

Regulation of Glycolysis:

- When ATP is needed, glycolysis is activated
- When ATP levels are sufficient, glycolysis activity decreases
- Control points:
  1. Hexokinase
  2. Phosphofrucktokinase-1
  3. Pyruvate kinase

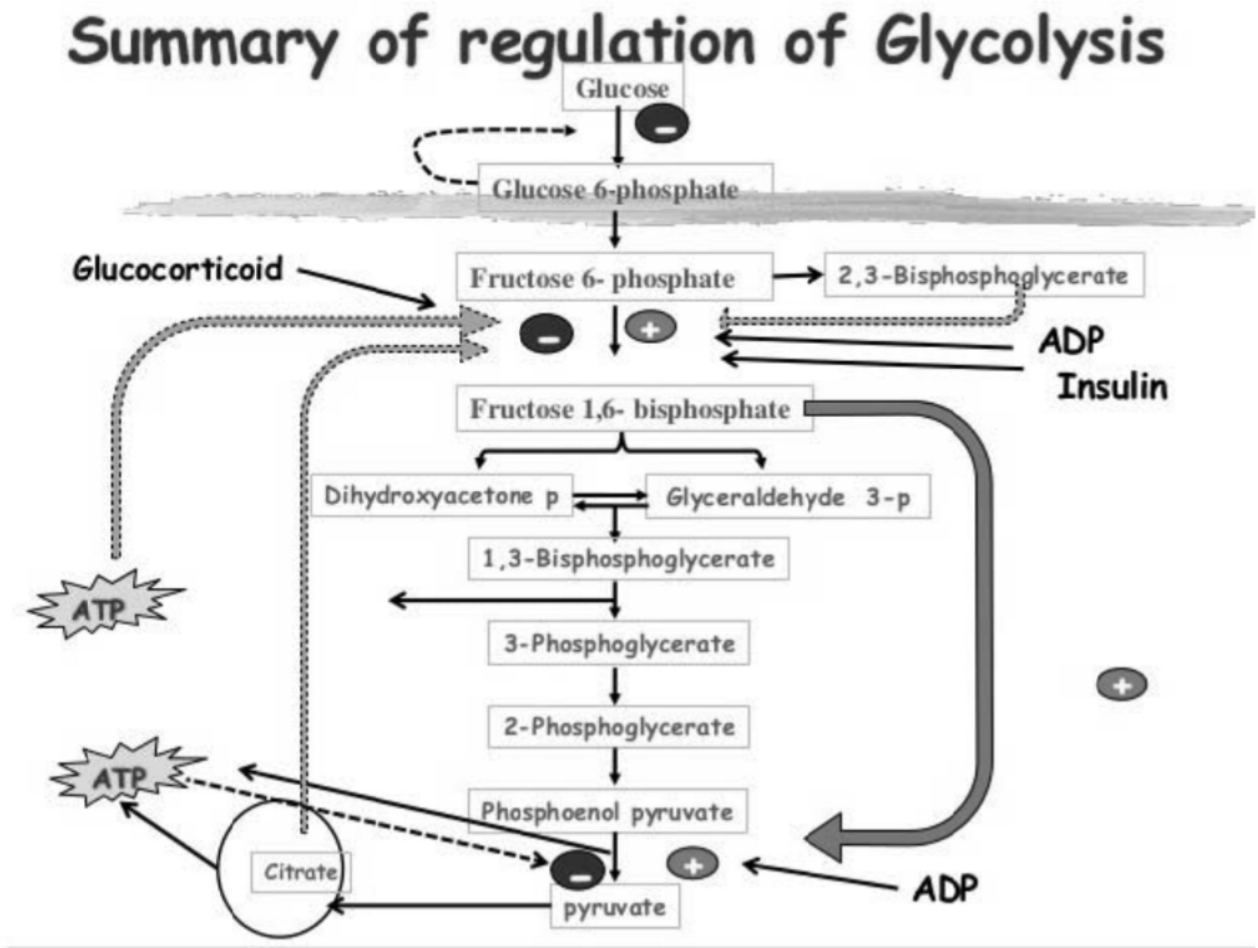

(A)
Initial phase of glycolysis Substrates from different sources are channeled into triose phosphate. For each molecule of sucrose that is metabolized, four molecules of triose phosphate are formed. The process requires an input of up to 4 ATP.
CYTOSOL
Sucrose
UDP
Invertase
Sucrose synthase
Glycolysis
PLASTID
Starch
Starch phosphorylase
Amylase
Glucose
Fructose
UDP-Glucose
ATP
Hexokinase
ADP
PPi
UTP
UDP-Glucose pyrophosphorylase
Glucose-1-P
Phosphoglucomutase
Phospho-gluco-mutase
Glucose
Glucose kinase
Glucose-6-P
Fructose-6-P
Hexose phosphates
Hexose phosphate isomerase
AMYLOPLASTS
PPi-dependent phosphofructokinase
ATP-dependent phosphofructokinase
Fructose-1,6-bisphosphate
Aldolase
CHLOROPLASTS
Triose phosphates
Glyceraldehyde-3-phosphate
Triose phosphate isomerase
Dihydroxyacetone phosphate
NAD+
NADH
Glyceraldehyde-3-phosphate dehydrogenase
1,3-Bisphosphoglycerate
Phosphoglycerate kinase
3-Phosphoglycerate
Phosphoglycerate mutase
2-Phosphoglycerate
Enolase
Phosphoenolpyruvate
PEP carboxylase
Oxaloacetate
Pyruvate kinase
Malate dehydrogenase
Pyruvate
Malate
Lactate dehydrogenase
Lactate
Pyruvate decarboxylase
Acetaldehyde
Alcohol dehydrogenase
Ethanol
Fermentation reactions
To MITOCHONDRION
Energy-conserving phase of glycolysis Triose phosphate is converted to pyruvate. NAD+ is reduced to NADH by glyceraldehyde-3-phosphate dehydrogenase. ATP is synthesized in the reactions catalyzed by phosphoglycerate kinase and pyruvate kinase. An alternative end product, phosphoenolpyruvate, can be converted to malate for mitochondrial oxidation; NADH can be reoxidized during fermentation by either lactate dehydrogenase or alcohol dehydrogenase.

Regulation of Glycolysis takes place at three irreversible reactions

1. Hexokinase (feed back inhibition) or Glucokinase (insulin)

2. Regulation of Phosphofructokinase (Allosteric Activator/Inhibitor)

3. Regulation of Pyruvate kinase (Allosteric Activator/ inhibitor)



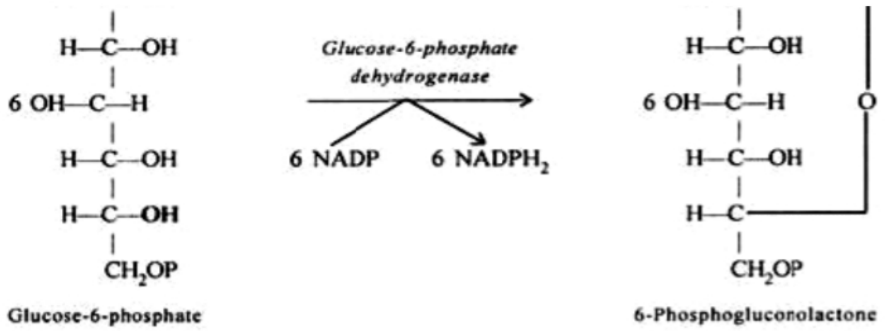
Pentose Phosphate Pathway (Warburg-Dicken's Pathway):

It involves the oxidation of Glucose-6-Phosphate to 6-Phosphogluconic acid which in turn is converted into pentose phosphates. In this pathway glucose-6-phosphate is directly oxidised without entering glycolysis, hence it is also known as Direct Oxidation Pathway or Hexose Monophosphate Shunt.

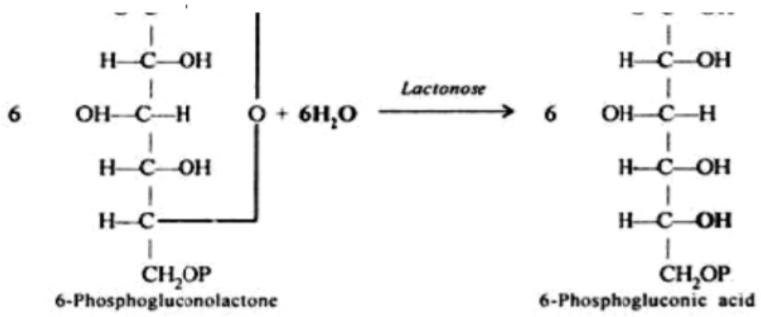
Reactions of Pentose Phosphate Pathway:

Starting from 6-molecules of glucose-6-phosphate, the various reactions of this pathway (Fig. 16.18) can be summarised as follows:

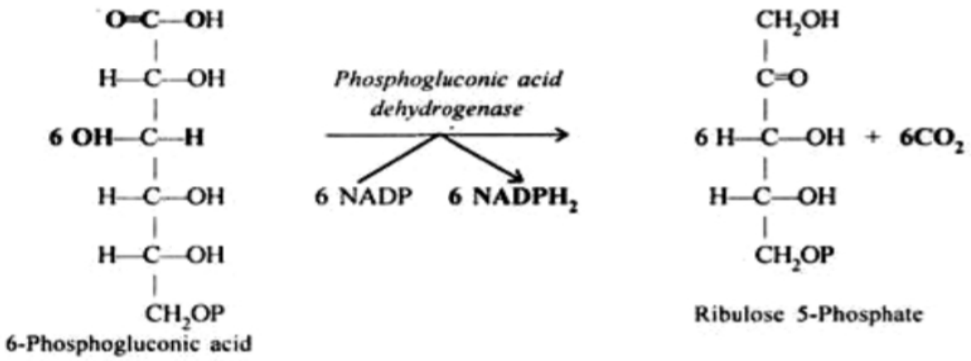
(1) 6 molecules of glucose-6-phosphate in the presence of coenzyme NADP are converted (oxidised) into 6 molecules of 6-phosphogluconolactone by the enzyme glucose-6-phosphate dehydrogenase. 6 molecules of NADP are reduced in the reaction which is reversible.



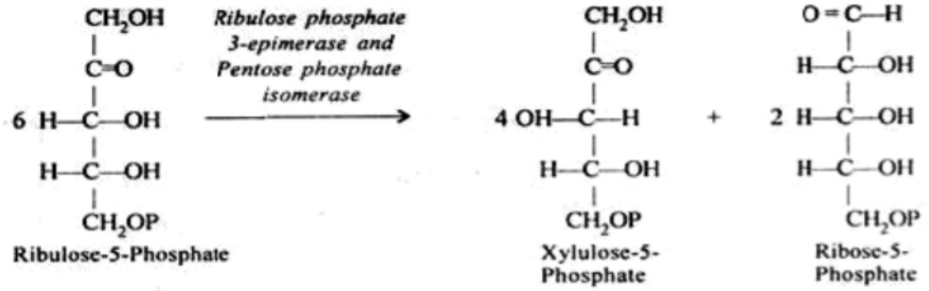
(2) 6-Phosphogluconolactone is hydrolysed by the enzyme Lactonase to produced 6 molecule of 6-phosphogluconic acid.



(3) 6-Phosphogluconic acid is oxidatively decarboxylated by the enzyme 6-Phosphogluconic acid dehydrogenase. 6 molecules of NADP are reduced, 6 molecules of CO_2 are released and 6 mols, of Ribulose-5-Phosphate are produced.

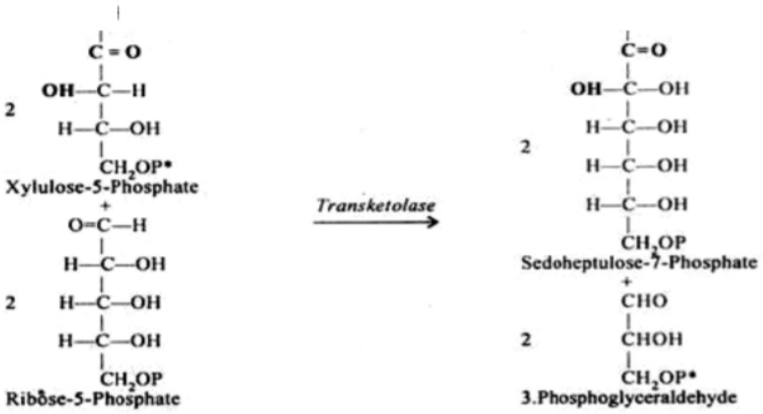


(4) 6 mols. of Ribulose-5-P isomerise into 4 mols. of XyIuIose-5-Phosphate and 2 mols. of Ribose-5-Phosphate in the presence of Ribulose phosphate-3-epimerase and Pentose phosphate isomerase

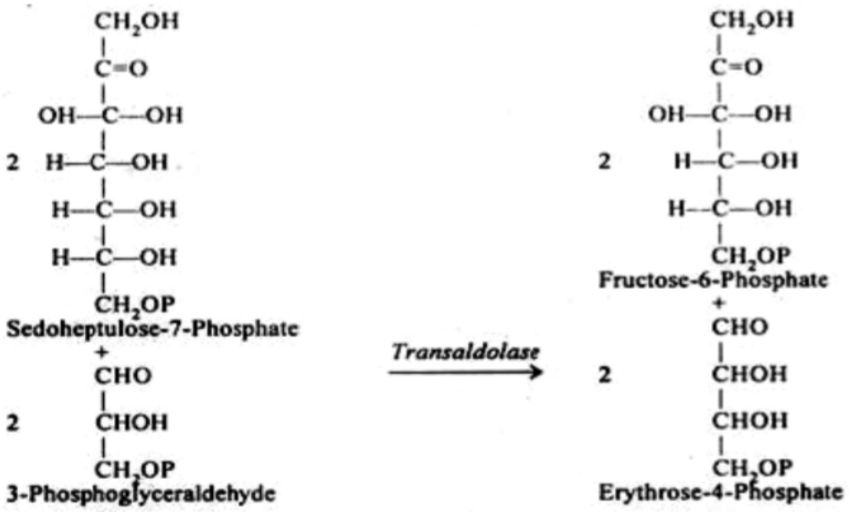


respectively.

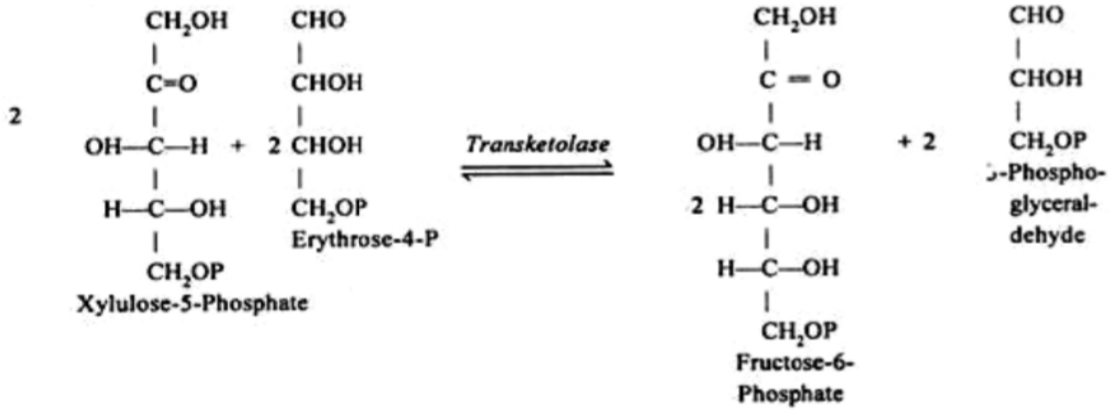
(5) 2 mols. of xylulose-5-Phosphate and 2 mols. of Ribose-5-phosphate combine in the presence of Transketolase to form 2 mols. of Sedoheptulose-7-Phosphate and 2 mols. of 3-Phosphoglyceraldehyde.



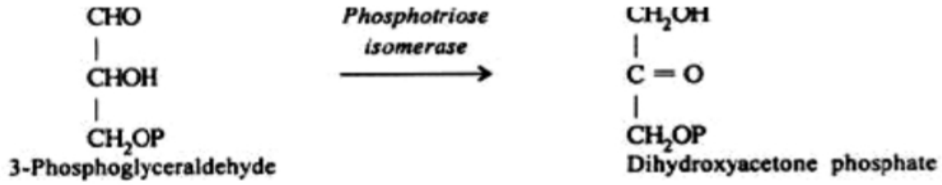
(6) 2 mols. of Sedoheptulose-7-Phosphate and 2 mols. of 3-Phosphoglyceraldehyde combine in the presence of Transaldolase to form 2 mols. of Fructose-6-Phosphate and 2 mols. of Erythrose-4-Phosphate (4-carbon atoms sugar).



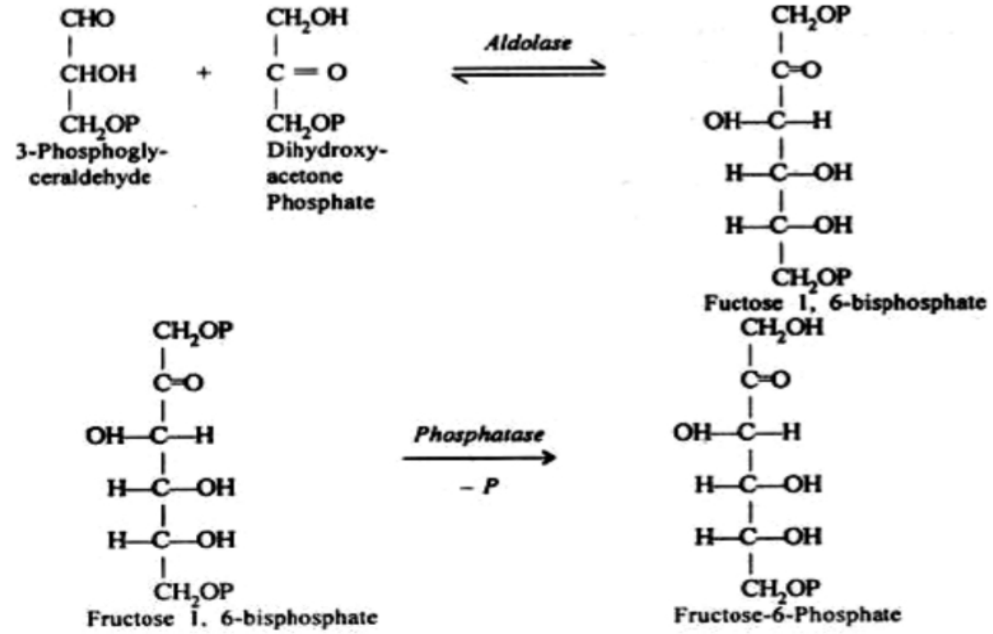
(7) 2 mols. of Erythose-4-Phosphate react with remaining two mols. of xylulose-5-Phosphate (see reaction No. 4 and 5) in the presence of Transketolase to form 2 mols. of Fructose- 6-Phosphate and 2 mols of 3-Phosphoglyceraldehyde.



(8) One mol. of 3-phosphoglyceraldehyde isomerises into dihydroxyacetone phosphate. The enzyme is Phosphotriose isomerase.



(9) Remaining one mole, of 3-Phosphoglyceraldehyde unites with Dihydroxyacetone phosphate in presence of Aldolase to form one mol. of Fructose 1, 6-bisphosphate. The latter, in the presence of Phosphatase forms one mol. of Fructose 6-Phosphate.

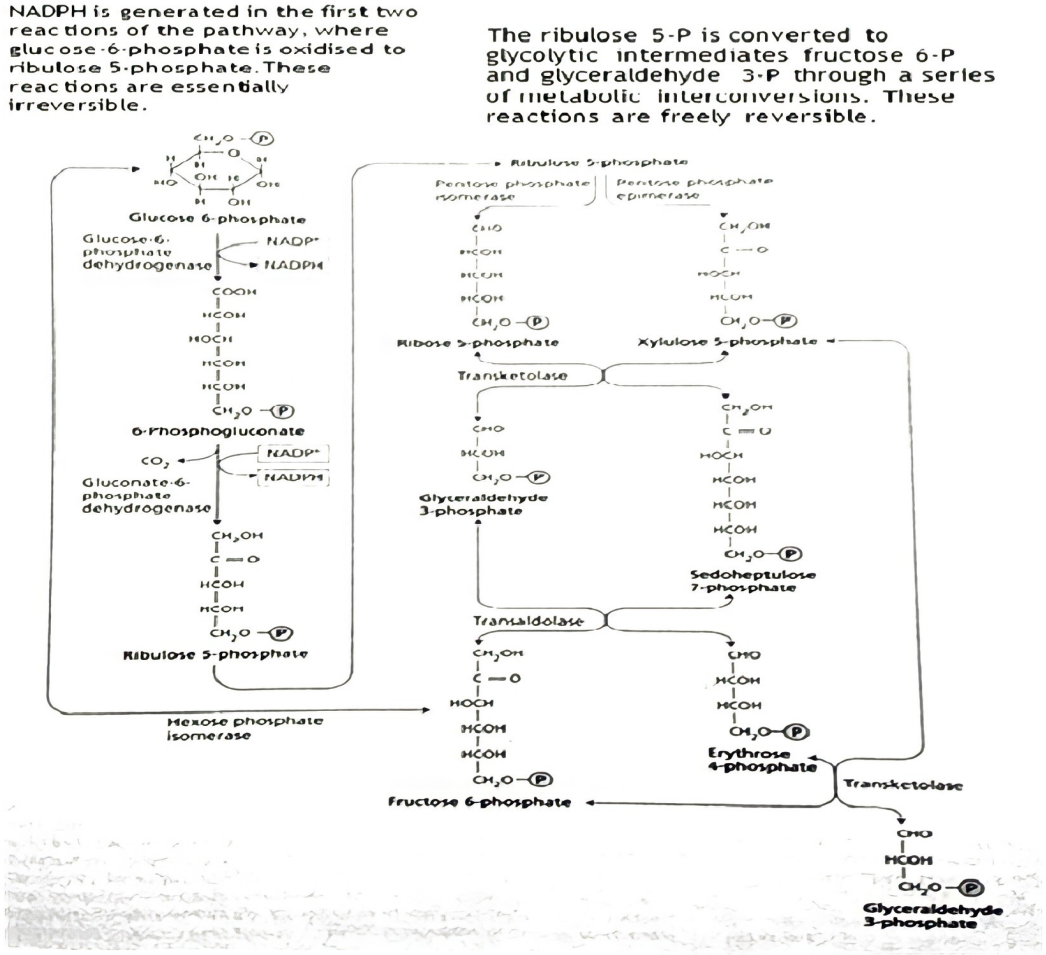


(10) 5 molecules of Fructose-6-phosphate produced in reactions 6, 7 and 9, isomerise into 5 mols. of Glucose-6-P in presence of Phosphohexose isomerase.

To summarise, 6 mols. of Glucose-6-P which enter into this pathway after oxidation produce 6 mols. of CO_2 (Reaction No. 3. CO_2 comes from C-No. 1 of the glucose molecule) and 12 mols. of reduced coenzymes $NADPH_2$ (reaction 1 and 3) while 5 mols of Glucose-6-Phosphate are regenerated (Reaction No. 10).

$6\ (Glucose\text{-}6\text{-}P) + 12\ NADP^+ \rightarrow 5\ (Glucose\text{-}6\text{-}P) + 12\ (NADPH + H^+) + 6\ CO_2$

All the enzymes of pentose phosphate pathway are present in cytosol.



Significance of Pentose-Phosphate-Pathway:

(i) It provides alternative route for carbohydrate breakdown.

(ii) It generates NADPH molecules which are used as reductants in biosynthetic processes under conditions when NADPH molecules are not generated by photosynthesis. It is therefore, especially important in non-photosynthetic tissues such as in differentiating tissues, germinating seeds and during periods of darkness. Production of NADPH is not linked to ATP generation in pentose phosphate pathway.

(iii) It provides Ribose sugars for the synthesis of nucleic acids.

(iv) It plays important role in fixation of CO_2 in photosynthesis through Ribulose-5-Phosphate. (Ribulose 1, 5-bisphosphate derived from Ribulose-5-Phosphate is the primary acceptor of CO_2 in photosynthesis).

(v) It provides Erythrose-4-phosphate which is required for the synthesis of shikimic acid. The latter is precursor of aromatic ring compounds.

2. Oxidative Decarboxylation Pyruvic Acid:

Now, pyruvic acid generated in cytoplasm through glycolysis is transferred to mitochondria. This is initiation of second phase of respiration. As soon as, pyruvic acid enters the mitochondria, one of the three carbon atoms of pyruvic acid is oxidised to carbon dioxide in a reaction called oxidative decarboxylation.

Here, pyruvate is first decarboxylated, and thereafter oxidised by enzyme pyruvate dehydrogenase. This enzyme is made up of a decarboxylase, lipoic acid, TPP, transacetylase and Mg^{+2}.

Acetyl Co-A acts as substrate entrant for Krebs cycle.

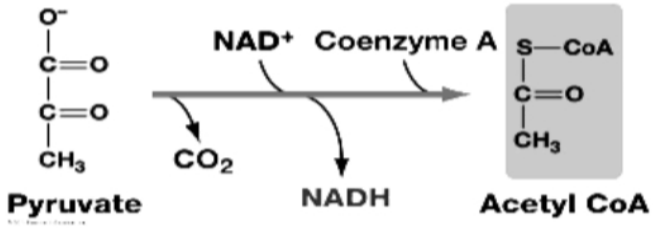
The equation is as follows:

$$\text{Pyruvate} + \text{NAD+} + \text{Co-A} \xrightarrow[\text{dehydrogenase}]{\text{pyruvate}} \text{Acetyl Co-A} + \text{NADH} + H^{-} + CO_2^{-}$$

Acetyl Co-A can enter into mitochondria while pyruvic acid cannot.

Pyruvate oxidation, which follows glycolysis, is essentially the conversion of **pyruvate** molecules to **carbon dioxide**, **acetyl coenzyme A**, and **NADH**. The last step of glycolysis produces two **pyruvate** molecules in the cytosol, which are then brought to the mitochondrial matrix via active transport.

Acetyl Co-A can enter into mitochondria while pyruvate acid cannot.



3. Krebs Cycle:

Sir Hans Adolf Krebs, discovered role of pyruvate in conversion of glucose hydrogens into fumarate. He discovered, in 1937, tricarboxylic acid cycle (i.e., TCA cycle), also known as Citric acid cycle or Krebs cycle. Citric acid cycle occurs in matrix of mitochondria. This cycle involves two decarboxylations and four dehydrogenations.

Various steps of these reactions are as follows:

The starting point of Krebs cycle is entrance of acetyl Co-A into a reaction to form citric acid. Krebs elucidated this cycle, and explained how pyruvate is broken down to $CO_2$and H_2O. For this pioneer work Krebs was awarded Nobel Prize in 1953.

In the first reaction of Krebs cycle, one molecule of acetyl Co-A combines with 4-carbon oxaloacetic acid (OAA); with the result 6-carbon citric acid is produced, and Co-A is released. This reaction is catalysed by enzyme citrate synthase.

$$\underset{(4C)}{\text{Acetyl Co-A + oxaloacetic acid}} \xrightarrow[\text{enzyme}]{\text{citrate synthase}} \underset{(6C)}{\text{Citric acid + Co-A}}$$

Now, citrate (citric acid) is isomerised to isocitrate (isocitric acid).

$$\text{Citric acid} \xrightarrow[\text{enzyme}]{\text{aconitase}} \text{Cis-aconitic acid + } H_2O$$

$$\text{Cis-aconitic acid + } H_2O \xrightarrow[\text{enzyme}]{\text{aconitase}} \underset{\text{(Isocitrate)}}{\text{Isocitric acid}}$$

Cis-aconitic acid is converted into isocitric acid with the addition of water in the presence of iron containing enzyme aconitase.

$$\underset{\text{(Isocitrate)}}{\text{Isocitric acid + NAD}^+} \xrightarrow[\text{dehydrogenase}]{\text{isocitrate}} \underset{\text{(oxalosuccinate)}}{\text{Oxalosuccinic acid + NADH + H}^+}$$

$$\underset{\text{(oxalosuccinate)}}{\text{Oxalosuccinic acid}} \xrightarrow[\text{decarboxylase}]{\text{oxalosuccinic}} \text{α-Ketoglutaric acid + } CO_2$$

$$\text{α-ketoglutaric acid + Co-A + NAD}^+ \xrightarrow[\text{dehydrogenase}]{\text{α- ketoglutaric}} \text{Succinyl Co-A + NADH + H}^+ + CO_2$$

$$\text{Succinyl Co-A + } H_2O + \text{GDP + iP} \xrightarrow[\text{thiokinase}]{\text{succinic}} \text{Succinic acid + Co-A + GTP}$$

$$\text{GTP + ADP} \longrightarrow \text{GDP + ATP (substrate phosphorylation)}$$

$$\text{Succinic acid + FAD} \xrightarrow[\text{dehydrogenase}]{\text{Succinic}} \text{Fumaric acid + } FADH_2$$

$$\text{Fumaric acid + } H_2O \xrightarrow[\text{enzyme}]{\text{Fumarase}} \text{Malic acid}$$

$$\text{Malic acid + NAD}^+ \xrightarrow[\text{dehydrogenase}]{\text{Malic}} \text{Oxaloacetic acid (OAA) + NADH + H}^+$$

During citric acid cycle (Krebs cycle) 3 molecules of NAD^+ and one molecule of FAD (Flavin Adenine Dinucleotide) are reduced to produce NADH and $FADH_2$, respectively.

During citric acid cycle NADH and FADH, are produced. Now, they are linked with electron transport system (ETS) and produce ATP by oxidative phosphorylation.

This may be summarised in following equation:

$$\text{Pyruvic acid + 4NAD}^+ + \text{FAD + 2}H_2O + \text{ADP + iP}$$

$$\downarrow \text{ mitochondrial matrix}$$

$$3CO_2 + \text{4NADH + 4H}^+ + FADH_2 + \text{ATP}$$

In the end of Krebs cycle, glucose molecule is completely oxidised. From one glucose molecule, two pyruvic acid molecules are formed. After oxidation of one pyruvic acid molecule, three CO_2 molecules are released. Thus, in all 6 molecules of CO_2 are released.

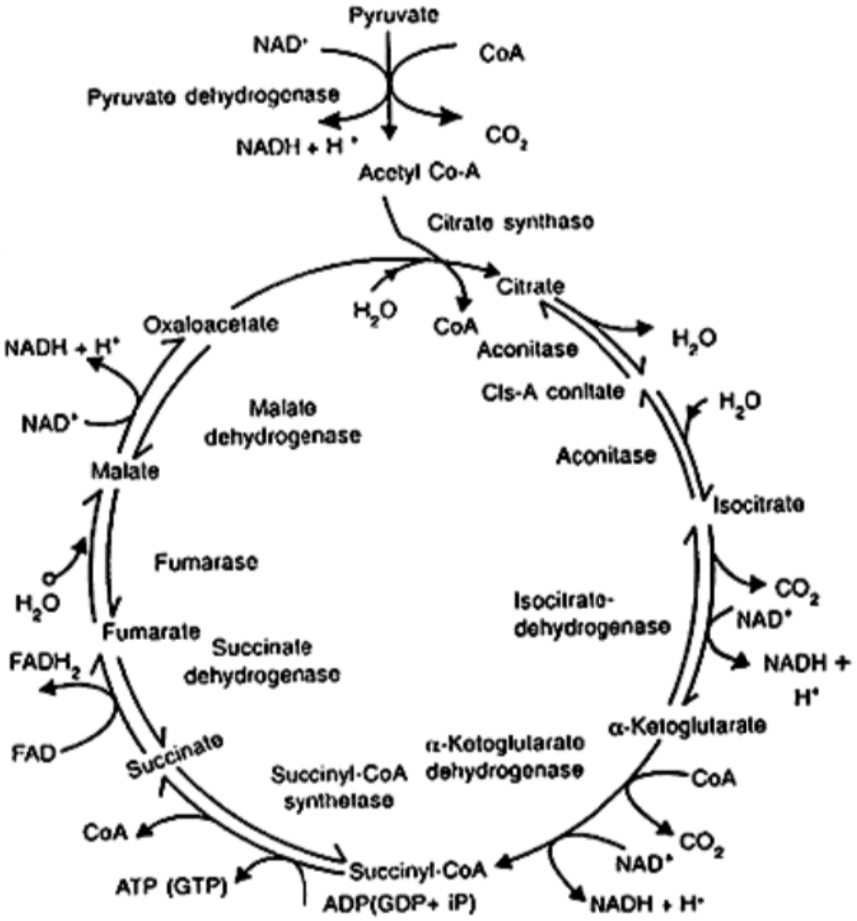


Fig. 4.3. Krebs cycle or citric acid cycle.

Regulation of the Citric Acid Cycle

- Pathway controlled by:

  (1) Allosteric modulators

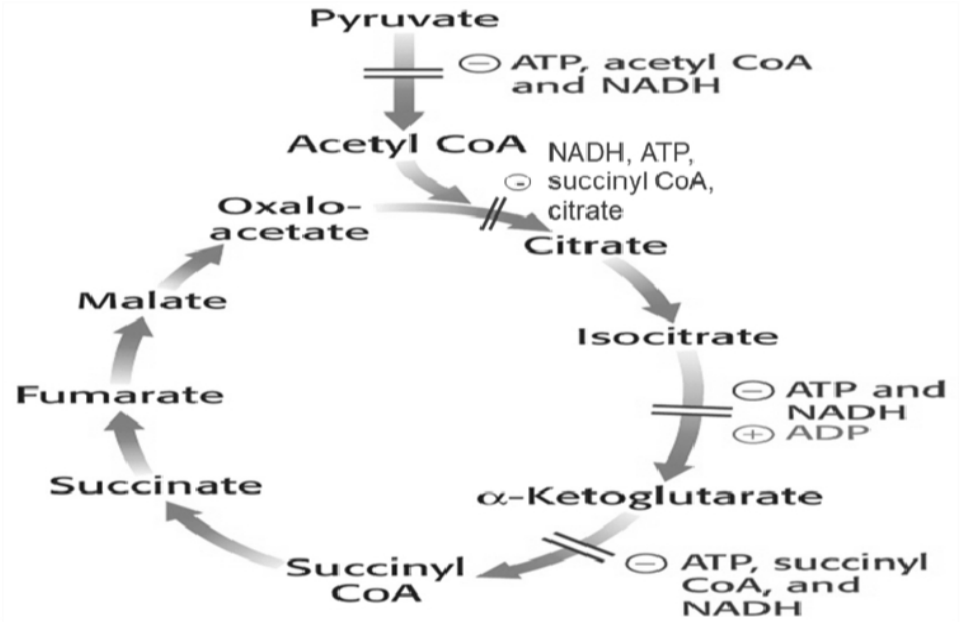
  (2) Covalent modification of cycle enzymes

  (3) Supply of acetyl CoA (pyruvate dehydrogenase complex)

Three enzymes have regulatory properties

- *citrate synthase* (is allosterically inhibited by NADH, ATP, succinyl CoA, citrate – feedback inhibition)
- *isocitrate dehydrogenase* (ICDH) (allosteric effectors: (+) ADP; (-) NADH, ATP. Bacterial ICDH can be covalently modified by kinase/phosphatase)
- *α-ketoglutarate dehydrogenase complex* (inhibition by ATP, succinyl CoA and NADH

Regulation of Kreb's cycle



Electron Transport System (ETS):

By the end of Krebs cycle, glucose molecule oxidises completely, but the energy does not release till NADH and $FADH_2$ oxidise through electron transport system (ETS). The metabolic pathway through which electron passes from one carrier to another, is called electron transport system (ETS). The electron transport system is also known as electron transport chain or mitochondrial respiratory chain.

The electron transport system consists of a series of coenzymes and cytochromes that take part in passage of electrons from a chemical to its ultimate acceptor. The passage of electrons from one-enzyme or cytochrome to the next takes place with a loss of energy at each step. Electron transport system is operative in the inner mitochondrial membrane.

The electron carriers include flavins, iron sulphur complexes, quinones and cytochromes. Most of them are prosthetic groups of proteins.

Electron transport system in mitochondria consists of four complexes which are found in bases of stalked particles in the inner mitochondrial membrane, and also ubiquinone (UQ) or coenzyme Q and cytochrome c which are not bound to stalked particles but act as mobile electron carriers between the complexes.

Complex-I:

Consists of NADH-dehydrogenase or NADH-Q reductase which contains a flavoprotein FMN (flavin mononucleotide) and is associated with iron-sulphur (Fe-S) proteins. This complex is responsible for passing electrons (also protons) from mitochondrial NADH to ubiquinone (UQ), located within inner mitochondrial membrane.

$$NADH + H^+ + FMN \longrightarrow FMNH_2 + NAD^+$$

$$FMNH_2 + 2Fe^{3+}\,S \longrightarrow FMN + 2Fe^{2+}\,S + 2H^+$$

$$2Fe^{2+}\,S + Q + 2H^+ \longrightarrow 2Fe^{3+}\,S + QH_2$$

Complex-II:

Consists of succinate dehydrogenase which contains a flavoprotein FAD (flavin adenine dinucleotide) in its prosthetic group and is associated with non heme iron-sulphur (Fe S) proteins.

This complex receives electrons (also protons) from succinic acid (which is oxidised in Krebs cycle to form fumaric acid) and passes them to ubiquinone (UQ). Ubiquinone also receives reducing equivalents via $FADH_2$ that is generated during oxidation of succinate, through the activity of energy succinate dehydrogenase, in Krebs cycle.

$$FADH_2 + 2Fe^{3+}\ S \longrightarrow 2Fe^{2} + S + 2H^{+} + FAD$$

Complex-III:

$$2Fe^{2+}\ S + Q + 2H^{+} \longrightarrow 2Fe^{3+}\ S + QH_2$$

Consists of ubiquinol, cytochrome c and cytochrome bc_1 .The reduced ubiquinone is called ubiquinol. Here ubiquinol is oxidised with the transfer of electrons to cytochrome c via cytochrome bc_1. Cytochrome c is a small protein attached to outer surface of the inner mitochondrial membrane and acts as a mobile carrier for transfer of electrons between complex III and complex IV.

This complex is called QH_2-cytochrome c reductase complex. This bears three components, i.e., cytochrome b, non-heme iron sulphur (Fe – S), and cytochrome c_1. Coenzyme Q is also involved between Fe-S and cytochrome c_1.

The equations are as follows:

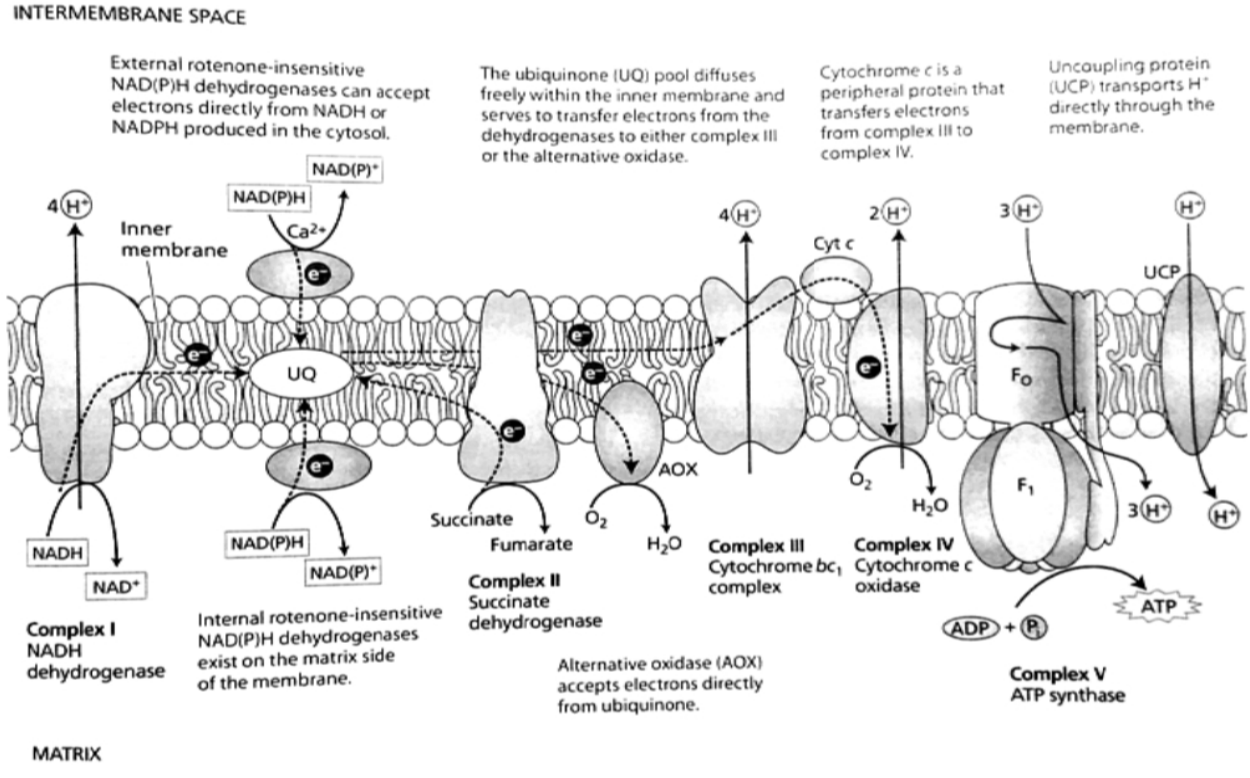
$$QH_2 + 2Fe^{3+}\ cy\ b \longrightarrow Q + 2H^{+} + 2Fe^{2+}\ cy\ b$$

$$2Fe^{2+}\ cy\ b + 2Fe^{3+}\ S \longrightarrow 2Fe^{3+}\ cy\ b + 2Fe^{2+}\ S$$

$$2Fe^{2+}\ S + Q + 2H^{+} \longrightarrow 2Fe^{3+}\ S + QH_2$$

$$QH_2 + 2Fe^{3+}\ cy\ c_1 \longrightarrow Q + 2H^{+} + 2Fe^{2+}\ cy\ c_1$$

Now, cytochrome c, transfers electrons to cy c. Like coenzyme Q, cy c is also mobile carrier of electrons.



Complex-IV:
Is known as cytochrome c oxidase complex. This contains cytochromes a and a_3, along with two copper centres. This complex receives electrons from cytochrome c and passes them to 1/2 O. Two protons are needed and Hp molecule is formed (terminal oxidation). Here, O_2 is ultimate acceptor of electrons. It combines with protons to form metabolic water or respiratory water.

$$\underset{(Fe^{++})}{\text{2 Ferrocytochrome}} + 2H^+ + 1/2\ O_2 \rightleftharpoons \underset{(Fe^{+++})}{\text{2 ferricytochrome } c} + H_2O$$

Complex-V:
When electrons are transferred from one carrier to next carrier via complexes 1 to IV in electron transport system (ETS), they are coupled to ATP synthase enzyme complex for production of ATP from ADP and inorganic phosphate (iP).
Here, number of ATP molecules synthesised during ETS, depends on nature of electron donor. Oxidation of one molecule of NADH gives rise to 3 molecules of ATP, and one molecule of $FADH_2$ gives rise to 2 molecules of ATP. ATP synthase complex is called complex V.
During transportation of electrons, hydrogen atoms split into protons and electrons. The electrons are carried by cytochromes. Before last stage, where hydrogen atom is accepted by oxygen to form water, the electrons again recombine with their protons. Oxygen acts as final hydrogen acceptor.

Oxidative Phosphorylation:
The whole process, where oxygen effectively allows the production of ATP by phosphorylation of ADP, is called oxidative phosphorylation. In other words, synthesis of ATP is called phosphorylation, and as it takes place in presence of oxygen, it is called oxidative phosphorylation.

$$2H \rightleftharpoons 2H^+ + 2e^-$$

$$NAD^+ + H^+ + H^+ + e^- + e^- \longrightarrow NADH + H^+$$

The enzyme required for synthesis of ATP, is called ATP synthase. This is located in F_1, or head piece of $F_0 - F_1$ or elementary particles. ATP synthase enzyme becomes active in ATP formation, where there is a proton gradient saving higher concentration of H_2.
ATP synthase, also known as complex V consists of two major components, i.e., F_1, and F_0. The F_1 headpiece is a peripheral membrane protein complex and contains the site for ATP from ADP and inorganic phosphate (iP).

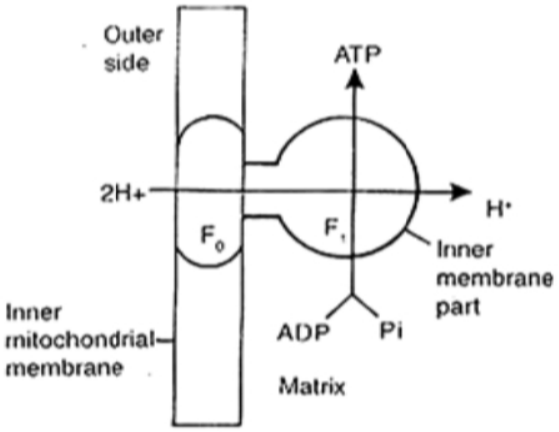


Fig. 4.5. Synthesis of ATP by inner membrane particles of mitochondrion.

Whereas, F_0 is an integral membrane mitochondrial-protein complex which forms the channel through which protons cross the inner membrane. The passage of protons through the channel is coupled to the catalytic site of the F_1 component for the production of ATP.
Oxidation of one molecule of $NADH_2$ produces 3 ATP molecules whereas a similar oxidation of $FADH_2$ produces 2 ATP molecules.

Net gain of ATP:

Complete oxidation of glucose to CO_2 and water shows that there is a net gain of 38 ATP. Each NADH + H^+ produces 3 ATP molecules, while $FADH_2$ forms only 2 ATP molecules at the end of reaction.

$$NADH + H^+ + 1/2\ O_2 + 3\ ADP + 3\ iP \longrightarrow NAD + 3\ ATP + H_2O$$

$$FADH_2 + 1/2\ O_2 + 2\ ADP + 2\ iP \longrightarrow FAD + 2\ ATP + H_2O$$

Thus, total gain of ATP in aerobic respiration is as follows:

However, in most eukaryotic cells, 2 molecules of ATP are required for transport of NADH produced in glycolysis into mitochondrion for further oxidation, and therefore, net gain of ATP is 36 molecules.

Significance of Krebs Cycle:

a. During Krebs cycle, carbon skeletons are obtained for use in growth and maintenance of the cell.
b. Many intermediate compounds are formed which are used in synthesis of other biomolecules, such as amino acids, nucleotides, chlorophyll, cytochromes and fats.
c. During this pathway amino acids are synthesised from α-ketoglutaric acid, pyruvic acid and oxaloacetic acid.
d. Here succinyl Co-A acts as starting molecule for synthesis of chlorophyll.
e. Krebs cycle is major pathway for generation of ATP molecules, which make energy currency of the cell.
f. Energy is released from glucose, and is used in various biochemical reactions.
g. Phenol, anthocyanin, etc., are produced from acetyl Co-A, whereas fatty acids are formed from glycerol.
h. Glutamic acid is formed from α-ketoglutaric acid; aspartic acid from oxaloacetic acid, and alanine from aspartic acid.
i. Amino acids are used in synthesis of proteins, nucleic acids, purines and pyrimidines.
j. Succinyl Co-A carries synthesis of pyrrole compounds of chlorophyll, cytochrome and phytochrome.
k. Krebs cycle is directly related to nitrogen metabolism, α-ketoglutaric acid, an intermediate of Krebs cycle is first acceptor molecule of NH_3 forming an amino acid, the glutamic acid. From glutamic acid various transamination reactions begin to form different amino acids which ultimately condense to form proteins.
l. Krebs cycle is also intimately related with fat metabolism. Dihydroxyacetone phosphate produced in glycolysis may be converted into glycerol via glycerol-3-phosphate and vice versa. After β-oxidation, fatty acids give rise to active 2-C units, the acetyl Co-A which enters the Krebs cycle.

Lipid metabolism

Digestion and Absorption:

Salivary juice produces a lipid hydrolyzing enzyme, especially in infants and children, known as lingual lipase. Lipids are not digested in the mouth, though lingual lipase is present because the high pH of the mouth does not favour the action of lingual lipase and also because the food remains here for a short period of time. As soon as the lipids enter the stomach, they get liquefied due to the heat of the stomach.

Further, the lipids get emulsified due to the peristaltic movements of the stomach. The gastric juice contains gastric lipase, which is inactive at the low pH of the stomach, whereas lingual lipase is active at this pH and it hydrolyses triacylglycerol's of short chain fatty acids (which are generally found in milk, so digestion in the stomach is seen only in children). The released fatty acids are absorbed via the stomach wall and enter the portal vein. The TAG with longer chain fatty acids dissolve and form fat droplets and finally enter the intestine.

In the intestine, the bile salts (sodium glycocholate and sodium taurocholate) and bile acids (cholic acid, chenodeoxycholic acid and cholesterol) help in emulsifying fats thereby making them susceptible to the digestive enzymes. The pancreatic secretions in the intestine contain pancreatic lipase which along with the help of a protein called co-lipase and lecithin acts on TAG at the water-oil interface.

Phospholipids are hydrolyzed by phospholipase A, B, C and cholesterol is hydrolyzed by cholesterol esterase.

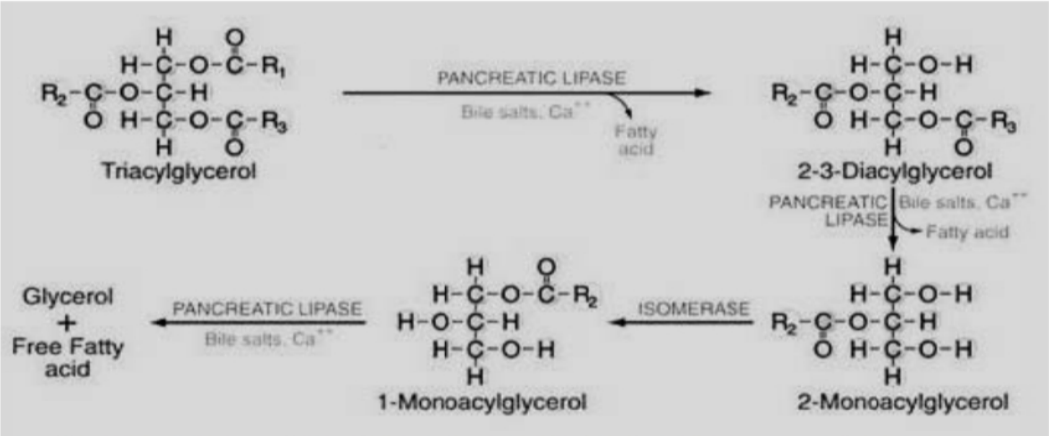
The end products of fat digestion are:
(1) Monoacylglycerols (MAG)

(2) Diacylglycerols (DAG)

(3) Triacylglycerol's (TAG)

(4) Free fatty acids and

(5) Glycerol.



Digestion of lipids takes place for a longer duration of time. Until and unless digestion of fats has not taken place, other food materials (carbohydrates and proteins) cannot be digested because the fats will cover the food and prevent enzymes reaching the food (hence take a fatty meal while going on a long journey).

The digested lipids enter the intestinal epithelium by diffusion or by a process called 'pinocytosis'. In the intestinal wall the free fatty acids and glycerol re-aggregate to form TAG i.e. here re-synthesis of digested TAG, phospholipids and cholesterol takes place (in order to maintain the concentration gradient of lipids).

The important pathways/cycles of lipid metabolism are briefly described:

1. Fatty Acid Oxidation:

The fatty acids in the body are mostly oxidized by α-oxidation. β-Oxidation may be defined as the oxidation of fatty acids on the p-carbon atom. This results in the sequential removal of a two carbon fragment, acetyl CoA (Fig. 67.10).

❖ **Knoop's β-oxidation of Fatty Acids:**

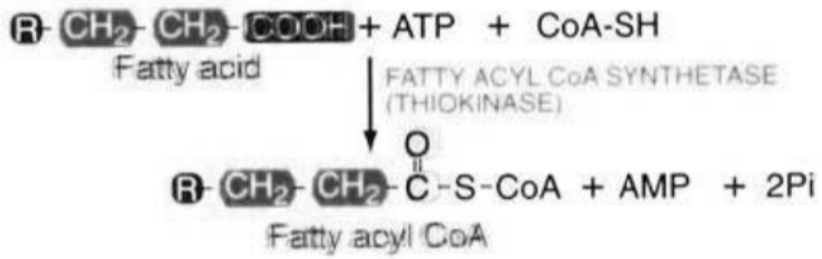
Oxidation of the fatty acids at the β-carbon atom to a carboxylic group is known as beta oxidation. This was proposed by the scientist Knoop, hence the name.

The steps involved in the β-oxidation of fatty acids are:

1. Activation of fatty acids
2. Formation of α-β unsaturated acyl-CoA (Enoyl-CoA)
3. Formation of β-hydroxy acyl-CoA
4. Formation of β-keto acyl-CoA
5. Thiolytic cleavage of keto acyl-CoA

1. Activation of fatty acids:

Oxidation of fatty acids can take place only if they are activated. The fatty acids are activated in the cytosol of the cell wherein the enzyme fatty acyl CoA synthetase (thiokinase) condenses the fatty acids with coenzyme-A by esterification which requires two high energy bonds.

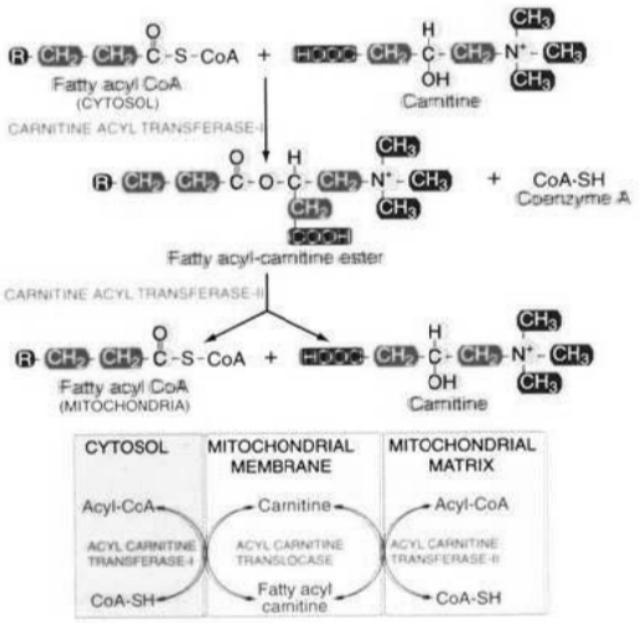


Further oxidation of fatty acids takes place in the mitochondrial matrix. The mitochondrial membrane is impermeable to the acylated fatty acids of the cytosol. Hence these are transported by carnitine mechanism after activation.

Carnitine mechanism:

Carnitine (beta-hydroxyl-gamma-trimethyl-ammonium butyrate) [$(CH_3)_3$-N^+– CH_2-CH(OH)-CH_2-COOH], is present in the inner mitochondrial membrane and it helps in the transport of fatty acids both form the cytosol to mitochondria and from mitochondria to the cytosol.

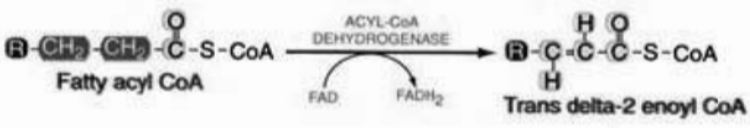
The mechanism of carnitine transfer is as follows:



Carnitine reacts with activated fatty acids in presence of an enzyme carnitine acyl transferase-I, forming fatty acyl-carnitine complex and CoA-SH. Now this complex is easily transported through the inner mitochondrial membrane through a transport protein called carnitine acyl carnitine translocate. In the inner surface of the membrane another enzyme carnitine acyl transferase-II hydrolyzes the fatty acyl carnitine to give fatty acyl-CoA and carnitine. Thereby carnitine is free for reutilization and fatty acyl- CoA undergoes further oxidation in the mitochondrial matrix.

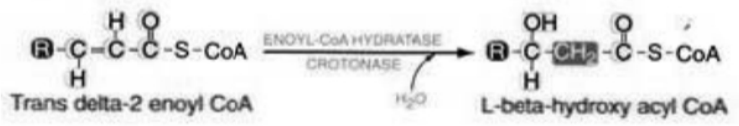
2. Formation of α-β unsaturated acyl-CoA (Enoyl-CoA):

The fatty acyl-CoA undergoes dehydrogenation at the α and β-carbon atoms forming trans alpha-beta unsaturated acyl-CoA. These hydrogen's are taken up by the coenzyme, Flavin Adenine Dinucleotide (FAD) which gets converted to $FADH_2$. Oxidation of $FADH_2$ through electron transport chain produces two ATPs. The enzyme is fatty acyl CoA dehydrogenase.



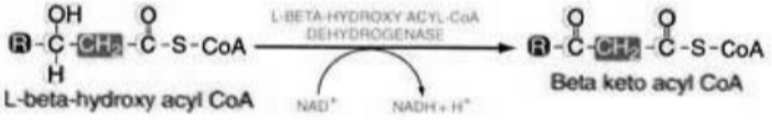
3. Formation of β-hydroxy acyl-CoA:

Enoyl-CoA is then hydrated by the enzyme crotonase (Enoyl-CoA hydratase) which adds water across the double bond. The product formed is P-hydroxy acyl-CoA.



4. Formation of β-keto acyl-CoA:

β-Hydroxy acyl-CoA undergoes another dehydrogenation of the P-oxidation process forming β-keto acyl-CoA. The enzyme is β-hydroxy acyl-CoA dehydrogenase and its redox potential allows it to use NAD^+as the coenzyme releasing NADH + H^+ that produces 3 ATPs upon oxidation through ETC.



5. Thiolytic cleavage of β-keto acyl-CoA:

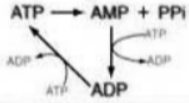
β-Keto acyl-CoA is then cleaved between the a and P-carbon atom releasing an acetyl-CoA and a fatty acyl-CoA shortened by two carbon atoms. The enzyme is β-keto thiolase that uses the coenzyme A for adding to the newly formed acyl-CoA.

The fatty acids undergo oxidative removal of two carbon units from the carboxylic side in the form of acetyl-CoA, which repeats till the fatty acid is completely converted to acetyl-CoA.

Calculation of Total ATP Produced When Palmitic Acid is Completely Oxidized to CO2 and H2O:

6 Carbon palmitic acid (i.e., the most abundant fatty acid in the human body) undergoes

7 such passes or cycles of P-oxidation, producing a total of 8 acetyl-CoA which are in turn oxidized through TCA cycle and the reducing equivalents produced are oxidized through ETC.

The number of ATPs produced by palmitic acid		
Name of the enzyme / process	**Number of reducing equivalents / ATP produced**	**Total number of ATP**
Fatty acyl CoA dehydrogenase	7 $FADH_2$ × 2	14
β-hydroxyacyl CoA dehydrogenase	7 NADH + H^+ × 3	21
	Total ATPs produced in β-oxidation	**35 ATP**
Acetyl CoA (8) oxidized through TCA cycle (each cycle=12 ATP)	8 × 12	96
Total ATP produced both in β-oxidation and TCA cycle		**131**
ATPs utilized	Activation step ATP → AMP 	2
	NET GAIN OF ATPs	**129**
Totally 129 ATP's are produced when palmitic acid is oxidized completely		

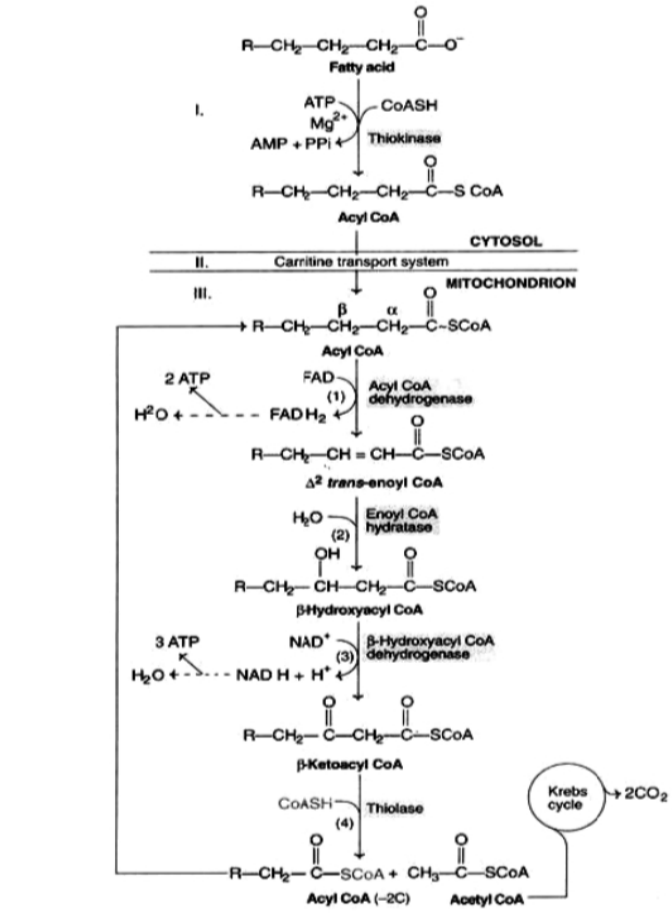


Fig. 67.10 : β-Oxidation of fatty acids : Palmitoyl CoA (16 carbon) undergoes seven cycles to yield 8 acetyl CoA [I-Activation; II-Transport; III-β Oxidation proper—(1) Oxidation, (2) Hydration, (3) Oxidation and (4) Cleavage]

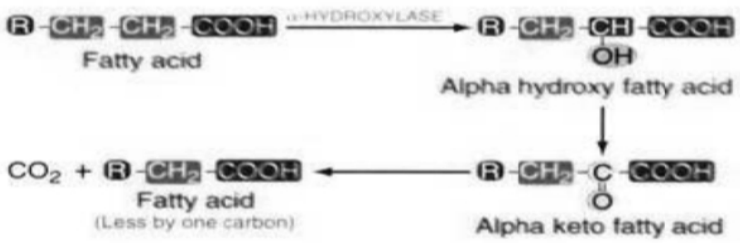
Fatty acids are oxidized by most of the tissues in the body. However, brain, erythrocytes and adrenal medulla cannot utilize fatty acids for energy requirement.

❖ α-Oxidation of Fatty Acids:

Oxidation of the fatty acids at the alpha carbon atom is known as alpha oxidation. During a-oxidation there is removal of one carbon atom at a time from the carboxyl end of the molecule. Alpha oxidation takes place for the alpha hydroxy fatty acids which are found in the brain.

During alpha oxidation, first a hydroxyl group is attached (if the fatty acid is devoid of alpha hydroxyl group) at the alpha carbon atom which is then oxidized to a keto group and then finally to a carboxylic group due to decarboxylation of the original carboxyl group giving rise to a fatty acid less by one carbon atom.

Characteristic Features of α-Oxidation:



1. It does not require CoA intermediates.
2. It does not generate high energy phosphate bonds.

Omega Oxidation:

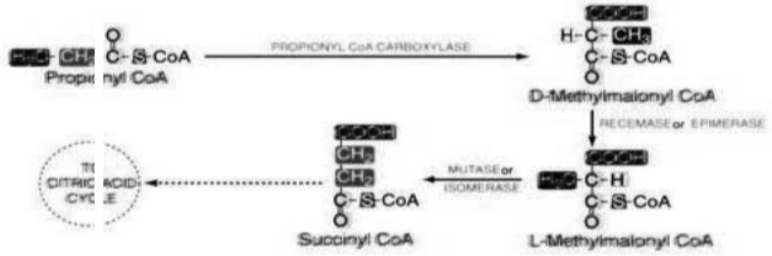
Oxidation of the omega carbon atom of the fatty acid is known as omega oxidation. During omega oxidation there is a successive removal of four

carbon atoms at a time. It predominantly occurs in the liver, whenever there is a high demand of energy as in the case of lactation, severe diabetics and starvation.

During omega oxidation first of all, with the help of hydroxylase enzyme a hydroxyl group is attached to the last carbon atom (-CH_3i.e., methyl group of the omega carbon atom), which is then further oxidized to a carboxylic group to form an alpha omega dicarboxylic acid. Now beta oxidation occurs from both the ends producing two acetyl-CoA at a time.

Oxidation of Fatty Acids with an Odd Number of Carbon Atoms:

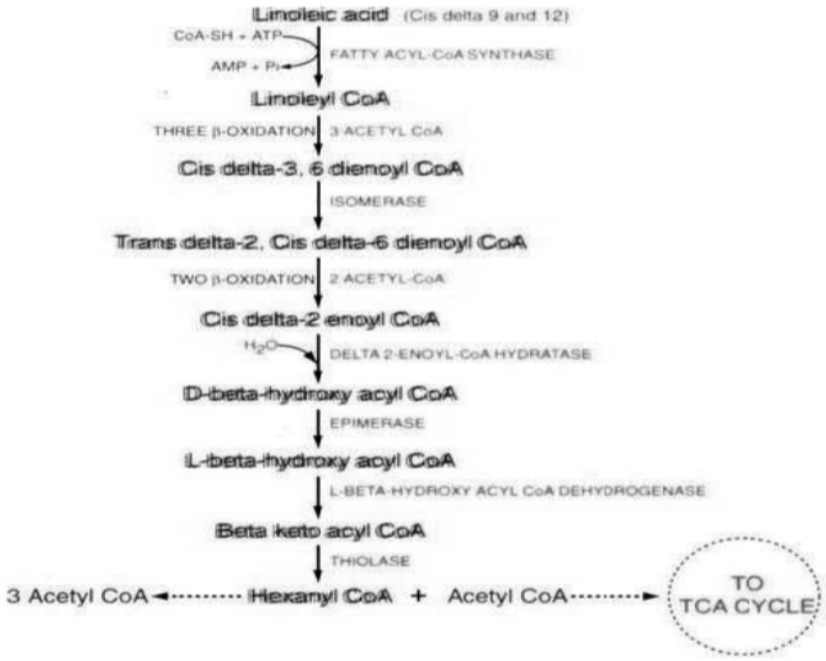
Fatty acids with an odd number of carbon atoms are normally oxidized by beta oxidation but the last cycle of the beta oxidation produces acetyl-CoA and propionyl-CoA (a 3 carbon moiety). This propionyl-CoA is further oxidized in the following manner:



Oxidation of Unsaturated Fatty Acids:

The 18 carbon linoleic acid is an unsaturated fatty acid, containing two cis double bonds (1) between carbon atoms 9 & 10 (cis Δ^9) and (2) between carbon atoms 12 & 13 (cis Δ^{12}). In its oxidation, three cycles of beta oxidation take place giving rise to 3 acetyl-CoA and cis- delta-3, 6-dienoyl-CoA, which is isomerized to trans-delta-2-cis-delta-6-dienoyl-CoA, which then undergoes two passes of beta oxidation forming two acetyl-CoA and nV-delta-2-enoyl-CoA.

This is converted to D-β-hydroxy acyl-CoA by the enzyme delta-2-enoyl-CoA hydratase. D-β-hydroxy acyl-CoA cannot be acted upon by the stereo-specific enzyme; L-P-hydroxy acyl-CoA dehydrogenase. Hence it is epimerized by an epimerase to give L-β-hydroxy acyl-CoA and then normal beta oxidation continues till the fatty acid is completely converted to acetyl-CoA.



2. Biosynthesis of Fatty Acids:

The dietary carbohydrates and amino acids, when consumed in excess, can be converted to fatty acids and stored as triacylglycerol's. De novo (new) synthesis of fatty acids occurs predominantly in liver, kidney, adipose tissue and lactating mammary glands.

The enzyme machinery for fatty acid production is located in the cytosomal fraction of the cell. Acetyl CoA is the source of carbon atoms while NADPH provides the reducing equivalents and ATP supplies energy for fatty acid formation.

The fatty acid synthesis may be learnt in 2 stages:

I. Conversion of acetyl CoA to malonyl CoA.

II. Reactions of fatty acid synthase complex.

I. Formation of malonyl CoA:

Acetyl CoA is carboxylated to malonyl CoA by the enzyme acetyl CoA carboxylase. This is an ATP-dependent reaction and requires biotin for CO_2 fixation. Acetyl CoA carboxylase is a regulatory enzyme in fatty acid synthesis.

II. Reactions of fatty acid synthase complex:

The remaining reactions of fatty acid synthesis are catalysed by a multifunctional enzyme known as fatty acid synthase (FAS) complex. In eukaryotic cells, including man, the fatty acid synthase exists as a dimer with two identical units. Each monomer possesses the activities of seven different enzymes and an acyl carrier protein (ACP) bound to 4′-phosphopantetheine.

Fatty acid synthase functions as a single unit catalysing all the seven reactions. Dissociation of the synthase complex results in loss of the enzyme activities. The sequence of reactions for the extra—mitochondrial synthesis of fatty acids (palmitate) is depicted in Fig. 67.11, and described below

1. The two carbon fragment of acetyl CoA is transferred to ACP of fatty acid synthase, catalysed by the enzyme, acetyl CoA-ACP transacylase. The acetyl unit is then transferred from ACP to cysteine residue of the enzyme. Thus ACP site falls vacant.

2. The enzyme malonyl CoA-ACP transacylase transfers malonate from malonyl CoA to bind to ACP.

3. The acetyl unit attached to cysteine is transferred to malonyl group (bound to ACP). The malonyl moiety loses CO_2 which was added by acetyl CoA carboxylase. Thus, CO_2 is never incorporated into fatty acid carbon chain.

4. β-Ketoacyl ACP reductase reduces ketoacyl group to hydroxyacyl group. The reducing equivalents are supplied by NADPH (from HMP shunt).

5. β-Hydroxyacyl ACP undergoes dehydration. A molecule of water is eliminated and a double bond is introduced between α and β carbons.

6. A second NADPH-dependent reduction, catalysed by enoyl-ACP reductase occurs to produce acyl-ACP. The four-carbon unit attached to ACP is butyryl group.

The carbon chain attached to ACP is transferred to cysteine residue and the reactions 2-6 are repeated 6 more times. Each time, the fatty acid chain is lengthened by a two-carbon unit (obtained from malonyl CoA). At the end of 7 cycles, the fatty acid synthesis is complete and a 16-carbon fully saturated fatty acid—namely palmitate—bound to ACP is produced.

7. The enzyme palmitoyl thioesterase separates palmitate from fatty acid synthase. This completes the synthesis of palmitate.

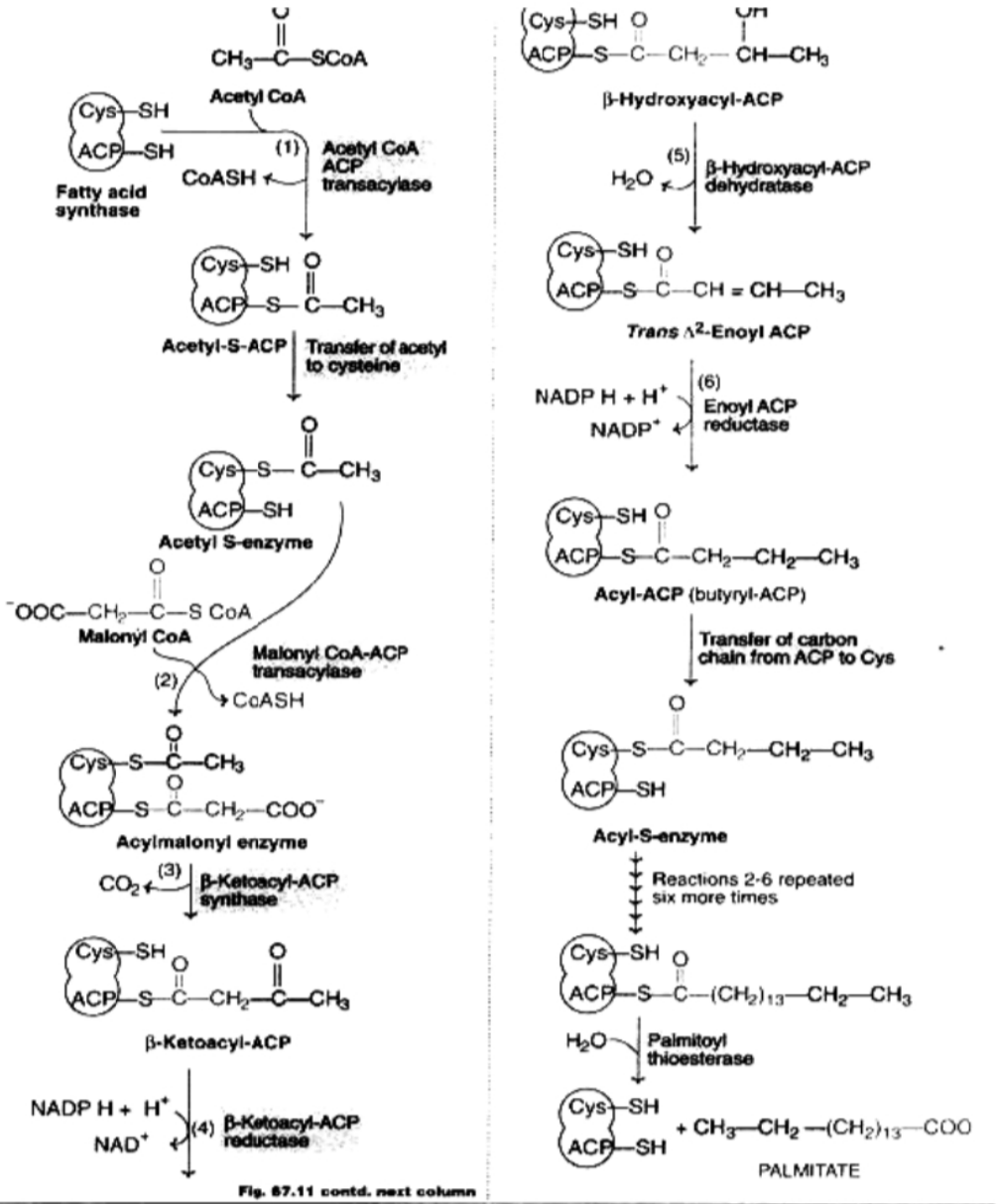


Fig. 67.11 contd. next column

Fig. 67.11 : Biosynthesis of long chain fatty acid–palmitate. (Cys–Cysteine; ACP–Acyl carrier protein; The pathway repeats 7 times to produce palmitate; the first two carbons at the methyl end are directly from acetyl CoA, the rest of the carbons come from malonyl CoA).

10. Nitrogen metabolism: Biological and non biological nitrogen fixation, nitrate and ammonium assimilation.

Nitrogen metabolism

All the living organisms are basically composed of carbon, hydrogen, oxygen, nitrogen and many other forms of chemical elements. These elements contribute to finally organize various biomolecules present in a cell. Nitrogen is next to carbon in importance in living organisms. In a living cell, nitrogen is an important constituent of amino acids, proteins, enzymes, vitamins, alkaloids and some growth hormones. Therefore, study of nitrogen metabolism is absolutely essential because the entire life process is dependent on these nitrogen-containing molecules. In this lesson, you will learn about various aspects of nitrogen metabolism including nitrogen fixation and nitrogen assimilation in plants.

Molecular nitrogen

Nitrogen is primarily present in the atmosphere freely as dinitrogen or nitrogen gas. It is present in the combined form as Chile saltpetre or sodium nitrate and Chile in South America is the major source of this nitrate nitrogen. Molecular Nitrogen or diatomic nitrogen (N2) is highly stable as it is triple bonded (NºN). Because of this stability, molecular nitrogen as such is not very reactive in the atmosphere under normal conditions. In the atmosphere molecular nitrogen is 78.03% by volume and it has a very low boiling point (-195.8 oC) which is even lower than oxygen. Proteins present in living organisms contain about 16% nitrogen.

Nitrogen fixation (biological and abiological)

The conversion of molecular nitrogen into compounds of nitrogen especially ammonia is called nitrogen fixation. Nitrogen fixation, is a reductive process i.e., nitrogen fixation will stop if there is no reducing condition or if oxygen is present. This nitrogen fixation may take place by two different methods – abiological and biological.

Abiological nitrogen fixation

In abiological nitrogen fixation the nitrogen is reduced to ammonia without involving any living cell. Abiological fixation can be of two types : industrial and natural. For example, in the Haber's process, synthetic ammonia is produced by passing a mixture of nitrogen and hydrogen through a bed of catalyst (iron oxides) at a very high temperature and pressure. In natural process nitrogen can be fixed especially during electrical discharges in the atmosphere. It may occur during lightning storms and nitrogen in the atmosphere can combine with oxygen to form oxides of

nitrogen.

Biological nitrogen fixation

Chemically, this process is same as abiological. Biological nitrogen fixation is reduction of molecular nitrogen to ammonia by a living cell in the presence of an enzyme nitrogenase. Nitrogen fixation of free living organisms and symbiotic nitrogen fixation Nitrogen fixation is a distinctive property possessed by a select group of organisms, because of the presence of the enzyme nitrogenase in them. The process of nitrogen fixation is primarily confined to microbial cells like bacteria and cyanobacteria. These microorganisms may be independent and free living.

Mechanism of Biological Fixation of Nitrogen

Nitrogen fixation requires

(i) the molecular nitrogen

(ii) a strong reducing power to reduce nitrogen like FAD (Flavin adenine dinucleotide)

(iii) a source of energy (ATP) to transfer hydrogen atoms to dinitrogen and

(iv) enzyme nitrogenise

(v) compound for trapping the ammonia formed since it is toxic to cells.

The reducing agent and ATP are provided by photosynthesis and respiration. The overall biochemical process involves stepwise reduction of nitrogen to ammonia. The enzyme nitrogenase is a Mo-Fe containing protein and binds with molecule of nitrogen (N_2) at its binding site. This molecule of nitrogen is then acted upon by hydrogen (from the reduced coenzymes) and reduced in a stepwise manner. It first produces dimide (N_2H_2) then hydrazime (N_2H_4) and finally ammonia ($2NH_3$). NH_3 is not liberated by the nitrogen fixers. It is toxic to the cells and therefore these fixers combine NH_3 with organic acids in the cell and form amino acids.

Molecular nitrogen is a very stable molecule. Therefore, sufficeient amount of cell energy in the form of ATP is required for stepwise reduction of nitrogen to ammonia.In legumes, nitrogen fixation occurs in specialized bodies called nodules. The nodules develop due to interaction between the bacteria Rhizobium and the legume roots. The biochemical steps for nitrogen fixation are same. However, legume nodules possess special protein called LEGHEMOGLOBIN. The synthesis of leghemoglobin is the result of symbiosis because neither bacteria alone nor legume plant alone possess the protein. Recently it has been shown that a number of host genes are involved to achieve this. In addition to leghemoglobin, a group of proteins called nodulins are also synthesized which help in establishing symbiosis and maintaining nodule functioning. Leghemoglobin is produced as a result of interaction between the bacterium and legume roots. Apparently, Rhizobium gene codes for Heme part and legume root cell gene codes for Globin moiety. Both the coded products together consitute the final protein leghemoglobin.

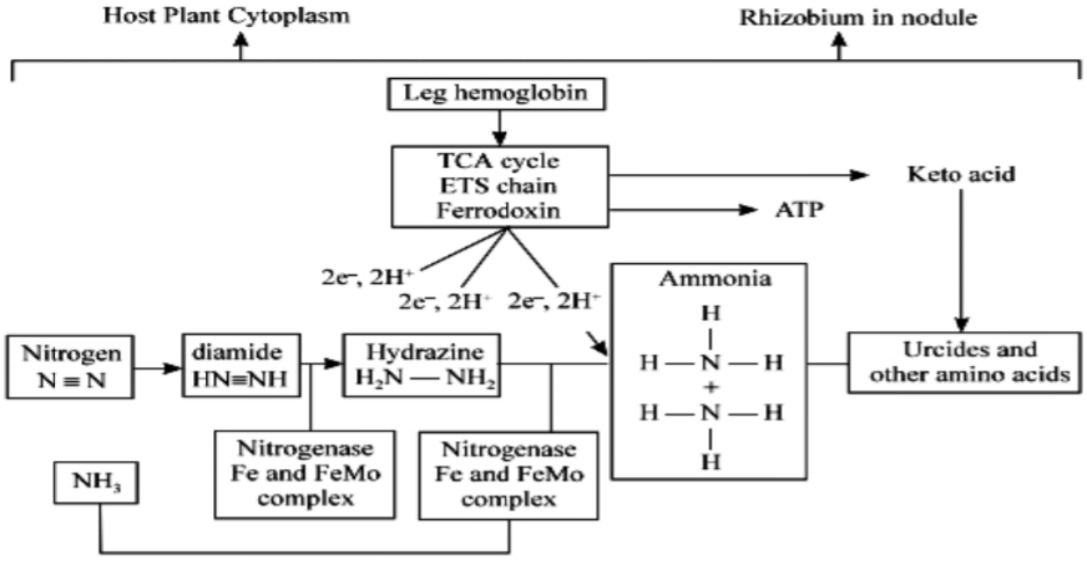


Fig. Simplified flow sheet of biochemical steps for nitrogen fixation

Leghemoglobin is considered to lower down the partial pressure of oxygen and helps in nitrogen fixation. However, this function is specific for legumes only because free living microbes do not possess nitrogen fixing leghemoglobin. Moreover, it has also not been found in cyanobacterial symbiosis with other plants.

Nitrate and ammonia assimilation by plants

As pointed in the previous section, nitrogen fixation is confined to selected microbes and plants. But all plants do require nitrogen because it has a role to play in the general metabolism. Therefore, plants which do not fix nitrogen, use other combined nitrogen sources such as nitrate and ammonia for carrying on metabolic activity. Nitrate is absorbed by most plants and reduced to ammonia with the help of two different enzymes. The first step conversion of nitrate to nitrite is catalyzed by an enzyme called nitrate reductase. This enzyme hasseveral other important constituents including FAD, cytochrome, NADPH or NADH and molybdenum.

The overall process of nitrate reduction take place in the cytosol and is an energy dependent reaction. The enzyme nitrate reductase has been studied in many plants and it is observed that the enzyme is continuously and synthesized and degraded. The enzyme nitrate reductase is inducible. This means that increase in nitrate concentration in the cytosol induces more of nitrate redutase to be synthesized. However, when excess NH_4^+ is produced then it has a negative effect on the synthesis of nitrate reductase. In plants, it has also been observed that light also increase nitrate reductase when nitrate is available. In the second step the nitrite so formed is further reduced to ammonia and this is catalyzed by the enzyme nitrite reductase. Nitrite present in the cytosol is transported into chloroplast or plastids where it is reduced to ammonia.

The enzyme nitrite reductase is able to accept electrons from sources such as NADH, NADPH or $FADH_2$. Besides, reduced ferredoxin has also been shown to provide electrons to nitrite reductase for reducing nitrite to ammonia. Ammonia so formed has to be utilized quickly by plants because accumulation of ammonia has a toxic effect. Some plants including algae leach out excess ammonia which can further be oxidized to nitrite and nitrate by microorganisms in the soil or water.

Amino acids synthesis by plants

As you have noticed that ammonia formation is achieved by plants either by (i.) nitrogen fixation or (ii) by reduction of nitrate to nitrite. Ammonium (NH_4^+) is the most reduced form of inorganic combined nitrogen. This ammonium now becomes the major source for the production of amino acids, which are the building blocks of enzymes and proteins. Amino acids have two important chemical groups. (i) amino group (NH) and (ii) carboxy1 group (-COOH).

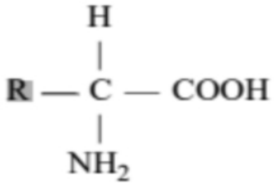


Fig. A typical amino acid with functional groups. R represents alkyl group

Ammonium so produced is the major source of amino group. However, the carboxyl group has to be provided by other organic molecule synthesized by the plants. There are two major reactions for amino acid biosynthesis in plants:

Reductive amination reaction

In this reaction, ammonia combines with a keto acid. The most important keto acid is the alpha ketoglutaric acid produced during the operation of Krebs cycle. The keto acid then undergoes enzymatic reductive amination to produce an amino acid.

$$\text{-ketoglutaric acid} + NH_3 \xrightarrow{\text{glutamic dehydrogenase}} \text{Glutamic acid}$$

(keto acid) (amino acid)

Similarly another amino acid called aspartic acid is produced by reductive amination of oxaloacetic acid. It has been noted that reductive amination respresents the major 'port of entry' for ammonia into the metabolic stream in plants. This initiates synthesis of glutamic acid followed by other amino acids.

Transamination reaction

This is another very important reaction for amino acid biosynthesis. The reaction involves transfer of amino group, from already synthesized amino acid, to the keto acid.

$$\text{-Ketoglutaric acid} + \text{Aspartic acid} \xrightarrow{\text{Transaminase}} \text{Glutamic acid} + \text{Oxaloacetic acid}$$

(Keto acid) (Amino acid) (Amino acid) (Keto acid)

In the above reaction, aspartic acid has transferred its amino group (NH_2) to the a-ketoglutaric acid to synthesize glutamic acid and release keto acid. The reaction is catalyzed by enzymes called transaminases. A large number of amino acids are synthesized by this transamination reaction. Amino acids are organic molecule containing nitorgen. The incorporation of amino group, from ammonium, into keto acids represents the major step for synthesis of nitrogenous organic biomolecules

11. Let's sum up

(i) Water potential is the chemical potential of water divided by the partial molal volume of water. The major factors influencing the water potential in plants are concentration, pressure,and gravity.

(ii) In higher plants water is absorbed through root hairs which are in contact with soil water and form a root hair zone a little behind the root tips. During absorption of water by roots, the flow of water from epidermis to endodermis may take place through three different pathways:(i) Apoplastic pathway, (ii) Trans-membrane pathway and (iii) Symplast pathway.

(iii)Three different theories of the mechanism by which ascent of water are brought about in plants. These hypotheses are (i) Root pressure (ii) Capillary action (iii) Cohesion-tension.

(iv) A cell wall can have up to three parts— middle lamella, primary wall and secondary wall. The middle lamella is laid down first, formed from the cell plate during primary cell wall is then deposited inside the middle lamella.

(v) Signal transduction is the process by which a chemical or physical signal is transmittedthrough a cell as a series of molecular events, most commonly protein phosphorylationcatalyzed by protein kinases, which ultimately results in a cellular response. Proteinsresponsible for detecting stimuli are generally termed receptors, although in some cases theterm sensor is used.

(vi) In plants, red and blue light are especially effective in inducing a photo-morphogeneticresponse. Phytochrome is a blue protein pigment responsible for the perception of light inphoto-physiological processes. It is possibly the only photoreceptor in photoperiodism and theflowering process.

(vii) Plants produce signaling molecules, called hormones that have profound effects ondevelopment at vanishingly low concentrations. Until quite recently, plant development wasthought to be regulated by only five types of hormones: auxins, gibberellins, cytokinins,ethylene, and abscisic acid.

Embryogenesis transforms a single-celled zygote into a multicellular, microscopic, embryonicplant. Differentiation is the process by which a cell acquires metabolic, structural, andfunctional properties that are distinct from those of its progenitor cell.

Germination is aprocess by which the embryo in the seed becomes activated and begins to grow into a new seedling.

(viii) Senescence is the process of aging in plants. Plants have both stress-induced and age-relateddevelopmental aging. Chlorophyll degradation during leaf senescence reveals the carotenoids,and is the cause of autumn leaf color in deciduous trees. Leaf senescence has the importantfunction of recycling nutrients, mostly nitrogen, to growing and storage organs of the plant.

(ix)Flower initiation takes place by the transformation of vegetative apex into a reproductivestructure. It signifies a transition from vegetative to the floral state. The shoot meristem isreduced and is also induced to develop sepals, petals, stamens and carpels in place of leaves.The ABC model of flower development in angiosperm demonstrates the presence of threeclasses of genes that regulate the development of floral organs. The genes are referred to asclass A genes, class B genes and class C gene.

(x) Stress is usually defined as an external factor that exerts a disadvantageous influence on theplant. In most cases, stress is measured in relation to plant survival, crop yield, growth(biomass accumulation), or the primary assimilation processes which are related to overallgrowth.

(xi)A buffer solution is an aqueous solutionconsisting of a mixture of a weak acid and itsconjugate base, or vice versa. pH is defined as the decimal logarithm of the reciprocal of thehydrogen ion activity, aH+, n a solution.

(xii) Enzymes are proteins that act as catalysts within living cells. Catalysts increase the rate atwhich chemical reactions occur without being consumed or permanently altered themselves.

(xiii) A chemical reaction is a process that converts one or more substances to another type ofsubstance.

(xiv) Carbohydrates are a group of organic compounds consisting of C, H, O usually in the ratio of1: 2: 1. Lipids are heterogeneous group of water insoluble compounds which are oily orgreasy in consistency but soluble in non-polar solvents. Proteins are organic nitrogenouscompounds in which a large number of amino acids are joined together by peptide linkages toform long polypeptide chains.

(xv) Photosynthesis is the process that gives life to all living beings. The plants convert lightenergy into life energy. It is the only biological process that makes use of sun's light energyfor driving the life machinery.

(xvi) In respiration carbohydrate is broken down, as a result the potential energy is transformed intokinetic form. Though it is a destructive or katabolic process, yet respiration is extremelybeneficial, because it releases the necessary energy for performing the life functions.

12. Suggested Reading

(i) Taiz, L., & Zeiger, E. Plant Physiology (4th ed.), 2006, Sinauer Associates, Inc. Publishers.

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(vi)Lehninger Principles of Biochemistry. Sixth Edition. 2013. David L. Nelson , Michael M.Cox. Freeman, Macmillan.Davies P.J. (ed.) Plant Physiology: Physiology, Bio-Chemistry & Molecular Biology, Academic Press.

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(x) Dainty, J. (1976) Water relations of plant cells. In Transport in Plants,Vol. 2, Part A: Cells (Encyclopedia of Plant Physiology, New

(xi)Series, Vol. 2.), U. Lüttge and M. G. Pitman, eds., Springer, Berlin, pp. 12–35.

13. Assignment

1. What will be the value of DPD in a fully turgid cell?
2. Where does photorespiration take place?
3. Discuss physiology of flowering with special reference to photoperiodism
4. Describe the mechanisms of enzyme actions.
5. Describe how plants use light and hormones to influence the germination of the seed.
6. How does phytochrome mediate the photomorphogenetic response?
7. Differentiate between cyclic and non-cyclic photophosphorylation.
8. Define buffer. Give an example.
9. Give an account of the secondary structure of protein.
10. What is Leghaemoglobin?
11. Write a short note on mode of action of auxin.
12. What do you mean by turgor pressure?
13. Explain the mechanism of water Absorption in plant.
14. What do you mean by Ascent of Sap?
15. What is the meaning of CAM? State the significance of it.
16. How does gibberellin induce α-amylase synthesis in aleurone layer of cereals?
17. Differentiate between apoplastic and symplastic pathway.

POST GRADUATE DEGREE PROGRAMME
(CBCS)
IN
BOTANY

SEMESTER - II

Course: BOTCOR T207

(Genetics, Cytogenetics, Plant Breeding and Biometry)

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING
UNIVERSITY OF KALYANI
KALYANI – 741235, WEST BENGAL

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Director's Messeage

Satisfying the varied needs of distance learners, overcoming the obstacle of distance and reaching the unreached students are the threefold functions catered by Open and Distane Learning (ODL) systems. The onus lies on writers, editors, production professionals and other personal involved in the process to overcome the challenges inherent to curriculam design and production of relevant Self Learning Materials (SLMs). At the University of Kalyani a dedicated team under the able guidance of the Hon'ble Vice-Chancellor has invested its best efforts, professionally and in keeping with the demands of PostGraduate CBCS Programmes in Distance Mode to devise a self-sufficient curriculum for each course offered by the Directorate of Open and Distane Learning (DODL) University of Kalyani.

Development of printed SLMs for students admitted to the DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode UGC, DEB Regulations, 2020 had been our endeavour. We are happy to have achieved our goal.

Utmost care and precision have been ensured in the development of SLMs, making them useful to the lesrners, besides avoiding errors as far as practicable. Further, suggestions from the stakeholders in this would be welcome.

During the production-process of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Manas Kumar Sanyal, Hon'ble Vice-Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticisms to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

Sincere gratitude is due to the respective chairpersons as well as each and every Members of PGBOS (DODL), University of Kalyani, Heartfelt thanks is also due to the Course Writers- faculty members at the DODL, subject-experts serving at University Post Graduate departments and also to the authors and academicians whose academic contributions have enriched the SLMs. We humbly acknowledge their valuable academic contributions. I would especially like to convey gratitude to all other University dignitaries and personnel involved either at the conceptual or operational level of the DODL of University of Kalyani.

Their persistent and co-ordinated efforts have resulted in the compilation of comprehensive, learners friendly, flexible text that meets curriculum requirements of the Post Graduate Programme through distance mode.

All the Self Learning Materials are self-writing and collected from e-Book, journals and websites.

Director
Directorate of Open & Distance Learning
University of Kalyani

SYLLABUS

COURSE – BOTCOR T207

Genetics, Cytogenetics, Plant Breeding and Biometry

(Full Marks – 75)

Course	Group	Details Contents Structure		Study hour
BOTCOR T207	**Genetics & Cytogenetics**	**Unit 1. Mendelian Inheritance**	1. Mendelian Inheritance: Meiosis; Chromosome theory of inheritance; Mendelian laws; Gene interactions.	1
		Unit 2. Non-Mendelian Inheritance	2. Non-Mendelian Inheritance: Organelle heredity; Infectious heredity; Maternal effects.	1
		Unit 3. Population Genetics	3. Population Genetics: Hardy-Weinberg principle; gene frequency in a population, genetic equilibrium, factors affecting gene frequency.	1
		Unit 4. Microbial Genetics	4. Microbial Genetics: Transformation, conjugation and transduction and their significance in gene mapping.	1
		Unit 5. Chromosome	5. Chromosome: Structure and nomenclature, centromere and telomere, chromosomal aberrations.	1
		Unit 6. Special Chromosomes	6. Special Chromosomes: Lampbrush, Polytene and B-chromosome.	1
		Unit 7. Sex Determination	7. Sex Determination: Sex determination in plants; dosage compensation; sex linked inheritance.	1
		Unit 8. Linkage and Crossing Over	8. Linkage and Crossing Over: Chiasma frequency and genetic map distance; Tetrad analysis; Centromere mapping with ordered tetrad.	1
		Unit 9. Transposable Elements	9. Transposable Elements: In bacteria (*IS* elements, composite transposons), maize (Ac and Ds elements), *Drosophila* (P-elements) and their genetic significance.	1
		Unit 10. Genetic Regulation	10. Genetic Regulation: Regulation of gene expression in prokaryotes and their viruses – *lac*, *trp* and *ara* operons of *E. coil*, Lambda lytic-lysogenic regulatory cascade; regulation of eukaryotic gene expression – brief account.	1

Course	Group	Details Contents Structure		Study hour
BOTCOR T207	**Plant Breeding & Biometry**	**Unit 11. Breeding methods**	1. Breeding methods: Introduction and conservation of germplasm, mass selection, pure line selection, clonal selection, hybridization, selection after hybridization (bulk, pedigree, recurrent), heterosis & inbreeding depression.	1
		Unit 12. Population samples	2. Population samples, sampling methods.	1
		Unit 13. Frequency distribution	3. Frequency distribution: histogram, normal curve, mean, median, mode, variance, standard deviation, standard error.	1
		Unit 14. Probability & test of significance	4. Probability & test of significance: $\chi 2$ test (detection of segregation ratio & linkage, test of independence); t-test (student & paired); analysis of variance (ANOVA).	1
		Unit 15. Correlation & regression	5. Correlation & regression	1

Content

COURSE – BOTCOR T207

Genetics, Cytogenetics, Plant Breeding and Biometry

Hard Core Theory Paper **Credits (A+B): = 3**

Group – A (Genetics & Cytogenetics)

Content Structure

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1. Introduction
2. Course Objective
3. Mendelian Inheritance: Meiosis; Chromosome theory of inheritance; Mendelian laws; Gene interactions.
4. Non-Mendelian Inheritance: Organelle heredity; Infectious heredity; Maternal effects.
5. Population Genetics: Hardy-Weinberg principle; gene frequency in a population, genetic equilibrium, factors affecting gene frequency.
6. Microbial Genetics: Transformation, conjugation and transduction and their significance in gene mapping.
7. Chromosome: Structure and nomenclature, centromere and telomere, chromosomal aberrations.
8. Special Chromosomes: Lampbrush, Polytene and B-chromosome.
9. Sex Determination: Sex determination in plants; dosage compensation; sex linked inheritance.
10. Linkage and Crossing Over: Chiasma frequency and genetic map distance; Tetrad analysis; Centromere mapping with ordered tetrad.
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13. Let's sum up
14. Suggested Readings
15. Assignments

1. Introduction

Genetics is the study of heredity and variations. Heredity and variations are controlled by genes—what they are, what they do, and how they work. Genes inside the nucleus of a cell are strung together in such a way that the sequence carries information, that information determines how living organisms inherit various features. This course helps the students in gaining sufficient knowledge to pursue academic career or work as an expert at national or international research laboratories.

2. Course Objectives

After completion of the course the learners will be able to:

- To know how the hereditary information in DNA controls what an organism looks like and how it works.
- Relate the structure and function of the DNA molecule to its functional role in encoding genetic material.
- An understanding of the DNA packaging and expression and regulation of gene at transcriptional and translational level.
- Be able to distinguish between maternal effect, sex-linked, and cytoplasmic modes of inheritance.
- Be able to look at a pedigree chart and discern the most likely mode of inheritance.
- To know how inheritance patterns are affected by position on chromosomes
- Gain an appreciation for how genes work together in biological processes.
- Describe variation both in DNA and chromosomal level.
- Explain about the fundamental process of biological systems.
- Apply the Hardy-Weinberg Law in analyzing population genetics for gene frequency, sex linkage, equilibrium, and heterozygote frequency.

3. Mendelian Inheritance: Meiosis; Chromosome theory of inheritance; Mendelian laws; Gene interactions.

Meiosis:

Meiosis is a type of cell division in which chromosome number is reduced to half following reduction division. The reduction of chromosome number is achieved due to a single sphase during the premeiotis interphase followed by two successive nuclear divisions. In general, meiosis takes place during gamete formation and hence it is confined to reproductive cells only.

Meiosis occurs during sexual life cycle of all eukaryoyes. Meiotic cell division is of three types: (i) Zygotic, (ii) Sporogenic and (iii) gametic – depending on stage where it occurs during sexual cycle.

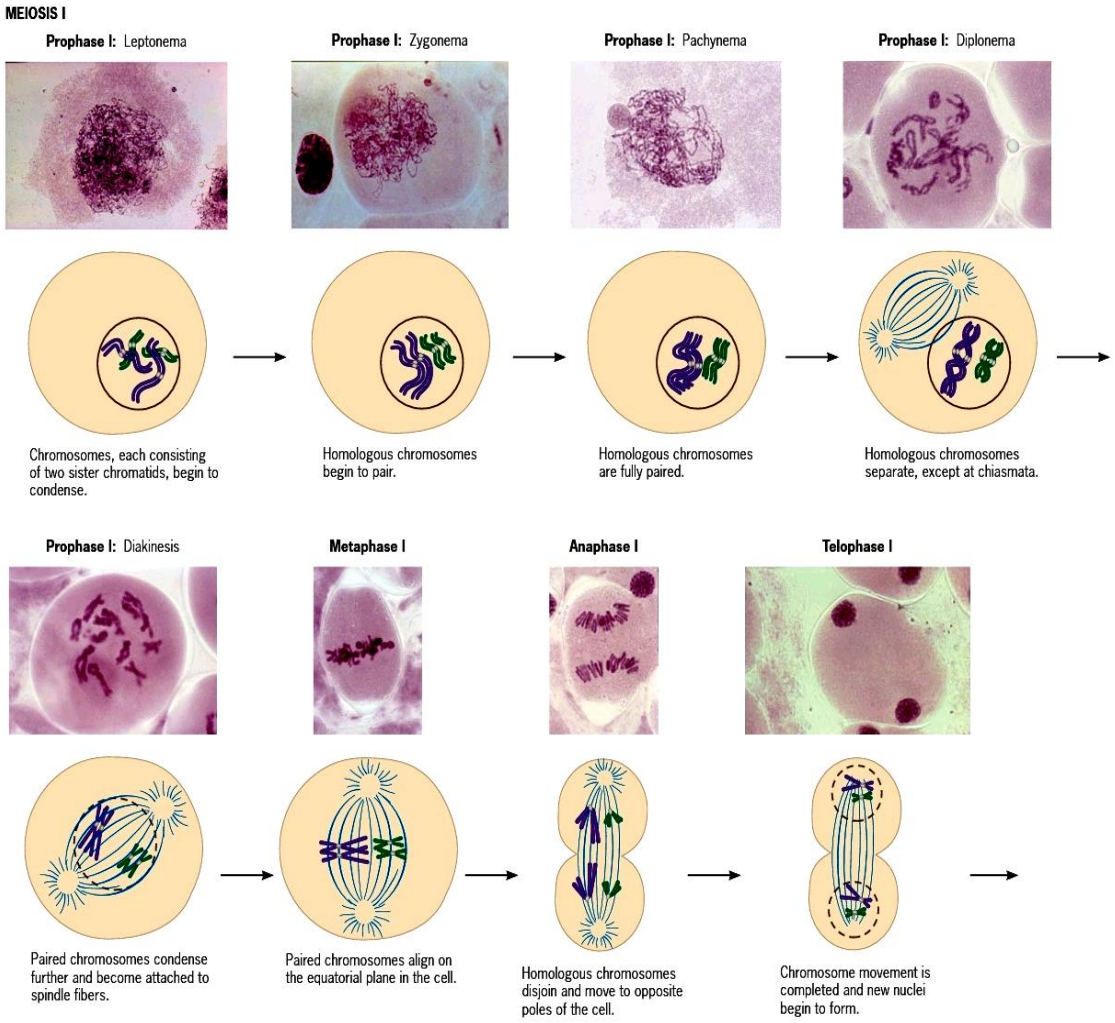
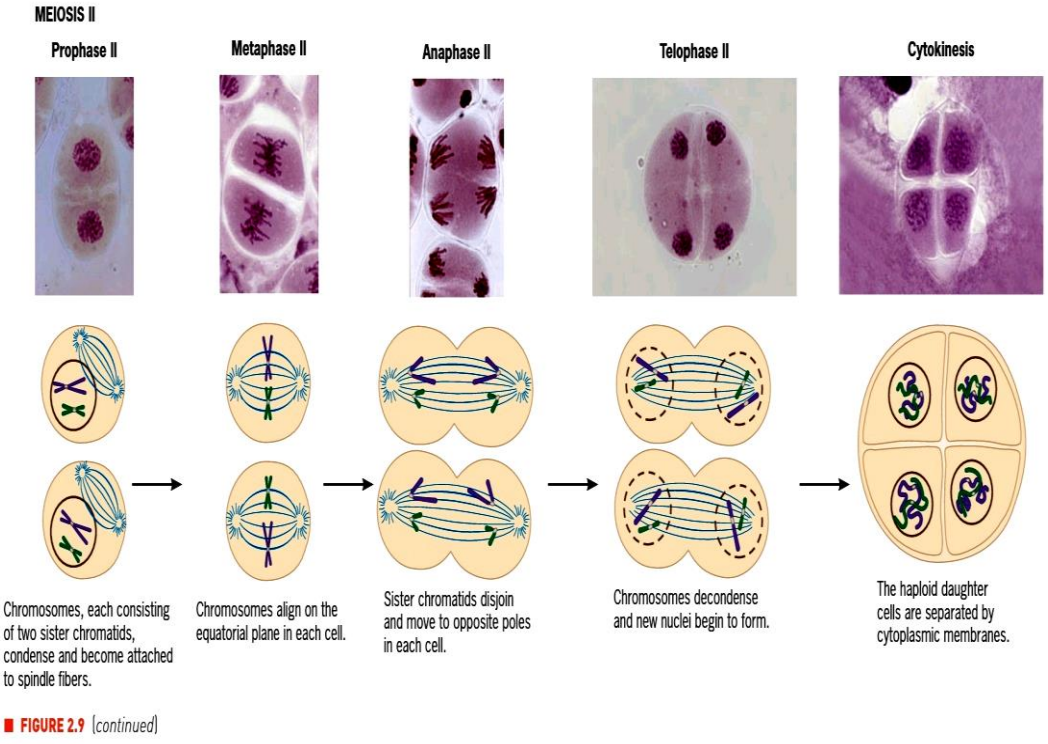


FIGURE 2.9 Meiosis in the plant *Lilium longiflorum*.



■ FIGURE 2.9 (*continued*)

Meiosis is divided into two parts: meiosis I and meiosis II. A the end of meiotic process, there are 4 daughter cells. During meiotic division the nucleus of each cell undergoes two successive divisions, referred to as the first and second meiotic division. Each of the two meiotic divisions is divided into prophase, metaphase, anaphase and telophase following the pattern of the mitotic cell division. The word first is added to the names of various stages of the first meiotic division to yield Prophase I, Metaphase I, Anaphase I, and Telophase I. Similarly, the word second is added to the various stages of second meiotic division to yield Prophase II, Metaphase II, Anaphase II and Telophase II:

First Meiotic Division:

The most significant events of the first meiotic division are (i) pairing between homologous chromosome (2) Crossing over between them during prophase I and (3) Separation of homologous chromosomes and their migration to the opposite poles of a cell during anaphase I. As a result, the two daughter nuclei produced by this division receive only

half chromosomes present in somatic cells. For this reason the first division is often referred to as the reduction division.

First Prophase:

This stage of meiotic division is the longest in duration, it is several times longer than mitotic prophase. The main events occurring during prophase I are (i) intimate pairing between homologous chromosomes. (ii) Condensation of Chromosomes, (iii) Crossing over between homologous chromosomes (iv) movement of chromosomes away from each other so that chiasma become observable, (v) Terminalization of chiasura, and (vi) Movement of chromosomes during chromosoma pairing and chiasma terminalization. Or the basis of these events, prophase I is divided into 5 stages (1) Leptotene (2) Zygotene (3) Pachytene (4) Diplotene and (5) Diakinesis.

Leptotene:

1. There is marked increase in total nuclear volume
2. There is chromosome condensation.
3. There is RNA synthesis as a result of which the volume of nucleous increases.
4. Proteins required for chromosome condensation are synthesized.

Zygotene:

1. Pairing between homologous chromosomes.
2. Completion of replication of the remaining 0.3% DNA of each nucleus, this DNA synthesis is referred to as 2DNA synthesis.
3. Synthesis of a specific nuclear protein.
4. Development of Synaptonemal complex.
5. Progressive condensation of chromosomes.

Pachytene:

1. There is a further condensation of chromosome pair become shorter and thicker.
2. As a consequence of synopsis between nomologerus chromosomes, the number of observable chromosomes is only half of the somatic chromatic chromosome number.
3. The haploid number of chromosome pairs commonly referred to as bivalents.

4. The nucleous is distinct and is quite large. It is associated with the nucleolar organizer region of the sat chromosome pair.
5. Crossing over between homologous chromosomes takes place during this stage.

Diplotene:

1. Homologous chromosomes of each bivalent begin to move away from each other.
2. The two homologues of each bivalent appears to be attached with each other at one or more points; these attachments are known as chiasmata.
3. As diplotene progresses, chiasmata slowly move towards the each of the homogous chromosomes, this movement is referred to as chiasma terminalization.
4. There is further condensation of chromosomes so that they become progressively shorter and thicker.

Diakinesis:

1. Chiasma terminalization is completed just before this stage.
2. Chromosomes become shorter and thicker due to the further condensation.
3. Towards the end of diakinesis bivalents move away from each other and spread toward the peripheri of cells.
4. Nucleolus and nuclear envelop disappear.
5. The spindle apparatus is organized.

First Metaphase:

1. Nucleolus disappears.
2. Nuclear envelope disintegrates.
3. Spindle apparatus is organized.
4. All the bivalents within a cell migrate to the equatorial plate. Centromeres of the two homologous chromosomes of each bivalent become attached to chromosomal fibers origination from opposite poles. Movement of bivalents on to the equatorial plate is primarily due to the shortening and elongation of chromosomal fibers.
5. Bivalents are arranged at the metaphase plate.
6. The part of bivalents lying on the equatorial plate is the central part of each bivalent, which consists of the telomere of the two homologous chromosomes attached almost end-to-end with each other.

First Anaphase:

1. Separation of the two homologous chromosomes of each bivalent marks the beginning of anaphase stage.
2. One chromosome from each bivalent begins to migrate to one pole, while other migrates to the opposite pole.
3. At the end of anaphase – I, one chromosome from each of the bivalents gather about one pole, while the second chromosome from each of the bivalents aggregate about the opposite pole. As a result, the number of chromosomes at each of the two poles of a cell is exactly half(h) of the somatic chromosome number (2n) and each pole receives one homologue from each of the bivalents present in a cell. Clearly, the reduction of chromosome number is not only a quantitative one but a qualitation as well. This stage comes to an end when the homologous chromosomes reach at the opposite pole.

First Telophase:

During meiotic telophase I (1) the chromosomes uncoil partially, while a complete uncoiling takes place during mitotic telephase, (2) Nuclear envelope becomes organized around the two groups of chromosomes (3) Nucleolus also reappears.

Cytokinesis:

In many species e.g. the cytoplasm of each cell divides into two halves by the end of first telophase so that in each of the two halves of a cell a single haploid nucleus is present. The two halves of each cell do not separate, but they stay together, this two celled structure is known as dyad. In some species, on the other hand (e.g. *Trillium*, cytokinesis occur only at end of second meiotic division.

Interphase:

In many species, the inter phase after first meiotic division is absent. In other species, where it occurs, it is very short duration. It is important to note that there is no DNA synthesis during this inter phase. In addition, chromosomes do not uncoil any further than they already have at the end of telophase I.

Second Meiotic Division: During second division of meiosis, the two haploid nuclei of each dyad divide synchronously. Ordinarily, the plane of second division is perpendicular to that of first one, but during megasporogenesis and as cospore development (in Ascomycetes) the two meiotic divisions occur in the same plane. During second division, the two sister chromatids of each chromosome separate and migrate to the opposite poles. As a result, the number of chromosomes in each of the two haploid nuclei remains the same (i.e. haploid) at the end of this division. Therefore, the second division of meiosis is often referred to as equational division.

The second meiotic division is also divided into 4 stages (1) Prophase II (2) Metaphase II, (3) Anaphase II and (4) Telophase II.

Second Prophase:

The stage is quite similar to that of mitosis; however, there are several significant differences between the two (1) During this stage there is no relational coiling between sister chromatids as a result of which (2) the two sister chromatids of each chromosome are easily visible. (3) The chromosomes are relatively much more condensed, hence they appear shorter and thicker, (4) There is further condensation of chromosomes so that they become more shorter and thicker. At the end of this stage, nucleolus and nuclear envelope disappear and spindle apparatus is organized. Following this, chromosomes migrate to the equatorial plate.

Second Metaphase:

This stage begins when the chromosomes become arranged on the equatorial plate. During this stage (1) nucleolus and (2) nuclear envelop are absent (3) spindle apparatus is present, and (4) Centromeres of all chromosomes are arranged in a single plane at the equatorial plate, (5) The two sister chromatids of each chromosomes are distinctly separated from each other, but they remain attached at the centromere, (6) Chromosomes become more condensed, thicker and shorter, (7) This stage is relatively quite short in a duration.

Second Anaphase:

Anaphase II begins and MII ends, when (1) Centromeres of chromosomes obserbably divide longitudinally and the two sister chromatids of each chromosome begin to separate and move away to the opposite poles.

It may be pointed out that the centromere of each chromosome is structurally divided into two during the first meiotic division itself. However, the centromeres of the two sister chromatids of each chromosome become functional and divide visibly only during A II.

Second Telophase:

Second telophase begins when sister chromatids of chromosomes reach the opposite poles. During this stage (1) the chromatids uncoil so that they assume the appearance of loose ball of thread, (2) Nucleolar envelope is recognized (3) nucleolus reappears.

Cytokinesis:

By the end of telophase II, cytoplasm of each of the 2-cells of a dyad divides into two parts. As a result, one parent cell produces 4 haploid daughter cells after completion of two meiotic divisions. The 4 daughter cells produced from a parent cell are present together, and are known as tetrad.

The 4 haploid cells produced by meiotic division of a single cell may differentiate into (animals) or spores (in plants). The spores generally give rise to gametophytic generation that may be independent (in case of lower plants) or totally dependent (higher plants) on the diploid seorophytic stage.

All living beings reproduce sexually, asexually and vegetatively. Reproduction usually results in the formation of offspring identical to his/her parents. Generally the resulting offspring most often do not resemble the parent fully. These variations may be due to the meiotic mechanism of transmission of a character which shows similarities as well as differences of characters from the parental generation. This phenomenon is popularly known as heredity. The subject genetic (from the Greek word geno-give birth) deals with the study of inheritance and science of variation occurred spontaneously. The term genetics was coined by W. Bateson (1905)

Genetics is barely one hundred years old. The first marked pioneer and experimental work in this field was started by Gregor Johan Mendal (1822-84). Therefore, the name “Father of genetics” was given to him.

Mendelian Inheritance:

The science of heredity starts with the experiments which the Austrian Monk, Greger Johan Mendel, made on pea plants. Mendal published his results in 1866 in the Journal. The Annual Proceedings of The Natural History Society of Brunn, in the paper entitled "Experiments on Plant Hybridization" but he received very little attention and his work was overlooked for a number of years. In 1900, the principles of heredity, which Mendel had discovered were independently, rediscovered by three biologists, Hugo de Vries in Holland, Carl Correns in Germany and Eric Von Taschamark in Austria. From this time on the science of heredity has made rapid progress. Mendel carried out a statistical study and discovered that individual traits are inherited as discrete factors which retain their physical identity is the offpring. Later Danish Botanist Wilnelm Johannsen (1909) defined as a gene.

Mendel's experiment

Mendel carried on a series of experiments in his garden with the common garden pea (*Pisum sativum*) and established some definite principles. These principles have since been tested and found correct in numerous cases and have thus become established as laws of Mendel.

He selected garden peas for his experiments because

1. The pea plants are self pollinated.
2. Life cycle is short.
3. The plants established constant and clear distinct features.
4. The hybrids were usually fertile.
5. Large number of offspring are produced that help in detecting the correct conclusions.

Mendel mainly considered 7 pairs of contrasting characters:

(i) Smooth and wrinkled sead.
(ii) Yellow and green seed coat.
(iii) Tall and dwarf plants.
(iv) Coloured and white flowers.
(v) Axial and terminal flower.
(vi) Inflated and constricted pods.

Mendel success was mainly due to the fact that:

(i) He always worked with pure homogenous materials.

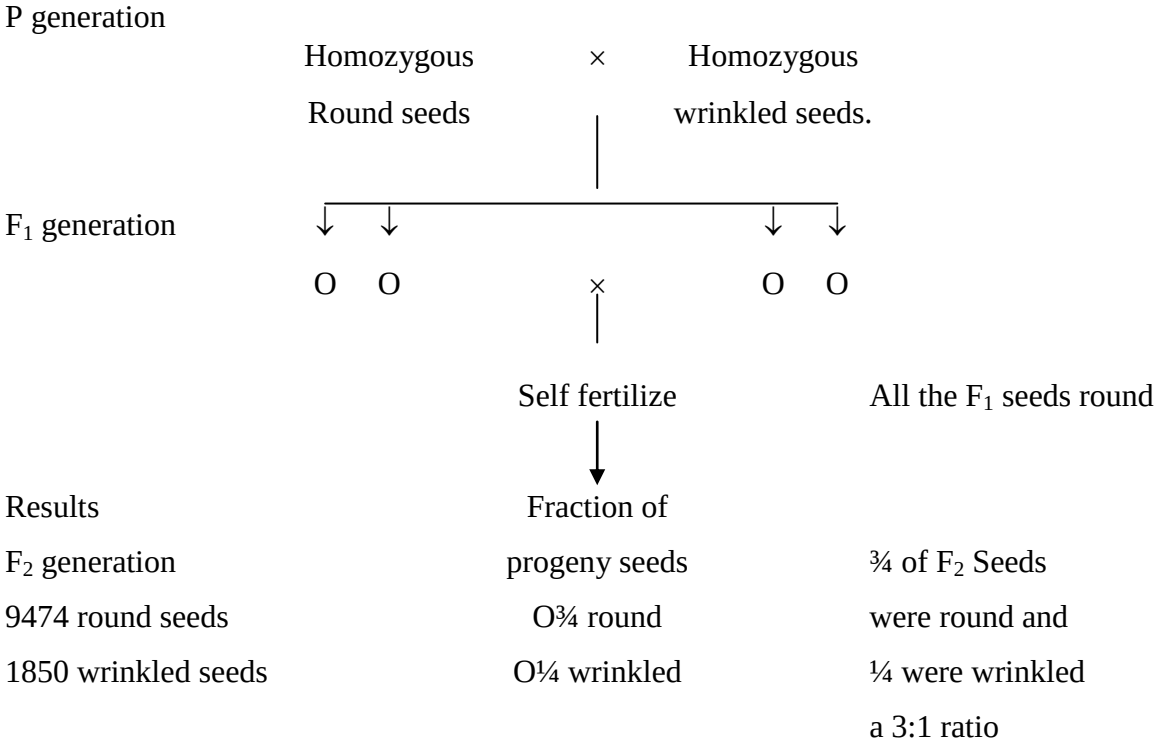
(ii) He considered with character independently.

(iii) The records of the offspring of each individual were kept separately.

(iv) The records were kept upto at least the third ficial generations.

Monohybrid crosses reveal the principle of segregation and the concept of Dominance:

Mendel began studying the results of monohybrid crosses – those between parents that differed in a single characteristic. In one experiment, Mendel crossed a pure breeding (homozygous) pea plant for round seed with that was pure breeding wrinkled seeds. This first generation of a cross is called the P (parental) generation.



Conclusion: The traits of the parent plants do not blend. Although F_1 plant displays the phenotype of one parent, both traits are passed to F_2 progeny in a 3:1 ratio.

The offspring from parents in the P generation are the F_1 (filial 1) generation. When Mendel examined the F_1 generation of this cross, he found that they expressed only one of the phenotypes present in the parental generation: all the F_1 seeds were round. Mendel carried out 60 such crosses and always obtained same result. He also conducted reciprocal crosses, in one cross, pollen was taken from a plant with round seeds and, in its reciprocal cross, and

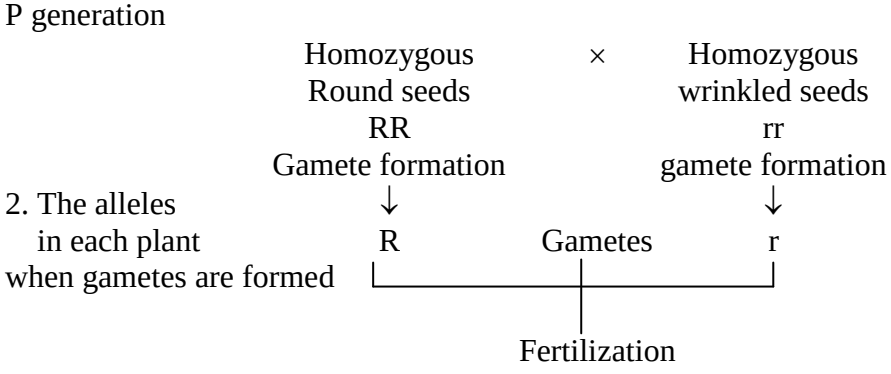
pollen was taken from a plant with wrinkled seeds. Reciprocal crosses gave the same result: all the F_1 seeds were round.

In the following spring, he planted the F_1 seeds, cultivated the plant that germinated from them and allowed the plants to self fertilize, producing a second generation the F_2 (filial^2) generation. Mendel counted 5474 round seeds and 1850 wrinkled seeds in F_2 generation. He noticed that the number of the round and wrinkled seeds constituted approximately a 3 to 1 ration, i.e., about ¾ of the F_2 seeds were round and ¼ were wrinkled. Mendel conducted monohybrid crosses for all 7 characteristics.

What Monohybrid cross reveal:

1. He concluded that each plant must therefore possess two genetic factors encoding a character. The genetic factors now called alleles is usually represented by R and the allele for wrinkled seed by r. The plants in the P generation of Mendel's cross possessed two identical alleles RR in the round-seeded parent and rr in the wrinkled seeded parent.
2. The second conclusion that Mendel drew from his experiment was that the 2 alleles in each plant separate when gametes are formed; a 1 allele goes to each gamete. When 2 gametes one from each parent fuses to produce a Zygote, the allele from the male parent unites with the allele from the female parent to produce the genotype of the offspring. Thus Mendel's F_1 plants inherited on R allele from the round seeded plant and an allele from the Wrinkled seeded plant. However, only the trait encoded by round alle (R) will be observed in F_1 progeny.

1. Mendel crossed a plant homozygous for round seeds (RR) with a plant homozygous for wrinkled seeds (rr)



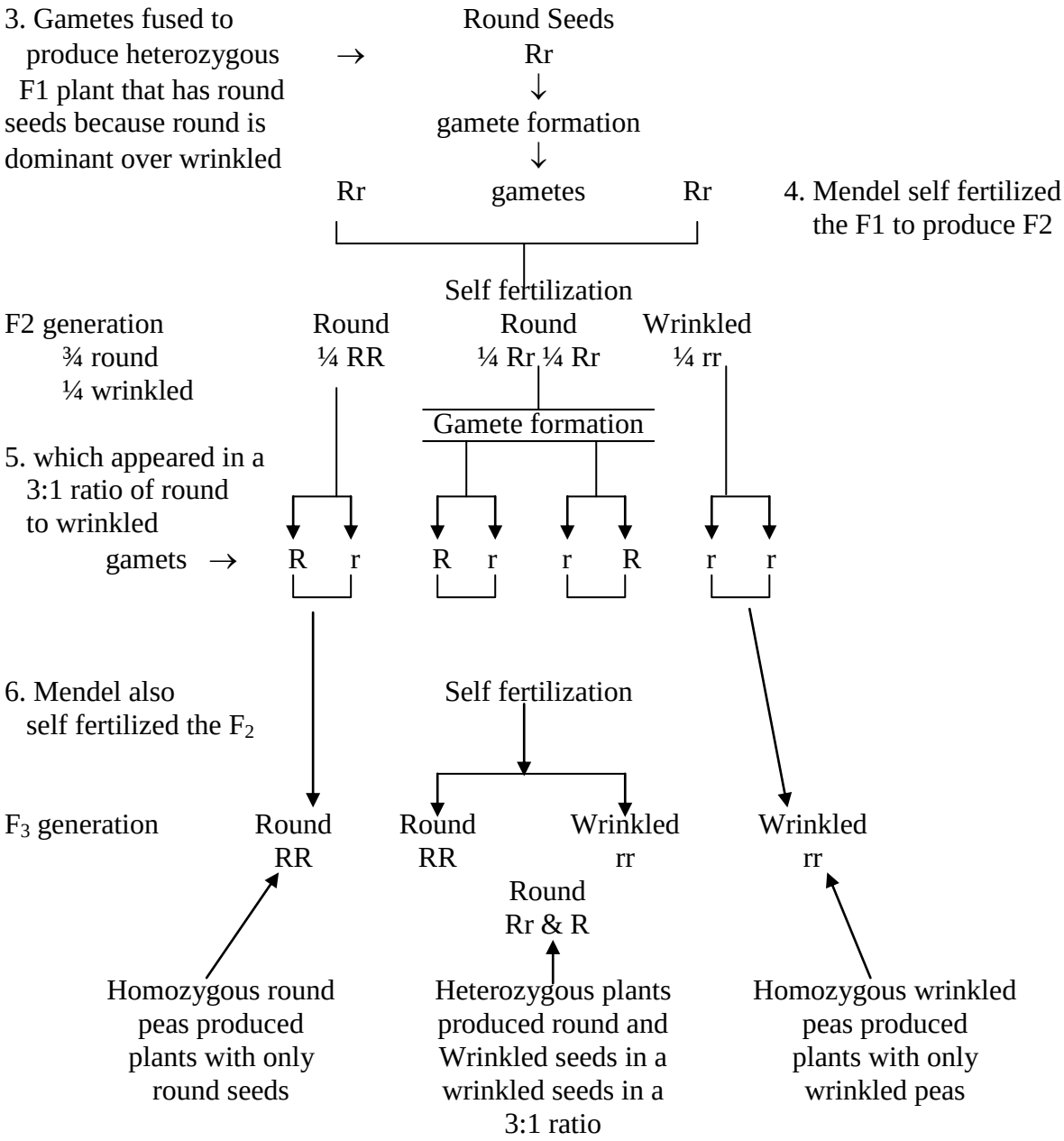


Fig 2. Mendel monohybrid crosses revealed the principle of segregation and concept of dominase

Those traits that appeared unchanged in the F_1 heterozygous offspring Mendel called dominant and those traits that disappeared in the F_1 heterozygous offspring called recessive. When dominant and recessive alleles are present together, the recessive albla is suppressed. The concept of dominance was the second important conclusion that Mendel derived from monohybrid cross.

The third conclusion of Mendel was that the gametes paired randomly to produce the following genotypes in equal proportions among the F_2 RR : Rr, rR, rr. Mendel observed in

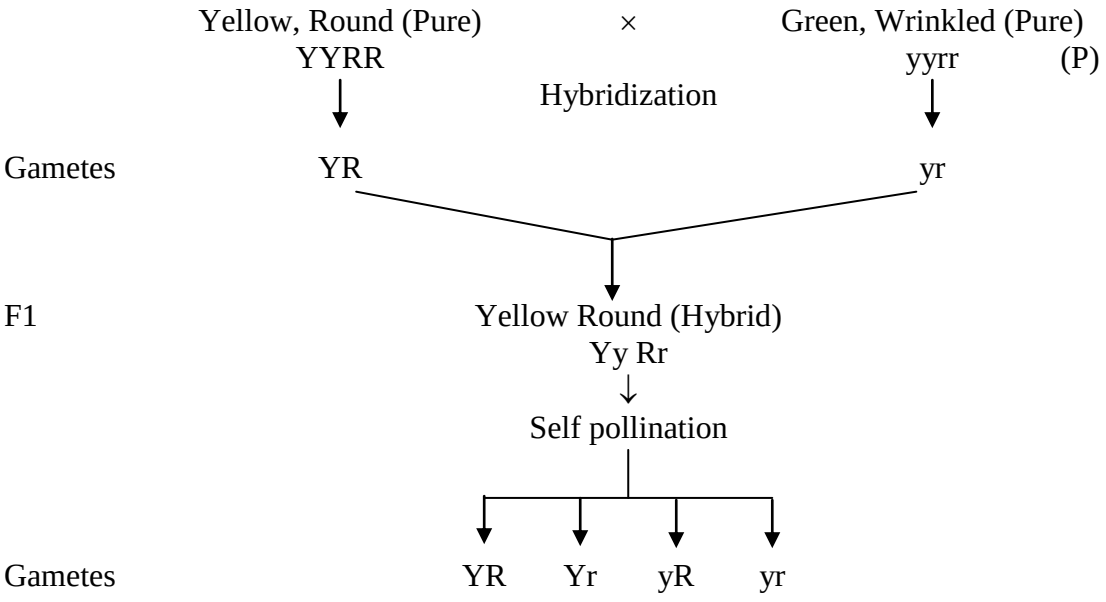
the F_2 could be obtained only if alleles of a genotype separated into gametes with equal probability.

Finally Mendel concluded from his monohybrid cross the principle of segregation which states that each individual diploid organism possesses two alleles for any particular characteristic. These alleles segregate when gametes are formed; one allele goes into each gamete. The concept of dominance states that, when the two alleles of a genotype are different, only the trait encoded by one of them – the dominant allele – is observed.

Dihybrid crosses reveal the Principle of Independent Assortment.

In addition to his work on monohybrid crosses, Mendel crossed varieties of peas that differed in 2 characteristics – a dihybrid cross.

Mendel raised separately two pure varieties of garden peas, one with yellow cotyledon, round seed and other with green cotyledon, wrinkled seed. From the cross between above mentioned two parental (P) generation plants, the offsprings in the F1 generation were all with yellow cotyledon and round seed. After self fertilization of F1 plants, the offsprings of F2 generation were of four types in the ratio 9:3:3:1 – (a) yellow cotyledon, round seed (b) yellow cotyledon round seed, (c) green cotyledon round seed and (d) green cotyledon, wrinkled seed.



O^- / O^+	YR	Yr	yR	yr
YR	YYRR	YYRr	YyRR	YyRr
Yr	YYRr	YYrr	YyRr	Yyrr
yR	YyRR	YyRr	yyRR	yyRr
Yr	YyRr	Yyrr	yyRr	yyrr

F_2

The offsprings showed that two pairs of contrasting character assort independently.

Chromosomal basis of Inheritance.

In 1902, Walter S. Sutton and T. Boveri proposed the chromosomal basis of Inheritance-theory. According to this theory the following points will be taken into consideration:

1. Chromosomes contain the genetic material which is transmitted from parent to offspring.
2. Chromosomes are replicated and transmitted in the following generation from parent to the offspring.
3. Most of eukaryotic cell contain nuclei which bears homologous pairs of chromosomes. One member of each pair is inherited from the father the other from the mother. During meiosis, one of the two members of each pair segregates into one of the daughter nucleus and the other segregates from different daughter nucleus. Ultimately the gametes contain one set of chromosomes.
4. During gamete formation, chromosomes segregate independently from each other.
5. Therefore, each parent is responsible to contribute one set of chromosomes to their offspring.
6. From the above theory we may conclude the relationship between Mendel's Law and chromosomal transmission.

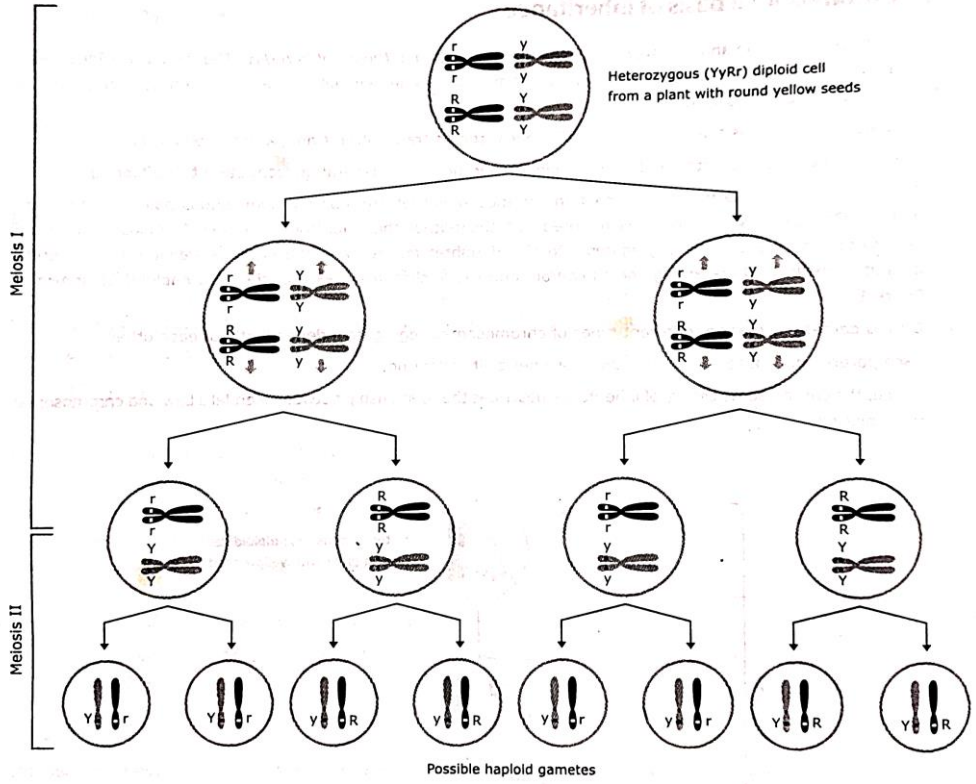


Fig: Segregation of homologous chromosome during meiosis explains Mendel's law of segregation

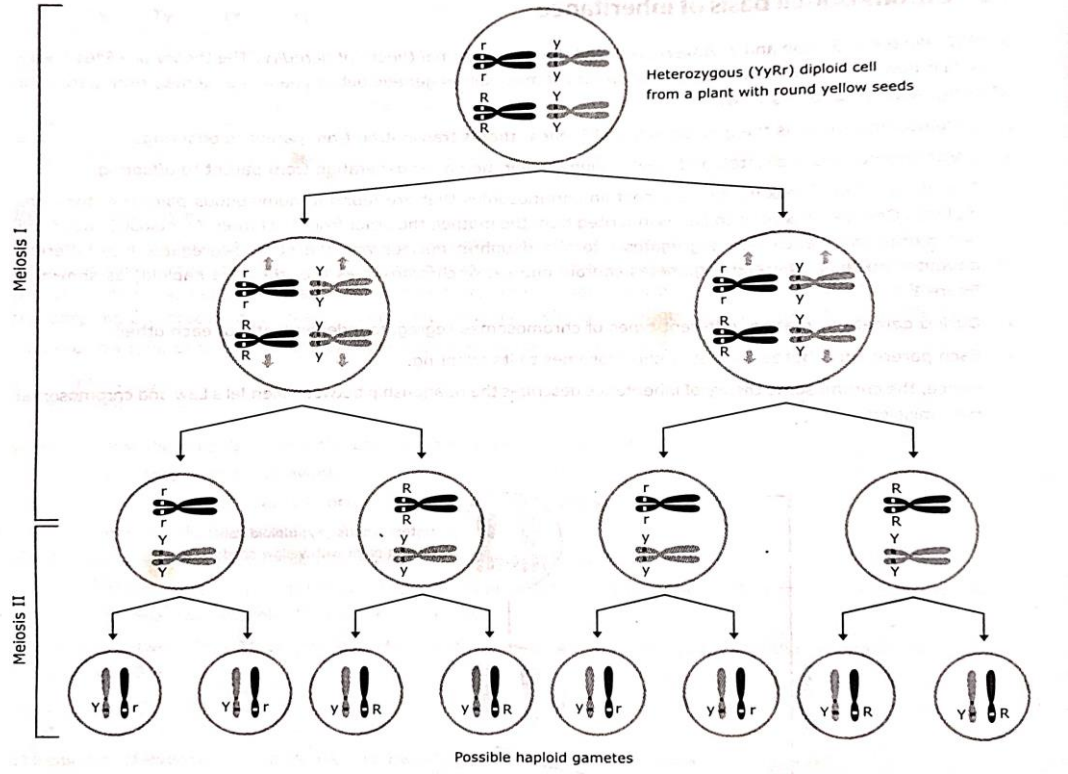


Fig: Random alignment of bivalents during prophase of meiosis I explains Mendel's law of independent assortment

Gene Interaction:

Mendelian genetics unable to explain all kinds of inheritance for which the phenotypic rations in some cases are completely different from Mendelian monohybrid ratio (3:1) or from dihybrid ratios (9:3:3:1). The phenomenon of two or more genes affecting the expression of each other in various ways in the development of single character of an organism is known as gene interaction.

Type:

There are two types of gene interactions: (1) Allelic or intra-allelic interactions i.e. these kinds of genetic interaction occurs between the alleles of a single gene and (2) Non allelic or inter allelic interaction i.e. these kinds of genetic interaction occurs where the development of single character occur due to 2 or more genes affecting the expression of each other in various ways.

The following table demonstrate different type of allelic and non-allelic gene interaction with example.

Incomplete Dominance:

The situation in which phenotype of a heterozygote is inter mediate between the two homozygote on some quantitative scale of measurement is called incomplete dominance.

In snapdragons, heterozygous have pink flower in contrast to red and white for the dominant and recessive homozygous respectively.

		Red		White
		RR		rr
Gametes	→	R		r
F1	→	Rr (Pink)		
F2	→	Pink	×	Pink
		Rr		Rr
Gametes	→	R, r		R,r

O^+ \ O^-	R	r
R	RR	Rr
r	Rr	rr

Result:

Genotype	Genotypic ratio	Phenotype	Phenotypic ratio
RR	1	Red	1
Rr	2	Pink	2
Rr		Pink	
Rr	1	White	1

Conclusion:

1. In the absence of complete dominance, every genotype has a distinguishable phenotype.
2. In the present case in the heterozygous condition, the tota product is intermediate between that of the dominant and recessive alleles.
3. The phenotypic ratio for the monohybrid cross between two heterozygote results 1:2:1 ratio instead of 3:1 for dominant alles.

   Therefore, a F_1 dihybrid showing incomplete alominance for both the characters will segregate in F_2 ratio (1:2:1) × (1:2:1)=1:2:1:2:4:2:1:2:1

Co-dominance: In this case both the alleles of a gene express themselves in the heterozygotes. Phenotypes of both the parents appear in F_1 hybrid rather than intermediate phenotype. Best example of codominance is documented in case of Human where MN blood group is controlled by a single gene. In this case only 2 alleles exist, M and N. mother with M blood group (genotype MM) and Father with N blood group (genotype NN) will have children with MN blood group. (genotype MN). Both phenotypes are identifiable in the hybrid are identifiable in the ratio 1M blood group: 2MN blood group: 1 N blood group.

Multiple Alleles

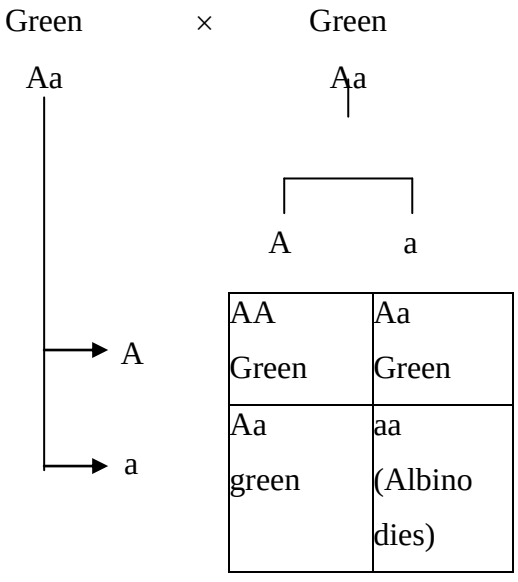
A gene for a particular character some bear more than two alleles occupying a same locus of a chromosome. These allelomorphs form multiple alleles.

Human ABO blood group system furnishes best example. The gene for antigen may occur into three possible allelic forms – I^A, I^B and i. The allele I^A for the antigen A is codominant with the allele I^B for the antigen B. Both are completely dominant to the allele I which fails to specify any detectable antigenic structure. Therefore, the possible genotypes of the 4 blood groups are shown in the following Table.

Blood groups	Genotypes
A	$I^A I^A$, IAi
B	$I^B I^B$, $I^B{}_i$
AB	$I^A I^B$
O	Ii

Lethal Factor (2:1)

The genes which are responsible for the death of a individual is called lethal factor. Following figure shows inheritance of lethal gene in barley.



- Recessive lethals are expressed only when they are in homozygous state and the heterozygotes remain unaffected.
- Dominant lethals are lost from the population because of death

- Balanced lethals are all heterozygous for the lethal genes both dominant and recessive heterozygotes will die.

Non–Allelic Interaction:

Complementary Gene action (9:7)

In this type of interaction certain character are produced due to interaction between 2 or more genes located on different loci inherited from different parents. For example in sweet pea (*Lathyrus odratus*) both the genes C and P are required to synthesize anthocyanin pigment causing purple colour. Due to absence of any one cannot produce anthecyanin with white colour. Therefore C and P are complementary to each other for anthocyanin formation.

Parents White × White

CCpp ccPP

↓ ↓

Gametes Cp cP

F_1 CcPp

Purple

↓

Selfing

↓

F_2

Genotype	Phenotype	Ratio
C – P	Purple	9
C – pp	White	3
Cc – PP	White	3
CCpp	White	1

7

Inheritance of Flower colour in *Lathyrus odoratus*.

Let us consider the example of summer squash where the rfuit colour is governed by 2 genes. The dominant gene W for white colour suppresses the expression of the gene Y which

controls yellow colour. So yellow colour appears only in absence of W. therefore W is epistatic Y. In absence of both W and Y, green colour develops.

Parents White Green

WWYY × WWyy

Gametes WY wy

F1 Ww Yy

White

↓

Selfing

F_2

Genotype	Phenotype	Ratio
W – y –	White	9
W – yy	White	3
wwY –	Yellow	3
WWyy	Green	3

12

Epistasis

Epistasis may be defined when one gene masks or inhibits the expression of another gene at distinct locus. Any gene when masks the expression of another non-allelic gene is called epistatic to that gene. The gene which is suppressed is called hypostatic.

The epistasis is of three types.

(a) Dominant epistasis (12:3:1)

(b) Recessive epistasis (9:3:4)

and (c) Duplicate recessive epistaris (9:7)

(a) Dominant epistasis (12:3:1)

When a dominant gene does not allow the expression of other non-allelic gene is called dominant epistasis.

(b) Recessive Epistasis or Supplementary Factor (9:3:4)

In present type of gene interaction homozygous recessive condition of a gene responsible for phenotypic expression irrespective of the allele of other gene pir.

Let us consider the grain colour in maize which is governed by two gens R (red) and Pr (purple). The recessive allele rr is epistatic to gene Pr.

Parents Red × White

RR pr pr rr Pr Pr

↓ ↓

Gametes Rpr r Pr

F1 Rr Pr pr

↓

Purple

↓

Selfing

↓

F_2

Genotype	Phenotype	Ratio
R – Pr.	Purple	9
R – pr pr	Red	3
Rr Pr	White	3
Rr pr pr	White	1

4

Inheritance of grain colour in Maize

(c) Duplicate recessive interaction (9:7)

In some cases both gene loci have homozygous recessive alleales and both of them are responsible for to produce identical phenotypes. The resulting F_2 ratio will be 9:7 instead of 9:3:3:1. In such case, the genotype aa BB, aa Bb, AA bb and aabb produce one phenotype.

Duplicate Gene interaction (15:1)

In some cases a character is controlled by 2 non-allelic genes whose dominant alleles produce the same phenotype where they are alone or together. For example, in *Capsella bursa-pastoris*, the presence of either gene A or gene B or both results in triangular capsule. The oval capsules are formed when both genes are in recessive form.

Parents Triangular Capsule × Oval capsule

AABB aabb

↓ ↓

Gametes AB ab

F1

Aa Bb

Triangular Capsule

↓

Selfing

F_2

Genotype	Phenotype	Ratio	
A – B -	Triangular	9	15
A – bb	Triangular	3	
Aa B	Triangular	3	
Ac b5	oval	1	

Inheritance of Capsule shape in *Capsella – bursa pastosis*

Inhibitory Factor (13:3)

Normally in case of this type of interaction gene itself has no phenotypic effect but inhibit the expression of another non allelic genes. For example in rice, purple leaf colour is due to gene P and p causing green colour. Another non-allelic dominant gene I inhibit the expression of P but is ineffective in recessive form (ii).

Parents Purple Green

pPiI pp II

Gametes Pi pI

F1

Pp Ii

Green

↓

Selfing

F_2

Genotype	Phenotype	Ratio	
P – I -	Green	9	13
ppI	Green	3	
Ppii	Green	1	
P – ii	Purple	3	

Inheritance of leaf colour in rice

Polymorphic gene (9:6:1)

In this type of gene interaction 2 non-allelic genes controlling a particular character to produce identical phenotypes when they are alone. Interestingly when both the genes are present together the phenotypic effect is amplified due to cumulative affect. This has been justified with inheritance of awn in barly.

Parents long awn × Awnless

AABB aabb

↓ ↓

Gametes AB ab

F1 Aa Bb

long Awn

↓

Selfing

F_2

Genotype	Phenotype	Ratio	
A – B -	Long awn	9	6
A – bb	Medium awn	3	
Aa – B -	Medium awn	3	
Aa bb	Awnlers	1	

Inheritance of awns in barley.

4. Non-Mendelian Inheritance: Organelle heredity; Infectious heredity; Maternal effects.

Non-Mendelian inheritance is any pattern of inheritance in which traits do not segregate in accordance with Mendel's laws. These laws describe the inheritance of traits linked to single genes on chromosomes in the nucleus.

Cytoplasmic Inheritance:

The inheritance of most of the characters of an individual is governed by nuclear genes. But in some cases, the inheritance is governed by cytoplasmic factors or genes. When the transmission of characters from parents to offspring is governed by cytoplasmic genes; it is known as cytoplasmic inheritance or **extra nuclear inheritance** or **extra chromosomal inheritance** or **non-mendelian inheritanc**e or **organellar inheritance**.

The first case of cytoplasmic inheritance was reported by Conens in 1909 in four 'o' clock (*Mirabilis jalapa*) for leaf colour. Later on, cytoplasmic inheritance was reported by various workers in various organisms.

TABLE 11.1. Differences between Mendelian inheritance and cytoplasmic inheritance

S.No. Particulars	*Mendelian Inheritance*	*Cytoplasmic Inheritance*
1. Governed by	Nuclear genes	Plasma genes
2. Segregation pattern	Distinct	Not distinct
3. Reciprocal differences	Not observed	Observed
4. Maternal effects	Not observed	Observed
5. Genes mapping	Easy	Difficult
6. Location of genes	Chromosomes	Chloroplasts or mitochondria

Plastid Inheritance:

Chloroplasts are the important plastids. Plastids have green pigments called chloroplasts. Plastids self-duplicate, have some amount of DNA and play an important role in cytoplasmic inheritance.

Some examples of plastid inheritance are given below:

(i) *Mirabilis jalapa:*

The first conclusive evidence of cytoplasmic inheritance was reported by Correns in 1909 for leaf colour in four 'o' clock plant (*Mirabilis jalapa*). This plant has three types of leaves, viz., green, white and variegated. Three types of results were obtained from crosses between these genotypes as given below –

1. When green was used as female and either green, white or variegated as male, all individuals in F1 were green.
2. When white was used as female and either green, white or variegated as male, all individuals in F1 were white.
3. When variegated was used as female and either green, white or variegated as male, various proportions of green, white and variegated individuals were obtained in F1 (Table 11.3).

TABLE 11.3. Inheritance of leaf colour in Mirabilis jalapa

Crosses between three leaf colours			*Expression of leaf colour*
Female		***Male***	***in F_1***
Green	×	Green	Green
	×	White	Green
	×	Variegated	Green
White	×	Green	White
	×	White	White
	×	Variegated	White
Variegated	×	Green	Green, white and variegated in various ratios in each cross
	×	White	
	×	Variegated	

The inheritance is governed by chloroplasts which are originated from proplastids. If the proplastids are normal, they will develop into normal chloroplasts and when proplastids are mutants, they will produce white chloroplasts. This suggests that green leaf branches have normal chloroplasts; white branches have mutant chloroplasts and variegated have a mixture of both normal and mutant chloroplasts.

Since cytoplasm is contributed to the zygote mainly by female parent, the plastids are transmitted to the zygote from the female parent. These plastids are responsible for variation in the crosses of green, white and variegated leaves.

(ii) Plastid inheritance in *Oenothera*:

Plastid inheritance in evening primrose (*Oenothera*) was reported by Renner. In some species of *Oenothera*, entire chromosome set of either pistillate or pollen parent of an individual is transmitted to the gametes. Thus gametes have either all chromosomes of female parent or of male parent. Such inheritance of chromosomes in block is due to complex series of reciprocal translocations.

Such inheritance is exhibited by the cross between two species, viz., *Oenothera muricata* and *O. hookeri*. Cross between *O. muricata* female and *O. hookeri* male produced normal green plants in F1.

However, the reciprocal cross (*O. hookeri* female x *O. muricata* male) resulted in yellow plants in F1 which were unable to survive. This suggests that muricata plastids can develop normally in the presence of hookeri nucleus, but hookeri plastids cannot develop in the presence of muricata nucleus.

Sometimes, hybrid between *O. muricata* and *O. hookeri* exhibits variegation in course of time like reciprocal cross. These yellow patches in later stage were explained due to the presence of hookeri plastids. Renner assumed that some plastids of hookeri pollen are transmitted to the hybrid. After multiplication they resulted in somatic segregation of plastids in the later stages of hybrid producing yellow sectors.

(iii) Iojap in Maize:

In maize, three types of leaves are found, viz., green, iojap (green and white stripes) and white. Crosses between green female and iojap male produced all green individuals in F, and a single gene segregation ratio, i.e., 3 green and 1 iojap in F2. However, the reciprocal cross (iojap female x green male) produced individuals with all the three phenotypes, viz., green, white and striped in F1 (Fig. 11.5).

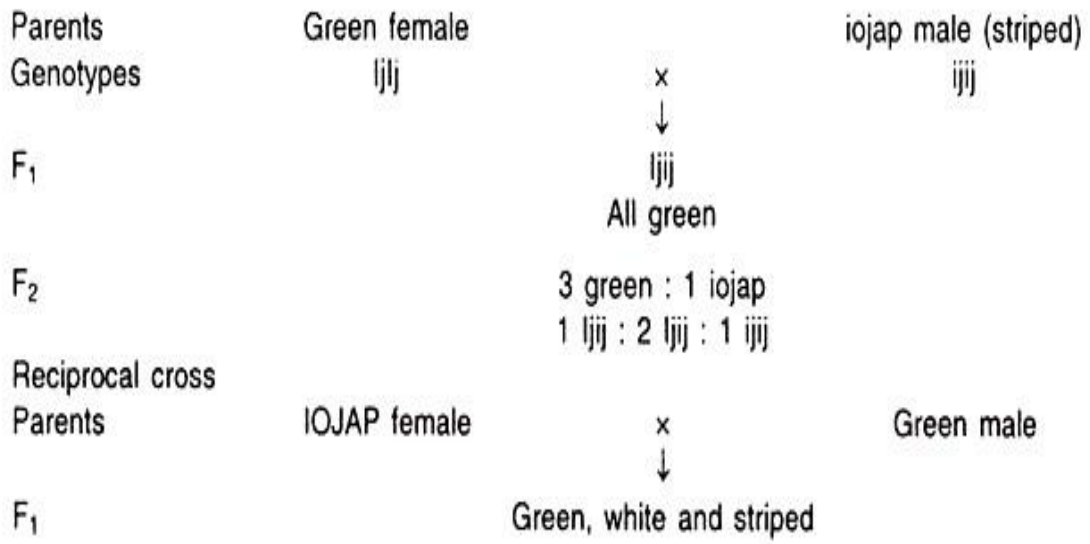


Fig. 11.5. Inheritance of IOJAP leaf colour in maize.

The iojap phenotype is governed by plastids. The green leaves have normal plastids; white leaves have mutant plastids and striped leaves have mixture of normal and mutant plastids. In a cross between iojap female and green male, there are three types of plastids, viz., green, white or both in the egg cell.

Depending upon the presence of these three types of plastids in the egg cell, a cross between iojap and green will produce three types of individuals, viz., green, white and striped in F1 because male parent does not contribute cytoplasm and thereby plastids to the zygote.

Significance of Cytoplasmic Inheritance in Plant Breeding:

1. Cytoplasmic inheritance has been useful in explaining the role of various cytoplasmic organelles in the transmission of characters in different organisms.

2. Studies of cytoplasmic inheritance have played key role in mapping of chloroplast and mitochondrial genome in several species, viz., yeasts, *Chlamydomonas*, maize, human, etc.

3. Development of cytoplasmic male sterility. CMS lines have been developed in several crops like maize, pearl millet, Sorghum, cotton, etc. Availability of CMS lines has facilitated

the production of hybrid seed in these crops at a cheaper cost than with hand emasculation and pollination method.

The CMS cytoplasm can be easily transferred to various agronomic bases for their use in the development of superior hybrids. Since CMS based hybrids have danger of uniformity, it is desirable to utilize various CMS sources.

4. Role of mitochondria in the manifestation of heterosis is gaining increasing importance these days.

5. Mutation of chloroplast DNA and mitochondrial DNA leads to generation of new variants. Some of such variants are of special significance especially in ornamental plants.

Mitochondrial Inheritance:

Mitochondria are the cytoplasmic organelles which contain various respiratory enzymes and are main source of energy for the cell. They are found in cell in variable numbers. The complexity of structure of the mitochondria and their similarity in some ways to plastids suggest the possibility that they may be inherited in the same way as the plastids.

The mitochondria have a double membrane and contain besides respiratory enzymes of electron transport chain (the cytochromes), their own genetic determinants (DNA). There is certain amount of electron micrographic evidence for the continuity of this cytoplasmic organelle through cell division.

Although most of the mitochondrial proteins and enzymes are produced by nuclear genes, yet nearly 20% of them result due to activity of mitochondrial genes.

The fact that mitochondrion contains its own DNA has led some to speculate that it evolved from symbiotic micro-organism that gradually lost the ability to exist independently. There is enough evidence in support of this. But, some people disagree with this. Mitochondria cannot be regarded truly autonomous cytoplasmic organelles as they require both their own genes and nuclear genes in order to exist.

The mitochondrial heredity has been exemplified by yeast (*Saccharomyces cereviceae*) and *Neurospora crassa*.

Ephrussi's Experiment with Yeast:

Certain strains of yeast (*S. cereviceae*) produce tiny colonies when grown on agar medium. Ephrussi (1953) observed that one or two out of every one thousand colonies were only about one-third or one- half of the diameter of the remainder. The small colonies are termed as petite colonies.

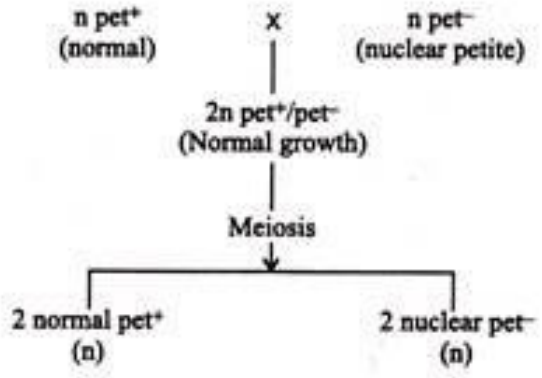
Cells from the normal large colonies, when spread on culture medium, further produced a small proportion of petite colonies and this happened so time after time. The cells from the small colonies were true breeding and they produced only petites.

Biochemical studies have established that the slow growth of petite colonies was due to the loss of aerobic respiratory enzymes particularly cytochrome a and b and enzyme cytochrome oxidase occurring in mitochondria of the cells and the utilization of the less efficient fermentation process by the cells.

The petite phenotype can result either from mutation of nuclear genes or from mitochondrial genes. Petite mutants resulted due to mutation in a nuclear gene follow Mendelian pattern of inheritance with segregation occurring in heterozygotes.

This type of petite mutation is called segregational petite or nuclear petite. When the individuals of petite colony are crossed to the individuals from normal large sized colony, normal zygotes are formed which produce normal cells vegetatively.

When meiosis takes place in diploid cells, haploid cells are recovered that will form petite and normal colonies in 1: 1 ratio as shown below:



Such any colonies are formed evidently due to mutant nuclear genes, they are called segregational petites.

Bores Ephrussi and his associates (1953) also found that in presence of small amount of acridine dyes such as acriflavine and euflavine many cytochrome deficient petite colonies developed which showed extra-chromosomal (Non Mendelian) inheritance for petite characters. They were called vegetative petites.

The rate of mutation was much higher at low concentrations than that normally expected for chromosomal mutation. The vegetative petites may arise directly from mutations in mitochondrial genes leading to defective mitochondria.

There are two classes of vegetative or extra-chromosomal petites:
(i) Neutral petites; and
(ii) Suppressive petites, which show different patterns of inheritance.

Neutral Extra-chromosomal or Vegetative Petites:

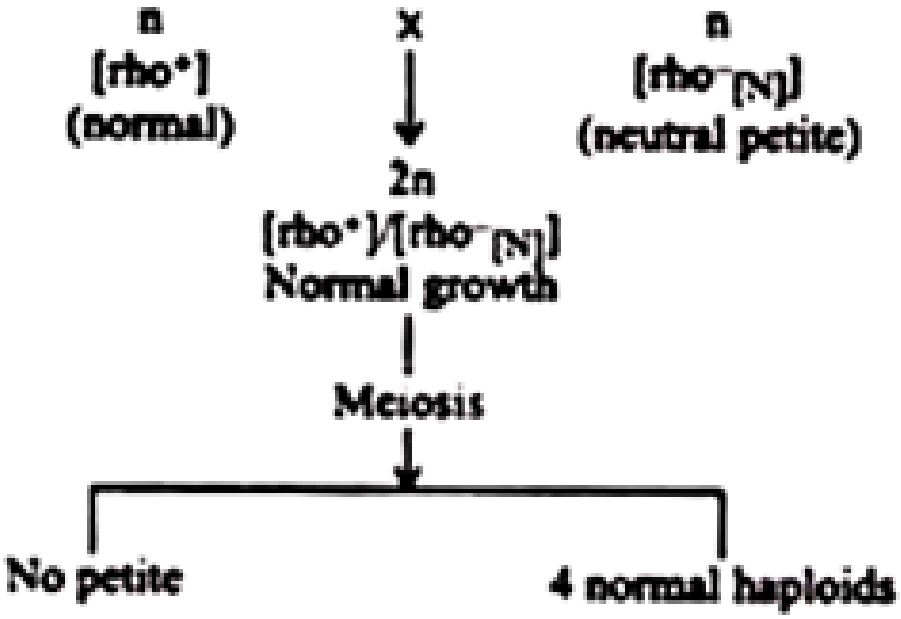
When a cross is made between wild type haploid yeast and neutral petite haploid yeast, normal diploid offsprings are obtained. The diploid individuals by budding process produce several normal diploids. When meiosis occurs in normal diploids, haploid ascospores are formed which produce normal haploid colonies.

If the determinants of this trait were chromosomal, one would expect normal and petite traits in 1:1 ratio in the population of haploid spore cells. This suggests that the inheritance is non-chromosomal.

The genetic basis of this type of inheritance can be explained assuming the presence of an extra-chromosomal or cytoplasmic factor [rho^{+}] in normal strain and missing or [$rho^{-}_{[N]}$] in neutral petite mutants. The neutral petites [$rho^{-}_{[N]}$] usually lack in mitochondrial DNA. Now, if the haploid neutral petite is crossed to haploid normal strain, the diploid would be normal. The normal condition in diploid cells appears because of normal mitochondria with [rho^{+}] factor contributed by normal haploid strain. These normal mitochondria replicate and are passed on to haploid spores after meiosis in diploid cells. The mitochondria contributed by

neutral petite mutant possibly do not replicate and gradually degenerate. So all the haploid spores and their descendants would be normal.

The pattern of inheritance is as follows:

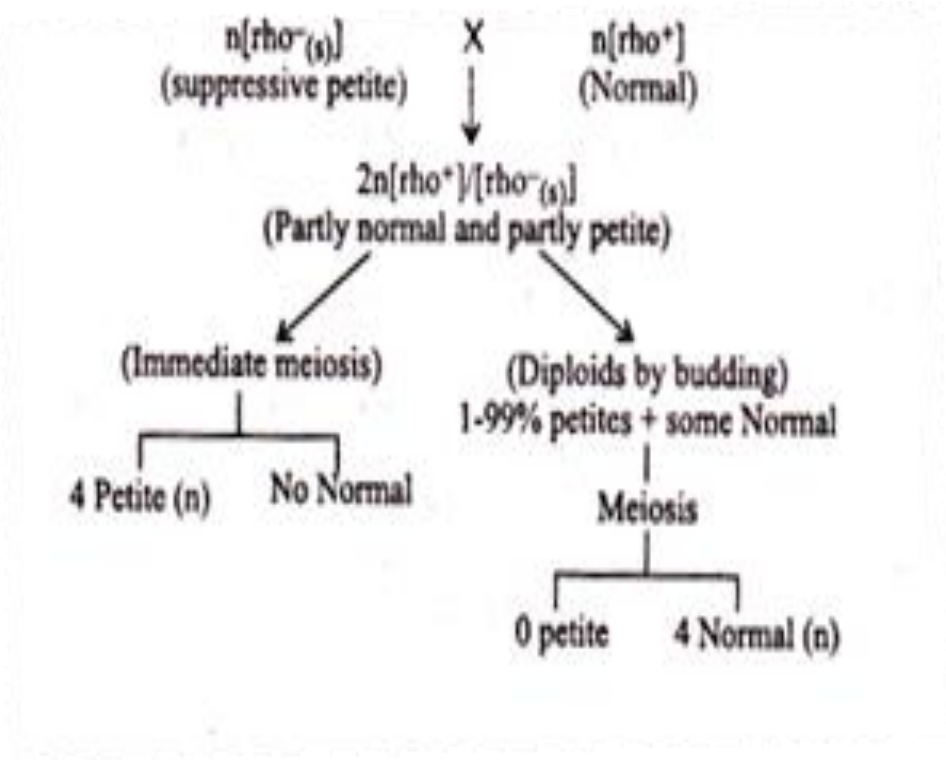


<u>Inheritance Pattern of Suppressive Petites:</u>

The suppressive petite mutant shows different behaviour than the neutral petite. When a cross is made between haploid cells of suppressive petite and haploid cells of normal strain, diploid cells are obtained which are in part normal and in part petite and as their name indicates, they can suppress normal aerobic respiration in presence of normal cytoplasm.

The normal diploids after meiosis produce normal haploid spores while the diploid petites after budding produce diploids which may be all petites or some normal and some petites. Normal diploids after sporulation produce only normal and no petite.

It is thus obvious that suppressive petites follow Non-Mendelian pattern of inheritance. The genetic basis of this type of inheritance can be explained by assuming the presence of an extra-chromosomal factor [rho^{+}] in normal strain and [$rho^{-}_{[s]}$] in suppressive petites.



The genetic cause for suppressive petite is mitochondrial mutation. Unlike neutral petites, the mitochondria of suppressive petites contain mutant DNA. The mutant mitochondria can replicate and can be passed on to the progeny cells which can, in turn, express mutant phenotype. In the cross in question it is the relative proportion of normal and mutant mitochondria that determines the phenotype of the particular cell.

The diploid cells and haploid spores would be normal if normal mitochondria predominated and they would show mutant phenotype if mutant mitochondria predominated. The lack of normal segregation and also the high mutability of normal colony cells provide good evidence that vegetative petite phenotype is due to extra-chromosomal or cytoplasmic genes.

The Poky Strain in *Neurospora*:

There are several examples for mitochondrial enzyme deficiency which are cases of extra-chromosomal inheritance. One of the classical examples of extra-chromosomal inheritance of plasma genes came from studies of *Neurospora*.

In this fungus, there is a slow growing mutant strain called poky. The mitochondria contain cytochromes a, b and c which are electron transport proteins necessary for oxidative phosporylation.

In poky strains, either cytochrome a or cytochrome b is absent but cytochrome c is present in excess. Poky differs from petite in that the two mutants are not deficient for the same enzymes. When poky as female parent was crossed with a normal strain as a male parent, the progeny were found to be poky.

In reciprocal cross (normal poky ♀× poky ♂), the progeny were normal. This Non-Mendelian uniparental inheritance suggested that the cytoplasm of female parent was important because the only difference between reciprocal crosses was in contribution of cytoplasm.

The male gametes in neurospora contribute negligible amount of cytoplasm just as in animals or higher plants. So, it is probable that the factor for pokyness resided somewhere in the cytoplasm. The segregation of poky from normal is never observed and the progeny of poky ♀× normal ♂ of will always be poky. Thus nuclear genotype has no effect on this particular phenotype.

Infectious heredity:

Infectious heredity is a form of non-Mendelian inheritance. Infectious particles such as viruses may infect host cells and continue to reside in the cytoplasm of these cells. If the presence of these particles results in an altered phenotype, then this phenotype may be subsequently transmitted to progeny. Because this phenotype is dependent only on the presence of the invader in the host cell's cytoplasm, inheritance will be determined only by the infected status of the maternal parent. This will result in a uniparental transmission of the trait, just as in extranuclear inheritance.

One of the most well-studied examples of infectious heredity is the killer phenomenon exhibited in yeast. Two double-stranded RNA viruses, designated L and M, are responsible for this phenotype. The L virus codes for the capsid proteins of both viruses, as well as an RNA polymerase. Thus the M virus can only infect cells already harbouring L virus particles. The M viral RNA encodes a toxin that is secreted from the host cell. It kills susceptible cells growing in close proximity to the host. The M viral RNA also renders the host cell immune to the lethal effects of the toxin. For a cell to be susceptible it must therefore be either uninfected or harbour only the L virus.

The L and M viruses are not capable of exiting their host cell through conventional means. They can only transfer from cell to cell when their host undergoes mating. All progeny of a

mating involving a doubly infected yeast cell will also be infected with the L and M viruses. Therefore, the killer phenotype will be passed down to all progeny.

Heritable traits that result from infection with foreign particles have also been identified in *Drosophila*. Wild-type flies normally fully recover after being anesthetized with carbon dioxide. Certain lines of flies have been identified that die off after exposure to the compound. This carbon dioxide sensitivity is passed down from mothers to their progeny. This sensitivity is due to infection with σ (Sigma) virus, a rhabdovirus only capable of infecting *Drosophila*.

Although this process is usually associated with viruses, recent research has shown that the *Wolbachia* bacterium is also capable of inserting its genome into that of its host.

Maternal Effects:

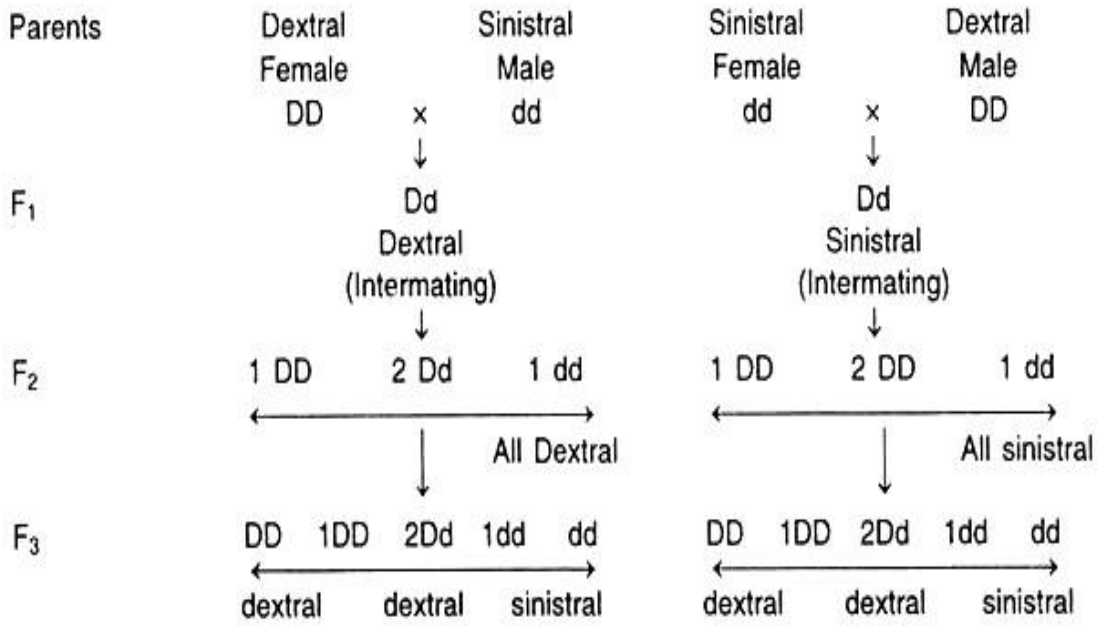
When the expression of a character is influenced by the genotype of female parent, it is referred to as maternal effect. Such characters exhibit clear-cut differences in F_1 for reciprocal crosses. Maternal effects are known both in plants and animals. Some examples of maternal effects are briefly presented below.

(i) Coiling Pattern of Shell in Snail:

The effect of maternal genotype on the coiling behaviour in water snail was studied by Sturtevant. There are two types of coiling pattern of shell in snail (*Limnaea peregra*), viz., right handed (dextral) and left handed (sinistral).

The coiling behaviour is controlled by a single gene. The dextral coiling behavior is governed by dominant allele D and sinistral by recessive allele d. When a cross is made between dextral female and sinistral male, it produces dextral snails in F_1 as well as in F_2.

However, in F_3 a segregation ratio of 3 dextral and 1 sinistral is observed. Similarly, when a reciprocal cross is made, i.e.,, sinistral as female and dextral as male, all the snails are sinistral in F_1 and dextral in F_2. Again in F_3 a ratio of 3 dextral and 1 sinistral is observed. This indicates that the inheritance of coiling direction in water snail depends on the genotype of female parent and not on its own genotype.



Maternal effect on coiling of water snail

Source: http://www.biologydiscussion.com/genetics/cytoplasmic-inheritance

Kappa Particles in *Paramecium*:

Kappa particles are found in certain killer strains of *Paramecium* and are responsible for production of substance paramycin, which is toxic to strains not possessing kappa (sensitive strain). The production of kappa particles is dependent on a dominant allele K, so that killer strains are KK or Kk and sensitive strains are ordinarily kk. In absence of dominant allele K, kappa particles cannot multiply and in absence of kappa particles, dominant allele K cannot produce them de novo. Consequently sensitive strains with genotypes KK or kk can be obtained. These will not carry any kappa particles. However, killer strain with genotype kk cannot be obtained, because even if kappa particles are present, these would be lost in absence of dominant allele. If *Paramecium* clones with genotypes KK or Kk are allowed to multiply asexually at such a fast rate, that division of kappa particles cannot keep pace with division of cells, kappa particles will be eventually lost. Consequently sensitive strains with dominant genotype (KK, Kk) having no kappa particles would be obtained.

If the killer (KK) and sensitive (kk) strains are allowed to conjugate, all exconjugants (the cells separating after conjugation) will have same genotype Kk. Phenotypes of these exconjugants will, however, depend upon duration for which conjugation is allowed. If conjugation does not persist long enough for exchange of cytoplasm, heterozygote (Kk) exconjugants will only have parental phenotypes. It means that killers will remain killers and sensitive will remain sensitive even after conjugation. If conjugation persists, sensitive strain will receive kappa particles and will become killer, so that exconjugants will be killers having genotype Kk.

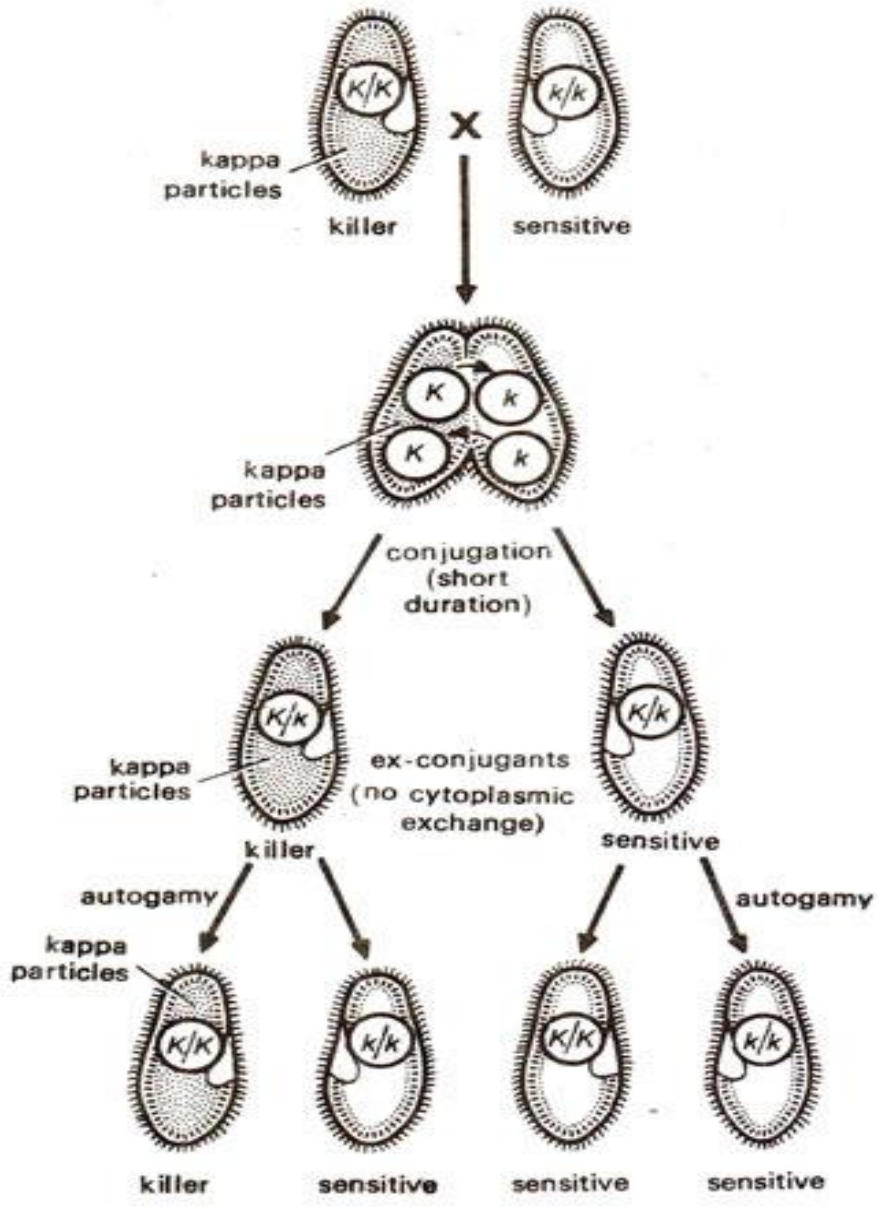


Fig. Results of a cross between a killer (KK) and a sensitive (kk) strain of *Paramecium*, when no cytoplasmic exchange is allowed.

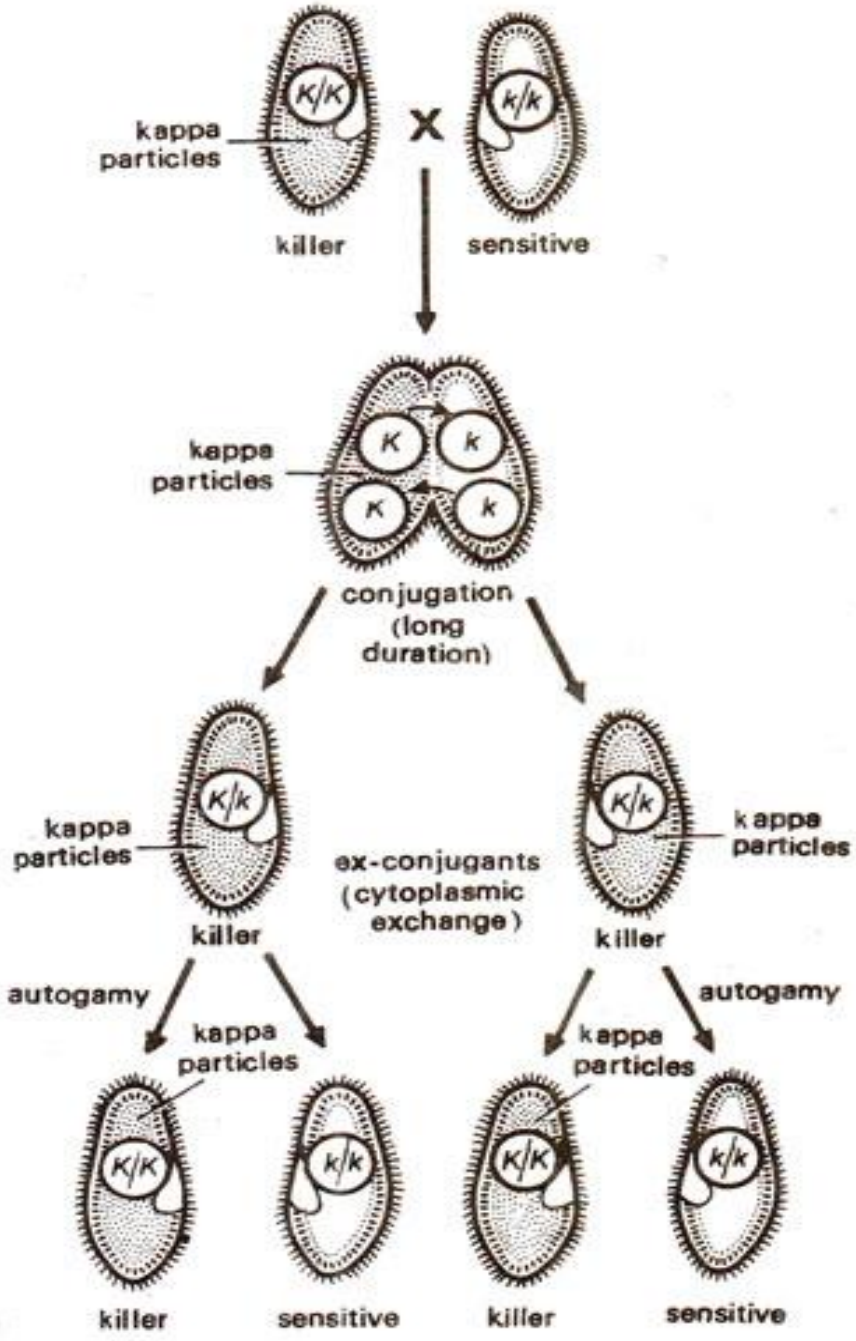


Fig. Results of a cross between a killer (KK)and a sensitive (kk)strain of *Paramecium*, when cytoplasmic exchange is allowed.

Source:https://biocyclopedia.com/index/genetics/maternal_effects_and_cytoplasmic_inheritance/kappa_particles_in_*paramecium*

5. Population Genetics: Hardy-Weinberg principle; gene frequency in a population, genetic equilibrium, factors affecting gene frequency.

Populations

A population is a group of organisms of the same species that are found in the same area and can interbreed. A population is the smallest unit that can evolve—in other words, an individual can't evolve.

Alleles

An allele is a version of a gene, a heritable unit that controls a particular feature of an organism.

For instance, Mendel studied a gene that controls flower color in pea plants. This gene comes in a white allele, w, and a purple allele, W. Each pea plant has two gene copies, which may be the same or different alleles. When the alleles are different, one—the dominant allele, W—may hide the other—the recessive allele, w. A plant's set of alleles, called its genotype, determines its phenotype, or observable features, in this case flower color.

Population genetics is the branch of genetics that deals with frequencies of alleles and genotypes in breeding populations. Population genetics examines allelic variation among individuals, the transmission of allelic variants from parents to offspring generation after generation, and the temporal changes that occur in the genetic makeup of a population because of systematic and random evolutionary forces.

Allele frequency

Allele frequency refers to how frequently a particular allele appears in a population. For instance, if all the alleles in a population of pea plants were purple alleles, W, the allele frequency of W would be 100%, or 1.0. However, if half the alleles were W and half were w, each allele would have an allele frequency of 50%, or 0.5.

The discipline deals with the study of alleales of genes and the forces responsible for maintaining or changing the frequencies of partiaulargenotype or alleles is known as population genetics.

The primary aim of population genetics is to understand the processes that shape a populations genepool. Firstly, we must quarry what effects reproduction and Mendelian principles ave on the genotype and allelic-frequencies. How do the segregation of alleles in gamete formation? We will get the answer of this question lias in the Hardy-Weinberg law in 1908, G.H. Hardy, a British mathematician, and W. Weinberg a German Physician, independently discovered a rule that relates allelic and genotypic frequencies in a population of diploid, sexually reproducing individuals.

The rule has three aspects.

1. The allelia frequencies at an autosomal locus in a population will not change from one generation to the next (allelic-frequency equilibrium)
2. The genotypic frequencies of a population can be determined in a predictable way by the allelic frequencies (genotypic-frequency equilibrium)
3. The equilibrium is neutral i.e. it will be reestablished within one generation of random mating at the allelic frequencies if all other requirements are maintained.

Hardey Weinberg principle:

1. The Hardey Weinberg law states when a population is in equilibrium, the genotypic frequency will be in the proportion P^2, 2pq, q^2.
2. Take two groups of population differing in a single gene i.e. one is AA and the other is aa.
3. Consider two genes or alleles ‘A’ and ‘a’ in a stable population, i.e. population is large, random mating (Panmictic) any individual has equal chance to mate with any of individual of opposite sex and there is no migration or mutation.
4. The frequency of each allele can be expressed as percentage fraction of 1. Thus if we consider ‘p’ is the frequency of A and q is the frequency of a, then the p+q must be 1.
5. The genotypes of individual resulting from the cross between A and a are as follow:

O^+ / O^-	A(P)	a(q)
A(P)	$AA(p^2)$	Aa(pq)
A(q)	Ha(pq)	$Aa(q^2)$

6. According to the theory of probability, due to random mating, the genotype frequency will be as follows:

   $(p+q) \times (p+q)=p^2+2pq+q^2$

   (p+q)=Total frequency of 2 alleles A and a.

   The equation is called Hardy-Weinberg equatic/equilibrium.

   According to the theory of probability the total frequency of p+q is always 1.

   So, $(p+q)^2=p^2+2pq+q^2=1$

Factors affecting gene frequency:

At the genetic level, a population may change because of (A) Mutations (B) Recombinations during Sexual Reproduction (C) Genetic Drift (D) Gene Migration (Gene Flow) (E) Natural Selection.

1. Mutation generally produce new alleles, through the mutation rate is too low.
2. Migration is responsible to change the frequencies bcause immigrants always beard different genetic makeup.
3. Selection generally reduces the survival and fertility ability of certain genotype.
4. Random genetic drift is responsible to cause minor deviations from the predicted frequencies.
5. Assortative mating of dissimilar as similar genotypes is responsible for increasing the homozygotes or heterozygate frequency.
6. Existence of sub populations increases the frequency of homozygotes which is the results of inbreeding.

(A) Mutations:

These are characterized by:

(i) These are sudden, large and inheritable changes in the genetic material.

(ii) Mutations are random (indiscriminate) and occur in all directions.

(iii) Most mutations are harmful or neutral. It is estimated that only one out of 1,000 mutations is useful.

(iv) Rate of mutation is very low, i.e., one per million or one per several million genie loci. But rate of mutation is sufficient to produce considerable genetic variability.

(v) Certain mutations are pre-adaptive and appear even without exposure to a specific environment. These express and become advantageous only when after exposure to new environment which only selects the pre-adaptive mutations that occurred earlier.

Significance of mutations:

(a) Mutations create and maintain variations within a population.

(b) These also introduce new genes and alleles in a gene pool.

(c) Accumulation of mutations over a number of generations may lead to speciation.

(B) Recombinations during Sexual Reproduction:

Recombination involves reshuffling of genes of chromosomes. Chances of recombination are more in those organisms which undergo sexual reproduction which involves gametogenesis followed by fertilization.

Sexual reproduction involves recombinations during three stages:

(i) Crossing over

(ii) By independent assortment of chromosomes

(iii) By random fertilization

Significance:

Due to recombination's, though only reshuffling of already existing characters takes place and no new genes are produced but it leads to redistribution of different traits to different individuals of a population. Different combinations bring diversity in genotype and phenotype of different organisms. So recombination is an agent of evolution.

(C) Genetic Drift:

It is the random change in the frequency of alleles occurring by chance fluctuations. It is characterized by:

(i) It is a binomial sampling error of the gene pool, i.e., that alleles which form the gene pool of the next generation are a sample of the alleles of present population.

(ii) Genetic drift always influences frequencies of alleles and is inversely proportional to the size of population. So genetic drift is most important in very small populations in which there

are increased chances of inbreeding which increases the frequency of individuals homozygous for recessive alleles, many of which maybe deleterious.

(iii) Genetic drift occurs when a small group separates from a larger population and may not have all the alleles or may differ from the parental population in the frequencies of certain genes. This explains for the difference between island populations and mainland population.

(iv) In a small population, a chance event (e.g. snow storm) may increase the frequency of a character having little adaptive value.

(v) Genetic drift can also operate through founder effect. In this, genetic drift can cause dramatic changes in the allele frequencies in a population derived from small groups of colonisers, called founders, to a new habitat.
These founders do not have all of the alleles found in their source population. These founders become quickly different from the parental population and may form a new species, e.g. evolution of Darwin finches on Galapagos Islands which were probably derived from a few initial founders.

(vi) Population bottleneck:
It is reduction in allele frequencies caused by drastic reduction in population size called population crash e.g. decrease in cheetah population in Africa due to over-hunting. As the given gene pool is limited, population bottleneck often prevents the species to reestablish its former richness so new population has a much restricted gene pool than the larger parent population.

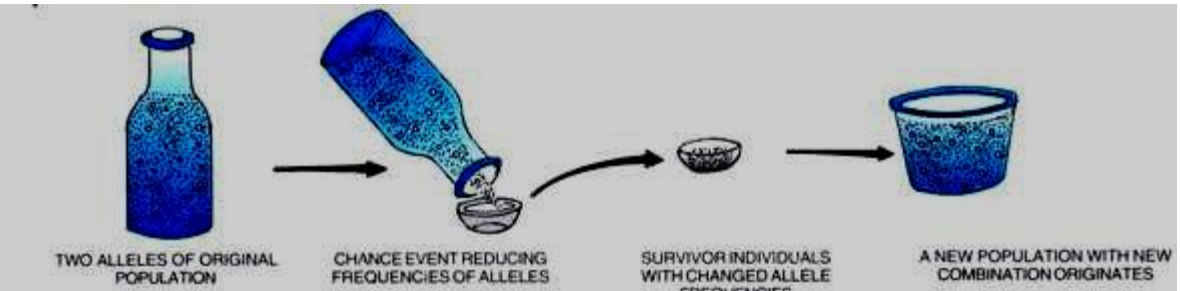


Fig. 7.47. Bottleneck effect on the frequencies of two alleles.

Source: http://www.yourarticlelibrary.com/biology/

(D) Gene Migration (Gene Flow):

Most populations are only partially isolated from other populations of same species. Usually some migration-emigration (moving out of some individuals out of a population) or immigration (entry of some members of a population into another population of same species) occurs between the populations.

Immigration results in the addition of new alleles into the existing gene pool and changes the allele frequencies. Degree of changes in allele frequencies depends upon the differences between the genotypes of immigrants and native population.

If there are no much genetic differences, then entry of a small number of migrants will not change the allele frequencies much. However, if the populations are genetically quite different, a small amount of immigration can result in large changes in allele frequencies.

If the migrating individuals interbreed with the members of local population, called hybridization, these may bring many new alleles into the local gene pool of the host population. This is called gene migration. If the inter specific hybrids are fertile, then these may initiate a new trend in evolution which lead to formation of new species.

(E) Natural Selection:

The process by which comparatively better adapted individuals out of a heterogeneous population are favored by the Nature over the less adapted individuals is called natural selection.

Types of Natural selection:

The three different types of natural selections observed are:

1. Stabilizing or balancing selection:

It leads to the elimination of organisms having overspecialized characters and maintains homogenous population which is genetically constant. It favors the average or normal phenotypes,

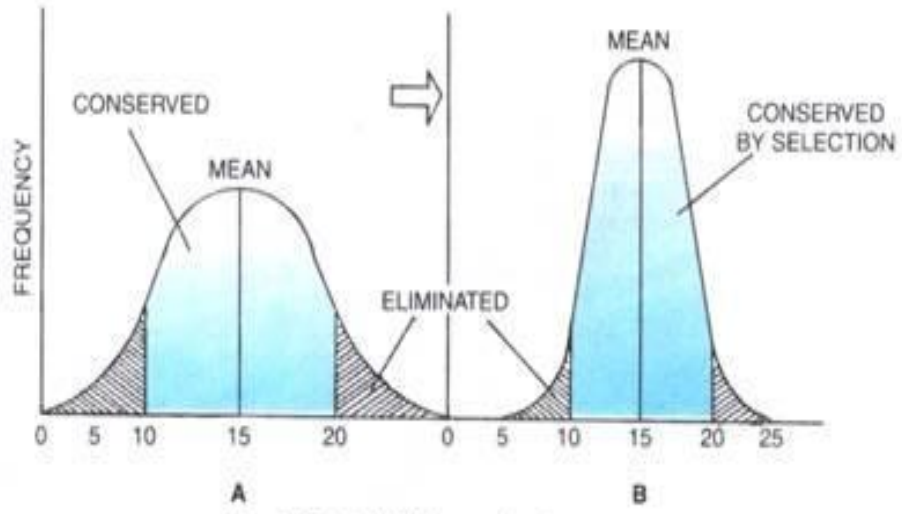


Fig. 7.49. Stabilizing selection.

Source: http://www.yourarticlelibrary.com/biology/

while eliminates the individuals with extreme expressions. In this, more individuals acquire mean character value.

It reduces variation but does not change the mean value. It results very slow rate of evolution. If we draw a graphical curve of population, it is bell-shaped. The bell-shaped curve narrows due to elimination of extreme variants.

Example:

Sickle-cell anaemia in human beings (Explained in Neo-Darwinism).

2. Directional or Progressive selection:

In this selection, the population changes towards one particular direction along with change in environment. As environment is undergoing a continuous change, the organisms having acquired new characters survive and others are eliminated gradually.

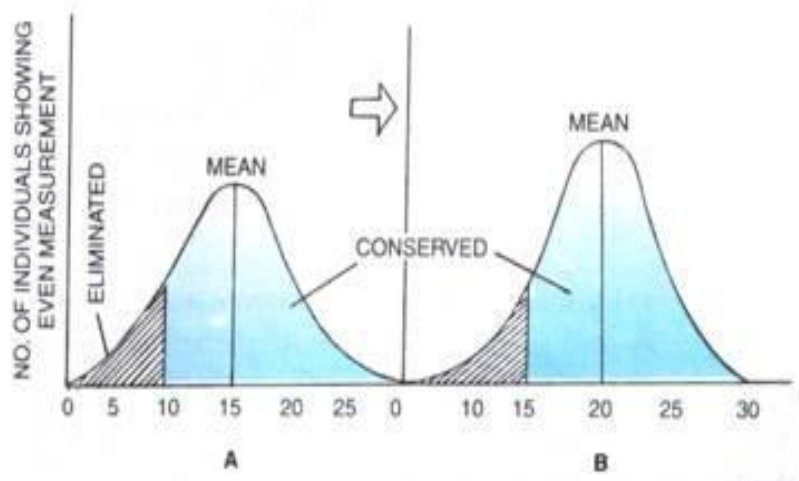


Fig. 7.50. Directional selection.

Source: http://www.yourarticlelibrary.com/biology/

In this, individuals at one extreme (less adapted) are eliminated while individuals at other extreme (more adapted) are favored. This produces more and more adapted individuals in the population when such a selection operates for many generations. In this type of selection, more individuals acquire value other than mean character value.

Examples:

Industrials melanism (Explained in Neo-Darwinism):

In this, number of the light coloured moths {*Biston betularia*) decreased gradually while that of the melanic moths (*B. carbonaria*) increased showing directional selection.

3. Disruptive selection:

It is a type of natural selection which favors extreme expressions of certain traits to increase variance in a population. It breaks a homogeneous population into many adaptive forms. It results in balanced polymorphism.

In this type of selection, more individuals acquire peripheral character value at both ends of the distribution curve. This kind of selection is rare and eliminates most of the members with mean expression so producing two peaks in the distribution of a trait.

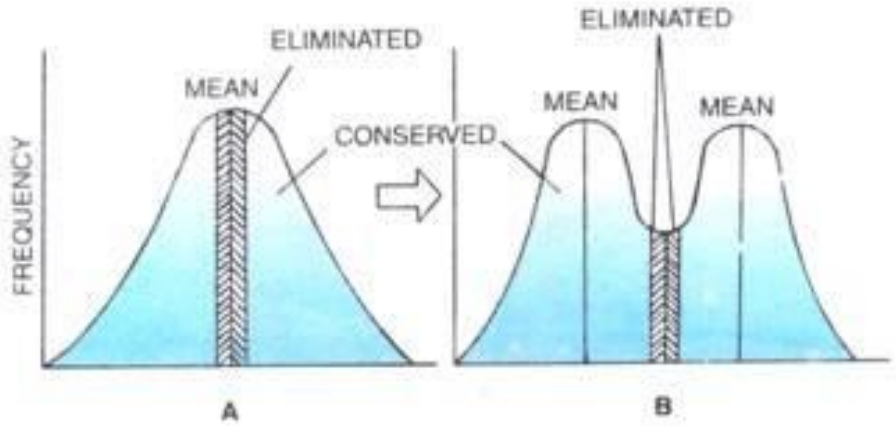


Fig. 7.51. Disruptive selection.

Source: http://www.yourarticlelibrary.com/biology/

Example:

In sea, the three types of snails i.e., white colored; brown colored and black colored are present. The white colored snails are invisible when covered by barnacles. The black colored snails are invisible when rock is bare. But brown colored snails are eaten by predators in both the conditions. So these are eliminated gradually.

6. Microbial Genetics: Transformation, conjugation and transduction and their significance in gene mapping.

Sexuality and recombination in bacteria involve transfer of genetic material from one bacterium to the other. There are three modes of transfer of DNA.

1. Transformation:

   It involves the uptake of naked DNA molecules from one bacterium (the donor

cell) to a recipient cell without coming in contact with each other.

2. Conjugation:

The is the process during which DNA from a (male) is transferred to the recipient (female) through a specialized sex pilues or conjugation tube when the two strains come in contact.

3. Transduction:

In this phenomenon the bacterial DNA is transferred from bacterial donor cell to another bacterial recipient cell by a bacteriophage.

1. Transformation

Transformation involves the uptake of naked DNA molecules from one bacterium to another bacterium. This was discovered by Griffith in 1928 in pathogenic strains of *Diplococcus pneumoniae*.

The uptake of DNA molecule by a recipient bacterium is an active energy-requiring process. It occurs naturally only those species possessing the enzymatic machinery in an active uptake process as well as recombination process. Therefore only competent cells which have a so called "competent factor" are capable as the recipients in transformation.

Mechanism of DNA transfer in bacteria by transformation:

(a) As DNA attaches to membrane bound ds-DNA binding protein.

(b) One of the two strands of the transforming DNA passes into the cell while the other strand is degraded by the enzyme nuclease.

(c) Single stranded exogenotes are unstable and are usually degraded unless they are integrated into the endogenote. By the process of homologous recombination the transformation DNA integrates into the bacterial chromosome. If the exoganote contains an allele of the endogenote, the resulting recombinant double helix would contain one or more mismatched pairs, and is referred to as a heteroduplex. These mismatches are repaired by DNA repair enzyme. The enzyme system preferentially replace the base in the new strand and by this mechanism resorting the genotype of recipient.

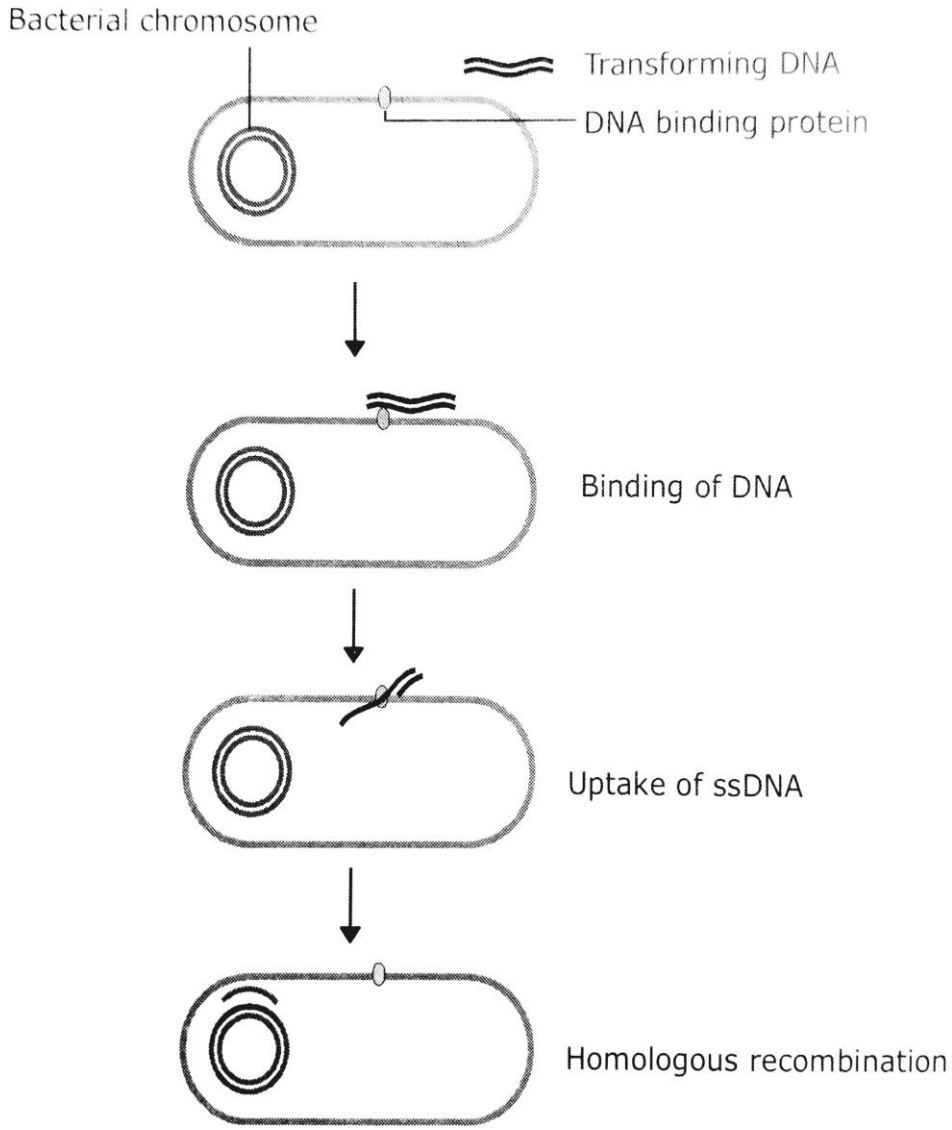


Fig.: Mechanism of transformation in bacterium

Transformation Mapping:

Transformation is used for gene mapping by transformation experiments is based on the principle that 2 markers transformation together if they are near enough to be carried on the same DNA fragment. Let us consider 2 genes A and B present on the bacterial chromosome. If 2 genes A and B are widely separated on the chromomosome then they can carry two different DNA fragments. If the 2 genes so near the chance of recombination will

be less. Thus the frequency of co-transformation is inversely proportional to the distance between 2 genes.

The chain of 20,000 nucleotide pairs in the average transforming DNA molecule certainly seems sufficiently long to contain within more than one gene. For example a transforming molecule carrying gene A mayalso carry gene B. if these genes are closely linked there is a good likelihood that transformation at the A locus produced by a single DNA molecule also produce transformation at the B locus. If A and B are not linked within one transforming DNA molecule, the frequency of double transformation will depend upon the resence of 2 different transforming DNA molecules. Thus closely linked genes will produce a much higher frequency of double transformants than those that are not linked or only distantly linked.

An illustration of how linkage is determined can be analyzed with following example. In *Bacillus subtilis*, 2 known genes affect the synthesis of tryptophan (trp_2^-) and tyrosine (tyr_1^-) respectively so that double mutants are unable to graw on a medium unsupplemented with both of these amino acids. This double mutant, however, may be transformed by the DNA of other strains so that both single so that both single (trp^+tyr^- and trp^-tyr^+) and double transformants (trp^+ tyr^+) can be selected and scored.

In one expt, the transforming donor DNA was given as a mixture of 2 separate type.

trp_2^+ tyr^- and trp_2^- try_1^+

to trp_2^- trp^- cells.

Number of single and double transformant classes arising from the transformation of trp_2 trp^- cells in <u>*Bacillus subtilis*</u>

Donor DNA		Recipient cells	Transformant classes	No. Colenies scored
$trp_2^+ tyr_1^-$			$trp^+ tyr^-$	190
	×	$trp_2^+ tyr1^-$	$trp^- tyr^+$	256
$trp_2^- tyr_1^+$			$trp^+ tyr^+$	2
			$trp^+ tyr^-$	196
$trp_2^+ tyr_1^+$	×	$trp_2^- tyr^-$	$trp^- tyr^+$	328
			$trp^+ tyr^+$	367

The extent of linkage can be calculated as follows: The frequency of recombination between these genes is then the proportion of recombinants among all transformants or

$$\frac{196+328}{196+328+367}=58.8$$

This value is called q value and thus represents the approximate linkage distance between trp_2 and tyr_1.

Conjugation:

In 1946, J. Lederberg and E. Tatum demonstrated experimentally that bacteria undergo conjugation. During conjugation, direct contact between donor and receipient bacteria leads to establishment of a cytoplasmic bridge between them and consequently transfer a part or whole genome from the donor cell to the recipient cell. The efficiency of a cell as donor cell is determined by the presence of self transmissible or cajugative plasmids called fertility plasmid or sex pilus. A recipient cell which received the DNA from the donor cell called transconjugant.

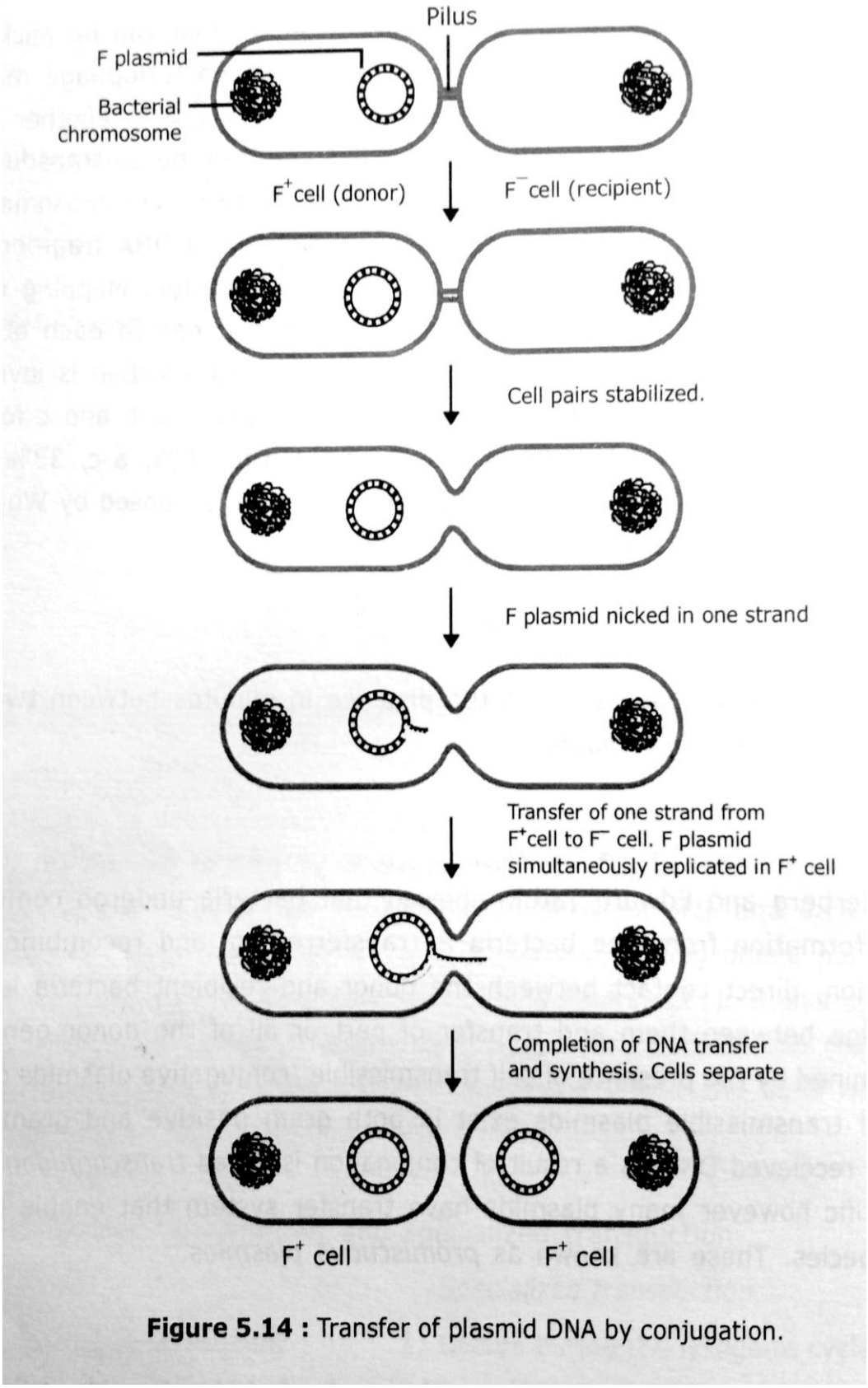


Figure 5.14 : Transfer of plasmid DNA by conjugation.

Fig.: Mechanism of conjugation

F^+ - F^- conjugation

1. The F plasmid of *E.coli* is the prototype for fertility factor bearing plasmids in Gram-genative bacteria. Generally these plasmids carry "tra" genes responsible for their own transfer and also for coding specialized timbrie (sex pilus). *E.coli* K12 has 1 to 3 piliper Cell.

2. The tra genes can be divided into 2 groups: those whose products are involved in mating pair formation (Mpt) and those whose products are involved in processing the plasmid DNA for transfer. The Mpt component includes a sex pilus that extrudes from the cell and holds mating between donor and recipient cell. The Mpt system also includes forming the channel in the membrane through which DNA and protein pass.

3. Each F^+ bacterium bears 1 to 3 sex pili that bind to a specific outer membrane protein of recipient bacteria to initiate mating of donor and recipient cell.

4. A intercellular cytoplasmic bridge is formed and one of the strand of the dsDNA of the F plasmid DNA is transferred from donor to recipient in the 5′ to 3′ direction.

5. The site on the plasmid DNA at which point transfer initiates is called the origin of transfer (Ori T).

6. Relaxes protein makes a single stranded cut at the Ori T site in the plasmid.

7. The transferred strand is then converted to circular double stranded F plasmid DNA in the recipient bacterium. Both the exoconjugate bacteria are F^+, and the F plasmid can therefore converted into F^+

Hfr – F^- conjugation:

1. When the F-plasmid containing the F factor, becomes integrated into the bacterial chromosome, the Cells are called Hfr (high frequency recombinant strain cells).

2. When the F – plasmid and bacterial chromosome carry DNA sequences that allow nutual recognition, then two types of DNA can combine. Such integration of F plasmid involves

breakage and rejoining of both molecular of DNA. This results from a recombination process between a homologous insertion sequence (LS) on both the F plasmid and the chromosome.

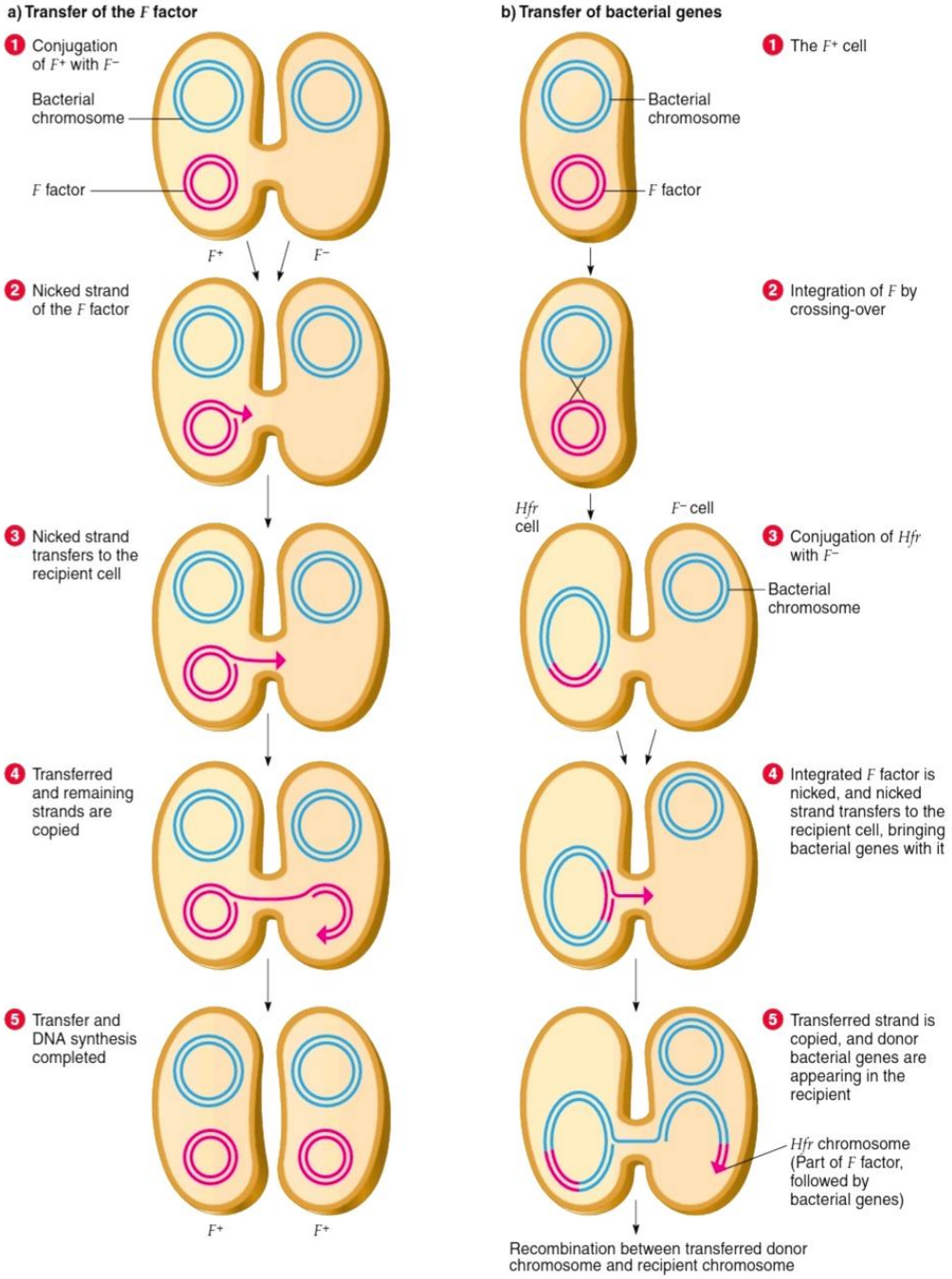
3. Once F plasmid is integrated with the bacterial chromosome, the whole chromosome acts as a big plasmid. The transfer of chromosomal genes to recipients occur at a relatively high frequency.

F′ ~ F⁻ conjugation:

Integrated F plasmids in Hfr strain sometimes be excised from bacterial chromosome and it excision precisely reverses the integration process, F+ cells are produced. Rarely excision occurs by recombinations involving insertion sequences or other genes on the bacterial chromosome. In such cases segments of the bacterial chromosome can become incorporate in hybrid F plasmids that are called F′ plasmid.

Figure 15.5

Transfer of genetic material during conjugation in *E. coli*. (a) Transfer of the *F* factor from donor to recipient cell during $F^+ \times F^-$ matings. (b) Production of *Hfr* strain by integration of *F* factor and transfer of bacterial genes from donor to recipient cell during *Hfr* $\times$ F^- matings.



Gene mapping through interrupted mating:

E. Wollman and F. Jacob crossed HfrH cells of genotype

$Hfr1^+$. thr^+ leu^+ azi – s T1 – s lac^+ gal^+ str – s

×

F–thr– leu– azi – r T_1r lac^- gal^- str – r

* thr and leu genes are responsible for the synthesis of the amino acid threonine and leucine respectively.

* Allele pairs, axi –s/azi r, T1s / T1-r and str-S/str-r control sensitivity (s) or resistance ® to sodiumazide, bacteriophage T1 and streptomycin respectively.

* Alleles lac^+ and lac^- and alleles gal^+ and gal^- govern the ability (+) or inability (–) to lactose and galactose respectively as energy source.

After varying times after Hf H and F^- cells were mixed to initiate matings samples were removed and agitated vigourously in a blender to break the conjugation tubes and separate the conjugating cells. The cells were then plated on medium containing the antibiotic streptomycin, but lacking the amino acids threenine and leucine. On this selective medium, only recombinant cells carrying the thr^+ and leu^+ genes of the Hfr H parent and the str-r gene the F^- parent can grow. The Hfr H donor cells are killed by the streptomycin; the F^- Cells can not grow without threconiuine and leucine. Colonues produced by thr^+ leu^+ str – r recombinants are replica plated to a series of plates containing diff-selective media to each of donor markers were present. The sense included medium containing (1) Sodium azide to score cells azi – r or azi – s (2) bacteriophage T1 to score cells as T1r or T1S (3) lactose as carbon source to score recombinants as lac^+ or lac^- and (4) gatactoz as the sole carbon source, to score recombinant colls as gal^+ or gal^-.

When the conjugating cells were interrupted at any time prior to 8 minutes after missing the Hfr H cells and the F^- Cells no thr^+ leu^+ str-r recombinants were selected. Recombinants (thr^+ ley^+ str-r) approved after 8½ minutes after missing Hfr H and F^- cells the Hfr H azi-s gene first approved among recombinants formed by conjugating cells that were separated by blending at about 9 minutes. The +IS, lac^+ and gal^+ markers first approved after 11, 18 and 25 minutes of mating. These results indicated that the Hfr H genes being transformed to the F^- Cells in a specific linear sequence.

The transfer of a complete chromosome from an Htr to F^- cell takes from 90–100 minutes depending on the strains.

Transduction:

Transduction in bacteria was discovered by N Zinder and J Lederberg (1952). In this mechanism, the bacteriophage carries the DNA of a donor bacteria to the another strain of bacteria faciliting subsequent recombination of gene markers of the two cells.

Two different types of Transduction are known:

1. Generalized Transduction
2. Specialized Transduction

1. Generalized Transduction: In this phenomenon, nearly random segment of bacterial DNA is wrapped up during phage maturation alongwith or in place of phage chromosome in a few progeny particles (the transducing particles). The transducing particles may contain only bacterial DNA or both phage and bacterial DNA.

Transduction experiment was performed by Zinder and Lederberg (1972). Two auxotrophic strains of *Salmonella typhimurium* LA22 and LA2 were placed in the two arms of Davis U-tube. These arms were separated by a glass filter at the bent of U which prevents the movement of bacteria but the DNA or phage particles can easily move. LA22 is a lysogenic strain and the phage P22 is ordinarily associated with it. LA2 is nonlysogenic strain to P22 phage.

Alternate suction and pressure was applied to one arm of the tube during incubation to mix the medium. Occasionally some prophages were passed into the arm of the U tube through the glass filter and infected LA2 non-lysogenic strain. Lysis of LA2 then occurred and new phage particles are produced which then again passed into the other tube of U and infect LA22. These phage particles carried DNA from LA2, some of which were wild type for mutant genes carried by the LA22 strain, thus LA22 becomes prototroph by recombination, whereas LA2 did not carry temperate phage and remained as auxotroph.

Here LA22 strain was transduced and the phage particles have transducing activity.

The strain LA2 is Ph^+ trp^+ met^- his^- whereas, LA22 is phe^- trp met^+ his^+. After transduction, LAZ strain remains as such, whereas LA22 becomes ph^+ trp^+ met^+ his^+ and transforms into prototrophi.

Zinder and Lederberg therefore proposed that the rare transducing phage particles can carry only small segment of bacterial chromosomal material.

The DNA fragment of donor cell may be integrated into the chromosomes of recipient cells. But sometimes it may remain free in the cytoplasm, it will not replicate and will be transmitted to only one progeny cell during each cell division. But genes located on the transduced segment are expressed even if they are not integrated. Cells carrying such nonintegrated transducing DNA segment are called abortive transductants.

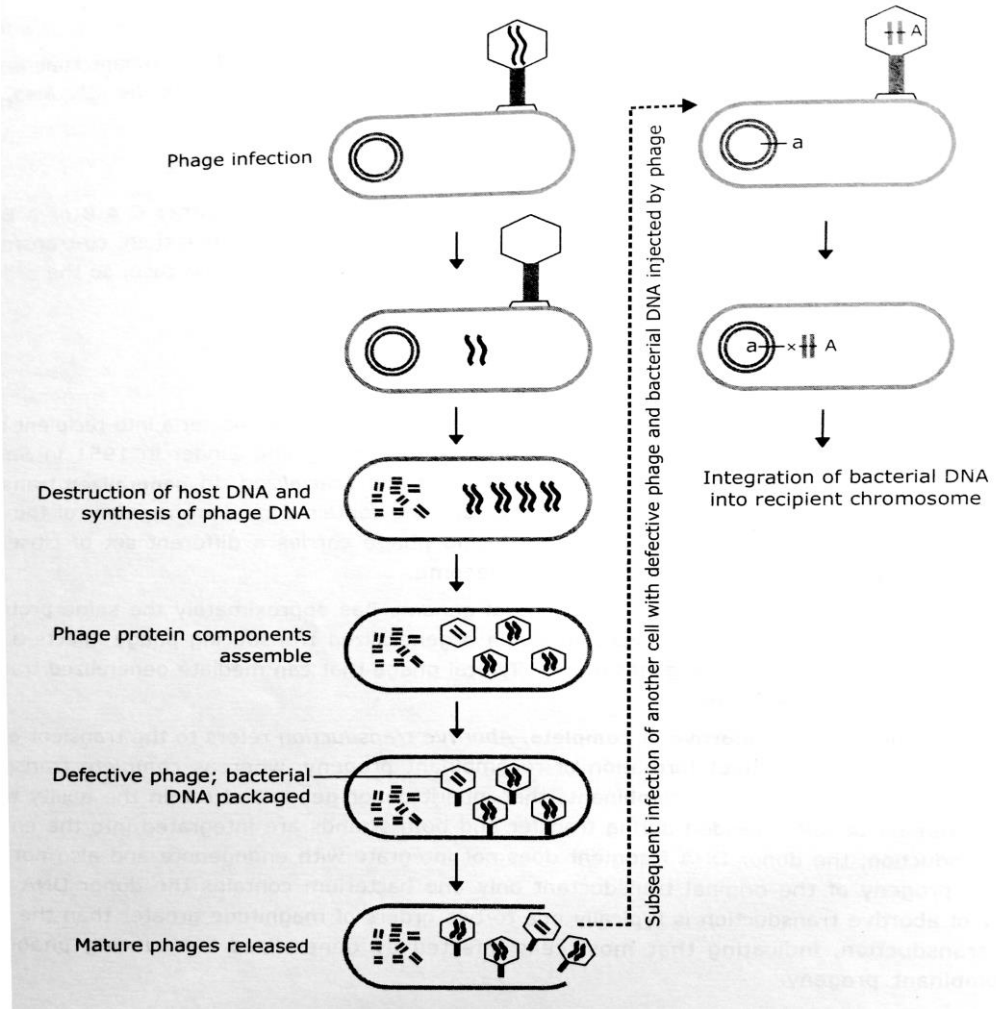


Fig.: Generalized transduction

Specialized transduction : Morse and Laderberg (1956) used wild type prototrophs as donors in X mediated transduction which could affect mutan recipients only by changing gal˜ but never transder various loci such as thr, trp, lac etc. This phenomenon is known as specialized transduction.

Specialized involves prophage integration, excision and transduction. Specialized transducing phages are formed only when lysogenic donor bacteria enter the lytic cycle and release phage progeny. There are two important features of specialized transduction.

1. Only bacterial genes that can be transduced are those very near the site at which the prophage is integrated. For example the only site at which λ phage integrates into the host chromosome is between the genes for galactose fermentation (gal) and biotin synthesis (bio). So, it the prophage disintegrates abnormally from the host chromosome, only the gal or bio genes could be transduced.

2. It results from defective excision of the prophage from the host chromosome.

Ultimately the specialized transducing phages contain both host and phage DNA linked to one single DNA molecule. They are rare recombinants which lack part of normal phge genome and contain part of the bacterial chromosome located adjacent to the prophage attachment site.

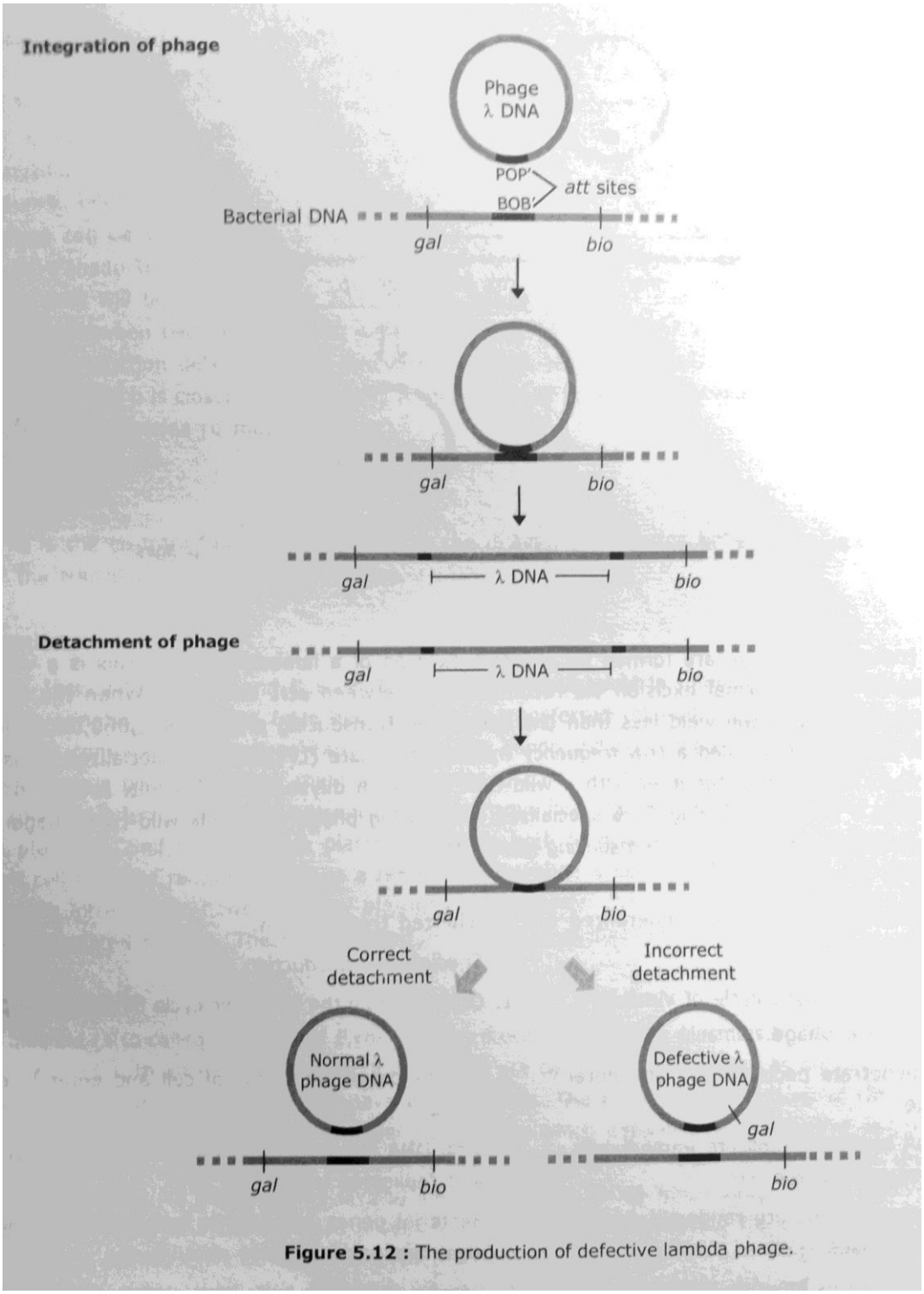


Figure 5.12 : The production of defective lambda phage.

Fig.: Specialized transduction

Transduction Mapping :

Generalized transducing particles are formed very infrequently (one out of perhaps every 10^6 phages emerging during the lysis of bacterial culture). In the analysis of transducing mapping data following principles are followed.

When two makers are followed during generalized transduction i.e. two factor transduction permit the establishment of relative cotransduction frequencies. Similarly more information on gene mapping was obtained by a three factor transduction.

An example of 3 factor transduction cross we can cite typical experiment in which the donor *E.coli* cells have the genotype $a^- b^+ c^-$ and the recipients have the genotype $a^- b^- c^-$. The donor is infected with P1, the P1 progeny are used to infect recipient cells and the recipient cells are then plated and subjected to selection for the presence of one of the donor markers (for example, the presence of a^+) Such recombinant recipient cells are said to have been transduced for atleast one marker, and they are now tested by replica plating to see whether they are transduced (recombination) for one or both of the other marker as well.

Therefore transduction data can supply co-transduction frequencies as well as gene order; to derive these frequencies. To give some actual data, an experiment was performed by Signer, Backwith and Brenner with $trpA^+$ $SupC^+$ $pyrF^+$ donor cells and $TrpA^-$ Supc pyr F^- recipient cells of *E.coli*, where typA is a gene involved in tryptophan synthesis, supc is an ochre~ suppressor gene and phy F is a gene involved in pyrimidne biosynthesis. P–1 mediated transductants for $supC^+$ were initially selected.

The transductants could be classed as follows:

1. Sup C^+ trp A^+ Pyr F^+ = 38
2. Sup C^+ trp A^+ Pyr F^- = 114
3. Sup C^+ trp A^- Pyr F^+ = 0
4. Sup C^+ trp A^- Pyr F^- = 453

= 603 Total

The marker order is at once recognized as being sup C trpA pyrF based on the genotype of the rarest class of transductants (Class 3). The supc+ and trpA+ markers are both transduced

in class (1) and (2) but not in (3) and (4), therefore the Sup C-trp A contransduction frequency is calculated as :

$$\frac{36+114}{603} = \frac{150}{603} = 0.25$$

Similarly contransduction frequency for Sup C and pys F is seen to be equivalent to the clas (1) frequency, namely,

$$\frac{36}{603} = 0.06$$

7. Chromosome: Structure and nomenclature, centromere and telomere, chromosomal aberrations.

Chromosome, the microscopic thread-like part of the cell that carries hereditary information in the form of genes. Chromosomes have special organisation, individuality and function. Their presence was first demonstrated in the eukaryotic cell by E. Strasburger in 1875 and these were first termed as chromosomes by W. Waldeyer in 1888.

This term is actually taken from Greek chromasoma which means “coloured bodies” (chroma = colour; soma = body) due to their marked affinity for basic dyes as a consequence of which they axe stained. This property is known as chromaticity.

Definition of chromosome:
“*DNA of eukaryotes such as plants and animals is arranged as a tightly packed thread-like structure known as a chromosome*.”
Or,
“A complex network of DNA and protein- coiled around each other and helps to fit DNA inside the nucleus is known as a chromosome.”

Staining the cell with certain types of stain (e.g., Aceto-orcein, Acetocamine, Feulgen’s stain)

shows that chromosomes are not visible in the interphase nucleus or metabolically active nucleus due to their high water content, but can be easily seen during cell division characteristics whether mitosis or meiosis.

During cell division, the chromosome undergoes dehydration, spiralisation and condensation. So they become progressively thicker and smaller and, accordingly, the satiability of chromosome also increases. Hence the chromosome becomes readily observable under microscope. Staining of chromosomes is generally carried out to make them visible under light microscope.

Chromosomes are capable of duplication and maintaining their morphologic and physiologic properties through successive cell divisions. It has also been demonstrated that the chro-mosome contains DNA, which in turn, carries the genes and thus plays a major role in heredity.

When reproduction of organism takes place, they are passed on to the next generation through the gametes. Besides, they play an important role in variation, mutation and evolution, and in their control of morphogenesis, multiplication and equilibrium of vital processes.

The term chromosome is mainly used to describe the chromosome of eukaryotic cell. The naked DNA of prokaryotes and DNA or RNA of viruses is sometimes broadly called prokaryotic chromosome and viral chromosome, respectively, due to their similarity in fundamental properties with eukaryotic chromosomes.

But the morphology and the organisation of eukaryotic chromosome is much more com-plex. The morphology of chromosomes in all eukaryotes is essentially similar—except some variations in number and size.

Most of the chromosomes in an eukaryotic cell are called autosomes which control all somatic characteristic of an organism [These are symbolized by ‘A’]. But, in addition, there are some other chromosomes which control some specialised characteristics of an organism and are called allosomes.

Sex chromosome (X and Y) for determination of sex, B-chromosomes, L-chromosomes, M-chromosomes, S-chromosomes and E- chromosomes are examples of allosomes. Autosomes are universally present in all eukaryotic-organisms, but allosomes may or may not be present in all organisms.

The number of chromosomes varies from species to species but it remains constant for a particu-lar species. The number of chromosomes serves as an aid in the determination of phylogenetic status, such as taxonomic position of plant and animal species.

In higher organisms, each somatic cell con-tains one set of chromosomes inherited from the maternal (female) parent or organ and a comparable set of chromosomes (homologous chromosomes or homologues) from the paternal (male) parent or organ.

The number of chro-mosomes in this dual set is called the diploid (2n) number. The suffix 'ploid' refers to chro-mosome "sets". Homologous chromosomes are two copies of a chromosome (one comes from the female and the other from the male parent or organ)—which are ordinarily identical in size and shape, gene content and gene order.

Sex cells or gametes—which contain half the number of chromosome set found in somatic cell—are referred to as haploid cells (n). A genome is a set of chromosomes corresponding to the haploid set of a species. The number of chromosomes in each somatic cell is the same for all members of a given species.

Chromosome number varies widely and may be very low or high in both plant and animals. In animal kingdom Ascaris megalocephala var univalens shows a single pair of chromosomes in the cells of the germ line.

But, since in the diploid soma the two chromosomes split into numerous small chromosomes, the single haploid chromosome has to be considered an aggregate chromosome or compound chromosome. It, for reasons unknown, maintains its unity under the conditions imposed by the cells of the germ line.

Again, the next lowest diploid chromosome number (2n) recorded so far in animals is four in *Mesotoma* (flat worm) and *Ophryotrocha puerilis* (Polychaeta). The highest diploid

chromosome number (2n) in animals is 254 in *Eupagurus schotensis* (a hermit crab). Belar (1926) has, in fact, recorded that *Aulacantha*, a radiolarian has as a diploid number (2n) approximately 1,600 chromosomes.

In plant kingdom, *Haplopappus gracilis*, a member of the family Asteraceae, has four chromosomes in its somatic tissues which is the lowest chromosome number known in plants. In *Ophioglossum reticulatum*, a member of eusporangiate primitive fern under pteridophyta, up to 1,260 chromosomes (2n) have been reported. This is the highest chromosome number known in plant.

The somatic chromosome number generally remains constant among individuals of the same species. But in many species, somatic cells of the same individual may exhibit different (2n, 4n, 8n etc.) chromosome numbers.

In such species, cellular differentiation is often accompanied in some cells with a phenomenon of endomitosis. Endomitosis means the duplication of chromosomes without division of the nucleus, resulting in increased chromosome number within a cell. Chromosome strands separate but the cell does not divide.

Endomitosis leads to the production of endopolyploid cells having 2n, 4n, 8n etc. chromosomes. In natural polyploid individuals, it becomes necessary to find out the ancestral chromosome number which is represented by x and is called as the basic number.
For example in common wheat *Triticum aestivum* 2n = 42; n = 21 and x = 7 showing that common wheat is a hexaploid (2n = 6x). The whole collection of chromosomes in the nucleus of an organism is referred to as chromosome complement.

The size of chromosome of a cell shows a remarkable variation depending upon the stage of cell division. Chromosomes are longest and thinnest during interphase. But on the onset of prophase there is a progressive decrease in size associated with an increase in thickness.

Chromosome are smallest during anaphase. But the measurement of chromosome size are practically taken during mitotic metaphase when they are very thick, quite short and well-spread.

The size of mitotic metaphase chromosome of various plants and animals varies from 0.5µ to 32µ in length and 0.2 µ to 3.0 µ in diameter. The giant chromosome found in the cells of salivary gland of *Diptera* are permanently in pre-metaphase stage and are easily visible in the interphase nucleus. These chromosomes are 300 µ in length and 10 µ in diameter.

Generally, plant chromosomes are longer than animal ones. In angiosperm, chromosomes of monocots are bigger than those of dicots and other plants. The longest metaphase chromosomes of plant are found in *Trillium*.

The size of each chromosome is 32 µ long. Again, chromosome size of *Trillium* is hundred times bigger than its closely related genus *Medeola*, size differences may be seen in the different species of a genus. For instance, the chromosomes of *Allium porrum* are half the size of the chromosome of *Allium sativum*.

The size of chromosome may vary in the different tissues within a single organism. For example, in plant Medeola, the root tip chromosomes are 50% bigger than the shoot tip chromosomes.

Among animals, grasshopper, crickets, mantids, newts and salamanders have big chromosomes. In animals, size variation of chro-mosome has also been reported in different varieties within a species.

For example, the chromosome size of chironomous thumii thumii (fly) differs from its closely related varieties. During embryogenesis of certain marine insects the size of chromosomes of the early blastula are smaller than those of the later stage of development.

The size, shape and number of the metaphase chromosomes constitute the karyotype which is distinctive for each species. When all chromosomes of a species are more or less equal in size, the karyotype is called symmetrical karyotype.

Asymmetrical karyotype refers to the chromosome of different size. In most organisms, all cells have the same karyotype. However, species that appear quite similar can have very different karyotypes—indicating that similar genetic potential can be organised on chromosomes in very different ways.

Variation in the size of the chromosome can be induced by some factors:

i. When the cell divides at low temperature, the size of chromosomes become short and more compact.

ii. When the pre-treatment of cells is done with colchicine, the chromosomes become slightly shorter in size.

iii. Repeated and rapid cell divisions tend to result in smaller chromosomes.

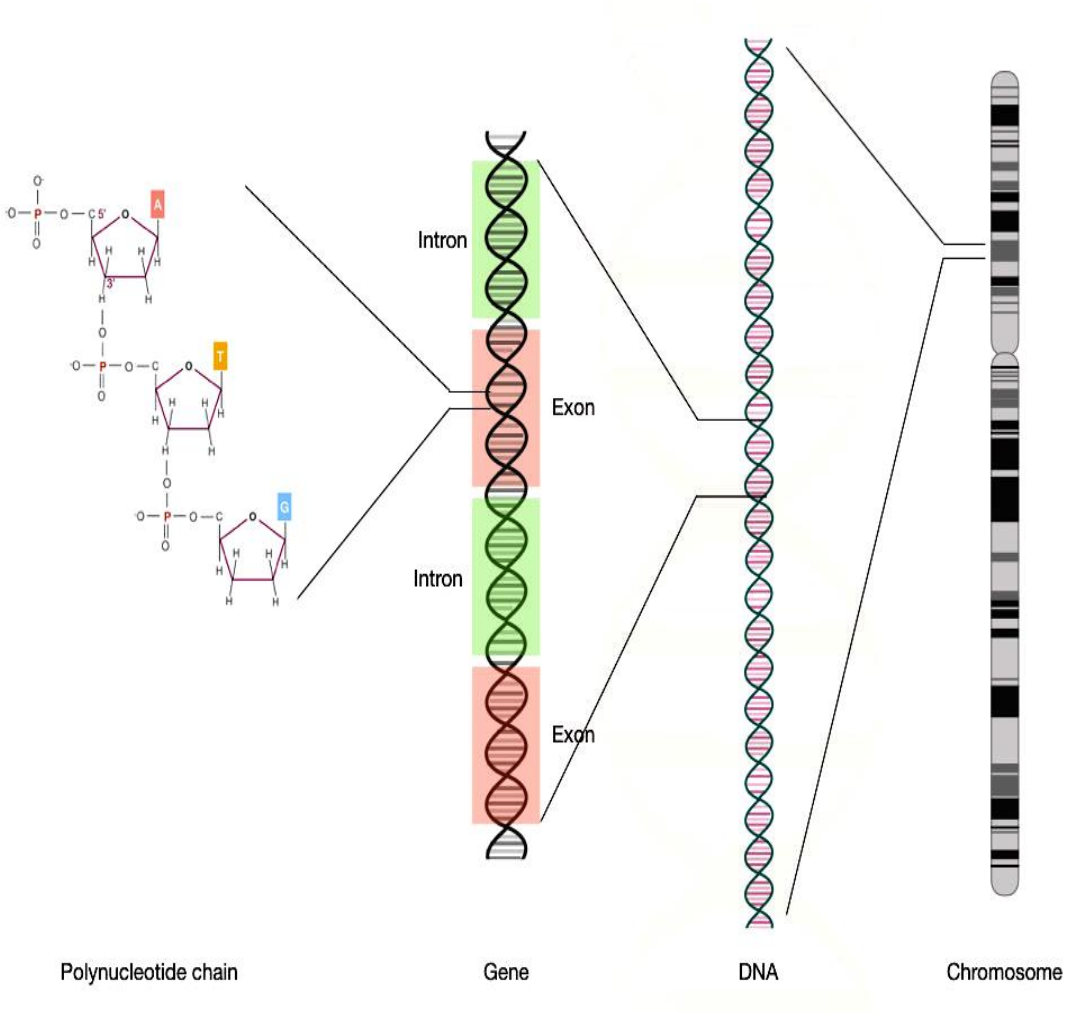


Fig.: The location of gene on a chromosome

Chromosome Structure:

Each cell has a pair of each kind of chromosome known as a homologous chromosome. Chromosomes are made up of chromatin, which contains a single molecule of DNA and associated proteins. Each chromosome contains hundreds and thousands of genes that can

precisely code for several proteins in the cell. Structure of a chromosome can be best seen during cell division.

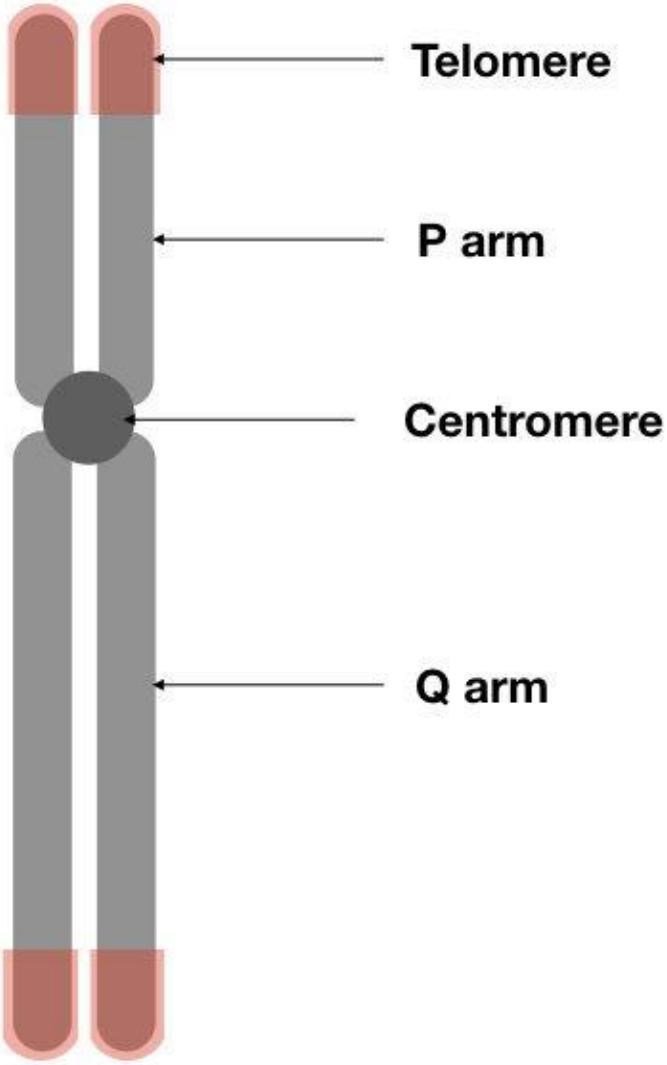
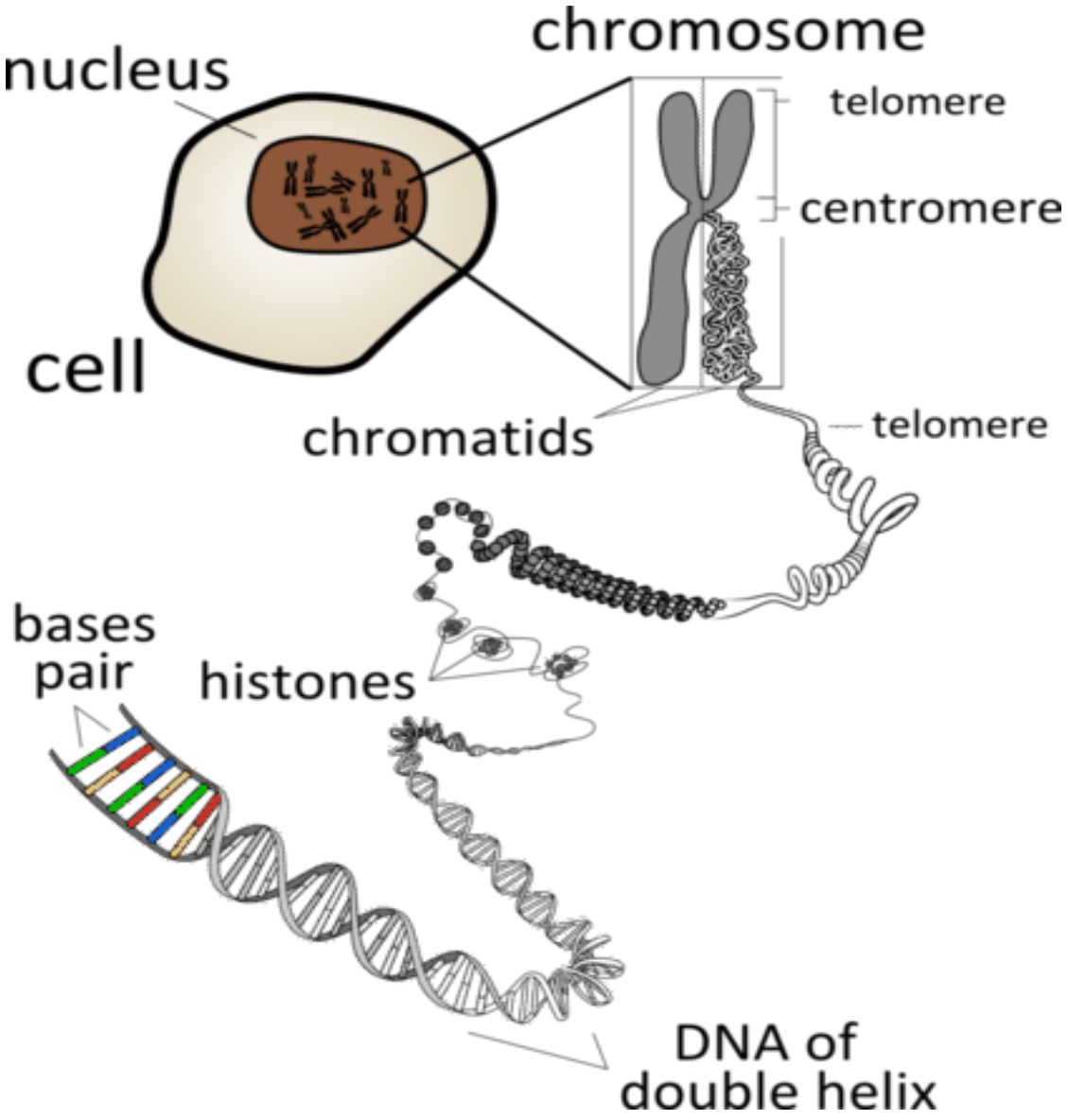


Fig.: The graphical representation of the structure of chromosome- centromere, arms and telomeres.

The "**p arm**" and "**q arm**" of the chromosome are attached to the centromeres. Although, based on the location of the centromere, the length of the arms varies.

Arms are the complex network of protein and DNA where genes are located.



Main parts of chromosomes are:

- **Chromatid:** Each chromosome has two symmetrical structures called chromatids or sister chromatids which is visible in mitotic metaphase.
    - Each chromatid contains a single DNA molecule
    - At the anaphase of mitotic cell division, sister chromatids separate and migrate to opposite poles

- **Centromere and kinetochore:** Sister chromatids are joined by the centromere.
    - Spindle fibres during cell division are attached at the centromere
    - The number and position of the centromere differs in different chromosomes
    - The centromere is called **primary constriction**
    - Centromere divides the chromosome into two parts, the shorter arm is known as **"p" arm** and the longer arm is known as **"q" arm**
    - The centromere contains a disc-shaped **kinetochore**, which has specific DNA sequence with special proteins bound to them
    - The kinetochore provides the centre for polymerisation of tubulin proteins and assembly of microtubules
- **Secondary constriction and nucleolar organisers:** Other than centromere, chromosomes possess secondary constrictions.
    - Secondary constrictions can be identified from centromere at anaphase because there is bending only at the centromere (primary constriction)
    - Secondary constrictions, which contain genes to form nucleoli are known as the **nucleolar organizer**
- **Telomere:** Terminal part of a chromosome is known as a telomere.
    - Telomeres are polar, which prevents the fusion of chromosomal segments
- **Satellite:** It is an elongated segment that is sometimes present on a chromosome at the secondary constriction.
    - The chromosomes with satellite are known as **sat-chromosome**
- **Chromatin:** Chromosome is made up of **chromatin.** Chromatin is made up of DNA, RNA and proteins. At interphase, chromosomes are visible as thin chromatin fibres present in the nucleoplasm. During cell division, the chromatin fibres condense and chromosomes are visible with distinct features.

- The darkly stained, condensed region of chromatin is known as **heterochromatin.** It contains tightly packed DNA, which is genetically inactive
- The light stained, diffused region of chromatin is known as **euchromatin.** It contains genetically active and loosely packed DNA
- At prophase, the chromosomal material is visible as thin filaments known as **chromonemata**
- At interphase, bead-like structures are visible, which is an accumulation of chromatin material called **chromomere.** Chromatin with chromomere looks like a necklace with beads
- It has been observed that the morphology of chromosome changes with the stage of cell division. During the prophase of meiosis, homologous chromosomes pair with each other at zygotene, the cell then enters the stage of pachytene where chromosomes become shortened and coiled.
- Pachytene stages are very useful for the study of chromosome morphology because they are longer than the chromosomes in mitotic metaphase, so that the structural details of chromosomes can be easily resolved.
- But the meiotic cell division as well as the pachytene stages are not readily available at any time for experimental purpose. On the other hand, mitotic metaphase is easily avail-able by arresting the divisional cycle with some chemical agents. Further, mitotic metaphase is also suitable as convenient stage for studies on chromosome morphology and some of the features are more clear during mitotic metaphase.
- At metaphase, each chromosome is made of two symmetrical structures, the chromatids. They are also called sister chromatids. Each chromatid contains a single DNA molecule. The chromatids are held together closely by the centromere and become separated at the start of anaphase when the sister chro-matids move to opposite poles.

Therefore, until two sister chromatids share the common centromere, they are called chromatids. But as soon as they are separated at anaphase and possess their own individual centromere, they are called chromosome instead of chromatid. Hence, from anaphase to next

G_1 phase, chromosomes have only one chromatid while from S phase to metaphase chromosomes have two (Fig. 13.2).

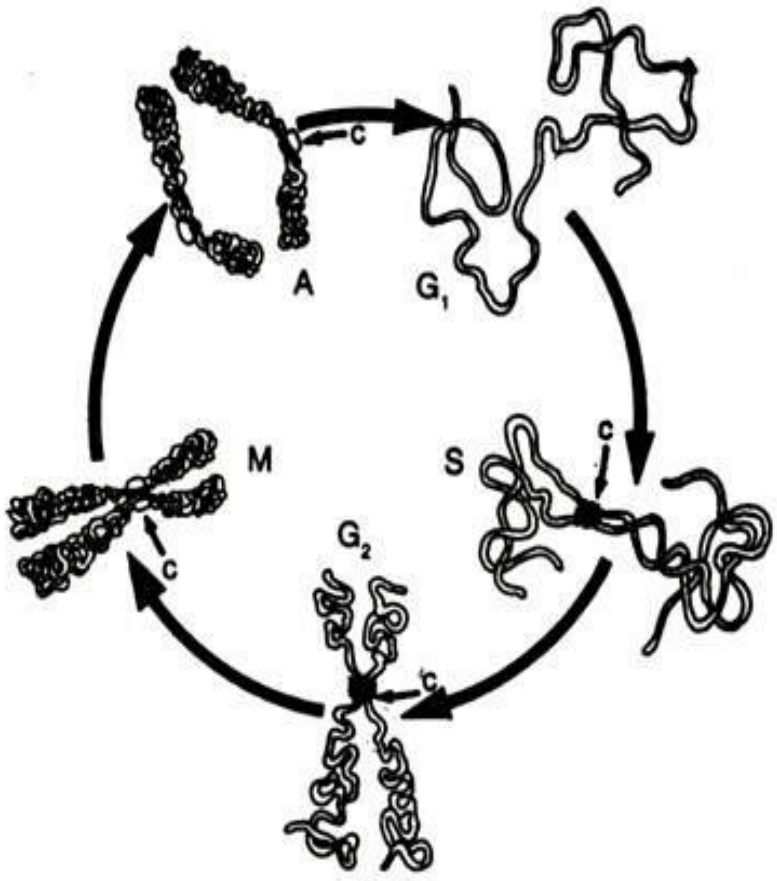


Fig. 13.2: The condensation and decondensation cycle of chromosomes.

The DNA present in each chromosome (made of a single chromatid) replicates during S phase to produce an identical copy of itself so that during G2 prophase and metaphase each chro-mosome is composed of two chromatids.

During prophase, and sometimes during in-terphase, the chromosomal material becomes visible as very thin threads which are called chromonemata and which represent chromatids in early stages of condensation. 'Chromatid' and 'chromonemata', therefore, are two names of the same structure. It is now accepted universally that chromatid is the structural and fundamental unit of chromosomes.

The region where two sister chromatids are held together is called the centromere. This region generally appears as a constricted or narrowed zone in the centromere, hence it is also

known as primary constriction. Sometimes centromere appears as gap during metaphase because it does not take up any stain.

Cen-tromere has a clear zone in which the fibrils remained uncoiled or less coiled than those in the rest chromosomes. The reduced similarity of centromere" is understandable as the chro-mosome region in centrosome is less coiled or uncoiled and is composed of heterochromatin.

At or near the centromere of each chro-mosome, the centromere is associated with a specialised structure called kinetochore. The structure of kinetochore is complex and is seen during late prophase. In ultra-thin sections of chromosomes, the kinetochore is seen as a stack of three layered proteinaceous disc like plates (Fig. 13.3).

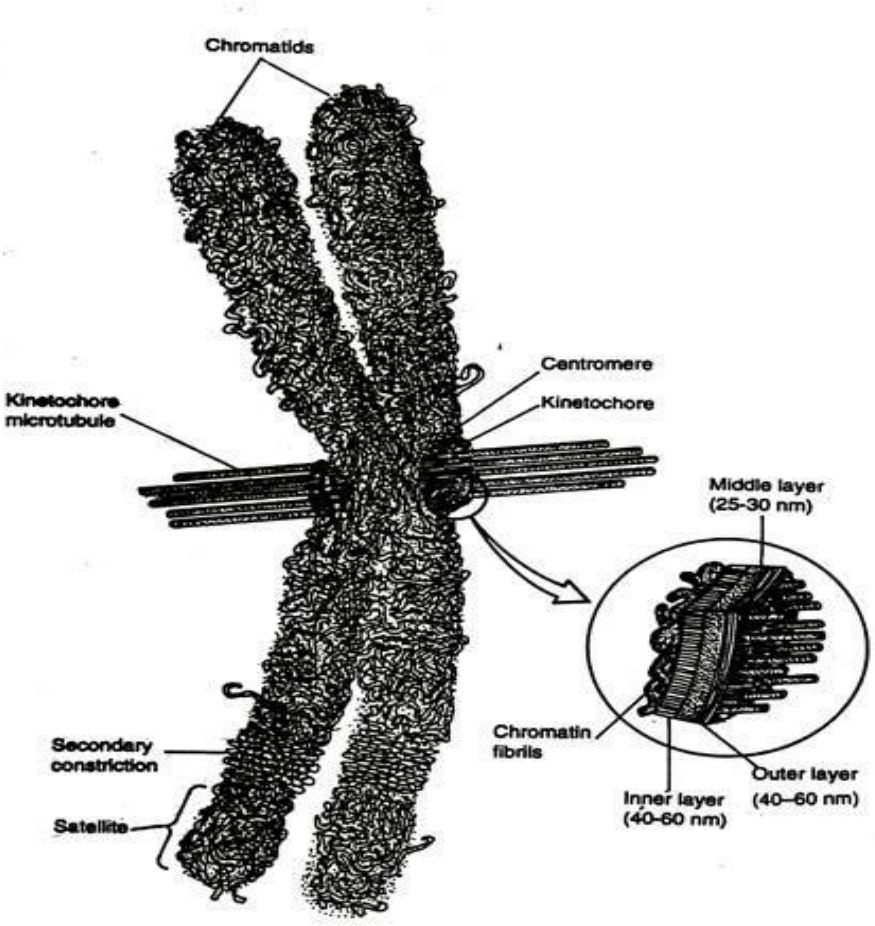


Fig. 13.3: Schematic diagram of metaphase chromosome showing the kinetochore.

Each chromosome in a genome can be dis-tinguished on the basis of the position of centromere which divides the chromosome into two arms of varying length.

Depending on the position of the centromere, chromosomes may be divided into four categories:

(i) metacentric, (ii) sub-metacentric, (iii) acrocentric and (iv) telocentric chromosome

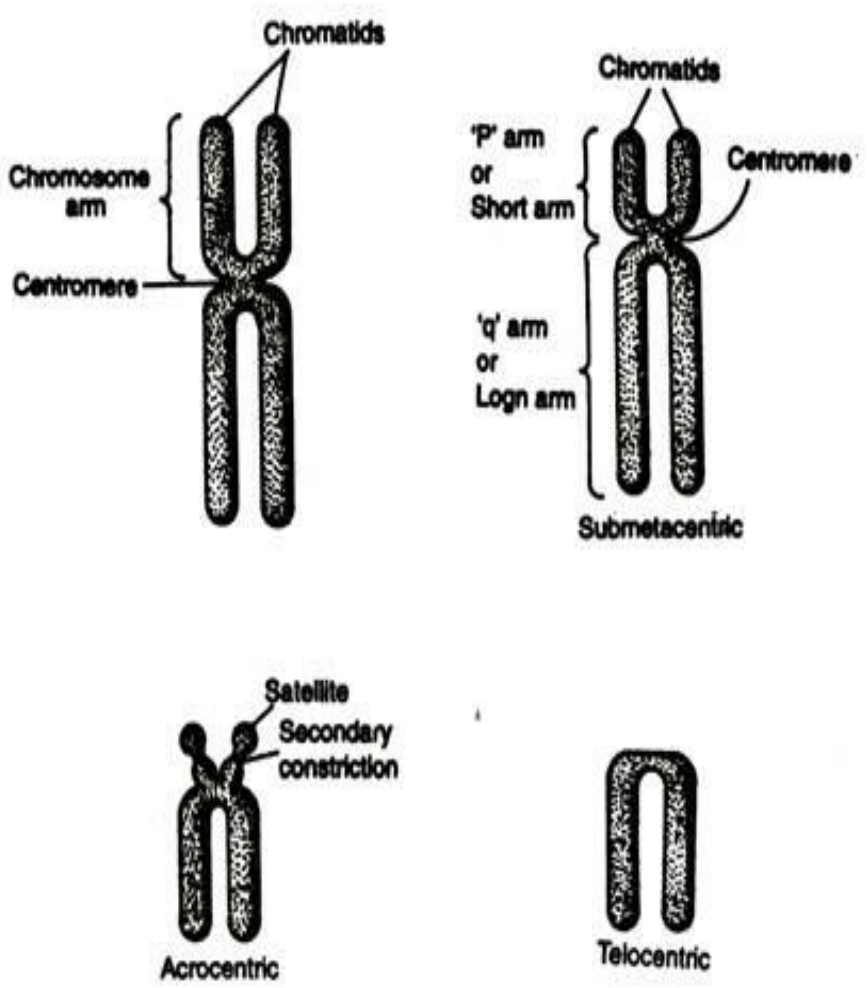


Fig. 13.5: Chromosomes are classified according to the position of the centromere.

In a metacentric chromosome it occurs at the centre, i.e., the centromere is median so that the two arms of such chromosomes are equal. The metacentric chromosomes look 'V'-shaped during anaphasic movement. In Trillium and Tradescantia, all the chromosomes are meta-centric.

The sub-metacentric chromosomes look 'L'-shaped in anaphase and the centromere is located on one side of the centred point, i.e., the centromere is sub-median so that it divides the centromere into two unequal arms.

In acrocentric or sub-telocentric or sub-terminal centromere, the centromere is situated almost near one end of the chromosome, i.e., centromere is sub-terminal in position and it gives two arms—one exceptionally short and the other long. Acrocentric chromosomes look 'J'-shaped in anaphase. Chromosomes of locust and some grasshoppers are acrocentric.

In some chromosomes, however, centromeres appear to be located at one end of the chromosome, i.e., in the position normally occupied by one of the two telomeres. In this type, one arm is more or less equal to the length of the chromosome, and, other arm beyond the centromere is represented simply by dot.

Acrocentric chromosome may appear 'rod- shaped' or T-shaped in anaphase. Telocentric chromosomes are of rare occurrence. Telocentric chromosome exists normally in certain species of holomastigote protozoa.

Telocentric chromosomes arising through a transverse fracturing of the centromere are believed to be unstable due to the centromere's irregular manner of division. This type of division is also known as mis-division of centromere—a process which leads to the forma-tion of isochromosome (those in which the two arms are of equal length and are genetically homologous with each other).

Misdivision of centromeres has been observed in Pea, *Datura*, Wheat and *Fritillaria*. Telocentric chromosomes have been experimentally produced in wheat, maize etc. Usually, each chromosome has only one cen-tromere but, in some species, each chromosome has more than one centromere. Again, in some cases the centromere is absent.

Hence, depending on the number of centromeres, chro-mosomes are classified as given:

(a) Acentric:
The chromosome is without any centromere. Acentric chromosome is very rare occurrence. It may arise due to unequal breaking of chromo-some arm into two so that only one part has the centromere while, in the other part, centromere is absent.

The part of chromosome having no centromere is called acentric fragment. Due to lack of centromere, acentric chromosomes are not able to attach with spindle fibres and they do not

take part in cell division. Ultimately, the cell eliminates the acentric fragments.

(b) Mono-Centric:

Mono-centric chromosomes have only one cen-tromere. It is a very common occurrence in most of the species.

(c) Dicentric:

A chromosome has two centromeres. Dicentric chromosome may be produced as a result of translocation, paracentric inversion etc. If the two centromeres tend to move to opposite poles during anaphase, the chromosome breaks. Rarely a new centromere may appear on the chromosome resulting in an abnormality. Such a centromere is called a neo-centromere. Di-centric chromosome is reported in the cells of wheat.

(d) Polycentric:

In addition to the shapely localised type of centromere described above, there exists a type of non-localised centromere where each chromo-some has more than two centromeres. Such chromosomes are called polycentric.

Polycentric chromosomes are found in plant *Luzula purpurea* (Family: Juncaceae), in the generative tissue of *Ascaris megalocephalla* univalens, and in Thyanta. In both the above cases, the centromeric property is confined to one or more definite locus of the chromosome so that such centromere is called localized centromere.

However, in many insects—e.g., most homopteran and hemipteran insects—the centromere activity is non-localized and spread over the entire chromosome length. In such cases, the centromere is called a diffused centromere.

Polycentric chromosome often breaks into a number of smaller fragments. Each small segment functions independently. For instance, in case of Ascaris megalocephala univalens, the zygotic cell contains only two chromosomes.

But during embryonic development these chromosomes break in the somatic cells so that the cell may have up to 42 chromosomes. However, the cell that will give rise to the generative cell contains only two chromosomes.

Non-staining gaps are seen in certain chromo-somes in addition to the primary constriction regions. These regions are called secondary constrictions (Sc). Generally, secondary constrictions are located in the short arm of chro-mosomes near end but in many chromosomes they are located in the long arm and sometimes they may be present on both arms.

Secondary constrictions are constant in their position and extent. These constrictions are useful in identifying particular chromosomes in a set.

The number of Sc in a genome varies from species to species. In some species, a somatic cell contains at least a pair of chromosomes with Sc while other chromosomes within the same cell are without Sc. In some other species, the number of chromosomes with Sc may be four (e.g., *Vicia hajastana*), six, eight, ten (e.g., human somatic cell).

Secondary constrictions are distinguished from primary constriction:

i) Sc is without kinetochore.

ii) Sc is not able to attach with spindle fibre during anaphasic movement.

iii) Sc shows the absence of marked angular deviation of the chromosomal segments during anaphase.

Karyotype:
Karyotype represents the chromosome consti-tution of a cell or an individual. It deals with the length of chromosome, the position of centromere, presence of secondary constriction, and the size of satellite of the somatic chro-mosome complement. Generally, karyotype is prepared from well-scattered chromosomes of mitotic metaphase plate.

The information regarding chromosome constitutions are obtained from hand drawing of the microscopic view of chromosomes with the help of camera lucida or drawing prism. Photomicrographs of metaphase plate are also used for the preparation of karyotype.

Karyotypes are presented by arranging the chromosomes in a descending order of length in a straight line. The longest chromosome is always placed on the right side and the smallest one of the right. All chromosomes in a karyotype do not bear the centromere at the same position. So, in a karyotype, chromosomes are grouped on the basis of the position of centromere and, in each group, descending order of length of chromosome is also maintained. Each chromosome of a karyotype is marked by a serial number from the extreme left to extreme right.

Broadly, karyotypes of different organisms may be classified into two categories:
i. Symmetrical Karyotype;
ii. Asymmetrical Karyotype.

A symmetrical karyotype has all metacentric chromosome of the same length. In case of asymmetrical karyotype (Fig. 13.10) variation of length of chromosome complement is found and the position of centromere may or may not be identical.

In certain case, karyotype is asymmetrical but the length of chromosome is sharply two types—some chromosomes are very long and some are very short. This type of asymmetrical karyotype is known as bimodal karyotype.

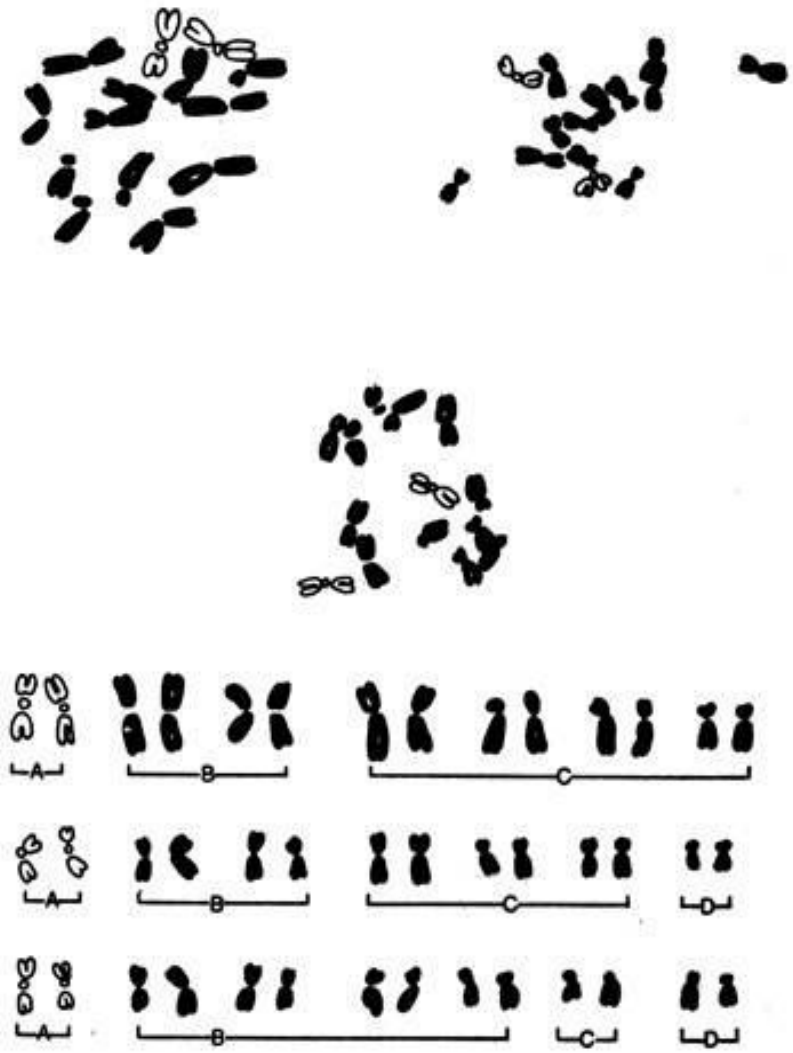


Fig. 13.10: Camera lucida drawing of chromosomes at metaphase and their Karyotype (asymmetric).

It is believed that symmetrical karyotype is the primitive form from which more advanced asymmetrical karyotype has been evolved. The karyotype of a species may be represented on graph or plain paper by bar diagram showing all morphological features of the chromosome. Such diagram is known as Idiotype or Idiogram (Fig. 13.11).

Idiogram is prepared from haploid chromosome complement of an organism. An idiogram gives the identical information like that of karyotype.

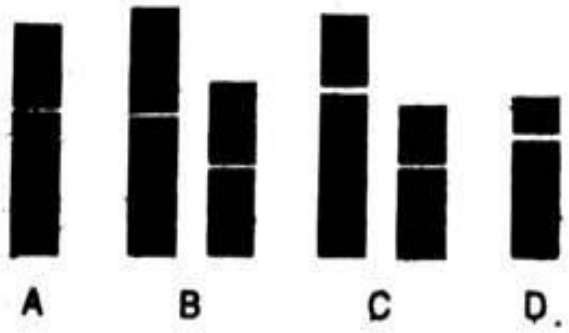


Fig. 13.11: Showing the idiogram of haploid set of chromosomes represented by bar.

Centromeres:

The centromere is a constricted region of the chromosome to which spindle fibers is attached and essential for proper chromosome movement in mitosis and meiosis. The centromeric region contains a disk-shaped structure of about 200 nm in diameter which is the site for microtubule attachment; this structure is called kinetochore. The kinetochore functions as the "microtubule organizing centre" (MTOC) of the chromosome. Kinetochore is the attachment site of spindle fibres and is composed of both DNA and protein. The DNA sequence within these regions is called CEN DNA. Because CEN DNA can be moved from one chromosome to another and still provide the chromosome with the ability to segregate, these sequences must not provide any other function. The yeast centromeric DNA contains three distinguishable sequences-

(i) Conserved element I (CDE I):

It is composed of 9 bp and is located at the left end of the centromere; it shows minor variations.

(ii) Conserved element II (CDE II):

This element is the middle region containing 80-90 bp. A=T rich sequences constitute more than 90% of this region.

(iii) Conserved element III (CDE III):

This element consists of 11 bp and is located at the right end of the centromere. It is a highly conserved sequence.

Mutations in the first two sub-domains have no effect upon segregation, but a point mutation in the CDE-III sub-domain completely eliminates the ability of the centromere to function during chromosome segregation. Therefore CDE-III must be actively involved in the binding of the spindle fibers to the centromere. The protein component of the kinetochore is only now being characterized. A complex of three proteins called CbfIII (Cbf-IIIA, Cbf-IIIB and Cbf-IIIC) binds to normal CDE-III regions. This protein complex has some motor activity due to which the centromeric region of the chromosome becomes attached to microtubules. Mitotic chromosome movement is inhibited when mutation occurs in the genes coding for CBF-III proteins.

Telomeres:

Telomeres are the region of DNA at the end of the linear eukaryotic chromosome that are required for the replication and stability of the chromosome. McClintock recognized their special features when she noticed, that if two chromosomes were broken in a cell, the ends were sticky and end of one could attach to the other and vice versa. Whatever their structure, telomeres must provide at least three important functions. They must (1) prevent deoxyribonucleases from degrading the ends of the linear DNA molecules, (2) prevent fusion of the ends with other DNA molecules, and (3) facilitate replication of the ends of the linear DNA molecules without loss of material.

The telomeres of eukaryotic chromosomes have unique structures that include short nucleotide sequences present as tandem repeats. Although the sequences vary somewhat in different species, the basic repeat unit has the pattern 5′ $T_{1–4}A_{0–1}G_{1–8}$-3′ in all but a few species. For example, the repeat sequence in humans and other vertebrates is TTAGGG, that of the protozoan *Tetrahymena thermophila* is TTGGGG, and that of the plant *Arabidopsis thaliana* is TTTAGGG. In most species, additional repetitive DNA sequences are present adjacent to telomeres; these are referred to as telomere-associated sequences. In vertebrates, the TTAGGG repeat is highly conserved.

Human telomeres contain the sequence TTAGGG repeated from about 500 to 5000 times. Certain bacteria possess telomeres in their linear genetic material which are of two types; one of the types is called a hairpin telomere. As its name implies, the telomeres bend around from

the end of one DNA strand to the end of the complimentary strand. The other type of telomere is known as an invertron telomere. This type acts to allow an overlap between the ends of the complimentary DNA strands.

The telomeres of humans and a few other species have been shown to form structures called t-loops, in which the single strand at the 3′ terminus invades an upstream telomeric repeat (TTAGGG in mammals) and pairs with the complementary strand, displacing the equivalent strand. The DNA in these t-loops is protected from degradation and/or modification by DNA repair processes by a telomere-specific protein complex called **shelterin**.

Shelterin is composed of six different proteins, three of which bind specifically to telomere repeat sequences. TRF1 and TRF2 bind to double-stranded repeat sequences, and POT1 (Protection Of Telomeres 1) binds to single-stranded repeat sequences. Subunits TIN2 and TPP1 tether POT1 to DNA-bound TRF1 and TRF2, and the TRF2-associated protein Rap1 helps regulate telomere length. Shelterin is present in sufficient quantities in most cells to coat all the single- and double-stranded telomere repeat sequences in the chromosome complement.

Telomere replication:

Telomerase

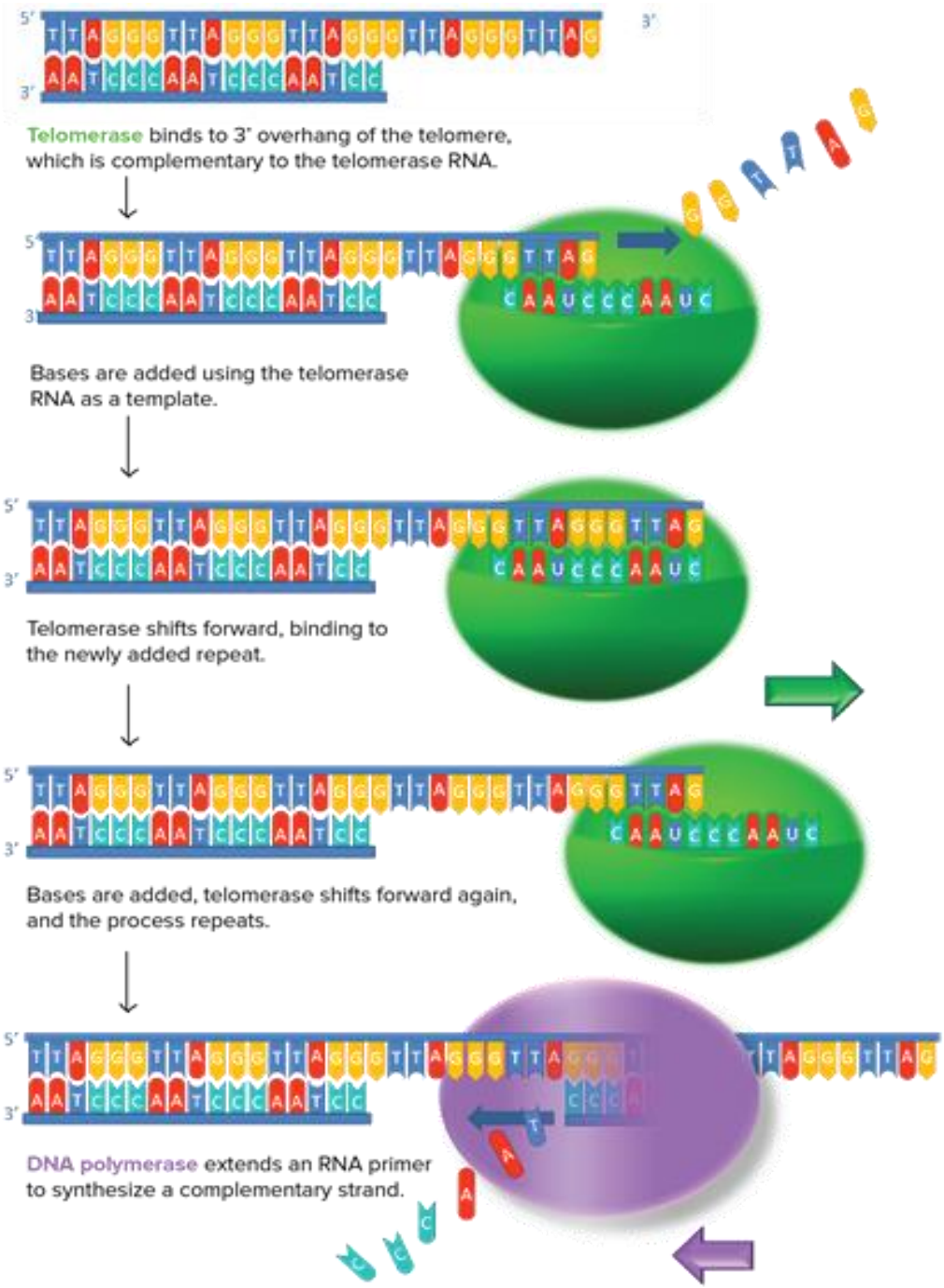
Some cells have the ability to reverse telomere shortening by expressing telomerase, an enzyme that extends the telomeres of chromosomes. Telomerase is an RNA dependent DNA polymerase, meaning an enzyme that can make DNA using RNA as a template.

How does telomerase work? The enzyme binds to a special RNA molecule that contains a sequence complementary to the telomeric repeat. It extends (adds nucleotides to) the overhanging strand of the telomere DNA using this complementary RNA as a template. When the overhang is long enough, a matching strand can be made by the normal DNA replication machinery (that is, using an RNA primer and DNA polymerase), producing double-stranded DNA.

The primer may not be positioned right at the chromosome end and cannot be replaced with DNA, so an overhang will still be present. However, the overall length of the telomere will

be greater. Telomerase is not usually active most somatic cells (cells of the body), but it is active in germ cells (the cells that make sperm and eggs) and some adult stem cells. These are cell types that need to undergo many divisions, or, in the case of germ cells, give rise to a new organism with its telomeric "clock" reset.

Interestingly, many cancer cells have shortened telomeres, and telomerase is active in these cells. If telomerase could be inhibited by drugs as part of cancer therapy, their excess division (and thus, the growth of the cancerous tumor) could potentially be stopped.



Chromosomal aberrations:

Cytogenetics of reciprocal translocation in plant species Gaudens and Velans complex

A translocation occurs when a portion of a chromosome is transferred to another location, either on the same chromosome or on some recipient chromosome not belonging to the same chromosome pair (i.e., the same homolog pair) as the donor chromosome. Two chromosomes that belong to the same chromosome pair, that is, that contain the same set of loci in the same order, are called homologous.

A **reciprocal translocation**, the most common kind of translocation, is an exact interchange of chromosomal segments between two chromosomes not belonging to the same chromosome pair.

In a translocation heterozygote two distinct pairs of homologous chromosomes have reciprocally exchanged nonhomologous segments between one member of each pair. As a result each of the affected chromosome pairs contain both homologous and nonhomologous segments. Put another way, each such pair has one translocated chromosome, and one normal (untranslocated) chromosome. More than two chromosome pairs can be altered in this way so that some or all of the chromosome pairs are composed of a translocated and an untranslocated member.

Organisms having chromosomes rearranged in this way are known as permanent translocation heterozygotes. Due to the way reciprocal translocations are processed during meiosis, all the translocated chromosomes pass to one gamete and all the normal chromosomes pass to the other. As a result, only two types of gametes are produced. One has all the translocated chromosomes and the other has all the normal ones (here "translocated" and "normal" are relative terms since in naturally occurring organisms it is usually unknown which of the two types was original). These two sets of chromosomes ("normal" and "translocated") are known as Renner complexes. In well- studied organisms of this type, the various Renner complexes have been assigned formal names.

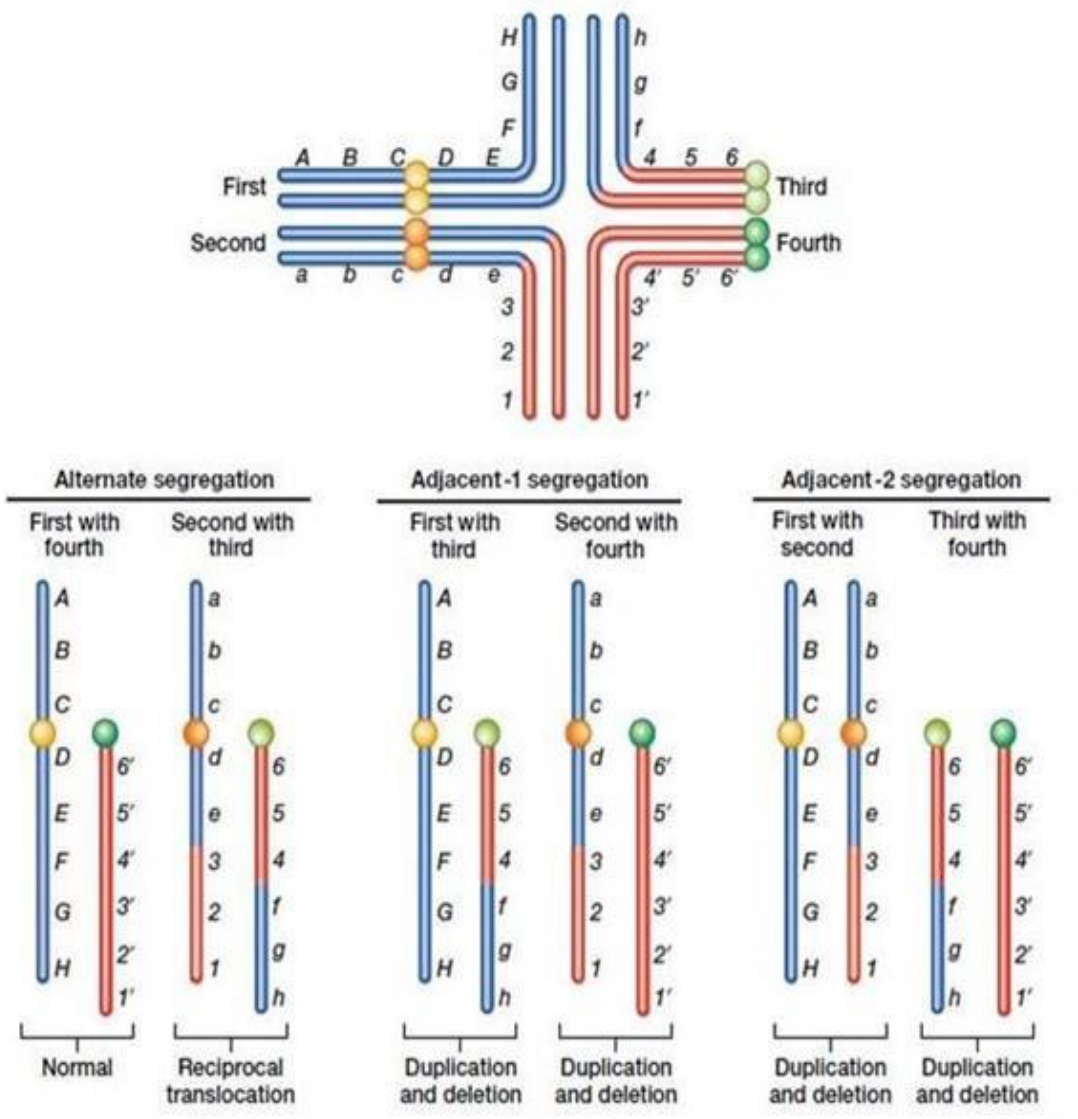


Fig.: Reciprocal translocation. Source: Snustad and Simmons, 2012, Principles of Genetics -6th ed.

Oenothera lamarckiana

In one of the best-known permanent translocation heterozygotes, the evening primrose *Oenothera lamarckiana*, the two Renner complexes are called velans and gaudens. So the karyotype of *O. lamarckiana* is designated as "velans/gaudens." Genetic interchange (crossing-over) between Renner complexes is very rare. So chromosomes in distinct complexes differ genetically in many respects.

Aneuploidization is a common stabilization process among permanent translocation

heterozygotes because there is an enhanced tendency to produce aneuploid gametes. The result is the production of numerous trisomic forms. But the most common stabilization process seen in organisms of this type occurs when forms with distinct Renner complexes hybridize to produce new forms. For example, hybridization of *O. lamarckiana* with another evening primrose, *O. strigosa*, which has the Renner complexes deprimens and strigens, yields four different types.

velans/deprimens, velans/strigens, gaudens/deprimens, gaudens/strigens

Some hybrids produced by such recombinations of Renner complexes are reproductively stable and others are not. By this means new stable types can be produced in a single generation. *O. lamarckiana* itself arose by such a process via hybridization between *O. biennis* and *O. hookeri*. This event occurred within the last two or three centuries in Europe where the two parental forms, both native to North America, were introduced. *O. lamarckiana* spread across Europe and later to North America. In fact, most of the 18 forms of European *Oenothera* that Renner (1942) treated as species arose in this way.

Robertsonian translocation (ROB) is a rare form of chromosomal rearrangement where the participating chromosomes break at their centromeres and the long arms fuse to form a single, large chromosome with a single centromere. In humans, Robertsonian translocations generally occur in the five acrocentric chromosome pairs, namely 13, 14, 15, 21 and 22. Other Robertsonian translocations can occur, but do not lead to a viable fetus. They are named after the American biologist William Rees Brebner Robertson Ph.D. (1881–1941), who first described a Robertsonian translocation in grasshoppers in 1916.They are also called whole arm translocations or centric fusion translocations. They are a type of chromosomal translocation.

A Robertsonian translocation is a type of translocation involving two homologous (paired) or non-homologous chromosomes (i.e., two different chromosomes, not belonging to a homologous pair). A feature of chromosomes that are commonly found to undergo such translocations is that they possess an acrocentric centromere, partitioning the chromosome into a large arm containing the vast majority of its genes, and a short arm with a much smaller proportion of genetic content. The short arms also join to form a smaller reciprocal product, which typically contains only nonessential genes also present elsewhere in the

genome, and is usually lost within a few cell divisions. This type of translocation is cytologically visible, and can reduce chromosome number (from 23 to 22 pairs, in humans) if the smaller chromosome that results from a translocation is lost in the process of future cellular divisions. However, the smaller chromosome lost may carry so few genes (which are, in any case, also present elsewhere in the genome) that it can be lost without an ill effect to the individual.

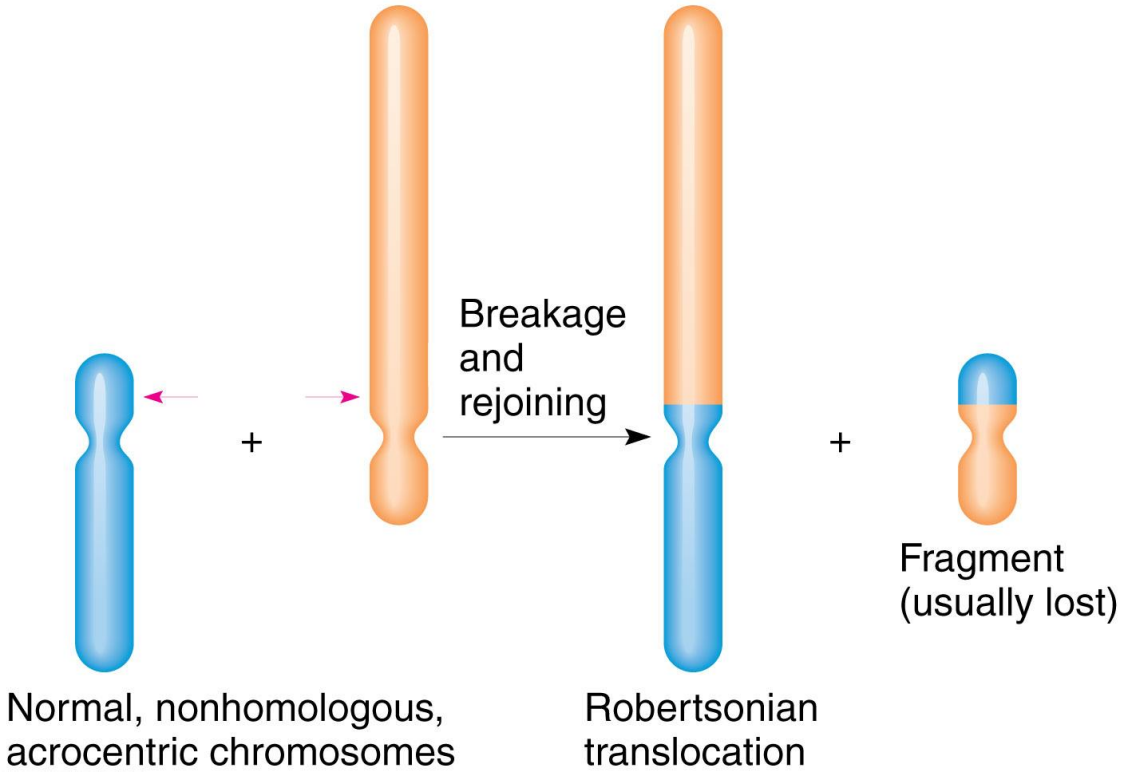


Fig. Robertsonian translocation. Source:
https://www.mun.ca/biology/scarr/Robertsonian_fusion.html.

Aneuploidy

Aneuploidy is defined as the loss or gain of one or few chromosomes compared to its somatic chromosome number of a species.

a) Additions to chromosome number:

i. **Trisomy [2n + 1]:** When somatic cells of an organism contain an extra chromosome (2n+1). The number of possible trisomies in an organism is equal to the haploid chromosome number.

Trisomics are of different types —

primary trisomics where extra chromosome is identical to two homologues.

secondary trisomics where the extra chromosome is an isochromosome with two genetically identical arms.

tertiary trisomies are the products of translocation. Double trisomies (2n + 1 + 1) are also available in nature.

The origin of the trisomics may be from the production of n + 1 types of gametes due to rare non-disjunction of a bivalent in a diploid or may also be produced by triploids through irregular meiosis.

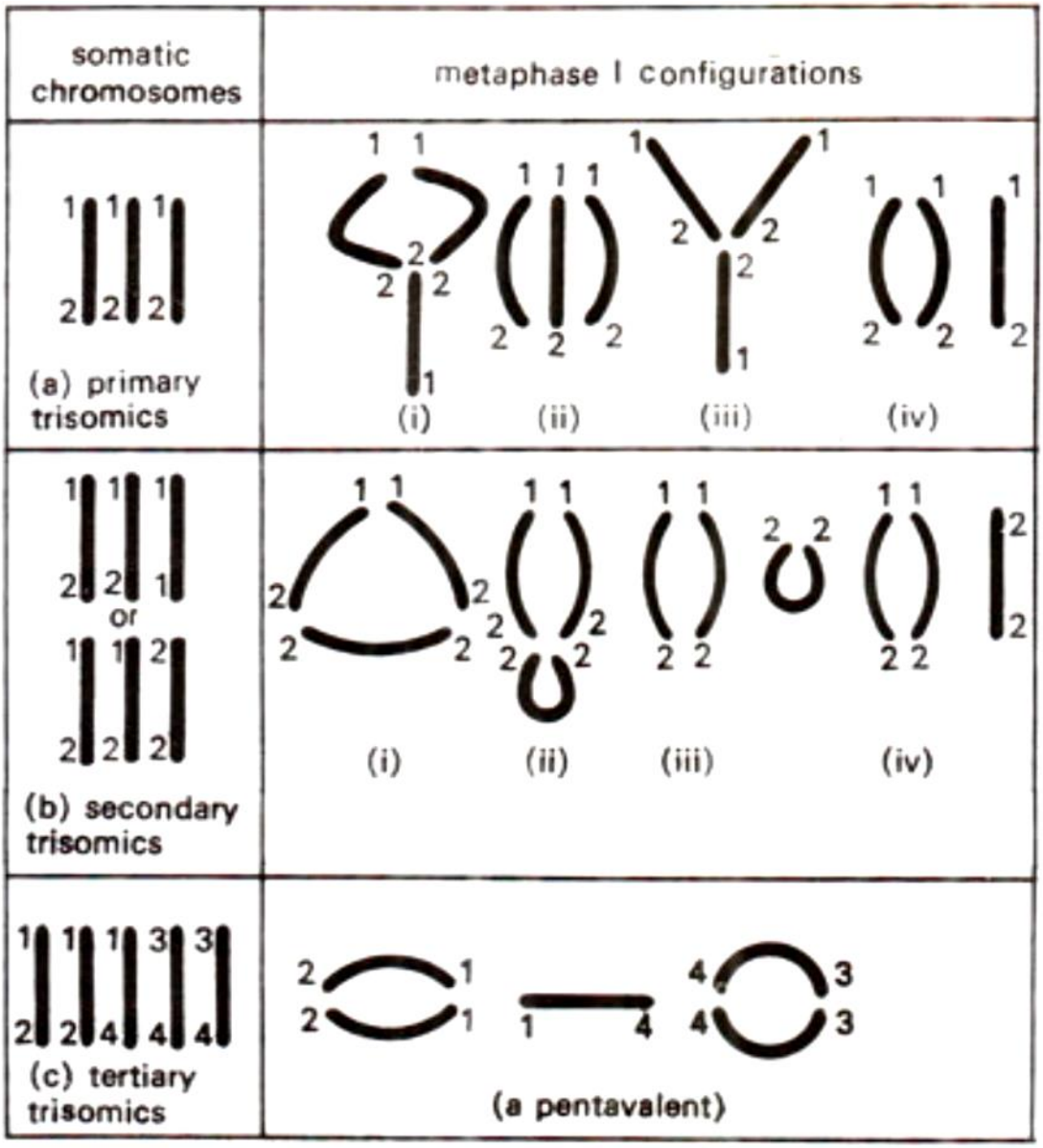


Fig.: Different types of Trisomics and their meiotic configuration at Metaphase I
Source: http://www.biologydiscussion.com/

In primary and secondary trisomics, the three homologues of the concerned chromosome may pair with each other to form a trivalent of different configurations at MI, or two of the chromosomes may form a bivalent leaving the additional chromosome as a univalent. In tertiary trisomic, some PMCs would show a dumbbell shaped figure containing five chromosomes at MI.

Gain of a complete chromosome causes a major imbalance, but its effects are not as deleterious as those due to the loss of a full chromosome. Therefore, trisomics are viable in diploid species, e.g. maize, tomato, pea, barley etc.

ii. **Tetrasomy [2n + 2]:** When somatic cells contain one pair of a chromosome in excess of the normal somatic compliment, i.e.,, chromosome compliment is present in four copies. Tetrasomics may be originated by selfing of trisomics. During meiosis, the four homologues of the tetrasomic set tend to form a quadrivalent.
The four homologues of the concerned chromosome may form one quardivalent, a trivalent and a univalent, or two bivalents at MI. Quadrivalents usually disjoin 2:2 at AI, but 3:1 disjunction may also take place.

b) Reductions in chromosome number:

iii. **Monosomy [2n − 1]:** When one chromosome is missing from the somatic complement. Monosomics of diploid species is not tolerated due to genetic imbalance but are fully viable in polyploidy species e.g., wheat, cotton, oats and tobacco. Double monosomics (2n – 1 – 1) as well as triple monosomics (2n – 1 – 1 – 1) may be obtained in polyploidy species.

The one chromosome remains unpaired during meiosis. The remaining chromosomes pair normally and produce of n – 1 types of gametes due to rare nondisjunction of a bivalent. In progeny of a monosomic, a mixture of disomic (2n), monosomies (2n – 1) and nuilisomics (2n – 2) is obtained.

iv. **Nullisomy [2n − 2]:** When one pair of chromosomes is missing from the somatic complement. Nullisomic has a strong deleterious effect on the organism, only viable in the highly polyploidy species e.g. hexaploid or bread wheat (*Triticum aestivum*) and oats (*Avena sativa*).

Cytologically, chromosome pairing is normal and at MI n-1 bivalents can be seen. These bivalents disjoin normally to produce all normal n-1 gametes.

Importance of Aneuploidy in Plants:

Aneuploids have played a role in evolution and have importance in plant breeding in addition to genetic analysis.

(a) Detecting linkage group:

The aneu-ploids have played an important role in locating a linkage group and a gene in a particular chromosome. Particularly nullisomics, mono-somies and trisomies have been used to determine linkage groups in tobacco, wheat, etc.

The study of aneuploidy has shown homoeology between A, B and D genomes of wheat. Identification of the chromosome involved in translocation has also been done with the help of aneuploids.

(b) Chromosome substitution in plant breeding:

The major contribution of aneuploids has been in the field of plant breeding. The substitu-tion of whole chromosome or part of the chro-mosome using aneuploids has been done. These substitutions resulted in significant modification of yield, resistance, lodging, etc.

(c) Speciation:

Aneuploidy can generate variation and source of speciation in vegetatively propagating species. In *Crepis*, aneuploid variations form a series X = 3, 4, 5, 6 and 7 among species. A very extensive aneuploid series has been observed in *Carex* (n = 6 to 56).

Aneuploidy in human:

- **Down syndrome:** A genetic chromosome 21 disorder causing developmental and intellectual delays. Caused by non-disjunction of the 21st chromosome. Typically associated with physical growth delays, characteristic facial features and mild to moderate intellectual disability.

- **Patau syndrome** Trisomy at chromosome 13 causing Intellectual disability and motor disorder, Structural eye defects, polydactyly, abnormal genitalia, kidney defects.

- **Edwards syndrome**

  Trisomy at chromosome 18, 47XX,+18, Multiple malformation of many organs lowest, Malformed ears, small mouth and nose, mental deficiency

- **Klinefelter syndrome:**

  47,XXY orXXY, Males with some development of breast tissue normally seen in females. Little body hair is present, and such person are typically tall, have small testes. Infertility results from absent sperm.

- **Turner syndrome**:

  45, X, Turner syndrome is associated with underdeveloped ovaries, short stature, webbed, and is only in women. Bull neck, and broad chest. Individuals are sterile, and lack expected secondary sexual characteristics.

Autosomes	Sex-Chromosomes
Numerical abnormalities	Turner syndrome
Trisomy	45, X
8 trisomy	46, Xi (Xq) and mosaics of Xq cell line
9 trisomy	46, Xdel (Xq) and mosaics Xq cell line
13 trisomy (Patau syndrome)	Mosaics 45X/46 XX 45X/47 XXX
18 trisomy (Edwards syndrome)	Mosaics 45, X/46 XY
21 trisomy (Down syndrome)	Other (del, Xp, X mosaics)
22 trisomy	Klinefelters syndrome
Polyploidy	47, XXY
Triploidy	48, XXXY
Tetraploidy	49, XXXXY
Structural abnormalities	Mosaics
Partial trisomy	Others
1 q trisomy	Polysomy X-females
2 q trisomy	47, XXX
3 q trisomy	48, XXXX
5 p trisomy	49, XXXXX
7 q trisomy	Mosaics
9, p, trisomy	Polysomy-Y
10q trisomy	47. XYY
14q trisomy	Others
22q trisomy	

Polyploids (three or more genomes):

The organisms having more than two sets of chromosomes by the addition of another chromosome is called polyploidy. It may arise as a result of abnormal mitosis where chromosomes divide by cytoplasm fails to divide during cytokinesis. The basic set of chromosomes undergoes multiplications. For example, in *Chrysanthemum* basic set is x = 9. Its species and hybrids show multiple of 9, such as 18, 27, 36, 45. In *Nicotiana* and *Solanum* basic set is x = 12 and multiple of somatic chromosome numbers are 24, 48 and 72 and in *Triticum* it is x = 7 and multiples are 14, 21, 42.

Origin of Polyploidy:

i) Polyploids are originated by failure of normal mitotic division in somatic cells.
ii) Polyploids may originate due to abnormal reduction divisions resulting
iii) It may naturally occur due to fertilization of egg by more than one male gamete.
iv) It may originate by artificial induction using colchicines or may be originated by cross hybridization between haploid and diploids.

Types of Polyploidy:

There are mainly four different types of Polyploidy, namely:

i) Autopolyploidy,
ii) Allopolyploids,
iii) Segmental allopolyploids and
iv) Auto-allopolyploids.

(i) Auto-Polyploidy:

When more than two genomes developed by the multiplication of chromosome number of some individual is called Autopolyploids. On the other hand autopolyploids are the individuals of which body cells contains more than two identical set of chromosomes derived by self-duplication. It arises due to failure of anaphase.

Examples:

Autotriploids (3n) – *Oenothera, Datura, Dahlia*, Rose etc. autotriploids are more vigorous, shows more perenation of the organs of vegetative propagation and are highly sterile.

Autotetraploids: (4n) – it contain four identical genomes and arise by fusion of two diploid gametes. It may results from duplication of somatic chromosomes. Autotetraploids show great adaptability, disease resistant, larger seeds, high vitamin-C content and low fertility. These are found in Apples, Grapes, Marigolds.

Meiosis in an Autopolyploid:

Meiotic behaviour in an autopolyploid such as autotetraploid is different than in a diploid. This is due to the presence of four homologous chromosomes of each kind.

Assuming that the primary material is a diploid species with 14 chromosomes (AA), these will form seven pairs (bivalents) at meiosis . In the tetraploid (AAAA) there will be four chromosomes of each type, and at meiosis, these seven groups of four chromosomes may form seven quadrivalents.

A quadrivalent is an association of four homologous chromosomes. Quadrivalents may be of different appearances. Sometimes, the homologous chro-mosomes are represented by an association of three chromosomes, called a trivalent and a univalent or by two bivalents.

As a rule, the average number of quadrivalents per cell is, therefore, lower than the medium possible num-ber. Autotetraploids of different species behave differently in this respect. Some of them have a very high frequency of quadrivalents as in *A. tuberosum*, in some cases bivalents are formed.

The occurrence of trivaients and univalents at meiosis in an autotetraploid leads to disturbances in chromosome distribution and to the formation of gametes with deviating chromosome numbers. This is the principal cause for the high degree of sterility in an autotetraploid.

Segregation of Genes in Autopolyploids:

The number of alleles of each gene is represented according to the ploidy level of the Polyploidy individual and gametes containing more than one allele of each gene (homo- or heterozygotic) may be produced.

According to the number of dominant and recessive alleles at a particular locus, the genotype of an autotetraploid may be quadriplex (AAAA or A_4), triplex (AAAa or A_3a), duplex (AAaa or A_2a_2), monoplex or simplex (Aaaa or Aa_3) and nulliplex (aaaa or a_4).

Autopolyploidy such as tetraploids shows the so called tetrasomic inheritance. The segregation of genes in autopolyploidy is affected by factors which play no essential role in diploid.

Among such factors are the number and position of chiasmata in the multivalents, the distance between particular locus and centromere, the behaviour of homologues in multivalent associations during anaphase I and the presence of univalents.

In auto-tetraploids, if it is assumed that the four homologous chromosomes are distributed to poles in 2:2 during anaphase I, theoretical seg-regation ratios for various autotetraploid genotypes of a locus may be calculated.

Table 11.3: Frequencies of the gamete types and zygote types of autotetraploid genotypes

Parent	Gametes				Zygotes					
Genotype	AA	Aa	aa	divisor	A^4	A^3a	A^2a^2	Aa^3	a^4	divisor
Quadriplex (AAAA)	1	–	–	1	1	–	–	–	–	1
Triplex (AAAa)	1	1	–	2	1	2	1	–	–	4
Duplex (AAaa)	1	1	1	6	1	8	18	8	1	36
Monoplex (Aaaa)	–	1	1	2	–	–	1	2	1	4
Nulliplex (aaaa)	–	–	1	1	–	–	–	–	1	1

Applications of Autopolyploidy in Crop Improvement

Autopolyploidy has found some valuable applications in crop improvement. These are briefly summarized below:

Triploids –

Triploids are produced by hybridization between tetraploid and diploid strains. They are generally highly sterile, except in a few cases. This feature is useful in the production of

seedless watermelons. In certain species, they may be more vigorous than the normal diploids, e.g., in sugarbeets. These two examples are described in some detail.

Seedless watermelons are grown commercially in Japan. They are produced by crossing tetraploid (4x, used as female) and diploid (2x, used as male) lines, since the reciprocal cross (2x x 4x) is not successful. The triploid plants do not produce true seeds; almost all the seeds are small, white rudimentary structures like cucumber (*Cucumis sativus*) seeds. But a few normal sized seeds may occur, which are empty. For good fruit setting, pollination is essential. For this purpose, diploid lines are planted in the ratio 1 diploid: 5 triploid plants. There are several, problems, viz., genetic instability of 4x lines, irregular fruit shape, a tendency towards hollowness of fruits, production of empty seeds and the labour involved in triploid seed production (by hand pollination). Recently, some diploid hybrids of watermelon ('ice-box type') have been developed that produce seedless fruits (all their seeds are like cucumber seeds).

Triploid sugarbeet produce larger roots and more sugar per unit area than do diploids, while tetraploids produce smaller roots and lower yields than diploids. Apparently, 3x is the optimum level of ploidy in sugarbeets.

Tetraploids –

Autotetraploids have been produced in a large number of crop species and have been extensively studied in several cases. Tetraploids may be useful in one of the following ways: (1) in breeding, (2) improving quality, (3) overcoming self-incompatibility, (4) making distant crosses and (5) used directly as varieties.

In banana (*M. sapientum*), autotetraploids are inferior to triploids in that they have weaker leaves and increased fertility. But they offer the only available chance of adding disease resistance to commercially successful varieties. In banana, autoteraploids are produced by chance fertilization of an unreduced triploid egg (AAA) by a haploid pollen from a disease resistant diploid parent. A large number of such tetraploids have been produced, but they have not yet gained any commercial success. This is an unusual case where auto tetraploidy is the only practical approach to breeding an otherwise successful triploid crop species.

Autotetraploidy is able to overcome self-incompatibility in certain cases, e.g., some genotypes of tobacco and white clover (*Trifolium repens*), *Petunia,* etc. Certain distant crosses are not successful at the diploid level, but are relatively successful at the autotetraploid level, e.g., 4x *Brassica oleracea* x *B. chinensis* is successful, but when *B. oleracea* is diploid it is unsuccessful. Similarly, autotetraploids of certain *Solanum* species produce hybrids with *S. tuberosum*, while the diploids do not.

Autotetraploids are larger in size and are more vigorous than diploids. Autotetraploid varieties of forage crops have been considerably successful. The most successful examples are tetraploid red clover (*Trifolium pratense*) and ryegrass (*Lolium perenne*). Other examples are tetraploids of alsika clover (*Trifolium hybridum,* Variety Tetra) and berseem (*Trifolium alexandrium*, variety Pusa Giant Berseem).

(ii) Allopolyploids:

Polyploidy may also result from doubling of chromosome number in hybrid which is derived from two or more distinctly different species. This brings two (or more) different sets of chromosome in hybrid. The doubling of chromo-somes in the hybrid, which gives rise to a Polyploidy, is called an allopolyploid.

An allopolyploid in which a sterile hybrid (AB) originating out of the combination of two different species, undergoes duplication of chromosome set, is known as amphidiploid (AABB).

Raphanobrassica is a classical example of amphidiploidy. In 1927, Karpechenko, a Russian scientist, reported a cross between *Raphanus sativus* (2n = 18) and *Brassica oleracea* (2n = 18) to produce F2 hybrids which were completely sterile.

This sterility was due to lack of chromosome pairing, since there is no homology between genomes from *Raphanus sativus* and *Brassica oleracea*. Among these sterile hybrids certain fertile plants were found. On cytological examination, these fertile plants were found to have 2n = 36 chromosomes, which showed normal pairing into 18 bivalents.

Thus in allopolyploids the paring is of autosyndesis type (paternal-paternal or maternal- maternal pairing) in contrast to allosyndesis (paternal-maternal pairing) in diploids and autopolyploid.

Of the allopolyploids, amphidiploid hybrids containing two sets of each species are of special importance because they are usually fertile, occur rather widely among angiosperms in nature, afford clues to the relationship of certain species, and open a new path to the improvement of cultivated plants.

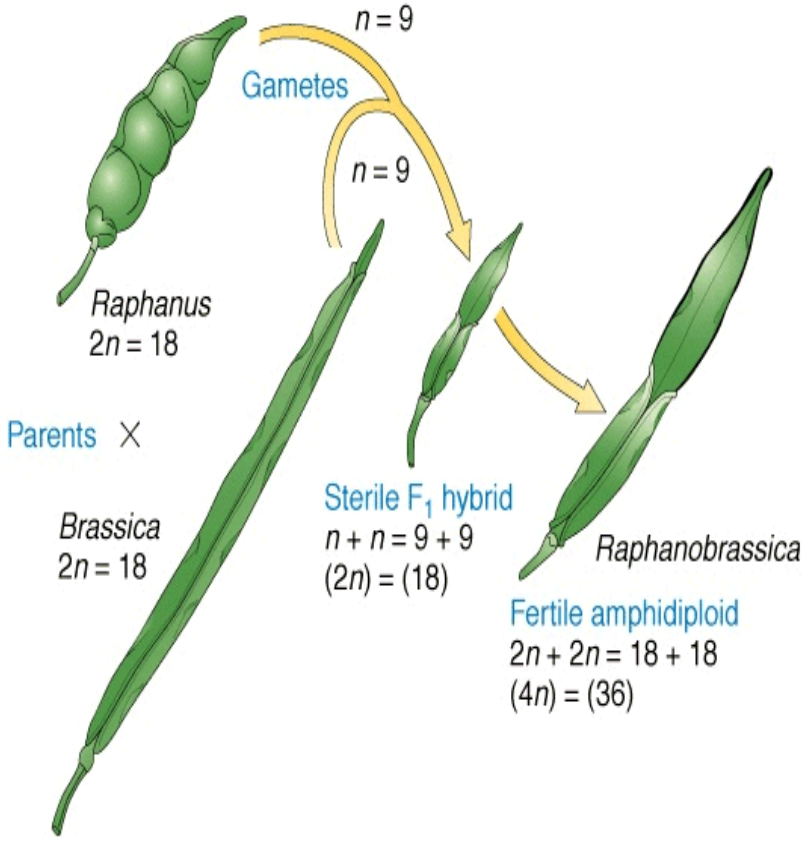


Fig. *Raphanobrassica*. Source: http://theelusiveguanaco.blogspot.com/2011/06/joy-of-chromosomes-part-2_16.html

Triticale is the most successful synthetic allopolyploid produced by crossing wheat (tetraploid or hexaploid) with rye. *Triticales* derived from tetraploid wheats have been the most successful, but those from hexaploid wheats may also become a successful crop species. The breeding strategy involves (l) production of a large number of triticale strains using different combinations (varieties as well as species) of wheat and, rye, (2) hybridization of these triticale strains among themselves, and (3) improvement of the defects of the triticale through selection.

One of the earliest known amphidiploid hybrids was the fertile *Primula kewensis*, with 36 somatic chromosomes. A cross between *P. floribunda* (2n = 18) and *P. verticillata* (2n = 18) had yielded the sterile diploid *P. kewensis* (2n = 18) with one genome from each parent species.

Amphidiploids sometimes arise in ways other than by somatic chromosome doubling. Diploid spores, and, therefore, diploid gametes may appear on failure of meiosis and union of two diploid gametes gives rise to tetraploid. Although the chance of obtaining such plants in this manner seems to be relatively small.

(iii) Segmental Allopolyploids:

In some allopolyploids the different genomes that are present are not quite different from one another, i.e., having partial homology with each other (616, 8282). Consequently, in this Polyploidy, chromosomes from different genomes do pair together to some extent and multivalents are formed. This means that segments of chromosomes and not the whole chromosome is homologous.

Such allopolyploids are called segmental allopolyploids (Stebbins). These chromosomes which are partially homologous and not completely homologous with each other are sometimes also described as homologous chromosomes. It is also believed that most of the naturally occurring Polyploidy are neither true autopolyploidy nor true allopolyploids.
Solanum tuberosum is the best example of segmental allopolyploid.

(iv) Auto-Allopolyploids:

When autopolyploidy is combined with allopolyploidy, autoallopolyploids are produced (AAAA6B). Polyploidy of this type are possible from hexaploid level upward as observed in *Nicotiana tabacum* and *Solanum nigrum*. Autoallopolyploids have importance in the evolution of certain plant species.

Role of Polyploidy:

Some of the important roles played by polyploidy are described below:

a) Role of Polyploidy in Plant Breeding:

When the techniques for artificial chromosome doubling became established, investigations on the origin of many of our economic plants were resumed. Many important crop plants like wheat, oat, sugarcane, cotton, tobacco as well as many fruits and vegetables are the Polyploidy of various degrees.

One of the important effects of polyploidy is the changes in the blooming season of the induced Polyploidy. As such, interspecific hybrids can be obtained of such species which otherwise remain isolated by seasonal isolation and different blooming season.

By artificial polyploidy induction, disease resistance and other desirable characters have been incorporated into some commercial crop plants. For example, *Nicotiana tabacum* is susceptible to TMV whereas *N. glutinosa* appears to be resistant.

The two tobacco species when crossed, the hybrids were found to be resistant but totally sterile. When the chromosomes were doubled it was possible to secure a fertile Polyploidy resistant to the virus. Many Polyploidy are selected and culti-vated because of their larger size, vigour and ornamental values. Several varieties of apples, pears and grapes have produced giant fruits which are of much economic value.

b). Role of Polyploidy in Evolution:

Polyploidy combined with interspecific hybridization provides a mechanism by which new species may arise in nature and play a role in evolution. Allopolyploidy can produce new species by combining new characters and stable in evolution. It has already been discussed under amphidiploidy how different types of new species may be evolved.

Among the inter-specific hybridization, the most important are *Primula kewensis* (n = 18) obtained by crossing *P. floribunda* (n = 9) and *P. verticillata* (n = 9), *Digitalis mertqnensis* (n = 56) obtained by crossing *D. pur*purea (n = 28) and *D. ambigua* (n = 28) and *Spartina townsendii* (n = 63) obtained from cross of *S. stricta* (n = 28) and *S. alterniflora* (n = 35).

Origin of some of the economically important plants like rice, wheat, cotton, tobacco is important in this aspect. The chromosome number of rice (*Oryza sativa*) is 2n = 24. It is an example of typical secondary allopolyploids with basic chromosome number x = 5.

The present cultivated variety of rice is actually produced by hybridization followed by aneuploidy and euploidy. The origin of wheat, cotton, Mustard, etc. are given below:

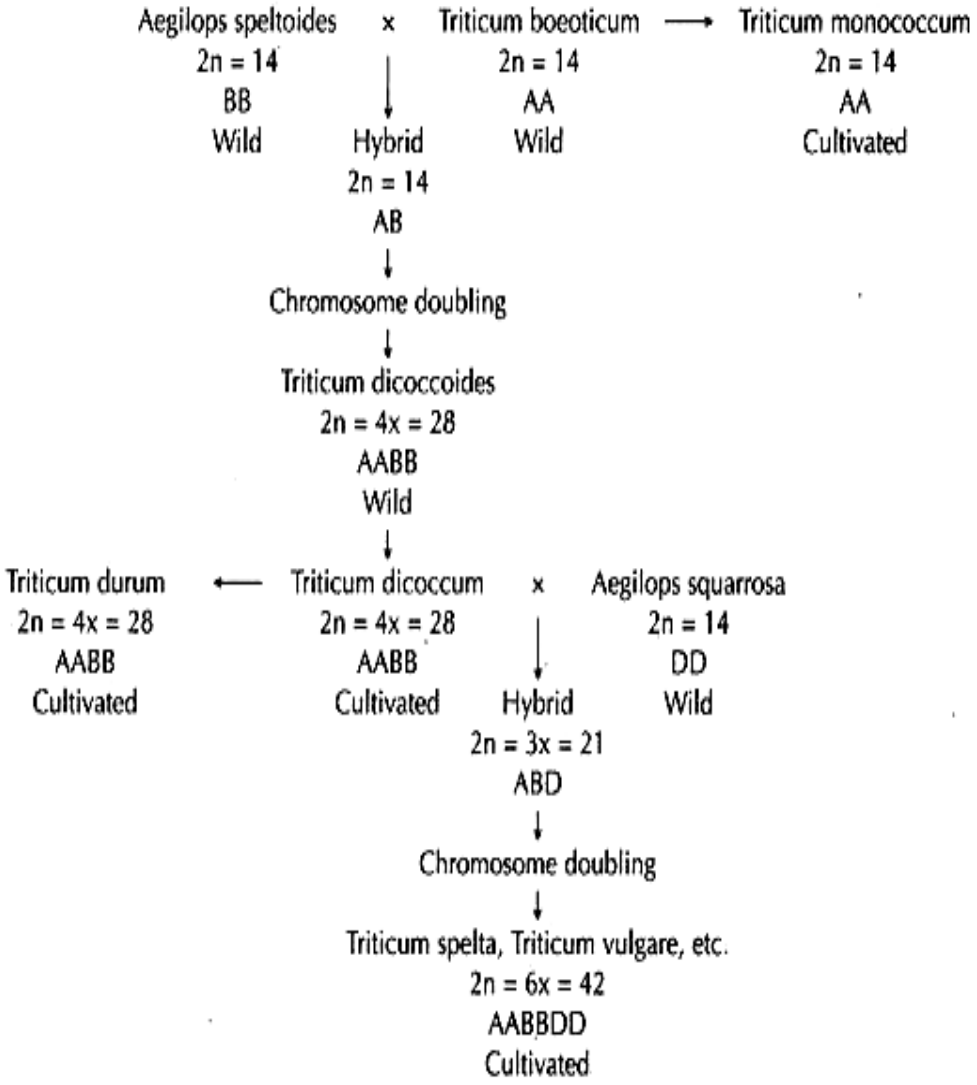


Fig. 11.12: Diagrammatic representation of the origin of tetraploid and hexaploid cultivated wheat from their wild ancestors

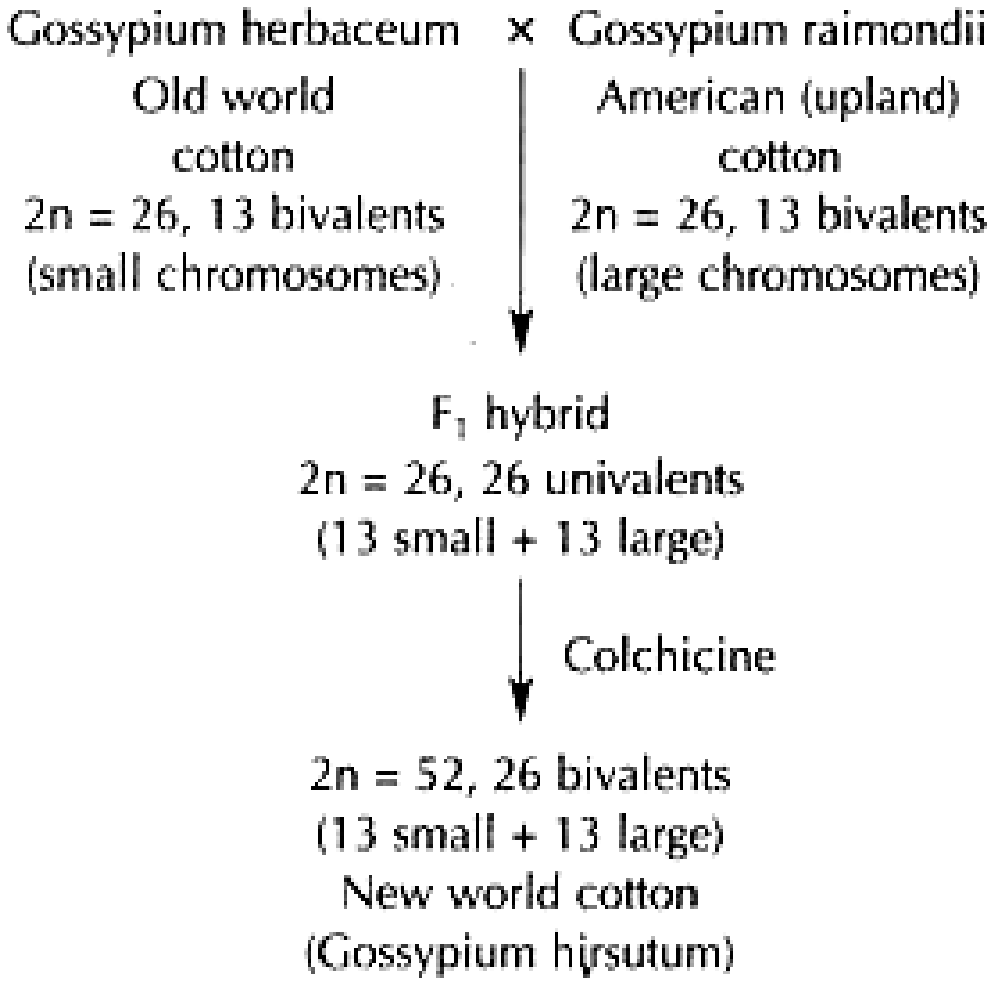


Fig. 11.13: Origin of new world cotton

Source: http://www.biologydiscussion.com/cell/notes-on-polyploidy-cell/38312

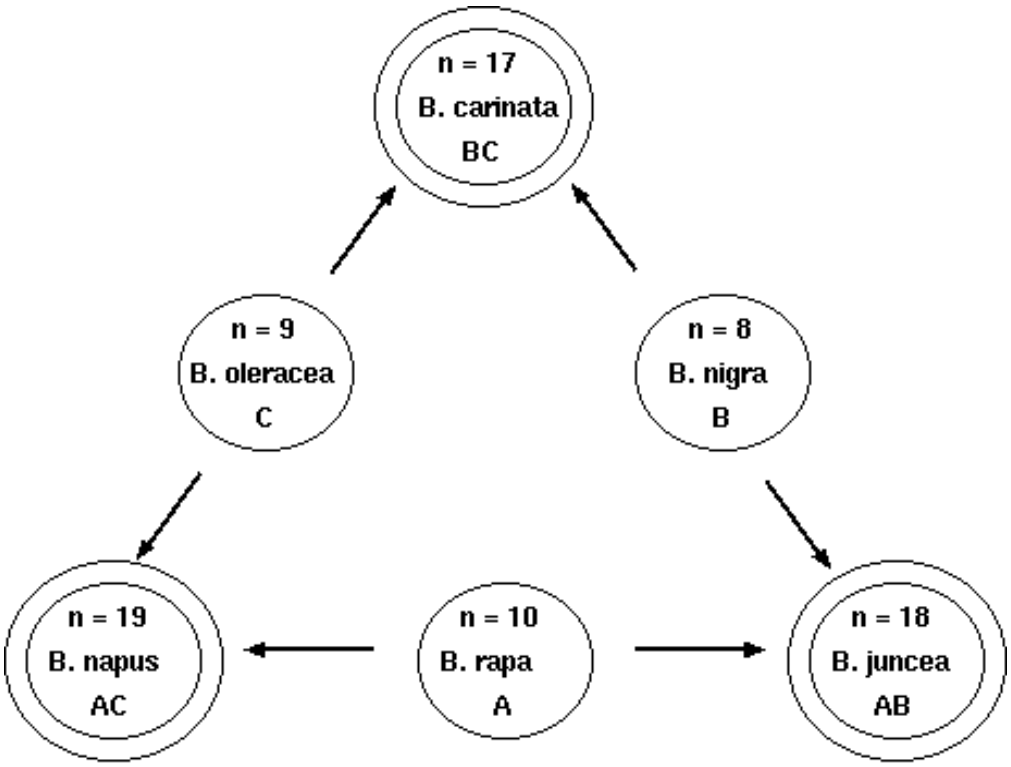


Fig. Origin of *Brassica* (triangle)

Source: http://www.biologydiscussion.com/genetics/cytoplasmic-inheritance

c) Media of Conservation of Characters:

Polyploidy plays an important role in conserving the characters. A recessive mutation in order to be expressed in an autotetraploid, all four genes must be in recessive condition which is a time requiring process. Thus the characters in a Polyploidy plant could be conserved.

d) Polyploidy and Geographical Distribution:

The Polyploidy plants can cope with diverse geographical areas than a diploid. Hence, the geographical distributions of Polyploidy plants are greater than diploids. Autopolyploidy cannot produce new species, but they can colonize a new environment easily. As allopolyploids contain different genomes, they can withstand different environmental condition.

Both these power of colonization and coping with a diverse environment of the Polyploidy plants, help their wide geographical distribution.

Limitation of Polyploidy:

Polyploidy has several limitations. Some important limitations of polyploidy in crop improvement are briefly presented below:

1. Limited use: The single species polyploidy has limited applications. It is generally useful in those crop species which propagate asexually like banana, potato, sugarcane, grapes etc.

2. Difficulty in maintenance: The maintenance of monoploids and triploids is not possible in case of sexually propagating crop species.

3. Undesirable characters: In bispecies or multispecies polyploids characters are contributed by each of the parental species. These characters may be sometimes undesirable as in case of *Raphanobrassica*.

4. Some other defects: Induced polyploids have several defects such as low fertility, genetic instability, low growth rate, late maturity, etc.

5. Chances of developing new species through allopolyploidy are extremely low.

8. Special Chromosomes: Lampbrush, Polytene and B-chromosome.

Lampbrush Chromosome:

These are the largest known chromosomes found in the yolk rich oocytic nuclei of certain vertebrates such as fishes, amphibians, reptiles and birds.

They can be seen with naked eye and are characterized by fine lateral loops, arising from the chromomeres, during first prophase (diplotene) of meiosis.

These loops give it a brush-like appearance; that is why these are called lampbrush chromosomes first discovered by Flemming in 1882 and were described in shark oocytes by

Ruckert (1892). Lampbrush chromosomes of certain urodele oocytes may reach upto 5900µ in length.

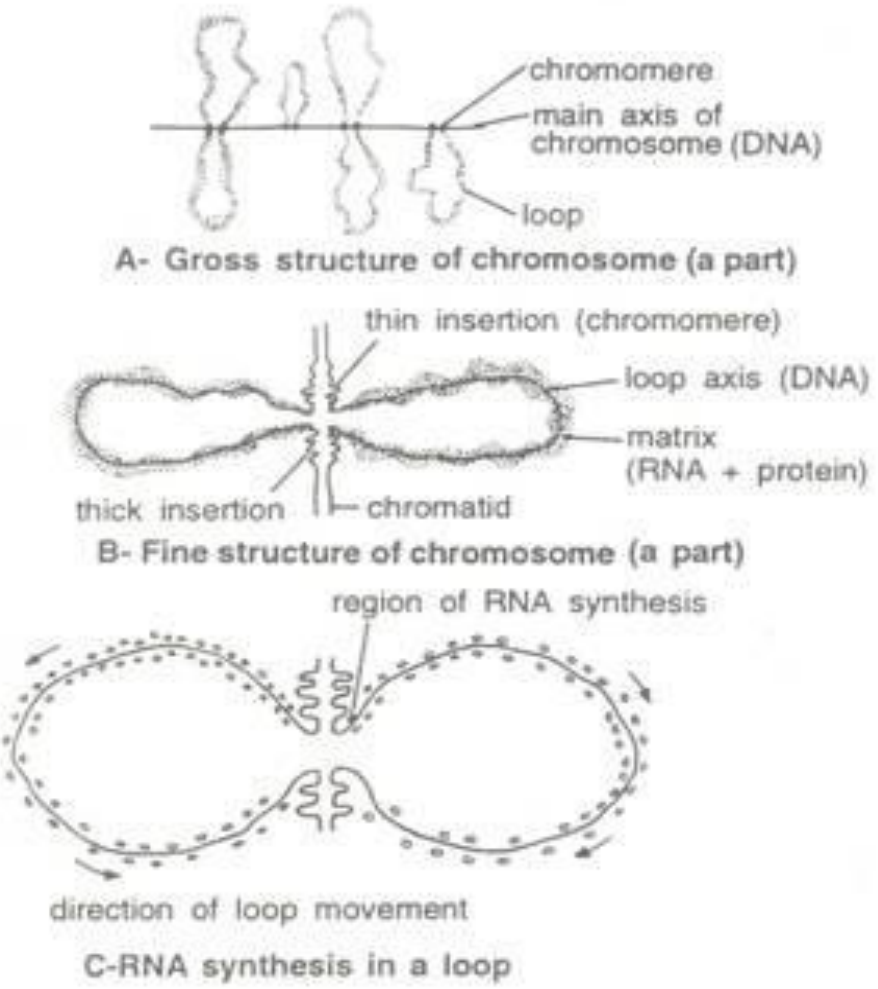
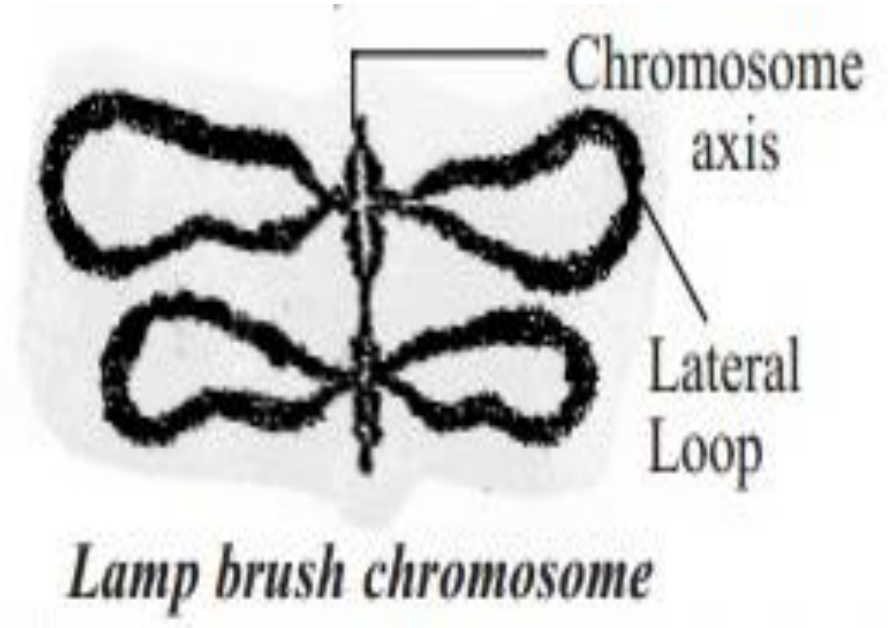


Fig. 11. Lampbrush chromosome.

It consists of longitudinal axis formed by a single DNA molecule along which several hundred bead-like chromomeres are distributed in a linear fashion. From each chromomere there emerge two symmetrical lateral loops (one for each chromatid), which are able to expand or contract in response to various environmental conditions.

About 5 to 10% of the DNA is in the lateral loops. Loop formation reduces the mass of the corresponding chromomeres, implying a spinning out of chromomere material into the lateral strands. The centromeres also have the appearance of elongate Feulgen-positive chromomeres but they characteristically lack lateral loops.



Lamp brush chromosome

Lampbrush lateral loops are formed of DNA, in chromomeres regions DNA is tightly folded and transcriptionally inactive. In lateral loops RNA synthesis is intense. Each loop in turn has an axis formed by a single DNA molecule, which is coated by a matrix of nascent RNA and proteins. The matrix is asymmetrical, being thicker at one end of the loop. RNA synthesis starts at the thinner end and progresses toward the thicker end.

Functions of Lampbrush chromosomes –

a) Synthesis of RNA: Functions of lampbrush chromosomes involve synthesis of RNA and protein by their loops. RNA is synthesized only at the thin insertion and then carried around the loops to the thick insertion. There it may be either destroyed or released into nucleus.

b) Formation of yolk material: There are some probabilities that lampbrush chromosomes help in the formation of certain amount of yolk material for the egg.

Polytene Chromosome:

Polytene chromosomes provided the first evidence that eukaryotic gene activity is regulated at the level of RNA synthesis. When dipteran chromosomes become polytenic, the DNA replicates by endomitosis, and the resulting daughter chromatids remain aligned side by side. There chromatids are visible during interphase and have a characteristic morphology of dark bands and alternating interbands. Within these chromosomes it is possible to observe the genetic activity of specific loci at local enlargements called puffs, which represent DNA

undergoing intense gene transcription. Puff distribution varies from one tissue to another and can be induced experimentally, indicating the cell specialization results from variable gene transcription. Polytene chromosomes constitute a valuable material for the study of gene regulation because their gene transcription can be visualized directly in the microscope.

Some cells of dipteran (flies, mosquitoes, midges) larvae become very large and have a high DNA content. The most prominent ones are located in the salivary gland, but other cells from the gut, fat body, and malpighian tubules of the larva also become 'polytenic'. (Polyteny differs from polyploidy, in which there is also excess DNA per nucleus, but in which the new chromosomes are separate from each other).

A polytene chromosome of *Drosophila* salivary glands has about 1000 DNA molecules arranged side by side which arise from 10 rounds of DNA replication (210 = 1024). Other dipteran species have even more DNA molecules per polytene chromosome, for example, *Chironomus* has 16,000.

In polytene cells, the chromosomes are visible during interphase, and the chromomeres (regions in which the chromatin is more tightly coiled) alternate with regions where the DNA fibres are folded more loosely. The alignment of many chromosomes gives polytene chromosomes their characteristic morphology, in which a series of dark bands alternate with clear zones called interbands. There are about 5000 bands in the *Drosophilla* genome. They have characteristic morphology and positions, which permit detailed chromosome mapping.
An additional characteristic of polytene chromosomes is that the maternal and paternal homologue remains associated side by side, in what is called 'somatic pairing'. This permits the identification of deletions, inversions, duplication as regions looped out of the chromosomes. The pericentromeric heterochromatin of all the *Drosophila* chromosomes coalesces in a chromocenter, where the chromosomes are joined together. The satellite DNAs of the chromocenter are underreplicated with respect to the rest of the chromosome, (i.e., they undergo fewer rounds of replication). Polytene cells are unable to undergo mitosis and are destined to die. Not all the cells in a dipteran larva have polytene chromosomes. Those destined to produce the adult structures after metamorphosis (imaginal discs) remains diploid.

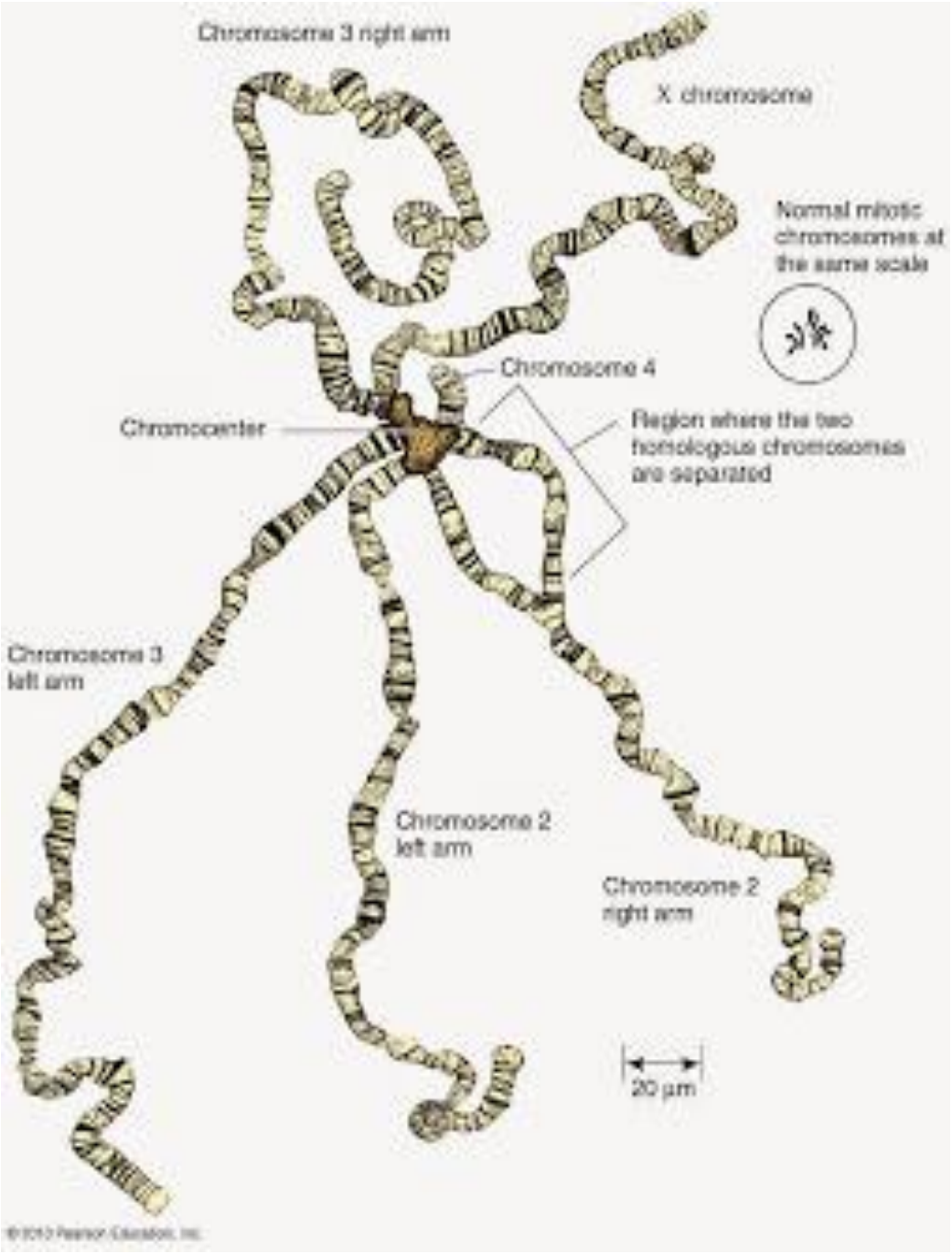


Fig. Polytene chromosome.

Source: https://www.mun.ca/biology/scarr/Polytene_Chromosomes.html

Polytene chromosomes have become even more important with the advent of recombinant DNA techniques because they make it possible to map any DNA segment to specific chromosomal loci by in sit hybridization. Polytene chromosomes are very suitable for in situ

hybridization because their 1000 DNA molecules are aligned side by side, thereby greatly facilitating the detection of single copy genes.

The bands have greatly helped in the mapping of chromosomes in cytogenetics studies. The bands occasionally form reversible "puffs" known as "chromosome puffs" or "Balbiani rings" which are associated with differential gene activation. A puff can be considered a band in which the DNA unfolds into open loops as a consequence of intense gene transcription, i.e., "puffs are sites of intense gene transcription".

In salivary glands, the appearance of some puffs has been correlated with the production of specific proteins which are secreted in large amounts in the larval saliva, e.g. *Chironomou*s has at the base of the salivary glands four specialized cells that contain cytoplasmic granules of a special secretary protein. The gene for this protein is located in a distinct puff that appears only in the four specialized cells. These results show that cell specialization results from variable gene transcription.

In *D. melanogaster* the giant chromosomes are found in the form of five long and one short strands radiating from a single more or less amorphous mass known as chromocentre. One long strand corresponds to the X-chromosome and the remaining four long strands are the arms of IInd and IIIrd chromosomes. The short strand which is small dot like is IVth chromosome. The centromeres of all there chromosomes fuse to form chromocentre. In the male files, the Y chromosome is also found fused within the chromocentre and is therefore not seen as a separate strand.

B chromosomes

B chromosomes are extra chromosomes to the standard complement that occur in many organisms. They can originate in a number of ways including derivation from autosomes and sex chromosomes in intra- and interspecies crosses.

In addition to the normal karyotype, wild populations of many animal, plant, and fungi species contain B chromosomes (also known as **supernumerary, accessory chromosomes**). These chromosomes are not essential for the life of a species, and are lacking in some (usually most) of the individuals. Thus a population would consist of individuals with 0, 1, 2, 3 supernumeraries.

Most B chromosomes are mainly or entirely heterochromatic, but some, such as the B chromosomes of maize, contain sizeable euchromatic segments. In general it seems unlikely that supernumeraries would persist in a species unless there was some positive adaptive advantage, which in a few cases has been identified. For instance, the British grasshopper *Myrmeleotettix maculatus* has two structural types of B chromosomes: metacentrics and submetacentrics. The supernumeraries, which have a satellite DNA, occur in warm, dry environments, and are scarce or absent in humid, cooler localities.

The evolutionary origin of supernumerary chromosomes is obscure, but presumably they must have been derived from heterochromatic segments of normal chromosomes in the remote past.

Function:

B chromosomes may play a positive role on normal A chromosomes in some circumstances. The B chromosomes suppress homologous pairing which reduces multiple pairing between homologous chromosomes in allopolyploids. Bivalent pairing is ensured by a gene on chromosome 5 of the B genome Ph locus. The B chromosomes also have the following effects on A chromosomes:

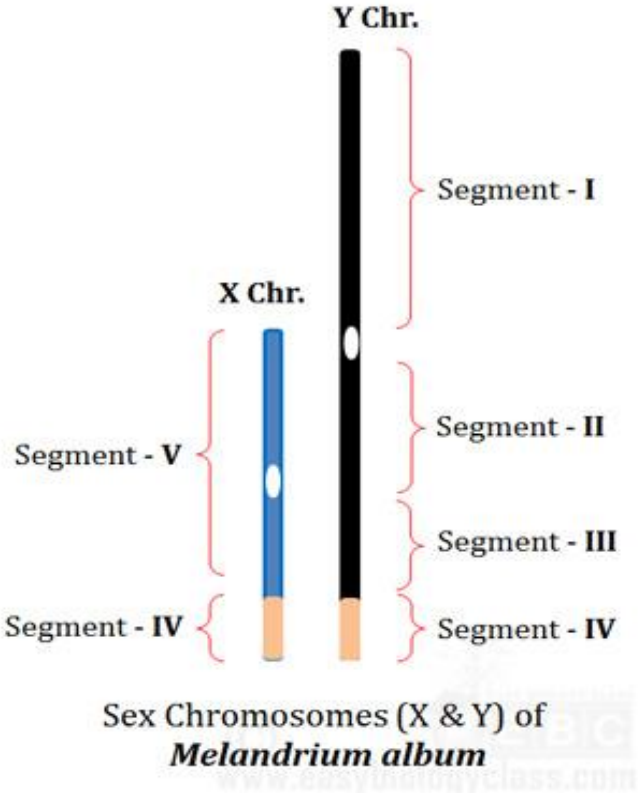
- increases asymmetry chiasma distribution
- increases crossing over and recombination frequencies: increases variation
- cause increased unpaired chromosomes: infertility
- B chromosomes have tendency to accumulate in meiotic cell products resulting in an increase of B number over generations. However this effect is counterbalanced for selection against infertility.

9. Sex Determination: Sex determination in plants; dosage compensation; sex linked inheritance.

Sex determination

A sex-determination system is a biological system that determines the development of sexual characteristics in an organism. Most organisms that create their offspring using sexual reproduction have two sexes. Occasionally, there are hermaphrodites in place of one or both sexes. There are also some species that are only one sex due to parthenogenesis, the act of a female reproducing without fertilization.

In many species, sex determination is genetic: males and females have different alleles or even different genes that specify their sexual morphology. In animals, this is often accompanied by chromosomal differences, generally through combinations of XY, ZW, XO, ZO chromosomes, or haplodiploidy. The sexual differentiation is generally triggered by a main gene (a "sex locus"), with a multitude of other genes following in a domino effect.



Sex Chromosomes (X & Y) of *Melandrium album*

Source: Snustad and Simmons, 2012, Principles of Genetics -6th ed.

The **XX/XY sex determination system** is the most familiar, as it is found in humans. The XX/XY system is found in most other mammals, as well as some insects. In this system, most females have two of the same kind of sex chromosome (XX); while, most males have two distinct sex chromosomes (XY). The X and Y sex chromosomes are different in shape and size from each other, unlike the rest of the chromosomes (autosomes), and are sometimes called allosomes. In some species, such as

humans, organisms remain sex indifferent for a time after they're created; in others, however, such as fruit flies, sexual differentiation occurs as soon as the egg is fertilized.

Sex determination in plants

The sexual phenotype of individuals is determined by sex chromosomes; males are heterogametic (XY) and females are homogametic (XX). Early cytogenetic studies of sex determining mutants in to conclude that the Y chromosome is divided into three regions relevant to sex expression: one required for the suppression of female development and two required for the promotion of male development. None of these regions would be necessary for the development of female reproductive organs, because these functions would reside on the X or autosomal chromosomes.

The mechanism of sex determination in *Coccinia indica*, a member of family Cucurbitaceae was studied in some detail by Prof. R.P. Roy and his co-workers at Patna University. They studied the sex in diploid, triploid and tetraploid plants with and without Y chromosome and observed that irrespective of the number of X chromosomes and/or autosomes, presence of a single Y chromosome gave a male individual.

Table 17.7. **The relation between chromosome constitution and sex in *Coccinia indica*.**

Chromosome constitution	X/A Ratio	Sex
2A + XX	1.00	Female
2A + XY	0.50	Male
2A + XYY	0.50	Male
3A + XXY	0.67	Male
3A + XXX	1.00	Female
4A + XXXX	1.00	Female
4A + XXXY	0.75	Male

Zea mays (maize) is an example of a monoecious species that produces only unisexual flowers in separate male and female inflorescences, referred to as the tassel and ear, respectively. Unisexuality in maize occurs through the selective elimination of stamens in ear florets (flowers) and by the elimination of pistils in tassel florets. The anther ear (an1) and

dwarf (d1, d2, d3, and d5) mutants of maize are recessive and masculinize ears by preventing stamen abortion in the female florets.

Sexual dimorphism in heterothallic species can be extreme, as exemplified by members of the genus *Micromitrium* (Bryophyte), in which the dwarf male gametophyte grows on the leaves of the markedly larger female plan.

Asparagus is a dioecious form. However, rarely female flowers bear rudimentary anthers and male flowers bear rudimentary pistils. Thus, rare male flowers having poorly developed pistils may set seeds. In one such case, when seeds obtained from a rare male flower, were raised into plants, male and female plants were found to be present in 3:1 ratio. When male plants raised thus were used to pollinate female flowers on female plants, only two-third of them showed segregation indicating that sex is controlled by a single gene. In this case, maleness should be dominant over femaleness and male plants should ordinarily be heterozygous.

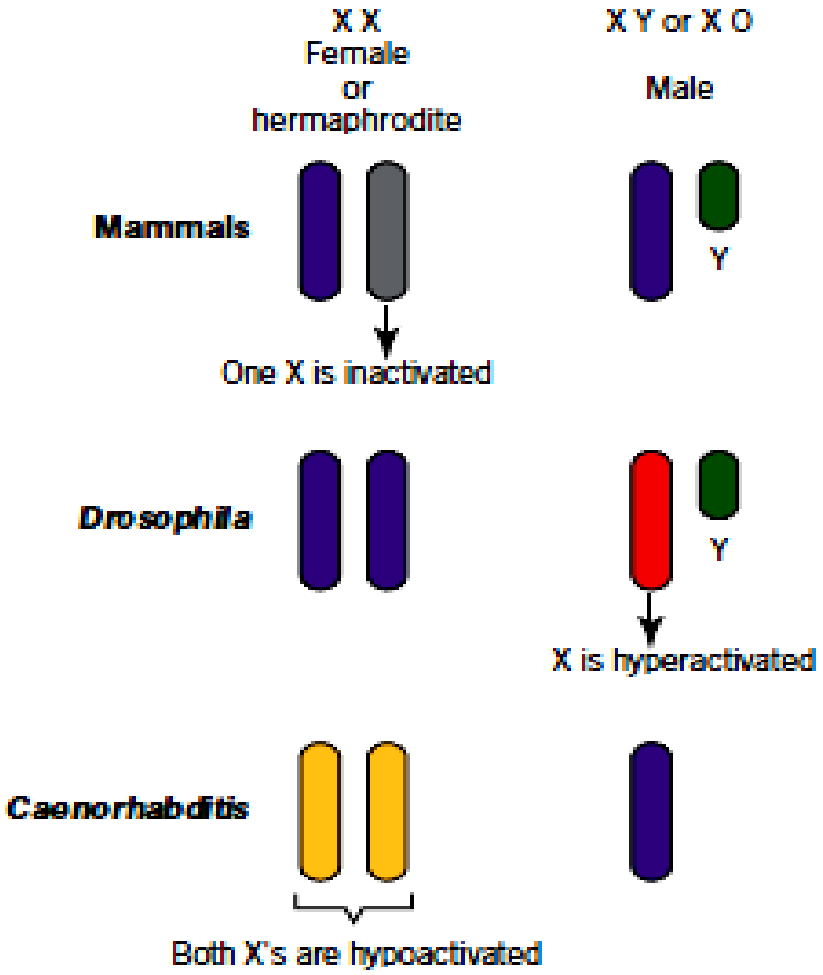
Dosage compensation

In species with XX-XY sex determination, the difference in the number of X chromosomes possessed by males and females presents a special problem in development. Because females have two copies of every X-linked gene and males have only one copy, the amount of gene product (protein) encoded by X-linked genes would differ in the two sexes: females would produce twice as much gene product as that produced by males. This difference could be highly detrimental because protein concentration plays a critical role in development. Animals overcome this potential problem through **dosage compensation,** which equalizes the amount of protein produced by X-linked genes in the two sexes. In fruit flies, dosage compensation is achieved by a doubling of the activity of the genes on the X chromosome of the male.

Normally, each gene is present in two copies. Departures from this condition, either up or down, can cause abnormal phenotypes, and sometimes even death. It is therefore puzzling that so many species should have a sex-determination system based on females with two X chromosomes and males with only one. In these species, how is the numerical difference of X-linked genes accommodated? A priori, three mechanisms may compensate for this difference: (1) each X-linked gene could work twice as hard in males as it does in females, or

(2) one copy of each X-linked gene could be inactivated in females, or (3) each X-linked gene could work half as hard in females as it does in males. Extensive research has shown that all three mechanisms are utilized, the first in *Drosophila*, the second in mammals, and the third in the nematode *Caenorhabditis elegans*.

These three different mechanisms of **dosage compensation**—inactivation, hyperactivation, and hypoactivation have an important feature in common: many different genes are coordinately regulated because they are on the same chromosome. This chromosome wide regulation is superimposed on all other regulatory mechanisms involved in the spatial and temporal expression of these genes.



Three different mechanisms of dosage compensation

Source: Snustad and Simmons, 2012, Principles of Genetics -6th ed.

Inactivation of X chromosomes in mammals

In mammals, X chromosome inactivation begins at a particular site called the X inactivation center (XIC) and then spreads in opposite directions toward the ends of the chromosome. Curiously, not all genes on an inactivated X chromosome are transcriptionally silent. One that remains active is called XIST (for X inactive specific transcript); this gene is located within the XIC. In human beings the XIST gene encodes a 17-kb transcript devoid of any significant open reading frames. It therefore seems unlikely that the XIST gene codes for a protein. Instead, the RNA itself is probably the functional product of the XIST gene. Though polyadenylated, this RNA is restricted to the nucleus and is specifically localized to inactivated X chromosomes; it does not appear to be associated with active X chromosomes in either males or females.

In mice, where fairly detailed experimental analysis has been possible, researchers have found that the homologue of the human XIST gene is transcribed during the early stages of embryonic development at a low level from both of the X chromosomes that are present in females. The transcripts from each of a female mouse's Xist genes are unstable and remain closely associated with their respective genes. As development proceeds, the transcripts from one of the genes stabilize and eventually envelop the entire X chromosome on which that gene is located; the transcripts from the other Xist gene disintegrate, and further transcription from that gene is repressed by methylation of nucleotides in the gene's promoter. Thus, in the female mouse, one X chromosome—the one whose Xist gene continues to be transcribed—becomes coated with Xist RNA and the other does not. The choice of the chromosome that becomes coated is apparently random.

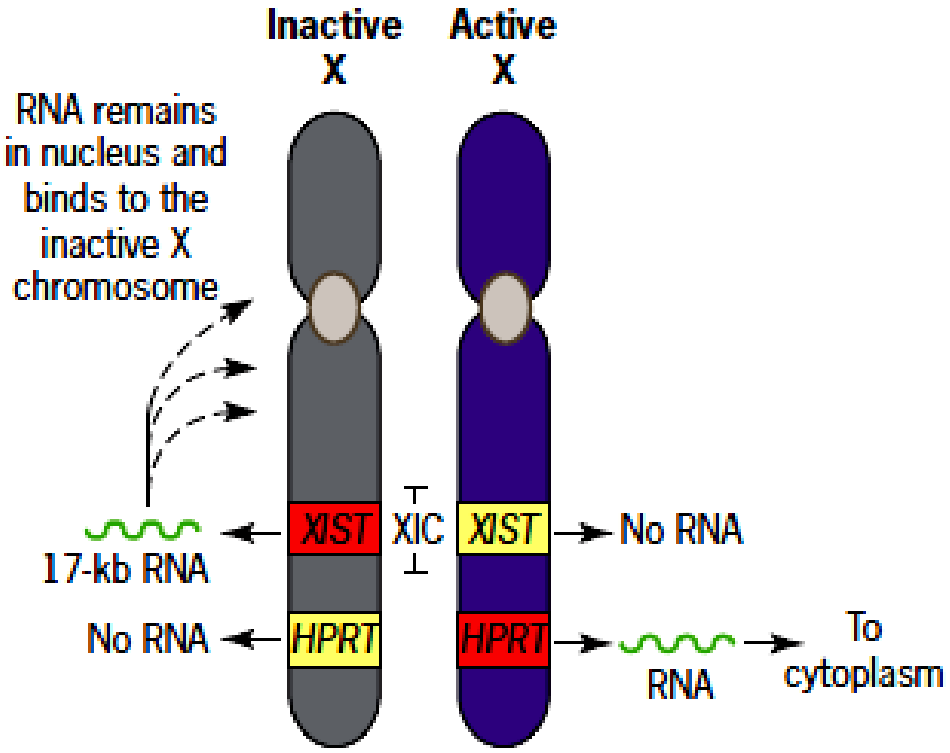
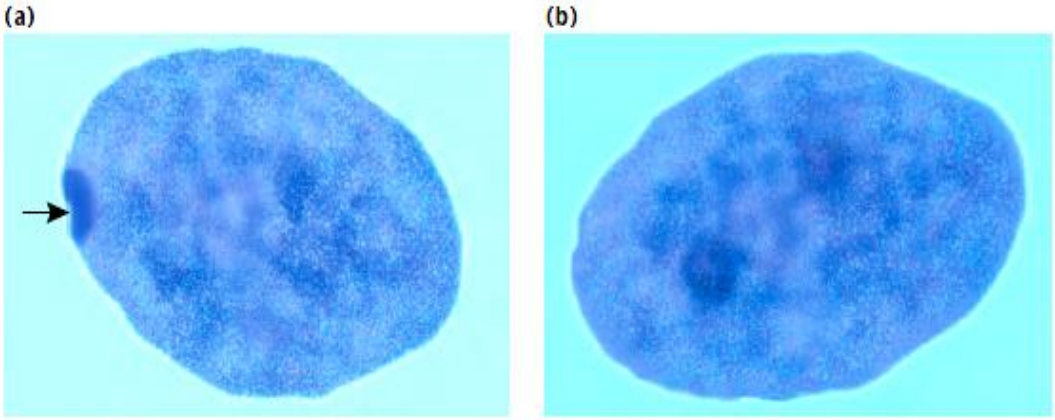


Fig. Inactivation of X chromosome. Source: Snustad and Simmons, 2012, Principles of Genetics -6th ed.

Lyon hypothesis:

In 1949, Murray Barr observed condensed, darkly staining bodies in the nuclei of cells from female cats; this darkly staining structure became known as a **Barr body.** Mary Lyon proposed in 1961 that the Barr body was an inactive X chromosome; her hypothesis has become known as the **Lyon hypothesis.** She suggested that, within each female cell, one of the two X chromosomes becomes inactive; which X chromosome is inactivated is random. If a cell contains more than two X chromosomes, all but one of them is inactivated. The number of Barr bodies present in human cells with different complements of sex chromosomes is shown in.



A Barr body is an inactivated X chromosome.

(a) Female cell with a Barr body (indicated by arrow). (b) Male cell without a Barr body**.**

The patchy distribution of color on tortoiseshell cats results from the random inactivation of one X chromosome in females.

Source: Benjamin A. Pierce, 2012, Genetics: A conceptual apporach -4th ed.

Hyperactivation of x chromosomes in *Drosophila*

In *Drosophila*, dosage compensation requires the protein products of at least five different genes. Null mutations in these genes result in male-specific lethality because the single X chromosome in males is not hyperactivated. Mutant males usually die during the late larval or early pupal stages. These dosage compensation genes are therefore called male-specific lethal (msl) loci, and their products are called the MSL proteins. Antibodies prepared against these proteins have been used as probes to localize the proteins inside cells. The remarkable finding is that each of the MSL proteins binds specifically to the X chromosome in males. These proteins do not bind to the other chromosomes in the male's genome, and they do not bind to any of the chromosomes, including the X's, in a female's genome. The binding of the MSL proteins to the male's X chromosome is facilitated by two types of RNA molecules called roX1 and roX2 (for RNA on the X chromosome) that are transcribed from genes on the X chromosome.

Sex linked inheritance

The inheritance of a trait (phenotype) that is determined by a gene located on one of the sex chromosomes is called sex linked inheritance. The expectations of sex-linked inheritance in any species depend on how the chromosomes determine sex. For example, in humans, males are heterogametic. It has one X chromosome and one Y chromosome. But females are homogametic. They two X chromosomes. In human males, the entire X chromosome is active. But one of a female's X chromosomes is largely inactive. Random inactivation of one X chromosome occurs during the early stages of female embryogenesis. Therefore, every cell that forms from a particular embryonic cell has the same X chromosome inactivated. This pattern of sex determination occurs in most vertebrates, but in birds and many insects and fish the male is the homogametic sex.

In general terms, traits determined by genes on sex chromosomes are not different from traits determined by autosomal genes. Sex-linked traits are distinguishable by their mode of transmission through successive generations of a family. In humans, it is called X-linked or Y-linked inheritance.

Characteristics of Sex Linked Inheritance:

1. It is criss-cross inheritance. Father does not pass the sex-linked allele of a trait to his son. The same is passed to the daughter, from where it reaches the grandson, i.e. diagynic. It is

because the males have only one X-chromosome which is transferred to the female offspring. Only Y-chromosome of the father is transferred to the male offspring but this sex chromosome does not carry many alleles.

2. Mother passes the alleles of sex-linked traits to both sons and daughters.

3. Majority of the sex linked traits are recessive.

4. Sex linked traits are more apparent in males than in females.

5. As many sex-linked traits are harmful, males suffer more from sex-linked disorders.

6. Females generally function as carriers of sex-linked disorders because recessive genes can express themselves in females only in the homozygous state.

I. X-linked recessive Traits: These are expressed in all heterogametic and homogametic which are homozygous for the recessive allele. An example is the sex-linked recessive is horns in sheep that appear only in males. The recessive phenotypes of such genes are more common in males than in females. The examples of X-linked recessive trait in human are Color blindness, Duchenne muscular dystrophy, Hemophilia.

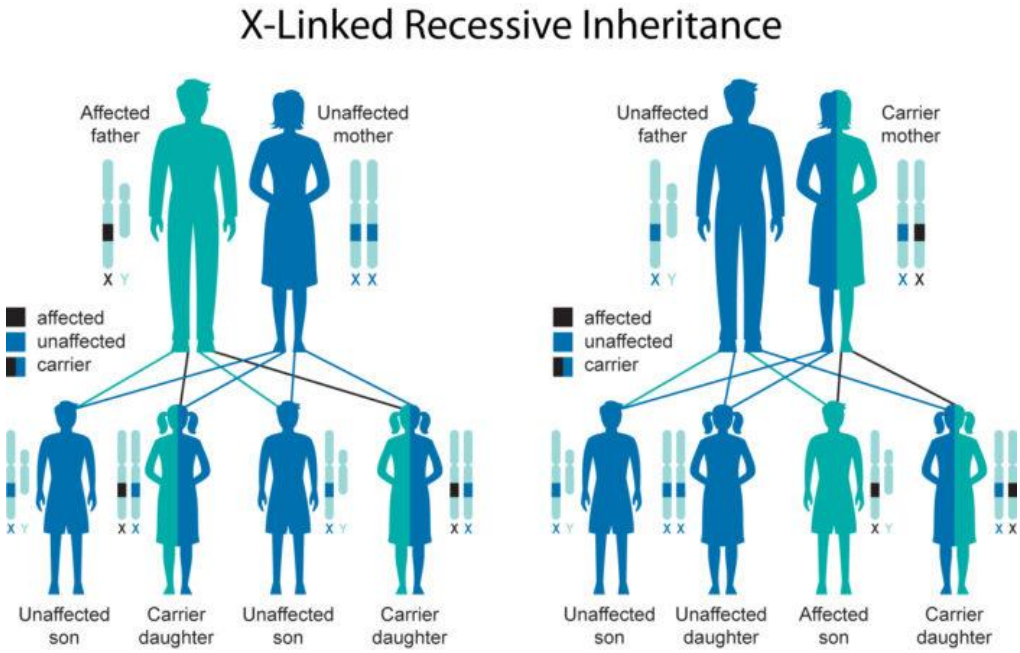


Fig. X-linked recessive inheritance. Source: https://www.nfed.org/learn/genetics-inheritance/

10. Linkage and Crossing Over: Chiasma frequency and genetic map distance; Tetrad analysis; Centromere mapping with ordered tetrad.

Linkage

Linkage is the phenomenon of certain genes staying together during inheritance through generations without any change or separation due to their being present on the same chromosome.

It was Morgan (1910) who clearly proved and defined linkage on the basis of his breeding experiments in fruitfully *Drosophila melanogaster*. In 1911, Morgan and Castle proposed chromosome theory of linkage. It states that

(i) Linked genes occur in the same chromosome.

(ii) They lie in a linear sequence in the chromosome.

(iii) There is a tendency to maintain the parental combination of genes except for occasional crossovers.

(iv) Strength of the linkage between two genes is inversely proportional to the distance between the two, i.e.,, two linked genes show higher frequency of crossing over if the distance between them is higher and lower frequency if the distance is small.

Linked genes are those genes which occur on the same chromosome while unlinked genes are the ones found on different chromosomes. Linked and unlinked genes can be easily known from breeding experiments. Unlinked genes show independent assortment, a di-hybrid ratio of 9:3:3:1 and the di-hybrid or double test cross ratio of 1:1:1:1 with two parental and two recombinant types.

The linked genes do not show independent assortment but remain together and are inherited en block producing only parental type of progeny. They give a dihybrid ratio of 3:1 and a test cross ratio of 1:1.

Types of Linkage:

Linkage is of two types, complete and incomplete.

1. **Complete Linkage:**

The genes located on the same chromosome do not separate and are inherited together over the generations due to the absence of crossing over. Complete linkage allows the combination of parental traits to be inherited as such. It is rare but has been reported in male *Drosophila* and some other heterogametic organisms.

2. **Incomplete Linkage:**

Genes present in the same chromosome have a tendency to separate due to crossing over and hence produce recombinant progeny besides the parental type. The number of recombinant individuals is usually less than the number expected in independent assortment. In independent assortment all the four types (two parental types and two recombinant types) are each 25%. In case of linkage, each of the two parental types is more than 25% while each of the recombinant types is less than 25%.

Linkage Groups:

A linkage group is a linearly arranged group of linked genes which are normally inherited together except for crossing over.

Significance of Linkage:

(i) Linkage plays an important role in determining the nature of scope of hybridization and selection programmes.

(ii) Linkage reduces the chance of recombination of genes and thus helps to hold parental characteristics together. It thus helps organism to maintain its parental, racial and other characters. For this reason plant and animal breeders find it difficult to combine various characters.

Crossing over

Crossing over is the exchange of genetic material between homologous chromosomes that results in recombinant chromosomes during sexual reproduction. It is one of the final phases of genetic recombination, which occurs in the pachytene stage of prophase I of meiosis during a process called synapsis. Synapsis begins before the synaptonemal complex develops and is not completed until near the end of prophase I. Crossover usually occurs when matching regions on matching chromosomes break and then reconnect to the other chromosome.

Crossing over was described, in theory, by Thomas Hunt Morgan.

1. Crossing over takes place during meiotic prophase, i.e.,, during pachytene. Each pair of chromosome has four chromatids at that time.
2. Crossing over occurs between non-sister chromatids. Thus one chromatid from each of the two homologus chromosomes is involved in crossing over.
3. It is universally accepted that crossing over takes place at four strand stage.
4. Each crossing over involves only two of the four chromatids of two homologus chromosomes. However, double or multiple crossing over may involve all four, three or two of the four chromatids, which is very rare.
5. Crossing over leads to re-combinations or new combinations between linked genes. Crossing over generally yields two recombinant types or crossover types and two parental types or non-crossover types.
6. Crossing over generally leads to exchange of equal segments or genes and recombination is always reciprocal. However, unequal crossing over has also been reported.
7. The value of crossover or recombinants may vary from 0-50%.
8. The frequency of recombinants can be worked out from the test cross progeny. It is expressed as the percentage ratio of recombinants to the total population (recombinants + parental types). Thus,

$$\text{Crossing over frequency (\%)} = \frac{\text{No. of recombinants}}{\text{Total progeny}} \times 100$$

Cases of two strand crossing over, somatic crossing over, sister strand crossing over and unequal crossing over is also known. However, frequency of such cases is extremely low, i.e., in fractions. Crossing over differs from linkage in several aspects.

Chiasma Frequency

In genetics during chromosomal crossover chiasma is the point where two homologous non sister chromatids exchange their genetic material which is DNA, as their genetic material is identical there is no noticeable change in daughter cells.

The chiasma becomes visible during prophase I of meiosis, more specifically during the diplotene stage. However, the actual crossover of genetic material occurs only during pachytene stage of prophase I.
Chiasma frequency is considered as the calculation of the level of genetic recombination of a population.

Chiasma frequency is calculated by,

$$f_c = 2 \times f_r$$

Here, f_c is the chiasma frequency, f_r is recombination frequency
Recombination frequency is given by

$$f_r = \frac{(N \times 100)}{N_p}$$

Here, N is the number of recombinants; N_p is the total number of progeny.

According to the given theory on the formations of the chiasmata, it is the cause of genetic crossover. The chiasmata can lead to the division and simultaneous exchange of the chromosomal segments but if crossover takes place, it is the result of the strain acted by the chiasma formation.

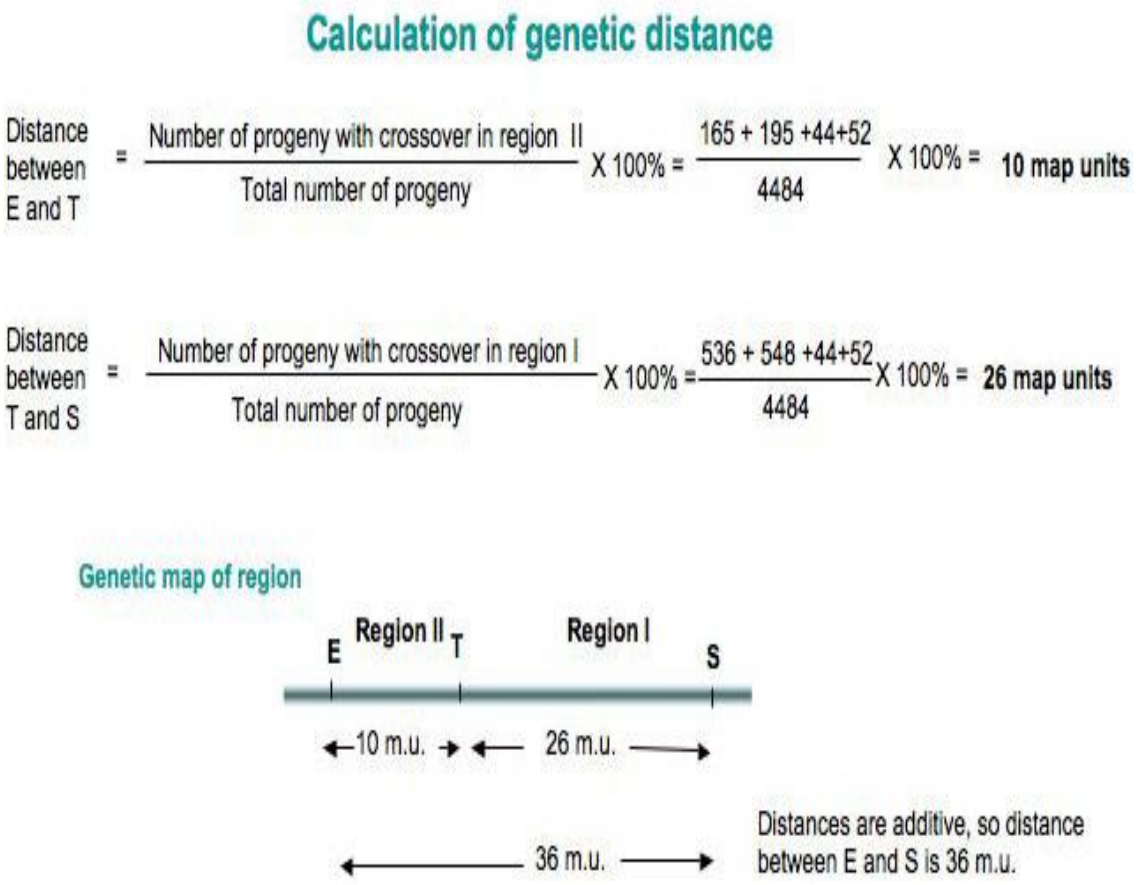
According to the hypothesis, adjacent loops should have some (sister chromatids separating) and reductional (sister chromatids not separating) separation of chromatids.

Chromosome pairs of different sizes are not affected by variation in minimum interchiasma distances. However, the average interchiasma distance increases with the bivalent length. According to the minimum-inter chiasma distance data, chiasma interference is complete over a chromosomal fragment that is about 60 Mb in length.

Genetic map distances

Genetic map distances are the distance between two points on the genetic map of a chromosome is the average number of crossovers between them.

The typical unit of genetic linkage is the centimorgan (cM). A distance of 1 cM between two markers means that the markers are separated to different chromosomes on average once per 100 meioses.



Tetrad analysis

The tetrad is the four spores produced after meiosis of a yeast or other Ascomycota, *Chlamydomonas* or other alga, or a plant. After parent haploids mate, they produce diploids. Under appropriate environmental conditions, diploids sporulate and undergo meiosis. The meiotic products, spores, remain packaged in the parental cell body to produce the tetrad.

If the two parents have a mutation in two different genes, the tetrad can segregate these genes as the parental ditype (PD), the non-parental ditype (NPD) or as the tetratype (TT).

Parental ditype: a tetrad type containing two different genotypes, both of which are parental. A spore arrangement in Ascomycetes that contains only the two non-recombinant-type ascospores.

Non-parental ditype: a non-parental ditype (NPD) is a spore that contains only the two recombinant-type ascospores (assuming two segregating loci). A tetrad type containing two different genotypes, both of which are recombinant.

Tetratype: a tetrad containing four different genotypes, two parental and two recombinant. A spore arrangement in Ascomycetes that consists of two parental and two recombinant spores indicates a single crossover between two linked loci.

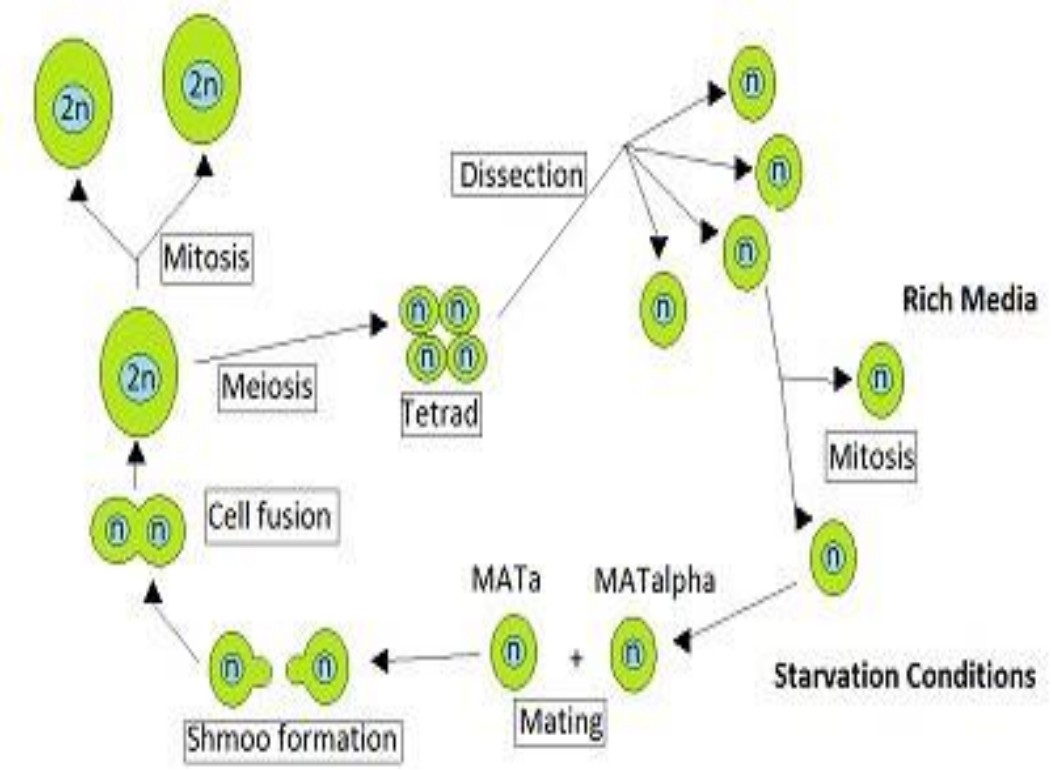


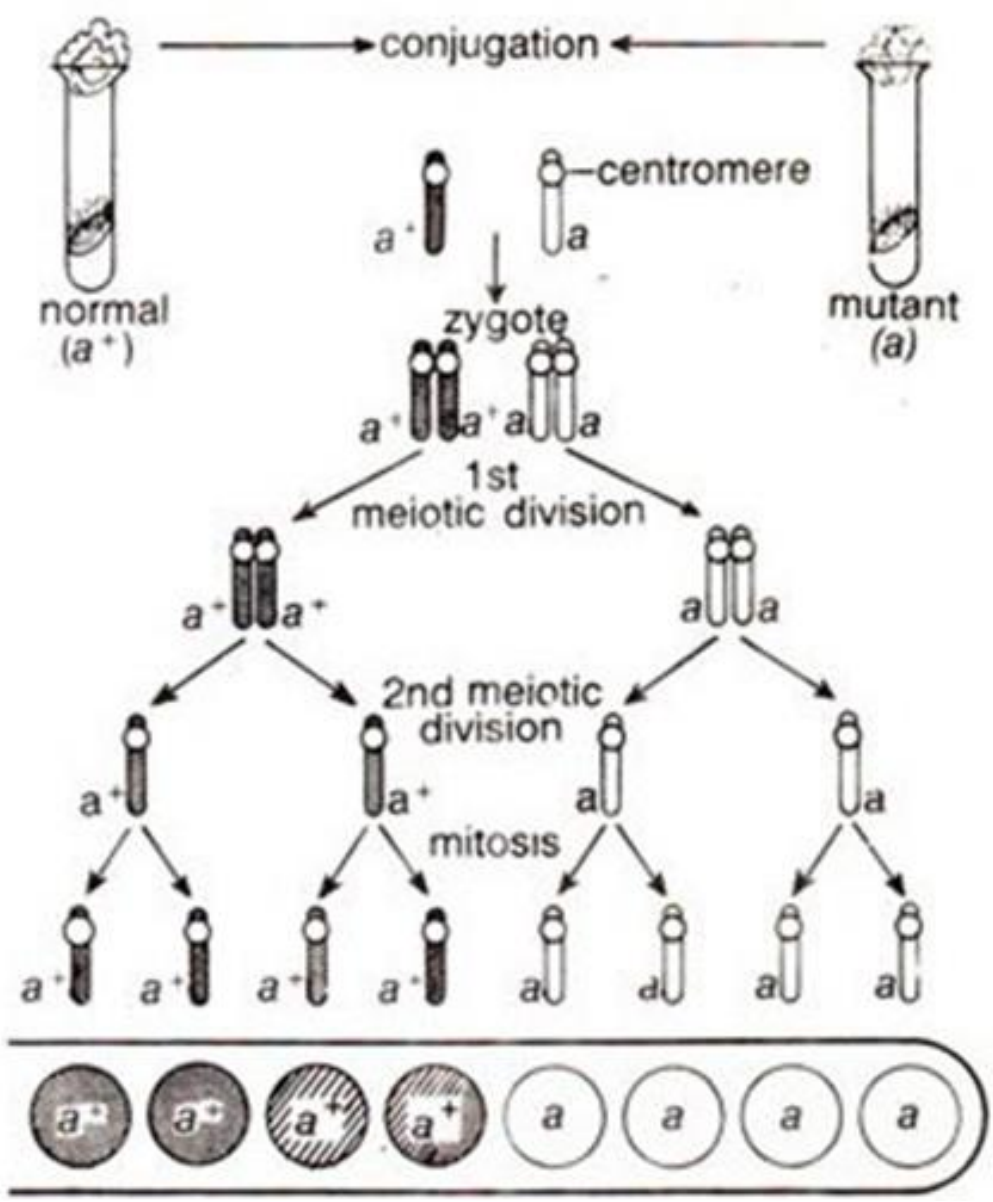
Fig.: Tetrad analysis. Source: Peter J. Russell IGenetics 3rd ed.

Centromere mapping with ordered tetrad. Analysis of ordered tetrads

Crossing over between centromere and gene (first and second division segregations).

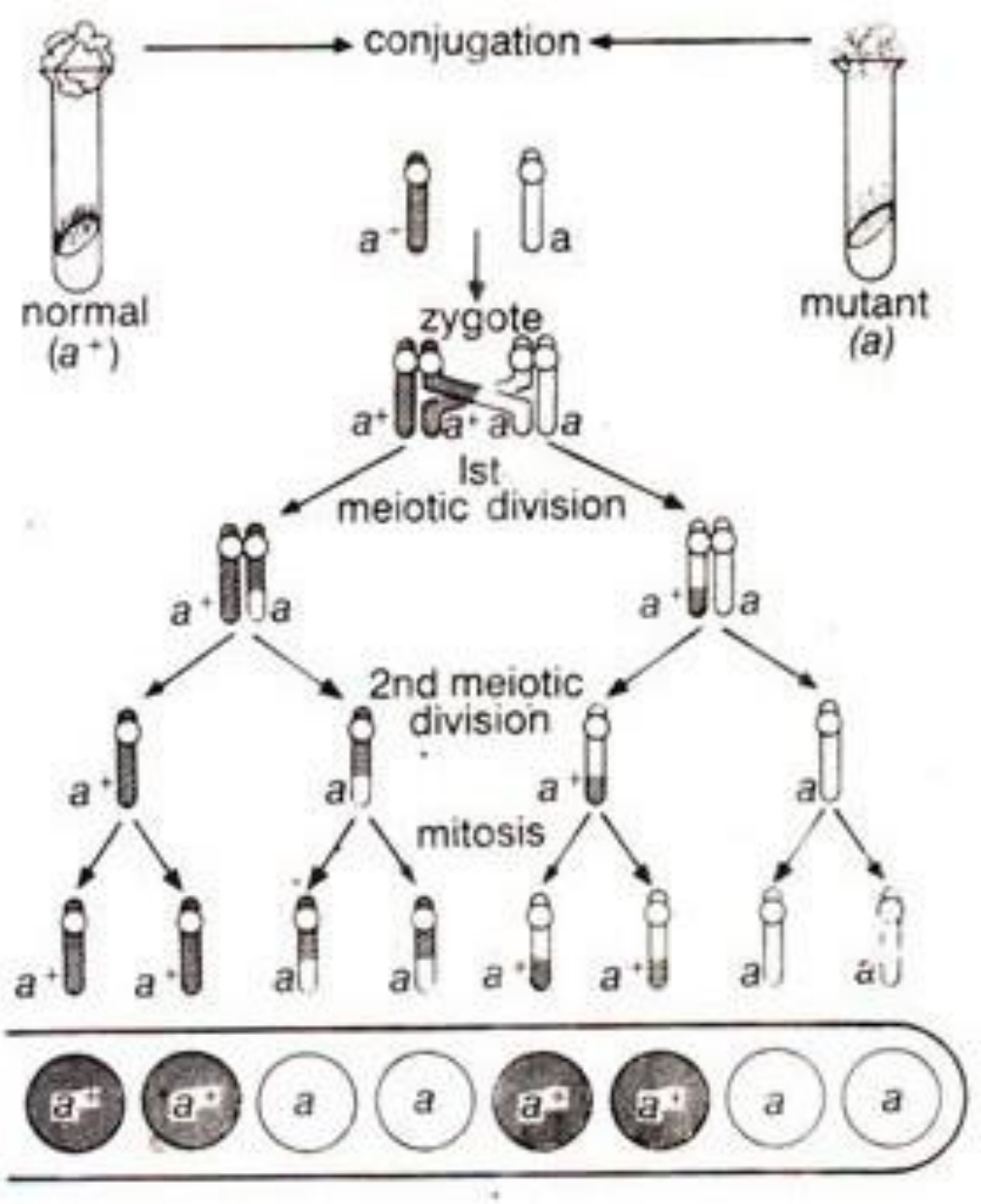
In *Neurospora*, products of meiosis are present in a linear order, so that ordered tetrads are obtained. Such a situation is not found in *Chlamydomonas* although tetrad analysis can be conducted. Due to ordered tetrads in *Neurospora*, a cross between normal (a+) and mutant (a) strain, will give rise to a linear arrangement of four normal spores (a+) at one end followed by four mutant spores (a) at the other end. Such an arrangement would be disturbed if crossing over occurs between centromere and the gene, because crossing over occurs at four strand and not at two strand stage. If crossing over at two strand stage was possible, it would again lead to four normal spores followed by four mutant spores. Therefore, by analysing linear arrangement, a 4:4 arrangement will suggest absence of crossing over and a paired arrangement (24: 2a : 24 :2a or 2A :4a :2A or 2a :4A :2a) will suggest that crossing over has taken place between the gene and corresponding centromere. When crossing over is

absent leading to 4:4 arrangement, this is described as First Division Segregation and when crossing over takes place leading to paired arrangement, it is described as Second Division Segregation.



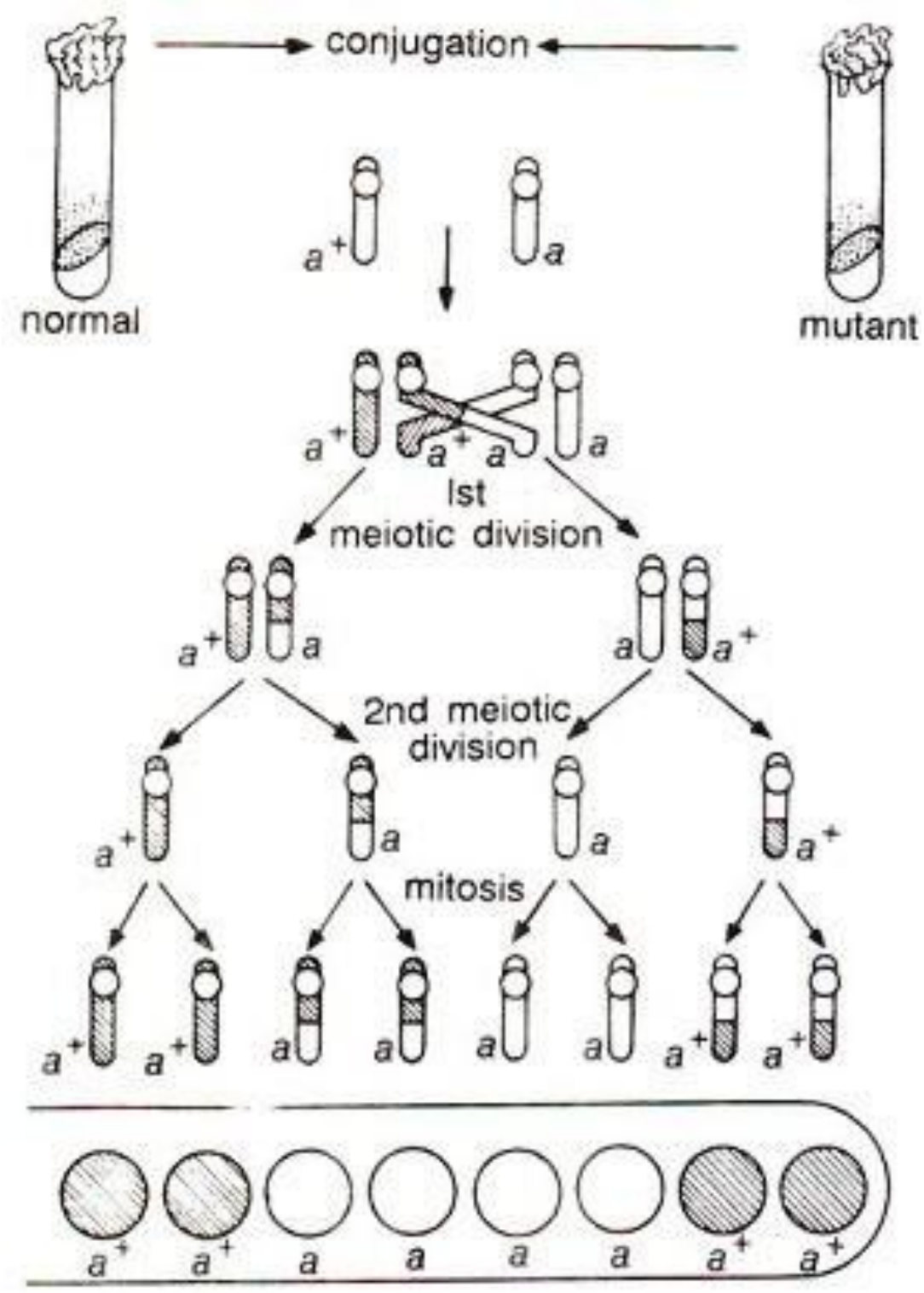
Source: Source: Peter J. Russell IGenetics 3rd ed.

Results showing first division segregation in a cross between normal (a+) and mutant (a) strains of *Neurospora*, in which crossing over between the gene and centromere does not take place.



Source: Source: Peter J. Russell IGenetics 3rd ed.

Results of a linear order of ascospores (2:2:2:2) showing second division segregation in a cross between normal (a+) and mutant (a) strains of *Neurospora*, in which crossing over takes place between the gene and the centromere.



Source: Source: Peter J. Russell IGenetics 3rd ed.

Results of a linear order of ascospores (2:4:2) which is different than the one shown in Figure. but still showing second division segregation in a cross between normal (a+) and

mutant (a) strains of *Neurospora*, in which crossing over takes place between the gene and the centromere. Frequency of recombination between centromere and gene. In *Neurospora*, since crossing over between a gene and centromere leads to second division segregation, the relative proportion of asci exhibiting second division segregation will give an estimate of crossing over. This will not be possible in *Chlamydomonas* due to absence of ordered tetrads. As an illustration, in an experiment in *Neurospora*, cross between albino (al) and wild type (al+) gave first division segregation (al al al+ al+) in 129 asci and second division segregation (al al+ al al+) in 141 tetrads. Since only two of the four strands undergo crossing over in second division segregation, recombination between centromere and the gene is-

$$\frac{1}{2} \times \frac{141}{(129+141)} = 0.26 \text{ or } 26 \text{ per cent.}$$

If a gene is located far away from centromere, crossing over may take place between the gene and centromere in each and every tetrad so that 100% tetrads should exhibit second division segregation. However, since in such cases, double crossovers, and multiple crossovers are also possible, some of the tetrads due to double crossover and other even number of crossover events, will give rise to first division segregation. Thus, second division segregation will never reach 100% level and in practice does not exceed 67% giving rise to only 33% recombination frequency (½ x 67, since only two of the four chromatids are involved in crossing over). This 33% recombination frequency will actually represent 50 units or more. Therefore, recombination frequencies should not be estimated over long distances, but should be estimated over small distances to avoid underestimation due to double and even multiple crossovers.

11. Transposable Elements: In bacteria (IS elements, composite transposons), maize (Ac and Ds elements), *Drosophila* (P-elements) and their genetic significance.

Transposable elements are mobile DNA sequences found in the genome of all biological organisms. Generally transposable elements are able to insert at different locations. There are different types of transposable elements and encode a variety of functions which are not

directly related to transposition. Irrespective of variations, generally many transposable elements have certain common features which are as follows:

1. Short flanking direct repeats.
2. Terminal inverted repeats.
3. Absence of origin of replication.
4. Presence of gene which encodes the enzyme transposase.

IS elements are autonomous unit. Normally it is constituted of bacterial chromosome or plasmid and is responsible for encoding protein molecules needed for transposition.

Different IS element bears different nucleotide sequence. Besides of difference in nucleotide sequence it bears same common feature.

1. Presence of inverted repeats containing ≈ 50 base pairs at each end of IS sequence.
2. Protein coding region is located in between the inverted repeats. This region is responsible for encoding 1 or 2 enzymes required for transposition.
3. Another important feature of IS element is the direct repeat sequence whose 5′ and 3′ repeats consisting of 5 – 11 base pairs. This region has been generated from the target site DNA binding insertion of a mobile element. During insertion process a second direct repeat adjacent to the IS sequence is created on the other end.

Simplest mechanism of IS element insertion is that the IS element is first excised and then it is inserted to a new position of a bacterial chromosome by a non replicative process i.e. the transposing element moves as a physical entity directly from one site to another site.

The steps of insertion are as follows:

(a) The enzymes which catalyzes the transcription of IS element is called transposase. These molecules bind to the inverted repeat sequences located at each end of the IS element in the donor DNA and cleave the DNA precisely from the existing element.

(b) Transpasase makes staggered cuts in the target DNA

(c) This enzyme then ligates the 3′ termini of the IS element to the 5′ ends of the cut donor DNA.

(d) The enzyme DNA polymerase, encoded by the host cell then extends the 3′ end of

the target site and the gap is filled informing a 3′×5′ phosphodiester bond. Thus an another short repeat is generated and two short direct repeats are observed on the two ends of IS element after insertion into bacteria.

Bacterial Transposons:

A genetic element that can move from one position to another position of a DNA molecule is called transposon. In bacteria transposable elements broadly are of two kinds.

(a) Replicative transposition:

In this type of mechanism, the element is duplicated during the reaction, so that the transposing entity is a copy of the original element. These are also called complex transposon.

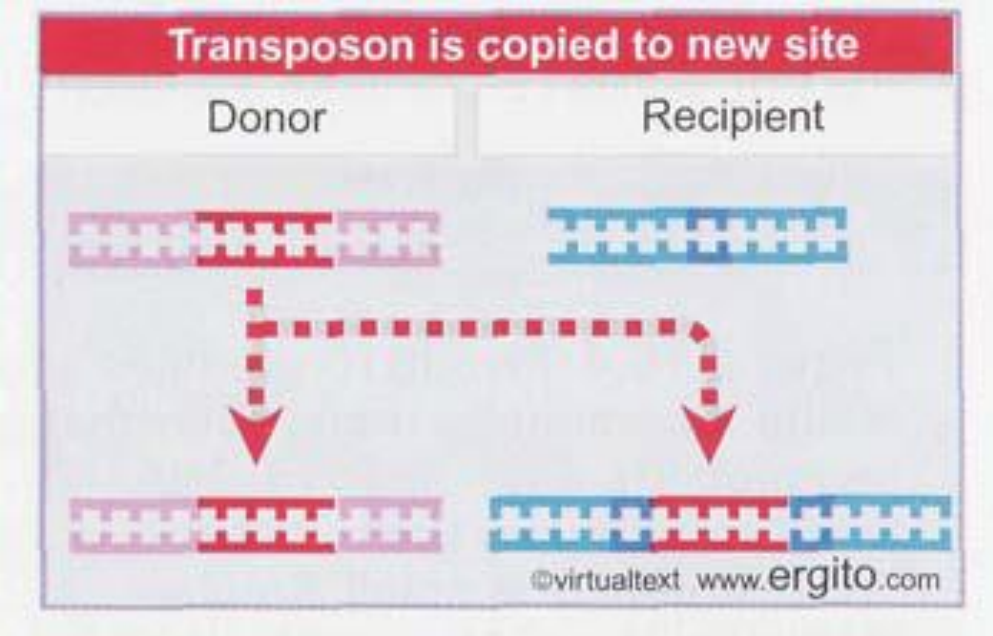


Fig.: Replicative transposition creates a copy of the transposon, which inserts at a recipient site. The donor site remains unchanged, so both donor and recipient have a copy of the transposon.

(b) Non replicative transposition:

In non replicative transposition, the transposable element moves from one site to another. There is no replication of transposable element, although short sequences in the target DNA are replicated, generating flanking direct repeats. Non-replicative

transposition requires only that transposable element and the target DNA be cleaved and joined together.

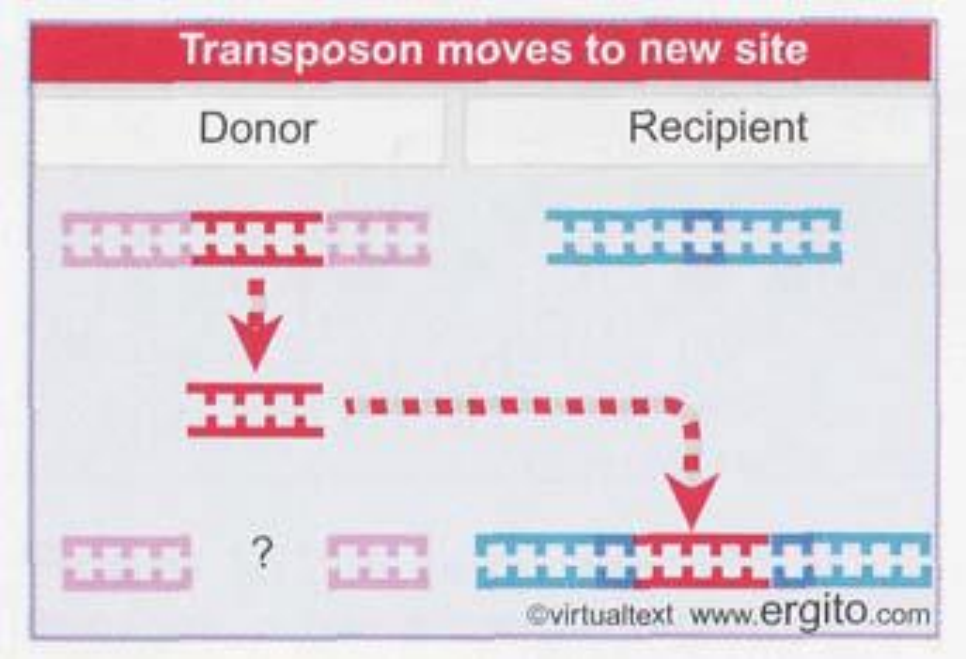


Fig.: Nonreplicative transposition allows a transposon to move as a physical entity from a donor to a recipient site. This leaves a break at the donor site, which is lethal unless it can be repaired.

Composite Transposon:

A composite transposon consists of two inverted repeats from two separate transposon moving together as one unit carrying the DNA between them. For example, if a segment of DNA is flanked at both ends by two additional insertion sequences, the enzyme transposase will move any segment of DNA surrounded by a part of inverted repeats that it recognizes. Each insertion sequence may be move independently or the whole sequence between two outermost inverted repeat as a unit and resulted to develop a composite transfoson. For example Tn5 (Kanamycin resistance), Tn 9 (Chloram phenicol resistance) and Tn 10 (tetracycline resistance). The IS elements of Tn 5 and Tn 10 are named as IS 5 and IS 10 respectively.

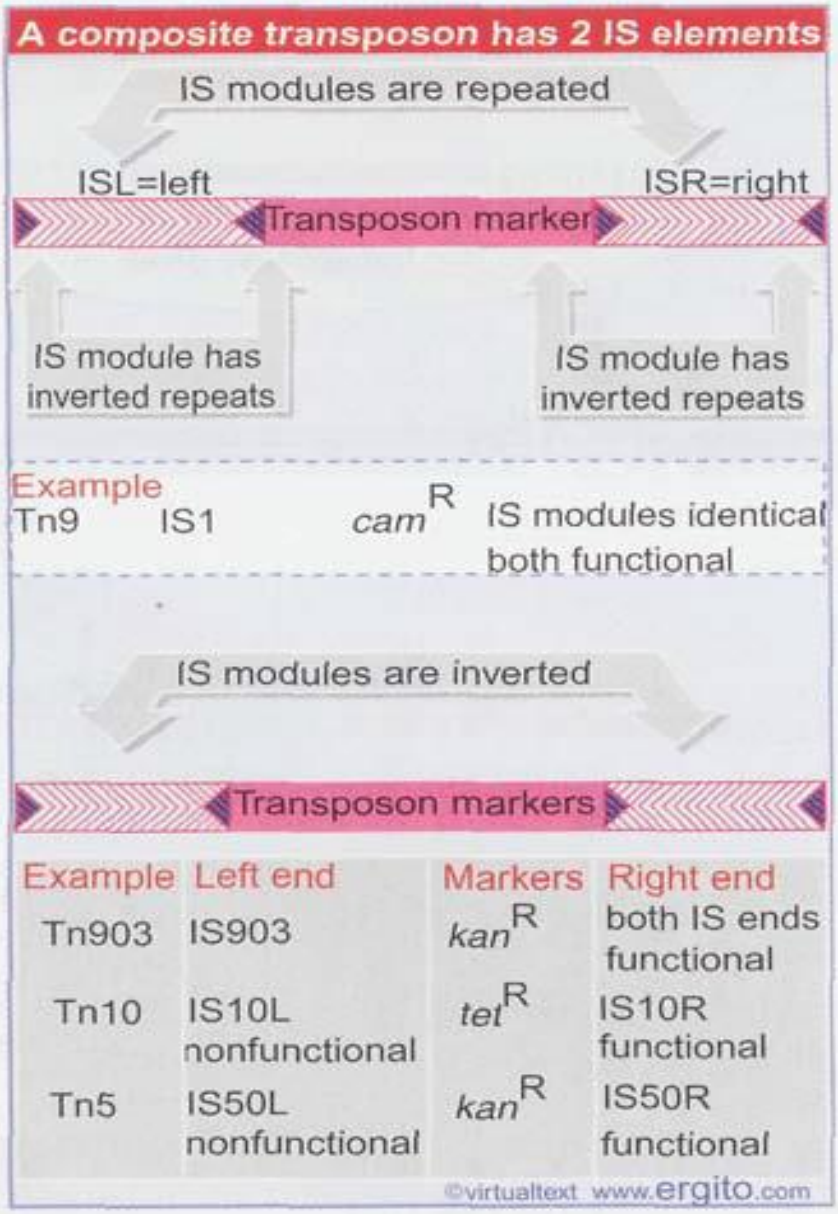


Figure 16.3 A composite transposon has a central region carrying markers (such as drug resistance) flanked by IS modules. *The modules have short inverted* terminal repeats. If the modules themselves are in inverted orientation, the short inverted terminal repeats at the ends of the transposon are identical.

Transposition in Eukaryotes:

AC – DS elements in Maize

Originally the discovery of mobile elements was made by Barbara Meclintock in maize. The notice for the first time that one strain of corn had the strange characteristic that a particular chromosome no. 9 tended to break at a fairly high rate at the same site due to spontaneous mutation. She termed this a mutable sites are actually locations where transposable elements have been inserted into the chromosome. She published her findings in cold Spring Harbour Sysposium on quatitative Biology in 1951. At that geneticists were unable to accept that the genetic material can move from one place to another place. Instead they were confident to believe that the genetic material always occupied a fixed position.

In the 1960, bacteria and bacteriophages were shown to possess transposable elements. After 35 years, the significance of early discoveries was finally recognized in 1983, when she was awarded the Nobel Prize in Physiology and Medicine. Mc Clintock's discovery of transposable elements had its genesis in the early work of Rollius A. Emerson on the maize genes that caused variegated (multi coloured) Kernels. Most corn Kernals are either wholly pigmenter or colourless (yellow), but Emerson noted that some yellow Kernels had spots or streaks of colour. He proposed that Kernels resulted from an unstable mutation i.e. a mutation in the wild type gene for pigment produced of colourless Kernel but, in some cells, the mutation reverted back to the wild type, causing a spot of pigment. However, Emerson didn't know why these mutations are unstable.

Mc Clintock discovered that the cause of the unstable mutation was a gene that moved. She noticed that chromosome breakage in maize often occurred at a gene that she called Dissociation (DS) but only if another gene, the activator (AC), also was present, DS and AC exhibited unusual patterns of inheritance; occasionally, the genes moved together. Mc Clintock called these moving genes controlling elements, because they controlled the expression of other gene.

1. DS/AC family of transposons in corn is simple and conservative. The DS locus could be transposed to different locations within the corn genome. DS means dissociation element because the locus was known to frequently cause chromosoma breaks. The DS elements vary in size and are defective, which are derived from AC (activator) element by deletion

of all or part of the transposae enzyme (which is essential for transposition). Therefore, DS element cannot move without AC element. The AC and DS elements do not need to be on the some chromosome for transposition to occur.

2. The AC element consists of 5 exons but is actually a single gene, the product is transposasae. On the 2 sides bear inverted 11 bp repeats like a typical transposon and target sequence of 8bp duplicated at the site of insertion and is called autonomous element.

3. DS element vary in length and sequence but related to AC. The ends are with same inverted repeats as that of AC element. This phenomenon is called non autonomous element.

4. Each Kernel in an ear of corn is a separate individual originating from the two haploid maternal nuclei and a haploid pollen grain i.e. triploid.

5. The pigment (anthocyanin) containing allele can be designated as ‘C’ which is dominant over colourless allele “c”. Therefore CC and Cc are purple while cc is colourless. When DS is transposed into C allele, the Cc genotype would be colourless. If C allele moves to a new location, the ‘C’ allele becomes functional again. All cells will have the genotype Cc will be purple. The presence of these pigmented cells surrounded by colourles cells produce a purple spot or streak on the Kernel to become variegated appearance phenotype.

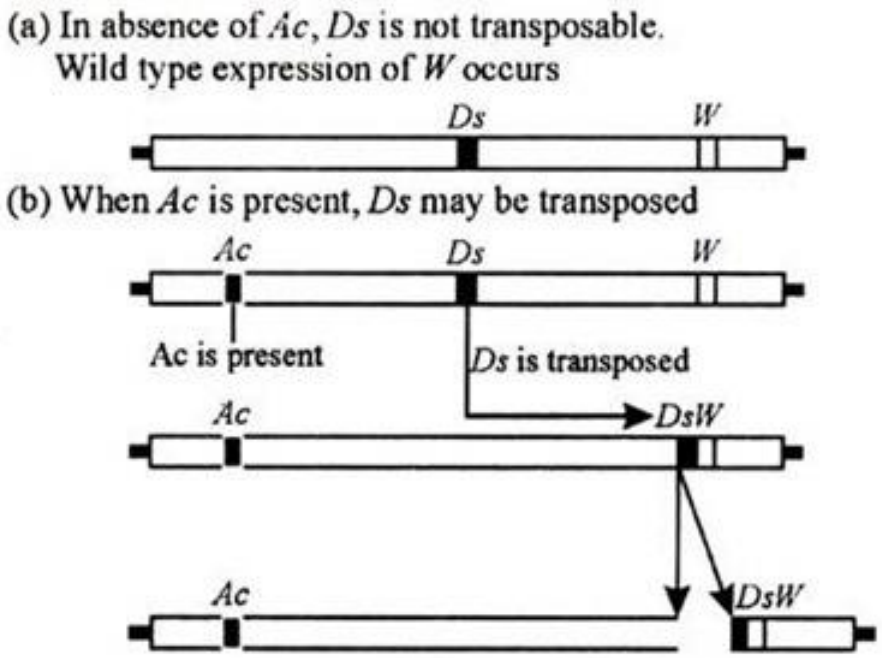


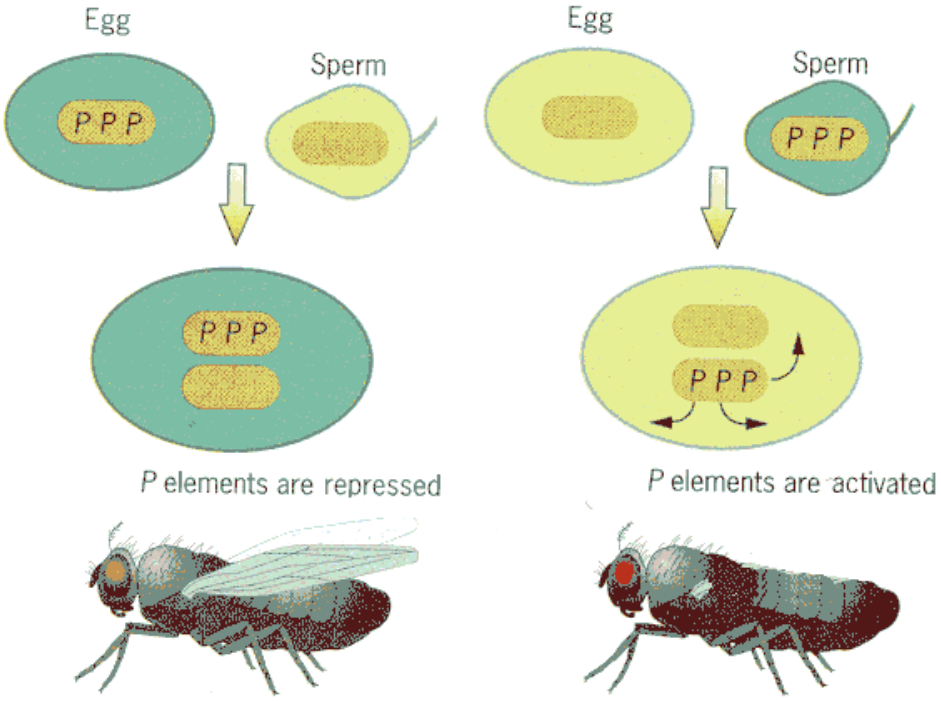
Fig. 6. Effects of Transposition involving *Ac-Ds* system in maize.

Source: http://www.biologydiscussion.com/biotechnology/transposons

P-element in *Drosophila*:

In 1977, Margeret and Kidwell discovered that crosses between certain strain of *Drosophila melanogaster* produce hybrids which showed frequent mutation, chromosome breakage and sterility. The term hybrid dysgenesis was used to denote abnormalities.

Kidwell and her colleagues classified this *Drosophila* strain as "P" and "M" crosses between two different 'P' strain or between two different M strains produce normal hybrids. But when male of P strain is crossed with a M strain females, produce hybrid dysgenesis.



Source: http://www.mun.ca/biology/scarr/P-element_hybrid_dysgenesis.htm

		M	P
Female Parent	M	Normal	Dysgenic
	F	Normal	Normal

They suggested from these findings that the chromosome of P strain carry genetic factors that are activated when they enter the eggs of M – females one activated these factors induce mutation and chromosome breakage.

The hybrid dysgenesis syndrome is marked by temperature – dependent sterility, elevated mutation rates and increased chromosome rearrangement and recombination.

This element is 2967 bp long and features a31-bp invested repeat at each end. The element encodes a transposase. Pelement do not utilize an RNA intermediate during transposition and can insert at many different positions in the *Drosophila* chromosomes.

Hybrid dysgenesis manifests when crossing P strain males with M strain females and not when crossing P strain females (females with autonomous Pelements) with M strain males. The eggs of P strain females contain high amount of repressor protein that prevents transcription of the transposase gene. The eggs of M strain mothers, which do not contain repressor protein, allow transposition of Pelements from the sperm of fathers.

Genetic Significance:

1. Transposable elements have played important role in genome evolution.
2. Transposable elements can be thought of as genomic parasites that provide no benefit to the cell and may even be harmful.
3. Transposable elements are responsible to induce mutations and chromosome rearrangements some biologists assumes that they exist because they generate variation, which facilitates evolutionary adaptation.
4. Regardless of evolutionary reasons for existence, some transposable elements have clearly evolved to serve useful purposes for their host cells. These transposons are sometimes referred to as demisticated, implying that their parasitic tendencies have been replaced by properties useful to the cell.
5. The evolution of new genes through transposons.

12. Genetic Regulation: Regulation of gene expression in prokaryotes and their viruses – *lac*, *trp* and *ara* operons of *E. coil*, Lambda lytic-lysogenic regulatory cascade; regulation of eukaryotic gene expression – brief account.

Concept:

Generally, in each cell, DNA has coded the genetic information for the synthesis of protein which is required at different times during cell division. Inspite of enormous genetic information, not all the genes function all the time. Normally in bacterial genome out of 4000 genes only a fraction is expressed at a given time. Similarly in case of human genome out of

1,00,000 genes a small fraction of genes are expressed at a specific time. Moreover, products of certain genes are needed only for certain types of cell. Therefore it is very clear to us that genes are selectively activated on special occasion and for a limited time based on demand. All cell types are able to synthesis some enzymes constitutively i.e. all the time. For other enzymes mRNA transcription is initiated only on demand and is switched off when requirement is fulfilled. The control of gene expression on protein synthesis is called gene regulation.

The mechanisms of gene control can influence the efficiency of different genes of the genome. Regulation of gene activity is controlled by the following features:

1. Quantitative difference in the amounts of protein/enzymes produced by different times.
2. The amount of protein/enzyme produced by a gene at different by different tissues.

During last few years extensive research has been carried out on gene activity at any of the following levels.

1. Transcription level i.e. at the time of synthesis of primary RNA transcript.
2. Post-Transcriptional level i.e. at the time of protein synthesis.
3. Translational level i.e. at the time of formation of chains into a functional protein.

The gene regulatory mechanisms in prokaryotes and eukarotes differed which are as follows:

Table: Basic differences in prokaryotic and Eukaryotic gene regulation

	Prokaryotes	Eukaryotes
1.	Regulation involves operons and operators	Different of eukaryotic cells have different regulatory elements.
2.	TATA box absent	TATA box is the regulatory element used by different types of cells.
3.	Regulatory proteins are not found.	The gene is expressed only after all the necessary regulatory proteins bind to the TATA box.

Gene regulation in Prokaryotes:

Strategies of Gene regulation:

In bacterial organisms the protein synthesis is generally controlled at transcription level. This regulation may be positive or negative.

Positive regulation:

In this mechanism, an inducer molecule activates the promoter which promotes transcription of mRNA. There is no inhibitor – inducer interaction. The positive regulation of gene action is called induction and the substance that induces gene action is called indicer.

Positive regulation takes place in any one two ways:

- Transcription will continue until a molecular signal binds to the activator molecule and causes it to dissociate from DNA.
- The activator – molecular signal complex binds to promoter. If the signal molecule dissociates, the activator falls off DNA, stopping the transcription.

Negative regulation:

In this case an inhibitor or repressor is produced in the cell. The binding of inhibitor to the repressor binding site suppresses transcription of relevant gene. A signal molecule or an inducer is required to remove the inhibitor and simultaneously to initiate transcription. This negative regulation of gene expression is known repression.

It should be kept in mind that positive and negative regulations are not mutually exclusive. Same systems are both positively and negatively regulated. A catabolic system may be regulated either positively or negatively.

Mechanisms of gene regulation at Transcription levels:

Induction and Repression:

A set of genes will be switched on when there is necessary to metabolise a substrate. When the genes are switched on enzymes are synthesized and this phenomenon is called induction.

Likewise, when a metabolite needed by bacterium is provided in excess in the medium, bacterium stops the synthesis and the gene is associated with the metabolism is turned off and this phenomenon is called free back repression.

1. Induction and inducible system (inducible operon):

In genetic induction the genes are switched on to produce mRNA, required for the synthesis of enzymes. The substance which induces the gene for protein synthesis or enzyme production is known as inducer. The phenomenon of induction can be demonstrated by growing *E.coli* is different nutritional media as follows:

- When *Escherichia coli* are in a culture medium containing glycerol, they produce all those enzymes which are needed for the breakdown of glycerol. The synthesis of all other enzymes is maintained at the minimum level.
- When *E.coli* cells are grown in a medium containing lactos, the synthesis of enzyme β. galactosidase increases many folds. This enzyme hydrolyses lactose intoglucose and galactose. The synthesis of other enzymes is reduced.
- Ehen *E.coli* cells are grown on glucose medium, they contain just traces of β. Galactosidase enzyme. If these bacteria are transferred into a medium containing lactose, the concentration of this enzyme increases manifold that enables them to metabolise lactose. Here substrate lactose has acted as inducer activating the specific gene to synthesize required enzyme.

The enzyme whose synthesis can be induced by adding substrate are called inducible enzymes. The gene complex responsible for the synthesis of inducible enzyme is called 'inducible system'.

2. Repressible system:

The enzyme whose full level of transcription occurs until such time as their effect by reaches a critical concentration at which transcription is inhibited. Such effector is called co repressor and such regulator system is called repressible system.

e.g. Truptophan synthesis. In absence of tryptophan the enzymes are not synthesized at full level.

∴ (i) Inducible system are off until effector is present

(ii) Repressible systems are on until effector is present.

Operon concept:

In 1961, Francis Jacob and Jacques Monod published a paper in the 'Proceedings of the French Academy of Science" demonstrating that two genes are involved in lactose

metabolism were coordinately regulated by a genetic element located adjacent to them. The term operon and operator were first introduced in this paper.

Operon is defined as a group of contiguous genes in a bacterial chromosome that are transcribed into a single mRNA molecular Jacob and Monod explained regulation of the activity of these genes by a model known as operon model. The operon model for lactose catabolism is called lac operon. It consists of two kinds of genes.

1. Structural genes: These genes are segments of DNA that are associated with the synthesis of those enzymes that are needed for catabolism of lactose. In the lac operon there are 3 structural genes.

- lac Z gene for β galactosidase enzyme
- lac Y gene for galactosidase permease enzyme
- lac A gene for thiogalactoside transacetylase.

The structural genes are those segments of DNA which carry soaler for synthesis of proteins. These genes determine the primary structure of polypeptide chain by controlling the sequence of amino acids during protein synthesis.

2. Control genes: These genes control the actively of structural genes either by induction or suppression. These genes are

- Regulator gene: The regular gene produces some specific enzymes which acts as a repressor substance. In case of lac operon, the regulator gene codes for a protein which strongly binds to the operator gene and suppresses its activity.
- Promoter gene: The promoter gene(p) is the DNA segment at which RNA polymerase binds and initiates the transcription of the structural genes.
- Operator gene: The operator gene(O) is the segment of DNA which exercises a control over transcription. The repressor substance produced by the regulator gene binds with the operator gene. It lies close to the structural gene.

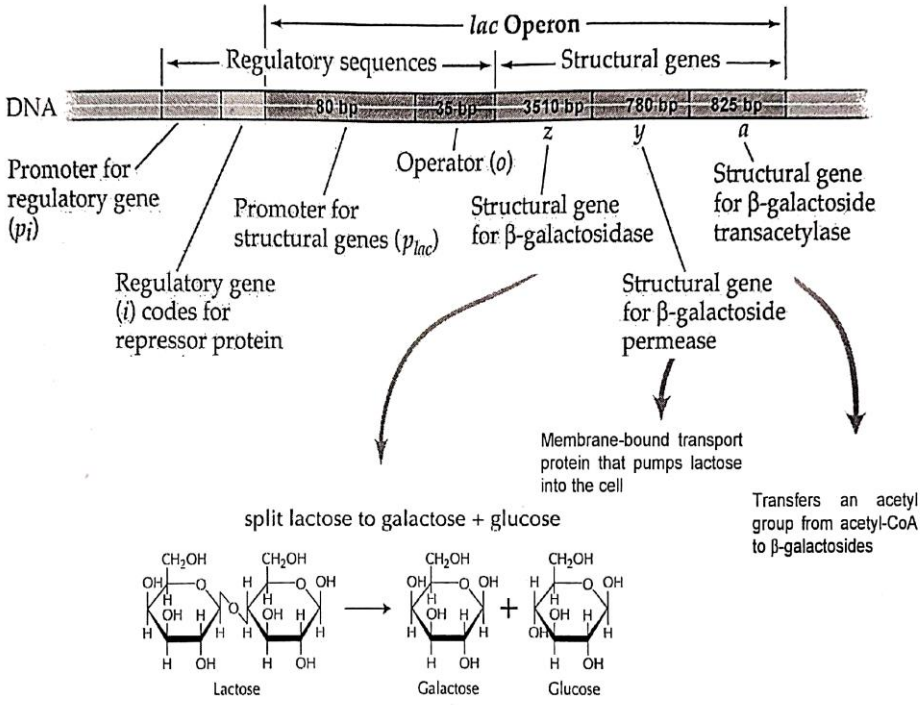
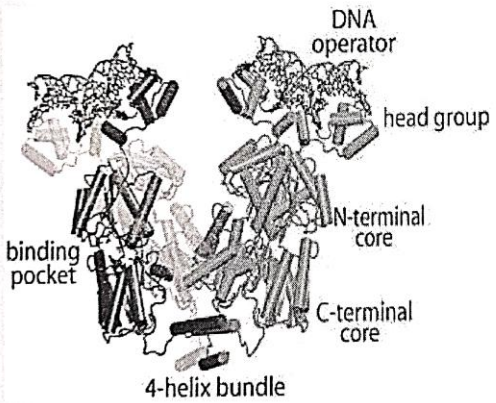
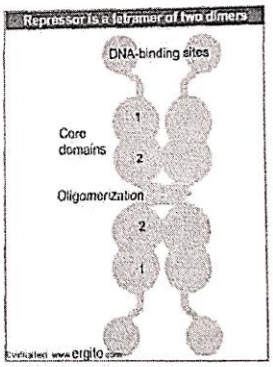


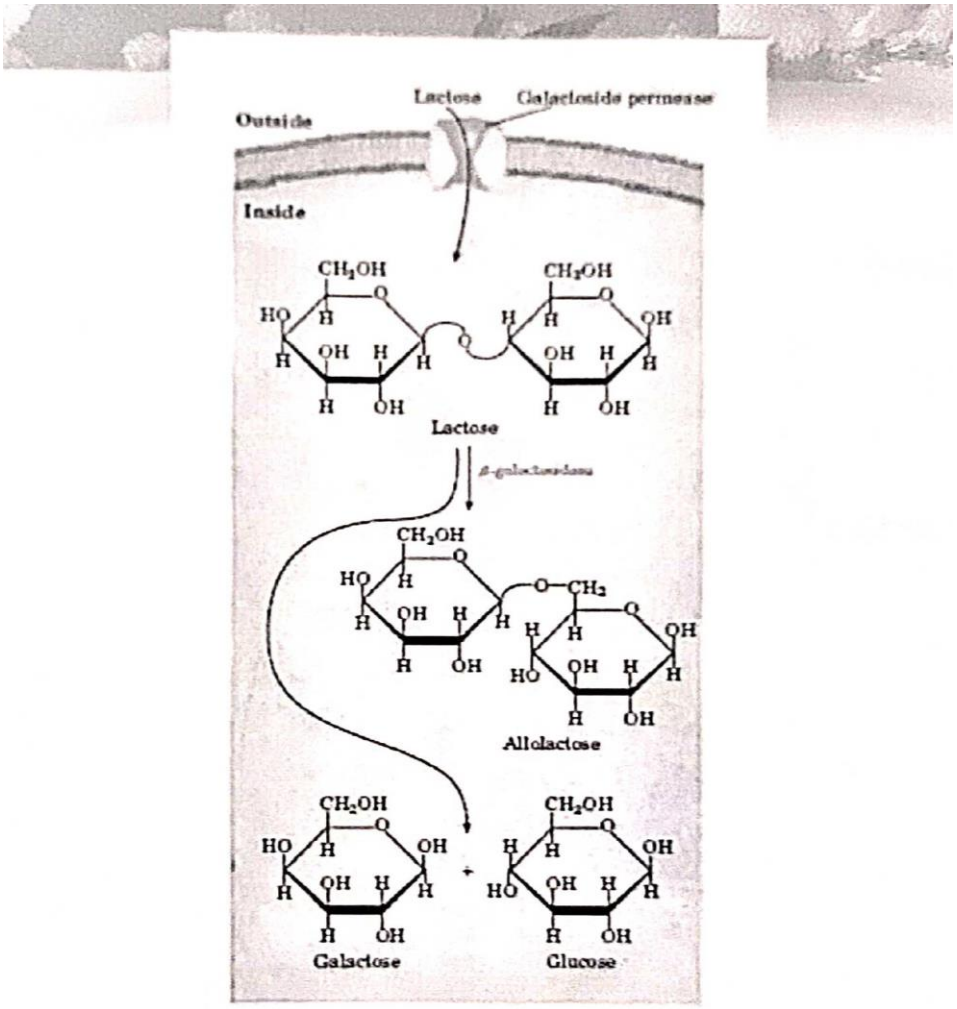
Figure: Lac operon of *Escherichia coli* (**Jacob-Monod model, 1965**)





***lac* repressor- a tetramer (152 kDa= 4 x 38 kDa)**

A **structural gene** is a gene that codes for any RNA or protein product other than a regulatory factor (i.e. regulatory protein). It may code for a structural protein, an enzyme, or an RNA molecule not involved in regulation.



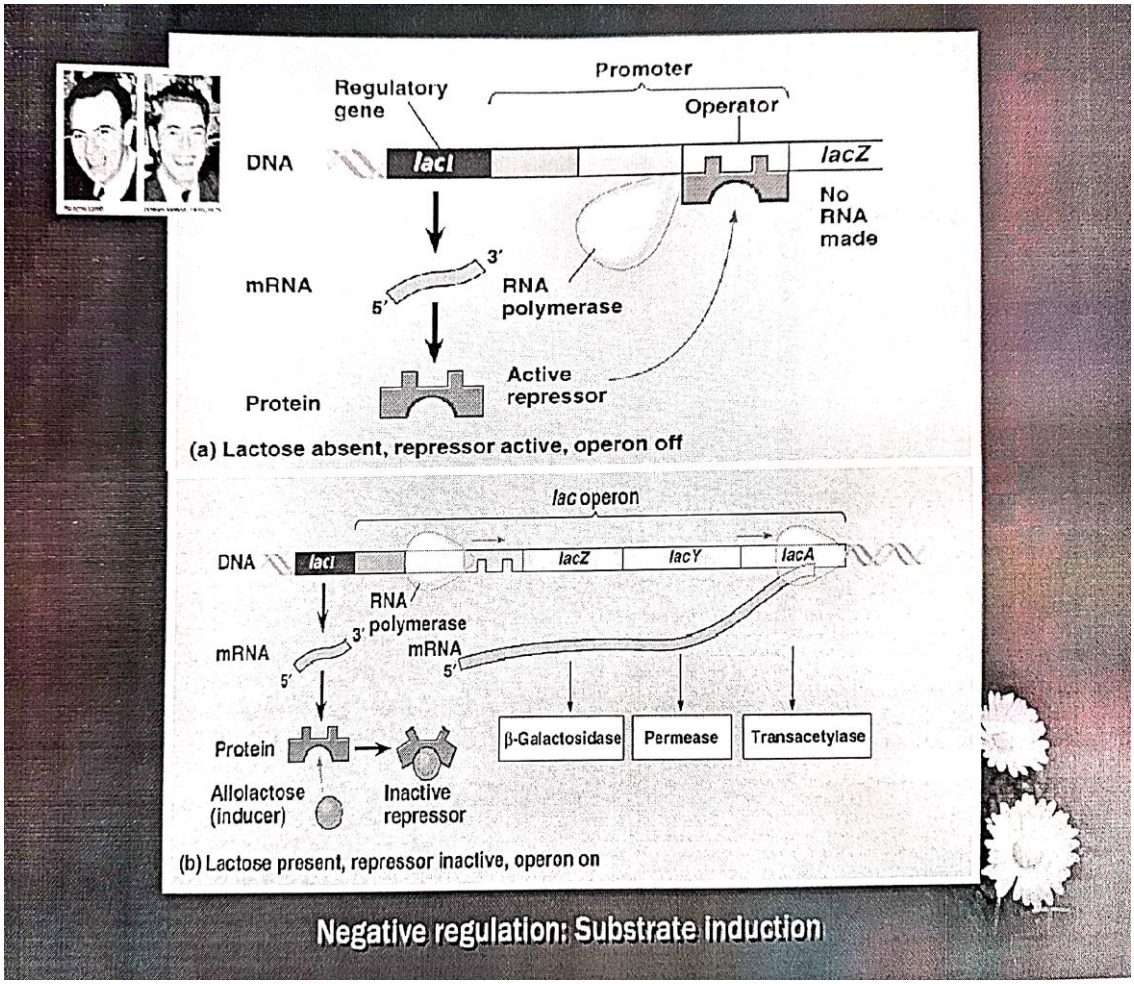
Functions of the enzyme β-galactosidase

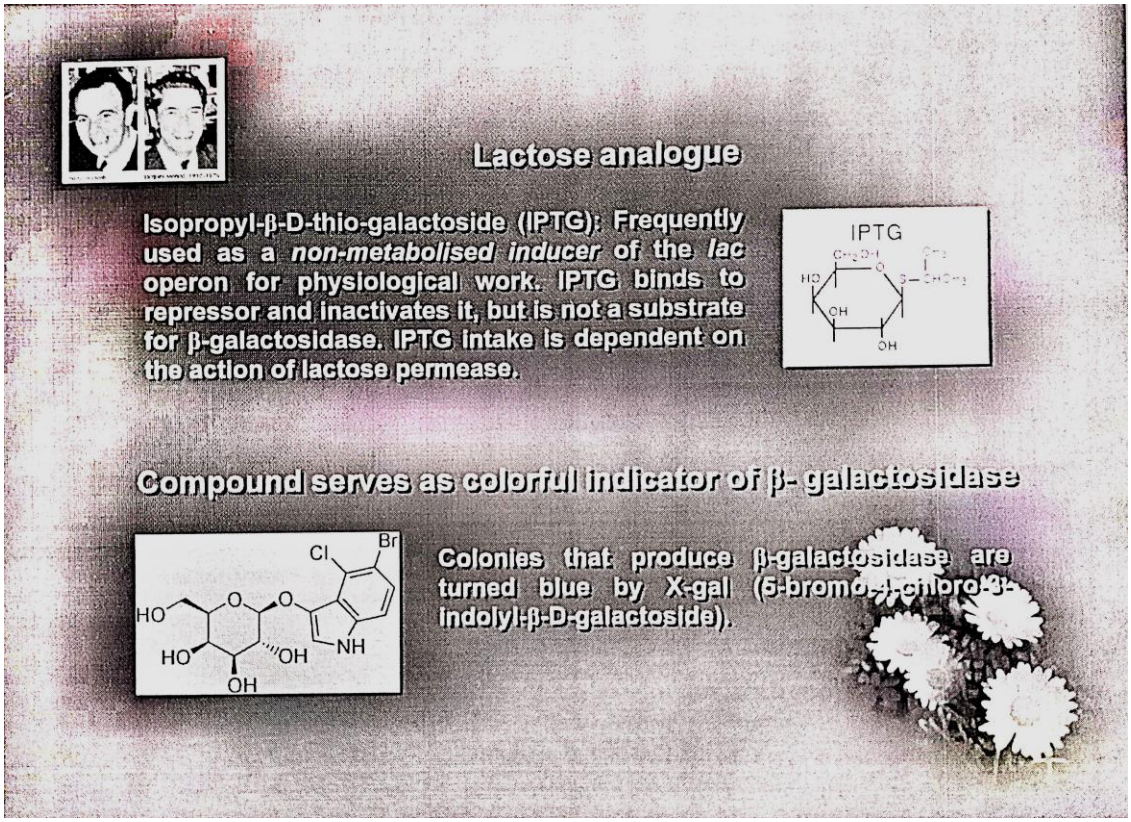
Negative control of lac operon:

Lac repressor is synthesized through the activity of the lac1 gene called the regulator gene. This repressor is an allosteric protein and plays important role in negative control of lac operon as follows:

(i) It can bind to the lacDNA at the operator site.

(ii) It can bind to the inducer. In the absence of inducer DNA binding site of repressor becomes functional. Then the repressor protein binds to the DNA at the operator site of the locus and blocks the transcription of the lac genes by RNA polymerase. Thus lac enzyme synthesis is inhibited.


Regulatory gene
Promoter
Operator
DNA
lacI
lacZ
No RNA made
mRNA
3′
5′
RNA polymerase
Protein
Active repressor
(a) Lactose absent, repressor active, operon off
lac operon
DNA
lacI
lacZ
lacY
lacA
RNA polymerase
mRNA
3′
5′
mRNA
5′
Protein
β-Galactosidase
Permease
Transacetylase
Allolactose (inducer)
Inactive repressor
(b) Lactose present, repressor inactive, operon on
Negative regulation: Substrate induction


Lactose analogue
Isopropyl-β-D-thio-galactoside (IPTG): Frequently used as a *non-metabolised inducer* of the *lac* operon for physiological work. IPTG binds to repressor and inactivates it, but is not a substrate for β-galactosidase. IPTG intake is dependent on the action of lactose permease.
IPTG
Compound serves as colorful indicator of β- galactosidase
Cl
Br
HO
O
O
NH
HO
OH
OH
Colonies that produce β-galactosidase are turned blue by X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside).

Positive regulation of lac operon:

It is additional regulatory mechanism which allows lac operon to function in the presence of glucose, an alternative and preferred energy source to lactose. Small molecules of cyclic AMP are distributed on bacteria Cyclic AMP is synthesized enzymatically by adenyl cyclase. Its concentration is regulated indirectly by glucose metabolism.

1. When bacteria are growing in culture medium containing glucose, the cAMP concentration is quite low.
2. In a medium containing any other carbon source that can not enter the biochemical pathway and metabolise glucose, the concentration of cAMP becomes high. It regulates the activity of the lac operon as well as of some other operons.
3. *E.coli* contains a protein called catabolic activator protein. It is encode by a gene Crp.
4. Mutants of eigher Crp or adenyl cyclase genes are unable to synthesize lac mRNA, it means both CAP and cAMP are needed for lac mRNA synthesis.
5. CAP and cAMP bind together cAMP – CAP unit. The complex unit regular lac system. It is a positive regulator.
6. In the absence of cAMP–CAP Complex, RNA polymerase binds weakly to promoter. This weak binding fails to initiate transcription.
7. CAMP – Crp Complex binds to a base sequence in DNA in the promoter region to initiate transcription.

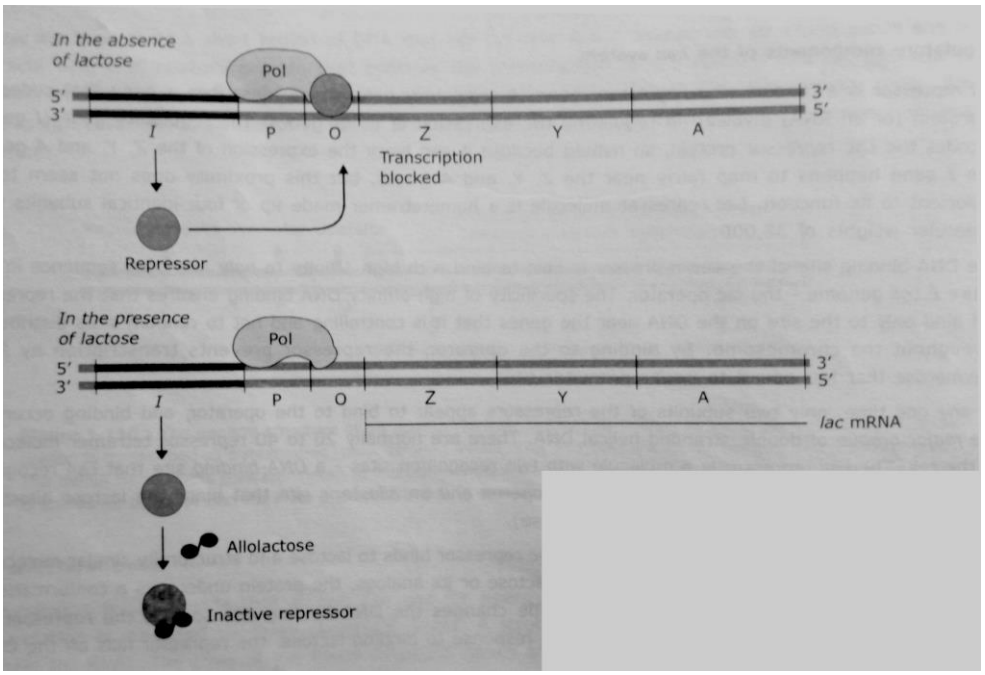
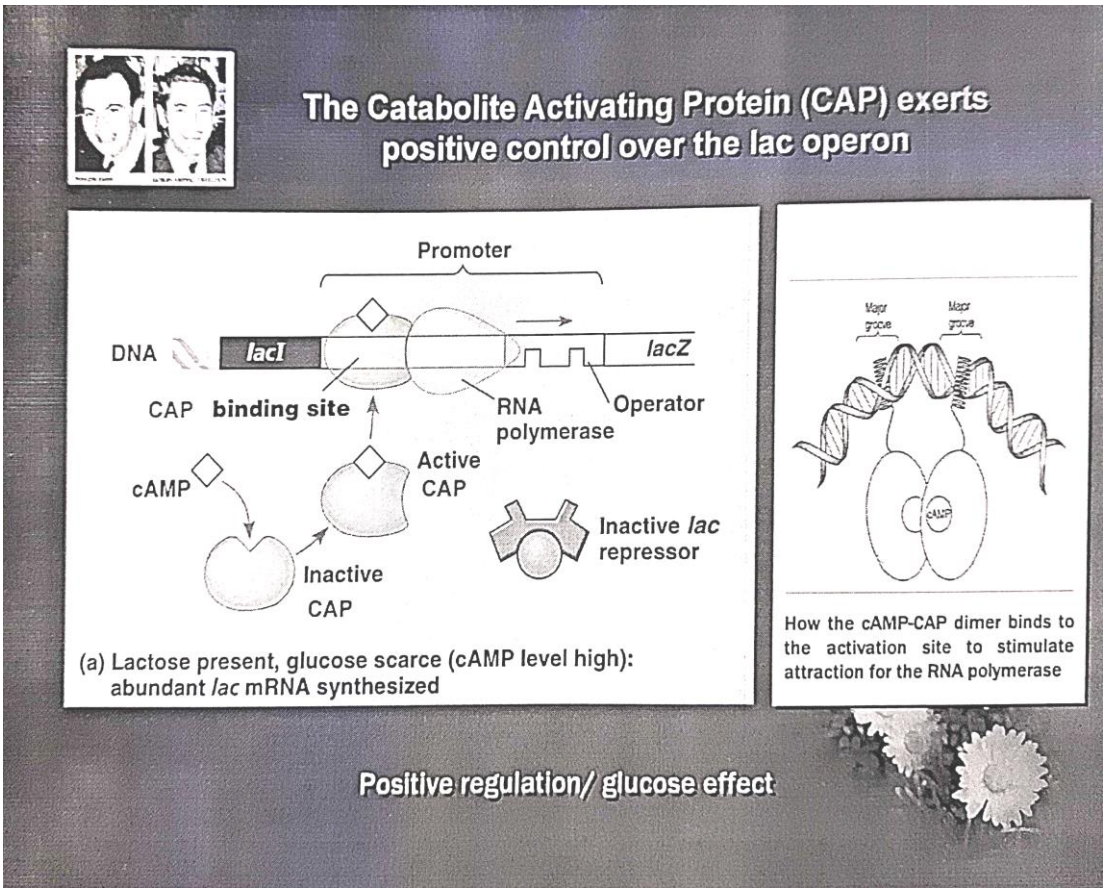
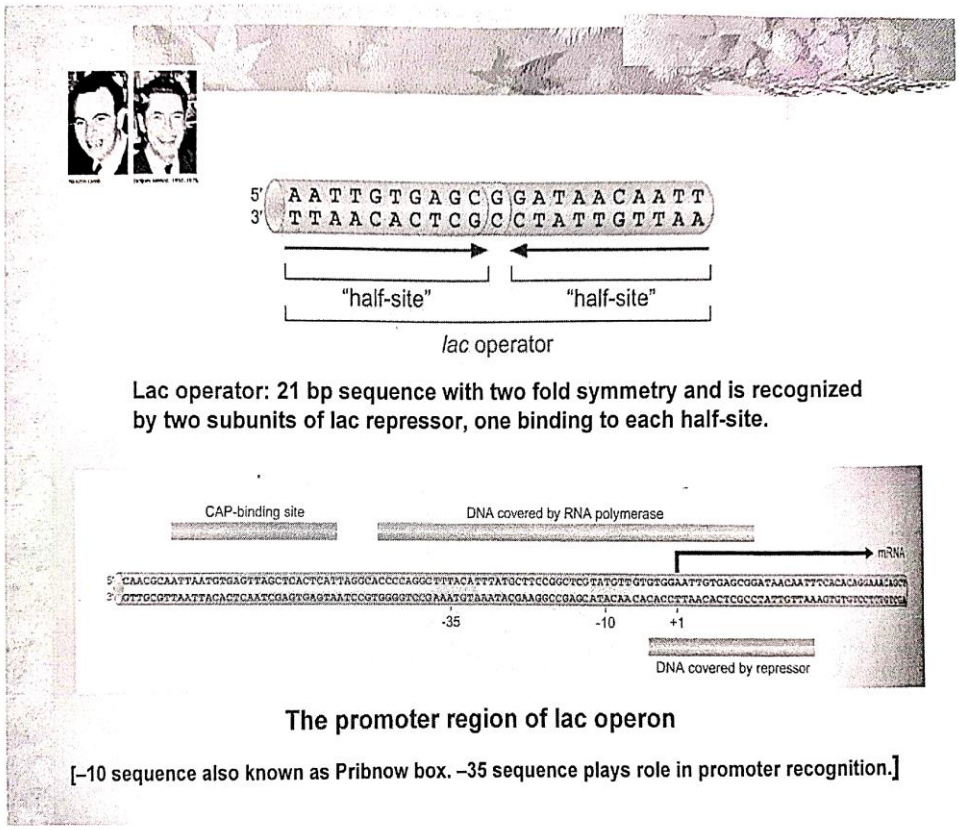
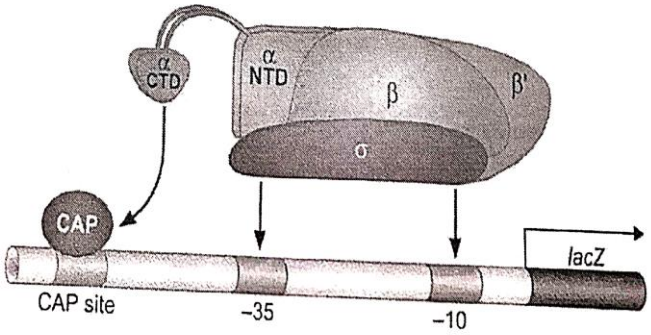


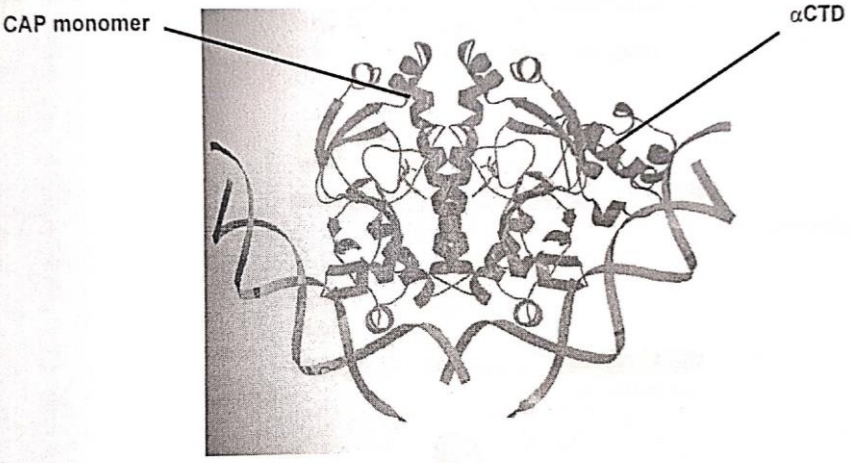
Fig.: Regulation of *lac* operon.


The Catabolite Activating Protein (CAP) exerts positive control over the lac operon
Promoter
DNA
lacI
lacZ
CAP
binding site
RNA polymerase
Operator
Active CAP
cAMP
Inactive CAP
Inactive lac repressor
(a) Lactose present, glucose scarce (cAMP level high): abundant lac mRNA synthesized
Major groove
Major groove
cAMP
How the cAMP-CAP dimer binds to the activation site to stimulate attraction for the RNA polymerase
Positive regulation/ glucose effect

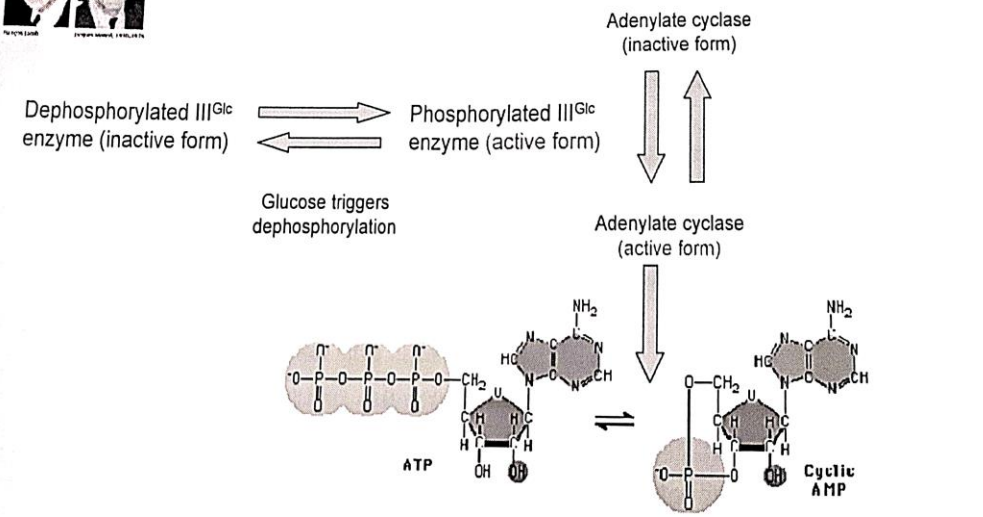

5' AATTGTGAGCGGATAACAATT
3' TTAACACTCGCCTATTGTTAA
"half-site"
"half-site"
lac operator
Lac operator: 21 bp sequence with two fold symmetry and is recognized by two subunits of lac repressor, one binding to each half-site.
CAP-binding site
DNA covered by RNA polymerase
mRNA
-35
-10
+1
DNA covered by repressor
The promoter region of lac operon
[–10 sequence also known as Pribnow box. –35 sequence plays role in promoter recognition.]



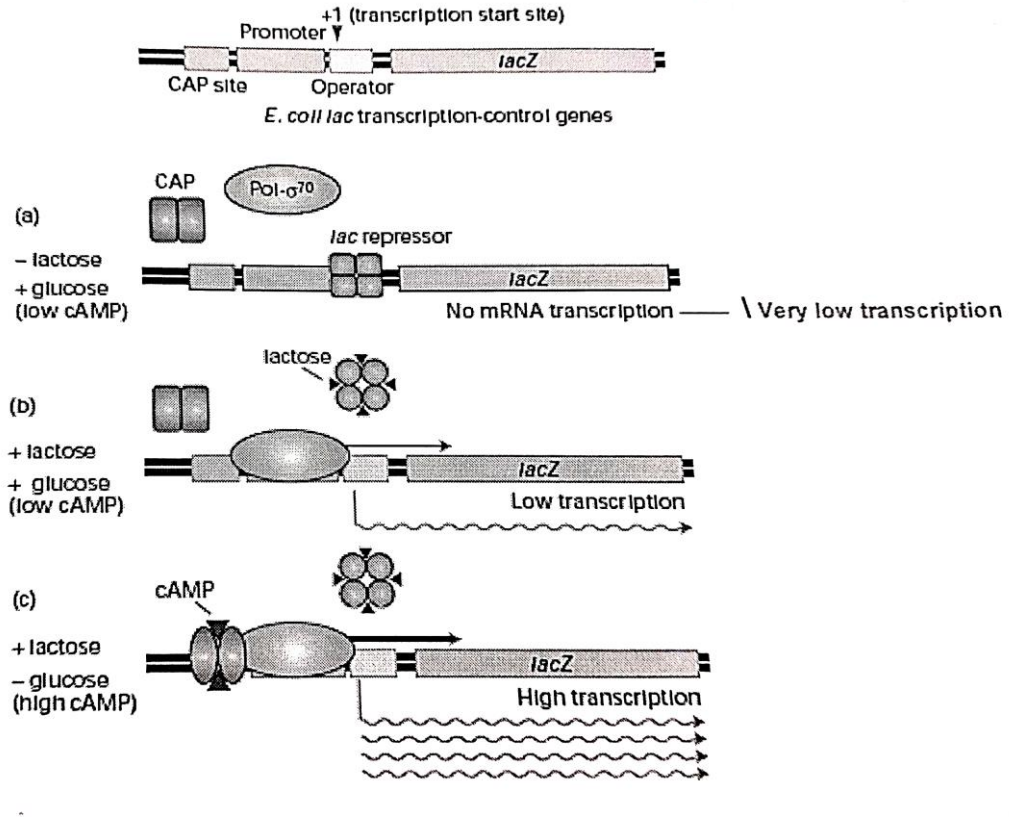
Activation of the lac promoter by CAP. CAP is recognized by the CTDs (C-terminal domain) of the α subunit. The αCTD also comes in contact with DNA, adjacent to the CAP site, when interacting with CAP. The σ factor is involved in stable binding of RNA polymerase specifically to promoter DNA. –10 sequence also known as Pribnow box. –35 sequence plays role in promoter recognition.



Structure of CAP- αCTD DNA complex [CAP as dimer. The αCTD of RNA polymerase is shown bound to an adjacent stretch of DNA and interacting with CAP. One molecule of ATP remains bound to each monomer of CAP].



Glucose concentration affects the activity of adenylate cyclase



lac Operon has three operator sites for the *lac* repressor

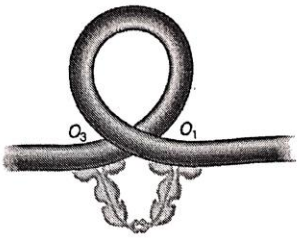
Detail genetic and crystallographic studies have shown that the binding of the *lac* repressor is more complex than originally thought

In all, three operator sites have been discovered

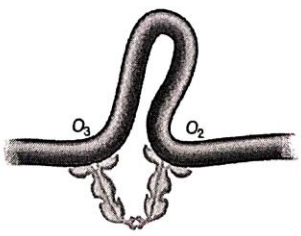
- O_1 → Next to the promoter
- O_2 → Downstream in the *lac*Z coding region
- O_3 → Slightly upstream of the CAP site

- According to the hypothesis *lac* repressor must bind to two of the three operators to cause repression
  - It can bind to O_1 and O_2, or to O_1 and O_3
    - But not O_2 and O_3
  - If either O_2 or O_3 is missing maximal repression is not achieved

- Binding of the *lac* repressor to two operator sites requires that the DNA form a loop
  - A loop in the DNA brings the operator sites closer together
    - This facilitates the binding of the repressor protein



lac repressor tetramer



lac repressor tetramer

Tryptophan Operon: A Repressible Operon

Tryptophan operon (trp operon) in *E.coli* responsible for controlling synthesis of amino acid toyptophan. Synthesis of tryptophan is carried out by five enzymea. The genes encoding these enzymes are located adjacent to one another in succession and are named trpE, trpD, trpE, trpB and trpA. Genes promoter, operator and two regions are called leader and attenuator which are located close to trp E gene. Trp R is a repressor gene and is present far from this gene cluster.

The regulation of trp operon takes place in the following steps:

1. Trytophan operon is switched on the normal transcription when amino acid trytophan is not accumulated in the nutrient medium.
2. When trytophan accumulates in the medium the operon is switched off.

The regulatory protein of trp operon is synthesized from the trpR gene. Mutations either in the operator gene or in trp R gene are responsible to cause constitutive initiation of trp RNA. Such phenomenon is called aporepressor. The aporepressor and tryptophan molecule unit and binds to operator causing switch off. Such phenomenon is called trp repressor.

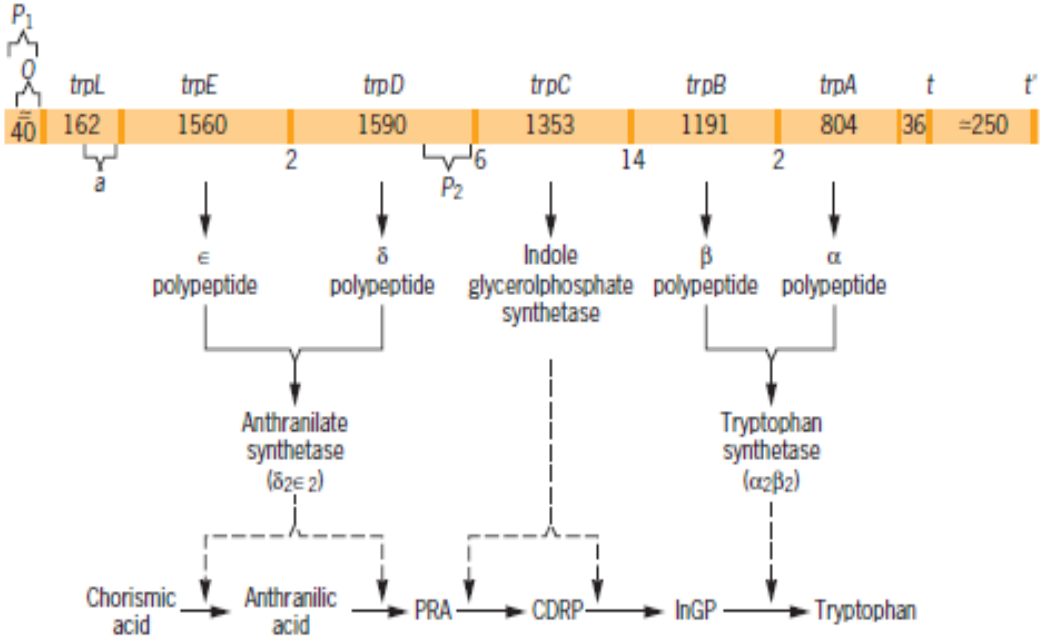


Fig. Organization of the trp operon in *E. coli.*
Source: Snustad and Simmons, 2012, Principles of Genetics -6th ed.)

Attenuation:

In most cases the regulato mechanisms of trp operon control the initiation of transcription. It has been observed experimentally that the regulatory site of trp operon is situated between promoter/operator of the first structural gene trpE. This site is called leader sequence. It is a non-coding DNA segment which is transcribed into leader mRNA at 5′ end of mRNA. This leader mRNA is about 162 nucleotides long and exercises an additional control on the transcription of polycistronic trp mRNA. This effer of leader sequence is called attenuation because it reduces i.e. attenuated the mRNA synthesis.

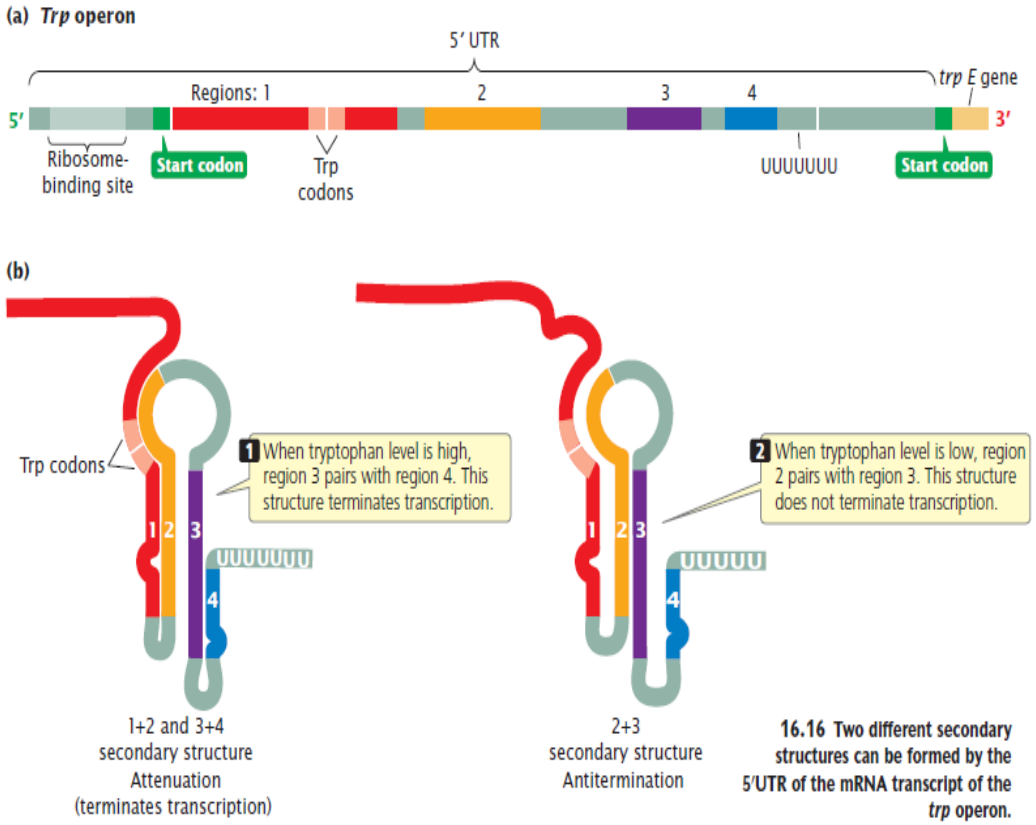


Fig. Attenuation of the trp operon. Source: Benjamin A. Pierce, 2012, Genetics: A conceptual apporach -4th ed.)

ara (arabinose) operon in *E.coli*:

E.coli can use arabinose as a carbon source by converting it into Xylulose-5-phosphate, an intermediate in the pentose phosphate shunt. It requires three enzymes viz. arabinose isomerase, ribulose kinase and ribulose-5-epimerase which are encoded by the genes ara A, ara B and ara D respectively.

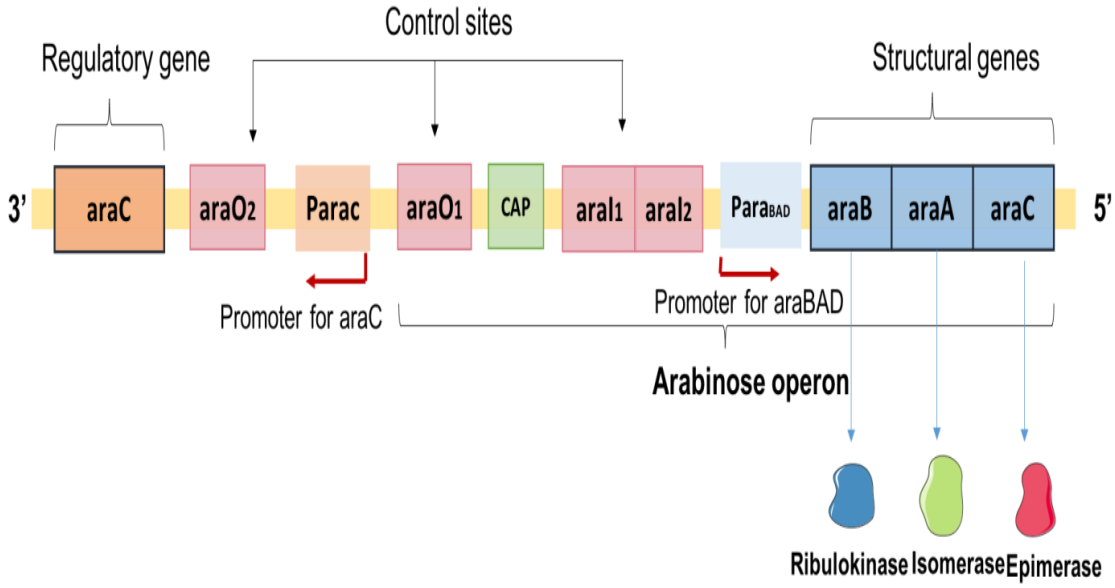


Fig. Structure of the ara operon. Source: https://en.wikipedia.org/wiki/L-arabinose_operon

Components of ara operon:

1. The ara operon constitutes three genes viz ara A, ara B ara D.
2. There is a regulatory site containing operators, ara O_1 and ara O_2.
3. Besides it includes another binding site for the ara C gene product (Ara C) called ara I (inducer) and a promoter (P_{BAS}) adjacent to ara I.
4. The ara C gene is present nearby and transcribed from its own promoter (Pc) near ara O_1, in opposite direction from ara BAD gene.
5. There is also a CAP – binding site adjacent to ara operon promoter (P_{BAD}). The binding site is antered ~92 bp away from the start point.

Mechanism:

1. One regulatory protein mediates both positive and negative control. The regulatory protein is Ara C encoded by ara C gene.

2. The Ara C protein regulates its own synthesis by represent in transcription of its gene. This phenomenon is called autoregulation. Ara C binding at ara O1 and repressing the transcription of ara-C gene when its concentration exceeds about 40 copies per cell. RNA polymerase synthesized ara C mRNA in a direction opposite to the ara BCD gene transcription.

3. When arabinose levels are low and glucose levels high, ara C protein binds to both ara I and ara O_2 and brings these sites together to form a DNA loop of about 210 base pairs. The operon repressed in this state. Ara C protein also binds to ara O_1 repressing further synthesis of Ara C.

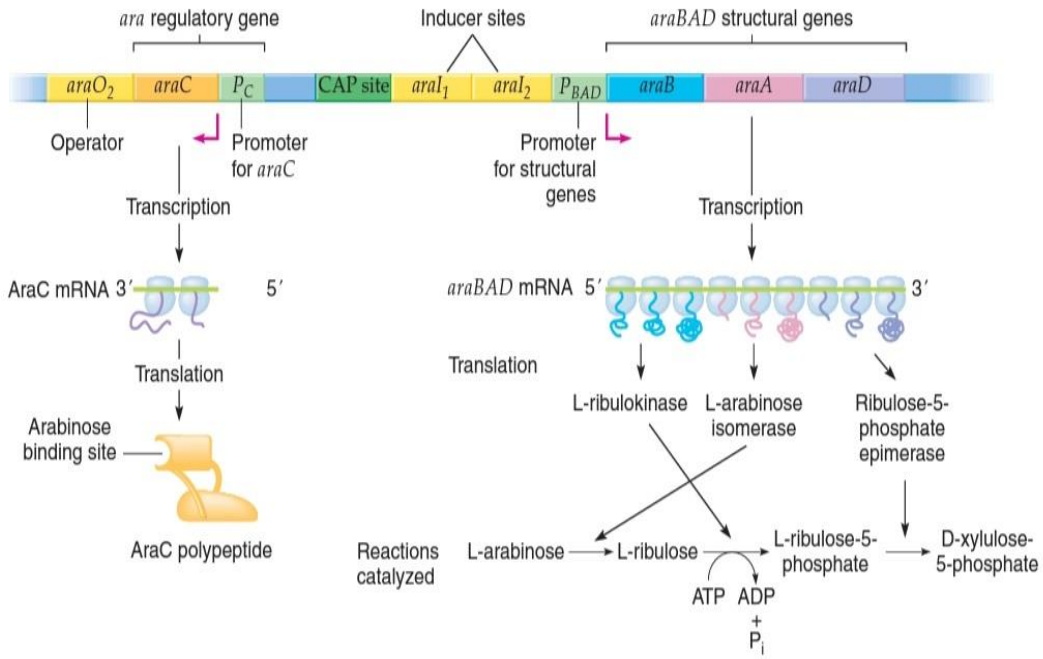
4. When glucose is absent or its concentration is very low but arabinose concentration is high, Ara C protein binds to arabinose, its conformation changes to an activator. The DNA loop is opened and the Ara C protein acts in concert with CAP – cAMP binding to induce transcription of ara BAD genes.

5. When arabinose and glucose both are abundant or absent, the ara BAD transcription remain inhibited.

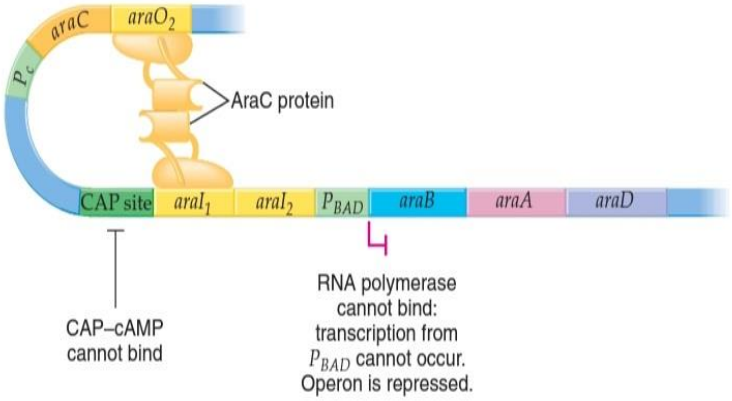
Figure 17.20

Regulation of the *ara* operon of *E. coli*.

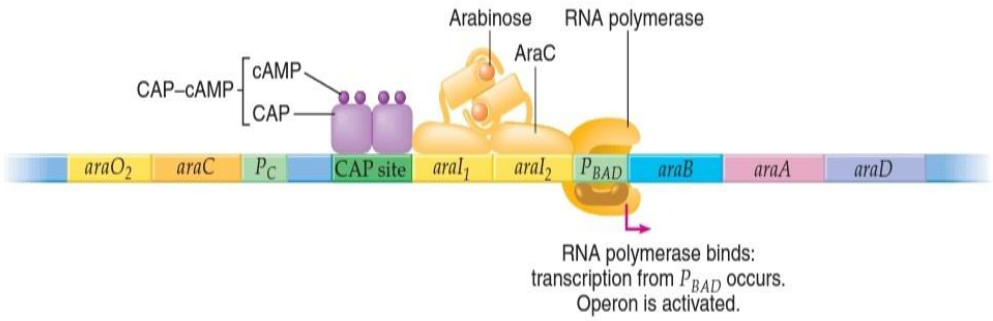
a) Organization of the *ara* operon



b) Arabinose and glucose absent: transcription of structural genes is inhibited



c) Arabinose present and glucose absent: transcription of structural genes is induced

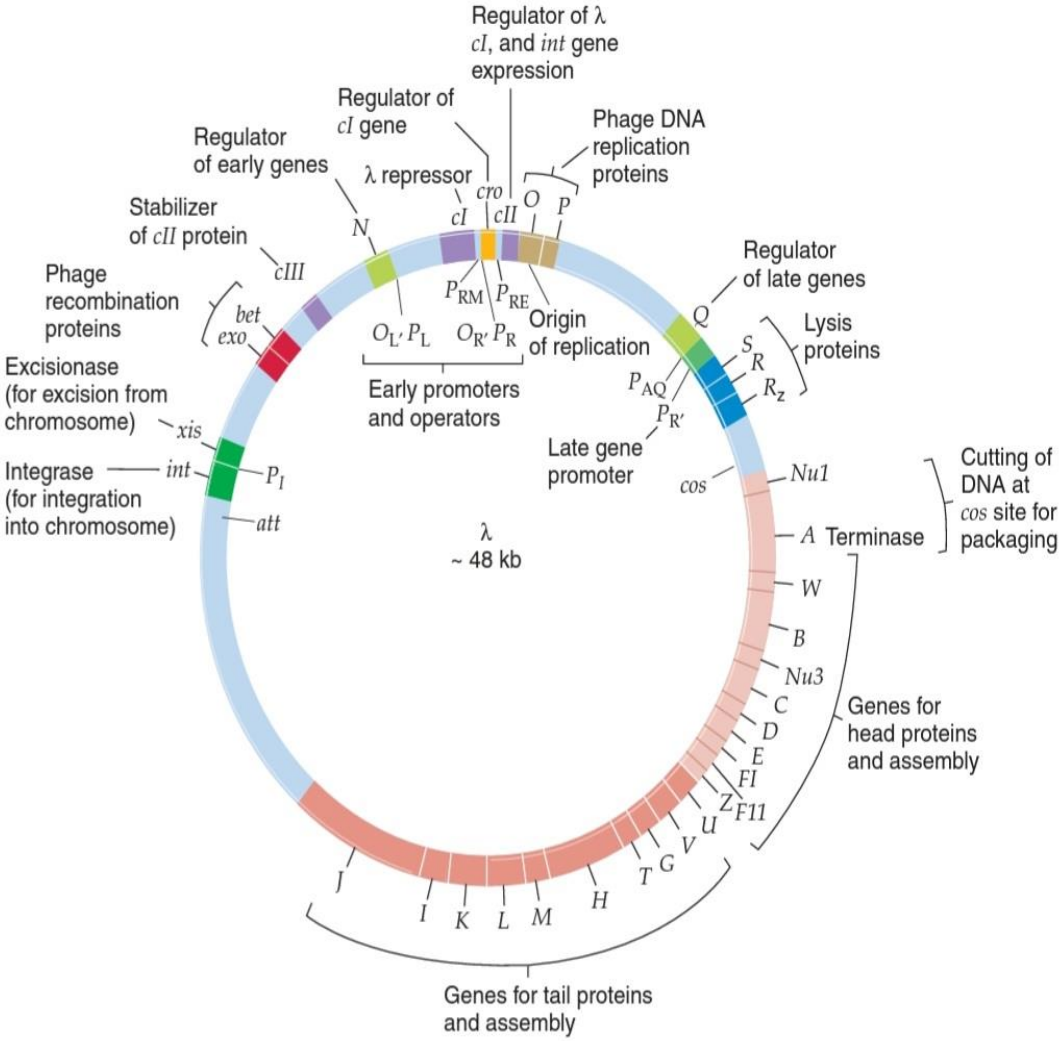


Gene regulation in Bacteriophages:

Gene expression in bacteriophages is also regulated through operons. There are promoter and reprenor genes, whose products regulate the activity of structural genes. For example, whether a phage is going to follow lytic or lysogenic pathway is determined by genetic switch involving repressor gene cI and cro gene. Switiching on of cro gene initiates lytic pathway, while turning on of repressor gene cI leads to lysogenic pathway.

Figure 17.21

A map of phage λ, showing the major genes. (Promoters discussed in text: P_L = promoter for leftward transcription of the left early operon, P_R = promoter for rightward transcription of the right early operon, P_{RE} = promoter for repressor establishment, and P_{RM} = promoter for repressor maintenance.)



Regulation of lytic Pathway:

When repressor gene is inactive two promoter genes P_1 (promoter left) and P_R (promoter right) are transcribed.

1. Gene P_R synthesizes cro protein which switches on early gene transcription during lytic pathway.

2. Gene P_L transcribes lamda or cl repressor protein-N. it allows RNA synthesis to proceed over certain transcription terminators. This process is called antiminator and gene P_L as antiterminator gene.

The product of both these genes turn on various genes of lytic pathway. These promote synthesis of coat protein, replication of viral DNA, assembly of new phage particles and lysis of bacterial cells.

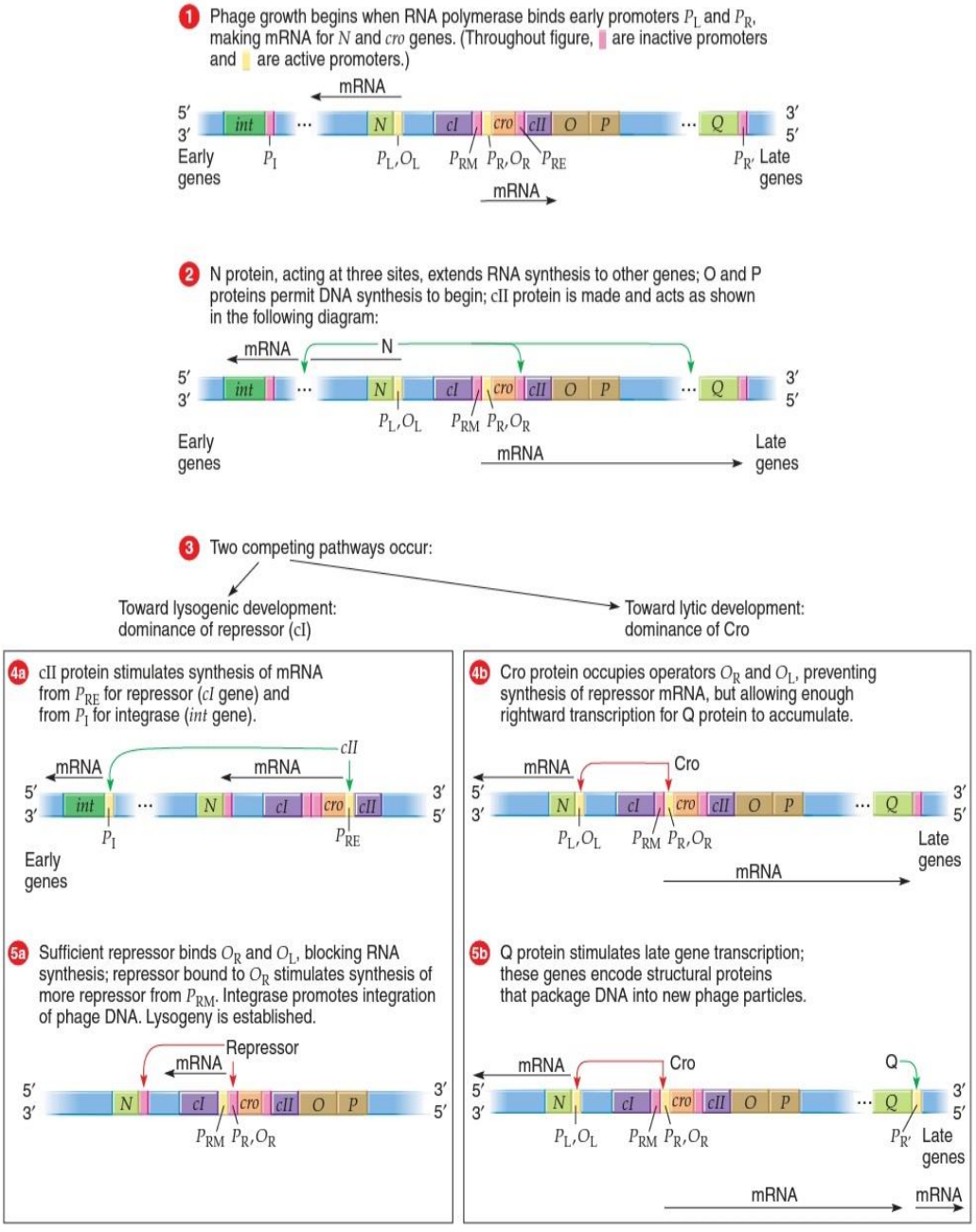
Regulation of lysogenic Pathway:

Lysogenic pathway is initiated by proteins produced by CII and CII genes. These activate transcription of CI gene which produces X – repressor protein. It binds to two operator O_L and O_R and also inactivates P_L and P_R. as a result, transcription of cro and N genes are blocked.

Thus in bacteriophages genes related to lytic or lysogenic pathways are organized into operons. Like bacterial operons, these operons are controlled through the interaction of regulatory proteins with operators, and these operators lie adjacent to clusters of structural genes.

Figure 17.22

Expression of λ genes after infection of *E. coli*, and the transcriptional events that occur when either the lysogenic or lytic pathway is followed. In the figure, stimulation of transcription is indicated by green arrows, and repression of transcription by red arrows.



Gene Regulation in Eukaryotes:

There are certain basic similarities between prokaryotic and eukaryotic regulation of gene expression. However, the transcriptional regulation is much more complex in eukaryotes especially multicellular eukaryotes. There are several major differences between the two, because:

1. Eukaryotes do not have typical operons as found in bacteria.
2. Eukaryotes have many more genes than prokaryotes. Their expression is regulated differently in different tissues and at different stages of development and differentiation.
3. Transcriptional regulation in eukaryotes involves many more regulatory proteins and DNA control elements such as promoters, enhancers, activators, insulators, mediators and other upstream or downstream nucleotide sequences.
4. Some eukaryotic regulatory proteins interact directly with DNA but in prokaryotes, many regulatory proteins act on DNA indirectly, via interactions with other proteins.
5. Expression of eukaryotic genes requires several activators. These may bind to upstream regions of promoter or to enhancer sequences several kilobases away from the promoter, or enhancer may be lie downstream of their target gene.
6. The regulatory proteins, transcription factor etc. are synthesized by ribosomes in the cytoplasm but transcription of RNA occurs in the nucleus. Therefore, the regulatory proteins are to be transported into the nucleus.
7. In eukaryotes, DNA is wrapped around octomeric histone protein complex and form bead-like structures, the nucleosomes. Nucleosomes do not allow DNA transcription. Removal of histones is essential for transcription to start.
8. In eukaryotes, long sections of DNA are frequently folded tightly into heterochromatin. In heterochromatic stage, they become inaccessible to RNA polymerase and transcription factors.
9. Introns are present in most eukaryotic genes but only few prokaryotic genes.
10. In eukaryotes, RNA is synthesized in the nucleus and is transported through

nuclear pores to the cytoplasm where it is translated.

11. Eukaryotes possess mechanism for rearrangement of DNA segments in a controlled manner by increasing number of copies of genes (genes amplification).
12. Regulatory regions are much larger than those of prokaryotes and these may be away from promoters.
13. In eukaryotes, most genes are subjected to positive control. Repressors are rare and usually behave differently to those in bacteria.

Levels of Gene Regulation in Eukaryotes:

In eukaryotes, gene expression is regulated at the following levels:

1. Genome level
2. Transcription of RNA
3. Processing of RNA transcript
4. Transport or translocation of RNA out of nucleus
5. Degradation of mature RNA
6. Translation of mRNA
7. Modification of processing of proteins
8. Degradation of protein products

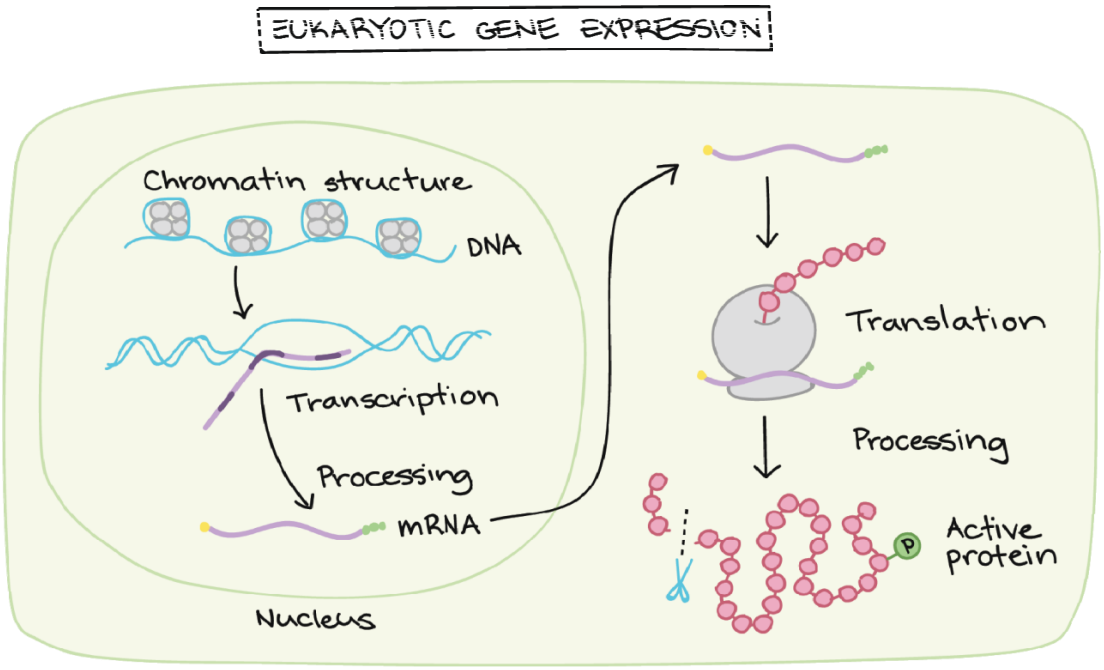
Eukaryotic gene expression can be regulated at many stages

In the articles that follow, we'll examine different forms of eukaryotic gene regulation. That is, we'll see how the expression of genes in eukaryotes (like us!) can be controlled at various stages, from the availability of DNA to the production of mRNAs to the translation and processing of proteins. Eukaryotic gene expression involves many steps, and almost all of them can be regulated. Different genes are regulated at different points, and it's not uncommon for a gene (particularly an important or powerful one) to be regulated at multiple steps.

- **Chromatin accessibility**: The structure of chromatin (DNA and its organizing proteins) can be regulated. More open or “relaxed” chromatin makes a gene more available for transcription.

- **Transcription**: Transcription is a key regulatory point for many genes. Sets of transcription factor proteins bind to specific DNA sequences in or near a gene and promote or repress its transcription into an RNA.

- **RNA processing**: Splicing, capping, and addition of a poly A tail to an RNA molecule can be regulated, and so can exit from the nucleus. Different mRNAs may be made from the same pre-mRNA by alternative splicing.



Source: https://www.khanacademy.org/science/biology/gene-regulation

Transcription: The key control point

Transcription is the process where a gene's DNA sequence is copied (transcribed) into an RNA molecule. Transcription is a key step in using information from a gene to make a protein. If you're not familiar with those ideas yet, you might consider watching the central dogma video for a solid intro from Sal.

Gene expression is when a gene in DNA is "turned on," i.e., used to make the protein it specifies. Not all the genes in your body are turned on at the same time, or in the same cells or parts of the body.

For many genes, transcription is the key on/off control point:

- If a gene is not transcribed in a cell, it can't be used to make a protein in that cell.
- If a gene does get transcribed, it is likely going to be used to make a protein (expressed). In general, the more a gene is transcribed, the more protein that will be made.

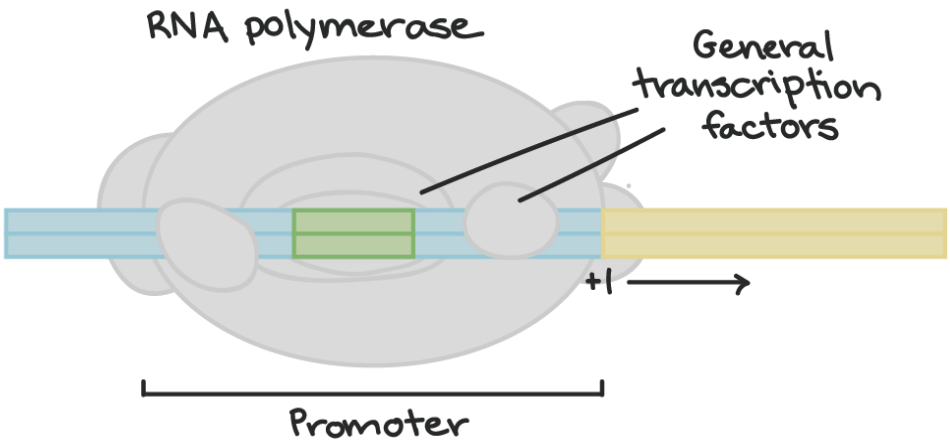
Various factors control how much a gene is transcribed. For instance, how tightly the DNA of the gene is wound around its supporting proteins to form chromatin can affect a gene's availability for transcription.
Proteins called transcription factors, however, play a particularly central role in regulating transcription. These important proteins help determine which genes are active in each cell of your body.

Transcription factors

What has to happen for a gene to be transcribed? The enzyme RNA polymerase, which makes a new RNA molecule from a DNA template, must attach to the DNA of the gene. It attaches at a spot called the promoter.

- In bacteria, RNA polymerase attaches right to the DNA of the promoter. You can see how this process works, and how it can be regulated by transcription factors, in the lac operon and trp operon videos.

- In humans and other eukaryotes, there is an extra step. RNA polymerase can attach to the promoter only with the help of proteins called basal (general) transcription factors. They are part of the cell's core transcription toolkit, needed for the transcription of any gene.



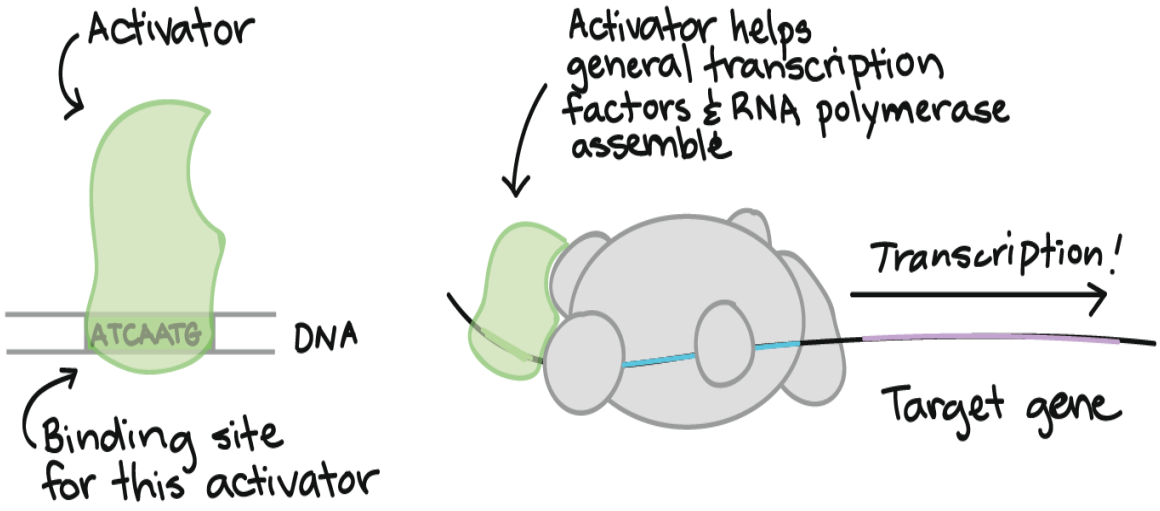
Source: https://www.khanacademy.org/science/biology/gene-regulation

How do transcription factors work?

A typical transcription factor binds to DNA at a certain target sequence. Once it's bound, the transcription factor makes it either harder or easier for RNA polymerase to bind to the promoter of the gene.

Activators

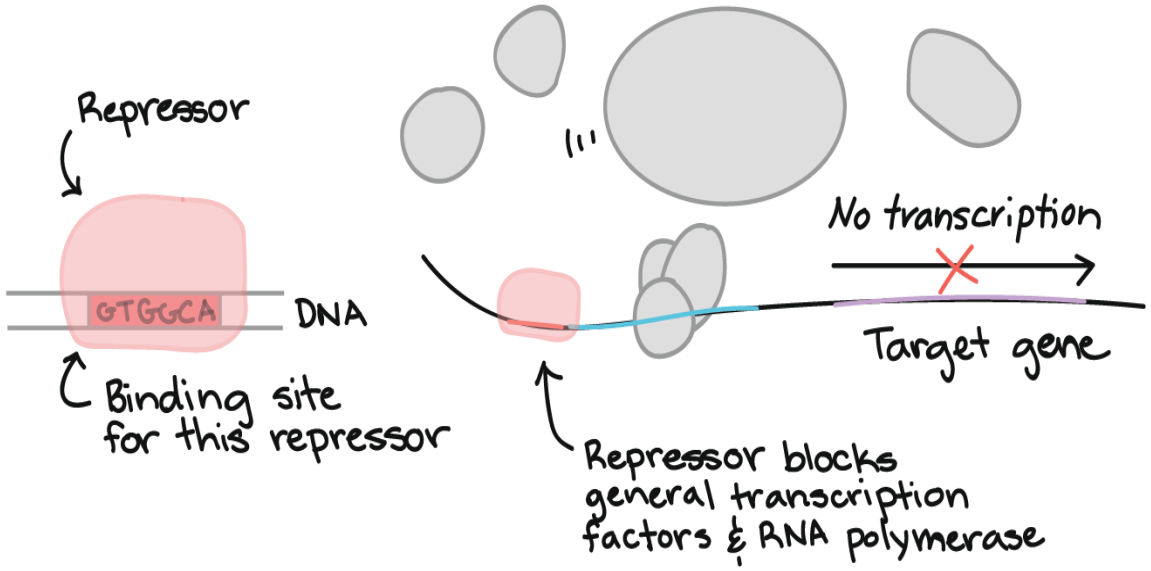
Some transcription factors activate transcription. For instance, they may help the general transcription factors and/or RNA polymerase bind to the promoter, as shown in the diagram below.



Source: https://www.khanacademy.org/science/biology/gene-regulation

Repressors

Other transcription factors repress transcription. This repression can work in a variety of ways. As one example, a repressor may get in the way of the basal transcription factors or RNA polymerase, making it so they can't bind to the promoter or begin transcription.



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Regulation of RNA processing

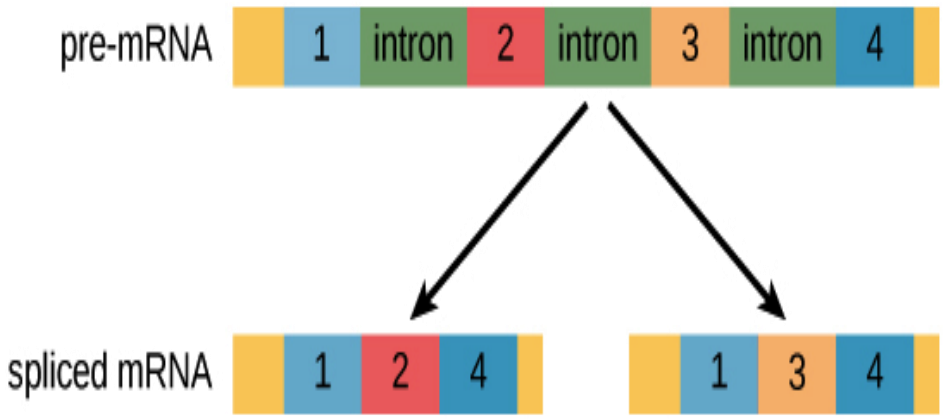
When a eukaryotic gene is transcribed in the nucleus, the primary transcript (freshly made RNA molecule) is not yet considered a messenger RNA. Instead, it's an "immature" molecule called a pre-mRNA.

The pre-mRNA has to go through some modifications to become a mature mRNA molecule that can leave the nucleus and be translated. These include splicing, capping, and addition of a poly A tail, all of which can potentially be regulated – sped up, slowed down, or altered to result in a different product.

Alternative splicing

Most pre-mRNA molecules have sections that are removed from the molecule, called introns, and sections that are linked or together to make the final mRNA, called exons. This process is called splicing.

In the process of alternative splicing, different portions of an mRNA can be selected for use as exons. This allows either of two (or more) mRNA molecules to be made from one pre-mRNA.



Alternative splicing, Source: https://www.khanacademy.org/science/biology/gene-regulation

Alternative splicing is not a random process. Instead, it is typically controlled by regulatory proteins. The proteins bind to specific sites on the pre-mRNA and "tell" the splicing factors which exons should be used. Different cell types may express different regulatory proteins, so different exon combinations can be used in each cell type, leading to the production of different proteins.

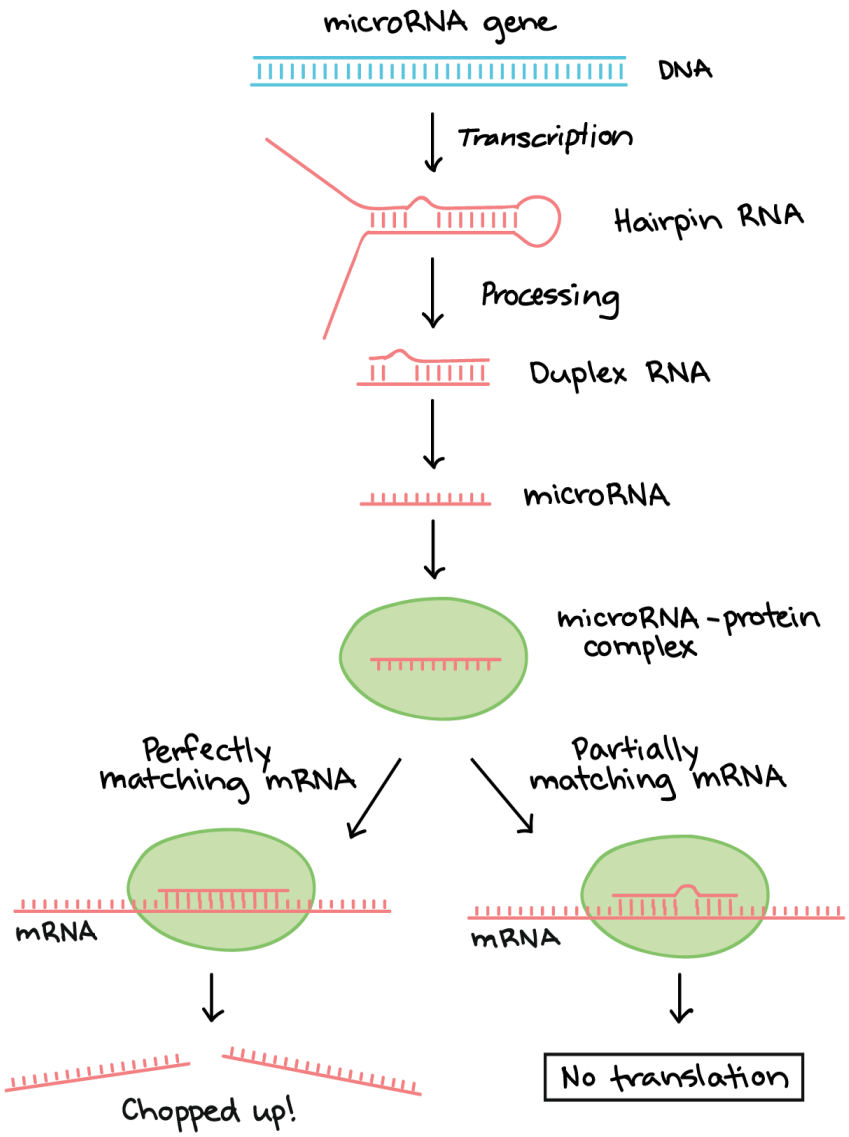
Small regulatory RNAs

Once an mRNA has left the nucleus, it may or may not be translated many times to make proteins. Two key determinants of how much protein is made from an mRNA are its "lifespan" (how long it floats around in the cytosol) and how readily the translation machinery, such as the ribosome, can attach to it.

A recently discovered class of regulators, called small regulatory RNAs, can control mRNA lifespan and translation. Let's see how this works.

microRNAs

MicroRNAs (miRNAs) were among the first small regulatory RNAs to be discovered. A miRNA is first transcribed as a long RNA molecule, which forms base pairs with itself and folds over to make a hairpin.



Source: https://www.khanacademy.org/science/biology/gene-regulation

Next, the hairpin is chopped up by enzymes, releasing a small double-stranded fragment of about 22nucleotides .One of the strands in this fragment is the mature miRNA, which binds to a specific protein to make an RNA-protein complex.

The miRNA directs the protein complex to "matching" mRNA molecules (ones that form base pairs with the miRNA). When the RNA-protein complex binds.

- If the miRNA and its target match perfectly, an enzyme in the RNA-protein complex will typically chop the mRNA in half, leading to its breakdown.
- If the miRNA and its target have some mismatches, the RNA-protein complex may instead bind to the mRNA and keep it from being translated.

These are not the only ways that miRNAs inhibit expression of their targets, and scientists are still investigating their many modes of action.
What do miRNAs actually do in organisms? Their direct role is to reduce the expression of their target genes, but they may play this role to produce many different outcomes.
For instance, in mice, a specific miRNA plays a key role in the development and function of the vascular (circulatory) system. Mice without function of this miRNA had defects in heart development and were unable to survive. Changes in expression levels of miRNAs are also associated with human diseases, including various types of cancer and cardiac hypertrophy.

Temperature: the heat shock genes
When organisms are subjected to the stress of high temperature, they respond by synthesizing a group of proteins that help to stabilize the internal cellular environment. These **heat shock proteins**, found in both prokaryotes and eukaryotes, are among the most conserved polypeptides known.

In *Drosophila*, for example, one of the heat-shock proteins called HSP70 (for heat-shock protein, molecular weight 70 kilodaltons) is encoded by a family of genes located in two nearby clusters on one of the autosomes. Altogether, there are five to six copies of these hsp70 genes in the two clusters. When the temperature exceeds 33ºC, each of the genes is transcribed into RNA, which is then processed and translated to produce HSP70 polypeptides. This heat induced transcription of the hsp70 genes is mediated by a polypeptide called the heat-shock transcription factor, or HSTF, which is present in the nuclei of

Drosophila cells. When *Drosophila* is heat stressed, the HSTF is chemically altered by phosphorylation. In this altered state, it binds specifically to nucleotide sequences upstream of the hsp70 genes and makes the genes more accessible to RNA polymerase II, The sequences to which the phosphorylated HSTF binds are called heat-shock response elements (HSEs).

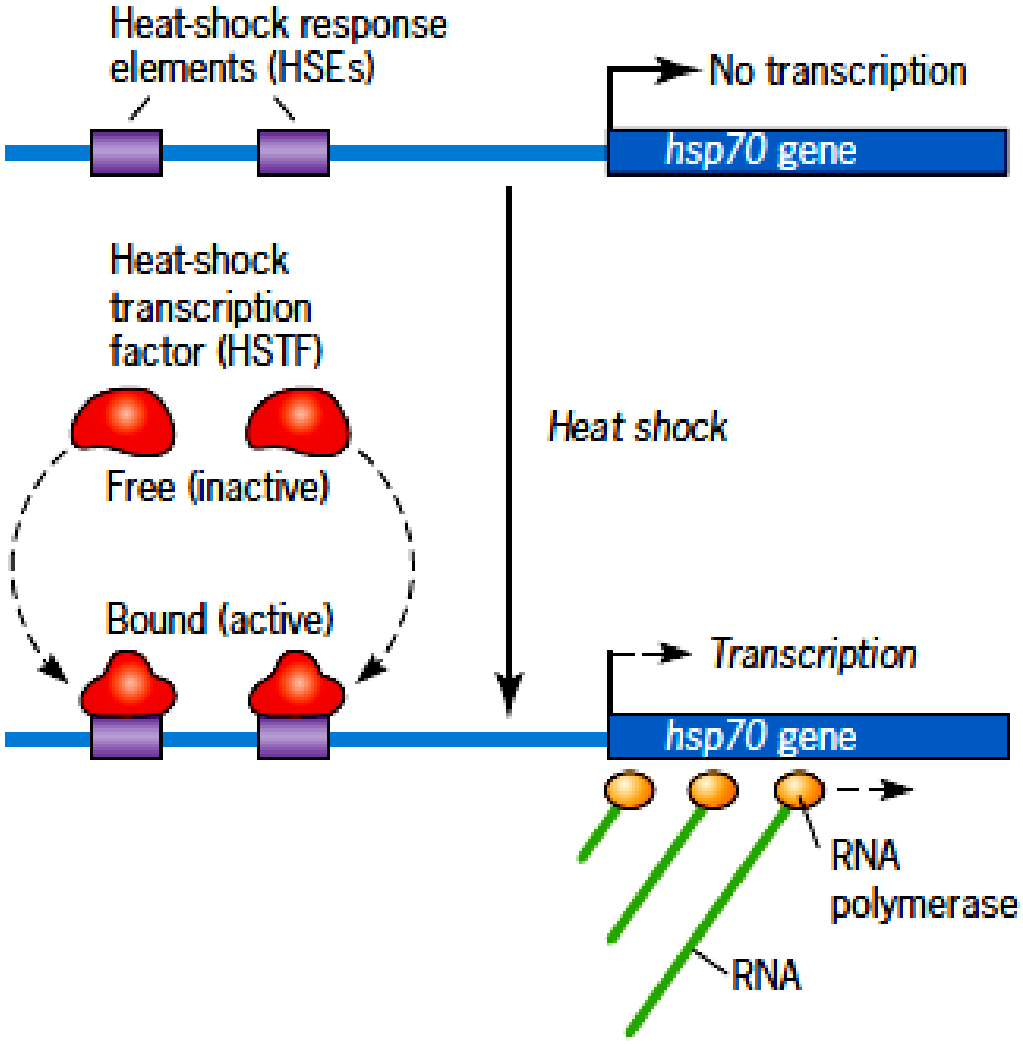


Fig. Induction of transcription from the Drosophila hsp70 gene by heat shock
Source: Snustad and Simmons, 2012, Principles of Genetics -6th ed.)

Signal molecules: genes that respond to hormones

In multicellular eukaryotes, one type of cell can signal another by secreting a **hormone.** Hormones circulate through the body, make contact with their target cells, and then initiate a series of events that regulate the expression of particular genes. In animals there are two general classes of hormones. The first class, **the steroid hormones**, are small, lipid-soluble molecules derived from cholesterol. Because of their lipid nature, they have little or no trouble passing through cell membranes, examples are estrogen and progesterone. Once these hormones have entered a cell, they interact with cytoplasmic or nuclear proteins called hormone receptors. The receptor/hormone complex that is formed then interacts with the DNA where it acts as a transcription factor to regulate the expression of certain genes.

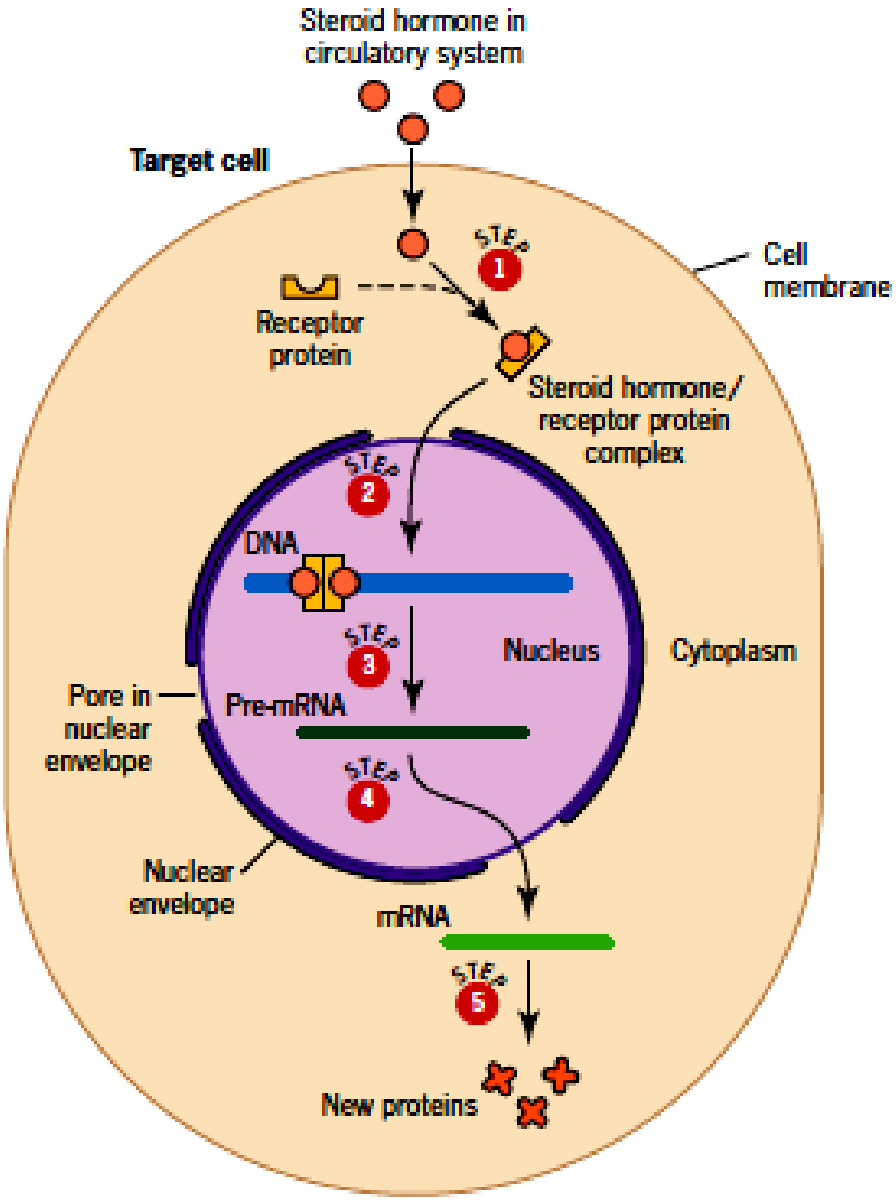


Fig. Signal transduction pathway. Source: Snustad and Simmons, 2012, Principles of Genetics -6th ed.)

DNase I Hypersensitivity

Several types of changes are observed in chromatin structure when genes become transcriptionally active. As genes become transcriptionally active, regions around the genes become highly sensitive to the action of DNase I. These regions, called **DNase I hypersensitive sites**, frequently develop about 1000 nucleotides upstream of the start site of transcription, suggesting that the chromatin in these regions adopts a more open configuration during transcription. This relaxation of the chromatin structure allows regulatory proteins access to binding sites on the DNA. Indeed, many DNase I hypersensitive sites correspond to known binding sites for regulatory proteins. At least three different processes affect gene regulation by altering chromatin structure: (1) the modification of histone proteins; (2) chromatin remodeling; and (3) DNA methylation. Each of these mechanisms will be discussed in the sections that follow:

Histone Modification

Histones in the octamer core of the nucleosome have two domains: (1) a globular domain that associates with other histones and the DNA and (2) a positively charged tail domain that probably interacts with the negatively charged phosphate groups on the backbone of DNA. The tails of histone proteins are often modified by the addition or removal of phosphate groups, methyl groups, or acetyl groups. These modifications have sometimes been called the **histone code**, because they encode information that affects how genes are expressed.

Methylation of histones: One type of histone modification is the addition of methyl groups to the tails of histone proteins. These modifications can bring about either the activation or the repression of transcription, depending on which particular amino acids in the histone tail are methylated. A common modification is the addition of three methyl groups to lysine 4 in the tail of the H3 histone protein, abbreviated H3K4me3 (K is the abbreviation for lysine). The H3K4me3 modification is frequently found in promoters of transcriptionally active genes in eukaryotes.

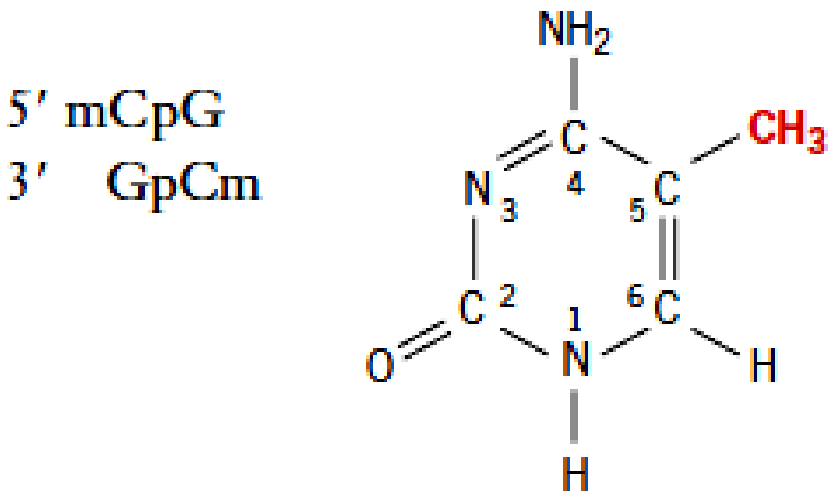
Acetylation of histones: Another type of histone modification that affects chromatin structure is acetylation, the addition of acetyl groups (CH3CO) to histone proteins. The acetylation of histones usually stimulates transcription. For example, the addition of a single acetyl group to lysine 16 in the tail of the H4 histone prevents the formation of the 30 nm chromatin fiber causing the chromatin to be in an open configuration and available for transcription.

Chromatin remodeling

Experiments that assess the sensitivity of DNA to digestion with DNase I have established that transcribed DNA is more accessible to in transcribed DNA, the nucleosomes are altered by multiprotein complexes that ultimately facilitate the action of the RNA polymerase. This alteration of nucleosomes in preparation for transcription is called **chromatin remodeling.**

DNA Methylation

Another change in chromatin structure associated with transcription is the methylation of cytosine bases, which yields 5-methylcytosine. The methylation of cytosine in DNA is distinct from the methylation of histone proteins mentioned earlier. Heavily methylated DNA is associated with the repression of transcription in vertebrates and plants, whereas transcriptionally active DNA is usually unmethylated in these organisms. Abnormal patterns of methylation are also associated with some types of cancer.



Structure of 5-methylcytosine

DNA methylation is most common on cytosine bases adjacent to guanine nucleotides (CpG, where p represents the phosphate group in the DNA backbone); so two methylated cytosines sit diagonally across from each other on opposing strands: DNA regions with many CpG sequences are called **CpG islands** and are commonly found near transcription start sites. While genes are not being transcribed, these CpG islands are often methylated, but the methyl groups are removed before the initiation of transcription. CpG methylation is also associated

with long term gene repression, such as on the inactivated X chromosome of female mammals.

Imprinting

DNA methylation in mammals is also responsible for unusual cases in which the expression of a gene is controlled by its parental origin. For example, in mice, the Igf 2 gene, which encodes an insulin-like growth factor, is expressed when it is inherited from the father but not from the mother. By contrast, a gene known as H19 is expressed when it is inherited from the mother but not from the father. Whenever the expression of a gene is conditioned by its parental origin, geneticists say that the gene has been **imprinted**—a term intended to convey the idea that the gene has been marked in some way so that it "remembers" which parent it came from.

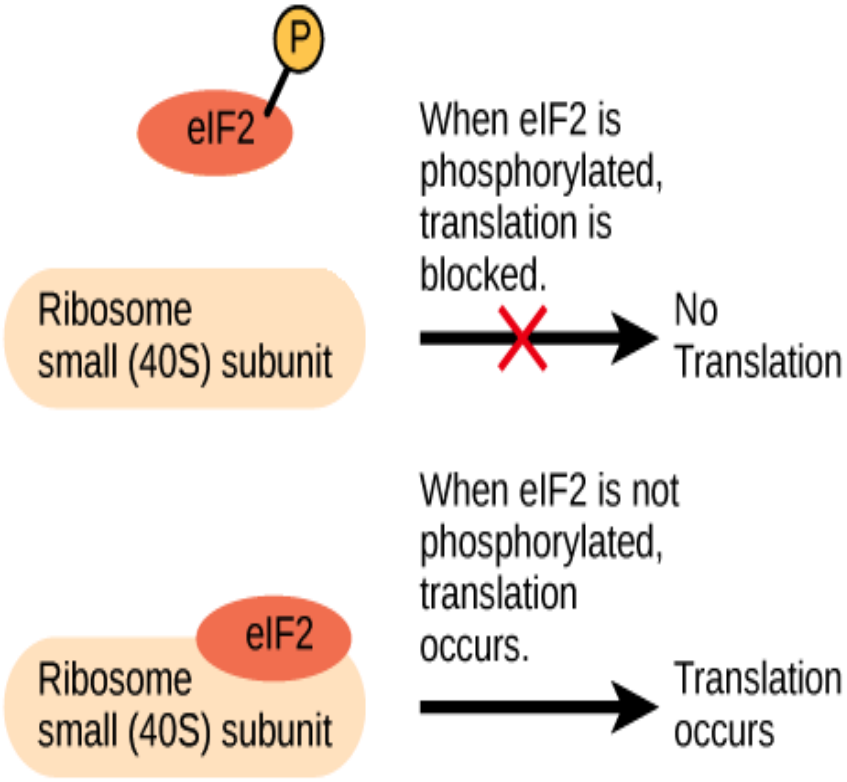
Regulation of translation

We already saw how miRNAs can inhibit translation, but there are a number of other ways that translation of an mRNA can also be regulated in a cell. One key step for regulation is translation initiation.

In order for translation to begin, the ribosome, an RNA-and-protein complex that houses translation, must assemble on the mRNA. This process involves many "helper" proteins, which make sure the ribosome is correctly positioned. Translation can be regulated globally (for every mRNA in the cell) through changes in the availability or activity of the "helper" proteins.

For example, in order for translation to begin, a protein called eukaryotic initiation factor-2 (eIF-2) must bind to a part of the ribosome called the small subunit. Binding of eIF-2 is controlled by phosphorylation, or addition of a phosphate group to the protein.

When eIF-2 is phosphorylated, it's turned "off"—it undergoes a shape change and can no longer play its role in initiation, so translation cannot begin. When eIF-2 is not phosphorylated, in contrast, it's "on" and can carry out its role in initiation, allowing translation to proceed.



Source: https://www.khanacademy.org/science/biology/gene-regulation

In this way, phosphorylation of eIF-2 acts as a switch, turning translation on or off. Inactivation of translation can be a good strategy in periods when the cell can't "afford" to make new proteins (e.g., when the cell is starved for nutrients).

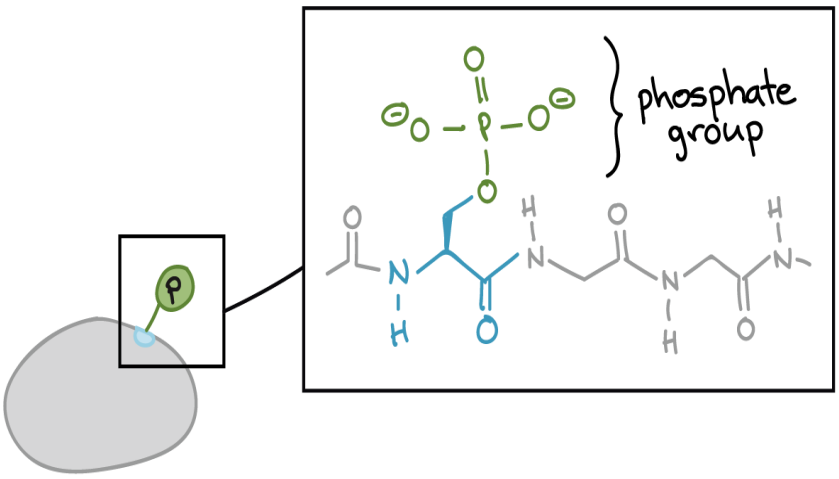
Proteins can be regulated after translation

There are also regulatory mechanisms that act on proteins that have already been made. In these cases, an "edit" to the protein – such as removal of amino acids, or addition of a chemical modification – can lead to a change in its activity or behavior. These processing and modification steps can be targets for regulation

Phosphorylation

One of the most common post-translational modifications is phosphorylation, in which a phosphate group is attached to a protein. The effect of phosphorylation varies from protein to protein: some are activated by phosphorylation, while others are deactivated, and others yet

simply change their behavior (interacting with a different partner, or going to a different part of the cell).



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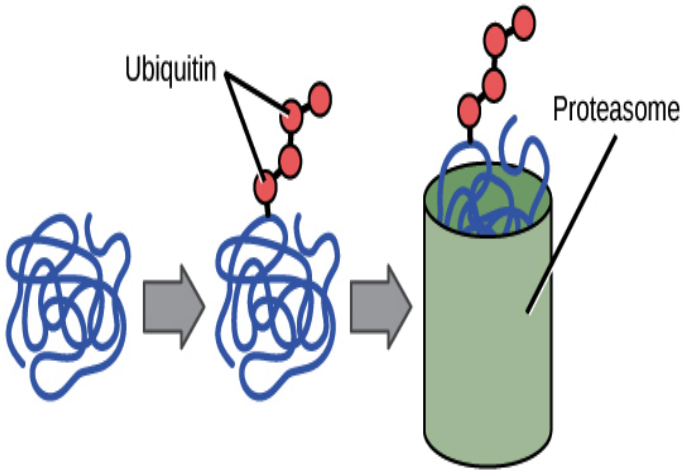
We saw one example of this above, when we examined how eIF-2 is inactivated by addition of a phosphate group (blocking translation). However, many different proteins can be selectively phosphorylated, producing various effects depending on the protein's role in the cell.

Ubiquitination

Proteins can be tagged for degradation by the addition of a chemical marker called ubiquitin. Ubiquitin-tagged proteins are taken to the proteasome, or "recycling center" of the cell, and broken down into their component parts. Ubiquitination is an important way of controlling the persistence of a protein in the cell.



Source: https://www.khanacademy.org/science/biology/gene-regulation

13. Let's sum up

- Centromeres are chromosomal regions where spindle fibers attach and telomeres consists tandemly repeated sequences that stabilize the ends of chromosomes. Telomerase is an enzyme makes DNA using RNA as a template.
- The mechanism by which sex is specified is termed sex determination. Sex may be determined by differences in specific chromosomes, genotypes, or environment. In Drosophila melanogaster, sex is determined by a balance between genes on the X chromosomes and genes on the autosomes. In humans, sex is ultimately determined by the presence or absence of the SRY gene located on the Y chromosome.
- Sex-linked characteristics are determined by genes on the sex chromosomes. Dosage compensation equalizes the amount of protein produced by X-linked genes in males and females.
- A polytene chromosome of *Drosophila* salivary glands has eukaryotic gene activity that is regulated at the level of RNA synthesis. The B chromosomes suppress homologous pairing which reduces multiple pairing between homologous chromosomes in allopolyploids. Deletion mapping has been used to reveal the chromosomal location of a gene.
- Linked genes do not assort independently. In a testcross for two completely linked genes (no crossing over), only nonrecombinant progeny are produced. When two genes assort independently, recombinant progeny and nonrecombinant progeny are produced in equal proportions. When two genes are linked with some crossing over between them, more nonrecombinant progeny than recombinant progeny are produced.
- In translocation heterozygotes, the chromosomes form cross like structures in meiosis, and the segregation of chromosomes produces unbalanced gametes. Aneuploidy usually causes drastic phenotypic effects because it leads to unbalanced gene dosage. All the chromosomes in an autopolyploid derive from one species; chromosomes in an allopolyploid come from two or more species.

- Organellar DNA controls plastidial inheritance in four o'clock plant, *Oenothera* and mitochondrial inheritance in yeast, maize.
- The rate at which individual genes are transferred during conjugation provides information about the order of the genes and the distances between them on the bacterial chromosome. Frequencies of the cotransformation of genes provide information about the physical distances between chromosomal genes. Phage genes can be mapped by infecting bacterial cells with two different phage strains and counting the number of recombinant plaques produced by the progeny phages.
- Transposable elements are mobile DNA sequences that insert into many locations within a genome and often cause mutations and DNA rearrangements. Transposons have played an important role in genome evolution.
- A population's genetic composition can be described by its genotypic and allelic frequencies. The Hardy–Weinberg law describes the effects of reproduction and Mendel's laws on the allelic and genotypic frequencies of a population.

14. Suggested Readings

1. Snustad, D.P. & Simmons, M.J. Principles of Genetics, John Wiley & Sons.
2. Pierce, Benjamin A. Genetics (2nd ed.), 2005, W.H. Freeman & Company.
3. Genetics by Monroe W. Strickbergar. (3rd edn). Macmillion Publishing Co., New York
4. Klug, W.S. & Cummings, M.R. Concepts of Genetics, 2003, Pearson Education.
5. Griffiths, A.I.F., Miller, J.H., Suzuki, D.T., Lewentin, C.R. & Gilbert, M.W. An Intrduction to Genetic Analysis, 2005 (8th ed.), W.H. Freeman & Company.
6. Russell, Peter J. 2006. iGenetics: A Molecular Approach. San Francisco: Benjamin Cummings.
7. B.D. Singh 1996. Fundamentals Of Genetics, Kalyani Publishers.
8. Bruce Alberts, Alexander Johnson, Julian Lewis, David Morgan, Martin Raff, Keith Roberts, Peter Walter. Molecular Biology of the Cell. 2015. 6th Ed: Garland Science.
9. Gerald Karp. Cell Biology. 2013. 7th Ed. International Student Version. Wiley.
10. Lewin, B. Genes VIII, 2004, Pearson Educational International.

11. Cooper, G.M. The Cell, A molecular approach (2nd ed.), 2000, ASM Press.
12. Gupta, P.K. Genetics, 2007, Rastogi Publications.
13. Kar, D.K. and Halder, S. Cell Biology, Genetics and Molecular Biology 2008, New Central Book Agency.
14. http://www.biologydiscussion.com/
15. https://en.wikipedia.org/

15. Assignments

i. Explain molecular organization of centromeres.
ii. What are chromosomal structural aberrations? Describe meiosis in heterozygotes for such aberrations.
iii. Write short notes on any four of the following:
    a. Dosage compensation. b. Polytene chromosomes
iv. Describe meiotic behaviour of trisomics. How are they useful in assigning genes to chromosomes?
v. Describe meiosis in an autotetraploid.
vi. Write short notes on any four of the following:
    a. Uses of allopolyploids. b. Primary and secondary trisomics. c. Allotetraploid and amphidiploids. d. Telomere. e. sex-influenced characters.
vii. Explain the multiple allelic inheritance and its significance.
viii. Give an account on Genetic map distances.
ix. Describe the methods of gene mapping in bacteriophages.
x. With the help of one suitable example for each, describe the genetic basis of mitochondrial and chloroplast related characters.
xi. Write a short note on the following:
    a. Distinction between cytoplasmic and nuclear types of inheritance. b. attenuation at *trp*. c. *IS* elements.
xii. Write short note on Lampbrush chromosome.
xiii. What is Segmental Allopolyploids?
xiv. Describe the Role of Polyploidy.

xv. Explain Robertsonian translocation.

xvi. Briefly describe telomere replication.

xvii. What is Replicative transposition?

xviii. Write short note on Non–Allelic Interaction.

All the materials are self-written and collected from ebook, journals and websites.

COURSE – BOTCOR T207

(Plant Breeding & Biometry)

Core Theory Paper **Credit: (Groups A+B) = 3**

Group – Group B (Plant Breeding & Biometry)

Content Structure

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1. Introduction

Plant breeding is an art and science, which tells us ways and means to change the genetic architecture of plants so as to attain a particular objective. Plant breeding can be accomplished through many different techniques ranging from simply selecting plants with desirable characteristics for propagation, to more complex molecular techniques Plant

breeding has been practiced for thousands of years, since near the beginning of human civilization. It is now practiced worldwide by individuals such as gardeners and farmers, or by professional plant breeders employed by organizations such as government institutions, universities, crop-specific industry associations or research centers. International development agencies believe that breeding new crops is important for ensuring food security by developing new varieties that are higher-yielding, resistant to pests and diseases, drought-resistant or regionally adapted to different environments and growing conditions.

From this course, you will get the detail knowledge about various breeding approaches and biostatistical experiments relating to estimation and hypothesis testing.

2. Course Objectives

You should gather knowledge after studying the course:

1. To know achievements about various breeding methodologies
2. Details explanation about Mass Selection Breeding
3. Gathering knowledge about disease resistance of plant

In this course you will learn how to effectively collect data, describe data, and use data to make inferences and conclusions about real world phenomena. After finishing this course, you should be able to:

1. Recognize the importance of data collection and its role in determining scope of inference.
2. Choose and apply appropriate statistical methods for analyzing one or two variables
3. Interpret statistical results correctly, effectively, and in context.
4. Understand and critique data-based claims.

3. Breeding methods: Introduction and conservation of germplasm, mass selection, pure line selection, clonal selection, hybridization, selection after hybridization (bulk, pedigree, recurrent), heterosis & inbreeding depression.

What is plant breeding?

Plant breeding is the genetic improvement of the crop in order to create desired plant types that are better suited for cultivation, give better yields and are disease resistant. Conventional plant breeding is in practice from 9,000-11,000 years ago. Most of our major food crops are derived from the domesticated varieties.

But now due to advancements in genetics, molecular biology and tissue culture, plant breeding is being carried out by using molecular genetics tools. Classical plant breeding includes hybridization (crossing) of pure lines, artificial selection to produce plants with desirable characters of higher yield, nutrition and resistance to diseases.

When the breeders wish to incorporate desired characters (traits) into the crop plants, they should increase yield and improve the quality. Increased tolerance to salinity, extreme temperatures, drought, resistance to viruses, fungi, bacteria and increased tolerance to insect pests should also be the desired traits in these crop plants.

Historical Background:

1. R. Camerarius produced the first artificial hybrid plant of maize in 1694.

2. Kolreuter (1733-1806) produced successful hybrids through artificial crosses in many plants.

3. The discipline of plant breeding witnessed great advances with the increased knowledge in the field of genetics.

4. Shull (1908, 1909) while investigating effect of inbreeding and cross-breeding in maize gave the concept of heterosis which has resulted in manifold increase in agricultural production.

5. Male Sterility in plants was reported by Kolreuter in 1763 which led to the economic exploitation of heterosis.

6. Alphonse de Condolle in 1882 was the first to give an account of the history and origin of cultivated plants.

7. N.I. Vavilov in 1925 proposed eight centres of origin of crops. These centres provided the regions of immense genetic resources of cultivated plants which existed there.

8. Monkambu Sambasivan Swaminathan (M.S. Swaminathan) initiated collaboration with Dr. Borlaug which reached the highest point into the "Green Revolution" through introduction of Mexican varieties of wheat in India.

Institutes Engaged in Plant Breeding at National and International Level:

1. International Rice Research Institute (IRRI), Philippines.
2. International Maize and Wheat Improvement Centre (CIMMYT), Mexico.
3. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad.
4. International Potato Centre (CIP), Peru.
5. International Board of Plant Genetic resources (now International Plant Genetic Resources Institute; IBPGR, now IPGRI).
6. Central Potato Research Institute (CPRI), Kufri (Shimla).
7. Indian Agricultural Research Institute (IARI), New Delhi.
8. Sugarcane Breeding Institute (SBI), Coimbatore.
9. Jute Agriculture Research Institute (JARI), Barackpore.
10. Indian Grassland and Fodder Research Institute (IGFRI), Jhansi.
11. Forest Research Institute, Dehradun.
12. Central Arid Zone Research Institute (CAZRI), Jodhpur.

Why breed plants?

The reasons for manipulating plant attributes or performance change according to the needs of society. Plants provide food, feed, fiber, pharmaceuticals, and shelter for humans. Furthermore, plants are used for aesthetic and other functional purposes in the landscape and indoors.

- Addressing world food and feed quality needs
- Addressing food supply needs for a growing world population
- Need to adapt plants to environmental stresses
- Need to adapt crops to specific production systems
- Developing new horticultural plant varieties
- Satisfying industrial and other end-use requirements

Germplasm

It is impossible to improve plants or develop new cultivars without genetic variability. Once the objectives have been determined, the breeder then assembles the germplasm to be used to initiate the breeding program. Sometimes, new variability is created through crossing of selected parents, inducing mutations, or using biotechnological techniques. Whether used as such or recombined through crossing, the base population used to initiate a breeding program must of necessity include the gene(s) of interest. That is, you cannot breed for disease resistance, if the gene conferring resistance to the disease of interest does not occur in the base population.

Plant introduction:

Plant introduction is a process of introducing plants (a genotype or a group of genotypes) from their own environment to a new environment.

The process of introduction may involve new varieties of crop or the wild relatives of crop species or totally a new crop species for the area.

Introduction may be classified into two categories:

Primary Introduction:

When the introduced variety is well suited to new environment then it is released for commercial cultivation without any alteration of genotype. For example, dwarf wheat varieties like 'Sonara-64', 'Lerma rojo' and dwarf rice varieties like 'Taichung Native 1', 'IR-8' are the examples of primary introduction.

Secondary Introduction:

When the introduced variety is subjected to Selection or used in hybridization programme with local varieties to get the improved varieties with some new characters introduced called secondary introduction. For example, the varieties like 'Kalyan Sona' and 'Sonalika' of wheat have been selected from material introduced from CIMMYT, Mexico.

The process of plant introduction is the successful compliance of two important aspects, viz., domestication and acclimatization. Domestication is the process of bringing of a wild species under cultivation by making them changed in behaviour suitable for new environment.

Acclimatization is the ability of a crop to become adapted to a new climatic and edaphic condition.

The process of acclimatization leads to increase the frequency of most adapted genotypes which depends on three factors:

(a) Breeding system,

(b) Genetic variation,

(c) Duration of the crop.

Purpose of Plant Introduction:

(a) Use in Agriculture, Forestry and Industry:

New varieties of plants or crops are introduced from various places for use as food, fibre, wood or medicinal purposes as well as the breeding material for hybridisation work.

(b) For Aesthetic Interest:

Various ornamental plants are introduced for beautification.

(c) For Germplasm Conservation:

The spread of high yielding varieties causes a danger to old varieties to get lost from an area. But germplasm collection and conservation help to maintain lines, clones, mutants, cultivars, etc. from as many sources as possible.

(d) For Studying Origin and Distribution:

The distribution of crop plants and their various forms in different parts of the world gives an idea of their origin and evolution.

Agencies of Plant Introduction in India:

The central plant introduction agency in India is National Bureau of Plant Genetic Resources (NBPGR), which has its headquarters at New Delhi, but has substations for testing the plant materials. NBPGR has the gene bank for long term storage and future use; it helps to assess the introduced plant material, coordinates the work of other agencies and imparts training in plant collection, introduction and maintenance in India.

The Substations under **NBPGR** are:

Shimla:

It represents the temperate zone of approx. 2300 m above sea level, place for germplasm collection in northern hills, station for acclimatization of material introduced from temperate zone and high altitude.

Jodhpur:

Exclusively meant for exploring and acclimatizing plant material for arid zone, this is under Central Arid Zone Research Institute.

Shillong:

This centre has been created for collection of germplasm from North-East India.

Other agencies engaged for this purpose are:

Botanical Survey of India:

This body is meant for introduction of medicinal plants and also plants for botanical importance.

Achievements of Plant Introduction:

The process of introduction from the pre-historic times helped the whole world to achieve newer crop species to new places as well as the new varieties of crop plants.

The examples of achievements are listed below:

(A) New Crop Species:

The crops like potato, maize, groundnut, chillies; coffee, rubber, guava, grape, pineapple, many ornamentals like Gulmohor, Phlox, Salvia, Aster-all have been introduced in India from outside.

(B) New Crop Varieties:

(a) Direct Multiplication and Released as New Varieties:

Dwarf wheat varieties like 'Sonora 64', 'Lerma Rojo' and dwarf rice varieties like TN-1′, 'IR-8' are introduced in India and used directly. There are many more examples of direct release of varieties in other crop plants like oat, soya-bean, tomato, cauliflower, onion, etc.

(b) Selection of Desirable Varieties:

Many varieties have come up after selection from introduced varieties, e.g., 'Kafyan Sona', 'Sonalika' were selected after introduction of Mexican varieties.

Mass selection:

Here a large number of plants having similar phenotype are selected and their seeds are mixed together to constitute a new variety. Thus the population obtained-from selected plants will be more uniform than the original population. However they are genotypically different.

Steps

1st year: From the base population select phenotypically similar plants, which may be 200 2000. Harvest the selected plants as a bulk.

2nd year: The bulk seed is divided into smaller lots and grown in preliminary yield trial along with control variety. Dissimilar phenotypes are rejected. Higher yielding plots are selected.

3rd to 6th years: With the selected lots conduct yield trials along with appropriate check or control. Select the best one and release it as a variety.

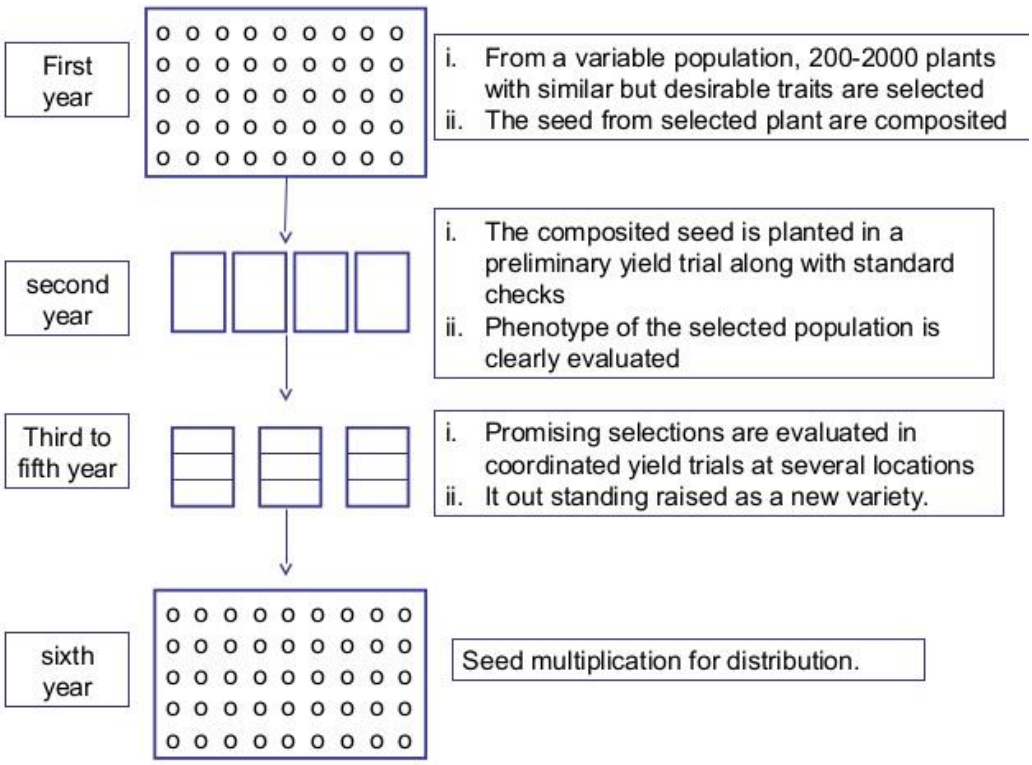


Fig.: Mass selection steps

Merits of Mass Selection

1. Since a large number plant is selected, the variety is more stable in performance over different environments as it is more adapted than a single pure line. So the varieties developed through mass selection are more widely accepted than pure line.
2. Extensive and prolonged field trials are not necessary. This reduces the time and cost needed for developing a new variety. This method is also less labour consuming.
3. Mass selection retains considerable genetic variability, so another mass selection after few years improves the variety.
4. This method can be applied to cross pollinated crops.

Demerits of Mass Selection

1. The varieties developed through mass selection show variation and are not uniform as pure line varieties, and strict selection for uniformity would lead to inbreeding depression.
2. The improvement of variety through mass selection is poorer than through pure line selection.

3. When the mass selection is done without progeny test, the homozygosity of the selected plants is not assured. In the self-pollinating species as there are chances for cross pollination to some extent, some plants must be heterozygous. In this method the genetic superiority either may be due to environment or due to genotype.
4. Pure line selection is more used than mass selection, only improvements of local old varieties are done through this method.
5. In seed certification programme, it is more difficult to identify the mass selected variety than the pure line.
6. This method utilizes only the variability already exists in the population, improvement is done only through selection. So the limitation is that it cannot generate new genetic variability.

Achievements:

Mass selection is effective when the population has the following characters:
(a) High genetic variability for different traits.
(b) The characters should be highly heritable in nature.
(c) The crop is grown under low population density.
(d) If only one particular character is chosen.

At present use of mass selection is limited to purification of pure line varieties of self-pollinated crops, because the superiority of the pure lines would be lost quickly if their purity is not maintained through mass selection.
Although no proper records are available, but the following varieties in different crops have been developed by mass selection in India:

Maize — Jaunpur local, Tinpakhia, Basri

Pearl Millet — Bajra-207, Bajri-28-15, Bichpuri local, Pusa moti

Mustard — Gurgaon brown sarson

Sorghum — RSI, T22

Cotton — Dodahatti local, Dharwar American, Combodian cotton

Pureline selection:

A large number of plants are selected from a self-pollinated crop. The selected plants are harvested individually. The selected individual plants are grown in individual rows and evaluated and best progeny is selected, yield tested and released as a variety.

Characteristics of purelines

i. All plants within a pure line have the same genotype.
ii. The variation with in a pureline is environmental and nonheritable.
iii. Purelines become genetically variable with time due to natural hybridization, mutation and mechanical mixtures.

General steps for making a pureline selection

1st year: From the base population select best looking plants having the desirable characters. Harvest them on single plant basis.

2nd year: The selected single plants are grown in progeny rows and estimate the performance. Reject unwanted progenies.

3rd year: Repeat the process of second.

4th year: Grow the selected single plants in replicated preliminary yield trial along with suitable check or controI variety.

5th year: Conduct regular comparative yield trial along with check variety and select the best culture.

6th year: Conduct multilocation trial in different research stations along with local check.

7th year: Conduct Adaptive Research Trial in farmer's field. Fix the best yielder and release it as a variety thro' Variety Release committee.

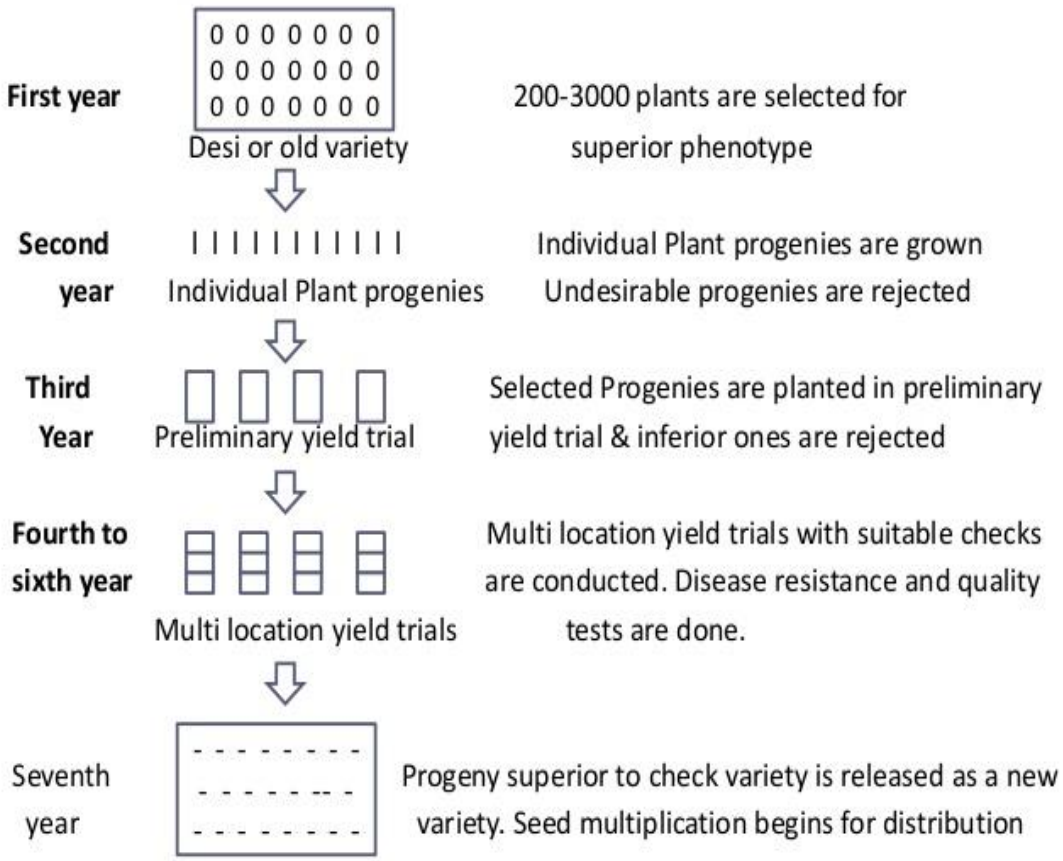


Fig. Pure line selection steps

Merits:

1. Pure line selection can achieve maximum possible improvement over the original variety. This is because the variety is the best pure line present in the population.
2. The pure line variety is extremely uniform since all the plants in the variety have the same genotype. Such uniformity is liked both by the farmers and the consumers.
3. Due to extreme uniformity, the variety is easily identified in seed certification programme.

Demerits:

1. As the pure line variety is genetically more homogeneous, the adaptability to various environments is less.
2. This procedure requires more time, space, labour and more expensive yield trials.
3. The upper limit of improvement is set by the genetic variation present in the original population.

4. Once a pure line is developed, the variability is frozen and further selection becomes less effective.

Achievements:

Pure line selection is the most extensively used breeding method in early days of crop improvement in India because at that time many genetically variable 'desi' varieties were available.

A large number of improved varieties were developed by this method in many self-pollinated crops:

Wheat — NP4, NP6, NP12, Pb-8, CI3, K46

Rice — M-351, Vidisa 60-1, Patni 6, Aispuri, BP 53

Cotton — Coimbatore 2, Gadag 1, MCU1

Barley — C-251, C-50, K-12

Tobacco — NP28, NP63, NP70

Comparison between pure line and mass selection

Pureline selection	Mass selection
The new variety is a pureline	The new variety is a mixture of purelines.
The new variety is highly uniform. In fact, the variation within a pureline variety is purely environmental.	The variety has genetic variation of quantitative characters, although it is relatively uniform in general appearance
The selected plants are subjected to progeny test	Progeny test is generally not carried out
The variety is generally the best pureline present in the original population. The pure line selection brings about the greatest	The variety is inferior to the best pureline because most of the purelines included in it will be inferior to the best pure line

improvement over the original variety	
Generally, a pure line variety is expected to have narrower adaptation and lower stability in performance than a mixture of pure lines	Usually the variety has a wider adaptation and greater stability than a pureline variety
The plants are selected for the desirability. It is not necessary they should have a similar phenotype	The selected plants have to be similar in phenotype since their seeds are mixed to make up the new variety
It is more demanding because careful progeny tests and yield trials have to be conducted.	If a large number of plants are selected, expensive yield trials are not necessary. Thus it is less demanding on the breeder.

Clonal selection:

A clone may be defined as a group of plants derived from a single plant by vegetative propagation.

The clone can be characterised by the following characters:

(a) Clone is homogeneous,

(b) Individuals of a clone are either homozygous or heterozygous,

(c) Clones are stable in nature,

(d) Variability can be induced through mutation,

(e) Clone is propagated vegetatively.

Selection Procedure:

In view of these considerations, in the earlier stages of clonal selection, when selection is based on single plants or single plots, the emphasis is on the elimination of weak and undesirable plants or clones. The breeder cannot reasonably hope to identify superior' genotypes at this stage.

First Year

From a mixed variable population, few hundreds to few thousand desirable plants are selected. A rigid selection can be done for simply inherited characters with high heritability. Plants with obvious weaknesses are eliminated.

Second Year

Clones from the selected plants are grown separately, generally, without replication This is done in view of the limited supply of the propagating material for each clone, and because of the large number of clones involved. The characteristics of clones will be clearer now than in the previous generation when the observations were based on individual plants. The number of clones is drastically reduced and inferior clones eliminated.

Third Year

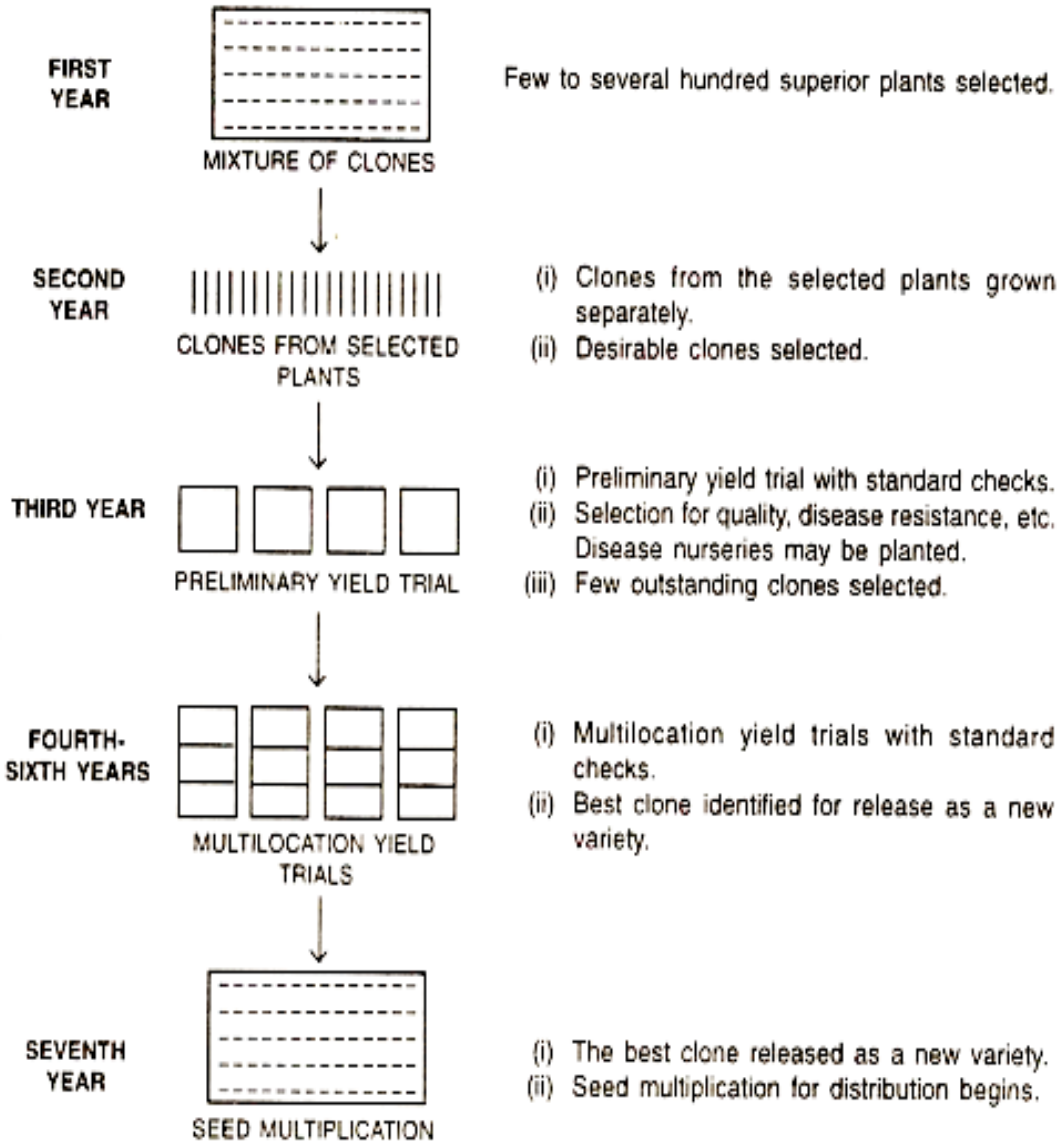
Replicated preliminary yield trial is conducted. Suitable, checks included for comparison. Few superior performing clones with desirable characteristics selected for multilocation trials. At this stage, selection for quality is also done.

Fourth to Sixth Years

Replicated yield trials are conducted at several locations along with a suitable check. The yielding ability, quality and disease resistance, etc. of the clones are rigidly evaluated. The best clone that is superior to the check in one or more characteristics is identified for release as a new variety.

Seventh Year

The superior clone is multiplied released as a new variety.



Merits of Clonal Selection

[1] It is the only method of selection applicable to clonal crops. It avoids inbreeding depression, and preserves the gene combinations present in the clones.

[2] Clonal selection, without any substantial modification, can be combined with hybridization to generate the variability necessary for selection.

[3] The selection scheme is useful in maintaining the purity of clones.

Demerits of Clonal Selection

[1] This selection method utilizes the natural variability already present in the population; it has not been devised to generate variability.

[2] Sexual reproduction is a prerequisite for the creation of variability through hybridization.

Hybridization

Individual produced as a result of cross between two genetically different parents is known as hybrid. The natural or artificial process that results in the formation of hybrid is known as hybridization.

The production of a hybrid by crossing two individuals of unlike genetical constitution is known as hybridization. Hybridization is an important method of combining characters of different plants. Hybridization does not change genetic contents of organisms but it produces new combination of genes.

The first natural hybridization was recorded by Cotton Mather (1716) in corn. The first artificial interspecific plant hybrid was produced by Thomas Fairchild in 1717. It is commonly known as 'Fairchild Mule'.

Hybridization was first of all practically utilized in crop improvement by German botanist Joseph Koerauter in 1760. Mendel onward, the hybridization had become the key method of crop improvement.

Objectives of Hybridization:

I. To artificially create a variable population for the selection of types with desired combination of characters.
II. To combine the desired characters into a single individual, and
III. To exploit and utilize the hybrid varieties.

Types of Hybridization:

Hybridization may be of following types:

(i) Intra-varietal hybridization:

The crosses are made between the plants of the same variety.

(ii) Inter-varietal or Intraspecific hybridization:

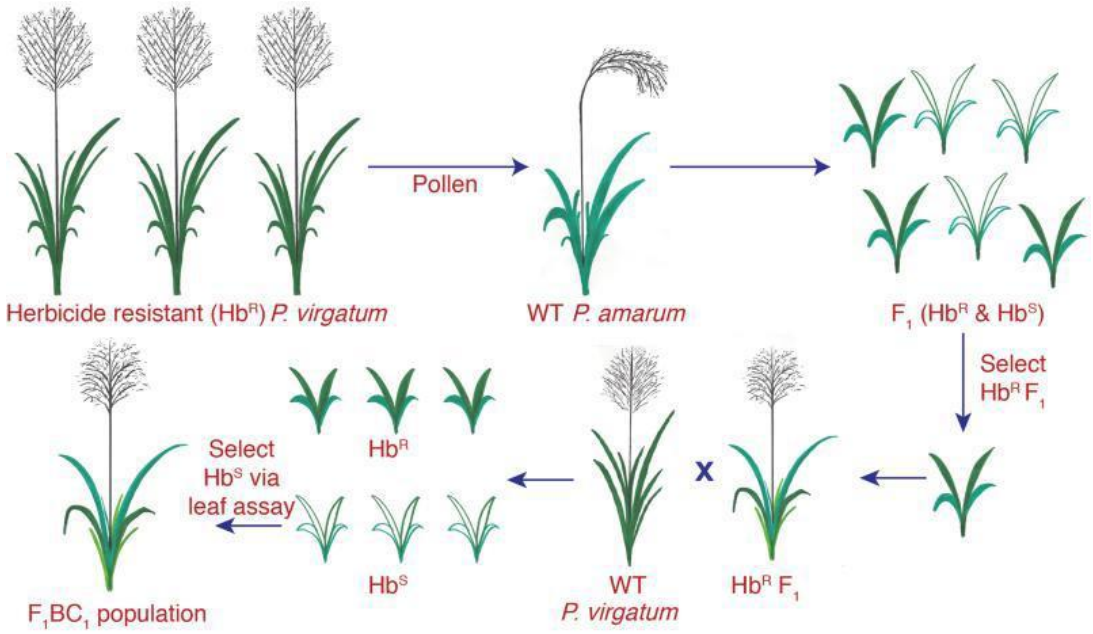
The crosses are made between the plants belonging to two different varieties.

(iii) Interspecific hybridization or intragenric hybridization:

The crosses are made between two different species of the same genus.

(iv) Introgressive hybridization:

Transfer of some genes from one species into the genome of the other species is known as introgressive hybridization. The crosses between different species of the same genus or different genera of the same family are also known as distant hybridization or wide crossing. Such crosses are called distant crosses.



Procedure of Hybridization:

It involves the following steps:

(i) Selection of parents:

The selection of parents depends upon the aims and objectives of breeding. Parental plants must be selected from the local areas and are supposed to be the best suited to the existing conditions.

(ii) Selfing of parents or artificial self-pollination:

It is essential for inducing homozygosity for eliminating the undesirable characters and obtaining inbreeds.

(iii) Emasculation:

It is the third step in hybridization. Inbreeds are grown under normal conditions and are emasculated. Emasculation is the removal of stamens from female parent before they burst and shed their pollens.

It can be defined as the removal of stamens or anthers or the killing of the pollen grains of a flower without affecting in any way the female reproductive organs. Emasculation is not required in unisexual plants but it is essential in bisexual or self-pollinated plants.

Various methods used for emasculation are:

a) Hand Emasculation or Forceps or Scissor Method:

This method is generally used in those plants which have large flowers. In this method the corolla of the selected flowers is opened and the anthers carefully removed with the help of fine-tip forceps.

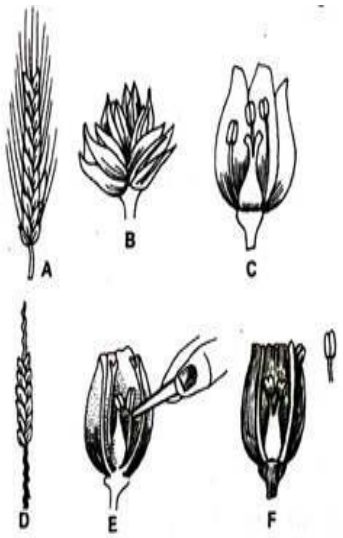


Fig. 6. (A-F) Emasculation in wheat. (A) Spike of spikelets, (B) spikelet, (C) Floret, (D) Upper and lower spikelets removed, awns removed, upper portion of florets cut, (E) Anthers removed with the help of fine-tip forceps. (F) Removed anther.

Following are the important precautions while performing this method:

i. Flowers should be selected at proper stage.

ii. Stigma should be receptive and anthers should not have dehisced.

iii. All the anthers should be removed from the flowers without breaking.

iv. Stigma and ovary of the flower should not be damaged.

b) Hot Water Treatment:

Removal of stamens with the help of forceps is very difficult in minute flowers. In such small hermaphrodite flowers (e.g., Bajra, Jowar) emasculation is done by dipping the flowers in hot water for certain duration (1-10 minutes) of time. This method is based on the fact that gynoecia can withstand the hot temperature at which the anthers are killed. In this method an equipment is used which is placed on a simple heavy stand.

It consists of a cylindrical metallic container of 60 cm length, with one hole of 5 cm to 16 cm diameter on one end to pass over a bajra or jowar head. After inserting the panicle inside the container a cork is fitted in the hole to close it. A 35 cm long rubber tube or belt is stretched over the side of the container, and when in use this tube is tied around the peduncle of the head. To measure the temperature, in the upper side of the container a thermometer is placed. In the field water is carried in a thermos jug.

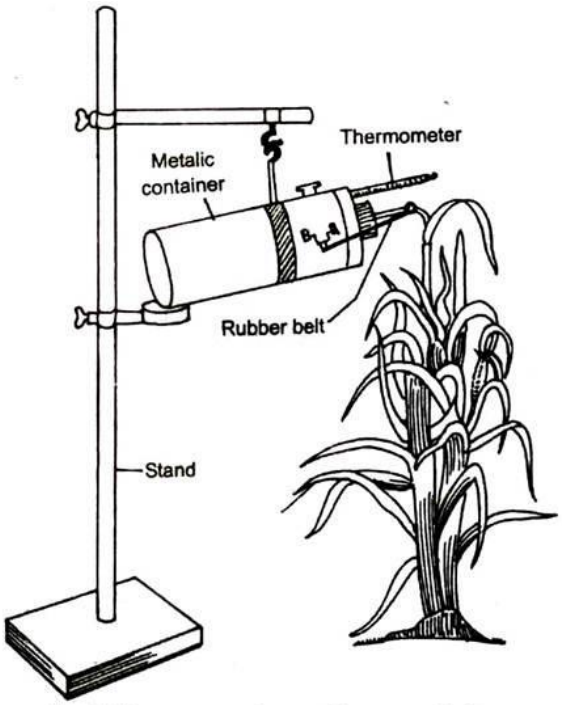


Fig. 7. Hot water equipment for emasculation.

The panicle is inserted in the container prior to blooming for a particular duration of time. It has been observed that pollen grains of rice are killed by immersing the inflorescence for 5 to 10 minutes in the hot water maintained at 40-44°C in a thermos flask.

c) Cold Water Treatment:

Like hot water cold water also kills pollen grains without damaging the gynoecium. In rice 0-6°C temperature is maintained to kill the pollen grains. This method is less effective than hot water treatment.

d) Alcohol Treatment Method:

This method is not commonly used for emasculation because duration of treatment is an important factor since a very short duration is required failing which even the gynoecium may be damaged. Flowers or inflorescences are immersed in alcohol of a suitable concentration for a brief period. In alfa-alfa, a treatment of even 10 seconds with 57 % alcohol is sufficient to kill the pollen grains.

e) Suction Method:

It is a mechanical method and is suitable for the crops having minute flowers. In this method the amount of pressure is applied in such a way that only anthers are sucked out and other

parts of the flower like gynoecium remain intact. However, in this method 10-15% self-pollination takes place. It is one of the major drawbacks of this method.

f) Male Sterility or Self-incompatibility Method:

Emasculation option can be eliminated by the use of male-sterile plants, In some self-pollinated plants for example, Sorghum, Onion, Barley etc. anthers are sterile and do not produce any viable pollens! Similarly self-incompatibility may also be used to avoid emasculation.

g) Chemical Gametocides:

Certain chemicals are capable of causing male sterility, when sprayed before flowering e.g., 2, 4-D, naphthalene acetic acid (NAA), maleic-hydrazide (MA), tribenzoic acid etc. FW450 in cotton may be used for bringing about emasculation.

(iv) Bagging:

It is the fourth step and completed with emasculation. The emasculated flower or inflorescence is immediately bagged to avoid pollination by any foreign pollen. The bags may be made of paper, butter paper, glassine or fine cloth. Butter paper or vegetable parchment bags are most commonly used.

Fig. 8. Different methods of Bagging.

The bags are tied to the base of the inflorescence or to the stalk of the flower with the help of thread, wire or pins. The bagging is done with the emasculation in bisexual plants and before the stigma receptivity and dehiscence of the anthers in unisexual plants. Both male and female flowers are bagged separately to prevent contamination in male flowers and cross-pollination in female flowers.

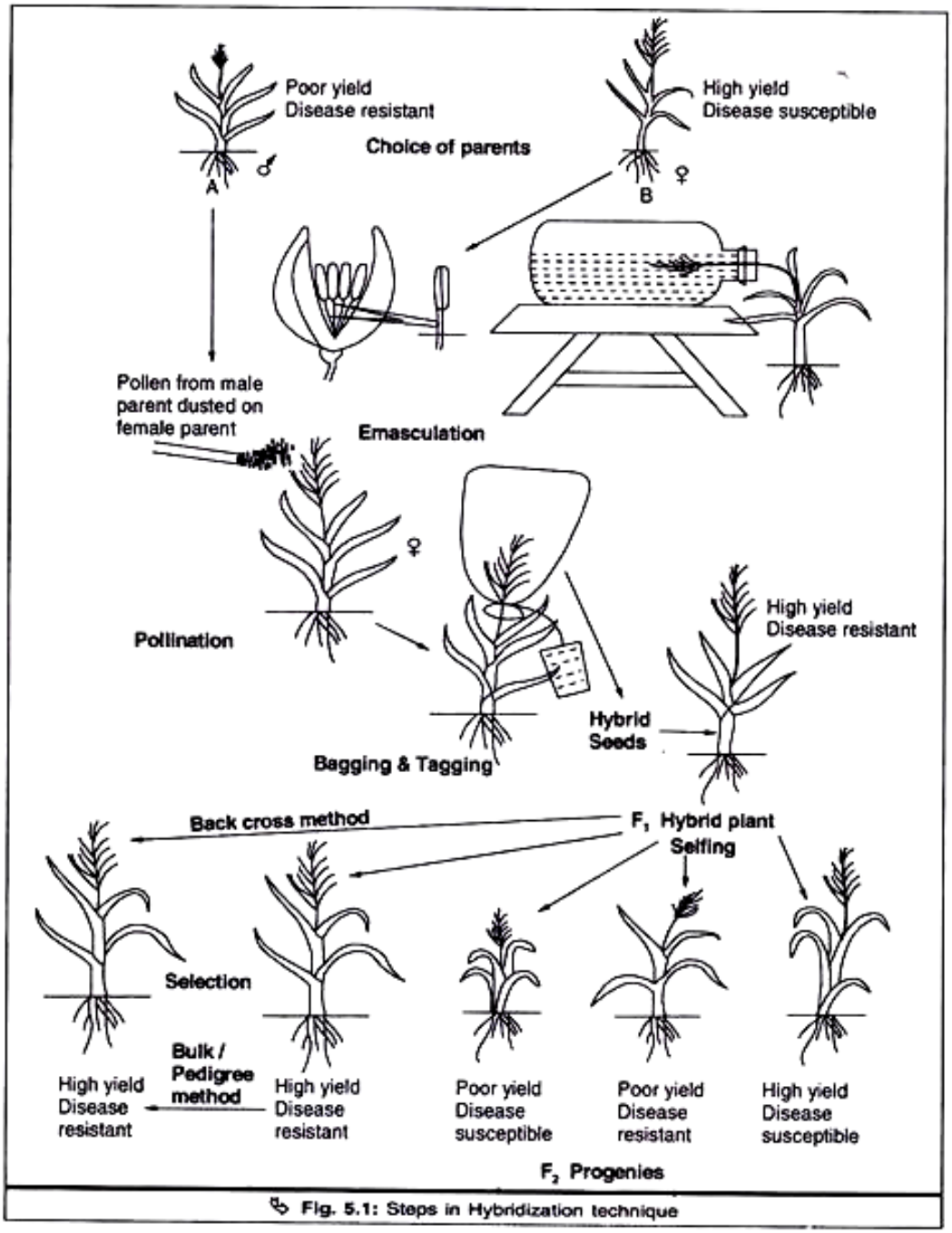


Fig. 5.1: Steps in Hybridization technique

(v) Tagging:

The emasculated flowers are tagged just after bagging. Generally circular tags of about 3 cm or rectangular tags of about 3 x 2 cm are used. The tags are attached to the base of flower or inflorescence with the help of thread.

The information on tag must be as brief as possible but complete bearing the following information:

i. Number referring to the field record
ii. Date of emasculation
iii. Date of crossing
iv. Name of the female parent is written first followed by a cross sign (x) and then the male parent, e.g., C x D denotes that C is the female parent and D is the male parent.

(vi) Crossing:

It is the sixth step. It can be defined as the artificial cross-pollination between the genetically unlike plants. In this method mature, fertile and viable pollens from the male parent are placed on the receptive stigma of emasculated flowers to bring about fertilization.

Pollen grains are collected in petridishs (e.g., Wheat, cotton etc.) or in paper bags {e.g., maize) and applied to the receptive stigmas with the help of a camel hair brush, piece of paper, tooth pick or forceps. In some crops (e.g., Jowar, Bajra) the inflorescences of both the parents are enclosed in the same bag.

(vii) Harvesting and Storing the F_1 Seeds:

Crossed heads or pods of desirable plants are harvested and after complete drying they are threshed. Seeds are stored properly with original tags.

(viii) Raising the F_1 generation:

In the coming season, the stored seeds are sown separately to raise the F_1 generation. The plants of F_1 generation are progenies of cross seeds and therefore are hybrids.

Selection after hybridization:

There are several methods of improvement of self-fertilized crops by hybridization. These are:

a. Pedigree method
b. Bulk method
c. Recurrent

Pedigree Method:

Record of the ancestry of an individual selected plant for various generations is known as pedigree. A selection method, which is used in segregating population of self-pollinated species and keeps proper record of plants and progeny selected in each generation, is known as pedigree breeding. This method is widely used for the development of varieties in self-pollinated crops.

In this method individual plants are selected till the progenies become homozygous. Selection for plants in the desired combination of characters is started in the F_2 generation and continued in succeeding generations until genetic purity is reached.

First Year:
Plants are chosen for hybridization and F1 seeds are produced.

Second Year (F1 generation):
F1 plants are space planted to produce maximum number of F2 seeds (see Fig. 9).

Third Year (F2 generation):
2000-10000 F2 plants are space planted. About 200-500 desirable superior plants are selected.

Fourth Year (F3 generation):
Selected superior plants in III year are space planted to study the individual plant. 3 to 5 best plants in these rows are selected and harvested (F4)

Fifth Year and Sixth Year (F4, F5 generation):
Process is continued as in F3 generation. Normally 20-50 families may be retained at the end of F5 generation.

Seventh Year (F6 generation):
Due to successive self-pollination most of the lines become homozygous and uniform. The plants uniform in desired characters are harvested and the seed, bulked together to constitute the variety.

Eighth Year (F7 generation):

Preliminary yield trials are conducted.

Ninth to Eleventh year (F8 – F10 generation):

Trials of superior lines are confirmed. During the testing period observations are made on height, tendency to lodge, maturity, disease resistance and quality.

Twelvth to Thirteenth Year (F11, F12 generation):

Seeds are multiplied and distributed to the farmers.

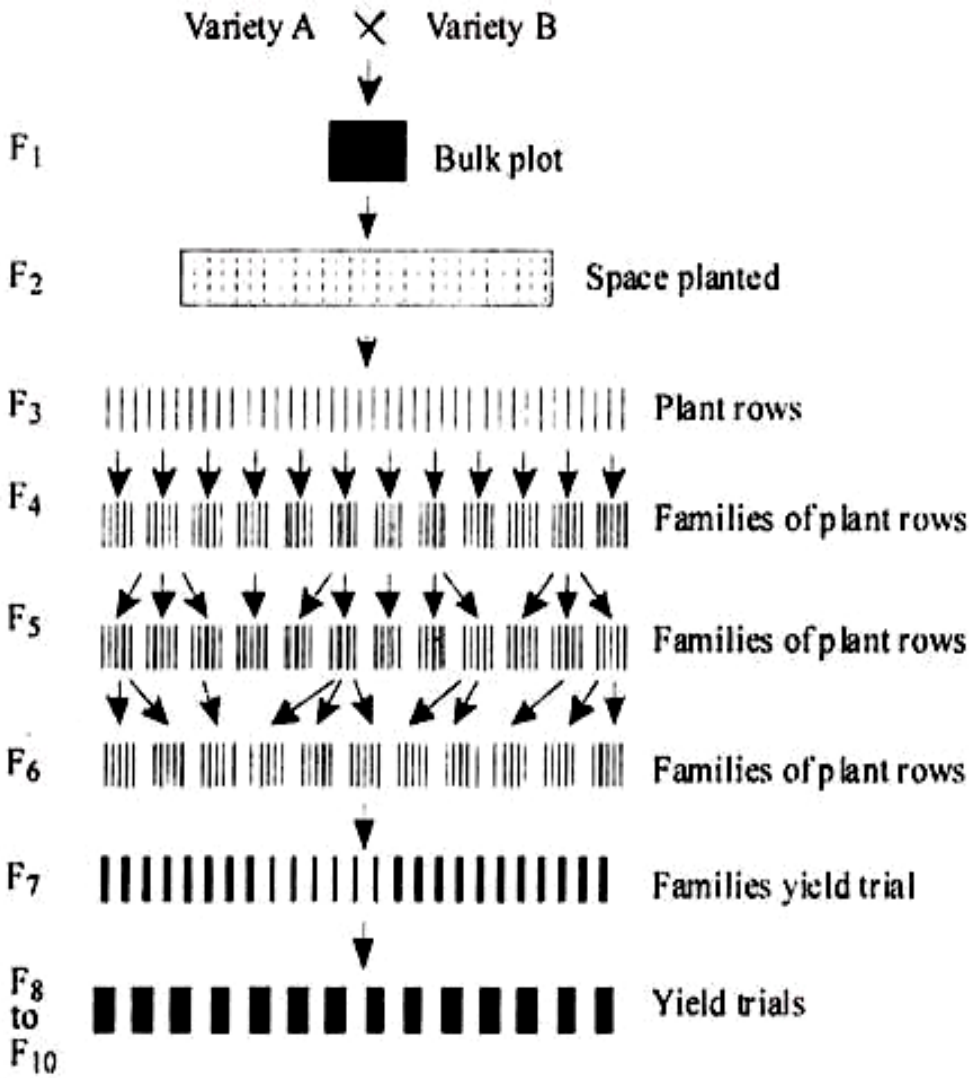


Fig. 9. Different steps involved in pedigree method.

Merits:

1. It is the quickest method.
2. Plant breeders can also obtain the genetic information.
3. There are chances of recovering transgenic segregation by this method.

Demerits:

1. Maintenance of accurate pedigree record is not easy. It takes much time.
2. Selected material becomes so large that handling of the same becomes very difficult.
3. Success of this method depends upon the skill of the breeder.

Bulk Method

A selection procedure which is used in segregating population of self-.pollinated species in which material is grown in bulk plot from F2to F5 with or without selection, next generation is grown from bulk seed and individual plant selection is practiced in F6 or later generations is called bulk method or breeding.

This method is also known as the mass or population method. Nilsson-Eule of Sweden was first to use the bulk method and it is in use ever since. This method differs from the pedigree method in that no selection is practiced in F_2-F_5 generations.

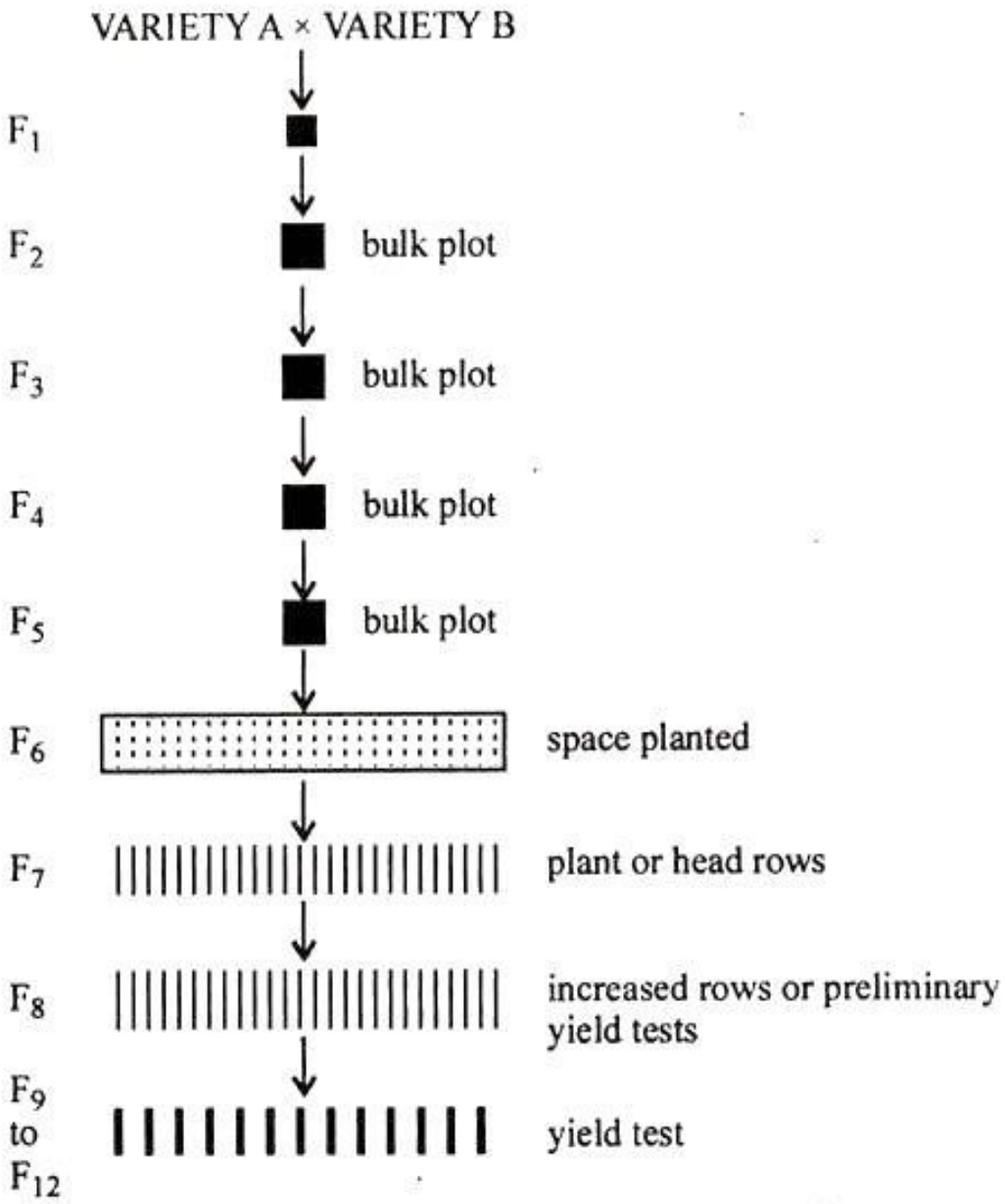


Fig. 10. Procedure of Bulk breeding method.

The method is as follows:

First Year:

Plants are chosen for hybridization and F1 seeds are produced.

Second Year (F1 generation):

50-100 F1 plants are grown and their F2 seeds are harvested in bulk,

Third Year (F2 generation):

F2 plants are grown and their F2 seeds are harvested in bulk.

Fourth Year (F3 generation):

F3 plants are grown and their F4 seeds are harvested in bulk.

Fifth Year (F4 generation):

FA plants are grown and their F5 seeds are harvested in bulk.

Sixth Year (F5 generation):

F5 plants are grown and their F6 seeds are harvested in bulk. (The process may be repeated until the desired period of homozygosity is achieved. In general bulk period is allowed up to F5 generation)

Seventh Year (F6 generation):

Seeds are space planted and single plant selection is done (F7generation).

Eighth Year (F7 generation):

The progeny of each single plant is grown separately and superior progeny are selected and isolated (F8).

NinthX Year (F8 generation):

Preliminary yield test are conducted (F9).

Tenth – Thirteenth Year (F9-F12 generations):

Multi-locations field trials are carried out, best performing strain is multiplied for seed distribution.

Merits:

(i) The bulk method is simple, convenient, inexpensive and less labour consuming (no pedigree record is to be kept).

(ii) During early segregating generations, very little work and attention is needed, which gives the breeder more time to concentrate on other breeding projects.

(iii) Selection is done by nature only and it increases the frequency of superior types in the population.
(iv) This method is suitable for studies on the survival of genes and genotypes in populations.

Demerits:

(i) This method takes much longer time to develop a new variety.
(ii) The breeder is enabling to exercise his skill and judgement in selection and therefore the method is less satisfying to him.
(iii) Information on the inheritance of characters cannot be obtained.
(iv) This method is totally dependent on natural selection to select the superior types. These types may not be necessarily the best yielding types.

Recurrent selection

Recurrent selection is defined as reselection generation after generation, with intermating of selected plant to produce the population for the next cycle of selection. The idea of this method was to ensure the isolation of superior inbreds from the population subjected to recurrent selection. The isolation of an outstanding inbred line depends on two factors:

1) The proportion of superior genotypes present in the base population from which lines are isolated and
2) The effectiveness of selection during the inbreeding of desirable genes.

Types of Recurrent Selection:

These are four types of recurrent selection.

i) Simple Recurrent Selection (SRS):

A type of recurrent selection that does not include tester is referred as simple recurrent selection. It is also known as phenotypic recurrent selection.

Procedure of Simple Recurrent Selection:

1) A number of plants with desirable phenotype are selected and self-pollinated in the 1st year.
2) In the second year separate progeny rows are grown from selfed seed of the selected plants.
3) The progenies are inter crossed in all possible combination by hand.

4) Equal amount of seed from each cross is composited to produce the next generation. This complete the original selection cycle.
5) In the third year, bulked seeds are grown and superior plants are selected and selfed, like first year.
6) In the fourth year, progeny of selected plants are grown from selfed seed and intermating is done like first year.
7) The crossed seed is composited in equal quantity for use in the next cycle of selection. This completes first cycle of simple recurrent selection. Thus selection cycles may be repeated till the desired improvement is achieved.

Recurrent Selection for General Combining Ability (GCA):

Recurrent Selection for SCA:

It was originally proposed by Hull in 1945, a form of recurrent selection that is used to improve the SCA of a population for a character by using homozygous tester is referred as (RSSCA) recurrent selection for specific combining ability. It is also known as half site recurrent selection with homozygous tester.

Reciprocal Recurrent Selection (RRS):

A form of recurrent selection used to improve both GCA and SCA of a population for a character using two heterozygous testers is known as RRS. It is also known as recurrent reciprocal half sib selection. Comstock et al in 1949, proposed this method.

Main Features of these Methods:

1) It is used for improvement of polygenic characters.
2) Selection is made on the basis of test cross performance.
3) Two heterozygous tester are used as a source of population.
4) It is used for improving population for GCA and SCA for specific characters.
5) It is equally effective with incomplete, complete and over dominance.
6) It is used for improvement of those characters, which are governed by both additive and non-additive gene action.
7) This method also requires three seasons for completion of each cycle of selection.

Merits:

1) Recurrent selection is an efficient breeding method for increasing the frequency of superior genes for various economic characters.

2) It helps in breaking repulsion phase of linkage.

3) It helps in maintaining high genetic variability due to repeated intermating of heterozygous population.

Demerits:

1) It is not directly used for the development of new varieties.

2) This method involves lot of selection crossing and selfing work.

3) It permits selfing, which leads to loss of genetic variability.

Heterosis

When two homozygous inbreeds (a true breeding line obtained by continuous inbreeding) of genetically unlike constituents are crossed together, the resulting hybrids obtained from the crossed seeds are usually robust, vigorous, productive and taller than the either parents.

This increased productivity or superiority over the parents is known as heterosis or hybrid vigour. Heterosis can be defined as the superiority of F1 hybrid over both the parents in terms of yield and some other characters.

History of Heterosis:

Heterosis has been known since the art of hybridization came into existence. Koelreuter (1763) was the first to report hybrid vigour in the hybrids of tobacco, *Datura* etc. Mendel (1865) observed this in pea crosses. While discussing the work on maize during a lecture at Gottingen (West Germany), Dr. G.H. ShuII (1914) proposed the term heterosis (Gr. heteros different and osis = condition). Poweri (1944, 45) reported that the crossing, however, may result in either weak or vigorous hybrids as compared to parental inbreeds.

Hybrid vigour is used as synonym of heterosis. It is generally agreed that hybrid vigour describes only superiority of the hybrid over the parents while heterosis describes the other situation as well i.e., crossing over may result in weak hybrids e.g., many hybrids in tomato are earlier (vegetative phase is replaced by reproductive phase). However, Whaley (1944) was of the opinion that it would be more appropriate to term the developed superiority of the hybrids as hybrid vigour and to refer to the mechanism by which the superiority is developed

as heterosis. Smith (1955) opined that the use of heterosis and hybrid vigour as synonyms is highly desirable on the basis of their long usage.

Types of Heterosis:

Heterosis is of two types:

1. True heterosis:

It is inherited.

It can be further divided into two types:

(a) Mutational true heterosis:

It is the sheltering or shadowing of the deleterious, un-favourable, often lethal, recessive mutant genes by their adaptively superior dominant alleles.

(b) Balanced true heterosis:

It arises out of balanced gene combinations with better adaptive value and agricultural usefulness.

2. Pseudo-heterosis:

Crossing of the two parental forms brings in an accidental, excessive and un-adaptable expression of temporary vigour and vegetative overgrowth. It is also called luxuriance.

Manifestation of Heterosis:

1. Increased Yield:

Increase in yield which may be measured in terms of grain, fruit, seed, leaf, tuber or the whole plant is one of the most important manifestations of heterosis.

2. Increase in Size and General Vigour:

Heterosis results in more vigorous growth which ultimately leads to healthier and faster growing plants with increase in size than the parents.

3. Better Quality:

In many cases heterosis yields better quality which may be accompanied with higher yield.

4. Greater Adaptability:

Hybrids are generally more adapted to environmental changes than the inbred lines due to heterozygosity.

5. More Disease Resistant:

Heterosis sometimes results into development of more disease resistant character in the hybrids.

6. Increased Reproductive Ability:

Hybrids exhibit heterosis by expressing high fertility rate or reproductive ability, which is ultimately expressed in yield character.

7. Increase in Growth Rate:

In many cases the hybrids show faster growth rate than the parents, but that does not always produce larger plant size than the parents.

8. Early Flowering and Maturity:

In many cases the hybrids may show early-ness in flowering and maturity than the parents, for some crops these are the desirable characters for crop improvement. All these manifestations of heterosis can be traced at all levels of hybrid plant organisation.

Molecular Level:

Heterosis is manifested in increased rate of DNA reduplication, transcription and translation influencing the formation of genetic information, enzymatic activity, other regulatory mechanisms and also hybrid protein molecule formation.

Functional Level:

Heterosis is expressed as an effective regulation in metabolic processes and morphogenesis in hybrid organism.

Cellular Level:

Due to change in electro-kinetic properties of hybrid cell nuclei, the heterosis is manifested by increased mitosis.

Organism Level:

Heterosis is expressed as increased growth and differentiation of vegetative organs, synthesis and accumulation of nutritional substances and utilisation of metabolic process for yield formation.

Genetic Basis of Heterosis:

There are two main theories to explain the genetic cause of heterosis.

(A) Dominance Hypothesis:

This hypothesis was proposed by Davenport and further expanded by others. This hypothesis suggests that at each locus dominant allele has the favourable character, whereas the recessive allele has the unfavourable character.

When they are combined together; i.e., in heterozygous condition in the hybrids, the favourable characters get expressed whereas the unfavourable characters are masked. So the heterosis results from the masking of harmful effects of recessive alleles by their dominant alleles.

Dominance Hypothesis has Assumptions:

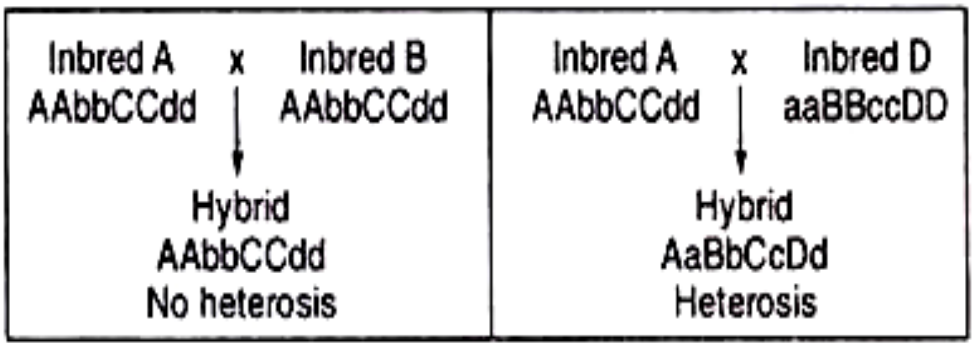
(a) Dominant genes are beneficial and recessive genes are deleterious.

(b) The loci show addition effects, non-allelic interactions are absent.

(c) No recombination barrier between the genes.

With the help of following example heterosis can be explained:

In a cross between Inbred A (AAbbCCdd) with Inbred B (AAbbCCdd), there will be no heterosis in F1 hybrid, there is no masking of recessive gene in hybrid. But in another cross, Inbred A (AAbbCCdd) is crossed with Inbred D (aaBBccDD), where the F1 hybrid is (AaBbCcDd) with all the genes having dominant allele.



As a result the harmful effects of a, b, c, d are hidden by the dominant alleles A, B, C and D. Thus some parents produce heterotic progeny while others do not. Generally parents of diverse or different origin are more likely to produce heterotic progeny than those of similar origin.

Objection:

1. Failure in Isolation of Inbreds as Vigorous as Hybrids:

According to dominance hypothesis it should be possible to get the inbred line with all the dominant genes. Such inbreds should be as vigorous as the F1 hybrids, but such inbreds have not been isolated.

2. Symmetrical Distribution in F2:

According to dominance hypothesis, the quantitative characters should not show symmetrical distribution as because dominant and recessive alleles should segregate in the proportion of 3: 1, but generally the F2 shows symmetrical distribution.

Above two objections can be explained by linked genes. Many of the quantitative characters are governed by linked genes together, so to get the inbred line with all dominant genes require several precisely placed crossovers. In another explanation it can be showed that if the number of genes governing the quantitative characters is large, symmetrical distribution would be obtained even without linkage.

(B) Over-dominance Hypothesis:

This hypothesis was independently proposed by East and Shull. This is sometimes known as single gene heterosis, super-dominance, and cumulative action of divergent alleles and stimulation of divergent alleles. According to this hypothesis, heterozygotes are superior to both the homozygotes.

So the heterozygote Aa would be superior to both the homozygotes AA and aa. Consequently, heterozygosity is essential for the cause of heterosis. In case of maize, the gene ma affects maturity. The heterozygote Ma/ma is more vigorous with late maturity than the homozygotes Ma/Ma or ma/ma.

Another proposal by East was that there are several alleles, e.g., a1, a2, a3, a4…….. etc. with increasingly different functions. Heterozygotes between more divergent alleles would be more heterotic than those involving less divergent genes, e.g., a1a4 is more heterotic than a1a2, a2a3, a3a4, etc. In these cases due to presence of divergent alleles the hybrids have the capacity to perform different functions which is not possible by any of the heterozygotes.

Objection:

1. There are many examples where the superiority is due to the epistatic affect of several non-allelic genes, not due to over-dominance (which is the interaction between allelic genes).
2. There is another objection against over-dominance hypothesis that there are many examples where the homozygotes are superior to the heterozygotes.

Physiological Basis of Heterosis:

Hybrid vigour, the product of heterotic mechanism, is essentially a physiological manifestation.

This better physiological efficiency of hybrids is derived chiefly from:

1. Better initial growth.
2. Greater uptake followed by better utilisation of nutrients by hybrids.

The initial growth activities include the different physiological processes during germination:

(a) Efficient water absorption,
(b) Better activity of enzymes,
(c) Rapid mobilization and utilization of stored food matter,
(d) Transformation and building up of active protoplasmic synthesis.

Applications of Heterosis in Plant Breeding:

Heterosis is observed in almost every crop species studied, the application of this phenomenon for its commercial exploitation depends on the expression of the degree of heterosis. This phenomenon is commercially used to produce hybrid or synthetic varieties, which needs the maximisation of its expression and also fixation.

Maximization of hybrid vigour (HF1) can be achieved by increasing either ‘d’ (directional dominance) or ‘y’ (initial differences in gene frequency between parents), i.e., choosing genetically divergent parents, HF1 = $\sum dy^2$.

Fixation of hybrid vigour is needed for its commercial application which can be done by vegetative propagation, or by stable apomictic reproduction, or by transferring heterozygosity to polyploid or fixation by obtaining structural heterozygotes.

Fixation of heterosis in crops like potato, sweet potato, sugarcane, sugarbeet and many ornamental plants can be achieved by vegetative propagation as there seeds are not essential. Incorporation of genes conditioning vegetative apospory or diploid parthenogenesis in hybrid seed crops may lead to a permanent heterozygote advantage.

Heterozygosity can be maintained or saved from being lost due to segregation by converting diploid heterozygotes into tetraploid or hexaploid. Incorporation of gaudensvelans combination helps to survive only the heterozygotes not the homozygous combination — such mechanism may be introduced in crop plants, thereby hybrid vigour can be fixed with great success.

Heterosis or hybrid vigour have been commercially utilised in both cross pollinated and in some self-pollinated species. In most of the cases the utilisation of this heterosis phenomenon is not successful because of difficulty in production of large quantities of hybrid seeds. This is particularly difficult in self-pollinated species.

Few examples where the heterosis has been utilised for improvement of the crop plant are:

Crop Species:

Asexually propagated species and also cross pollinated species like maize, jowar, bajra, sunflower, legume, cotton, etc.

Vegetable Crops:

Tomato, brinjal, onions, cucurbits, etc.

Fruits:

In almost all the fruit trees.

Inbreeding Depression

Inbreeding depression is the reduced biological fitness in a given population as a result of inbreeding, or breeding of related individuals. Population biological fitness refers to an organism's ability to survive and perpetuate its genetic material. Inbreeding depression is often the result of a population bottleneck. In general, the higher the genetic variation or gene pool within a breeding population, the less likely it is to suffer from inbreeding depression.

Inbreeding depression seems to be present in most groups of organisms, but varies across mating systems. Hermaphroditic species often exhibit lower degrees of inbreeding depression than outcrossing species, as repeated generations of selfing is thought to purge deleterious alleles from populations. For example, the outcrossing nematode (roundworm) Caenorhabditis remanei has been demonstrated to suffer severely from inbreeding depression, unlike its hermaphroditic relative C. elegans, which experiences outbreeding depression.
It is mating between individuals related by descent or having common ancestry. (Brother - Sister mating or sib mating). The highest degree of inbreeding is obtained by selfing.

4. Population samples, sampling methods.

Meaning of Sampling:

In biological experiment, it is not possible to collect complete information about a population. If the number of pods/plant is to be collected from a field then it is really time consuming and also rarely possible to do it.

Then few plants are taken into account for studying the whole population of plant in that field. The method by which only few items are selected from the population in such a way so that they will represent the population in unbiased way is called sampling.

The size of sample is an important factor in statistical analysis which depends on the number of sampling units selected from a population for investigation. The size should not be too big or too small. Taking only 10 values from a plot of 1000 plants will give erroneous result and also it is difficult to handle with 1000 number of values.

Population sampling is the process of taking a subset of subjects that is representative of the entire population. The sample must have sufficient size to warrant statistical analysis.

Why Sampling is Essential?

A. Sampling saves time, the data can be collected and summarised more quickly with a sample than a complete count of the whole population.

B. In case of infinite population, sampling is the only method for statistical analysis.

C. Sampling reduces the cost of experiment because only a few selected items are studied in sampling.

Types of Sampling:

1. **Non-Probability Sampling**

In this type of population sampling, members of the population do not have equal chance of being selected. Due to this, it is not safe to assume that the sample fully represents the target population. It is also possible that the researcher deliberately chose the individuals that will participate in the study.

Non-probability population sampling method is useful for pilot studies, case studies, qualitative research, and for hypothesis development.

This sampling method is usually employed in studies that are not interested in the parameters of the entire population. Some researchers prefer this sampling technique because it is cheap, quick and easy.

2. Probability Sampling

In probability sampling, every individual in the population have equal chance of being selected as a subject for the research.

This method guarantees that the selection process is completely randomized and without bias.

The most basic example of probability sampling is listing all the names of the individuals in the population in separate pieces of paper, and then drawing a number of papers one by one from the complete collection of names.

The advantage of using probability sampling is the accuracy of the statistical methods after the experiment. It can also be used to estimate the population parameters since it is representative of the entire population. It is also a reliable method to eliminate sampling bias.

Limitations of Sampling:

A. If the sampling is not done properly, i.e., if it is biased then it misleads which results in false, inaccurate interpretation.

B. There may be personal biasness during sampling or choice of method of sampling which may also lead to erroneous interpretation.

Criteria for Good Sampling:

A. Selected samples from the population should be homogenous and should not have any differences when compared with the population.

B. Reasonable number of items is to be included in the sample to make the result more reliable.

C. The selected sample should have the similar characteristics as the original population from which it has been selected.

D. The individual items composing the sample should be independent from each other.

E. The number of observations included in a sample should be more to make the results more reliable.

Methods of Sampling:

The proper method of selection of samples and the relation between the sample and population is the matter which determines the method of sampling. There are various methods of sampling; it totally depends upon a statistician which method will be applicable for proper selection of method of sampling

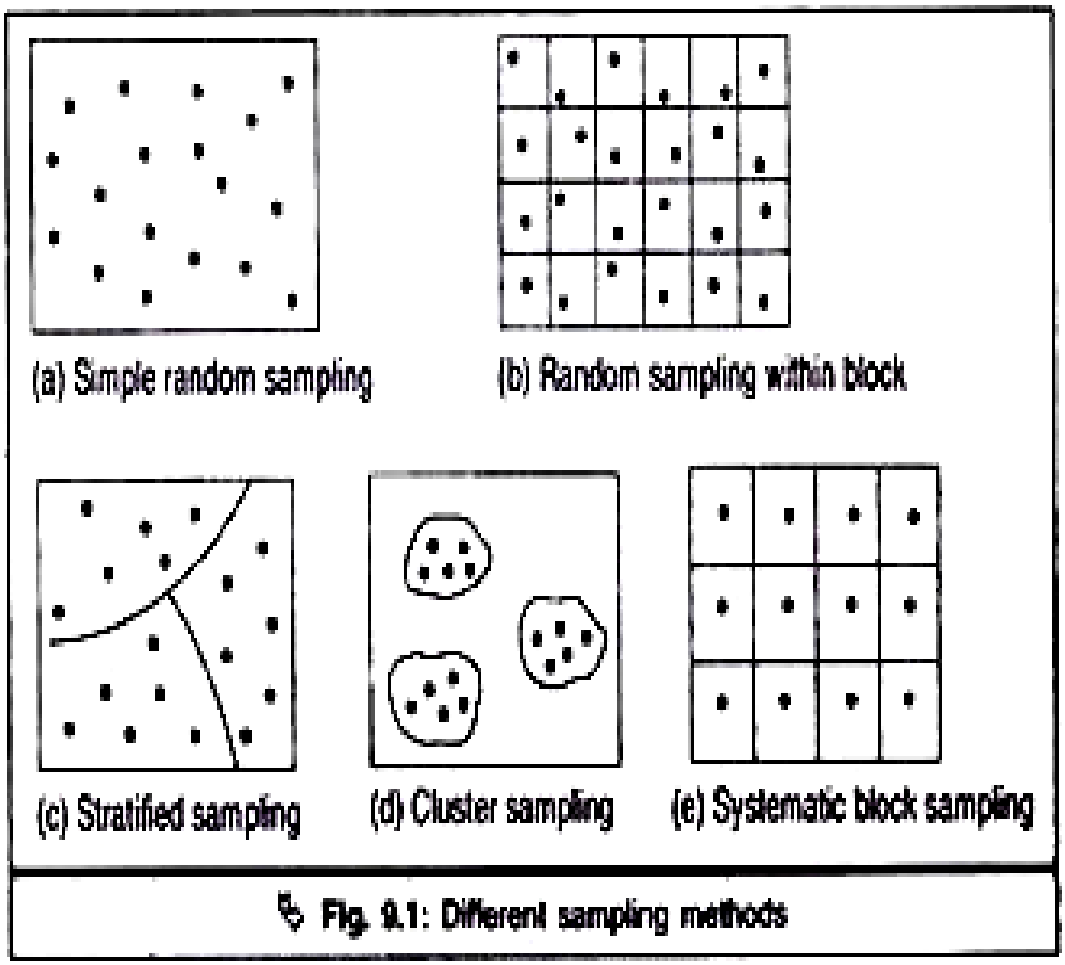


Fig. 9.1: Different sampling methods

Different Sampling Methods

A. Simple Random Sampling:

This method is followed where each item of the population has an equal chance of being included in the sample. Random sampling suggests that selection should be done without any biasness.

To ensure the randomness of selection one can adopt either lottery method or refer to table of random numbers. Lottery method is the simplest and most popular method, all the items are numbered and slips of identical size and shape are made. All are shuffled together and selection is done blindly.

Table of random numbers can be used in place of blind selection. For this purpose, Random Number Table (5 digit) prepared by Snedecor and Cochran (1988) can be used either horizontally or vertically for selection of sample without biasness. This method of sampling

is more scientific because there are less chances of personal biasness in sampling from the population and chances of selection of every item are equal.

B. Systematic Sampling:

This method is applied when the population is large, scattered and not homogeneous.

Systematic procedure follows to choose a sample by taking every K the individual, where K refers the sample interval calculated by the formula:

K = Total population/Sample size desired

Example:

20% sample to be taken from 1000 individual of a population,

K= 1000/20% of 1,000 = 5

So the, First sample will be the 5th individual,

Second sample will be the 10th individual,

Third sample will be the 15th individual, and like this way.

C. Stratified Sampling:

This method of sampling is followed when the population is not homogeneous, hence the population is first divided into several homogeneous groups or strata and the sample is drawn from each stratum at random. This method is useful as it represents the proportionate representative sample from each group and it gives greater accuracy.

D. Cluster Sampling:

A cluster is randomly selected group. This method is used when the units of population are natural groups such as school, hospitals, etc. The technique of cluster sampling allows small number of target population and the data provided statistically valid at 95% confidence limits.

E. Non-Random Sampling:

This method is called as judgement sampling. In this method the selection process of sample is somewhat subjective. The choice of sample items depends exclusively on the judgement of the investigator.

The investigator exercises his judgement in the choice and includes those items in the sample which he considers most typical for his investigation. For example, from a rice field one investigator may select only the healthy plants for artificial inoculation of a pathogen.

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5. Frequency distribution, histogram, normal curve, mean, median, mode, variance, standard deviation, standard error.

Biometry:

Statistics is a branch of science which deals with methods of collection, classification and analysis, i.e., drawing inferences from collected data; then testing of hypothesis and making comment on these.

The statistics can be applied in all scientific branches like social, physical and biological. The use of statistics in Biology is known as Biostatistics or Biometry. It deals with the application of different statistical methods and analysis of data collected from biological system.

Application of Biometry:

In biological system, it is very difficult to draw any concrete conclusion about any happening, as there is much difference among the individuals. Such as in the same plant, the leaves are all different; again in a field, all the same kind of plants are not identical; in the same place, the same crop does not respond equally in different plots. So it is very difficult to reach at a definite and reliable conclusion unless we take the help of statistical analysis of the observed data.

In case of plant breeding, the application of biometry has immense importance, as the statistical analysis only helps to conclude about a population of plant; such as:

(a) How the character, which is acquired by the population, is distributed, how the character is being inherited, etc.

(b) Whether two characters in a population are correlated or not, if correlated, how much they are correlated.

(c) In case of plant breeding whether Mendelian Genetics is being followed or not, can be tested by chi-square test.

(d) The application of fertilizer or irrigation or any kind of cultural practice can be effectively justified by application of test of significance.

(e) The laws of probability is of importance in genetics for forecasting the chance of obtaining certain result from a cross and elucidating the operation of genetic principle.

(f) The effects of two or more different fertilizer application on the same field can be analysed by analysis of variance.

(g) Study of alleles of genes in populations and the factors which maintain or change the frequencies of particular genotype in populations can be studied.

Limitation of Biometry:

(a) All the conclusions about statistical analysis depend on the availability of sample data. If the sampling is biased, the analysis will be eronotic.

(b) Statistics can be used and applied only on collective data, not on individual data.

(c) Statistical laws are always true in case of large population on the average, not on the small sample.Frequency distribution

For any statistical analysis, the handling with raw data requires some treatment, i.e., the classification of data to organise the available values in a more compact way. The frequency distribution presents the data very concisely indicating how frequently a variable occurs in a group of study.

Construction of Frequency Distribution Table:

If there are repetitions in individual values or items of any investigation, suitable frequency table can be formed. These frequency tables may be discrete or may be continuous in nature. The available raw data at first should be converted into arrayed data. For biostatistics the raw data are arranged in ascending order to make it arrayed data (Example-2).

Example 1:

Following raw data is obtained in an investigation. 100 pea plants bore pods ranging from 15 to 41 in a garden of pea plants (see Raw Data Table A):

Raw Data Table A:

33	31	28	15	17	17	16	18	16	18	20	22	24
25	31	27	30	29	33	28	20	22	23	25	41	39
30	36	37	27	33	28	31	29	32	31	29	34	19
22	25	40	19	21	24	30	26	37	27	28	32	32
31	29	34	21	23	25	40	26	38	27	26	33	28
34	29	30	30	35	29	23	29	26	38	27	32	28
34	35	29	30	33	32	35	29	24	26	38	27	36
28	34	29	35	30	33	32	36	37				

Arrayed Data Table B:

15	16	16	17	17	18	18	19	19	20	20	21	21
22	22	22	23	23	23	24	24	24	25	25	25	25
26	26	26	26	26	27	27	27	27	27	27	28	28
28	28	28	28	28	29	29	29	29	29	29	29	29
29	30	30	30	30	30	30	30	31	31	31	31	31
32	32	32	32	32	32	33	33	33	33	33	33	34
34	34	34	34	35	35	35	35	36	36	36	37	37
37	38	38	38	39	39	40	40	41				

Then for discrete frequency distribution table, the values of variables are written in one column and the repetition of that value is written against it which is the frequency (Table 9.1, 9.2).

Table 9.1 : Determination of frequency of each variable

No. of Pods Variable	Tally mark	Repetition number of Plants or Frequency
15	I	1
16	II	2
17	II	2
18	II	2
19	II	2
20	II	2
21	II	2
22	III	3
23	III	3
24	III	3
25	IIII	4
26	𝍸	5
27	𝍸 I	6
28	𝍸 II	7
29	𝍸 IIII	9
30	𝍸 II	7
31	𝍸	5
32	𝍸 I	6
33	𝍸 I	6
34	𝍸	5
35	IIII	4
36	III	3
37	III	3
38	III	3
39	II	2
40	II	2
41	I	1

Table 9.2 : Variables and respective frequency			
No. of Pods (Variables)	No. of Plants (Frequency)	No. of Pods (Variables)	No. of Plants (Frequency)
15	1	29	9
16	2	30	7
17	2	31	5
18	2	32	6
19	2	33	6
20	2	34	5
21	2	35	4
22	3	36	3
23	3	37	3
24	3	38	3
25	4	39	2
26	5	40	2
27	6	41	1
28	7		$\Sigma f = 100$

But for continuous frequency distribution table, the values are grouped in fixed interval and then the frequency within that interval is observed and noted.

The number of classes or the range of class interval is an important factor for making this kind of frequency distribution table. There is no fixed rule for how many classes can be formed, generally it depends on observation of available data, minimum 3 classes and maximum 20 classes can be formed.

The size of class interval also depends on the range of data and the number of classes, it is equal to the difference between highest and lowest value divided by the number of classes,

i – H-L/K

where i = class interval K.

H = Highest value

L = Lowest value

K = number of classes.

Overlapping Frequency Distribution Table:

Values of variables are grouped in such a fashion that the upper limit of one class interval is represented in next class interval. In an example (Example 2), number of pods ranges from 15 to 41, the classes may be 15-17, 17-19, 19-21, etc. (Table 9.3).

Table 9.3 : Overlapping frequency table

No. of Pods in class interval	No. of plants in frequency
15–17	3
17–19	4
19–21	4
21–23	5
23–25	6
25–27	9
27–29	13
29–31	16
31–33	11
33–35	11
35–37	7
37–39	6
39–41	4
	$\Sigma f = 100$

Non-Overlapping Frequency Distribution Table:

Values of variables are grouped in such a fashion that the upper level of one class interval does not overlap the preceding class interval. In the above example, number of pods ranges from 15 to 41, the classes can be prepared like 15-17, 18-20, 21-23, etc. (Table 9.4).

Table 9.4 : Non-overlapping frequency table	
No. of Pods in class interval	No. of plants in frequency
15–17	5
18–20	6
21–23	8
24–26	12
27–29	22
30–32	18
33–35	15
36–38	9
39–41	5
	$\Sigma f = 100$

Cumulative Frequency Distribution Table:

Cumulative frequency is determined by adding the frequency of a class interval with the frequency of the preceding class interval. The cumulative frequency table can be prepared from both the overlapping and non-overlapping frequency distribution table (Table 9.5, 9.6).

Table 9.5 : Overlapping cumulative frequency distribution table		
Class interval	**Frequency**	**Cumulative frequency**
15–17	3	3
17–19	4	3 + 4 = 7
19–21	4	7 + 4 = 11
21–23	5	11 + 5 = 16
23–25	6	16 + 6 = 22
25–27	9	22 + 9 = 31
27–29	13	31 + 13 = 44
29–31	16	44 + 16 = 60
31–33	11	60 + 11 = 71
33–35	11	71 + 11 = 82
35–37	7	82 + 7 = 89
37–39	6	89 + 6 = 95
39–41	5	95 + 5 = 100

Table 9.6 : Non-overlapping cumulative frequency distribution table		
Class interval	**Frequency**	**Cumulative frequency**
15–17	5	5
18–20	6	5 + 6 = 11
21–23	8	11 + 8 = 19
24–26	12	19 + 12 = 31
27–29	22	31 + 22 = 53
30–32	18	53 + 18 = 71
33–35	15	71 + 15 = 86
36–38	9	86 + 9 = 95
39–41	5	95 + 5 = 100

Relative Frequency:

This is calculated from the cumulative frequency against the total population or sample.

Relative frequency of a class = Cumulative frequency of that class/Total no. of sample.

Class Limit:

It is defined as two boundaries of a class, i.e., the highest and lowest values of a class, which can be represented by L1 and L2.

Mid-Value of Class-Interval:

The central point of a class interval is called its mid-value or mid-point of that class, which is obtained by using the following formula.

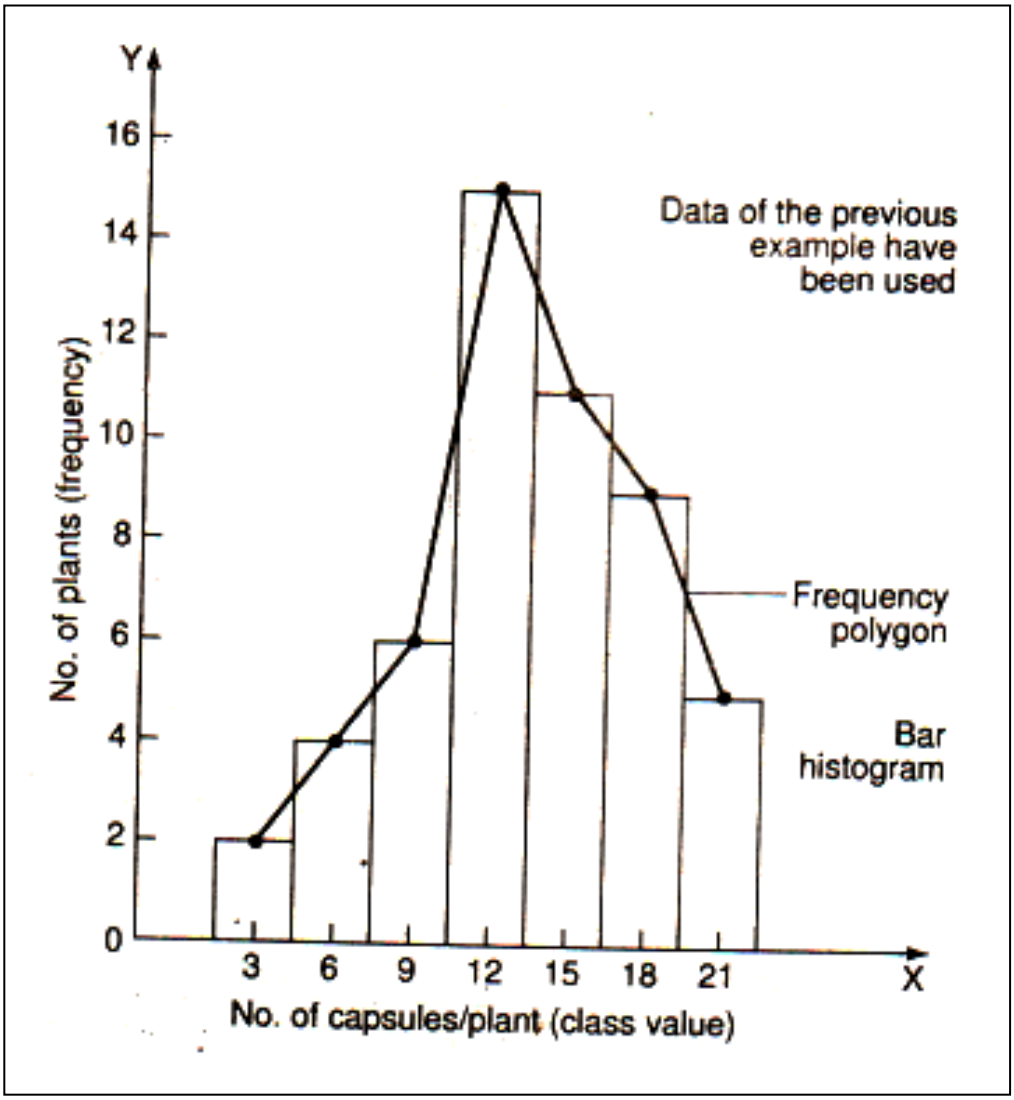
Mid – value of a class = L1 + L2/2

Frequency distribution

The diagrams commonly used to depict statistical data, given in the form of frequency distribution are: 1. Histogram, 2. Frequency polygon, 3. Ogive.

1. **Histogram**: It is the most common form of diagrammatic representation of a grouped frequency distribution. It consists of a set of adjoining rectangles drawn on a horizontal base line with area proportional to the class frequencies.

Uses: (a) give a visual representation of the relative size of the various groups; (b) the surface of the tops of rectangles gives an idea of nature of frequency curve for the population; (c) may be used to find out the mode graphically.



2. **Frequency polygon**: It is alternative to histogram and derived by joining the mid-points of the tops of consecutive rectangles.

3. **Ogive**: It is the graphical representation of cumulative frequency distribution and hence called cumulative frequency polygon.

Normal Distribution:

If we observe in any population any attribute is distributed mostly near the mean value and equally distributed to the higher and lesser value gradually in decreasing order then the distribution pattern is called normal distribution.

When this kind of normally distributed attribute is plotted graphically with the help of available data, the normal distribution pattern gives a bell shaped symmetrical curve which is called ‘normal distribution curve’. In this curve the mean value lies in the peak of the curve.

Properties of Normal Distribution Curve:

1. It is a continuous bell shaped curve which is associated with continuous variable.

2. There is only one maximum peak (unimodal). The normal curve is symmetrical and asymptotic (touches at infinity).

3. The height of normal curve is maximum at its Mean. Mean, Median and Mode coincides in normal curve.

4. The peak divides the distribution in two equal halves.

5. Most of the observations are clustered around the Mean and there are relatively a few observations at the extremes.

6. The normal distribution curve has a fixed mathematical characteristic feature independent of the scale (Unit of measurement) of magnitude.

Skewness and Kurtosis:

In normal distribution, most of the cases fall in the middle but there are cases in which central tendency do not exhibit normal behaviour.

There are two types of divergence from normal distribution:

(i) Skewness and

(ii) Kurtosis

Skewness means that the curve is not symmetrical. In a skewed distribution, the Mean, Median and Mode do not coincide it pulls the Median and Mean away from Mode either left or right. In a skewed distribution, the frequency curve is not bell shaped and values do not lie on both sides of measure of central tendency equally. Here Mean Median and Mode fall at different points.

In symmetrical distribution curve, Mode coincides with Mean and Median. In positively skewed curve, the value of Mean and Median lie away from Mode values (right hand), the values are greater than Mode. In negatively skewed curve, the value of Mean and Median lie left hand to Mode value, the values are lesser than the Mode value.

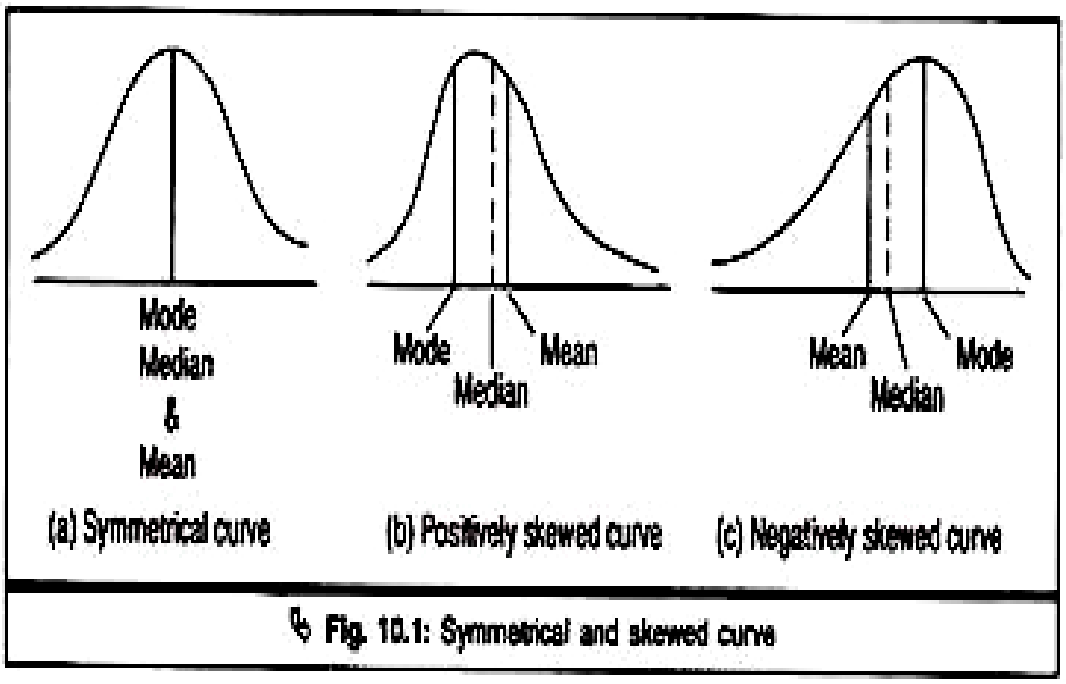


Fig. 10.1: Symmetrical and skewed curve

Measures of Central Tendency

Mean:

(a) Arithmetic mean

It is most commonly used of all the averages. It is the value which we get by dividing the aggregate of various items of the same series by the total number of observations.

When observations are denoted by x values showing x1, x2, x3… xn; the total number of observations is calculated by summing up the observations and dividing the sum by the total number of observations (n)

Find out the average pod length of the plant.

$$\bar{x} = \frac{x_1 + x_2 + x_3 + \cdots + x_n}{n}$$

Example 1: The pod length of ten pods of a plant shows following data:

5.2 cm,	5.3 cm,	5.6 cm,	5.7 cm,	5.4 cm,
5.2 cm,	5.3 cm,	5.3 cm,	5.4 cm,	5.2 cm.

Find out the average pod length of the plant.

$$\bar{x} = \frac{\sum x}{n} = \frac{5.2 + 5.3 + 5.6 + 5.7 + 5.4 + 5.2 + 5.3 + 5.3 + 5.4 + 5.2}{10} \text{cm}$$

$$= \frac{53.6}{10} \text{cm} = 5.36 \text{cm}$$

When the series is discrete, each value of the variable is multiplied by their respective frequencies, sum of all values is divided by total number of frequencies. Variable x has the values like x1, x2, x3, …, xn and their frequencies are f1 , f2, f3, …, f respectively.

$$\bar{x} = \frac{f_1 x_1 + f_2 x_2 + f_3 x_3 + \cdots + f_n x_n}{f_1 + f_2 + f_3 + \cdots + f_n} = \frac{\sum fx}{\sum f}$$

Then Arithmetic Mean:

When the series is continuous, the arithmetic mean is calculated after taking the midpoint value of class intervals.

$$\bar{x} = \frac{\sum fm}{\sum f}$$

where, $\bar{x}$ = Arithmetic mean

$\sum fm$ = Sum values of midpoint value multiplied by their frequencies

If = Sum of frequencies

m = Mid points of various class intervals.

Example 2:

An observation on 32 Balsam plants shows the following data. Calculate the arithmetic mean.

No. of flowers/plant (x)	4	5	6	7	8	9
No. of plants (f)	3	5	6	9	5	4

No. of flowers / plant (x)	No. of plants (f)	f × x
4	3	12
5	5	25
6	6	36
7	9	63
8	5	40
9	4	6
	$\Sigma f = 32$	$\Sigma fx = 212$

$$\bar{x} = \frac{\sum fx}{\sum f} = \frac{212}{32} = 6.62 \text{ (approx.)}$$

The average number of flowers/plant is 6.62.

No. of pods/plant	Mid points of class (m)	No. of plants frequency (f)	m.f.
15-17	16	5	80
18-20	19	6	114
21-23	22	8	176
24-26	25	12	300
27-29	28	22	616
30-32	31	18	558
33-35	34	15	510
36-38	37	9	333
39-41	40	5	200
		$\Sigma f = 100$	$\Sigma mf = 2,887$

$$\text{Arithmetic Mean} = \bar{x} = \frac{\Sigma mf}{\Sigma f} = \frac{2,887}{100} = 28.87.$$

Merits, Demerits and Uses of Arithmetic Mean:

Merits:

1. It has the simplest formula to calculate and it is easily understood.
2. It is rigidly defined mathematical formula the same result will come on repeated calculations.
3. The calculation is based on all the observations.
4. It is least affected by sampling fluctuation.
5. The arithmetic mean balances the value on either side.
6. It is the best measure to compare two or more series.
7. Arithmetic mean is totally dependent on values not on the position.

Demerits:

1. It cannot be calculated if all the values are not known.
2. The extreme values have greater effect on mean.
3. The qualitative data cannot be measured in this way.

Uses:

1. The arithmetic mean is mostly used in practical statistics.

2. Mean helps to calculate many other estimates in statistics.

3. The arithmetic mean is most popular method of any measurement used by common people to get the average of any data.

(b) Geometric Mean:

The geometric mean is defined as the n-th root of the product of n observations.

$$\text{Geometric Mean (GM)} = \sqrt[n]{x_1 \cdot x_2 \cdot x_3 \cdots x_n}$$

Where n = number of observations; x1, x2, x3… xn = variable values.

When n is small then the above formula can be applied but in case of large 'n' number the logarithms are used to find out the GM

$$GM = \text{Anti log}\frac{\log x_1 + \log x_2 + \log x_3 + \cdots + \log x_n}{n} = \text{Anti log}\frac{\log x}{n}$$

Example 3:

Find out the geometric mean of the following seeds, x denotes the weight of each seed in mg.

5 mg, 7 mg, 8 mg, 6 mg and 4 mg.

x	log x
5 mg	0.70
7 mg	0.85
8 mg	0.90
6 mg	0.78
4 mg	0.60
	$\Sigma \log x = 3.83$

$$\frac{\Sigma \log x}{5} = \frac{3.83}{5} = 0.77$$

Antilog of $\frac{\Sigma \log x}{5}$, i.e., antilog of 0.77 = 5.89 mg

So the geometric mean of seed weight = 5.89 mg.

This mean is based on all observations, rigidly defined, less affected by extreme values. This mean is difficult to understand, compute and interpret.

This mean is mostly helpful in averaging ratios, percentage and determining ratio of change. This mean is important in construction of index number.

(c) Harmonic Mean:

When the variables are expressed in ratios or rates, the proper average to be calculated through harmonic mean. The harmonic mean is defined as the reciprocal of arithmetic mean of the reciprocal of the given values.

The harmonic mean is applicable only in restricted field such as oxygen consumption/hour, calorie requirement/hour, CO_2 evolution/hour, flow of sap/min, etc.

$$\text{Harmonic mean (HM)} = \frac{n}{\frac{1}{x_1}+\frac{1}{x_2}+\frac{1}{x_3}+\cdots+\frac{1}{x_n}} = \frac{n}{\sum\left(\frac{1}{x}\right)}$$

Where n = Total number of observation; x1, x2, x3 are the values of variables.

Example 4:

In a particular experiment, 5 different sets of *Hydrilla* plants showed O_2, evolution/hour, was recorded.

2.5 c.c./hour, 1.8 c.c./hour, 2.0 c.c./hour

2.2 c.c./hour, 2.4 c.c./hour.

$$HM = \frac{5}{\frac{1}{2.5}+\frac{1}{1.8}+\frac{1}{2.0}+\frac{1}{2.2}+\frac{1}{2.4}} = \frac{5}{.4+.55+.5+.45+.41} = \frac{5}{2.3} = 2.17 \text{ c.c./hour.}$$

So, harmonic mean of the observation is 2.17 c.c./hour. This HM determination is based on all the observations of a series. It gives more weightage to the smaller items and also not much affected by sample fluctuation.

It is not very easy to calculate and also the positive and negative, both values, cannot be computed.

Mode:

Most frequent value in a series. Mode cannot be determined from a series of individual observations unless it is converted into either a discrete or continuous series. In a discrete series the value of the variable against which the frequency is the largest would be the modal value.

For example, 2, 4, 4, 4, 6, 9, 3, 2, 4, 6, 11, 13 mode is 4 as it is occurring maximum.

For example, 5, 3, 6, 3, 5, 10, 7, 2 mode is 3 and 5 such series is known as bi-modal series.

Similarly in a continuous frequency distribution the class interval having the maximum frequency would be the modal class.

Mode can be determined from grouped data using the following formula:

$$\text{Mode} = l_1 + \frac{\Delta_1}{\Delta_1 + \Delta_2} \times i,$$

where l_1 = lower limit of modal class, Δ_1 = difference of frequencies between modal class and the preceding class, Δ_2 = difference of frequencies between modal class and post modal class and i = class interval.

For example:

Class interval (Marks obtained)	*Class value*	*Frequency*
1–9	5	3
10–20	15	5
21–29	25	12
30–40	35	15
41–49	45	25
50–60	55	40
61–69	65	18
70–80	75	11
81–89	85	3
90–100	95	2

Modal class (*maximum frequency*) is 50–60.

$$\text{Mode} = l_1 + \frac{\Delta_1}{\Delta_1 + \Delta_2} \times i$$

$l_1 = 50, \Delta_1 = 40 - 25 = 15, \Delta_2 = 40 - 18 = 22, i = 10.$

$$\text{Mode} = 50 + \frac{15}{15 + 22} \times 10 = 50 + 4.054 = \mathbf{54.05.}$$

Merits of Mode:

i. It is simple and easily understood.

ii. Mode is not affected by the values of extreme items provided they follow to the natural law relating to extremes.

iii. For determination of mode all values in the series are not considered.

Demerits of Mode:

i. As mode is not based on all observations of a series, therefore, it is not rigidly defined.

ii. Mode is not capable of further mathematical treatment.

iii. Mode may be unrepresentative in many cases and it may be impossible to set a definite value of mode as in a set of observations 2 or 3 or more modal values may occur.

Median:

Median is the value of the middle item of a series when arranged in order of magnitude (ascending or descending order).

M = size of (n+ 1)/2 th item, where M stands for median and n for the number of items.

For example,

from ungrouped data: 3, 15, 6, 9, 21, 7, 12, 10

arrange the series is magnitude: 3, 6, 7, 9, 10, 12, 15, 21

$n = 8$ (*the series is even*)

$$M = \text{size} = \frac{(8+1)}{2} = 4.5.$$

In such case 4th and 5th items would be added and divided by 2.

$$\therefore \text{ Median} = \frac{9+10}{2} = 9.5.$$

For example,

series: 2, 12, 9, 6, 17, 5, 8, 14, 23, 27, 11

arrange in magnitude: 2, 5, 6, 8, 9, 11, 12, 14, 17, 23, 27

$n = 11$ (*odd series*),

$$\text{Median} = \text{size of } \left(\frac{11+1}{2}\right) \text{th item} = \text{size of 6th item} = 11.$$

Median from grouped data can be calculated from the following formula:

$$\text{Median} = l_1 + \frac{N/2 - F}{fm} \times i,$$

Where, l_1 = lower limit of median class,

N = total frequency,

F = cumulative frequency,

f_m = frequency of the median class and

i = class interval of median class.

For example, find out median value from the following distribution:

Age group of children	*No. of children (frequency)*	*Cumulative frequency*
1–3	5	5
4–6	10	15
7–9	12	27
10–12	35	62
13–15	20	82
16–18	17	99
19–21	9	108
	N = 108	

Median = the value of $\left(\frac{n+1}{2}\right)$ th item $= \frac{108+1}{2} = 54.5,$

which lies in 7–9 class. Therefore, 7–9 is the median class.

Applying the formula

$$M = 7 + \frac{54.5 - 27}{12} \times 2 = 11.58.$$

Another formula can be used

$$M = l_1 + \frac{l_2 - l_1}{f_1}(m - c),$$

l_1 = lower limit of the median class, l_2 = upper limit, f_1 = frequency of median class, m = middle item; c = cumulative frequency of the group preceding the median group.

$$M = 7 + \frac{9-7}{12}(54.5 - 27) = 7 + \frac{2}{12}(27.5) = 11.58.$$

Merits of Median:

i. Easily calculated and better understood and not affected by the values of the extreme items.

ii. It can be located merely by inspection in many cases.

iii. It gives best result in a study of those phenomena which are incapable of direct quantitative measurement, for example, assessment of madness among mentally retarded patients. In such case it is possible to arrange a group of patients in ascending or descending order in relation to the degree of madness and thus to locate a person whose madness can be said to be average.

Demerits of Median:

i. Median may not be representative of a series in many cases.

ii. It is not suitable for further algebraic treatment.

iii. When median has to be calculated in continuous series it requires interpolation.

iv. Median ignores the values of extremes in the series.

v. Median is likely to be affected by the fluctuations of sampling.

Example 1:

Find out mean, mode and median from the following data and draw the frequency distribution curve:

Solution:

No. of capsules/plant [Class interval]	*Class value (x)*	*No. of plants [frequency]*	*Cumulative frequency*	fx
1–5	3	3	3	9
6–10	8	7	10	56
11–15	13	12	22	156
16–20	18	20	42	360
21–15	23	27	69	621
26–30	28	32	101	896
31–35	33	21	122	693
36–40	38	16	138	608
41–45	43	10	148	430
46–50	48	6	154	288
51–55	53	2	156	106
		N = 156		Σfx = 4223

Mean $= \bar{x} = \frac{\Sigma fx}{n} = \frac{4223}{156} = 27.07.$

Mode = Modal class 26–30.

Mode $= l_1 + \frac{\Delta_1}{\Delta_1 + \Delta_2} \times i = 26 + \frac{5}{5+11} \times 4 = 27.25.$

Median = the value of $\left(\frac{n+1}{2}\right)$**th item** $= \left(\frac{156+1}{2}\right) = 78.5$**, thus median class is 26–30.**

Median $= l_1 + \frac{l_2 - l_1}{f_1}(m - c) = 26 + \frac{30-26}{21}(78.5 - 69) = 26 + \frac{4}{21}(9.5)$ **= 27.81.**

Variance:

Variance is a measure of variation and is the sum of square of deviation (d) divided by the number of degree of freedom (n — 1).

Variance of the sample $S^2 = \sum fd^2/(n-1)$

It is also denoted by σ^2.

Standard Deviation:

A statistical measuring the spread or variability of the sample around the mean or in other words it may be defined as the measure of dispersion of different variables around the central value.

It is square root of variance:

$$\sigma = \sqrt{\frac{\Sigma(x_i - \bar{x})^2}{n-1}} = \sqrt{\frac{\Sigma d^2}{n-1}}, \text{ or, } \sqrt{\frac{\Sigma f d^2}{n-1}}.$$

Merits:

1. The calculation is based on all observations.
2. It is more rigidly defined.
3. Less affected by fluctuations of sampling compared to other measures of dispersion.
4. It summarizes the deviation of large number of observations from mean and is expressed as one unit of variation.

Demerits:

1. It requires a lengthy calculation, i.e., squaring of deviations and then again square root of summed up values.
2. Not very simple to understand.
3. The calculation gives more weightage to extreme values.

Uses of Standard Deviation:

1. It helps in correlating and comparing of different samples.
2. It helps in finding the suitable size of sample for valid conclusion.
3. It helps in finding the standard error which determines whether the difference between means of two similar samples by chance or real.
4. The value of mean and standard deviation help to comment on the population on the basis of observation of sample (Fig. 10.3).

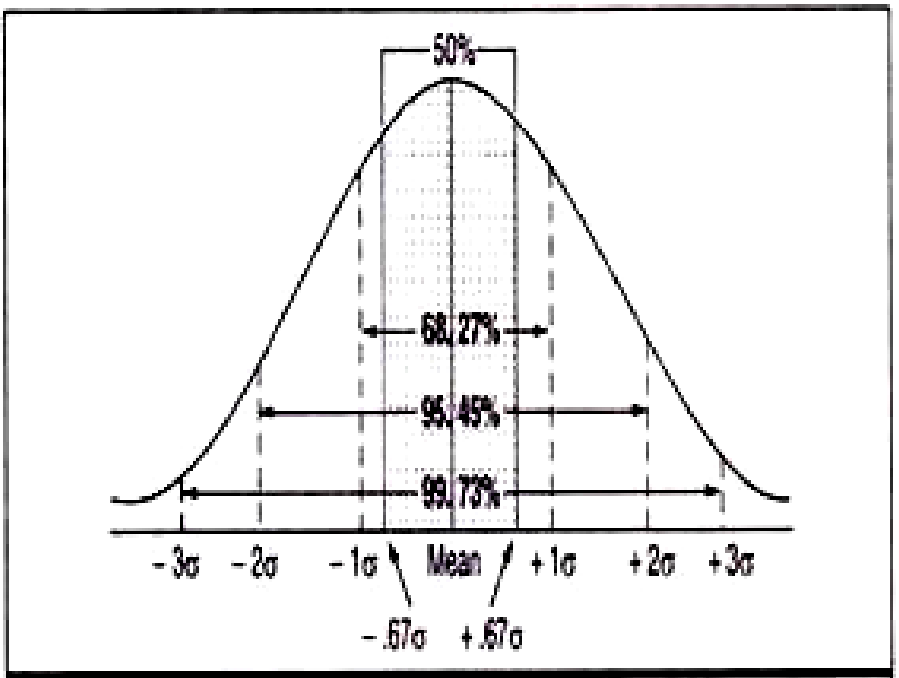


Fig. 10.3: Relationship between Normal Distribution Curve and Standard Deviation

(a) 50% of total observations lie in an area bounded by a distance of 0.6745 σ on both side of the mean.

(b) Mean ± 1σ covers the 68.27% area of the curve.

(c) Mean ± 2σ covers the 95.45% area of the curve.

(d) Mean ± 3a covers the 99.73% area of the curve.

Coefficient of Variation:

In measurement of dispersion, we use the units which are used for observation. But if we want to compare the dispersion of two different characters in the same population, then the calculation of coefficient of variation is needed.

This measurement is expressed in percentage ignoring the units. For example, from the same population if we like to study the length of pod and number of seeds/pod and to compare the dispersion of both the two characters, we must calculate the coefficient of variation.

Standard deviation expressed as percentage of the mean and is denoted by the formula:

Coefficient of variation = Standard deviation/Mean x 100

$$\text{or, c.v.} = \frac{\sigma}{\bar{X}} \times 100 \text{ or } \frac{s}{\bar{X}} \times 100 \text{ in case of samples or population respectively.}$$

This coefficient of variation is also helpful to get an idea or compare two different populations about the dispersion of a character. More the c.v., more is the inconsistency about the dispersion of character.

Example 7:

In two different populations (Batch I and Batch II) the seed number/fruit is calculated:

Batch I 7, 9, 6, 8, 6, 5, 7, 8, 6, 8

Batch II 10, 8, 9, 10, 11, 10, 5, 6, 4, 7

Batch I

Seed number/fruit (x)	Frequency (f)	f × x	Deviation (|d|)	Deviation^2 (d^2)	f × d^2
5	1	5	2	4	4
6	3	18	1	1	3
7	2	14	0	0	0
8	3	24	1	1	3
9	1	9	2	4	4
	$\Sigma f = 10$	$\Sigma fx = 70$			$\Sigma fd^2 = 14$

$$\bar{x} = \frac{\Sigma fx}{n} = \frac{70}{10} = 7$$

$$\sigma = \sqrt{\frac{\Sigma fd^2}{n-1}} = \sqrt{\frac{14}{9}} = 1.25$$

$$\text{c.v.} = \frac{\sigma}{\bar{x}} = \frac{1.25}{7} \times 100 = 17.8\%$$

Batch II

Seed number/fruit (x)	Frequency (f)	f × x	Deviation (|d|)	Deviation² (d²)	f × d²
4	1	4	4	16	16
5	1	5	3	9	9
6	1	6	2	4	4
7	1	7	1	1	1
8	1	8	0	0	0
9	1	9	1	1	1
10	3	30	2	4	12
11	1	11	3	9	9
	$\Sigma f = n = 10$	$\Sigma fx = 80$			$\Sigma fd^2 = 52$

$$\bar{x} = \frac{\Sigma fx}{n} = \frac{80}{10} = 8$$

Standard error:

Standard error is the approximate standard deviation of a statistical sample population. Standard error is a statistical term that measures the accuracy with which a sample represents a population. In statistics, a sample mean deviates from the actual mean of a population; this deviation is the standard error.

The formula for the standard error of the mean is:

$$\sigma_M = \frac{\sigma}{\sqrt{N}}$$

where σ is the standard deviation of the original distribution and N is thesample size (the number of scores each mean is based upon).

Solved Problem:

Find out mean, standard deviation, mean deviation, coefficient of variation and standard error from the given sample:

Class value (x)	*Frequency* (f)	fx	$(x - \bar{x}) = d$	fd	d^2	fd^2
48	8	384	−4.75	+38.00	22.56	180.50
50	32	1600	−2.75	+88.00	7.56	242.00
52	75	3900	−0.75	+56.25	0.56	42.18
54	52	2808	+1.25	+65.00	1.56	81.25
56	28	1568	+3.25	+91.00	10.56	295.75
58	5	290	+5.25	+26.25	27.56	137.81
	n = 200	Σfx = 10,550		Σfd = 364.50		979.49

$$\text{Mean } \bar{x} = \frac{\Sigma fx}{n} = \frac{10,550}{200} = \mathbf{52.75.}$$

$$\text{Mean deviation} = \frac{\Sigma fd}{n}$$

[Note: The positive signs exactly cancel negative sign of deviations and the summation of deviations gives a value of zero and, therefore, the spread of distribution cannot be shown. In calculation of mean deviation, the algebraic signs may be ignored.]

$$= \frac{364.50}{200} = 1.8225 = \mathbf{1.82.}$$

$$\text{Standard deviation } \sigma = \sqrt{\frac{\Sigma fd^2}{n-1}} = \sqrt{\frac{979.49}{200-1}}$$

$$= \sqrt{4.9221} = 2.2185 = \mathbf{2.22.}$$

$$\text{Coefficient of variation (CV)} = \frac{\sigma}{\bar{x}} \times 100 = \frac{2.22}{52.75} \times 100$$

$$= 4.2085 = \mathbf{4.21\%.}$$

$$\text{Standard error} = \frac{\sigma}{\sqrt{n}} = \frac{2.2}{\sqrt{200}} = 0.1556 = 0.16.$$

Therefore, mean is represented as $52.75 \pm \mathbf{0.16.}$

6. Probability & test of significance: $\chi 2$ test (detection of segregation ratio & linkage, test of independence); t-test (student & paired); analysis of variance (ANOVA).

Probability is a number expressed in a quantitative scale. When one event will not occur at all then the probability of that event is 0, and if there is any event which will happen positively without fail then the probability of that event is 1. But in biological science, mostly we find the probability of any event lies between impossibility to certainty i.e., the value ranges from 0 to 1.

Mathematically probability can be explained in the following way:

If an event can happen in 'a' number of ways, and fails to happen in 'b' number of ways, then the probability of its happening 'p' is written as.

$$p = \frac{a}{a+b} \quad \text{or} \quad p = \frac{\text{Number of events occurring}}{\text{Total number of events}}$$

Similarly **probability of failure** of any event is denoted as 'q', where,

$$q = \frac{b}{a+b} \quad \text{or} \quad q = \frac{\text{Number of events not happening}}{\text{Total number of events}}$$

Therefore, $p + q = \frac{a}{a+b} + \frac{b}{a+b} = 1$

So, if the probability of happening any event is 0.7, then the probability of not happening of that event is 0.3.

Events:

The results of any experiment in all possible forms are said to be events of that experiment. Such as, throwing of a dice has 6 possible outcomes, either 1 or 2 or 3 or 4 or 5 or 6. All these six outcomes are called events of that single experiment.

Null Event:

When there is no chance of getting an event is called null or impossible event. It is symbolically denoted by ϕ. Such as, survival of any human being forever is an impossible event or null event.

Sure Event:

If the likelihood of occurrence of any event is sure then the event is called sure event. Such as, the death of a human being is a sure event.

Equally likely events:

If the likelihood of the occurrence of every event in an experiment is same then those are called as equally likely events. Such as, when a dice is thrown, there is no biasness, there are the possibilities of coming any number 1 to 6 in equal probability, so the events are equally likely events.

Mutually Exclusive Events:

If in an experiment the occurrence of one event prevents or rules out the happening of all other events, then these are called as mutually exclusive events. Such as, when a coin is tossed either ‘head’ or ‘tail’ will come.

The occurrence of one event affects the occurrence of another event, both events cannot occur together, i.e., occurrence of ‘head’ rules out getting ‘tail’ in the same trial. Here the events are connected by the words ‘either’ or ‘or’.

Simple Event and Compound Event:

Any event having only one sample point of a sample space is called simple event and if any event is decomposable into a number of simple events then it is called as compound event.

Such as, if a bag contains 4 white and 6 red balls, and if one ball is drawn then it is simple event, but if two balls are drawn together then the events will be — ‘both the balls are white’,

‘both the balls are red’, ‘one ball is white and another ball is red’ — these are compound events.

The compound events may be of two types:

Independent Event:

Two or more events are said to be independent events when the outcome of one event does not affect or is not affected by the other events. For example, if a coin is tossed twice, the result of second tossing would in no way be affected by the result of first tossing, so these are independent events.

Dependent Event:

The occurrence or non-occurrence of one event in one trail affects the probabilities of other events in other trails are called dependent events. For example, the probability of drawing a queen from a pack of 52 cards is 4/52, but if the card drawn for the first time (queen) is not replaced then the probability of second drawing of a queen is 3/51, as the pack now contains 3 queens and 51 cards.

Addition and Multiplication Rules:

Probability is estimated usually on the basis of following two rules of chances:

1. Addition rule
2. Multiplication rule

Addition Rule:

This rule is applied when two events are mutually exclusive, i.e., both events cannot occur simultaneously. The birth of a male child excludes the birth of a female child in the same trial. The probability that one of several mutually exclusive events will occur is the sum of the probabilities of the individual events.

Mathematically, when two events A and B are mutually exclusive, the chance of occurrence or probability of occurrence of either A or B can be calculated from the following formula:

$p (A \text{ or } B) = p(A) + p(B)$

This rule is applicable to any number of mutually exclusive events as follows:

$p(E_1 \text{ or } E_2 \text{ or } E_3 \ldots E_n) = p(E_1) + p(E_2) + p(E_3) + \ldots + p(E_n)$

Example 1:

If a dice is rolled, what is the probability of getting either 3 or 5?

Probability of getting 3 is $p(3) = 1/6$

Probability of getting 5 is $p(5) = 1/6$

$\therefore$ Probability of getting either 3 or 5 is $p(3) + p(5) = 1/6 + 1/6 = 1/3$

Example 2:

What is the probability of getting a king or a joker from a pack of 54 cards?

Probability of getting a king is $p(K) = 4/54 = 2/27$

Probability of getting a joker is $P(J) = 2/54 = 1/27$

So, the probability of either a king or a joker is

$p(K \text{ or } J) = p(K) + p(J) = 2/27 + 1/27 = 3/27 = 1/9$

Addition rule changes when the events are not mutually exclusive, i.e., if two events A and B can occur simultaneously in few cases, then the rule becomes modified in the following way:

$p(A \text{ or } B) = p(A) + p(B) - p(A \text{ and } B)$

Example 3:

What is the probability of getting a king or club from a pack of 52 cards?

In this example, getting a king and a club are not mutually exclusive events as there will be one king which is king of club. So the chance or probability of getting that event should be subtracted.

$p(\text{King or Club}) = p(\text{King}) + p(\text{Club}) - p(\text{King and Club})$

Probability of King = $p(\text{King}) = 4/52 = 1/13$

Probability of Club = p(Club) = 13/52 = 1/4

Probability of King and Club = p (King and Club) = 1/52

So, p(King or Club) = (1/13 + 1/4) – 1/52 = 4/13

Multiplication Rule:

(a) When the Events are Independent:

Probability of two or more independent events occurring together is the product of the probabilities of individual events.

Symbolically, if p(A) and p(B) are the probabilities of two respective events A and B, and the happening of these two events are independent then the probability of happening both the events together can be calculated with the following formula:

p(A and B) = p(A) x p(B)

Thus the rule may be extended to any number of independent events like E_1, E_2, E_3… E_n, and the formula will be as follows:

$p(E_1 \text{ and } E_2 \text{ and } E_3 \ldots \text{ and } E_n) = p(E_1) \times p(E_2) \times p(E_3) \times \ldots \times p(E_n)$

Example 4:

If two dice are thrown simultaneously what is the probability of getting 3 in both the dice?

The probability of getting 3 in first dice is p(A) = 1/6

The probability of getting 3 in 2nd dice is p(B) = 1/6

So, the probability of getting 3 in both the dice is

p(A and B) = p(A) × p (B)= 1/6 × 1/6 = 1/36

(b) When the Events are Dependent:

When the probability of happening one event is affected by the occurrence of another event then it is called conditional probability. Such as, conditional probability of happening A, when B has already happened, is denoted as p(A/B); conditional probability of B, and A has already happened, is denoted as p(B/A).

When the two events A and B are occurring simultaneously but any one event has conditional probability then the multiplication rule will be written as:

p(ab) = p(A)p(B/A) or p(B)-p(A/B)

where p(A/B) = Conditional probability of A given that B has happened

p(B/A) = Conditional probability of B given that A has happened

Example 5:

Four cards are drawn consecutively four times from a pack of 52 cards. Find the chances of drawing an ace, a king, a queen and a jack. The cards are not replaced after each withdrawal.

Probability of drawing an ace = p(A) = 4/52

Probability of drawing a king = p(K) = 4/51

Probability of drawing a queen = p(Q) = 4/50

Probability of drawing a jack = p(J) = 4/49

So, the combined probability

p(A and K and Q and J) = p(A) x p(K) x p(Q) x p(J)

= 4/52 x 4/51 x 4/40 x 4/49 = 0.317

Example 6:

Four cards are drawn in four consecutive drawals from a pack of 52 cards without replacing the cards after each drawal. What is the probability of drawing a king in each drawal?

The probability of getting a king in 1st drawal = 4/52

The probability of getting a king in 2nd drawal = 3/51

The probability of getting a king in 3rd drawal = 2/50

The probability of getting a king in 4th drawal = 1/49

So, the combined probability of getting a king in 4 consecutive drawals is

4/52 x 3/51 x 2/50 x 1/49 = 1/270725

Test of Significance:

In biological research when we compare any character of two samples, we calculate the significance of difference in the mean and variance to draw a meaningful conclusion.

χ2 test:

It is used for testing the agreement of observed frequencies with those expected upon a given hypothesis or in other words it can be said that it is test of deviation between theoretical and observed frequencies and to see whether the deviation is significant or not. If the deviation is significant then the assumed hypothesis on which the test is performed is rejected.

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

O = Observed frequency;
E = Expected frequency;
Σ = Summation over all classes.

Salient Features of χ2 test:

1. Complete agreement of observed and expected frequencies will give χ2 -value as zero; but due to chance deviation (from sample fluctuations) positive values will be scored.

2. The use of χ2 test requires that the frequency in any class is not 5 or less.

3. The test is applicable only to comparisons of observed and expected values of absolute frequencies.

Applications of χ2 test:

1. Testing goodness of fit.
2. To determine genetic ratios [gene(s) involved for a particular trait] from F2/BC1 segregating data.
3. Estimation of linkage.
4. Use in testing independence of classifications.

5. Use in testing heterogeneity of data.

4. Testing Goodness of Fit of χ2 test:

Example 1:

A chlorophyll mutant (viridis) was spotted in M_2 generation of black cumin (*Nigella sativa* L.) following treatments of dry seeds with 5kR gamma-rays. The mutant was crossed with pollens from normal plants and F_1 progenies were raised. All F1's were normal and on selfing 72 normal and 8 viridis were obtained in F2 segregating population.

Question:

State the nature of inheritance of the mutant trait.

Solution:

(a) First see how many phenotypic classes are there. In the present case there are 2 classes—normal and mutant. Therefore, the hypothesis to be assumed will be from the following genetic ratios: 1:1, 3:1, 9:7, 13:3, 15:1.

(b) Add the total observations and select ratio which will be closest to observe values (as goodness of fit of χ2 test is performed).

(c) As the trait concerned is qualitative, χ2 test of goodness of fit has to be applied to ascertain inheritance of the mutant trait.

(d) Hypothesis considered in the present case is 15:1. Therefore, it has to be seen whether 15:1 is good-fit or not.

	Normal	*Mutant*	*Total*
Observed	72	08	80
Expected	75	05	80
$O - E = d$	−3	3	
$(O - E)^2 = d^2$	9	9	
$\frac{(O-E)^2}{E} = \frac{d^2}{E}$	$\frac{9}{75}$	$\frac{9}{5}$	
	0.12	1.80	

$$\chi^2 = \sum \frac{(O - E)^2}{E} = 0.12 + 1.80 = 1.92.$$

As there are two phenotypic classes, degree of freedom will be n – 1 =1 [degree of freedom is the choice of the experiment; n = number of classes in the present case).

Comment: The χ^2 value for 1 DF has been found to be 1.92, which is much less than the χ2-value given at table (3.84) at 5% (standard check) for 1 DF. Therefore, the deviation that occurred between observed and expected values is not significant and considered to be good-fit at the probability level of 0.10 to 0.20 (from χ^2-table). Thus, the assumed hypothesis is accepted (15:1).

Inference:

1. 15:1 genetic ratio indicated that the nature of inheritance of chlorophyll trait (present case) is digenic (15 : 1 is a modification of Mendel's di-hybrid ratio), that is, 2 genes (two pairs of alleles) are involved for the trait.
2. As the mutant crossed with normal gave normal at F_1 and in F_2 frequency of mutants was much lower than normal it may be concluded that mutation was recessive in nature.

Thus, the mutant trait was disgenic recessive to normal.

Note: Why it is $(O - E)^2/E$?

1. To eliminate negative sign, the deviation is squared.
2. As the deviation from expected is tested, therefore, deviation is divided by expected.

Example 2:

A brown seed-coat (bb) mutant of Nigella sativa was crossed with plants having black seed-coat (BB). The F_1 plants were black seeded and were crossed with brown seed-coat mutants and in BC_1 generation 32 black seed-coat and 28 brown seed-coat plants were observed.

Question:

Test the hypothesis at 5% level and comment on the nature of inheritance of the mutant trait (brown seed-coat trait).

Solution:

1. As there are only two phenotypic classes and the F_1's were crossed with recessive parent so the assumed hypothesis can only be 1:1.
2. Instead of F_2 generation the data has been obtained from BC_1 segregating population.

	Phenotypic classes		
	Brown seed-coat	*Black seed-coat*	*Total*
Observed	28	32	60
Expected	30	30	60
$(O - E) = d$	−2	+2	
$(O - E)^2 = d^2$	4	4	
$\frac{(O-E)^2}{E} = \frac{d^2}{E}$	$\frac{4}{30}$	$\frac{4}{30}$	
$\chi^2 = \sum \frac{(O-E)^2}{E}$	0.13	0.13	
$\chi^2 = 0.26$ at 1 DF			

The calculated $\chi 2$-value 0.26 at 1 DF is much less than the $\chi 2$-table value (3.86) at 5% for 1 DF. Thus, the observed and expected values are good-fit (p-value 0.50 to 0.70) and, therefore, the deviation between them is not significant and consequently the assumed hypothesis is accepted.

Inference:

As the hypothesis 1 : 1 is accepted, it can be concluded that the mutant trait is monogenic (one pair of alleles) recessive to normal.

In practical classes seeds are given in petri-plates to test goodness of fit of a particular data. For some data careful test should be conducted.

Example 3:

Black seeds	Brown seeds	Total
18	22	40

Solution:

Let the assumed hypothesis be 1:1. Therefore, expected frequencies will be 20 for black seeds and 20 for brown seeds.

$$\frac{(O-E)^2}{E} = \frac{4}{20} \text{ and } \frac{4}{20} = 0.2 \text{ and } \dot{0}.2; \quad \chi^2 = 0.2 + 0.2 = 0.4.$$

As χ2-value 0.4 at 1 DF is much less than table value 3.87 at 1 DF, the deviation between expected and observed is not significant rather good-fit at the probability level of 0.50 to 0.55. Therefore, the hypothesis 1:1 is accepted and seed-coat colour is controlled by one pair of alleles.

But for the same set of data—hypothesis 9:7 may be considered.

	Black seeds	*Brown seeds*
Observed	18	22
Expected	17.5	22.5
$\frac{(O-E)^2}{E}$	$\frac{(0.5)^2}{17.5} = 0.014$	$\frac{(0.5)^2}{22.5} = 0.011$
$\chi^2 = 0.025$		

In this case also the hypothesis 9:7 is accepted as the deviation between observed and expected values are not significant and are good-fit at 0.80 to 0.90 probability level. Acceptance of this hypothesis is indicated that the seed-coat colour had a digenic mode of inheritance.

Inference:

9:7 is the correct hypothesis as it shows lesser deviation between observed and expected values than 1:1. Thus, assumption of hypothesis in $\chi 2$– test of goodness of fit should be based on close fit of expected with observed values.

Test of Linkage following the use of $\chi 2$-Test:

Example 4:

A tall homozygous pea plant (TT) bearing yellow pods (YY) was crossed with a dwarf plant (tt) having green pods (yy).

The F_1 plants raised were all tall and yellow poded and on selfing of these plants F_2 plants were developed in the following frequencies:

Tall Yellow–120	*Tall Green–10*
Dwarf Yellow–10	*Dwarf Green–20*

Question:

Comment on the assortment of T and Y genes.

Solution:

$\chi 2$-test of goodness of fit will be applied to test the hypothesis 9:3:3:1 is the assumed hypothesis as it is a data from di-hybrid cross.

	Phenotypic classes				
	Tall Yellow	*Tall Green*	*Dwarf Yellow*	*Dwarf Green*	*Total*
Observed	120	10	10	20	160
Expected	90	30	30	10	160
$(O - E)$	30	−20	−20	10	
$(O - E)^2$	900	400	400	100	
$\frac{(O-E)^2}{E}$	$\frac{900}{90}$	$\frac{400}{30}$	$\frac{400}{30}$	$\frac{100}{10}$	
$\chi^2 = \sum \frac{(O-E)^2}{E}$	10.0	13.33	13.33	10.0	
$\chi^2 = 46.66$					

Therefore, $\chi 2 = 46.66$ at 3 DF (4 phenotypic classes; n-1 =3).

As computed $\chi 22$-value 46.66 at 3 DF is much higher than table value 7.8 at 3 DF, the deviations between observed and expected values are not good-fit and, therefore, the assumed hypothesis 9 : 3 : 3 : 1 is not accepted.

This significant deviation may be due to failure of independent assortment of T and Y genes as the consequence of linkage. High frequency of parental combinations than recombinant also indicated the possibility of linkage.

Linkage can be estimated from F^2 data by the following formula:

$P^2 = E – M/N$,

where P = linkage value, E = sum of end classes (parental classes), M = sum of middle classes (recombinant classes) and N = number of progenies.

$p^2 = 140 – 20/160 = 0.75$; $P = \sqrt{0.75} = 0.8660$.

Therefore, percentage of linkage in the given data is 86.6. Recombination value is thus 13.4%. As 1% recombination is considered as 1 map unit, therefore, T and Y genes are 13.4 map units distance apart.

χ^2 Test of Independence:

Another common use of the χ^2 Test is in testing Independence of classifications in what are known as contingency table. Data are set out in a table with rows and columns, i.e., each observation is assigned to one of the cells in the table.

For example, if there are r rows and c columns, the table is generally called r x c contingency table, where r and c may represent any number and the simplest table of this kind is 2 x 2 contingency table.

			Total
	a	b	a + b
	c	d	c + d
Total	a + c	b + d	a + b + c + d

$$DF = (r - 1)(c - 1).$$

χ^2-value for 2 × 2 table is given by the following formula:

$$\chi^2 = \frac{(ad - bc)^2(a + b + c + d)}{(a + b)(b + d)(d + c)(c + a)}.$$

For a 2 x 2 table, there is only one degree of freedom, i.e., only one of the four cell frequencies can be arbitrarily given if the row and column total remain fixed. It is, therefore, necessary to make a correction of formula, so that its approximation to the continuous chi-square distribution can be improved. This is known as Yates correction for continuity.

$$\chi^2 = \frac{N\{(ad - bc) - N/2\}^2}{(a + b)(b + d)(d + c)(a + c)}.$$

Example 8:

In a survey of 200 boys of which 75 were intelligent, 40 had skilled fathers; while 85 of the unintelligent boys had unskilled fathers. Do these figures, support the hypothesis that skilled father have intelligent boys?

Solution:

The data are shown in the following 2 x 2 table:

Intelligence of sons	*Skill of fathers*		*Total*
	Skilled	*Unskilled*	
Intelligent	40	35	75
	(*a*)	(*b*)	
Unintelligent	40	85	125
	(*c*)	(*d*)	
Total	80	120	200

Null hypothesis—The two attributes skill of father and intelligence of son are independent (As it is a test of independence—the assumed hypothesis is considered to be independent between attributes).

$$\chi^2 = \frac{N\{(ab - bc) - N/2\}^2}{(a+b)(b+d)(d+c)(a+c)}$$

$$= \frac{200\left[(40 \times 85) - (35 \times 40) - \frac{200}{2}\right]^2}{80 \times 120 \times 75 \times 125}$$

$$= 8.02 \text{ at } 1 \text{ DF } [(r-1)(c-1) = (2-1)(2-1)] = 1.$$

As the observed value 8.02 at 1 DF is greater than the table value (3.84 at 1 DF) at 5% level of significance, there exist significant deviation between observed and expected cell frequencies and, therefore, the assumed hypothesis is rejected.

Inference:

By the use of χ^2-test of independence it can be concluded that skilled fathers have intelligent sons (the attributes are dependent).

Example 9:

Anaphase I chromosomal segregation in *Allium cepa* (2n = 16) showed seasonal variation and the data obtained has been represented in 2 x 2 contingency table. Test whether the attributes are independent or not.

	Anaphase I separations		
Seasons	8 : 8	9 : 7	*Total*
Summer	65(a)	35(a)	
Winter	80(c)	20(d)	100
Total	145	55	200

Solution

Null hypothesis—Anaphase I separation of chromosomes is independent of seasons:

$$\chi^2 = \frac{N\{(ad - bc) - N/2\}^2}{(a+b)(b+d)(d+c)(a+c)}$$

$$= \frac{200\{(65 \times 20) - (35 \times 80) - 200/2\}^2}{100 \times 55 \times 100 \times 145}$$

$$= 6.42 \text{ at } 1 \text{ DF.}$$

The calculated χ^2-value 6.42 at 1 DF is greater than the table value 3.87 (1 DF at 5% level of significance) and, therefore, the hypothesis is rejected as there exist significant deviation between observed and expected cell frequencies.

Inference:

Anaphase I separation of *Allium cepa* chromosomes varied with seasons (the traits are dependent to one another).

Example 10:

500 PMCs of *Nigella sativa* (black cumin) were assessed in 2 different seasons (summer and winter) for studying anaphase I segregation of chromosomes (2n = 12) and the data obtained have been tabulated in r x c contingency table. Test the independence of the characters (anaphase I chromosome segregation and seasons,).

Seasons	*Anaphase I chromosome separation*			*Total*
	4 : 8	6 : 6	5 : 7	
Summer	42	137	61	240
	a	b	c	
Winter	58	113	89	260
	d	e	f	
Total	100	250	150	500

Solution:

Null hypothesis—Anaphase I chromosome segregation is independent to seasons.

The expected value for each class can be calculated from the following formula:

E = r x c / G, where r = row, c = column and G = grand total.

Expected values:

$$a = \frac{100 \times 240}{500} = 48; \qquad b = \frac{250 \times 240}{500} = 120;$$

$$c = \frac{150 \times 240}{500} = 72; \qquad d = \frac{100 \times 260}{500} = 52;$$

$$e = \frac{250 \times 260}{500} = 130; \qquad f = \frac{150 \times 260}{500} = 78.$$

$$\chi^2 = \frac{(42-48)^2}{48} + \frac{(137-120)^2}{120} + \frac{(60-72)^2}{72} + \frac{(58-52)^2}{52} + \frac{(113-130)^2}{130} + \frac{(89-78)^2}{78}$$

$$= 0.75 + 2.41 + 2.0 + 0.69 + 2.22 + 1.55$$

$$= 9.62 \text{ DF} = (r-1)(c-1) = (2-1)(3-1) = 2.$$

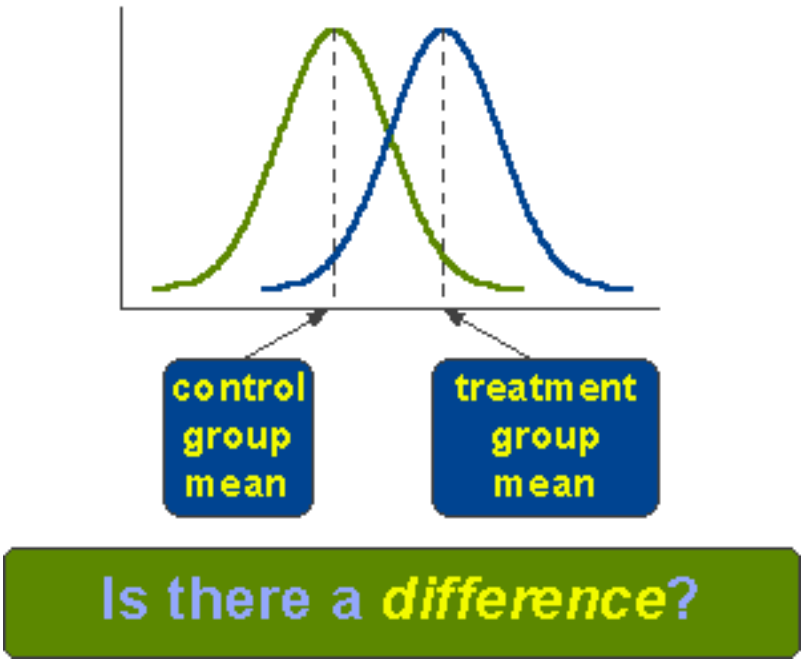
The calculated χ^2-value 9.62 at 2 DF is higher than table value (5.9 at 2 DF) at 5% level of significance, thereby indicating that the expected and observed frequencies are not good-fit rather showed significant deviations. Thus, the hypothesis is rejected.

Inference:

Anaphase I chromosome segregation is dependent to seasons.

Student's 't'-Test:

To test the significance of difference of means of two samples, W.S. Gosset applied a statistical tool called 't'-test. According to nick name of Gosset, the test has been named as Student's 't'-test.



In this test we make a choice between two alternatives:

1. To accept the null hypothesis (no difference between the two means);

2. To reject the null hypothesis (the difference between the means of two samples significant).

The test is applied to small sample (n<30), and samples must be drawn randomly from normal population. The 't' is defined as quantity representing the difference between the sample mean or true means or population mean expressed in terms of the standard error.

T = Difference between sample means/Standard error of the difference between means

$$t = \frac{\bar{x}_1 - \bar{x}_2}{SE_D}$$

$\bar{x}_1$ and $\bar{x}_2$ = Mean of two samples

SE_D = Standard error of the difference between means

Degree of Freedom:

To calculate the standard error of the difference, the degree of freedom can be obtained by one less than the number of observations of sample. In case of unpaired 't' test, i.e., when the comparison between two samples is done, degree of freedom is calculated by the formula:

$(n_1 + n_2) - 2$, where n_1 = no. of observations in sample I

n_2 = no. of observations in sample II.

Whereas, in case of paired 't' test, the degree of freedom is (n – 1), as there is the same number of observations.

Determination of Significance:

Probability of occurrence of any calculated value of 't' is determined by comparing it with the value given in the 't'-table corresponding to the calculated degree of freedom, derived from the number of observations in the samples under study. If the calculated value of 't' exceeds the value given at p = 0.05 (5% level) in the table, it is said to be significant. If the calculated value of 't' is less than the value given in 't'-table, it is not significant.

The 't'-test procedure is done to locate the observed value of 't' in the student's 't'-distribution curve. The 't' distribution curve is a symmetrical curve with mean zero, it extends to infinity on either sides. When degree of freedom is less in number, 't'- distribution resembles to normal distribution curve. If the calculated value of 't' is near the centre then the data is regarded as compatible with H_0, which concludes that the observed deviation is due to chance factor or only due to sampling. If the 't' value is situated at tail region then $H_0$is not accepted.

Unpaired t-Test:

This test is applied to unpaired data of independent observations made on individuals of two different or separate groups or samples drawn from two different populations.

Steps to be followed for calculation of 't':

1. The means of two samples are calculated and the differences between the means of two samples are calculated.

$$(\overline{x}_1 - \overline{x}_2)$$

2. The standard error of the difference between two means is calculated.

$$\sigma_d = \sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}$$

where, $\sigma_1^2 = \dfrac{\sum fx_1^2 - \dfrac{(\sum fx_1)^2}{n_1}}{n_1 - 1}$ and $\sigma_2^2 = \dfrac{\sum fx_2^2 - \dfrac{(\sum fx_2)^2}{n_2}}{n_2 - 1}$

3. 't' value is calculated by the ratio between the observed difference of means and its standard error.

For Large Sample,

$$\sigma_d = \sqrt{\frac{\Sigma(x_1 - \bar{x}_1)^2 + \Sigma(x_2 - \bar{x}_2)^2}{n_1 + n_2 - 2}} \quad \text{or} \quad \sqrt{\frac{\Sigma x_1^2 - \dfrac{(\Sigma x_1)^2}{n_1} + \Sigma x_2^2 - \dfrac{(\Sigma x_2)^2}{n_2}}{n_1 + n_2 - 2}}$$

4. The calculated 't' value is compared with 't' table to find out the significance at that particular degree of freedom.

Practical sheet for 't' analysis:

No. of Replicates	Sample 1 x_1	x_1^2	Sample 2 x_2	x_2^2
1				
2				
3				
4				
5				
6				
—				
—				
—				
n				
	$\Sigma x_1 =$	$\Sigma x_1^2 =$	$\Sigma x_2 =$	$\Sigma x_2^2 =$

$$\bar{x}_1 = \qquad \bar{x}_2 =$$

$$\frac{(\Sigma x_1)^2}{n_1} = \qquad \frac{(\Sigma x_2)^2}{n_2} =$$

$$\sigma_d = \sqrt{\frac{\Sigma x_1^2 - \frac{(\Sigma x_1)^2}{n_1} + \Sigma x_2^2 - \frac{(\Sigma x_2)^2}{n_2}}{n_1 + n_2 - 2}}$$

Small Sample **Large Sample**

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sigma_d \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \qquad t = \frac{\bar{x}_1 - \bar{x}_2}{\sigma_d}$$

Example 1:

In an experiment to find out the effect of a hormone spray on the seed yield of dwarf French bean, the following results were obtained. Analyse the data using the 't' test for inference on the effect of hormonal spray on the seed yield.

No. of seeds/plant

Control :	30	35	31	36	38	32	25	39	26	27	40	41	35	38	32
	31	38	30	35	36	25	28	29	37	36	36	30	31	34	33
	35	40	30	32	28	26	29	30	30	35	36	37	38	39	30
	31	39	40	35	36										
Treated :	35	38	40	45	40	42	46	48	49	50	35	38	45	47	45
	46	50	51	46	41	42	48	49	51	52	50	53	54	55	43
	45	50	44	51	52	53	52	48	50	49	47	49	50	49	51
	52	50	51	52	40										

Control (x_1)					← No. of seeds/plant →	Treated (x_2)			
x_1	f	fx_1	x_1^2	fx_1^2	x_2	f	fx_2	x_2^2	fx_2^2
25	2	50	625	1250	35	2	70	1225	2450
26	2	52	676	1352	38	2	76	1444	2888
27	1	27	729	729	40	3	120	1600	4800
28	2	56	784	1568	41	1	41	1681	1681
29	2	58	841	1682	42	2	84	1764	3528
30	7	210	900	6300	43	1	43	1869	1849
31	4	124	961	3844	44	1	44	1936	1936
32	3	96	1024	3072	45	4	180	2025	8100
33	1	33	1089	1089	46	3	138	2116	6348
34	1	34	1156	1156	47	2	94	2209	4418
35	6	210	1225	7350	48	3	144	2304	6912
36	6	216	1296	7776	49	5	245	2401	12005
37	2	74	1369	2738	50	7	350	2500	17500
38	4	152	1444	5776	51	5	255	2601	13005
39	3	117	1521	4563	52	5	260	2704	13520
40	3	120	1600	4880	53	2	106	2809	5618
41	1	41	1681	1681	54	1	54	2916	2916
					55	1	55	3025	3025
	$\Sigma f=50$	$\Sigma fx_1=1670$		$\Sigma fx_1^2=56726$		$\Sigma f=50$	$\Sigma fx_2=2359$		$\Sigma fx_2^2=112499$

$$\bar{x}_1 = \frac{\Sigma fx_1}{\Sigma f} = \frac{1670}{50} = 33.4$$

$$\text{Variance, } \sigma_1^2 = \frac{\Sigma fx_1^2 - \frac{(\Sigma fx_1)^2}{\Sigma f}}{\Sigma f - 1}$$

$$= \frac{56726 - \frac{(1670)^2}{50}}{49}$$

$$= \frac{56726 - 55778}{49} = \frac{948}{49} = 19.347$$

$$\bar{x}_2 = \frac{\Sigma fx_2}{\Sigma f} = \frac{2359}{50} = 47.18$$

$$\text{Variance, } \sigma_2^2 = \frac{\Sigma fx_2^2 - \frac{(\Sigma fx_2)^2}{\Sigma f}}{\Sigma f - 1}$$

$$= \frac{1112499 - \frac{(2359)^2}{50}}{49}$$

$$= \frac{1112499 - 111297.62}{49} = \frac{1201.38}{49} = 24.518$$

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sigma_d} = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}} = \frac{|133.4 - 47.28|}{\sqrt{\frac{19.347}{50} + \frac{24.518}{50}}} = \frac{13.88}{\sqrt{0.387 + 0.49}} = \frac{13.88}{\sqrt{0.877}} = \frac{13.88}{0.936} = 14.829$$

Degrees of freedom = n1 + n2 – 2 = 50 + 50 – 2 = 98

Calculated t = 14.829

Tabulated t = 3.37 (p = 0.001)

Since the calculated t (14.829) far exceeds the tabulated t (3.37) with 98 d.f. at p = 0.001, the null hypothesis stating that there is no difference in the two sample means, is rejected. Alternatively there is a highly significant difference between the two sample means, i.e., the hormonal spray has a very significant effect on the seed yield.

Example 2:

Two varieties of potato plants (A and B) yielded tubers as shown in the following table. Does the mean weight of tubers of the variety A significantly differ from that of variety B.

Variety A : 2.2 2.5 1.9 2.6 2.3 1.8 2.0 2.1 2.4 2.3 kg/plant

Variety B : 2.8 2.5 2.7 3.0 3.1 2.3 2.4 3.2 2.5 2.9 kg/plant

Variety A		Variety B	
x_A	x_A^2	x_B	x_B^2
1.8	3.24	2.3	5.29
1.9	3.61	2.4	5.76
2.0	4.00	2.5	6.25
2.1	4.41	2.5	6.25
2.2	4.84	2.7	7.29
2.3	5.29	2.8	7.84
2.3	5.29	2.9	8.41
2.4	5.76	3.0	9.00
2.5	6.25	3.1	9.61
2.6	6.76	3.2	10.24
$\sum x_A = 22.10$	$\sum x_A^2 = 49.45$	$\sum x_B = 27.40$	$\sum x_B^2 = 75.94$

$$\bar{x}_A = \frac{22.10}{10} = 2.21 \qquad \bar{x}_B = \frac{27.40}{10} = 2.74$$

$$\sum x_A^2 - \frac{(\sum x_A)^2}{n_A} = 49.45 - \frac{(22.1)^2}{10} = 49.45 - 48.84 = 0.61$$

$$\sum x_B^2 - \frac{(\sum x_B)^2}{n_B} = 75.94 - \frac{(27.4)^2}{10} = 75.94 - 75.08 = 0.86$$

$$n_A + n_B - 2 = 10 + 10 - 2 = 20 - 2 = 18$$

Pooled variance,

$$\sigma_d^2 = \frac{0.61 + 0.86}{18} = \frac{1.47}{18} = 0.082$$

Pooled standard deviation,

$$\sigma_d = \sqrt{\sigma_d^2} = \sqrt{0.082} = 0.286$$

$$\therefore \quad t = \frac{|\bar{x}_A - \bar{x}_B|}{\sigma_d\sqrt{\frac{1}{10} + \frac{1}{10}}} = \frac{2.74 - 2.21}{0.286\sqrt{\frac{1}{5}}} = \frac{0.53}{0.286 \times 0.45} = \frac{0.53}{0.129} = 4.108$$

Calculated t = 4.108

Tabulated t = 3.92 (0.001 p) for 18 d.f.

As the calculated t (4.108) for the difference of the two sample means is greater than the tabulated t (3.92) for 18 d.f. at $p \leq 0.001$, the difference between the two means is therefore highly significant. The null hypothesis stating that the two sample means are not different is rejected at $p \leq 0.001$.

Example 3:

Application of fertilizer (NPK) was tested on the yield of rice grown in 10 plots. Another set of 10 plots of similar size and conditions was taken as the control. Determine whether the fertilizer has any significant effect on the yield of rice.

	NPK applied			Control	
Plot Nos.	Rice yield (x_1) (Quintal/hectare)	x_1^2	Plot Nos.	Rice yield (x_2) (Quintal/hectare)	x_2^2
1	16	256	1	10	100
2	14	196	2	12	144
3	18	324	3	11	121
4	15	225	4	9	81
5	13	169	5	13	169
6	17	289	6	13	169
7	16	256	7	12	144
8	15	225	8	14	196
9	14	196	9	13	169
10	13	169	10	11	121
Total	$\Sigma x_1 = 151$	$\Sigma x_1^2 = 2305$	Total	$\Sigma x_2 = 118$	$\Sigma x_2^2 = 1414$

$$\therefore \bar{x}_1 = \frac{\Sigma x_1}{n_1} = \frac{151}{10} = 15.1 \qquad \therefore \bar{x}_2 = \frac{\Sigma x_2}{n_2} = \frac{118}{10} = 11.8$$

$$\Sigma x_1^2 - \frac{(\Sigma x_1)^2}{n_1} = 2305 - \frac{(151)^2}{10} \qquad \Sigma x_2^2 - \frac{(\Sigma x_2)^2}{n_2} = 1414 - \frac{(118)^2}{10}$$

$$= 2305 - 2280.1 = 24.9 \qquad = 1414 - 1392.4 = 21.6$$

Pooled variance,

$$\sigma_d^2 = \frac{\Sigma x_1^2 - \frac{(\Sigma x_1)^2}{n_1} + \Sigma x_2^2 - \frac{(\Sigma x_2)^2}{n_2}}{n_1 + n_2 - 2} = \frac{24.9 + 21.6}{10 + 10 - 2} = \frac{46.5}{18} = 2.58$$

$$\sigma_d = \sqrt{2.58} = 1.61; \quad |\bar{x}_1 - \bar{x}_2| = |15.1 - 11.8| = 3.30$$

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sigma_d \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} = \frac{3.30}{1.61\sqrt{\frac{1}{10} + \frac{1}{10}}} = \frac{3.30}{1.61\sqrt{0.2}} = \frac{3.30}{1.61 \times 0.45} = \frac{3.3}{0.72} = 4.58$$

Tabulated t = 3.92 (p = 0.001) for 18 df. is found to be less than the calculated t (4.58). Thus the difference between the means of two samples is highly significant. The null hypothesis stating that the two means have no difference is rejected at p = 0.001.

Paired 't'-Test:

Paired 't'-test is applied when each individual gives a pair of observations. Here the paired data of independent observation from one sample only to be compared.

This kind of observations is made available in biological sciences, such as:

(a) To study the effect of fertilizer, pesticide, drug on plants.

(b) To compare the effect of two different fertilizers or drugs.

(c) To compare the result of two techniques or the accuracy of two different instruments.

Steps to be followed for calculation of 't':

1. The difference in each set of paired observations are made.

$$\bar{x}_1 - \bar{x}_2 = D$$

2. The mean of difference $\bar{D}$ is calculated,

$$\bar{D} = \frac{\sum D}{n}.$$

3. Standard deviation of the difference (S_D) and then standard error of mean from the same is calculated,

$$SE_D = \frac{S_D}{\sqrt{n}}, \quad \text{where} \quad S_D = \sqrt{\frac{\sum D^2 - \frac{(\sum D)^2}{n}}{n-1}}$$

4. The ‘t’ value is determined by substituting the above values in the formula,

$$t = \frac{\bar{D}}{\text{Standard error of difference}(SE_{\bar{D}})} \quad \text{or} \quad t = \frac{\bar{D}}{\frac{S_D}{\sqrt{n}}}$$

5. The calculated value of ‘t’ is compared with ‘t’-table to find out the significance at that particular degree of freedom.

Example:

Determine the effect of a new growth regulator (x) on the callus growth of Nicotiana in laboratory condition. The observations are made on particular 10 calli.

Callus no.	1	2	3	4	5	6	7	8	9	10
Initial wt. of callus (g)	2.9	2.7	2.8	2.8	2.9	2.9	2.8	3.0	2.6	2.8
Final wt. of that callus (g)	3.0	2.8	2.9	2.8	3.2	3.1	3.0	3.2	2.7	2.9
Difference (D)	0.1	0.1	0.1	0	0.3	0.2	0.2	0.2	0.1	0.1
D^2	0.01	0.01	0.01	0	0.09	0.04	0.04	0.04	0.01	0.01

$$\Sigma D = 1.4 \qquad \Sigma D^2 = 0.26 \qquad n = 10$$

$$\bar{D} = \frac{1.4}{10} = 0.14$$

$$\text{Standard deviation of the difference} = \sqrt{\frac{\sum D^2 - \frac{(\sum D)^2}{n}}{n-1}}$$

$$= \sqrt{\frac{0.26 - \frac{1.96}{10}}{9}} = \sqrt{\frac{.06}{9}} = \sqrt{.0066} = .08$$

$$SE_D = \frac{.08}{\sqrt{10}} = \frac{.08}{3.16} = .025$$

$$t = \frac{\bar{D}}{SE_D} = \frac{0.14}{.025} = 5.6.$$

Tabulated t = 4.78 at p .001 with 9 degrees of freedom.

The calculated 't' far exceeds the tabulated 't' at p = 0.001 with 9 degrees of freedom. So the result is highly significant, i.e., the hormone has the positive effect on callus growth.

Analysis of variance (F test):

The analysis of variance is based on F-statistics which is a ratio of variances. The ratio of variances due to treatments and variances due to random differences within the treatment is calculated and the estimate, known as the 'F', is then used for comparison.

It is a collection of statistical models used to analyze the differences among group means and their associated procedures (such as "variation" among and between groups), developed by statistician and evolutionary biologist Ronald Fisher.

ANOVA statistical significance result is independent of constant bias and scaling errors as well as the units used in expressing observations.

Example:

Four rice varieties were grown in 4 replications in randomized block design and their yield/plot was assessed. From yield data do you consider that the mean yield of varoieties differ among themselves?

Solution:

A_5	C_{10}	B_2	D_{10}
B_4	A_6	C_{12}	D_9
D_9	A_5	B_2	C_{11}
C_{12}	B_3	A_7	D_8

Significance:

No biasness over the experiment—Wide range of treatments with no restriction on number of replications.

Demerits:

Sectoral representation of some variety/treatments (that is, the treatments were not distributed uniformly) may occur.

Yield of the varieties—kg/plot.

Replication	*Varieties*				
	A	B	C	D	
1	5	2	10	10	27
2	6	4	12	9	31
3	5	2	11	9	27
4	7	3	12	8	30
	23	11	45	36	115 (Grand Total)

In the present case null hypothesis is assumed as:

(a) Varietal means are same, i.e., the varieties do not differ among themselves in yield.

(b) Varieties do not differ in replication.

First Step:

Add row and column totals to find out grand total = 115(T).

CF (Correction factor) = $T^2/n = (115)^2/16 = 826.56$.

Second Step:

Grand total sum of squares = $5^2 + 6^2 + 5^2 + 7^2 + \ldots\ldots + 9^2 + 9^2 + 9^2 + 8^2 = 1003$.

Third Step:

(Total SS = Grand total SS – CF) = 1003 – 826.56 = 176.44.

Fourth Step:

SS due to variety (column) = $23^2 + 11^2 + 45^2 + 36^2/4$ – CF =166.19.

It is divided by 4 as each value is sum of four items.

Fifth Step:

SS due to replication (row) = $27^2 + 31^2 + 27^2 + 30^2 /4$ – CF = 3.19.

Sixth Step:

SS due to error

= Total SS – (SS of variety + SS of replication)

= 176.44 — (166.19 + 3.19) = 7.06.

Anova Table

Sources	df	SS	MSS	*F-value*	*Table Value*		
					5%	1%	0.1%
Variety	$3(n_1)$	166.19	55.40	71.03***	3.9	7.0	13.9
Replication	$3(n_1)$	3.19	1.06	1.36			
Error	$9(n_2)$	7.06	0.78				
Total	15						

$$\text{MSS} = \text{mean sum of squares} = \frac{\text{SS}}{\text{df}}$$

$$\text{Calculated F-value} = \frac{\text{MSS of source}}{\text{MSS of error}}$$

Results indicated that the varieties varied significantly among themselves at 0.1% level of significance; however, the varieties did not varied in replication.

Critical Difference (CD):

The square root of the error mean square measures the standard error per plot due to uncontrolled environmental effects. The varietal means were obtained from four plots (replication) and, therefore, standard error of varietal mean in the present case will be
$\sqrt{0.78/4} = 0.44$.

The standard error of difference of means of two varieties will be:

$0.44 \times \sqrt{2} = 0.44 \times 1.41 = 0.6204$.

From the value of standard error of difference we can calculate the value of the difference which will be just significant at a chosen level of significance. The difference is known as the

critical difference for the particular level of significance (generally 5% level of significance is considered adequate).

Hence, CD is t-value x standard error of difference (5%) for 9 DF
= 2.26 (t-value at 5% for 9 DF in the present case) x 0.6204
= 1.4.

That is, if the difference between 2 varieties is 1.4, then it is significant.

	A	B	C	D
Total	23	11	45	36
Mean	5.75	2.75	11.25	9.0
CD at 5%	1.4			

From CD-value it is apparent that yield varied significantly between the varieties.

7. Correlation and Regression

Correlation:

Association between variables or attributes or characteristics at a given time is known as correlation.

Example:

(i) The amount of rainfall and yield of a certain crop;
(ii) Age of husband and wife;
(iii) Height and weight of students and
(iv) Different concentrations of mutagen and their effect on seed germination frequency.

In plant breeding the breeders targets improvement of yield. Relationship between yield and yield related traits (plant height, number of primary branches/ plant; total branches/plant, number of capsules/plant, capsule length, seeds/capsule, 100-seed weight, etc.) and between the yield related components can be worked out through correlation studies.

Significant correlation obtained will be helpful for selection and ascertaining the model plant type for the concerned species.

Precisely correlation may be defined as movement of one variable tend to be accompanied by corresponding movements in the other. Such simultaneous movement of two variables can be graphically plotted using value of one variable on x-axis and the other variable along y-axis. Such representation of variables indicates the nature of association between the attributes and is called as scattered diagram or correlation chart.

Correlation may:

(a) Positive Correlation:

Increase in plant height is related to increase in number of branches per plant. On the scattered diagram the dots (each pair of observation) representing the variables are in a linear path diagonally across the graph paper from bottom left-hand corner to the top right.

(b) Negative Correlation:

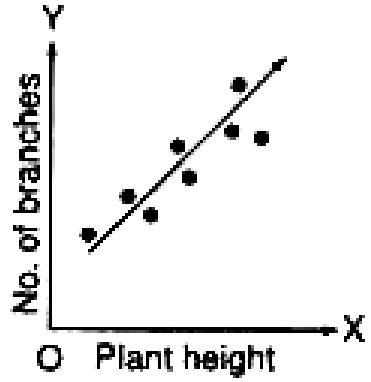
For example, increase in plant height of a species is related to decrease in branch number per plant. The pattern of dots be such as to indicate a straight line path from the upper left-hand corner to the bottom right.

(c) Zero Correlation:

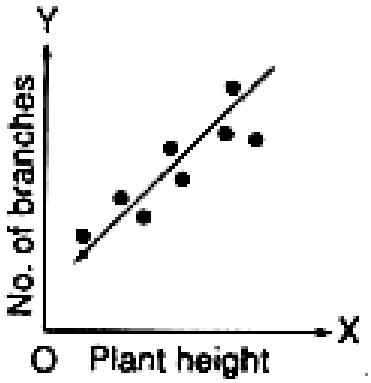
The dots are scattered and do not indicate any straight line.

(d) Perfect Correlation:

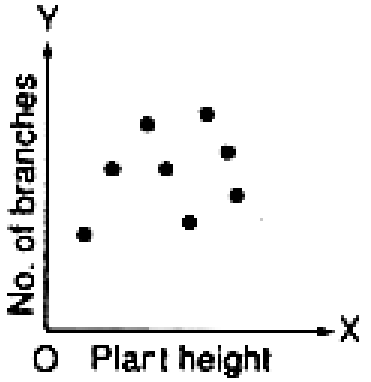
When the dots lie exactly on a straight line.



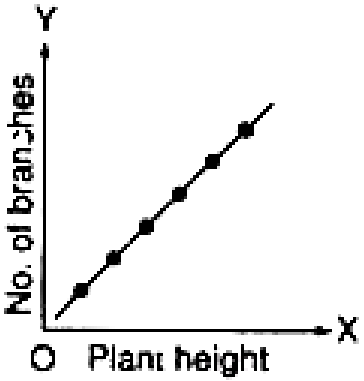
Positive correlation



Negative correlation



Zero correlation



Perfect correlation

In the present example height of plants represented independent variable and on the other hand the variable which changes with the change in the independent variable is called dependent variable (branches/plant).

It is customary to use the horizontal axis (x-axis) for the independent variable and the vertical axis (y-axis) for dependent variable.

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The degree of relationship between 2 attributes can be determined by calculating a coefficient called as correlation coefficient. The correlation coefficient is expressed by the letter ‘r’. r varies from 0 to 1 and can be + (positive correlation) or — (negative correlation). Practically, r is never zero or 1 (complete/absolute).

Whenever correlation coefficient analysis is made, r-value ranges from 0 to 1 but it is necessary to compare the calculated r-value with table value at specific degree of freedom. If the value is significant, i.e., if the calculated r-value is greater than table value, then only we can say that the two attributes are statistically associated to one another. Degree of significance level has also to be assessed (5%, 1% and 0.1% levels).The correlation coefficient is expressed by 'r' which varies from 0 to 1.

$$r = \frac{\Sigma(x - \bar{x})(y - \bar{y})}{\sqrt{\Sigma(x - \bar{x})^2 \Sigma(y - \bar{y})^2}} = \frac{\Sigma d_x d_y}{\sqrt{\Sigma d_x^2 \Sigma d_y^2}},$$

where x and y are the variables.

$$\Sigma d_x d_y = \Sigma xy - \frac{\Sigma x \Sigma y}{n};$$

$$\Sigma d_x^2 = \Sigma x^2 - \frac{(\Sigma x)^2}{n};$$

$$\Sigma d_y^2 = \Sigma y^2 - \frac{(\Sigma y)}{n}.$$

In correlation degree of freedom is n — 1, where n represents pairs of observations.

Example 1:

Ten plants have been assessed in sesame (Til) for plant height (cm) and number of branches per plant. From the given data do you consider that there exist correlation (significant) between the variables?

Solution:

No. of plants	*Plant height (cm) x variable*	*Branches per plant y variable*	x^2	y^2	xy
1	10	12	100	144	120
2	15	16	225	256	240
3	20	20	400	400	400
4	22	25	484	625	550
5	30	35	900	1225	1050
6	35	40	1225	1600	1400
7	40	45	1600	2025	1800
8	45	50	2025	2500	2250
9	50	52	2500	2704	2600
10	55	60	3025	3600	3300
$n=10$	$\Sigma x = 322$	$\Sigma y = 355$	$\Sigma x^2 = 12484$	$\Sigma y^2 = 15079$	$\Sigma xy = 13710$

$$r = \frac{\Sigma d_x \Sigma d_y}{\sqrt{\Sigma d_x^2 \Sigma d_y^2}}$$

$$\Sigma d_x d_y = \Sigma xy - \frac{\Sigma x \Sigma y}{n} = 13710 - \frac{322 \times 355}{10} = 2279.$$

$$\Sigma d_x^2 = \Sigma x^2 - \frac{(\Sigma x)^2}{n} = 12484 - \frac{(322)^2}{n} = 2115.6.$$

Inference: The calculated value 0.93 for 9 DF is higher than the tabulated value at 5%, 1% and 0.1% levels and it can be suggested that the two variables are positively and significantly correlated between them.

The calculated value 0.996 for 9 DF is higher than the tabulated value at 5%, 1% and 0.1% levels and hence it can be suggested that the two variables are positively and significantly correlated between them at 0.001 probability level.

The r-value can be represented as 0.996*** to show the level of significance.

Thus, selection of plants with enhance no. of primary branches will facilitate selection of plants with increase no. of umbels/plant.

How to prepare Correlation Table from Experimental Data:

Following data has been given

a. Plant height and number of primary branches/plant = 0.65

b. Plant height and total branches per plant = 0.57.

c. Height and number of capsules per plant = 0.81**.

d. Height and yield = 0.62.

e. Primary branches and total branches = 0.35.

f. Primary branches and capsules per plant = 0.80**.

g. Primary branches and yield = 0.87***.

h. Total branches and number of capsules = 0.52.

i. Total branches and yield = 0.43.

j. Capsules per plant and yield = 0.82**.

Attributes	*Plant height*	*Primary branches/ plant*	*Total branches per plant*	*Capsules per plant*	*Yield*
Plant height		0.65	0.57	0.81**	0.62
Primary branches per plant			0.35	0.80**	0.87***
Total branches per plant				0.52	0.43
Capsules/plant					0.82**

Inference:

Interrelationship between four yield related traits and their association with yield have been documented in tabular form. Result indicated positive and significant correlation between

height and capsules/plant (1% level), primary branches/plant and capsules/plant (1% level), primary branches and yield (0.1% level) and capsules/plant and yield (1% level).

Thus, plants having higher number of primary branches with enhanced capsule number should be the selection indices for higher yield in the plant species.

Partial Correlation:

X_1 and X_2 estimated by taking into account the effect of a 3rd variable X_3 and is denoted as $r_{12.3}$.

Partial correlation provides better relationship between the two variables X_1 and X_2 and is given by the formula:

$$r_{12.3} = \frac{(r_{12} - r_{13}r_{23})}{\sqrt{(1 - r_{13}^2)(1 - r_{23}^2)}}$$

r_{12}, r_{13} and r_{23} are the estimates of simple correlation coefficients between the variables X_1 and X_2, X_1 and X_3 and X_2 and X_3, respectively.

Multiple Correlation:

Estimate of joint influence of two or more variables on a dependent variable is called multiple correlation. Such an estimate helps in understanding the dependence of one variable, say x_1 on a set of independent variable say X_2, X_3.

$$R_{1.23}^2 = \frac{[(r_{12}^2 + r_{13}^2) - 2(r_{12} \cdot r_{13} \cdot r_{23})]}{(1 - r_{23}^2)}.$$

The square root of $R^2_{1.23}$ is the estimate of multiple correlation coefficient. $R^2_{1.23}$ is coefficient of determination.

Linear Regression Analysis:

The statistical analysis employed to find out the exact position of the straight line is known as Linear regression analysis. From simple correlation analysis if there exist relationship between independent variable x and dependent variable y then the relationship can be expressed in a mathematical form known as Regression equation.

From regression equation we can work out the actual value of y variable (dependent) based on X variable (independent) and such values plotted graphically will give precise nature of the straight line (point of interception to y-axis can be noted).

Simple regression equation Yx = a + bx, where a and b are constant which minimize the residual error of Y. Y is the dependent variable.

The constants a and b can be obtained from the formula:

$$b = \frac{\Sigma dxdy}{\Sigma dx^2}, \quad \text{where } \Sigma dxdy = \Sigma xy - \frac{\Sigma x \Sigma y}{n};$$

$$\Sigma dx^2 = \Sigma x^2 - \frac{(\Sigma x)^2}{n}.$$

$$a = \bar{y} - b\bar{x}.$$

Example:

From the data find out the regression equation and draw a regression line on the graph paper.

No. of Branches (x)	No. of Capsules (y)	x^2	xy
2	4	4	8
5	10	25	50
8	15	64	120
10	20	100	200
15	25	225	375
20	30	400	600
25	40	625	1000
$\Sigma x = 85$	$\Sigma y = 144$	$\Sigma x^2 = 1443$	$\Sigma xy = 2353$

Solution:

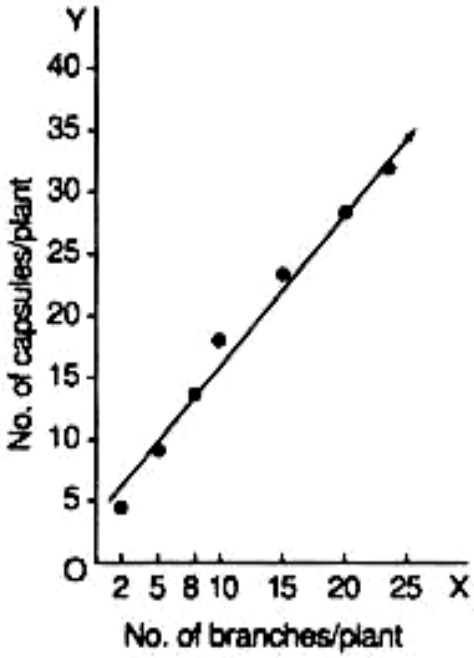
$$\bar{x} = \frac{85}{7} = 12.14; \quad \bar{y} = \frac{144}{7} = 20.57.$$

$$b = \frac{\Sigma xy - \frac{\Sigma x \Sigma y}{n}}{\Sigma x^2 - \frac{(\Sigma x)^2}{n}} = \frac{2353 - \frac{85 \times 144}{7}}{1443 - \frac{(85)^2}{7}} = 1.48.$$

$$a = \bar{y} - b\bar{x} = 20.57 - (1.48)(12.14) = 2.6.$$

Therefore, the regression equation $y_x = a + bx$, $y_x = 2.6 + 1.48x$.

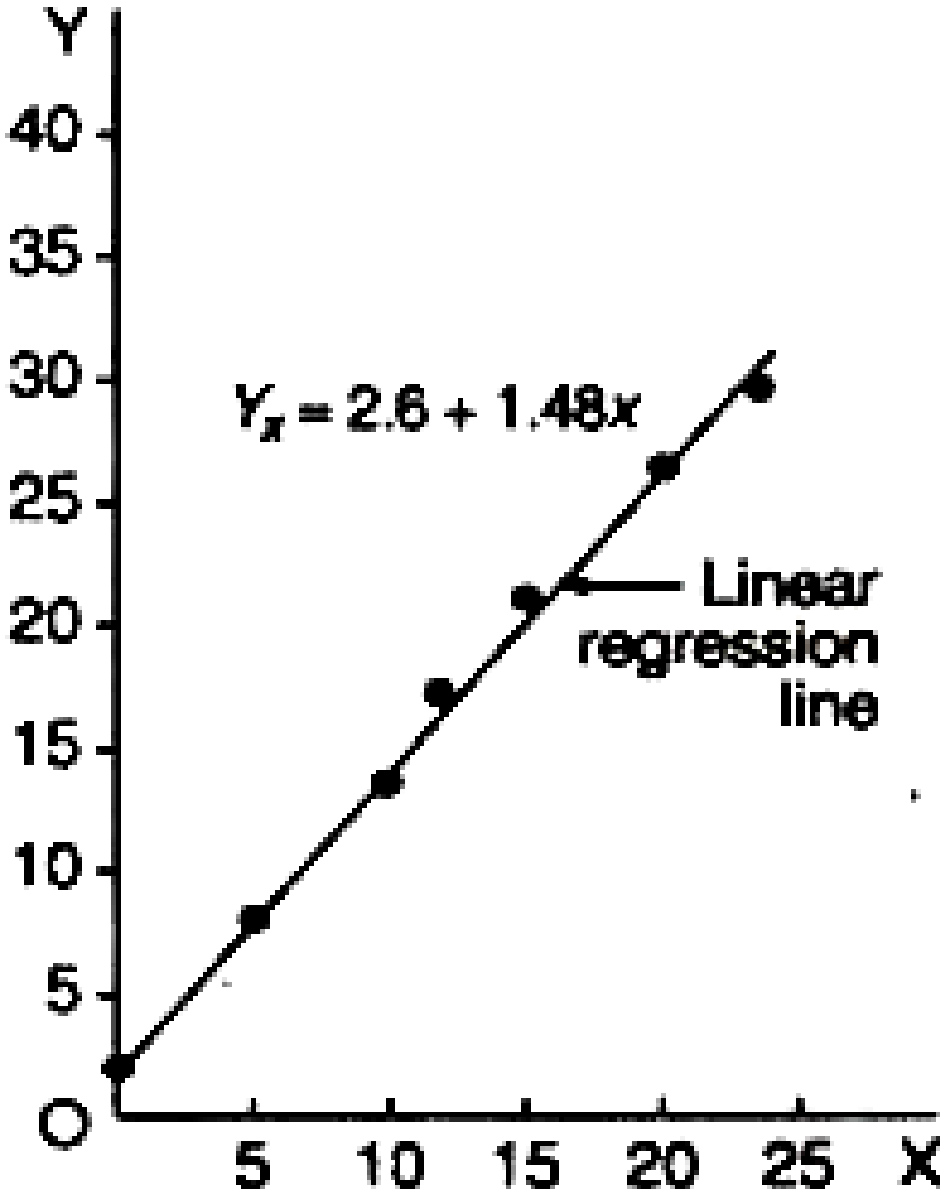
Using the regression equation $y_x = 2.6+1.48x$ the actual values of dependent variable can be worked out.



If $x = 0$, $y = 2.60$
$x = 2$, $y = 5.56$
$x = 5$, $y = 10.00$
$x = 8$, $y = 14.44$
$x = 10$, $y = 17.40$
$x = 15$, $y = 24.80$
$x = 20$, $y = 32.20$
$x = 25$, $y = 39.60$

Using data of the given example the straight line is drawn but the point of interception to y-axis is lacking and, therefore, precise nature of the straight line is not understood. However, from the straight line it is evident that the variables were significantly and positively correlated between themselves.

These set of values plotted graphically will give a straight and the precise nature of the straight line can be obtained from x = 0, y = 2.6 (point of interception to y- axis can be found out).



8. Let's sum up

- Plant breeding is the genetic improvement of the crop in order to create desired plant types that are better suited for cultivation, give better yields and are disease resistant.
- Plant introduction is a process of introducing plants from their own environment to a new environment. The central plant introduction agency in India is National Bureau of Plant Genetic Resources (NBPGR), which has its headquarters at New Delhi.

- In mass selection, a large number of plants having similar phenotype are selected and their seeds are mixed together to constitute a new variety whereas in pure line selection, The selected individual plants are grown in individual rows and evaluated and best progeny is selected, yield tested and released as a variety.
- Individual produced as a result of cross between two genetically different parents is known as hybrid. The natural or artificial process that results in the formation of hybrid is known as hybridization.
- Heterosis can be defined as the superiority of F1 hybrid over both the parents in terms of yield and some other character and inbreeding depression is the reduced biological fitness in a given population as a result of inbreeding.
- Then few plants are taken into account for studying the whole population of plant in that field. The method by which only few items are selected from the population in such a way so that they will represent the population in unbiased way is called sampling.
- Mean is the value which we get by dividing the aggregate of various items of the same series by the total number of observations. Mode is the Most frequent value in a series and median is the value of the middle item of a series when arranged in order of magnitude.
- In biological research when we compare any character of two samples, we calculate the significance of difference in the mean and variance to draw a meaningful conclusion.
- $\chi 2$ test is used for testing the agreement of observed frequencies with those expected upon a given hypothesis.
- The analysis of variance is based on F-statistics which is a ratio of variances. The ratio of variances due to treatments and variances due to random differences within the treatment is calculated and the estimate, known as the 'F', is then used for comparison.
- Association between variables or attributes or characteristics at a given time is known as correlation. The statistical analysis employed to find out the exact position of the straight line is known as Linear regression analysis.

9. Suggested Readings

1. Chaudhuri, H.K. Elementary Principles of Plant Breeding, Latest Ed., Oxford & IBH.
2. Singh, B.D. Plant Breeding, Principles & Methods (7th ed.), 2005, Kalyani Publishers.
3. Barley, N.T.J. Statistical Methods in Biology, Latest Ed., Cambridge University Press.
4. Roy, D. Plant Breeding: Analysis & Exploitation of Variation, 2000, Narosa Publishing
5. Kar, D.K. and Halder, S. Plant Breeding & Biometry, 2006, New Central Book Agency.
6. Datta, Animesh K. Basic Biostatistics & its Application 2006, New Central Book Agency.
7. http://www.biologydiscussion.com/
8. https://en.wikipedia.org/wiki/Plant_breeding

10. Assignments

1. What is meant by sample?
2. What is normal curve?
3. Short note on a) CV, b) Standard error, c) ANOVA, d) Gene for gene hypothesis.
4. Write the differences between pureline selection and mass selection.
5. Describe the merits and Demirits of mean, median and mode.
6. Why CD is important?
7. How can you differentiate Student's t test from F-test?
8. What does 't-test' signify?
9. Mention the equation for simple regression analysis.
10. How can you calculate CD from ANOVA table?
11. What are the measures of dispersion?

12. How can you calculate mode from grouped data with regular class interval?
13. What is mean shift?
14. What is the genetical significance of χ^2-test analysis?
15. Mention the names of test of significance.
16. What is ‘r’ and how it varies?
17. State two applications of student ‘t’ test.
18. What is degree of freedom?
19. Write notes on the following:
    i. Probability
    ii. Paired t-test
    iii. Phenotypic correlation analysis

All the materials are self writing and collected from e-book, journals and web sites

POST GRADUATE DEGREE PROGRAMME
(CBCS)
IN
BOTANY

SEMESTER – II

Course: BOTGEC T
(Plants in Human Welfare)

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING
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University of Kalyani, Kalyani-741235, West Bengal and

Director's Message

Satisfying the varied needs of distance learners, overcoming the obstacle of distance and reaching the unreached students are the threefold functions catered by Open and Distance Learning (ODL) systems. The onus lies on writers, editors, production professionals and other personnel involved in the process to overcome the challenges inherent to curriculum design and production of relevant Self Learning Materials (SLMs). At the University of Kalyani a dedicated team under the able guidance of the Hon'ble Vice-Chancellor has invested its best efforts, professionally and in keeping with the demands of Post Graduate CBCS Programmes in Distance Mode to devise a self-sufficient curriculum for each course offered by the Directorate of Open and Distance Learning (DODL), University of Kalyani.

Development of printed SLMs for students admitted to the DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode DEB Regulations, 2020 had been our endeavour. We are happy to have achieved our goal.

Utmost care and precision have been ensured in the development of the SLMs, making them useful to the learners, besides avoiding errors as far as practicable. Further suggestions from the stakeholders in this would be welcome.

During the production-process of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Manas Kumar Sanyal, Hon'ble Vice- Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticism to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

Sincere gratitude is due to the respective chairpersons as well as each and every member of PGBOS (DODL), University of Kalyani. Heartfelt thanks are also due to the Course Writers-faculty members at the DODL, subject-experts serving at University Post Graduate departments and also to the authors and academicians whose academic contributions have enriched the SLMs. We humbly acknowledge their valuable academic contributions. I would especially like to convey gratitude to all other University dignitaries and personnel involved either at the conceptual or operational level of the DODL of University of Kalyani.

Their persistent and co-ordinated efforts have resulted in the compilation of comprehensive, learner-friendly, flexible texts that meet the curriculum requirements of the Post Graduate Programme through Distance Mode.

All the Self Learning Materials are-self writing and collected from e-book, journals and websites.

Director
Directorate of Open and Distance Learning
University of Kalyani

SYLLABUS
COURSE–BOTGEC T
Plants in Human Welfare

(Full Marks–50) **Credit: 4**

Course	Group	Details Contents Structure		Study hour
BOTGEC T	Plants in Human Welfare	**Exploitation of Microorganisms and their Products:**	**Unit1.** Health care products (antibiotics, interferons, vaccines, hormones, vitamins etc.); enzymes (amylase, protease); biofertilizers; food & dairy products.	**1**
			Unit2. Microbial production of organic acids (citric acid, acetic acid); amino acids (glutamic acid, lysine).	**1**
			Unit 3. Microbial production of alcoholic beverages (bear & wine); biofuels (ethanol, methane, biogas, biohydrogen).	**1**
			Unit 4. Microbes in biological warfare; microbial leaching (copper, uranium); role in biosorption, biotransformation of xenobiotics; microorganisms in the recovery of precious metals.	**1**
			Unit 5. Microbes in composting and biopesticide formulation; microbes in bioremediation & biopolymer production; microbes in single cell proteins & single cell oil, Microbial Enhanced Oil Recovery (MEOR).	**1**
		Fungi:	**Unit 6.** General & unique characters, nutrition, thallus structure, spores, basic idea of different groups; Mushrooms: basidiocarp, ascocarp.	**1**
			Unit 7. Macroscopic & microscopic features, Cultivation procedure of edible mushrooms; beneficial & harmful fungi.	**1**
		Algae:	**Unit 8.** Understanding algae as a plant group; Societal issues involving algae.	**1**
		Bryophyte, Pteridophyte & Gymnosperm:	**Unit 9.** Brief introduction of the plant groups and evolutionary importance, economy and livelihood of the modern-day people with the representative taxa of Bryophyte, Pteridophyte.	**1**
			Unit 10. Brief introduction of the plant groups and evolutionary importance, economy and livelihood of the modern-day people with the representative taxa of Gymnosperm.	**1**
		Plant and Medicine:	**Unit 11.** Introduction, source and medicinal uses of the following plant-derived pharmaceutical compounds - (artemisinin, aspirin, atropine, campothecin, cannabadiol, ephedrine, digoxin, diosgenin, galanthamine,	**1**

			Unit 12. Introduction, source and medicinal uses of the following plant-derived pharmaceutical compounds - L-dopa, morphine, codeine, quinine, colchicine, vincristine, vinblastine, podophyllotoxin, taxol). Importance of phytopharmaceuticals	**1**
			Unit 13. Classification of plant secondary metabolites; Exploration of secondary metabolites in therapeutics.	**1**
		Plant Tissue Culture:	**Unit 14.** Concept of cellular totipotency; culture media; organogenesis;	**1**
			Unit 15. Somatic embryogenesis; haploid plant production and micropropagation.	**1**
		Genetically Modified Crops:	**Unit 16.** Recombinant DNA technology and its use in crop improvement.	**1**
		Plants in Forensic Investigation:	**Unit 17.** Introduction; use of different branches of Botany (Palynology; Plant Anatomy including Dendrochronology in forensic investigation.	**1**
			Unit 18. Introduction; use of different branches of Botany Ecology; Limnology; Plant systematic & Taxonomy in forensic investigation.	**1**
			Unit 19. Introduction; use of different branches of Botany Molecular Biology; Plant Biotechnology; Bioinformatics etc in forensic investigation.	**1**
		Intellectual Property Rights (IPR):	**Unit 20.** Definition; Differentiating between Intellectual Property and Physical Property; Types of Intellectual Property Rights; Importance of IPR.	**1**

Content

COURSE – BOTGEC T
Plants in Human Welfare

Theory Paper **Credit: 4**

Content Structure

1. Introduction

Biology is the youngest of the formalized disciplines of natural science. Progress in physics and chemistry proceeded much faster than in Biology. Applications of physics and chemistry in our daily life also have a higher visibility than those of biology. However, twentieth century and certainly twenty-first centuries has demonstrated the utility of biological knowledge in furthering human welfare, be it in health sector or agriculture. The discovery of antibiotics, and synthetic plant -derived drugs, anesthetics have changed medical practice on one hand and human health on the other hand. Life expectancy of human beings has dramatically changed over the years. Agricultural practices, food processing and diagnostics have brought socio -cultural changes in human communities.

2. Course Objectives

After completion of the course the learners will be able to:

- describe the Mushroom cultivation process and their uses;
- explain the Characteristics of different plant groups;
- discuss the role of plant in forensic science;
- explain the concepts of genetically modified crops;
- Identify IUCN categories plant.

3. Exploitation of Microorganisms and their Products:

Health care products (antibiotics, interferons, vaccines, hormones, vitamins etc.); enzymes (amylase, protease); organic acids (citric acid, acetic acid); amino acids (glutamic acid, lysine); alcoholic beverages (bear & wine); biofuels (ethanol, methane, biogas, biohydrogen); biofertilizers; food & dairy products; microbes in biological warfare; microbial leaching (copper, uranium); role in biosorption, biotransformation of xenobiotics; microorganisms in the recovery of precious metals; microbes in composting and biopesticide formulation; microbes in bioremediation & biopolymer production; microbes in single cell proteins & single cell oil, Microbial Enhanced Oil Recovery (MEOR).

1. Health care products (antibiotics, interferons, vaccines, hormones, vitamins etc.)

1.1 Antibiotics

Antibiotics are the secondary metabolites of one organism which inhibit the growth of other organisms at very low concentrations. They can be obtained either from natural sources, viz, microbes, or can be synthesized chemically. They are used widely in different fields like medicine, veterinary, agriculture, etc.

The first antibiotic, penicillin, was discovered by Alexander Fleming in 1929, when he was working at St. Mary's Hospital, London. He observed the inhibition of growth of *Staphylococcus aureus* in one of the Petri plates by a contaminating microorganism which was later identified as *Penicillium notatum*. The inhibition of growth of the microbe happened due to the secretion of a chemical by the mold. The chemical was named the penicillin for which Alexander Fleming and Chain, a biochemist, shared the noble prize in 1941. During World War II the demand for penicillin to treat wound infections led to the development of a production process for penicillin and the beginning of the era of antibiotic research, which is known as to the golden era of industrial microbiology. Since the discovery of penicillin, thousands of different antibiotics produced by different groups of microbes like fungi, actinomycetes and bacteria have been isolated and identified.

Table: Number of antibiotics produced by different groups of microorganisms:

Taxonomic Group	Number of antibiotics
Fungi	1600
Bacteria (other than actinomycetes)	950
Actinomycetes	4600

Out of them, only 123 have been currently produced by fermentation. In addition, more than 50 antibiotics are produced as semi-synthetic compounds; three antibiotics chloramphenicol, phosphonomycin, and pyrrolnitrin are produced completely synthetically.

Classification of antibiotics: Although antibiotics are classified according to their antimicrobial spectrum, mechanism of action and producer organism, and manner of biosynthesis, they differ in their molecular weight, chemical structure, elemental composition, and physicochemical characteristics. These properties are also taken into consideration for the classification of antibiotics. Based on the similarity in their chemical structure Berdy et al., 1985 classified antibiotics into:

1. Aminoglycoside antibiotics: They contain amino sugars linked together by glycoside linkage. Important antibiotics belonging to the group include streptomycin, neomycin, kanamycin, paromomycin, gentamycin, tobramycin, and amikacin.

2. Antifungal antibiotics: They include two different categories of antibiotics:

(i) Polyenes with a large ring containing a conjugated double bond system. Nystatin and amphotericin B are the

important antibiotics belonging to this category. (ii) Other antifungal antibiotics include 5-fluorocytosine, clotrimazole, and griseofulvin.

3. Chloramphenicol: It forms a separate group by itself and contains nitrobenzene derivative of dichloroacetic acid.

4. Macrolide antibiotics: They contain a macrocyclic lactone ring to which sugars are bonded. The important antibiotics of this group are spiramycin, oleandomycin, and erythromycin.

5. B-lactam antibiotics: They have an ß-lactam ring in their chemical structure. The natural penicillin, the semisynthetic penicillins, and cephalosporins belong to this group.

6. Peptide antibiotics: They are made up of peptide-linked amino acids of both dextro and laevo forms. These include bacitracin, niacin, gramicidin, and polymyxin.

7. Tetracycline (quinines) antibiotics: They are the derivatives of the polycyclic naphthacene carboxamide. The important antibiotics include tetracycline, chlorite tetracycline, oxytetracycline, demeclocycline, and minocycline.

8. Unclassified antibiotics: They differ widely in their chemical structure and, therefore, are not grouped in the above-described major groups. The important antibiotics include cycloserine, fusidic acid (steroid), novobiocin, prosinomycin, spectinomycin, and Vancomycin.

Antibiotics are also classified based on the target organism.

1. Antibacterial - if they are capable of inhibiting bacteria.
2. Antifungal - if they are active against fungi.
3. Antiprotozoan - If they are active against protozoa.

Mechanism of action: Though a very large number of antibiotics have been discovered, less than 1% have been of practical value in medicine. However, the useful antibiotics have made a dramatic impact on the treatment of infectious diseases. Further, many antibiotics are made more effective by chemical modifications in the laboratory, these are called semisynthetic antibiotics. Some antibiotics can stop only growth and are incapable of killing the organism. Such antibiotics are called static (bacteriostatic or fungistatic), while the antibiotics which kill organisms are called bactericidal or fungicidal.

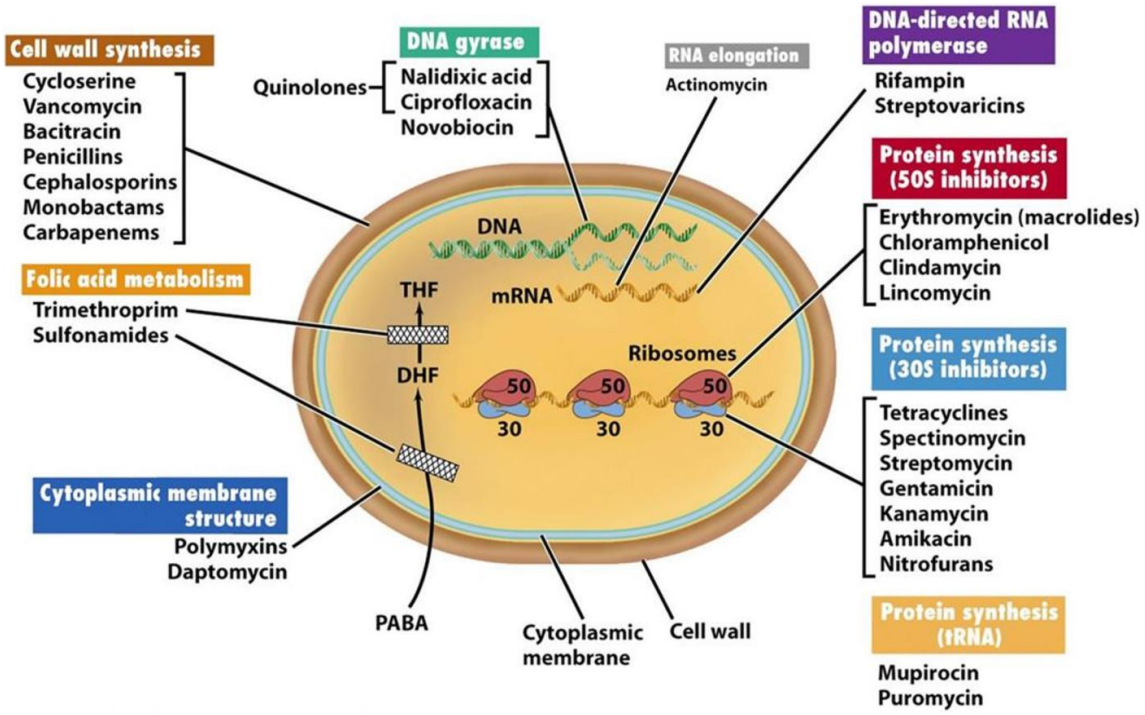


Fig: Mode of actions of different antibiotics.

The sensitivity of microorganisms to antibiotics and other chemotherapeutic agents varies. Gram (+) positive bacteria

are usually more sensitive to antibiotics than gram (-) negative bacteria, although some antibiotics act as only one gram (-) negative bacteria. An antibiotic that acts on both gram (+) positive and gram (-) negative bacteria is called a broad-spectrum antibiotic, which generally finds wider medical use than a narrow-spectrum antibiotic, which acts only on a single group of organisms, either gram (+) ve or gram (-) ve. An antibiotic with a limited spectrum of activity may, however, be quite valuable for the control of specific microorganisms that fail to respond to other antibiotics. For instance, vancomycin, a glycopeptide, is a bactericidal agent that acts against gram (+) positive bacteria such as Staphylococcus, Bacillus, and Clostridium.

Antibiotics and other chemotherapeutic agents are classified based on their mode of action into five types.

Table: Classification of antibiotics based on mode of action

Process inhibited	Antibiotic
Cell wall inhibition	Cycloserine Vancomycin Bacitracin Penicillins (β-lactams) Cephalosporins Monobactam Carbapeneme
Cytoplasmic membrane	Polymyxins
Protein synthesis	Tetracycline Spectinomycin Streptomycin Gentamycin Tobramycin Kanamycin Amikacin Nitrofuran Mupirocin Puromycin
Nucleic acid synthesis	Nalidixic acid Norfloxacin Novobiocin Rifampicin
Folic acid metabolism	Trimethoprim Sulfonamides

Out of these antibiotics, fermentative production of penicillin, cephalosporins, tetracyclines, erythromycin, streptomycin, bacitracin, chloramphenicol, fusaric acid, interferon, and nisin are described.

Penicillin

Chemically the natural penicillin is 6-amino penicillanic acid (6 APA), which consists of a thiazolidine ring with a condensed B-lactam ring. The various penicillins differ primarily in. the nature of the R-side chain which is attached by an amide linkage to the chemical nucleus of the molecule. Fleming's original *Penicillium notatum* strain, when grown on his medium produced penicillin-F, which is known as 2-pentenyl penicillin. Subsequently, P. chrysogenum

proved to be a better fungus and more suitable for submerged fermentation.

If penicillin fermentation is carried out without the addition of a side-chain precursor, the natural penicillins are formed from which only benzylpenicillin can be isolated. However, the desired penicillin can be obtained by adding a suitable side-chain precursor into the medium. Such penicillins are called semi-synthetic penicillins. Penicillin-G and Penicillin-V are generally produced commercially. When compared to natural penicillins, semisynthetic penicillins have improved characteristics *viz*., acid stability, resistance to plasmid or chromosomally coded B-lactamases, expanded antimicrobial effectiveness, and are, therefore, extensively used in therapy.

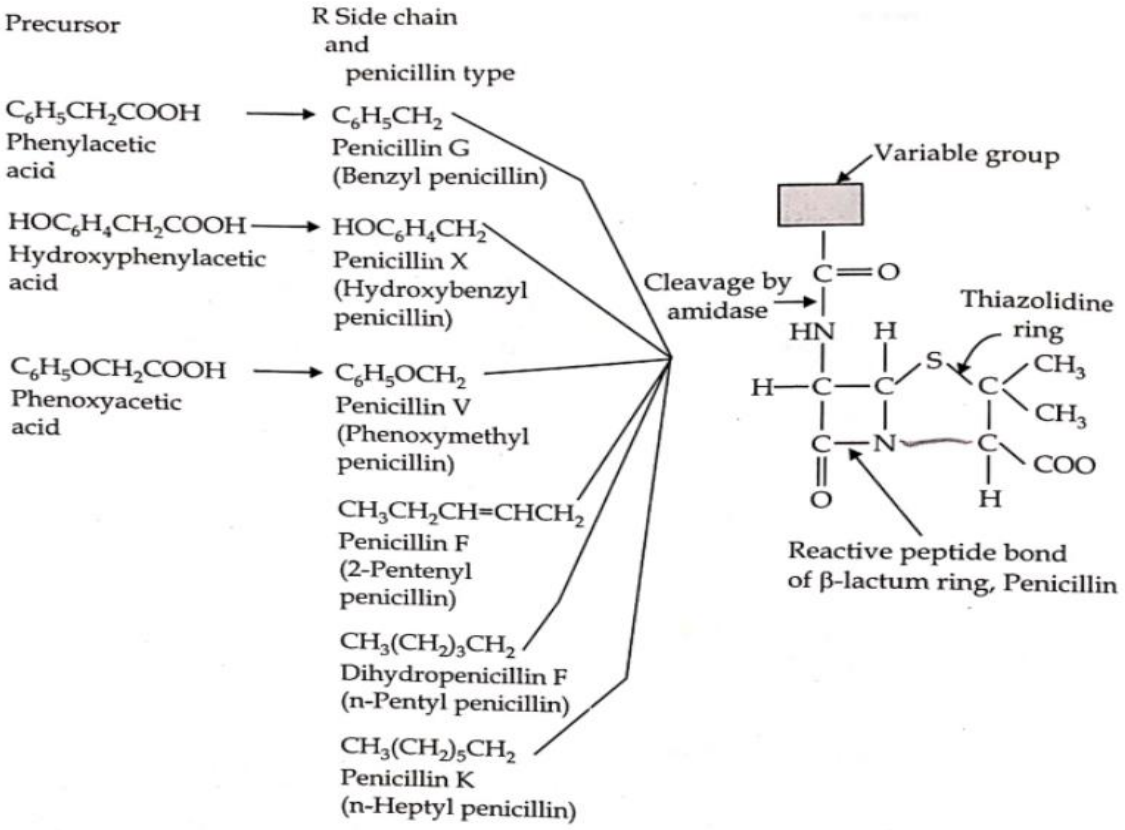


Fig: Different penicillin with precursor and R side chain

Biosynthesis of penicillin: The B-lactam thiazolidine ring of penicillin is formed by the condensation of L-cystine and L-valine. The biosynthesis occurs in a non-ribosomal process using dipeptide composed of (a-a-AAA) and a-cystine or a breakdown product of cystathionine. Subsequently, L_{v} valine is connected via epimerization reaction resulting in the formation of the tripeptide. The first product of cyclization of the tripeptide which can be isolated is penicillin N but the biochemical reactions leading to intermediate are not understood. Benzylpenicillin is produced in exchange for a - a - AAA with the activated phenylacetic acid.

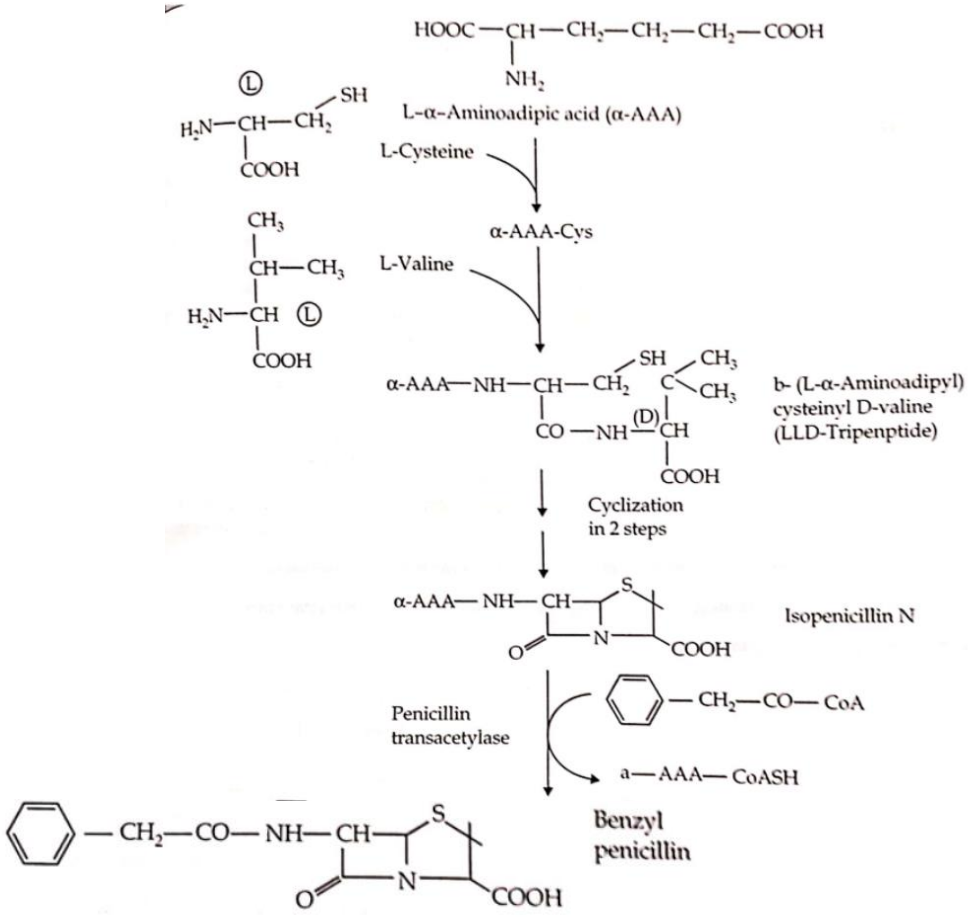


Figure: Biosynthesis of penicillin.

About 38% of the penicillin produced commercially is used in human medicine. 12% in veterinary medicine and 43% as starting materials for the production of semi-synthetic penicillin.

Fermentation: Penicillin fermentation is an aerobic process with a volumetric oxygen absorption rate of .4-0.8mm min^ - The required aeration rate varies according to the strain, the type of fermenter used, and the impeller system. However, the aeration rate varies between 0.5 and 1.0 vvm. It is produced by a fed-batch submerged fermentation in a stirred tank fermenter. This process can be described under the following headings.

1. Strain development,
2. Inoculum production,
3. Inoculation,
4. Penicillin production
5. Extraction and purification

Strain development: The variety of molds that yield a greater amount of penicillin is called a high-yielding strain. They are generally developed from the wild *P. chrysogenum* by a process called sequential genetic selection. This process consists of stepwise development of improved mutant by treating the wild strain of *P. chrysogenum* with a series of mutagenic agents or exposing to ultraviolet radiation either individually or in combination, such as X-rays and chemical mutagens, which is called strain improvement. Strain development is a laborious and time-consuming process. The selected mutant possesses a greater capacity for antibiotic production than the wild type. Details of some high-yielding strains which are developed from wild *P. chrysogenum* (NRRL 1951) are shown in the table.

The expanded role for penicillins came from the discovery that different biosynthetic penicillins can be formed by the addition of side-chain precursors to the fermentation medium and that natural penicillins can be modified chemically

to produce penicillins with improved characteristics. Most penicillins are now semisynthetically produced by chemical modification of natural penicillin obtained by fermentation using strains of P chrysogenum. Modification is achieved by removing their natural acyl group, leaving 6 APA to which other acyl groups can be added to confer new properties. This is achieved by passage through a column of immobilized penicillin acylase usually obtained from E. coli at neutral pH. Penicillin G for example converted to 6-APA and phenylacetic acid. The 6-APA is then ethically acylated with an appropriate side chain to produce semi-synthetic penicillin. Hetacillin, bacampicillin, epicillin, pivampicillin, and talampicillin are converted to ampicillin in the body. These penicillins exhibit various improvements including resistance to stomach acids to allow oral administration, to penicillinase, and an extended range of activity against gram (+) positive bacteria.

Table: Significant stages in strain improvement program in *P.chrysogenum*

Strain	Method	Penicillin (mg ml^{-1})	Characteristics
P. notatum (Fleming)	**P. chrysogenum**	3	
NRRL 1951	Original isolate	60	Yellow pigment
NRRL 1951.B25	Selection	125	Yellow pigment
X-1612	X rays	300	Yellow pigment
WIS Q 176	UV-light	800–1000	Yellow pigment
B 13-D10	UV-light	-	Pigment free
WIS 47-638	Selection	-	Pigment free
WIS 47-1564	Selection	800–1000	Pigment free, 95-100% G*
WIS 48-701	Selection	-	Pigment free, 95-100% G*
WIS 49-133	Nitrogen mustard gas	1500–3000	Pigment free, 95-100% G*
WIS 49-2166	Nitrogen mustard gas	1500–3000	Pigment free, 95-100% G*
WIS 50-535	Nitrogen mustard gas	1500–00	Pigment free, 95-100% G*
WIS 51-20	Selection	2000–3000	Pigment free, 95-100% G*
EIS.1	NM & Selection	7000	Pigment free, Penicillin G

It has been reported that most of the high-yielding strains of *P. chrysogenum* are genetically unstable. Genetic instability increases with the increase in the yield. However, it can be controlled to some extent by following suitable preservation methods. The following preservation methods are generally adopted for storing high yielding strains of *P. chrysogenum*. A spore suspension is stored in a frozen state under liquid nitrogen.

2. A spore suspension can be lyophilized in an appropriate medium.

3. A spore suspension is mixed with a sterile finely divided inert material like soil or sand and desiccated.Inoculum production: The microorganism which is used in fermentation is called the inoculum. A high yielding strain of *P. chrysogenum* is generally employed as inoculum. process is a strain of the fungus is subcultured from stock culture for inoculum development. Spores from the primary source are suspended in water or a dilute solution of a nontoxic wetting agent such as 1:10000 sodium lauryl sulfate. The spores are then added to flasks or bottles of wheat bran plus nutrient solution and these are incubated for five to seven days at 24 degrees C to provide heavy sporulation. The entire process is repeated several times to have more sporulation.

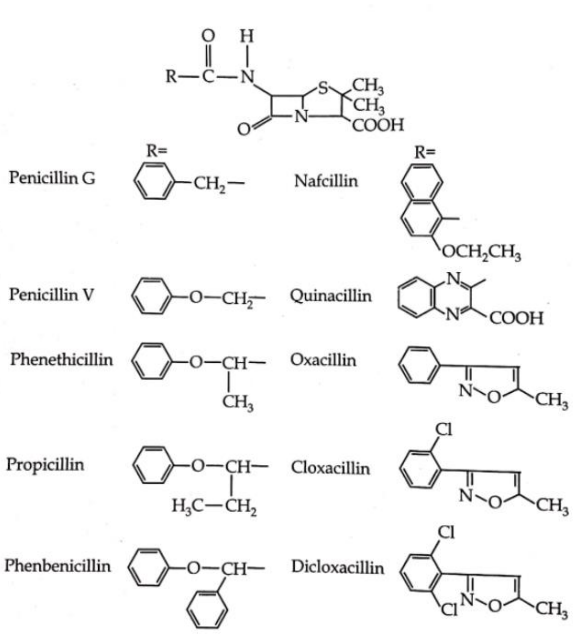


Fig: Structure of some clinically useful penicillin.

The resulting spores are used directly to inoculate inoculum tanks or stirred fermenters. The incubation temperature is maintained at 24-27^ (for 2 days with agitation and aeration to facilitate heavy mycelial growth, which may be added to a second or even a third stage fermentation. The resulting inoculum which is employed in a production tank is tested both by microscopic examination and by the subculturing method. Many sporulation media have been designed to obtain a large number of spores. The one developed by Moyer and Coghill (1946) is most extensively used and is given below table.

Inoculation: Introduction of pure inoculum into the production tanks or fermenters is called inoculation. This is done by any one of the following three methods.

Table: characteristic of clinically used semi-synthetic Penicillins.

Name	Method of preparation	Specific activity against		Acid stability	Resistance to staphylococcal penicillinase
		Gram (+) cocci	Gram (–) rods		
Amoxicillin	Semi synthetic	+	+	+	Low
Ampicillin	Semi synthetic	+	+	+	Low
Azidocillin	Semi synthetic	+	+	+	Low
Azolocillin	Semi synthetic	+	Some	+	Low
Bacampicillin	Semi synthetic	+	+	+	Low
Carbenicillin	Semi synthetic	+	Some	-	Low
Cloxacillin	Semi synthetic	+	Nil	+	High
Cyclacillin	Semi synthetic	+	+	-	High
Dicloxacillin	Semi synthetic	+	Nil	+	High
Epicillin	Semi synthetic	+	+	++	Low
Flucloxacillin	Semi synthetic	+	Nil	+	High
Hetacillin	Semi synthetic	+	+	+	Low
Mecillinam	Semi synthetic	+	+	+	High
Metamacillin	Semi synthetic	+	+	+	Low
Methicillin	Semi synthetic	+	-	+	High
Mezlocillin	Semi synthetic	+	+	+	Low
Nafcillin	Semi synthetic	+	Nil	+	High
Oxacillin	Semi synthetic	+	Nil	+	High
Penicillin G	Natural	+	Nil	–	Nil
Penicillin V	Natural	+	Nil	+	Nil
Phenethicillin	Semi synthetic	+	Nil	+	Nil
Pirbenicillin	Semi synthetic	+	+	+	Medium
Pivampicillin	Semi synthetic	+	+	+	Low
Pivmecillinam	Semi synthetic	+	+	+	High
Propicillin	Semi synthetic	+	Nil	+	Low
Sulbenicillin	Semi synthetic	+	Some	+	Low

1. Dry spores may be used as inoculum: Since the spores of *P. chrysogenum* are hydrophobic, either spores are

blown deep into the medium or a wetting agent such as sodium lauryl sulfate is used.

2. Suspension of ungerminated spores: This suspension is made by using 1:10000 sodium lauryl sulfate solution. This suspension is fed to the fermenter by suitable techniques like spray guns or pipettes. This is followed by agitation and aeration of the fermentation medium to achieve equal and uniform distribution of the spores in the entire medium.

Table: Composition of Moyer and Coghill 1946 sporulation medium

Component	Concentrating (glt^{-1})
Glycerol	7.5
Cane molasses	7.5
Corn steep liquor	2.5
Mg SO_4. $7H_2O$	2.5
KH_2PO_4	0.050
Peptone	0.060
NaCl	5.00
Fe-tartarate	0.005
$CuSO_4.5H_2O$	0.004
Agar	2.50
Distilled water	1.0

3. Feeding the fermentation tanks with pre-germinated spores or mycelial pellets which are prepared by the germination of spores. Pellets are generally fed to the fermentation medium after two or three days of spore inoculation.

Fermenters with a capacity of 40,000 to 2 lakhs liters are generally employed for the production of penicillin. Due to difficulties with the oxygen supply larger tanks are not employed. Some manufacturers use Walsh of fermenters or airlift fermenters, but this is only possible in mutants that generate low viscosity. Depending upon the production strain, the operational temperature is maintained between 25°-27°C.

Medium: The medium employed for penicillin production should be suitable to achieve

1. An abundant growth of the mycelium.
2. Maximum accumulation of the antibiotic.
3. Easy and inexpensive extraction and purification of the antibiotic. Carbon source is generally supplied in the form of lactose. Glucose, sucrose, glycerol, and sorbitol can also be employed as carbon sources. A nitrogen source is generally supplied in the form of ammonium sulfate or

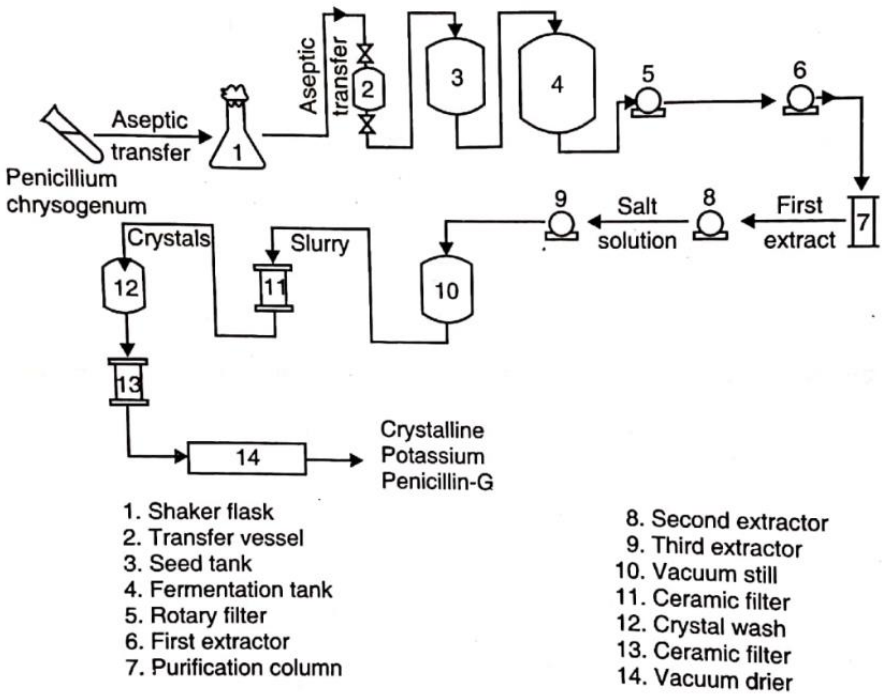


Fig: Flow sheet for large-scale production of Penicillin

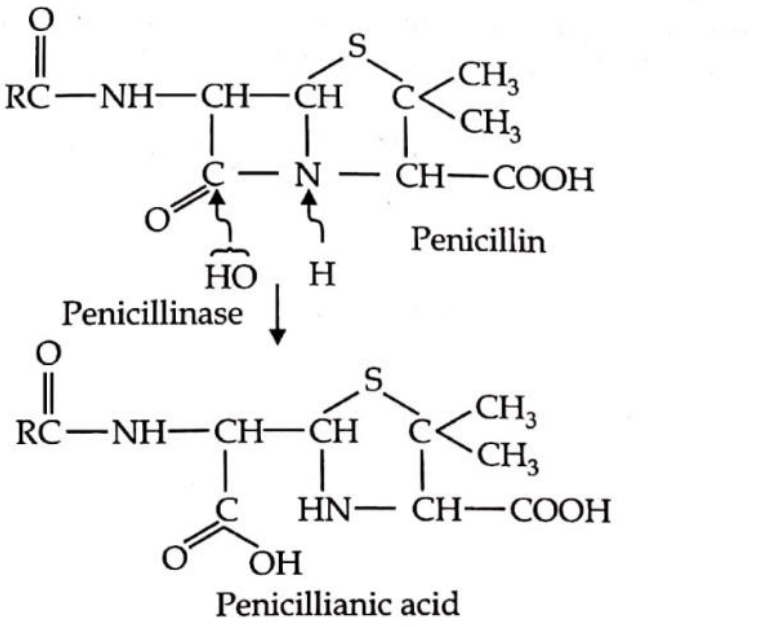
ammonium acetate or ammonium nitrate. Abundant formation of mycelium and spores takes place when a medium contains corn-steep liquor because it contains important amino acids required for mycelial growth. Potassium, phosphorus, magnesium, sulfur, zinc, and copper are supplied in the form of salts. Potassium and phosphorus are supplied in the form of potassium dihydrogen phosphate, and magnesium, iron, and copper are supplied in the form of sulfates. All these elements may be present in corn steep liquor. Penicillin-F and penicillin-K are the naturally produced penicillins synthesized by *P. notatum* and P. chrysogenum, respectively, in the absence of a precursor. But, if phenylacetic acid is supplied in the medium *P. chrysogenum* produces penicillin-G instead of penicillin-K. Similarly, desired Penicillin yields with time are linear from approximately 48 to 96 hours. The final penicillin yield is in the range of 3 to 5% which largely depends upon the amount of carbohydrate consumed during the fermentation process, which is approximately equal to 1500 international units per millilitre. Sylvester and Coghill (1954) have estimated that to produce 1000 gallons of fermented culture, which is capable of yielding 2.2-2.7 kg of penicillin by the submerged culture method requires approximately 227 kg of nutrients, 3400 kg of steam, 45460 It of water, 1000 kWh of electricity and 7075 m^3 of air.

Table: Composition of Jacksons 1958medium.

Component	Concentration (In Percentage)
Corn steep liquor	3.5
Lactose	3.5
Glucose	1.0
Calcium carbonate	1.0
Potassium dihydrogen phosphate	0.4
Edible oil	0.25

Penicillin easily gets carboxylate to form penicillin acid which is biologically inactive by the action of the enzyme penicillinase. The enzyme penicillinase is widely distributed among different microorganisms. These organisms may enter the fermenter at any stage and may convert penicillin into penicillin acid Thus, in penicillin fermentation and contamination is the main constraint. Hence, one has to be careful in preventing contamination. This was one of the main problems during the early times of penicillin production when fermentation was carried out in bottles and contamination in one bottle may destroy penicillin in the entire batch of bottles.

In the typical penicillin fermentation, there is a growth of 10 hrs duration with a doubling time of 6 hrs during which the greater part of the cell mass is formed. The oxygen supply in the growing culture is critical since the increasing viscosity hinders oxygen transfer. After the growth phase, the culture proceeds to actual penicillin production. The growth is sharply reduced by feeding with various culture medium components. The production phase can be extended to 120 180 hrs. Penicillin production by continuous fermentation has been attempted but it has been difficult due to the instability of the production strains. A batch fill and draw system has been suggested as an alternative. In this process, 20-40% of the fermentation contents are drawn off and replaced with a fresh nutrient solution. This process may be repeated up to 10 without affecting yield.



The carboxylation of penicillin by the action of penicillinase

iii) Extraction and purification: After it is assessed that a sufficient amount of penicillin has been produced during the fermentation process, it is extracted and then purified. The entire process is carried out in three different stages. They are:

(a) Separation of mycelium

(b) Extraction of penicillin and

(c) Treatment of crude extract

(a) Separation of mycelium: Mycelium is separated from the medium by employing a rotatory vacuum filter. This process should be performed carefully to avoid contaminating microorganisms that produce penicillinase enzyme, degrading the penicillin.

(b) Extraction of penicillin: The penicillin is excreted into the medium and less than 1% remains as mycelium bound. Extraction of penicillin is carried out by employing the counter-current extraction method. The pH of the

liquid after the separation of the mycelium is adjusted to 2.0 to 2.5 by adding phosphoric or sulphuric acid. This treatment converts penicillin into an anionic form. The liquid is immediately extracted with an organic solvent such as amyl acetate or butylacetate or methyl isobutyl ketone. This step has to be carried out quickly because penicillin is quite unstable at low pH values. Podbielniak counter-current extractor is used for this purpose. The penicillin is then back-extracted into the water from the organic solvent by adding enough potassium or sodium hydroxide which also results in the elevation of pH to 7.0 to 7.5. The resulting aqueous solution is again acidified and re-extracted with an organic solvent. These shifts between the water and the solvent help in the purification of penicillin. Finally, penicillin is obtained in the form of sodium penicillin. The spent solvent is recovered by distillation for reuse.

(c) Treatment of crude extract: The resulted sodium penicillin is treated with charcoal to remove pyrogens (fever-causing substances). It is also, sometimes, sterilized to remove bacteria by using a Seitz filter. Then, the sodium penicillin is prepared in crystalline form by crystallization. It may be packed powder in sterile vials or prepared in the form of tablets or the form of syrups for oral usage. The pharmaceutical grade may be used in the production of semi-synthetic penicillin.

Most penicillin are active against Gram-positive bacteria, the cell wall synthesis leading to the death of bacteria. which they inhibit 2. Used therapeutically in the treatment of infectious diseases of humans caused by Gram (+) positive bacteria,

1.2 Vaccine

Vaccine (L. Vacca = cow) is a preparation/suspension or extract of dead/attenuated (weakened) germs of a disease which on inoculation (injection) into a healthy person pro-vides temporary/permanent active/passive immunity by inducing antibodies formation. Thus antibody provoking agents are called vaccines.

The principle of immunisation or vaccination is based on the property of 'memory' of the immune systems. Vaccines also generate memory-B and T cells that recognize the pathogen quickly. In snake bites, the injection which is given to the patients contains pre-formed antibodies against the snake venom. This type of immunisation is called passive immunization. The process of introduction of vaccine into an individual to provide protection against disease is called vaccination. In vaccination, a preparation of antigenic proteins of pathogens or inactivated/weakened pathogens (vaccine), is introduced into the body.

These antigens generate the primary immune response, and the memory B and T cells. When the vaccinated person is attacked by the same pathogen, the existing memory T or B cells recognize the antigen quickly and attack the invaders with a massive production of lympho-cytes and antibodies.

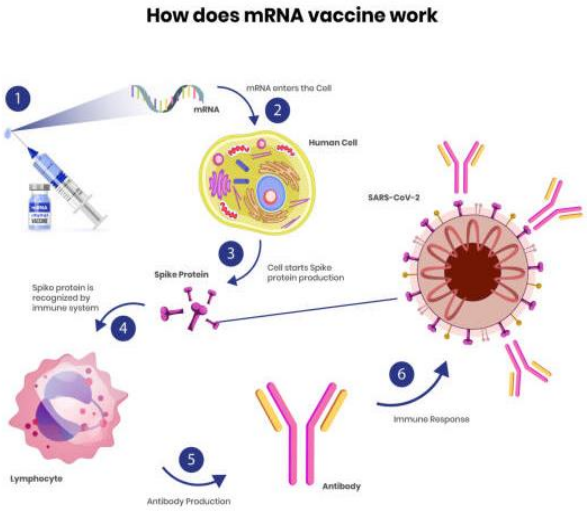


Fig: RNA vaccine work

Vaccination and immunisation are two different processes. Vaccination only refers to the administration of a vaccine

or toxoid, while immunisation is the process by which the body produces antibodies against the vaccine-preventable diseases through the administration of spe-cific vaccines. These are used to protect us and our domestic animals against viral and bacterial diseases.

Toxoid is a modified bacterial toxin that has been made nontoxic but retains the capacity to stimulate the formation of antitoxin.

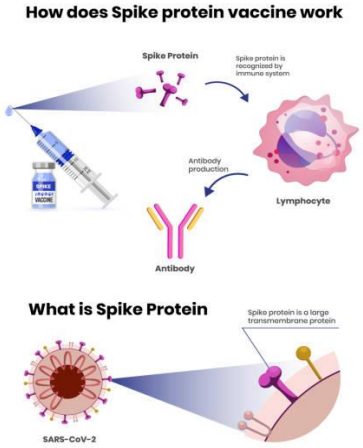


Fig: Spike protein vaccine work

Types of Vaccines:

There are several basic types of vaccines. Some vaccines are described here.

1. Attenuated whole-agent vaccines use living but attenuated (weakened) microbes. Examples of attenuated vaccines are the Sabin polio vaccine and those used against measles, mumps and rubella (MMR). The widely used vaccine against the tuberculosis bacillus and certain of the newly introduced, orally administered typhoid vaccines contain attenuated bacteria.

2. Inactivated whole-agent vaccines use microbes that have been killed. Inactivated virus vaccines used in humans include those against rabies, influenza and polio (the Salk polio vaccine). Inactivated bacterial vaccines include those for pneumococcal pneumonia, cholera, pertussis (whooping cough), and typhoid.

3. Toxoids which are inactivated toxins, are vaccines directed at the toxins produced by a pathogen. Examples.Vaccines against tetanus and diphtheria.

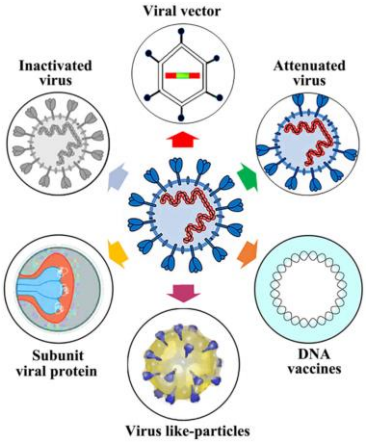


Fig: Type of vaccine.

4. Subunit vaccines use only those antigenic fragments of a microorganism that best stimulate an immune response. Subunit vaccines that are produced by genetic modification techniques, meaning that other microbes are programmed

to produce the desired antigenic fraction, are called recombinant vaccines. For example, the vaccine against the hepatitis B virus consists of a portion of the viral protein coat that is produced by genetically modified yeast.

5. Conjugated vaccines have been developed in recent years to deal with the poor immune response of children. The polysaccharides are combined with proteins such as diphtheria toxoid. This approach has led to the very successful vaccine for Haemophilic influenza type b, which gives significant protection.

6. Nucleic acid vaccines or DNA vaccines are among the newest and most promising vaccines, although they have not yet resulted in any commercial vaccine for humans. Ex-periments with animals show that plasmids of "naked" DNA injected into muscle result in the production of the protein encoded in the DNA.

These proteins stimulate an immune response. A problem with this type of vaccine is that the DNA remains effective only until it is degraded. Indications are that RNA, which could replicate in the recipient, might be a more effective agent.

Vaccines are also classified as follows.

1. **First-generation vaccines:** These are produced by conventional methods, e.g., smallpox vaccine, and Salk's polio vaccine.

2. **Second-generation vaccines:** These are prepared with the help of genetic engineer-ing techniques, e.g., vaccines against Hepatitis B and Herpes virus.

3. **Third-generation vaccines:** These are synthetic vaccines that are under trial.

Vaccines against Malaria, Leprosy, Herpes, Hepatitis C, AIDS, Dental caries, etc. are under study.

Immunization and Pregnancy:

The question of whether immunisation of a pregnant woman presents any danger for the fetus is frequently raised. Ideally, immunisation should be performed before gestation, since some vaccines are not perfectly safe during pregnancy. Pregnant women are, however, often vaccinated either because they travel to foreign coun-tries or when an epidemic occurs.

Vaccines that are safe during pregnancy are tetanus, influenza, inactivated poliomyeli-tis, cholera, and hepatitis B.

Vaccines that are to be avoided during pregnancy are the smallpox vaccine, oral polio-myelitis vaccine, and rubella vaccine.

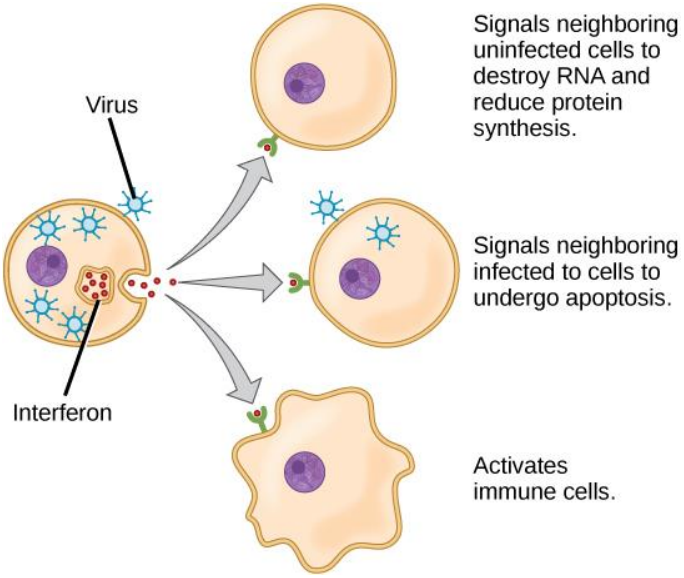
1.3 Interferon

Interferons are natural glycoproteins produced by virus-infected eukaryotic cells which protect host cells from virus infection. They were discovered by Isaacs and Lindenmann in 1957 in course of a study of the effect of UV-inactivated influenza virus on chick chorioallantoic membrane kept in an artificial medium.

They observed that the infected membrane produced a soluble substance in the medium which could inhibit the multiplication of active influenza virus inoculated in fresh chick chorioallantoic membranes. The substance was called interferon because it interfered with intracellular multiplication of viruses.

Later observations confirmed that such host-produced antiviral substances were common to many viruses.

Fig: Interferon produced by a virus-infected eukaryotic cell.



Viral interference is a phenomenon observed when multiplication of one virus is inhibited by another virus. For instance, when influenza-A virus is inoculated into the allantoic cavity of an embryonated egg followed after 24 hr by influenza-B virus, the multiplication of influenza-B virus is partly or completely inhibited. The reason why influenza-B virus cannot multiply is that the influenza-A virus infected cells produce interferon which partly or totally inhibits multiplication of B virus. The interferon also protects cells from influenza A virus.

1.3.1 Characteristics of Interferons:

An outstanding feature of interferons is that they are host-cell-specific and not virus-specific. This means that interferons produced by mouse or chicken will not protect human cells against the same virus which induced interferon in the experimental animals. On the other hand, an interferon produced by a virus X in an animal will protect the animal also from other viruses.

This is because interferons do not interact directly with the viruses. But they induce the virus infected cells to synthesize antiviral proteins which inhibit viral multiplication. These proteins have a wide inhibitory spectrum. As a result, not only the interferon-inducing virus, but others are also inhibited.

Types of Interferons

S.NO	Types	Source
1	α-interferon	Monocytes and B-Lymphocytes
2	β-interferon	Fibroblasts and epithelial cells
3	Y-interferon	T-cells

The reason why interferon produced by one species does not protect another species is that the same virus produces different interferons in different species. It has been observed that interferons produced by different host species

following infection by the same virus differ in molecular weight as well as in other properties, like isoelectric point etc. Not only different species produce different interferons, but different tissues of the same animal also produce different interferons.

All types of interferons are proteins having a comparatively low molecular weight ranging between 15,000 to 40,000 Daltons. Hence, they are non-dialyzable and destroyed by proteolytic enzymes. Interferons are fairly stable at low pH (pH2) and can withstand moderate temperature being stable at 37°C for an hour or so. They are produced in minute amounts by the infected cells as a longer precursor having 23 amino acid residues more than the mature molecule.

Human interferons are of three main types. These are called alpha interferons (α-IFN), beta-interferons (β-IFN) and gamma-interferons (γ-IFN). Alpha-interferon contains many subtypes. The total subtypes exceed 20 in number.

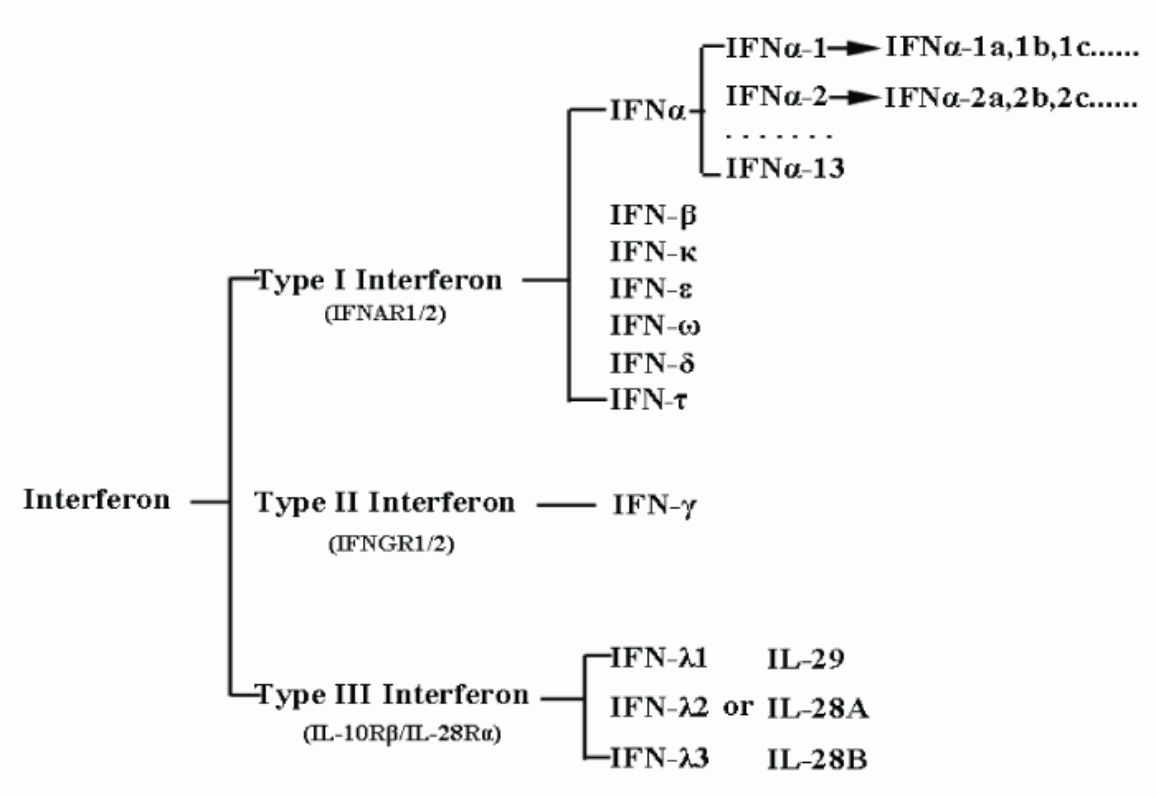


Fig: Types of interferons.

It is produced by the B-lymphocytes, monocytes and macrophages. β-IFN is produced by the fibroblasts in the connective tissues. γ-IFN is synthesized by the T-lymphocytes after they are activated by antigens. α-IFN has been shown to be coded by as many as 20 distinct chromosomal genes, indicating thereby that the subtypes of this interferon represent a family of closely related proteins.

β-IFN appears to be a glycoprotein. It is coded by a single human gene. All the genes of α-IFN and β-IFN are located on the short arm of human chromosome 9. α-IFN proteins are all 166 amino acid long (except one). They are non-glycosylated and the proteins are monomeric. The single β-IFN protein is also 166 amino acid long and a glycoprotein. It is dimeric.

1.3.2 Production of Interferons:

Interferons are produced by living animal cells, both in vivo as well as cultured cells. Interferon production and its antiviral activity require expression of cellular genes, and these functions are blocked by inhibitors of transcription and translation. Thus, virus-infected host cells fail to produce interferon in presence of actinomycin D, an inhibitor of eukaryotic RNA polymerase. When the inhibitor is added after 2 hr of infection, interferon production is not inhibited, suggesting that transcription is completed by that time.

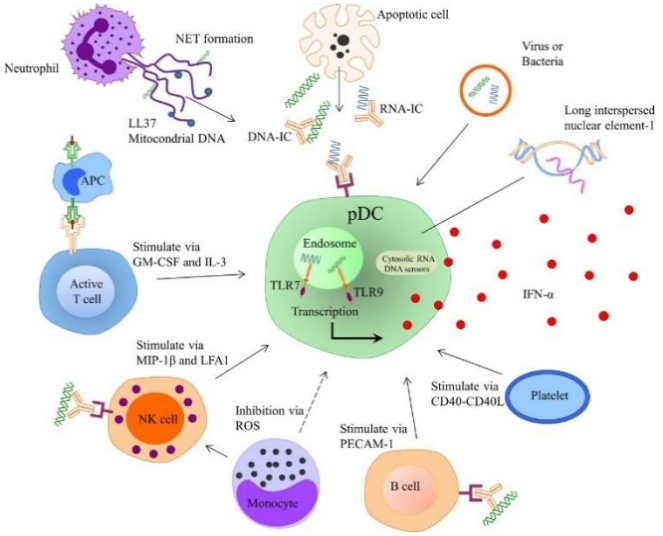


Fig: Production of Interferons.

Interferon production starts after the initiation of viral maturation and continues for 20 to 50 hr after that. Then the production stops, due to the formation of a repressor which presumably is formed or activated only when the interferon concentration in the producing cell exceeds a certain threshold concentration. Most of the interferon is transported from the producing cell to other neighbouring cells.

The substance is a virus that is responsible for interferon synthesis by the host cell and is known as an interferon inducer. The nature of this substance was identified by Merigan (1970) as double-stranded RNA. The activity seems to reside in polyribonucleotide with high helical content. The double-stranded RNA viruses — like reoviruses — can act as interferon inducers without replication. Single-stranded RNA viruses can act as inducers only after replication when they form double-stranded replicative intermediates. DNA viruses can also induce interferons, presumably due to overlapping transcription of viral DNA as observed in the case of vaccinia virus.

1.3.3 Double-Stranded RNA Formation

Fungal viruses which have mostly double-stranded RNA genomes are also efficient inducers of interferons. Some synthetic polymers containing riboinosinic acid, ribocytidylic acid (Poly I: C) as well as those containing riboadenylic acid and ribouridylic acid (Poly A: U) are also good inducers. All interferon inducers are characterized by high molecular weight, high density of anionic groups and resistance to enzymatic degradation. DNA and DNA-RNA hybrids have been found to be ineffective as interferon inducers.

The induction of interferon synthesis concerns α- and β-interferon's which belong to a single class, called Type I. Gamma-interferon belongs to a separate class, called Type II. The human Y-interferon is the single representative of its type. The gene coding the y-interferon protein is located on the long arm of chromosome 12. The gene has three introns, while the genes of α- and β- interferons are without any introns. Gamma-interferon (human) has 146 amino acids and is an N-glycosylated tetrameric protein. It is induced by antigenic stimulation of T-lymphocytes.

In presence of the inducer which is viral ds-RNA, the α- and β-interferon genes of the host chromosome(s) are activated to produce interferon mRNAs. Those are then translated intoα- and β- interferon proteins. These proteins at first accumulate in the producing cell and eventually leave the cell to reach neighbouring host cells.

As the interferon concentration in the producing cell rises above a threshold level, it activates another gene of the producing cell which codes for a repressor protein which feeds back and stops further synthesis of interferon. As a result, virus-infected cells generally produce only small quantities of interferons.

The interferon molecules that leave the producing cell reach the neighbouring uninfected host cells and interact with the cell membrane or nuclear membrane receptors of these cells. Thereby these cells are induced to synthesise antiviral proteins. These antiviral proteins are the actual agents that provide protection to these host cells against viral

infection.

1.3.4 Mechanism of Action of Interferons:

a) Type I interferons:

Type I interferons include α-IFN and β-IFN. These interferons do not interact with the viruses directly causing their inhibition, but they induce the formation of antiviral proteins which are activated to inhibit viral multiplications. These interferon-regulated proteins (IRPs) act presumably by blocking the synthesis of the macromolecular components necessary for viral multiplication.

Antiviral Action of Type I Interferons

Several interferons regulated host proteins (IRPs) have been identified, though all of them have not been fully characterized. Among the better known of these proteins are a protein kinase and an enzyme catalyzing the formation of a short polymer of adenylic acid, the 2′, 5′-oligoadenylate synthetase (2′-5′ A synthetase).

The protein kinase is induced by Type I interferons. It has to be activated by ds-RNA. The activated kinase catalyses phosphorylation of initiation factor (el F-2) thereby causing inhibition of protein synthesis.

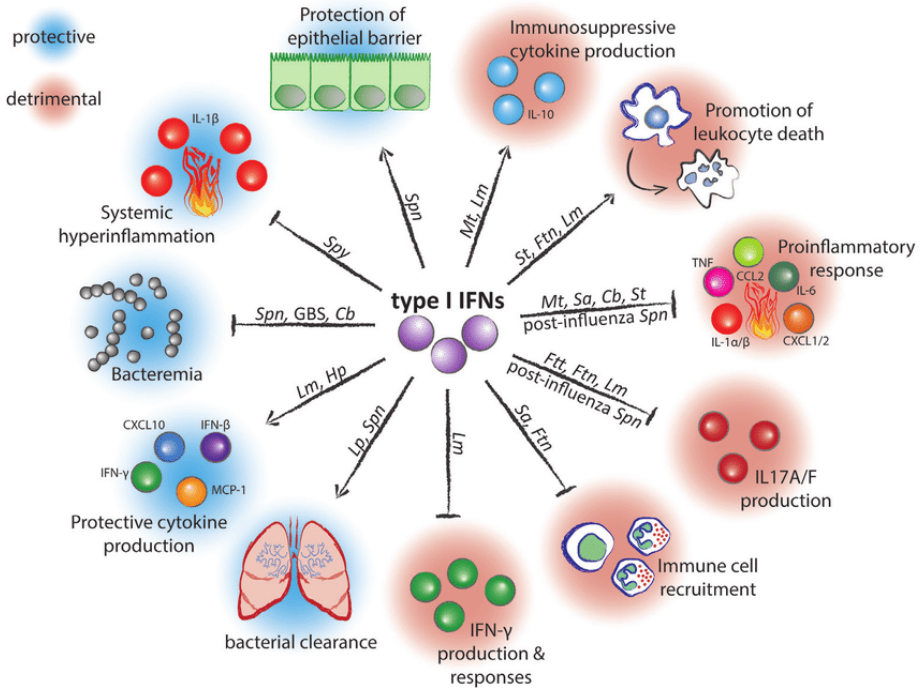


Fig: Mechanism of Action of type-1 Interferon.

Mechanism of Inhibition of Viral Protein Synthesis

The 2′-5′-oligoadenylate synthetase is an enzyme also induced by Type I interferons which requires activation by ds-RNA like the protein kinase. The activated synthetase acts as an activator of an endonuclease, RNase L. The activated RNAse degrades viral ss-RNA.

Another group of proteins, called Mx-proteins induced by α- and β-IFN are known to possess intrinsic antiviral activity, although the exact molecular mechanism by which they inhibit viral multiplication is not known. Mx-proteins have been reported to play a major controlling role in infections caused by influenza viruses in experimental animals as well as in humans.

b) Type II interferon:

Type II interferon includes g-IFN which is also known as immune IFN. Although g-IFN also possesses anti-viral activity, its major role is in the immunity through activation of cytotoxic T-lymphocytes which can destroy virus infected cells. Besides T-lymphocytes, other naturally occurring killer cells like macrophages and monocytes are also

activated by g-IFN.

Thus, in contrast to that of Type I interferons, the antiviral effect of g-IFN is expressed through activating the killer cells of the body which destroy the virus-infected cells.

Type II interferon induces the major histocompatibility antigens of human cells. Expression of these antigens is essential for immuno-potent cells to present foreign antigens to the T-lymphocytes during generation of specific immune responses.

IFN induced expression of these major histocompatibility antigens represents an important contribution of the antiviral activity of g-IFN through enhancement of the activity of cytotoxic T-lymphocytes. The activation of cytotoxic T-lymphocytes by y-IFN also implies its possible role in elimination of cancer cells which are recognized by the immune system of the body as foreign objects.

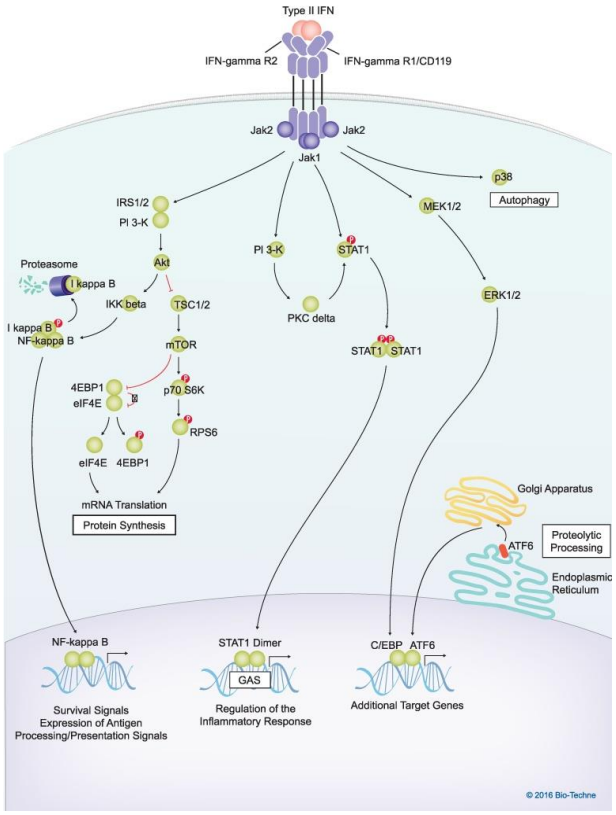


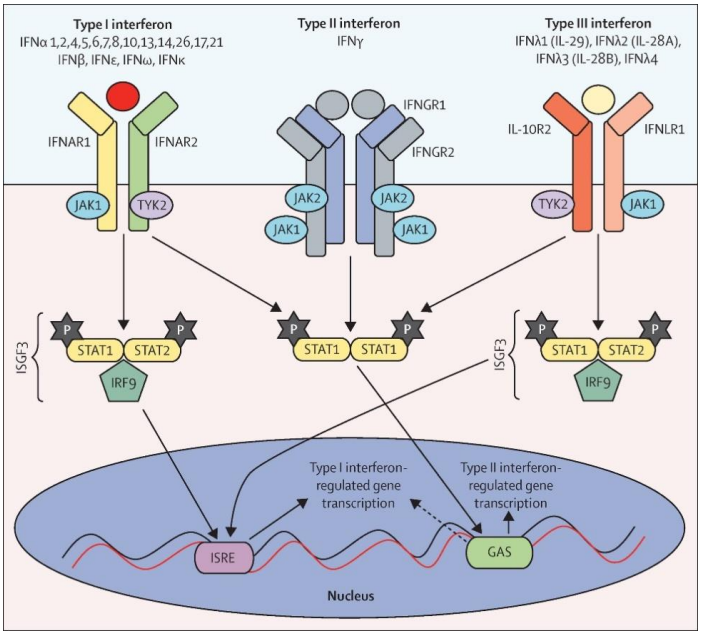
Fig: Type ll interferon mechanism.

1.3.5 Applications of Interferons:

Interferons could be ideal agents for combating viral diseases. They inhibit viral multiplication at such low concentration which is non-toxic to uninfected cells. One interferon can inhibit many viruses. But there are certain draw-backs which stand in their use.

Firstly, for application in humans, interferon must be of human origin, though interferons produced in monkey kidney cell cultures are also effective in humans. Interferons are produced in very small quantities and it is difficult to get them in sufficient quantity in pure form for clinical application. Another factor is that interferons are effective only for short periods and as such can be used against only acute infections, like influenza.

The difficulty of obtaining sufficient quantity of pure interferon for clinical use has been overcome by cloning the α-IFN and β-IFN human genes in bacteria and yeast. By growing these transgenic organisms in mass culture, it has been possible to obtain clinically usable interferons in sufficiently large quantities. Alpha-interferon has been marketed in 1984 under the trade name Intron A.



In the following years, this biotechnologically produced interferon has been approved for clinical use against diseases like genital herpes caused by herpes-virus, hepatitis B and C. Beta-interferon has also been biotechnologically produced and marketed under the trade name Betaseron. It has been used in a disease called multiple sclerosis. A recombinant g-interferon has been found effective against an inherited chronic disease, called granulomatous disease. The neutrophils of the affected individual are unable to kill the infectious bacteria. Application of y-IFN to such persons restores the ability of the neutrophils to kill bacteria. As the disease is chronic and inherited, the affected persons must take g-IFN throughout their life to remain normal.

Interferons are not only antiviral, but they have also anticancer activity. Clinical trials have shown that interferons have effect against only some types of tumours. Alpha-interferon has been approved for treating hairy-cell leukemia, and Kaposi's sarcoma, a cancer that occurs in AIDS patients.

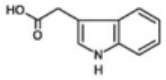
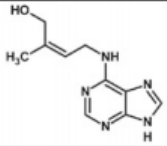
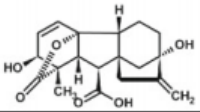
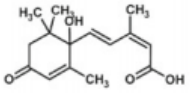
Gamma-interferon has been mainly used as an immuno-stimulant in cancer patients. Resistance against tumours in the body is controlled by the immune response against tumour antigens. The cytotoxic T-lymphocytes recognize these antigens as foreign and destroy them. Gamma-interferon can stimulate the cytotoxic function of T-lymphocytes and other natural killer cells of the body, thereby helping to control the tumour cells.

1.4 Hormones

Hormones are defined as chemical compounds that are produced in small amounts in a certain tissue controlling and regulating various functions related to growth, metabolism and reproduction in the receptive tissue. Plants produce different hor- mones, also called phytohormones, but the structures of these hormones are—in contrast to those of animals -rather simple and the molecules are not produced and stored in specific glands.The five classical phytohormone classes are auxins, cytokinins, gibberellins, abscisic acid and ethylene. More recently discovered phytohormones include strigo- lactones, brassinosteroids, jasmonate, salicylic acid, polyamines and nitric oxide.

Phytohormones in Microbes

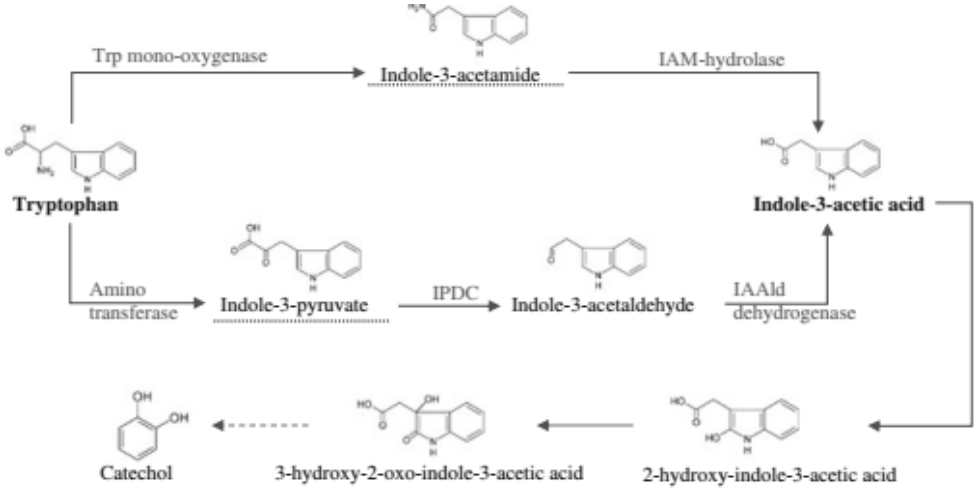
Phytohormones have also been detected and identified in the supernatant of culture medium of many soil and plant-associated bacteria and fungi. In these organisms, the phytohormones do not induce typical hormonal or major physiological changes. Microbial phytohormone production has been linked to changes in root architecture and plant growth promotion. However, the degree of proof for their involvement can vary a lot depending on the phytohormone and the studied microbial strain. The presence of a certain phytohormone in the supernatant of a microbial culture is not enough to prove a functional role of this molecule in its interaction with the plant.

Class	Structure [1]	Effect on plant
Auxin		Cell elongation and division Root initiation Apical dominance
Cytokinin		Inhibition of root elongation Stimulation of cell division Leaf expansion by cell enlargement Delay of senescence
Gibberellin		Seed germination Stem elongation Floral induction and fruit growth
Abscisic acid		Stomatal closure Inhibition of shoot growth Bud dormancy Abiotic and biotic stresses
Ethylene	$H_2C{=}CH_2$	Stress and ripening hormone Senescence and abscission Abiotic and biotic stresses

1.4.1 Auxin

This group of phytohormones has the ability to induce cell elongation in the subapicalregion of the stem. Besides this ability, auxins are involved in almost all aspects ofplantgrowthanddevelopmentsuchasstemandrootelongation,stimulation of cell division, lateral and adventitious root initiation, apical dominance, vascular tissuedifferentiation, gravitropism and phototropism.

Until now, six biosynthetic pathways have been described in microbes, with most pathways being suggested based on the presence of metabolic intermediates in the culture medium. Despite the multitude of pathways, there are apparently two dominant microbial pathways based on both the abundance and genetic evidence for these pathways: one via the intermediate indole-3-acetamide (IAM) and one via the intermediate indole-3-pyruvate (IPyA). In the IAM pathway, tryptophan is first converted by a tryptophan monooxygenase to IAM, which is then catalyzed to IAA by an IAM hydrolase. In the IPyA pathway abundant in beneficial plant-associated bacteria, tryptophan is in a first step transaminated to IPyA by an aromatic aminotransferase. In the second, rate-limiting step, IPyA is converted to indole-3-acetaldehyde (IAAld) by a decarboxylation reaction catalyzed by an IPyA decarboxylase (IPDC, encoded by the ipdC gene). Finally, IAAld is converted into IAA.



1.4.2 Cytokinins

Naturally occurring cytokinins (CK) are mostly derived from adenine and modified by substitutions at the N6, including the respective ribotides, ribosides and glycosides. CKs promote cell division and differentiation in

meristematic tissues both in plant roots and shoots. They are also involved in processes such as senescence delay, organ formation, root and root hair development and leaf expansion. The massive production of auxins and cytokinins by phytopathogenic bacteria or by the transfer of bacterial oncogenes into the infected plant exemplified by *Pseudomonas savastanoi* and *A. tumefaciens* are a strategy to induce tumor/gall formation. For the pathogen *R. fascians*, the fas operon, comprising six genes, is responsible for the biosynthesis of CKs necessary for leafy gall formation. . Also for many non-pathogenic bacteria, CK production has been demonstrated. Although plant growth-promoting action is claimed for CKs, firm evidence is lacking due to the absence of mutant strains defective in CK biosynthesis. However, it is proven that bacterial CKs are perceived by plant CK receptors, pointing towards the potential of bacterial CKs to influence CK signaling in plants.

1.4.3 Gibberellins

The class of gibberellins (GAs) is a broad group of more than 100 compounds that can be classified as tetracyclic diter- penoid acids, with ent-gibberellane as backbone. GAs are involved in developmental processes such as cell division and elongation during almost all stages of plant growth (from seed germination to fruit growth).Both in some fungi as bacteria, GA production has been detected and the biosyn- thetic pathways have been proposed and/or unraveled. In the fungal rice pathogen *Gibberella fujikuroi*, which is used to commercially produce GA3,In bacteria, genetic evidence for GA biosynthesis is minor. Operons containing genes encoding for putative enzymes involved in GA biosynthesis were identified in some Rhizobium and Bradyrhizobium strains. Some plant-associated microbes produce GAs as assessed by measuring the GA content of the culture medium. The best documented case for the role in plant growth promotion is the reversion of the dwarf phenotype of plants induced by GA inhibitors by inoculation with GA-producing *Azospirillum* strains. Since the identified blend of GAs in nodules resembles the GAs found in the culture medium of the rhizobial strains, it was suggested that nodule-derived GAs originated from the rhizobial strains.

1.4.4 Abscisic Acid

Abscisic Acid (ABA) induces stomatal closure and fruit ripening and inhibits seed germination. In addition it is involved in bud dormance and protective responses against abiotic stresses such as drought, salt stress and metal toxicity.Bacterial production of ABA has been reported for *A. brasilenseand B. japon- icum* strains, although the biosynthetic pathways are unknown. Since ABA inhibits cytokinin biosynthesis, bacterial ABA production can interfere with the cytokinin levels in plants. In addition under stress conditions, the bacterial ABA production might sustain the internal ABA pool in plants, alleviating the negative effects of the imposed stress.

1.4.5 Ethylene

The gaseous phytohormone ethylene is in- volved in physiological and developmental processes such as seed germination, cell expansion, senescence and abscission. It is sometimes called the ripening hormone since it induces fruit ripening. Ethylene is also involved in the plant defense responses against pathogens. It can affect the outcome of the jasmonate- dependent defense responses by acting synergistically with jasmonate on one branch of the pathway leading to resistance to necrotrophic pathogens. Microbial ethylene production has been reported but mainly for bacterial pathogens such as *Pseudomonas, Xanthomonas and Erwinia*. The ethylene produc- tion is contributing to the bacterial virulence by inducing hormonal imbalances in the plant. The suggested bacterial biosynthetic pathways are distinct from the plant biosynthetic pathway. In the first bacterial pathway, methionine is the precursor, while in the second pathway ethylene is produced from 2-oxoglutarate. The full understanding of the putative ethylene biosynthetic pathways and the role of ethylene production in disease are still unclear.

1.5 Vitamins:

Vitamins are organic compounds that perform specific biological functions for normal maintenance and optimal growth of an organism. These vitamins cannot be synthesized by the higher organisms, including man, and therefore they have to be supplied in small amounts in the diet.

Microorganisms are capable of synthesizing the vitamins. In fact, the bacteria in the gut of humans can produce some

of the vitamins, which if appropriately absorbed can partially meet the body's requirements. It is an accepted fact that after administration of strong antibiotics to humans (which kill bacteria in gut), additional consumption of vitamins is recommended.

Microorganisms can be successfully used for the commercial production of many of the vitamins e.g. thiamine, riboflavin, pyridoxine, folic acid, pantothenic acid, biotin, vitamin B12, ascorbic acid, P-carotene (pro-vitamin A), ergosterol (pro-vitamin D). However, from economic point of view, it is feasible to produce vitamin B12, riboflavin, ascorbic acid and p-carotene by microorganisms. For the production of ascorbic acid (vitamin C), the reader must.

Vitamin A

Vitamin A is a group formed by different retinoids, retinol, retinal, retinoic acid and retinyl esters. Vitamin A is involved in many functions such as in the immune system, vision, reproduction, cellular communication, cell growth and differentiation, and it is critical for the normal development and mainte- nance of the heart, lungs, kidneys and other organs. It is essential for vision since it is a key component of retinal receptors.

At the industrial scale, vitamin A is mainly produced chemically (2700 t per annum t/a), as is beta- carotene (400 t/aSome microbial processes have been put to efficient use, using the green microalga *Dunaliella* and the fungus *Blakeslea trispora* to produce beta-carotene. Under certain condi- tions, *Dunaliella* can accumulate more than 0.1 g g^{-1} dry cells (20–30% salts, nitrogen limited, 10,000 lux and 25–27°C for 3 months).*B. trispora* fermen- tation can produce 0.2 g g^{-1} of dry cells after seven days and whole cells are used directly as a feed additive; alternatively beta-carotene can be purified from them.*Candida utilis* and *Saccharomyces cerevisiae* have been modified to express bacterial carotenoid biosynthesis genes, affording beta-carotene titres between 0.1 and 0.4 mg g^{-1} dry cells. Escherichia coli has also been bioengineered to produce this carotenoid, using different strategies in which the expression of genes from *Enterococcus faecalis* and *Streptococcus pneumoniae* is the most productive. *S. cerevisiae* has been modified to express carotenogenic genes from *Xanthophyllomyces dendro- rorhous*, producing beta-carotene levels that reach 6.3 mg g^{-1} of dry cells.13,14 Currently, other hosts are under development to produce carotenoids, such as *Pichia pastoris* (from which, to date, only low amounts of beta-carotene have been produced: 0.34 mg g^{-1}dry cells) and *Yarrowia lipolytica*.

Vitamin D

Vitamin D is a fat-soluble compound derived from cholesterol and ergos- terol. On the one hand, cholesterol is modified metabolically to produce 7-dehydrocholesterol, which can be cleaved by UV-radiation to form chole-calciferol (vitamin D3). On the other hand, ergosterol can be transformed into ergocalciferol (vitamin D2). Vitamin D can be synthesized by most people through exposure to sun- light, but excessive radiation can be carcinogenic so it is prudent to coun- teract the exposure of the skin to sunlight with a complete diet including this vitamin. Currently it is common to satisfy the need for this vitamin by supplementing its levels in food to create fortified foods. Vitamin D is synthesized chemically from sterols, ergosterol and cholesterol, using UV radiation.

Ergosterol is produced by bioprocesses including generally recognized as safe (GRAS) yeasts such as *S. cerevisiae*, *Saccharomyces uvarum* and *C. utilis*. Fungi such as *Trichoderma*, *Cephalosporium* and *Fusarium* have also been investigated with regard to their capacity to accumulate ergosterol, but they afford lower production titres than *S. cerevisiae*.*S. cerevisiae* has been bioengineered to increase the accumulation of ergosterol by overexpressing different enzymes involved in the biosynthetic pathway and using molasses as a cheap carbon source. In addition, strains producing cholesterol instead of ergosterol have been developed by meta- bolic engineering of *S. cerevisiae*.

Vitamin E

The vitamin E group is formed by different molecules with antioxidant activities. There are eight chemical forms, four of tocopherol and four of tocotrienol. Tocopherol is a fat-soluble antioxidant involved in the regulation of reactive oxygen species (ROS) produced during fat oxidation. Thus, its activity diminishes the damage caused by free radicals to cells. Photosynthetic microorganisms are known to accumulate tocopherols and *Euglena gracilis* have been seen to be the best producer organisms, reaching 7.35 mg g^{-1} of dry cells, where 97% of tocopherols is in the alpha-isoform. Most genetic and metabolic engineering aimed at producing alpha- tocopherol has been developed in

Synechocystis sp., which has been used as a well-characterized model organism for this purpose, and strategies have been developed leading to a five-fold increase in tocopherol.

Vitamin K

VitaminKisafamilyofchemicallyrelatedcompounds:naphthoquinones.There are two kinds of naphthoquinones; phylloquinones, pro-duced by plants and cyanobacteria, and menaquinones pro-duced by bacteria.More than 90% of our dietary vitamin K comes from plant foods (espe- cially vegetables) but there is also a contribution by our intestinal bacteria, although this is very low.Certain fermented foods are enriched in this vitamin since some of the microorganisms involved in the fermentation process can produce and accumulate menaquinones. *Proprionibacterium* can produce cheese with a high content of the vitamin, while Bacillus sub- tilis can produce enriched fermented soy foods.Some microorganisms are able to produce this vitamin. For example, *Flavobacterium* sp. has been found to be a potent producer.*B subtilis*, and *Propionibacterium freudenreichii*have also been proposed as candidates for vitamin K production.

Vitamin B1

Vitamin B1, also called thiamine, is a water-soluble compound and there are five phosphate derivatives, including thiamine monophosphate (ThMP), thiamine diphosphate (ThDP), thiamine triphosphate (ThTP), adenosinethiamine triphosphate (AThTP) and adenosine thiamine diphosphate (AThDP).Recently, a patent protecting the metabolic engineering of bacteria to accu- mulate high amounts of thiamine in media has been developed using *B. subtilis*. The patent refers to species of Bacillaceae, Lactobacillaceae, Streptococcaceae, Corynebacteriaceae and Brevibacteriaceae, in which a microorganism containing a mutation that deregulates thiamine production and causes thiamine products to be released from the cell is described.

Vitamin B2

Vitamin B2 is also called riboflavin, which takes its name from its yellow colour (flavus). It is essential for the proper functioning of all the flavopro- teins, since riboflavin is the central component of the FAD and FMN co-factors. This compound is naturally pro- duced by several microorganisms such as ascomycetes fungi (*Ashbya gossypii*, *Eremothecium ashbyii*), by yeasts such as *Candida flaeri* and *Candida famata*, and also by bacteria such as *B. subtilis* and *Corynebacterium ammoniagenes*.

Vitamin B3

Vitamin B3 is a group formed of nicotinic acid, nicotinamide and other compounds such as inositol hexanicotinate, that exhibit a related biological activity.Unlike the other B vitamins, vitamin B3 can be generated by mammals via an endogenous enzymatic pathway from tryptophan and is stored in the liver, although it is also obtained from the diet. itamin B3 is also synthesized from tryptophan by intestinal bacteria . *Bacteroides fragilis* and *Prevotella copri* (Bacteroidetes); *Ruminococcus lactaris*, *Clostridium difficile* (Firmicutes); *Bifidobacterium infantis* (Actinobacteria); *Helicobacter pylori* (Proteobacteria); and *Fusobacterium varium* (Fusobacteria) possess a vitamin B3 biosynthesis pathway. Thus, many intestinal bacteria from various genera can produce vitamin B3, suggesting that both dietary and commensal bacteria-derived vitamin B3 are important for host immunity.

Vitamin B5

Vitamin B5 (pantothenic acid) is a precursor of coenzyme A (CoA), which is an essential cofactor for the TCA cycle and fatty acid oxidation. Vitamin B5 deficiency causes immune diseases such as dermatitis, as well as non-immune-related conditions such as fatigue and insomnia. Bacterial vitamin B5 is synthesized from 2-dihydropantoate and β-alanine via de novo synthesis pathways. Bacterial vitamin B5 exists as free pantothenic acid, which is directly absorbed in the large intestine, converted to CoA, and distributed in the same way as dietary vitamin B5. According to a genomic analysis, *Bacteroides fragilis* and *Prevotella copri* (Bacteroidetes); some *Ruminococcus* spp. (*R. lactaris* and R. torques) (Firmicutes); *Salmonella enterica* and *Helicobacter pylori* (Proteobacteria) possess a vitamin B5 biosynthesis pathway, indicating that intestinal commensal bacteria can produce vitamin B5. In contrast, most *Fusobacterium* (Fusobacteria) and *Bifidobacterium* spp. (Actinobacteria) and some strains of *Clostridium difficil*e, *Faecalibacterium* spp., and *Lactobacillus* spp. (Firmicutes) lack such a pathway, although some of them do express

pantothenic acid transporter to utilize vitamin B5 for energy generation.

Vitamin B6

Vitamin B6 exists in several forms, including as pyridoxine, pyridoxal, and pyridoxamine. These forms of vitamin B6 are precursors of the coenzymes pyridoxal phosphate (PLP) and pyridoxamine phosphate (PMP), which are involved in a variety of cellular metabolic processes, including amino acid, lipid, and carbohydrate metabolism.

Microbial vitamin B6 is synthesized as PLP from deoxyxylulose 5-phosphate and 4-phosphohydroxy-L-threonine or from glyceraldehyde-3-phosphate and d-ribulose 5-phosphate. In the large intestine, bacteria-derived PLP is converted to free vitamin B6, which is absorbed by passive transport, transported to the blood, and distributed throughout the body. Metagenomic analysis has shown that *Bacteroides fragilis* and *Prevotella copri* (Bacteroidetes), *Bifidobacterium longum* and, *Collinsella aerofaciens* (Actinobacteria), and *Helicobacter pylori* (Proteobacteria) possess a vitamin B6 biosynthesis pathway. Bacteroidetes and Proteobacteria likely produce vitamin B6 starting from deoxyxylulose 5-phosphate and 4-phosphohydroxy-l-threonine, whereas Actinobacteria likely start from glyceraldehyde-3-phosphate and d-ribulose 5-phosphate.

Vitamin B7

Vitamin B7 (biotin) is a cofactor for several carboxylases that are essential for glucose, amino acid, and fatty acid metabolism. Vitamin B7 is abundant in foods such as nuts, beans, and oilseed.Vitamin B7 is also produced by intestinal bacteria as free biotin synthesized from malonyl CoA or pimelate via pimeloyl-CoA. Metagenomic analysis has shown that *Bacteroides fragilis* and *Prevotella copri* (Bacteroidetes); *Fusobacterium varium* (Fusobacteria) and *Campylobacter coli* (Proteobacteria) possess a vitamin B7 biosynthesis pathway. Furthermore, vitamin B7 production appears to proceed in a cooperative manner among different intestinal bacteria; Bifidobacterium longum in the intestine produces pimelate, which is a precursor of vitamin B7 that enhances vitamin B7 production by other intestinal bacteria.

Vitamin B9

Vitamin B9 (folate), in its active form as tetrahydrofolate, is a cofactor in several metabolic reactions, including DNA and amino acid synthesis.Foods such as beef liver, green leafy vegetables, and asparagus contain high levels of vitamin B9. Vitamin B9 exists as both mono- and polyglutamate folate species in the diet. Intestinal bacteria synthesize vitamin B9 as THF from GTP, erythrose 4-phosphate, and phosphoenolpyruvate . Bacterial THF is directly absorbed in the colon via PCFT and distributed through the body by the blood. Metagenomic analysis has shown that *Bacteroides fragilis* and *Prevotella copri* (Bacteroidetes); *Clostridium difficile*, *Lactobacillus plantarum*, *L. reuteri*, *L. delbrueckii ssp. bulgaricus*, and *Streptococcus thermophilus* (Firmicutes), some species in *Bifidobacterium* spp (Actinobacteria); *Fusobacterium varium* (Fusobacteria) and *Salmonella enterica* (Proteobacteria) possess a folate biosynthesis pathway.

Vitamin B12

Vitamin B12 (cobalamin) is a cobalt-containing vitamin that, in its active forms of methylcobalamin and adenosylcobalamin, catalyzes methionine synthesis. Together with vitamin B6 and B9, vitamin B12 plays important roles in red blood cell formation and nucleic acid synthesis, especially in neurons.Bacterial vitamin B12 is synthesized from precorrin-2 to produce adenosylcobalamin (10), which is absorbed directly by the large intestine and distributed throughout the body; the mechanism underlying this absorption is currently unclear. Metagenomic analysis has predicted that Bacteroides fragilis and Prevotella copri (Bacteroidetes); Clostridium difficile, Faecalibacterium prausnitzii and Ruminococcus lactaris (Firmicutes); Bifidobacterium animalis, B.infantis, and B.longum (Actinobacteria); Fusobacterium varium (Fusobacteria) possess a vitamin B12 biosynthesis pathway.

2. Enzymes (amylase, protease)

2.1 Amylase

Amylases are a complex group of enzymes that hydrolyse polysaccharides like starch and glycogen into glucose. During the hydrolysis of 1, 4-glycoside, linkages present in the above polysaccharides are degraded which results

first in the formation of short-chain dextrins, then in maltose and glucose.

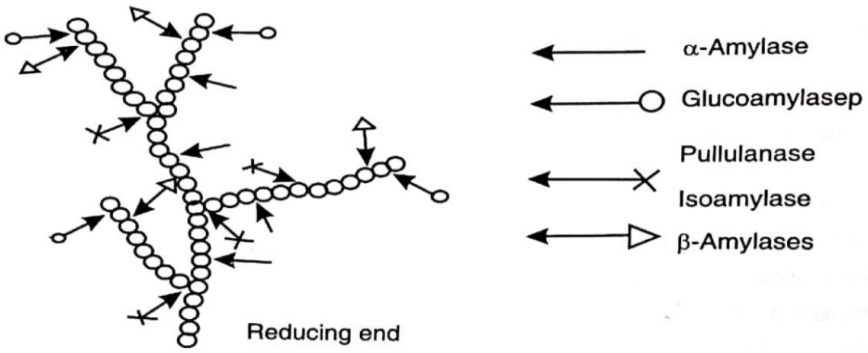


Fig: Mechanism of Starch Hydrolysis by Amylase

There are mainly two groups of amylases such as o-amylose and B-amylose. a-amylases are also called 1,4- a- glucan glucans hydrolase. Extracellular enzymes hydrolyze 1,4 glycosidic bonds. These enzymes are also called endoenzymes as they split the substratum in the interior of the molecule in a random fashion. On the other hand, B-amylases split the substratum from one end of the molecule in a successive fashion. On every alternate 1, 4 glycosidic bondsrelease maltose. These enzymes are also called Exo amylases. The other enzymes involved in the degradation of starch include amyloglucosidase (Aspergillusniger and Rhizopus niveus), isoamylase and pullulanase (*Klebsiella pneumonia* and *Bacillus acidopullulyticus*). The molecular weight of various a-amyloses do not differ considerably and requires calcium as a stabilizer.

Table: Different enzymes involved in starch degradation:

Endo-amylases (dextrinizing	Act on starch randomly releasing dextrins (a) α - amylases–Act on 1, 4 glycosidic bond (b) Debranching – Act on 1, 6 glycosidic bond (i) Isoamylase – Act on dextrins (ii) Pullulanase – Act on pullulan
Exo-amylases	-Act on from one end of starch (a) α -amyloglucosidase–Act on every 1, 4-glycosidic bond releasing glucose (b) β -amylase–Act on alternate 1, 4 glysidic bond releasing maltose (c) Maltose – Act on maltose releasing glucose

α-amylases are secreted by many bacteria and fungi. They are classified according to their:

1. Starch-liquefying or saccharogenic effect.
2. pH optimum.
3. Temperature range.
4. Stability.

Table: molecular weight of some α-amylases from different microorganisms

Organism	Molecular weight (in × 10^3)
Aspergillus oryzae	51–52
A. niger	58–61
Bacillus acidocaldarius	68
B. amyloliquefaciens	49
B. subtilis	24–100
Thermomonospora curvata	62

Saccharogenic amylases produce free sugars (B-amylose and glucoamylase), whereas starch liquifying amyloses (a-amylose) break down the starch polymer but do not produce free sugars. Bacteria which produce a-amylase are *Bacillus subtilis, B. cereus, B. amylo- liquefaciens, B. coagulans, B. polymyxa, B. stearothermophilus, B. caldolyticus, B. acidocaldarius, B. subtilis amylesacchuraticus, B. licheniformis, species of Lactobacillus, Micrococcus, Pseudomonas, Arthrobacter, Escherichia, Proteus, Thermomonospora* and *Serratia*. Some a-amylase producing fungi are species of Penicillium, Cephalosporium, Mucor, Candida, Neuraspora and Rhizopus. Although all the above bacteria and fungi are capable of producing a-amylase, the most important of them from which a-amylase are produced commercially through fermentation process include Bacillus amyloliquefaciens, *B. licheniformis* and *Aspergillus oryzae*. However, Bacillus are used much more extensively than those of Aspergillus. The most important areas of application for these two enzymes are precised in table 8.4.

Table: Important applications of α-amylase

Industry	Source		Applications
	Bacillus	Aspergillus	
Starch industry	+	--	Liquefaction of starch for production of glucose, fructose, maltose
Milling	--	+	Modification of deficient flour
Alcohol	+	+	Liquefaction of starch before the addition of malt for saccharification
Baked goods	--	+	Increase in the proportion of fermentable carbohydrates
Brewing	+	--	Barley preparation, liquefaction of additives
	--	+	Improved fermentability of grains, modification of beer characteristics
Paper	+	--	Liquefaction of starch without sugar production for sizing of paper
Textiles	+	--	Continuous desizing at high temperature
Feed industry	+	--	Improvement of utilization of enzymatically treated barley in poultry and calf raising
Sugar	+	--	Improvement of filterability of cane sugar juice via breakdown of starch in juice
Laundry and detergent	+	--	Increase in cleansing power for laundry soiled with starch, additive in dish washer detergents

2.1.1 Fungal α-amylase: Fungal α-amylase is produced commercially by employing either Aspergillus oryzae or Aspergillus niger. The stationary culture method is used when A. oryzar is employed, while the submerged culture method is used when Aspergillus niger is employed The process of the submerged culture method is only described here.

(a) Preparation of inoculum: Suitable and pure seed culture is generally selected as inoculum. In certain cases, mutants capable of giving higher yield are selected as inoculum.

Component	Amount (g liter^{-1})
Corn starch	24
Corn steep liquor	36
Potassium chloride	0.2
Sodium monohydrogen phosphate	47
Calcium chloride	1
$MgCl_2 \cdot 6H_2O$	0.2

(b) Preparation of medium: The following medium is generally employed for submerged fermentation.
Amylase biosynthesis is inhibited when there is glucose in the medium. The medium is steam sterilized. The sterilized medium is passed into a production fermenter for α-amylase

(c) Fermentation process: A cylindrical fermenter made up of stainless steel is production. Generally used in the fermentation process. It is equipped with an agitator, an aerating device, a cooling system and other ancillary equipment like a device for foam control, monitoring of pH, temperature and control of oxygen tension etc. sufficient quantity of pre-sterilized production medium is taken in the fermenter and is inoculated with spores of the selected species of the fungus. The spores are allowed to germinate and produce sufficient mycelium by controlling the fermentation conditions. Control of fermentation conditions play a vital role in the success of the process, which include pH, temperature, aeration, agitation, oxygen supply etc. The optimum pH for the fermentation is 7.0. Calcium carbonate is used as buffer to maintain pH. The fermentation process is generally operated at a temperature of 30 to 40°C. Aeration and agitation of the production medium is needed because of high viscosity of the medium due to the presence of mycelial mal. of the

(d) Harvest and recovery: The following steps are followed during the recovery enzyme after the completion of fermentation. In order to avoid denaturation of the enzyme, the fermentation broth is subjected to rapid cooling at 5°C temperature immediately and the enzyme is extracted. 1. Separation of fungal mycelium is accomplished by filtration of the refrigerated broth.

2 The suspended particles present in the broth are removed with flocculating agents like calcium phosphate.

3. The enzyme is precipitated, in order to get high degree of purity, by using acetone or alcohol or even inorganic salts like ammonium sulphate or sodium sulphate.

4. Sometimes fractional precipitation of the enzyme is done to obtain it in purest form

2.1.2 Bacterial α-amylase: Bacterial a-amylase is produced by Bacillus subtilis or B. amyloliquefaciens or B. licheniformis. For industrial production of bacterial a-amylase, nowadays submerged culture method is generally employed in many countries.

(a) Preparation of inoculum: Pure culture of any of the above-mentioned species of Bacillus is selected as inoculum. Mutants that produce 250 times greater yields than the wild strain are preferred as inoculum.

(b) Preparation of medium: The formulation of the production medium and control of fermentation conditions play a major role in the success of enzyme fermentation. The production medium should basically contain an energy source, a carbon source, a nitrogen source, and growth requirements such as essential amino acids or vitamins. For obtaining high yields of the enzyme, the production medium should also contain certain inducers like lactose.

Sometimes certain compounds like glucose present in the production medium act as a repressor for certain enzymes like α-amylase. In such conditions either the concentration of glucose should be kept low or it should be fed intermittently. The following production medium is generally employed in a submerged culture method (Table 8.5).

Table: Composition of medium for submerged fermentation.

Substances	Amount (in %)
Lactose	4.5
Ground soyabean meal	1.85
$MgSO_4 \cdot 7H_2O$	0.04
Hodag KG –1 antifoam	0.05
Amber BYF (Autolysed brewers yeast fractions)	1.50
Distillers dried solubles	0.70
N-Zamine (enzyme casein hydrolyzate)	0.65
Water	90.40

(c) Fermentation process: The type of fermenter that is used for fungal a-amylase is also used for bacterial a-amylase production. About 1000 to 30,000 gallons of production Water medium is taken in the fermenter and inoculated. The fermentation is continued for up to 4-6 days. The pH of the medium is maintained at 7.0. Calcium carbonate is used as a buffer for maintaining neutral pH. The temperature is maintained at 30-40°C. The production of a-amylase starts when the bacterial density reaches 10"-1010 cells ml-1 However, the enzyme production increases just before the growth rate of the microorganism decreases and spore formation begins.

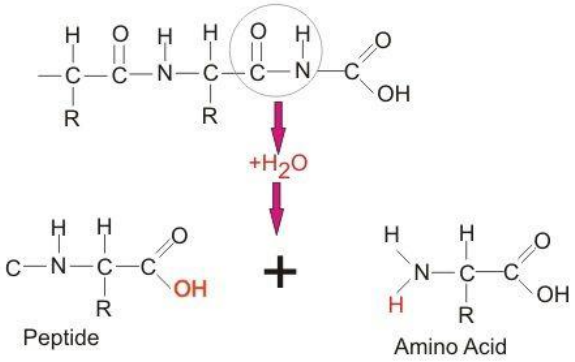
(d) Harvest and recovery: Bacterial c-amylase is harvested and recovered by the same method that is used for the recovery of fungal a-amylase. The most active liquid enzyme preparation contains 2% amylase protein and solid preparation contains 5% amylase.

2.2 Proteases

Proteolytic enzymes (proteases) are enzymes that break down protein. These enzymes are made by animals, plants, fungi, and bacteria. Proteolytic enzymes break down proteins in the body or on the skin. This might help with digestion or with the breakdown of proteins involved in swelling and pain. Some proteolytic enzymes that may be found in supplements include bromelain, chymotrypsin, ficin, papain, serrapeptase, and trypsin.

A protease (also called a peptidase or proteinase) is an enzyme that catalyzes (increases reaction rate or "speeds up") proteolysis, breaking down proteins into smaller polypeptides or single amino acids, and spurring the formation of new protein products. They do this by cleaving the peptide bonds within proteins by hydrolysis, a reaction where water breaks bonds. Proteases are involved in many biological functions, including digestion of ingested proteins, protein catabolism (breakdown of old proteins), and cell signaling.

In the absence of functional accelerants, proteolysis would be very slow, taking hundreds of years. Proteases can be found in all forms of life and viruses. They have independently evolved multiple times, and different classes of protease can perform the same reaction by completely different catalytic mechanisms.



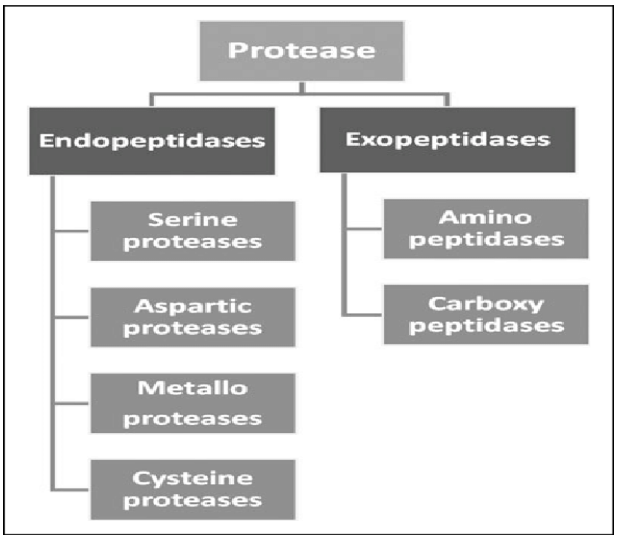
Hydrolysis of Peptide Bond

Based on catalytic residue

Proteases can be classified into seven broad groups:

- Serine proteases - using a serine alcohol
- Cysteine proteases - using a cysteine thiol
- Threonine proteases - using a threonine secondary alcohol
- Aspartic proteases - using an aspartate carboxylic acid
- Glutamic proteases - using a glutamate carboxylic acid
- Metalloproteases - using a metal, usually zinc
- Asparagine peptide lyases - using an asparagine to perform an elimination reaction (not requiring water)

Proteases were first grouped into 84 families according to their evolutionary relationship in 1993, and classified under four catalytic types: serine, cysteine, aspartic, and metallo proteases. The threonine and glutamic-acid proteases were not described until 1995 and 2004 respectively. The mechanism used to cleave a peptide bond involves making an amino acid residue that has the cysteine and threonine (proteases) or a water molecule (aspartic acid, metallo- and acid proteases) nucleophilic so that it can attack the peptide carbonyl group. One way to make a nucleophile is by a catalytic triad, where a histidine residue is used to activate serine, cysteine, or threonine as a nucleophile. This is not an evolutionary grouping, however, as the nucleophile types have evolved convergently in different superfamilies, and some superfamilies show divergent evolution to multiple different nucleophiles.



2.2.1 Peptide lyases

A seventh catalytic type of proteolytic enzymes, asparagine peptide lyase, was described in 2011. Its proteolytic mechanism is unusual since, rather than hydrolysis, it performs an elimination reaction. During this reaction, the catalytic asparagine forms a cyclic chemical structure that cleaves itself at asparagine residues in proteins under the right conditions. Given its fundamentally different mechanism, its inclusion as a peptidase may be debatable.

Proteases are involved in digesting long protein chains into shorter fragments by splitting the peptide bonds that link amino acid residues. Some detach the terminal amino acids from the protein chain (exopeptidases, such as aminopeptidases, carboxypeptidase A); others attack internal peptide bonds of a protein (endopeptidases, such as trypsin, chymotrypsin, pepsin, papain, elastase).

Table1: Function of proteolytic enzymes

Pancreatic proteolytic enzymes and their function	
Proteolytic Enzyme	**Function**
Trypsin	Cleaves bonds at lysine or arginine; cleaves pancreatic proenzymes
Elastase	Cleaves bond at aliphatic amino acid
Chymotrypsin	Cleaves bonds at aromatic or neutral amino acids
Carboxypeptidase A	Cleaves aromatic amino acids at terminal ends of peptides
Carboxypeptidase B	Cleaves arginine or lysine from terminal ends of peptides

2.2.2 Catalysis

Catalysis is achieved by one of two mechanisms:

- Aspartic, glutamic, and metallo-proteases activate a water molecule, which performs a nucleophilic attack on the peptide bond to hydrolyze it.
- Serine, threonine, and cysteine proteases use a nucleophilic residue (usually in a catalytic triad). That residue performs a nucleophilic attack to covalently link the protease to the substrate protein, releasing the first half of the product. This covalent acyl-enzyme intermediate is then hydrolyzed by activated water to complete catalysis by releasing the second half of the product and regenerating the free enzyme

2.2.3 Biodiversity of proteases

Proteases occur in all organisms, from prokaryotes to eukaryotes to virus. These enzymes are involved in a multitude of physiological reactions from simple digestion of food proteins to highly regulated cascades (e.g., the blood-clotting cascade, the complement system, apoptosis pathways, and the invertebrate prophenoloxidase-activating cascade). Proteases can either break specific peptide bonds (limited proteolysis), depending on the amino acid sequence of a protein, or completely break down a peptide to amino acids (unlimited proteolysis). The activity can be a destructive change (abolishing a protein's function or digesting it to its principal components), it can be an activation of a function, or it can be a signal in a signalling pathway.

Plants

Protease-containing plant-solutions called vegetarian rennet have been in use for hundreds of years in Europe and the Middle East for making kosher and halal Cheeses. Vegetarian rennet from Withania coagulans has been in use for thousands of years as a Ayurvedic remedy for digestion and diabetes in the Indian subcontinent. It is also used to make Paneer.

Plant genomes encode hundreds of proteases, largely of unknown function. Those with known function are largely involved in developmental regulation. Plant proteases also play a role in regulation of photosynthesis.

Animals

Proteases are used throughout an organism for various metabolic processes. Acid proteases secreted into the stomach (such as pepsin) and serine proteases present in the duodenum (trypsin and chymotrypsin) enable us to digest the protein in food. Proteases present in blood serum (thrombin, plasmin, Hageman factor, etc.) play an important role in blood-clotting, as well as lysis of the clots, and the correct action of the immune system. Other proteases are present in leukocytes (elastase, cathepsin G) and play several different roles in metabolic control. Some snake venoms are also proteases, such as pit viper haemotoxin and interfere with the victim's blood clotting cascade. Proteases determine the lifetime of other proteins playing important physiological roles like hormones, antibodies, or other enzymes. This is one of the fastest "switching on" and "switching off" regulatory mechanisms in the physiology of an organism. By a complex cooperative action, proteases can catalyze cascade reactions, which result in rapid and efficient amplification of an organism's response to a physiological signal.

Bacteria

Bacteria secrete proteases to hydrolyse the peptide bonds in proteins and therefore break the proteins down into their constituent amino acids. Bacterial and fungal proteases are particularly important to the global carbon and nitrogen cycles in the recycling of proteins, and such activity tends to be regulated by nutritional signals in these organisms. The net impact of nutritional regulation of protease activity among the thousands of species present in soil can be observed at the overall microbial community level as proteins are broken down in response to carbon, nitrogen, or sulfur limitation.

Bacteria contain proteases responsible for general protein quality control (e.g. the AAA+ proteasome) by degrading unfolded or misfolded proteins.

A secreted bacterial protease may also act as an exotoxin, and be an example of a virulence factor in bacterial pathogenesis (for example, exfoliative toxin). Bacterial exotoxic proteases destroy extracellular structures.

Viruses

The genomes of some viruses encode one massive polyprotein, which needs a protease to cleave this into functional units (e.g. the hepatitis C virus virus and the picornaviruses). These proteases (e.g. TEV protease) have high specificity and only cleave a very restricted set of substrate sequences. They are therefore a common target for protease inhibitors.

2.2.4 Uses

Main article: Proteases (medical and related uses)

The field of protease research is enormous. Since 2004, approximately 8000 papers related to this field were published each year. Proteases are used in industry, medicine and as a basic biological research tool.

Digestive proteases are part of many laundry detergents and are also used extensively in the bread industry in bread improver. A variety of proteases are used medically both for their native function (e.g. controlling blood clotting) or for completely artificial functions (e.g. for the targeted degradation of pathogenic proteins). Highly specific proteases such as TEV protease and thrombin are commonly used to cleave fusion proteins and affinity tags in a controlled fashion.

3. Organic acids (citric acid, acetic acid)

3.1 Citric acid

Citric acid is a weak organic tricarboxylic acid (TCA) found in citrus fruit (lemons, orange, tomatoes, beets, etc.). It is a natural intermediate in kreb's cycle. It is naturally non-toxic due to its widespread presence.

3.1.1 Microorganisms used for citric acid production

Large number of microorganisms including bacteria, fungi and yeasts have been employed to produce citric acid. The main advantage of using these microorganisms are- (i) Easy to handling, (ii) Its ability to ferment a variety of cheap raw materials, (iii) High yield.

Fungi – *Aspergillus niger, A. aculeatus, A. awamori, Penicillum janthinellum.*

Bacteria – *Bacillus licheniformis, Arthrobacter praffinens, Corynebacterium* sp.

Yeast – *Saccharomyces lipolytica, Canidida tropicalis, C. oleophila, C. citroformans*

Glucose is the predominant carbon source for citric acid production. The biosynthetic pathway for citric acid production involves glycolysis wherein glucose is converted to two molecules of pyruvate. Pyruvate in turn forms acetyl CoA and oxaloacetate which condense to finally give citrate.

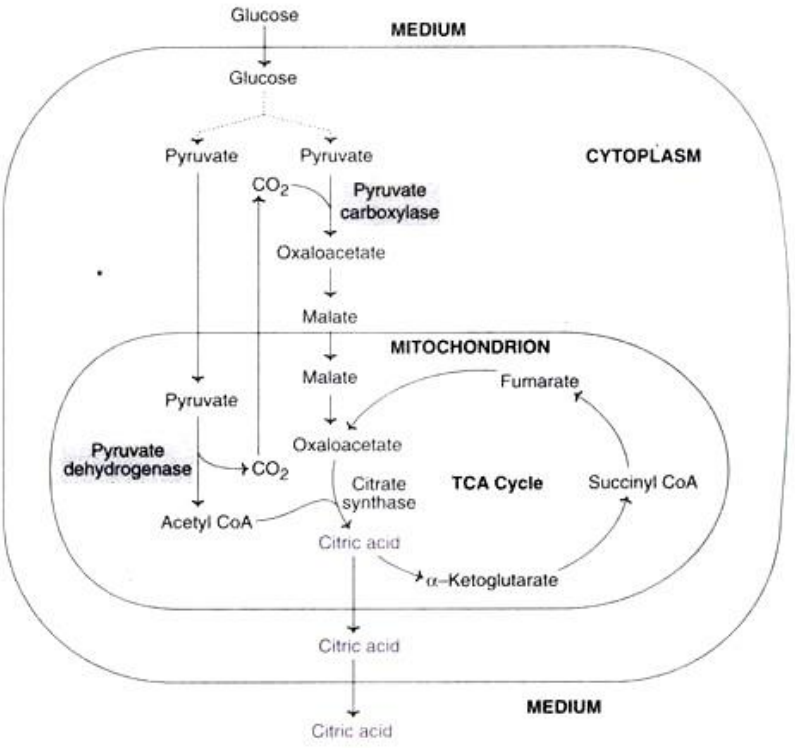


Fig: An outline of metabolic pathway for biosynthesis of citric acid

3.1.2 Production Processes for Citric Acid

There are two processes by which citric acid can be industrially produced — the surface process and submerged process

a) ***The surface process*:**This is characterized by growing the microorganisms as a layer or a film on a surface in contact with the nutrient medium, which may be solid or liquid in nature. Thus, the surface process has supported-growth systems.

b) ***The submerged process:***In this case, the organisms are immersed in or dispersed throughout the nutrient medium. There are two types of submerged fermenters (bioreactors) stirred bioreactors and airlift bioreactors.

3.1.3 Production of Citric Acid from Alkanes

Both yeasts and bacteria can be used for citric acid production from n-alkanes (C9-C23 hydrocarbons). The citric acid yield is better from hydrocarbons compared to sugars i.e. 145% of citric acid from paraffin. The most commonly used organism is *Candida lipolytica*. The fermentation can be carried out in batch, semi-continuous or continuous modes. The pH should be kept above 5. The major limitations of citric acid production from alkanes are—very low solubility of alkanes and increased production of unwanted isocitric acid.

3.1.4 Factors in the Regulation of Citric Acid Production

Strict maintenance of controlled nutrient conditions is very crucial for maximal production of citric acid.

a) Carbohydrate source: The concentration of carbohydrate significantly influences citric acid production. Ideally, the sugar concentration should be 12-25%. At a concentration less than 5% sucrose, citric acid formation is negligible, and increases as the concentration is raised to 10% and then stabilizes. It is believed that a high sugar concentration induces increased glucose uptake and consequently enhanced citric acid production

b) Trace elements: Certain trace elements (Fe, Cu, Zn, Mn, Mg, Co) are essential for the growth of A. Niger. Some of the trace metals particularly Mn^{2+}, Fe^{3+} and Zn^{2+} increase the yield of citric acid. These ions promote glycolysis and reduce respiration; both these processes promote citric acid production.

c) pH: The pH of the medium influences the yield of citric acid, and it is maximal when pH is below 2.5. At this pH,

the production of oxalic acid and gluconic acid is suppressed. Further, at low pH, transport of citric acid is much higher. If the pH is above 4, gluconic acid accumulates at the expense of citric acid. And when the pH goes beyond 6, oxalic acid accumulates. Another advantage with low pH is that the risk of contamination is very minimal, since many organisms cannot grow at this pH.

d) Dissolved O_2: The yield of citric acid production substantially increases when the dissolved O2 tension is higher. This can be achieved by strong aeration or by sparging with pure O2. It has been observed that sudden interruptions in O2 supply (as occurs during power breakdowns) cause drastic reduction in citric acid production without harming the growth of the organism.

e) Nitrogen source: Ammonium salts, nitrates and urea are the nitrogen sources used in the media for citric acid production. All the three compounds are equally good sources, as long as they do not adversely affect the pH of the medium. If molasses are used for nutrient supply, addition of extra nitrogen source is not required. However, some workers have shown that exogenous addition of ammonium ions stimulates citric acid production.

3.1.5 Application of citric acid

Food industries –

1. Canides – Prevent crystallization of sucrose, produce dark colour in hard candies, inversion of sucrose.
2. Dairy products – As emulsifier in ice creams and processed cheese, acidifying agent in many cheese products and as an antioxidant.
3. Jellies and jams – Gelling agent, provides the desired degree of tartness, tang and flavour.
4. Soft drinks and syrup – As acidulant in carbonated and sucrose based beverages, stimulates natural fruit flavour, incorporate tartness.
5. Pharmaceuticals – As effervescent in powders and tablets in combination with bicarbonates, solubilisation action for cathartics, antioxidant in vitamin preparations, acidulant in mild astringent formulations, anticoagulant.
6. As an emulsifying agent in ice-creams.
7. As an chelating agent.

3.2 Acetic acid

Acetic acid is colourless liquid with a sharp irritating smell. In aqueous solution it function as a weak acid. Pure acetic acid is called glacial acetic acid because it freezes at slightly below ordinary room temperature.Highly miscible with water, glycerol, ether, acetone and benzene.

3.2.1 Microorganisms used for citric acid production:

Although there are a large number of bacteria, as well as microorganisms, which have the ability to produce acetic acid in small amounts from various substrate, only relatively few bacteria possess the characteristics desired for vinegar production. The three important genera's are – *Acetobacterium aceti, A. xylinum, A. ascendens, Clostridium thermoaceticum, Gluconobacter, Acetobacter curvum, A. orleanense*.

3.2.2 Biosynthesis of acetic acid

Acetic acid is a product of incomplete oxidation of ethanol. Ethanol is first oxidized by alcohol dehydrogenase to acetaldehyde which then gets hydrated to form acetaldehyde hydrate. The latter is then acted upon by acetaldehyde dehydrogenase to form acetic acid.

For every molecule of ethanol oxidised, one molecule of acetic acid is produced. Thus, high- yielding strains can produce 11-12% acetic acid from 12% alcohol. For optimal production, adequate supply of oxygen is very essential. Insufficient O2, coupled with high concentration of alcohol and acetic acid result in the death of microorganisms. Surface fermentation or submerged fermentation processes can be carried out to produce acetic acid. Trickling generation process, a type of surface fermentation, is very commonly used.

3.2.3 Production of Vinegar

Vinegar is an aqueous solution containing about 4% by volume acetic acid and small quantities of alcohol, salts,

sugars and esters. It is widely used as a flavoring agent for processed liquid foods such as sauces and ketchups. The starting materials for vinegar production are wine, whey, malt (with low alcohol content). Vinegar production can be carried out either by surface process (trickling generator) or by submerged process.

Surface process: The fermentation material is sprayed over the surface which trickles through the shavings that contain the acetic acid producing bacteria. The temperature is around 30°C on the upper part while it is around 35°C on the lower part. Vinegar is produced in about 3 days.

Submerged process: The fermentation bioreactors are made up of stainless steel. Aeration is done by a suction pump from the top. The production rate inthe submerged process is about 10 times higher than the surface process.

3.2.4 Uses of acetic acid: Industrially, acetic acid is used in the preparation of

(i) Metal acetates – used in some printing processes.
(ii) Vinyl acetate – employed in the production of plastic.
(iii) Cellulose acetate – used in making photographic films and textiles.
(iv) Volatile organic esters (such as ethyl and butyl acetates), widely used as solvents for resins, paints, and lacquers.
(v) Biologically acetic acid is an important metabolic intermediate, and it occurs naturally in body fluids and in plant juices.
(vi) Acetic acid is largely used in the food industry as vinegar, and as an acidity regulator.

4. Amino acids

4.1 Glutamate:

L-Glutamic acid was the first amino acid to be produced by microorganisms. The original bacterium, *Corynebacterium glutamicum*, that was first used for large scale manufacture of glutamic acid continues to be successfully used even today. This was the birth of the use of monosodium glutamate (MSG) as a flavour-enhancing compound. The other important organisms (although used to a lesser extent due to low yield) employed for glutamic acid production belong to genera *Micro bacterium*, *Brevibacterium* and *Arthrobacter*. The successful commercialization of monosodium glutamate (MSG) with this bacterium provided a big boost for amino acid production and later with other bacteria like E. coli as well.

4.1.1 Biosynthesis of L-glutamic Acid:

Glutamic acid commercial production by microbial fermentation provides 90% of world's total demand, and remaining 10% is met through chemical methods. The pathway for the synthesis of glutamic acid with glucose as the carbon source. Glucose is broken down to phosphoenol pyruvate and then to pyruvate. Pyruvate is converted to acetyl CoA. Phosphoenol pyruvate (by the enzyme phosphoenol pyruvate carboxylase) can be independently converted to oxaloacetate. Both these carboxylation reactions are quite critical, and require biotin as the cofactor.

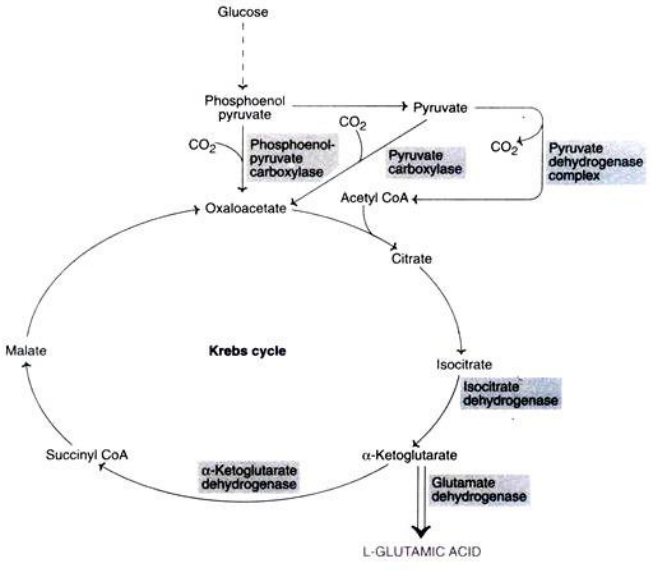


Fig: Biosynthesis of L-glutamic Acid

The next series of reactions that follow are the familiar citric acid (Krebs) cycle reactions wherein the key metabolite namely α-ketoglutarate is produced. In the routine citric acid cycle, α – ketoglutarate is acted upon by the enzyme α-ketoglutarate dehydrogenase to form succinyl CoA.

For the production of glutamic acid, α-ketoglutarate is converted to L-glutamic acid by the enzyme glutamate dehydrogenase (GDH). This enzyme is a multimer, each subunit with a molecular weight of 49,000. The reducing equivalents, in the form of NADPH + H+, are required by GDH. They are generated in the preceding reaction of Krebs cycle (catalysed by the enzyme isocitrate dehydrogenase) while converting isocitrate to α-ketoglutarate. The supply and utilization of NADPH + H+ occurs in a cyclic fashion through the participation of the two enzymes, namely isocitrate dehydrogenase and glutamate dehydrogenase.

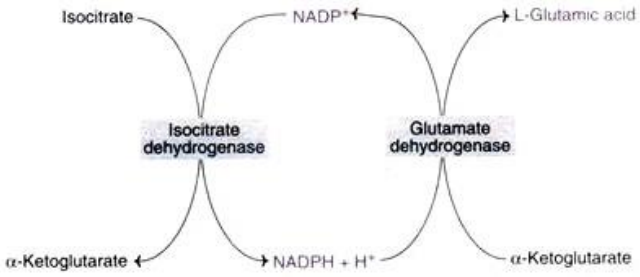


Fig: Biosynthesis of L-glutamic Acid-role of NADP+

Theoretically, one molecule of glutamic acid can be formed from one molecule of glucose. In practice, the conversion efficiency of glucose to glutamic acid was found to be around 70%.

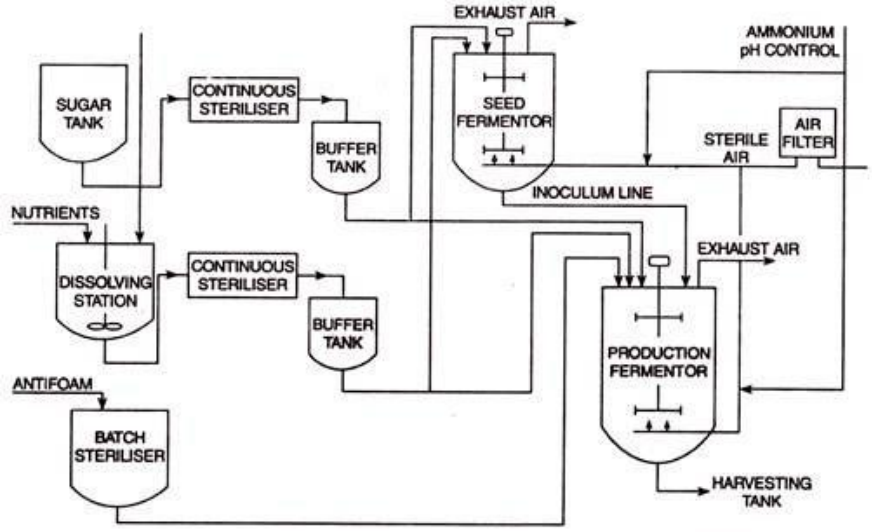


Fig: Flow diagram of commercial production method of Glutamic acid

4.1.2 Uses of Glutamic Acid:

As stated earlier, glutamic acid is widely used in the production of monosodium glutamate (MSG) which is commonly known as the 'seasoning salt'. The world production of glutamic acid is to the tune of 800,000 tonnes/year. Monosodium glutamate is condiment and flavour-enhancing agent, it finds its greatest use as a common ingredient in convenient food-stuffs.

4.2 Lysine:

Lysine is present at a low concentration in most of the plant proteins. Being an essential amino acid, supplementation of plant foods with lysine increases their nutritional quality. L-Lysine is predominantly produced by *Corynebacterium glutamicum* and to some extent by *Brevibacterium flavum* or *B. lactofermentum.*

4.2.1 Biosynthesis of L-lysine

The pathway for the synthesis of L-lysine is complex. This metabolic pathway is also involved in the formation of 3 other amino acids, namely methionine, threonine and isoleucine. As the glucose gets oxidised by glycolysis, phosphoenol pyruvate and pyruvate are formed. Both these metabolites can be converted to oxaloacetate, a key component of citric acid cycle. On transamination, oxaloacetate forms aspartate. The enzyme aspartate kinase converts aspartate to aspartyl phosphate which later forms aspartate semi-aldehyde.

Aspartate semi-aldehyde has two fates—the biosynthesis of lysine and formation of 3 other amino acids (methionine, threonine and isoleucine). When homoserine dehydrogenase acts on aspartate semi-aldehyde, it is diverted for the synthesis of 3 amino acids. The enzyme dihydrodipicolinate synthase converts aspartate semi-aldehyde (and pyruvate) to piperideine 2, 6-dicarboxylate.

There are two distinct enzymes succinylase variant (catalyses 4-step reaction) and dehydrogenase variant (catalyses a single step reaction) that can convert piperideine 2, 6-dicarboxylate to D, L-diaminopimelate which later forms L-lysine.

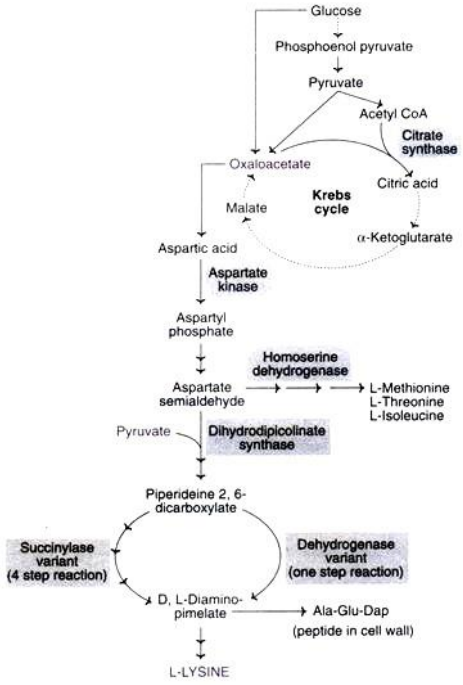


Fig: Biosynthesis pathaway of Lysine

4.2.2 Use of Lysine:

Lysine is a vital amino acid for humans. Since cereal proteins are often deficient in lysine, it is generally used as a supplement for nutritional requirement such as bread and other food stuffs for human being.

5. Alcoholic beverages (bear & wine)

5.1 Beer

The term beer is given to non-distilled alcoholic beverages made from partially germinated grains referred to as malt. The various beverages in this category include beer, ale, porter, stout, and malt tonics. The fermented product involving bottom yeast in the cold is called lager beer, while the beer involving top yeast and allowed to age at comparatively higher temperature are ale, stout, and porter. The ale contains a high level of hops and alcohol content

may be as high as 8.0%. Stout and porter employ heavy wort without malt adjuncts resulting in a dark-colored, heavy-bodied, and high alcohol content. The main ingredients of these fermentations are hops (offering beer a characteristic flavor and aroma), water, and yeast. The raw materials required for beer fermentation are: (i) Malt, (ii) Malt adjuncts, (iii) Microorganisms, (iv) Hops a ps, and (v) Water.

Malt: Malt is prepared carefully from selected barley. Barley is cleaned and steeped in water for two days. Then excess water is drained and allowed to germinate for up to 5-6 days to allow the formation of a short rootlet and acrospire. This step induces the activity of a-amylase, B-amylase, and protease enzymes as well as various flavor and color components. At the end of the incubation period, the temperature is raised just enough to kill the seed and at the same time not inactivate desired enzymes. This becomes green malt which is dried and stored for future fermentation use. At 2-3% moisture malt can be used. stored for several months. The preparation of good malt is a difficult task and requires careful selection of barley and close supervision of the malting process. Therefore, many leading brewing companies do not produce malt but rely on separate companies that specialized in this art. The production of dark-colored caramelized malt for brewing ale and stout are prepared by raising it to a higher temperature. Malt contributes amylases, proteases, starch, protein, additional yeast nutrients, growth factors, and flavor characteristics to the medium. Nearly 65% of malt constituents contribute to beer production, while the residue is separated and used as cattle feed or discarded

Malt adjuncts: Barley contains a considerable amount of protein, which forms a source of carbon and nitrogen, which may contribute to the dark color of the beer. It becomes unstable and filled with average taste. In order to decrease the carbon and nitrogen ratio, if they are in excess starch-containing malt adjuncts are added. The various sources of these malt adjuncts are grits or meals prepared from rice, degree corn, barley, wheat flour, prepared starches such as flaked corn, dextrose sugar a syrup. The starch of these adjuncts must first be gelatinized by boiling and saccharified by the amylases of malt. In the USA up to 60% may be used, while 40% is recommended in Europe. British beer is prepared from 75% malt and 25% malt adjuncts. On the other hand, in Germany, beer purity forbids the use of any adjuncts. Approximately 80-90% of the weight of these starch adjuncts is extracted into the fermentation medium.

Microorganisms: In beer brewing, both top-fermenting and bottom-fermenting strains d *Saccharomyces cerevisiae, S. carlbergensis, and S. uvarum*. employed. Top fermenting exhibits flotational flocculation behavior employed in ale and stout. The top yeast differs from the bottom yeast in their inability to ferment melibiose, on the other hand, better yeast performs sedimentary flocculation. Top yeasts are unable to ferment malt triose Yeasts are selected for their fermenting ability and their ability to flocculate at the proper time at the end of fermentation. Therefore, each industry selects its own strain. However, in fermentation, yeasts are selected from a previous fermentation, and inoculation of yeast is known as pitching. In order to avoid contamination, the yeast cell mass with tartaric acid, ammonium persulphate which inhibits the bacterial growth a washed phosphoric acid which makes the medium acidic (pH 2,5) and still some industries prefer to use fresh culture. 0.4536 kg of yeast is added as inoculum which yields approximately 1.39-1.84 kg at the harvest. Yeast is basically an aerobe and oxygen for its growth. By utilizing sugars, amino acids, and different minerals present need the wort, yeast grows at a faster rate which results in the bubbling of the fermentation medium.

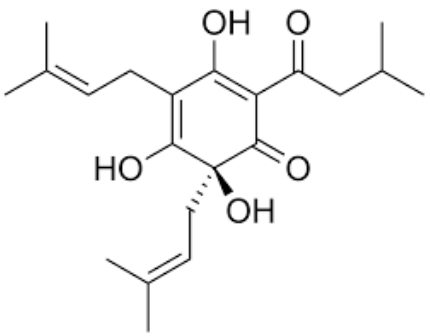


Fig: Structure of Humulones

Hops: Hops are the dried female flowers of *Hamulus lupulus* and *H. japonicas*Approximately 100 150g of hops per barrel of beer and up to 1kg per barrel of a are added. The hops provide the beer with its aromatic, pungent character and stabilizing effect. It contains A and B resins primarily humulones, lupus, and tannins which provide bitter flavor as well as preservative action against gram (+) positive bacteria. Tannins present in the bud help to coagulate protein

degradation products in the medium. The pectin of buds may contribute to the foam characteristics.

Water: Nearly 10-12 barrels are required to produce a barrel of beer obviously only a small portion of this water ends up in the finished product. The composition of water is very important in beer production as affects the flavor and properties of the finished product. In fact, the geographical location of a brewery often depends on the quality of the available water. Water containing more carbonates produces heavy flavored and dark-colored beer, while water without carbonates and the presence of sulfate results in the production of light beer, pale-ale with a light flavor and absence of harshness. The water with pH 6.5-7.0. carbonates of calcium, magnesium less than 100 ppm, trace amounts of magnesium, calcium (preferably as sulfate) 250-500 ppm, and calcium sulfate. sodium chloride 200-300 ppm and iron less than 1ppm are most suitable for beer production. The brewing is essentially divided into four main phases.

Beer The basic raw material for the brewing of beer worldwide is barley. The barley must be malted, a process whereby the barley starch is converted to a substrate (sugar) that is amenable to fermentation. Malt beverages are made by fermenting the malted barley, and these include beer, stout, porter, and malt liquor. Sake, an alcoholic beverage produced in Japan, is a product of malted rice. The starch present in barley, rice, and other grains is not available for fermentation unless the polymer is depolymerized to low molecular weight sugars.

The malting process is a series of manipulations that depolymerize starch to free ars. In the malting process, bar sag ley is soaked (steeped) in cold water so that the grains absorb sufficient water to germinate. The wet grain is then placed in rolling tubs or large open boxes in the presence of air Temperature and humidity are controlled to initiate germination of the grain. During germination, enzymes, including amylases and proteinases, are activated, and these cause the release of sugars and amino acids from the malting grain. The germination process is halted before sprouts develop, by drying the germinated grain in a kiln under a flow of warm air. The dried grain is then broken into small pieces in a grinding mill. Adjuncts such as corn grits or rice may be added at this stage and subjected to the milling process, which exposes the starch.

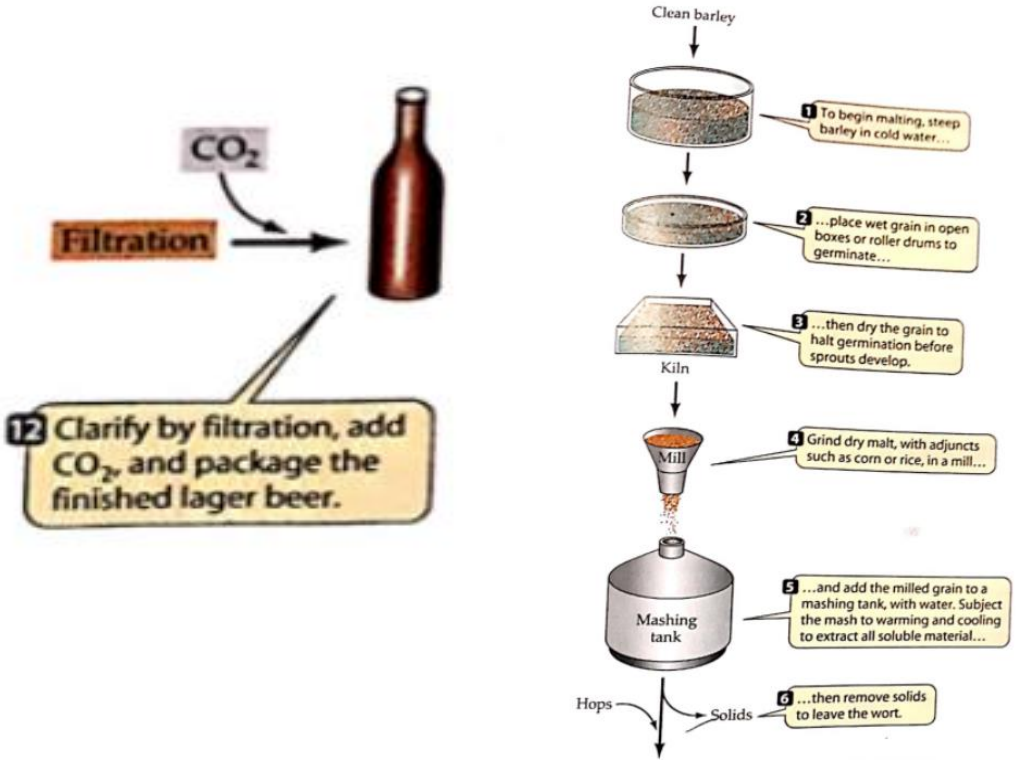
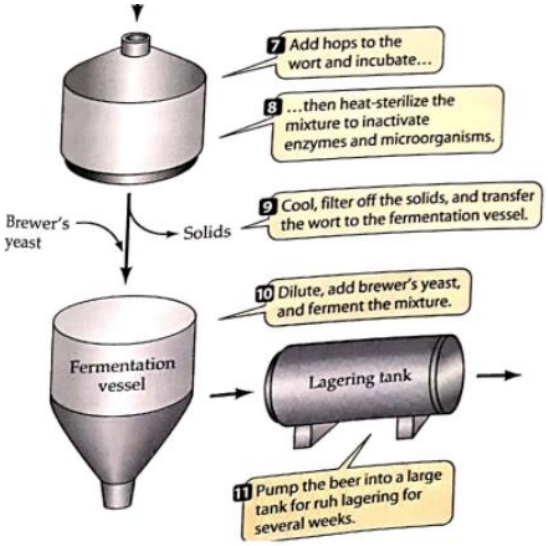


Fig: Flow chart of manufacture of beer.



The milled grain (mash) is placed in a mashing tank, and water is added. The mash is subjected to periods of warming and cooling to room temperature to extract all soluble material. During mashing, the proteinases and starch-hydrolyzing enzymes are very active. Following the mashing process.the solids are removed and the liquid remaining is the fermentable portion called wort. The wort is cooked in a brew kettle to halt enzymatic action and kill microorganisms. Hops are added, and the wort is heated to extract tannins and other flavoring agents from the hops. The wort is sterilized by live steam and filtered to remove hops and precipitates. The wort is then transferred to the fermentation vessel.

Quality beer production depends on careful attention to the details involved in the preparation of wort. During these processes, pH, length of heating/cooling cycles, removal of precipitates, and other factors are important in order to produce a desirable product. Another major concern in beer manufacturing is the quality of added water. It must be low in carbonates and calcium and properly balanced in other minerals. The brewing industry developed historically at sites where quality water was available, such as Pilsen, Czechoslo vakil; Munich, Germany; and Dublin, Ireland.

After the wort has been cooled and filtered, it is diluted with water. The fermentation is initiated either by a top-fermenting yeast (*Saccharomyces cerevisiae*) or a bottom-fermenting yeast (*Saccharomyces carlsbergensis*). Top fermenters, so-called because they are carried to the top (actually distributed throughout) by the CO produced during the fermentation, are used to produce ale. In contrast, the bottom fermenters, which tend to settle to the bottom, are used to produce beer. The top fermentation is carried out at 14 degrees C to 23 degrees C for 5 to 8 days. The ale is aged at 4 degrees to 8 degrees C The bottom fermentation for beer is carried out at 6 degrees C to 12 degrees C for about the same length of time as the ale. Following fermentation, the beer is pumped into a large tank for maturation. Yeast is added at about 1 million cells per ml, and the beer is allowed to "rest" for several weeks at 0.1°C. This process is called the rule (German, meaning "rest") or lagering.

The finished beer is clarified by centrifugation or filtration, CO is added, and the beer is packaged. Draft beer (kegs, bottles, or cans) is generally passed through membrane filters to remove all microorganisms. Prior to the availability of filter systems, bottled beer was pasteurized to prevent spoilage by acetic acid bacteria, and this pasteurization tended to produce off-flavors. Draft beer was considered superior because it was unpasteurized but was kept cold to prevent spoilage. Most European and American beer is a lager beer, although ales are increasingly being produced locally by microbreweries in the United States

Distilled Beverages Distilled spirits are extensions of the yeast brewing process just discussed. The fermented liquid is placed in a closed vessel with attached cooling coils. It is then heated, and the volatiles is condensed and collected.

Ethanol has a boiling point of 78.5°C, so it can be readily separated from the fermentation medium by distillation. The distillate obtained has a very high ethanol content and must be diluted before aging and bottling.
Gin, vodka, whiskey, rum, and brandy are examples of distilled alcoholic beverages. Gin and vodka are essentially distillate from the alcoholic fermentation of grain. Gin is produced when the alcohol is refluxed over juniper berries to extract the distinct aroma and flavor of the berries. Rum is manufactured by distilling fermented cane sugar molasses, and brandy is made from distilled wine. Distilled alcoholic beverages are 40% to 43% ethanol by volume (80 to 86 proof). Gin may be somewhat higher.

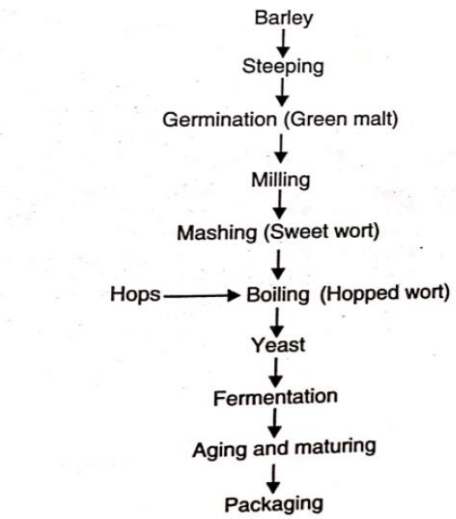


Fig: Flow chat of beer production.

Whiskey (spelled whisky in Scotland) is defined by the grain employed in the fermentation. Rye whiskey must have 51% or more rye grain in the malting process, and bourbon must have at least 51% corn. There are three types of Scotch whisky: malt whisky, grain whisky, and blended whisky. Malt whisky (single malt) is made from 100% malted barley. Grain whisky is produced from a variety of malted cereals (wheat, maize) that may or may not include malted barley. Blended whisky is a mixture of malt and grain whisky. Most Scotch whisky sold in the United States is blended, but single malt Scotch is growing in popularity. If a Scotch contains any whisky not made from malted barley, it cannot be labeled or sold as malt whisky. It is interesting to note that there are over 300 different single-malt
Scotches are available in Scotland. The manufacture of whiskey apparently originated in Ireland or Scotland and moved to the United States (bourbon), Canada, and Japan. Scotch and some bourbons are made by sour mash fermentation. A sour beer production encompasses five main stages. These include malting, the germination of barley grains; mashing, a stepwise heating process to promote starch hydrolysis; wort boiling. with hops; fermentation; and post-fermentation treatments.
Beer production encompasses five main stages. These include malting, the germination of barley grains; mashing, a stepwise heating process to promote starch hydrolysis; wort boiling. with hops; fermentation; and post-fermentation treatments.
Malting of barley: The barley grains are malted or soaked in water, to encourage germination. During germination, the endosperm (stored food) of the grain secretes berlin, a hormone that stimulates the growth of rootlets and the emerging stem. Gibberellin induces the aleurone (lining of the endosperm) to produce hydrolases, enzymes that break down starch to maltose, and proteins to amino acids for use by the growing plant. The hydrolases actually become

activated in the second stage, called mashing, when the grains a crushed and stirred in huge vats of water.

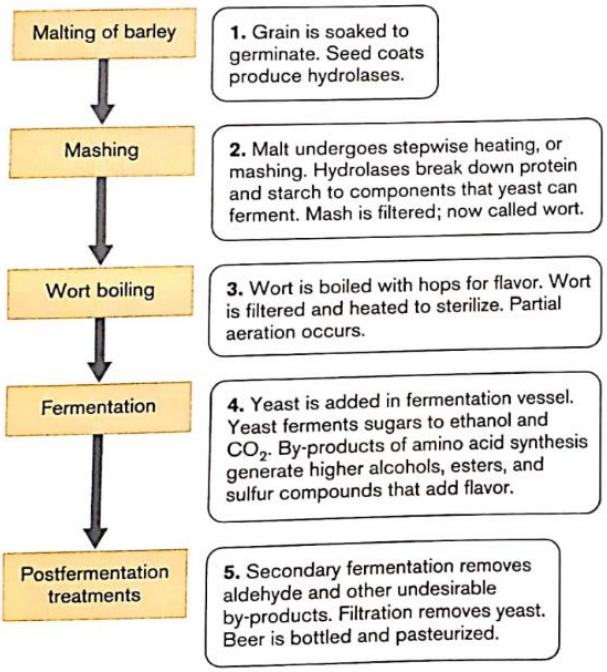
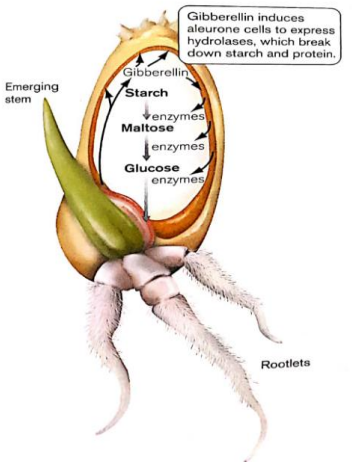


Fig: Processes of Beer production from barley and hop.

Mashing: While the grain is mashed, the temperature is raised in steps, each of which optimizes the activity of ad ferent hydrolase. At 52°C, the protein hydrolases and is activated. Then at 68°C, the starch hydrolases convert long-chain sugars to the disaccharide maltose. The final temperature (77°C) inactivates all enzymes; then the mash is cooled, pressed, and interred the liquid filtrate of the mash is called wort.

Wort boiling: The wort is supplemented with hops, a traditional herb used for centuries in Europe to contribute a de incentive flavor to the beer. After boiling with hops, the wort is again filtered.

Fermentation: The wort is inoculated with a special sat of Saccharomyces cerevisiae known as brewer's yeast and conducts ethanolic fermentation on the matone for hydrolyzed starch. At the same time, minor by-products, long-chain alcohols, impart good flavors. The time offered mentation is a key factor in the quality of the beer.



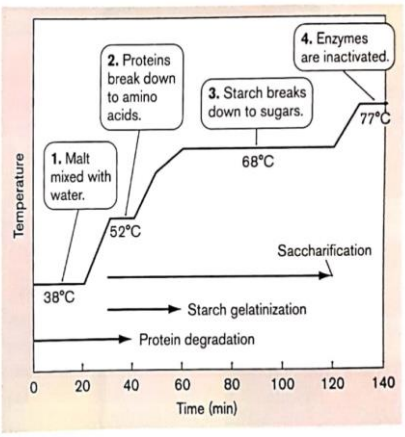


Fig: a) Melting the grain. **b) Mashing the wort.**

Post-fermentation treatment: This includes filtering the wort to remove the bulk of the yeast. The product, now recognizably beer, still contains undesirable levels of acetaldehyde and diacetyl generated by partial oxidation. These oxidized by-products can be reduced by the few remaining yeast cells during a period of secondary fermentation. During secondary fermentation, oxygen is completely excluded and the temperature is decreased to 15°C or lower. The best German beers.are aged at 2°C for several months.

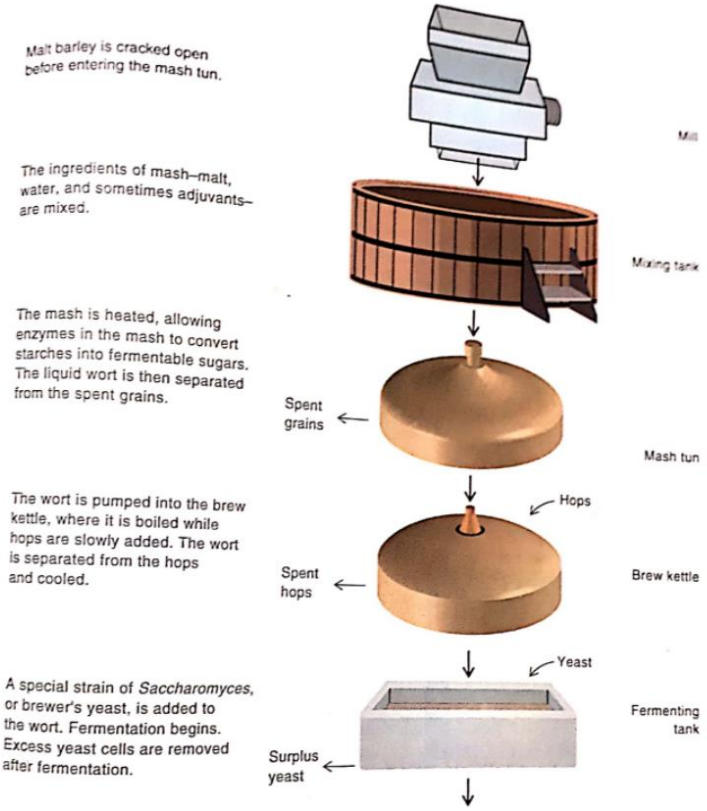
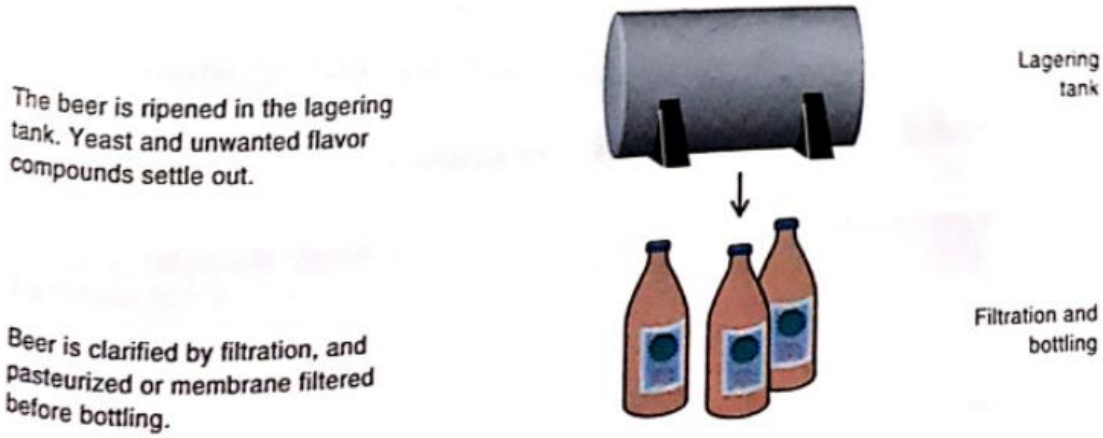


Fig: Commercial production of beer.



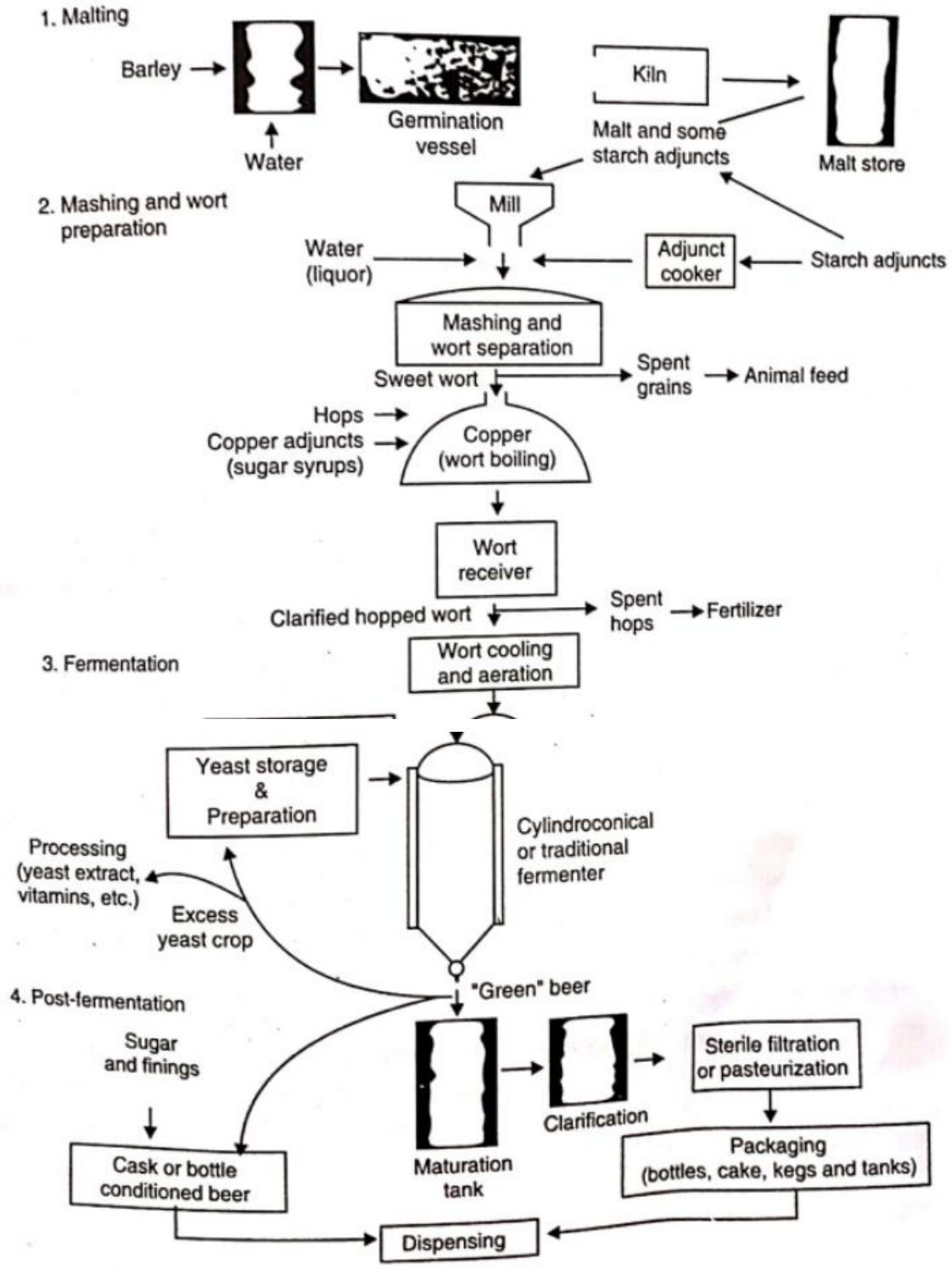


Fig: Commercial Production of beer.

5.2 Wine

Beverages Containing Alcohol

The alcoholic beverage industry is economically the most significant of all commercial processes that involve microorganisms. Alcohol-containing beverages such as beer and wine are as old as civilization itself. Mead is

considered to have been the first consumable alcoholic beverage. Mead is the name given to the product of honey fermentation and is the oldest word associated with drinking, it has been traced to the early Sanskrit language, quite likely, it originated when honey, which was gathered by the earliest recorded civilizations, became diluted with water, allowing natural fermentation to take place.

Alcoholic beverages are now produced worldwide from a variety of plant materials. Fruit sugars or grain polysaccharides are the major sources of fermentable substrates Wine, beer, and distilled beverages are the major alcoholic beverages produced commercially. Yeasts of the genus *Saccharomyces* are the organisms most involved in alcohol production. A discussion of the processes involved in the production of major alcoholic beverages follows.

Wine

There are many types of wine (such as grape, peach, pear, and dandelion), but by far the most favored is that made from grapes. The leading countries in wine production are Italy, Spain, France, Portugal, the United States, Australia, South Africa, and Argentina, Italy and Spain combined produce about 60% of the world's total. The best area for wine-producing vineyards is between 30 and 50° North or 30° and 40" South of the equator. In these areas of warm summer and mild winter climate, the grapes ripen slowly and yield superior wines. In the United States, California, New York, Washington, and Oregon have a favorable climate and soil for the growth of wine-producing grapes Wine occurs in two distinct types-white red.

Table: Major alcoholic beverages and sources of sugars for fermentation

Wine	Fruit sugars
Beer	Barley
whiskey	Rye, Corn, Barley
Tequila	Agave cactus
Mead	Honey
Sake	Rice

White wine is made from white grapes or can be made from red grapes provided the skins are removed from the must (must is crushed grapes ready for fermentation) prior to fermentation. Red wines gain the red pigmentation (and flavor), as they are partly fermented (3 to 10 days) in the presence of the red skins. During this period, the alcohol formed extracts and solubilities anthocyanin pigments present in the grape skin. Rosé (pink) wines are made from pink grapes or more often from red grapes but the time of exposure to the skin is for 12 to 36 hours, and much less of the anthocyanin pigments are solubilized. A dry wine is one in which means of the sugar has been fermented to ethanol. Sweet wine has more residual sugar. Fortified wine such as sherry or port contains higher levels of ethanol, and this is attained by supplementing them with ethanol that has been distilled from other wine or from fermented grain.

Yeasts are part of the natural microbiota of grapes. Crushed grapes will undergo a natural fermentation process that eventually produces wine. Natural fermentations are not favored commercially because the natural microbiota can be inconsistent and will not produce sufficient amounts of ethanol. Much of the wine produced by a natural fermentation is not potable. In commercial processes, the grapes are crushed and the must (crushed grapes) is treated with sulfur dioxide (SO) or sometimes pasteurized to destroy the natural microbiota. The must is then inoculated with a proprietary strain of *Saccharomyces* ellipsoids to bring about the desired fermentation. Laboratory-bred yeasts are favored because they have been selected to tolerate 12% to 15% ethanol, whereas the natural microbiota will cease fermenting when the ethanol content reaches 3.5% to 4%. Qualitywine contains 8% to 12% percent ethanol. White wine fermentations are quite direct. The grapes are destemmed, crushed, and skins and solids (pomace) removed. The must is treated with SO, and yeast is added. After fermentation, the wine is aged in casks, tanks, or bottles During a process called racking, red wine is drawn from the primary fermentation vat, placed in casks, and stored at a low temperature.

The aging process is typically much longer for red than white wines. Whereas white wines are ready for consumption within a year of production, most red wines require further aging. During the lengthy aging process used for some quality red wines, such as cabernet sauvignon, a second fermentation occurs. This is termed malo-lactic fermentation. The residual malic acid in the wine is fermented by lactic acid bacteria and converted to lactic acid and CO. This lowers the pH of the wine and improves the quality. After aging wines a clarified in a process called fining.

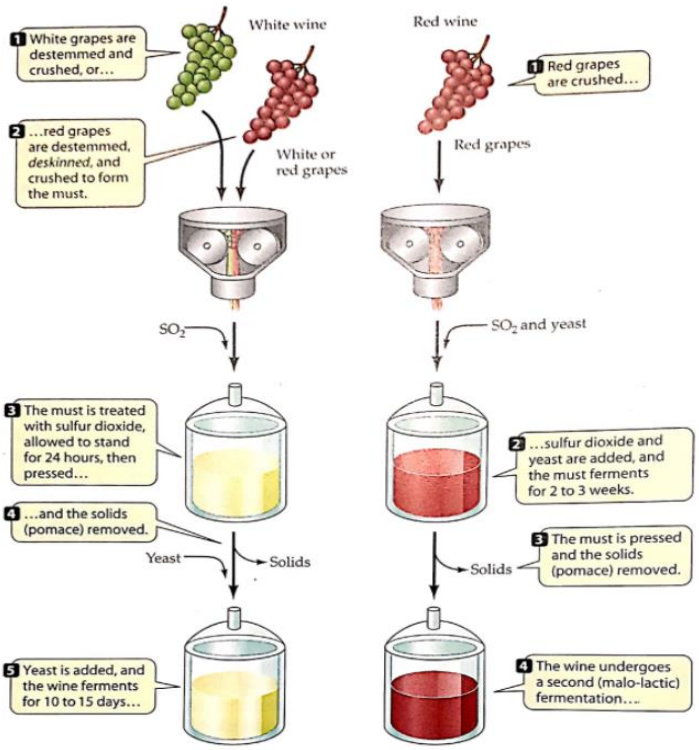
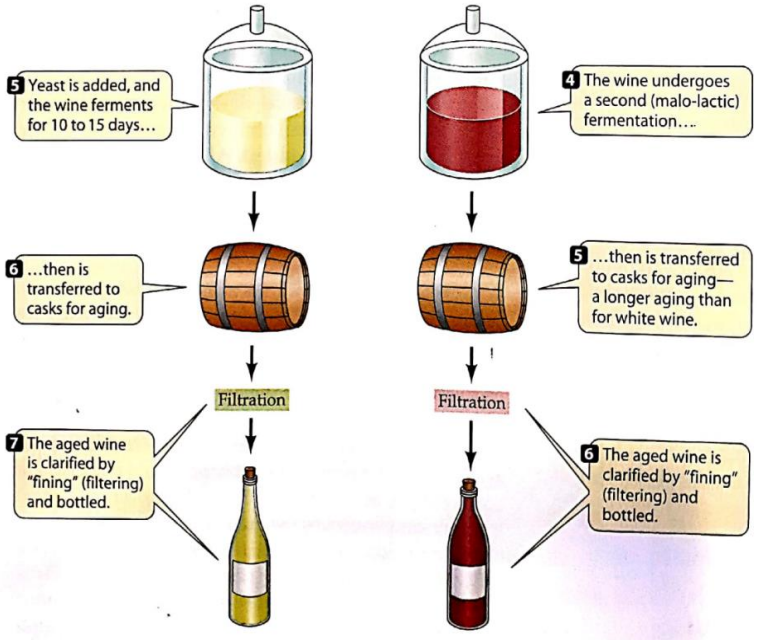


Fig: Manufacture of the wine flow chart for the production of white and red wine.



Fining can be accomplished by filtering through casein, diatomaceous earth or bentonite. A few wines are clarified by centrifugation.

Wine fermentations may be accomplished in tanks from 50 to 50,000 gallons, but it is essential that the temperature of the fermentation is maintained at 20°C to 24°C to produce quality wine. The heat generated during metabolism can raise temperatures above the tolerance level of the yeast. Aging is carried out at a lower temperature, as this improves flavour and aroma. Sparkling wines such as champagne are aged in bottles with a secondary yeast fermentation that generates carbonation (bubbles). Some champagne is carbonated by CO_2 injection after the fermentation. Champagne is aged in bottles that are slanted so that the cork faces down. This allows sediments to gravitate to the neck of the bottle. Periodically the neck is frozen, the bottle uncorked, and sediment removed. Wine lost during this process is replaced before recorking. All wines, including champagne, that are stoppered with a cork should be stored with the bottles lying on their side. This retains moisture in the cork and prevents air from entering. The presence of air allows the growth of vinegar bacteria that convert ethanol to acetic acid, thus "spoiling" the product.

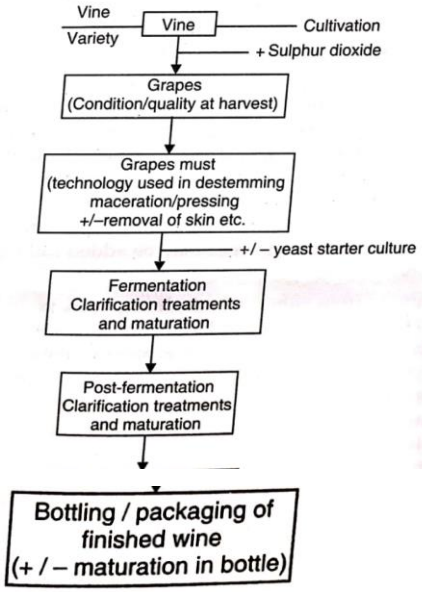


Fig: Flow diagram of wine production.

Importance of wines: Wines are reported to have medicinal properties and are responsible for improving the health of man which are as follows:

Table: Mineral content of different fruit wines.

Type of Beverages	Na	K	Mg	Ca	Fe	Cu	Zn	Mn
Apricot wine (New castle)	11	1481	18	71	2.72	0.96	1.92	0.88
Wild Apricot wine (Chudi)	43	2602	25	94	5.97	0.50	2.69	0.99
Apple wine (Golden delicious)	18	1044	16	144	3.68	0.21	0.76	0.84
Hard cidar (Golden delicious)	19	1069	17	97	3.03	0.19	0.91	0.82
Cidar (Apple juice concentrate)	61	1900	23	137	4.31	0.32	1.54	1.01
Pear wine (Sand pear)	87	1906	37	122	8.91	0.16	0.80	1.10
Plum wine (Santa Rosa)	20	1008	18	82	12.73	0.20	1.04	0.95

1. They contain antioxidants that are useful in preventing coronary heart diseases (CHD) by the following ways:
(i) Can prevent free radical damage to the tissues
(ii) Glutathione wheat
(iii) Acts as a chelating agent.
2. The wines increase the high-density lipoproteins (HDL) level in the body (also known as good cholesterol).
3. Provides nutrients and minerals.
4. Presence of glucose tolerant factor (GTF) in wine is a cure for diabetes.
5. Presence of resveratrol prevents cancer.
6. Different wines are a rich source of different minerals required by man.

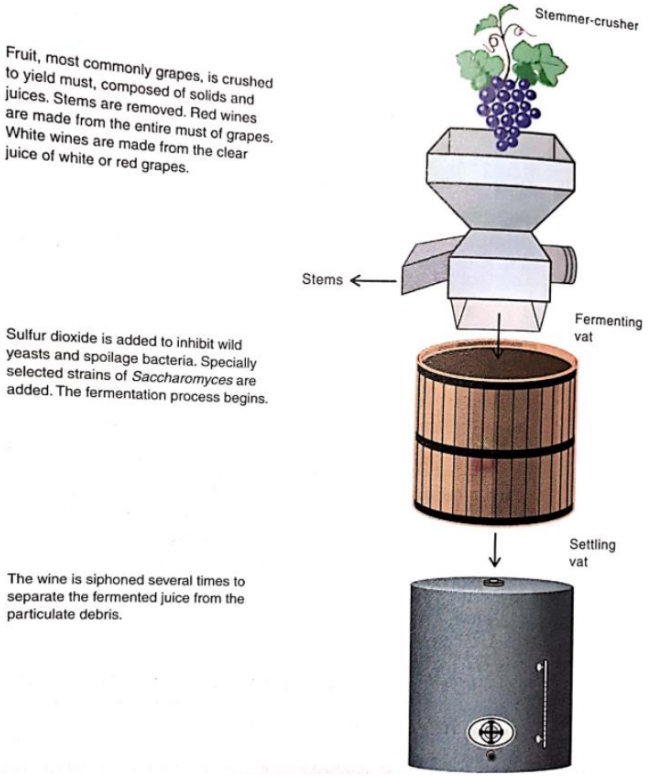
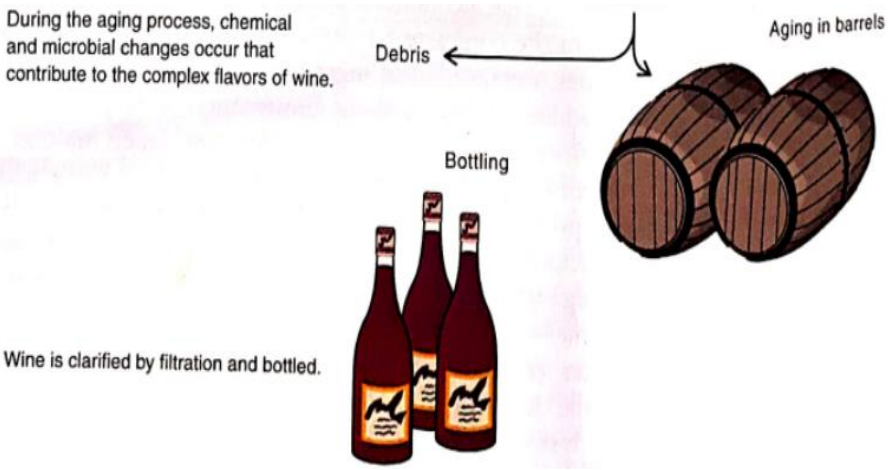


Fig: Commercial production of wine.



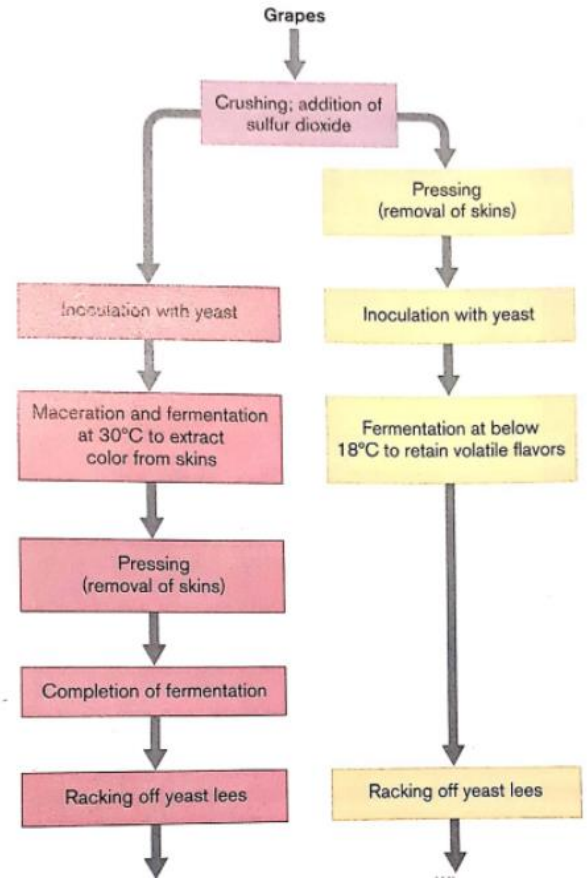
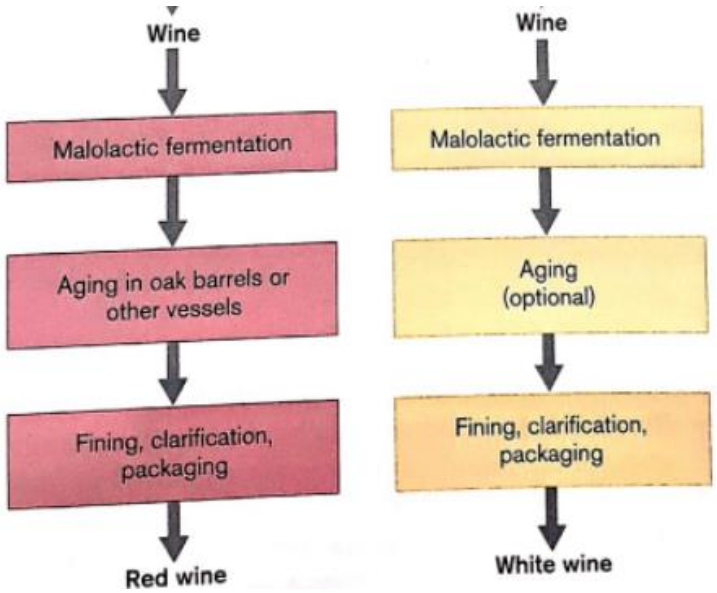


Fig: Production of red and white wine.

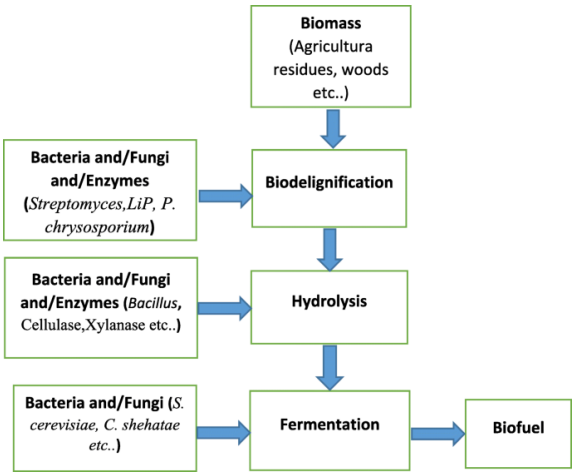


6. Biofuels

Biofuel is produced from living organisms and their waste. Ethanol, a commonly produced biofuel, is made from plants in the same way as the ethanol produced for alcohol. Some of the main plants used in ethanol production are sugar beet, corn, and sugar cane, due to the large presence of sugar which can then be easily fermented by microbes such as the yeast *Saccharomyces cerevisiae.*Biofuels have a vastly reduced impact on climate change compared to

fossil fuels. Studies have shown that biodiesel reduces hydrocarbon emissions by 75-90% and using it instead of diesel reduces emissions of carbon monoxide and smog-producing particulate matter by around 50%. It also eliminates all sulfur emissions.

Biofuel is regarded as one of the purest and most readily available fuels on the planet. Biofuels are made from biomass such as wood and straw, which is converted into a gaseous and liquid fuel by direct combustion of dry matter. Organic materials such as sludge, sewage, and vegetable oils matter, for example, can be turned into biofuels by a wet process such as digestion and fermentation.



However, biofuels are not perfect. It is expensive to convert plant material into useable ethanol. Also, there are ethical considerations. Large amounts of land must be used to produce fuel, which reduces the land available for food production, which is a problem in developing countries with large populations to feed. The amount of land needed also threatens vital ecosystems: In Brazil, there is a very real concern that rainforest will be cleared to grow sugar cane for this growing demand.

Using Microbes to Produce Better Biofuel

Micro-organisms are the subject of particular focus in several different scientific fields. They are abundant in most environments on earth and their use is driving a small but significant technological revolution. Microbes are being used to produce ethanol for biofuels which is produced from lignocellulose, a mixture of cellulose, hemicellulose, and lignin, which make up the plant cell wall.

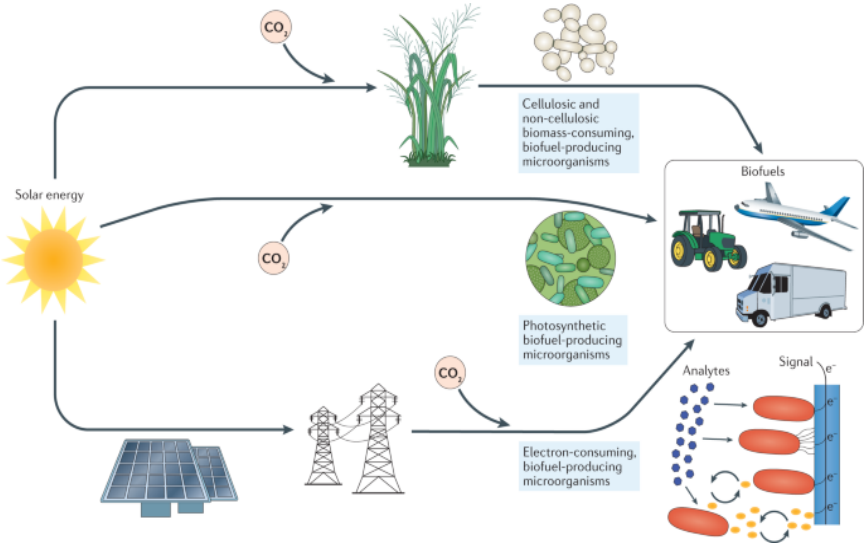


Fig: Biofuel production from microorganisms

The enzyme which breaks down cellulose is cellulase. Scientists have been investigating sources of this enzyme in several microbial species in diverse environments. Some of these environments are unusual, including the stomachs of termites, and soil found near volcanoes.

Sulfulobus solfatarticus is an archaeon found in volcanic pools near Mount Vesuvius. Researchers have recently been experimenting with genetic modification to improve this microbe's ability to produce the necessary enzymes.

The fungus *Trichoderma reesei* is found in soil globally. It feeds by secreting significant quantities of cellulase. Originally discovered during WWII, this fungus was responsible for "jungle rot" which broke down the cellulose in tents and uniforms of US soldiers. A Canadian company has genetically modified the fungus to produce larger quantities of cellulase and convert straw into glucose, which can then be turned into ethanol. They have managed to convert 75% of the straw into glucose.

Another possible solution is algae. These use photosynthesis to turn carbon dioxide into sugar, which they then use to produce lipids. Using small-scale laboratory bioreactors, scientists are using the lipids to create biodiesel and algal carbohydrates to produce bioethanol. If they can scale it up to industrial levels, the use of algae to produce biofuel may become a large part of the mix.

There is also the problem of waste. As the plant material used is inedible – for example, straw – this is reduced. A microbial system to produce biofuel is therefore less wasteful, more ethical, and cheaper. It also provides a significant reduction in greenhouse gas use and emission.

Microbes and Biofuel: Toward a Brighter Future

Climate change, the end of oil, overpopulation, and land use are existential problems for the human race. With innovative approaches such as the use of microbes to produce better biofuels, the mitigation of issues such as these is indeed possible. Much more research will be needed to provide these solutions on an industrial scale, but they are increasingly within our grasp as we move forward.

6.1 Ethanol Production:

Microbial production of one of the organic feed stocks from plant substances such as molasses is presently used for ethanol production. This alcohol was produced by fermentation in the early days but for many years by chemical means through the catalytic hydration of ethylene.

In modem era, attention has been paid to the production of ethanol for chemical and fuel purposes by microbial fermentation. Ethanol is now-a-days produced by using sugar beet, potatoes, com, cassava, and sugar cane.

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Both yeasts (*Saccharomyces cerevisiae, S. uvarum S. carlsbergensis, Candida brassicae, C. utilis, Kluyveromyces fragilis, K. lactis*) and bacteria (*Zymomonas mobilis*) have been employed for ethanol production in industries.

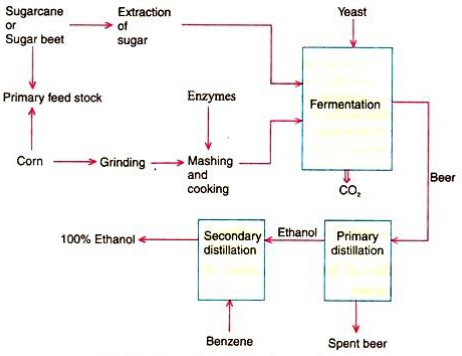


Fig: Ethanol production

The commercial production is carried out with *Saccharomyces cerevisiae*. On the other hand, uvarum has also largely been used. The *Candida utilis* is used for the fermentation of waste sulphite liquor since it also ferments pentoses.
Recently, experimentation with *Schizosaccharomyces* has shown promising results. When whey from milk is used, strain of *K. fragilis* is recommended for the production of ethanol. It is also found that *Fusarium*, *Bacillus* and *Pachysolen tannophilus* (yeast) can transform pentose sugars to ethanol.
Theoretically, it is interesting to note that fermentation process retains most of the energy of the sugar in the form of ethanol. The heat of combustion of solid sucrose is 5.647 MJ mol-1, the heat of combustion of glucose is 2.816 MJ mol^{-1} but the heat release is 1.371 MJ mol-1.

The equations are given below:

1st equation: Because $C_{12}H_{24}O_{11} \longrightarrow 4C_2H_5OH + 4CO_2$
(sucrose) (ethanol)
Hence, $5.647 \longrightarrow 4 \times 1.371 = 5.184$
i.e. 97% conversion

2nd equation: In this case,
$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2$
(glucose) (ethanol)
Hence, $2.816 \longrightarrow 2 \times 1.371 = 2.742$
i.e. 97% conversion

Thus, the above reactions show that 97% sugar transforms into ethanol. But in practice, the fermentation yield of ethanol from sugar is about 46% or one hundred grams of pure glucose will yield 48.4 grams of ethanol, 46.6 g of CO_2, 3.3 grams of glycerol and 1.2 g of yeast. It is noteworthy that the ethanol at high concentration inhibits the yeast. Hence, the concentration of ethanol reduces the yeast growth rate which affects the biosynthesis of ethanol.
It can produce about 10-12 % ethanol but the demerit of yeast is that it has limitation of converting whole biomass derived by their ability to convert xylulose into ethanol. The *Zymomonas* has a merit over yeast that it has osmotic tolerance to higher sugar concentration. It is relatively having high tolerance to ethanol and have more specific growth rate.

1. Preparation of Medium:

Three types of substrates are used for ethanol production:
(a) Starch containing substrate,
(b) Juice from sugarcane or molasses or sugar beet,
(c) Waste products from wood or processed wood. Production of ethanol from whey is not viable.
If yeast strains are to be used, the starch must be hydrolysed as yeast does not contain amylases. After hydrolysis, it is supplemented with celluloses of microbial origin so as to obtain reducing sugars. About 1 ton of starch required 1 litre of amylases and 3.5 litre of glucoamylases. Following steps are involved in conversion of starch into ethanol.

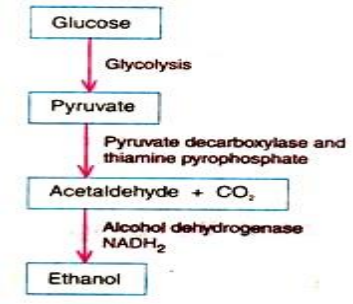


Fig: Biosynthesis of ethanol.

On the other hand, if molasses are used for ethanol production, the bagasse can also give ethanol after fermentation. Several other non-conventional sources of energy such as aquatic plant biomass, wood after hydrolysis with celluloses gives ethanol. Sulphite waste-liquor, a waste left after production of paper, also contains hexose as well as pentose sugar. The former can be microbially easily converted.

2. Fermentation:

The culture medium or supernatant is processed for recovery of ethanol. Ethanol is also produced by batch fermentation as no significant difference is found both in batch and continuous fermentation.

Although as stated earlier within 12h *Saccharomyces cerevisiae* starts producing ethanol at the rate of 10% (v/v) with 10-20g cells dry weight/lit. The reduction in fermentation time is accomplished use of ceil recycling continuously in fermentation.

3. Recovery:

Ethanol can be recovered upto 95 percent by successive distillations. To obtain 100 percent, it requires to form an azeotropic mixture containing 5 percent water. Thus, 5 percent water is removed from azeotropic mixture of ethanol, water and benzene after distillation. In this procedure, benzene water ethanol and then ethanol-benzene azeotropic mixture are removed so that absolute alcohol is obtained.

Neuberg's Fermentation:

Yeasts utilize pyruvate during fermentation resulting in the formation of an intermediary product acetaldehyde.

This is trapped by hydrogen sulfite to yield the acetaldehyde in precipitated form and fluid product formation is glycerol as shown below:

$$CH_3\,CHO + NaHSO_3 \rightarrow CH_2\text{-}CHOH\text{-}SO_3Na$$

Now in place of acetaldehyde, dihydroxyacetone phosphate acts as a hydrogen acceptor which is reduced to glycerol-3-phosphate.

After removal of phosphate i.e. dephosphorylation, it gives glycerol as given below:

$$C_6H_{12}O_6 + H_2SO_3 \rightarrow CH_2\text{-}CHOH\text{-}SO_3Na + \text{Glycerol} + CO_2$$

Neuberg's fermentation process is categorized as reward and third fermentation.

The first fermentation equation is given below:

$$2\text{Glucose} + H_2O \rightarrow C_2H_5OH + \text{acetate} + \text{glycerol} + 2CO_2$$

6.2 Methane Production

Methane is the most abundant constituent of biogas. It can also be directly used for various domestic and industrial purposes. The microbial generation of methane, appropriately referred to as methanogenesis from biomass occurs in four phases.

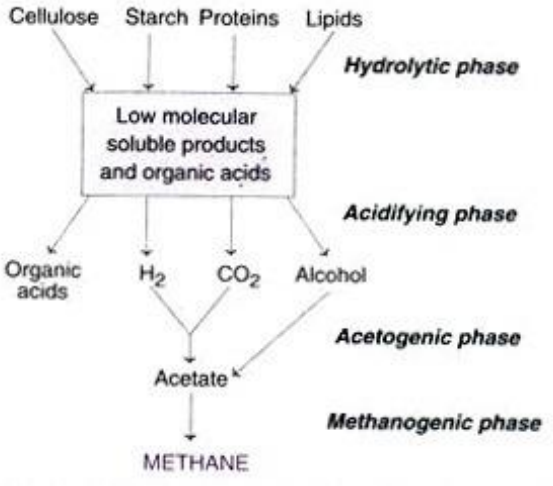


Fig: Microbial production of medicine

1. Hydrolytic Phase:

Certain facultative anaerobic bacteria hydrolyse the complex organic materials of the biomass (cellulose, starch, proteins, and lipids) to low molecular weight soluble products and some organic acids.

2. Acidifying Phase:

This phase is characterized by more formation of organic acids, besides H2, CO2, and alcohol.

3. Acetogenic Phase:

Acetogenic bacteria convert alcohol into acetate. These bacteria also generate acetate from H2 and CO2.

4. Methanogenic Phase:

This is the actual phase of methane gas formation. The methanogenic bacteria (e.g. Methanobacterium omelianskii, M. formicicum, M. bryantii, Methanosarcina barkeri) convert acetate, and CO2 and H2 into methane.

$CH_3COOH \rightarrow CH_4 + CO_2$

$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$

Some other substrates like formate and methanol can also be converted to methane.

$4HCOOH \rightarrow CH_4 + 3CO_2 + 2H_2O$

$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O$

The overall reaction of methane formation from glucose as the starting material may be represented as follows.

$C_6H_{12}O_6 \rightarrow 3CH_4 + 3CO_2$

The complex polysaccharides particularly lignin and cellulose due to their inefficient conversion, limit methane production. In the normal process of methanogenesis, approximately 50% of the complex polysaccharides contribute to methanogenesis.

Biogas production from biomass is an anaerobic process. The anaerobic digestion is usually carried out by using air tight cylindrical tanks which are referred to as anaerobic digesters. A digester may be made up of concrete bricks and cement or steel, usually built underground. The digester has an inlet attached to a mixing tank for feeding cow dung.

The methanogenic bacteria from another digester are also added with cow dung. The digester is attached to a movable gas holding or storage tank with a gas outlet. The used slurry (spent cow dung) comes out from the digester through an outlet. This can be used as manure. The anaerobic digester described above, is a low technology gobar gas plant, commonly used for domestic purposes in rural areas in India. The process of digestion usually takes about 2-3 weeks when cow dung is used as the substrate.

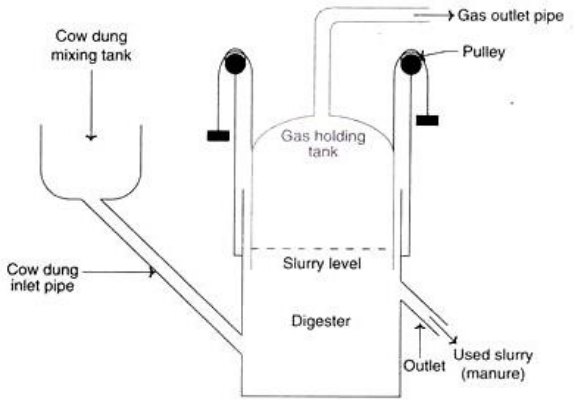


Fig: Diagrammatic representation of a biogas plant

Landfill Sites for Methane Production:

Landfill sites are low cost digesters built underground for the digestion of solid wastes (of industries and municipalities). As the anaerobic digestion of solid organic material occurs, methane gas is generated. It can be recovered by boring gas wells into the top of the landfill.

The factors affecting methane production, with special reference to biogas plant, are briefly described.

Factors Affecting Biogas (Methane) Production:

1. Temperature and pH:

The ideal temperature is 30-40°C, while the pH is 6-8, for good yield.

2. Slurry composition:

The ratio between solid and water composition in the slurry should be around 1: 1. A carbon nitrogen ratio of 30: 1 in the slurry results in optimal methane production. Good mixing and solubilization of the organic constituents is required.

3. Anaerobic conditions:

The digester should be completely airtight, so as to create suitable anaerobic conditions.

4. Presence of inhibitors:

Ammonium sulfate and antibiotics inhibit methane production. Agricultural wastes, pig and chicken manure (generating ammonia) and wastes from paper (rich in sulfate) inhibit biogas production.

6.3 Biogas Production

Biogas is the mixture of gases produced by the microorganisms. It is a renewable source of energy. Methane is the predominant gas present in the biogas mixture.

Certain bacteria grow under anaerobic conditions and produce a large amount of methane along with carbon dioxide and hydrogen. The bacteria which produce the gaseous mixture are collectively known as methanogens. Methanobacterium is one such methanogen.

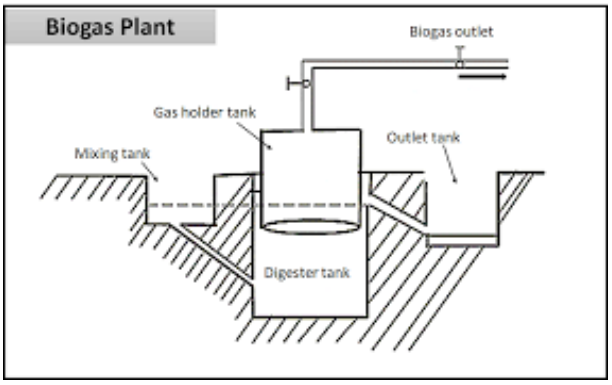
Methanobacterium is present inside the rumen of the cattle and the sludge produced during sewage treatment. The Methanobacterium present in the food of the cattle digests the cellulose present. The dung then produced by the cattle contains these methanogens which can be used for the production of biogas also known as the gobar gas.

Cattle dung is available in the rural area in very large quantities. Therefore, we can find biogas plants more often in rural areas. The biogas produced can be used for lighting and cooking purposes.

The conversion of waste into energy takes place in a biogas plant, by the activity of certain microbes.

Biogas Plant

The biogas plant consists of a source to supply the feedstock, a digestion tank for biogas production, a biogas recovery unit to isolate the produced biogas, and heat exchanger to maintain the temperature of the digester. The biowaste and the slurry of dung are fed into an anaerobic digester. The slurry is covered with a floating cover. The gas produced due to microbial activity makes the cover rise upwards. The produced biogas is supplied to the respective places through connected pipes and can be used for cooking and lighting. The used slurry is removed through an outlet and can be used as fertilizer later.

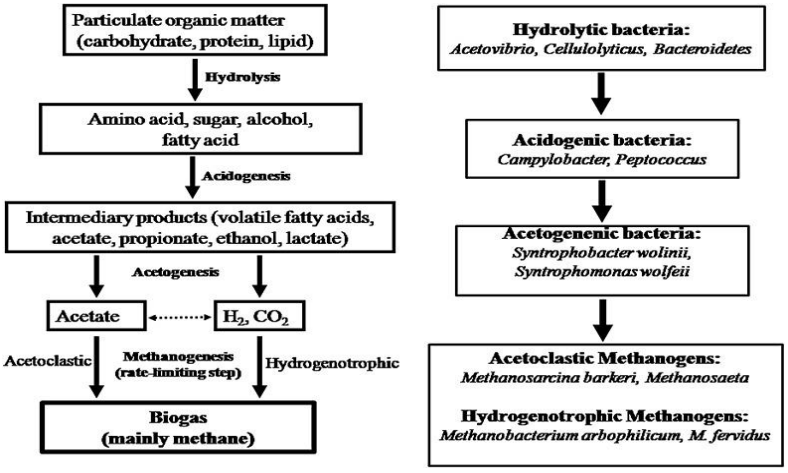


Biogas Production in Landfill

Apart from the biogas plant, the biogas is also produced in the landfills.

The organic matter naturally decomposes inside the landfill, i.e. inside a pit in the land, and biogas is produced by the activity of the microbes.

The Methanobacteria present in the organic waste decompose the waste and produce the mixture of gases known as the biogas. There is a network of interconnected pipes in the landfill to collect the gas produced. The composition of the gas varies after a certain time interval. After a year, the composition of methane and carbon dioxide is 60% and 40% respectively. This method is gaining acceptance due to the fact that it prevents the explosion caused by the collection of methane inside the landfill, and also prevents the loss of methane in the atmosphere. The biogas thus produced is used to create electricity.



Advantages of Biogas

- Biogas is a safe, cheap, renewable source of energy.
- Biogas can be burnt in stoves to provide heat.
- It is used for domestic and street lighting, and cooking.
- It is eco-friendly and does not cause any pollution.
- It is also used for driving engines.
- It is easy to generate, transport and store.
- It improves the sanitation of the surroundings.

The residue left after the production of biogas can be used as manure. The use of biogas is environment-friendly. It implies the conversion of animal and plant waste into useful energy, thereby, reducing the production of methane. This is because of the biogas combustion which results in a net decrease in the emission of greenhouse gases.

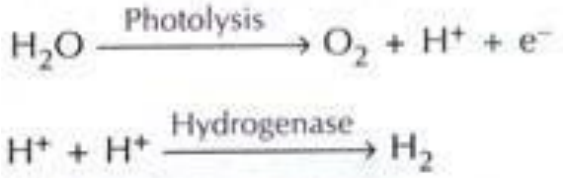
6.3 Biohydrogen Production

Hydrogen is a simple molecule which can be easily collected, stored (as a gas or liquid) and transported. It is highly combustible and can be used as a fuel or for the production of electricity. Hydrogen, on mixing with oxygen, provides around 30,000 calories per gram as compared to 11,000 and 8,000 calories per gram of gasoline and coal respectively.

Further, use of hydrogen is environmental friendly, since it is a non-pollutant. Hydrogen is truly a versatile fuel. It can be used for automobiles, aero planes, helicopters, buses, cars and scooters. Liquid hydrogen is considered to be an ideal fuel for subsonic and supersonic aircrafts world over.

6.3.1 By photosynthetic bacteria:

Biological production of hydrogen can be achieved by photolysis of water by photosynthetic algae and bacteria, a phenomenon referred to as bio-photolysis. Certain microalgae, and cyanobacteria (e.g. *Chlorella, Chlamydomonas, Scenedesmus, Microcystis, Oscillatoria, Anebaena*) can generate molecular hydrogen. Water is the source of raw material.



The action of hydrogenase can be inhibited by creating oxygen pressure. This condition favours release of free hydrogen. Isolated chloroplasts along with the bacterial enzyme hydrogenase have also been used for production of hydrogen.

6.3.2By fermentation:

It is possible to produce hydrogen from glucose, by bacterial action. However, the yield is less and uneconomical. Hydrogen can also be generated by anaerobic fermentation, by a process comparable to that of methane production. This is also not economical, besides being low in efficiency. Photosynthetic bacterium Rhodospirillium can be used to produce hydrogen from organic wastes.

6.3.3By legume crops:

The leguminous plants convert N_2 to NH_3 and H_2. This reaction is catalysed by the enzyme nitrogenase. In the normal circumstances, this H_2 gas, a byproduct of nitrogen metabolism is lost in the soil. It is estimated that from a soybean crop in one hectare field, about 30 billion m^3 hydrogen is generated and lost annually. As such, there are no methods available to trap such huge quantities of hydrogen produced in agricultural fields.

7. Biofertilizers

Chemical fertilizers are being used in increasing amounts in order to increase output in high yielding varieties of crop plants.However, chemical fertilizers cause pollution of water bodies as well as ground water, besides getting stored in crop plants.

Therefore, environmen-talists are pressing for switch over to organic farming. Organic farming is the raising of unpolluted crops through the use of manures, bifertilizers and biopesticides that provide optimum nutrients to crop plants, keeping pests and pathogens under control.

\Bio-fertilizers are micro-organisms which bring about nutrient enrichment of soil by enhancing the availability of nutrients to crops. The micro-organisms which act as bio-fertilizers are bacteria, cyanobacteria (blue green algae) and mycorrhizal fungi. Bacteria and cynobacteria have the property of nitrogen fixation while mycorrhizal fungi preferentially withdraw min-erals from organic matter for the plant with which they are associated.

Nitrogen fixation is the process of conversion of molecular or dinitrogen into nitrogen compounds. Insoluble forms of soil phosphorus are converted into soluble forms by certain micro-organisms. This makes the phosphorus available to the plants. Phosphate is also solubilised by some bacteria and by some fungi that form association with plant roots. The various bio-fertilizers are as follows.

7.1 Free Living Nitrogen Fixing Bacteria:

They live freely in the soil and perform nitrogen fixation. Some of them are saprotrophic, living on organic remains, e.g., *Azoto- bacter, Bacillus polymyxa, Clostridium, Beijerinckia*. They are further distinguished into aerobic and anaerobic forms.

The property of nitrogen fixation is also found in photoautotrophic bacteria, e.g., *Rhodopseudomonas, Rhodospirillum, Chromatium*. Inoculation of soil with these bacteria helps in increasing yield and saving of nitrogen fertilizers. For example, Azotobacter occurring in fields of Cotton, Maize, Jowar and Rice, not only in-creases yield but also saves nitrogen fertilizer to the tune of 10-25 kg/ha. Its inoculation is available under the trade name of azotobactrin.

7.2Free Living Nitrogen Fixing Cyanobacteria:

A number of free living cyanobacteria or blue-green algae have the property of nitrogen fixation, e.g., Anabaena, *Nostoc, Aulosira, Totypothrix, Cylindrospermum, Stigonema. Cyanobacteria* are photosynthetic. Therefore, they add

organic matter as well as extra nitrogen to the soil.

Aulosira fertilissima is consid-ered to be the most active nitrogen fixer of Rice fields in India (Aiyer et al, 1972). *Cylindrospermum licheniforme* grows in Sugarcane and Maize fields. Cyanobacteria are an extremely low cost biofertilisers. In Tamil Nadu, the technique of cyanobacteria inoculation to rice fields is being followed. Phosphate, Molybdenum and Potassium are supplied addi-tionally.

7.3 Loose Association of Nitrogen Fixing Bacteria:

Certain nitrogen fixing bacteria like *Azospirillum* live around the roots of higher plants without developing any intimate relationship. It is often called rhizosphere association. The bacteria obtain some plant exudate and use the same as part of their food requirement. The bacteria fix nitrogen and exude a part of the fixed nitrogen for use by the plant. The phenomenon is termed as associative mutualism (= associative symbiosis).

7.4 Symbiotic Nitrogen Fixing Bacteria:

They form a mutually beneficial association with the plants. The bacteria obtain food and shelter from plants. In return, they give a part of their fixed nitrogen to the plants. The most important of the symbiotic nitrogen fixing bacteria is Rhizobium (pi Rhizobia). It forms nodules on the roots of legume plants. There are about a dozen species of Rhizobium which form association with different legume roots, e.g., R. leguminosarum, R. lupini, R. trifolii, R. meliloti, R. phaseoli.

These bacteria, also called rhizobia, live freely in the soil but cannot fix nitrogen except for a strain of Cowpea Rhizobium (Me Comb et al, 1975). They develop the ability to fix nitrogen only when they are present inside the root nodules. In the nodule cells, bacteria (bacteroids) lie in groups surrounded by membrane of the host which is lined by a pink-red pigment called leghaemoglobin. Presently cultures of Rhizobium specific for different crops are raised in the laboratory.

Frankia, a nitrogen fixing mycelial bacterium (actinomycete), is associated symbiotically with the root nodules of several nonlegume plants like Casuarina, Alnus (Alder) Myrica, Rubus etc. Leaves of a few plants (e.g., Ardisia) develop special internal cavities for pro-viding space to symbiotic nitrogen fixing bacteria, Xanthomonas and Mycobacterium. Such leaves are a constant source of nitrogen fertilizer to the soil.

7.5 Symbiotic Nitrogen Fixing Cyanobacteria:

Nitrogen fixing cyanobacteria (blue- green algae) form symbiotic association with several plants, e.g., cycad roots, lichens, liverworts, Azolla (fern). Out of these, Azolla-Anabaena association is of great importance to agriculture.

Azolla pinnata is a small free floating fresh water fern which multiplies rapidly, doubling every 5-7 days. The fern can coexist with rice plants because it does not interfere with their growth. In some South-East Asian countries, especially China, the rice fields are regularly provided with *Azolla.*

Anabaena azollae resides in the leaf cavities of the fern. It fixes nitrogen. A part of the fixed nitrogen is excreted in the cavities and becomes available to the fern. The decaying fern plants release the same for utilization of the rice plants. When field is dried at the time of harvesting, the fern functions as the green manure, decomposing and enriching the field for the next crop.

7.6 Microphos Biofertilizers

They release phosphate from bound and insoluble states, e.g., Bacillus polymyxa, Pseudomonas striata, Aspergillus species.

7.7 Mycorrhiza

It is a mutually beneficial or symbiotic association of a fungus with the root of a higher plant. The most common fungal partners of mycorrhiza are Glomus species. Mycorrhizal roots show a sparse or dense wooly growth of fungal hyphae on their surface. Root cap and root hairs are absent.

The shape is irregular, tuberous, nodulated or coralloid. The fungus remains restricted to the cortex of the root. The vascular strand and growing point are not affected. Mycorrhiza often remains in the upper layers of the soil where organic matter is abundant. Depending upon the residence of the fungus, mycorrhizae are of two types—ectomycorrhiza and endomycorrhiza.

(a) Ectomycorrhiza (= Ectotrophic Mycorrhiza):

The fungus forms a mantle on the surface of the root. Internally, it lies in the intercellular spaces of the cortex. The root cells secrete sugars and other food ingredients into the intercellular spaces for feeding the fungal hyphae. The exposed fungal hyphae increase the surface of the root to several times. They perform several functions for the plant—

(i) Absorption of water,

(ii) Solubilisation of organic matter of the soil humus, release of inorganic nutrients, absorption and their transfer to root,

(iii) Direct absorption of minerals from the soil over a large area and handing over the same to the root. Plants with ectomycorrhiza are known to absorb 2-3 times more of nitrogen, phosphorus, potassium and calcium,

(iv) The fungus secretes antimicrobial sub-stances which protect the young roots from attack of pathogens. Ectomycorrhiza occurs in the trees like Eucalyptus, Oak (Quercus), Peach, Pine, etc. The fungus partner is generally specific. It belongs to basidiomycetes.

(b) Endomycorrhiza (- Endotrophic Mycorrhiza):

Fewer fungal hyphae lie on the surface. The remaining live in the cortex of the root, mostly in the intercellular spaces with some hyphal tips passing inside the cortical cells, e.g., grasses, crop plants, orchids and some woody plants. In seedling stage of orchids, the fungal hyphae also provide nourishment by forming nutrients rich cells called pelotons. Intracellular growth occurs in order to obtain nourishment because unlike ectomycorrhiza, the cortical cells do not secrete sugars in the intercellular spaces.

The hyphal tips passing into cortical cells either produce swollen vesicles or finely branched masses called arbuscules. Therefore, endomycorrhiza is also called VAM or vesicular-arbuscular mycorrhiza. The major benefits of VAM to the plant are the supply of inorganic nutrients as well as enhanced water absorption. Phosphate which is mostly present in the unavailable form in the soil, becomes abundantly available to the plant. A single fungus may form mycorrhizal association with a number of plants, e.g., Glomus.

7.8 Importance of Bio-fertilizers:

(i) They increase the yield of plants by 15-35%.

(ii) Bio-fertilizers are effective even under semi-arid conditions,

(iii) Farmers can prepare the inoculum themselves,

(iv) They improve soil texture,

(v) Bio-fertilizers do not allow pathogens to flourish,

(vi) They produce vitamins and growth promoting bio-chemical's,

(vii) They are non-polluting.

8. Food and dairy products

8.1 Food Products

8.1.1 Fruits and Vegetables:

Fruits and vegetables are generally contaminated by bacteria including species of *Bacillus, Enterobacter, Lactobacillus, Leuconostoc, Pseudomonas, Sarcina, Staphylococcus, Streptococcus* etc.

Various moulds and yeasts also inhabit the fruits and vegetables. Fruits and vegetables get rotten as a result of the microbial degradation of pectin, the substance responsible for maintaining the firmness and texture of fruits and vegetables.

Microbes produce pectin esterases and a polygalacturonases enzymes that hydrolyse pectins resulting in the formation of soft rots in fruits and vegetables. 20% of the harvested crops of fruits and vegetables are lost to spoilage mainly because of the activities of bacteria and micro-fungi.

(i) Contamination through infection:

Fruits and vegetables are normally susceptible to bacterial, fungal and viral infections. These infections invade the fruit and vegetable tissue during various stages of their development and result in the subsequent spoilage.

(ii) Contamination through post-harvest handling:

Usually, mechanical handling of fruits and vegetables during post-harvest period produce "breaks" in them which invite microbial invasion. Since the pH of the fruits is relatively acidic (i.e., high in sugar), they are more susceptible to fungi in contrast to vegetables which are more susceptible to bacteria because of their pH being slightly higher (5.0 to 7.0; less in sugar).

8.1.2. Cereals:

Cereals and cereal products contain microorganisms from insects, soil and other sources. Bacillus, Lactobacillus, Micrococcus, Pseudomonas, etc. are the bacteria which are generally found on freshly harvested grains.

Wheat flours are contaminated mostly by bacteria such as species of *Bacillus, Micrococcus, Sarcina, Serratia*, coliforms, etc. Moulds like *Aspergillus, Penicillium, Rhizopus, Neurospora, Endomyces* are also very common.

8.1.3 Microorganisms of Animal Food Products:

1. Meats:

The interior portions of meat are usually free of microbial contaminations if healthy animal is properly slaughtered. The fresh cut meat gets immediately contaminated with microorganisms derived from globes, hands, implements used to cut the meats, hides, hairs, intestines of animals, and the air of the slaughter house.

Each new surface of meat, resulting from a new cut, adds more microorganisms to the exposed tissue. The more common microorganisms occurring on fresh meats include both bacteria and moulds. Bacteria such as species of Bacillus, Clostridium.

Escherichia, Pseudomonas, *Lactobacillus*, *Micrococcus*. *Streptococcus*,*Sarcin*a.*Salmonella* occur most commonly. Moulds that contaminate fresh meat include *Cladosporium, Geotrichum, Mucor, Penicillium, Sporotrichum*, etc. Yeasts are less common in occurrence.

2. Eggs:

Clean eggs with un-cracked shell normally do not contain microorganisms within. Poor sanitary and storage conditions under which it is held determine its subsequent microbial content.

Bacteria and moulds may enter the egg through cracks in the shell. The microbial flora recovered from the egg shells generally include the species of bacteria Micrococcus, Pseudomonas, Streptococcus, Staphylococcus, Sarcina, and the moulds.

3. Poultry:

The surface of freshly dressed eviscerated poultry has microbial flora which is derived from the live birds or from the manipulations during killings, defeathering and evisceration. Species of Bacillus, Enterobacter, Escherichia, Proteus, Pseudomonas, Salmonella, and Staphylococcus constitute the major microbial flora on the skin of freshly dressed eviscerated poultry.

4. Fish:

The microbial flora of freshly caught fish usually reflects the microbial conditions of water from where they are harvested. Fish micro-flora includes bacteria like Alcaligenes, Micrococcus, Pseudomonas, Serratia, Vibrio, etc.

When the fish are cleaned and cut on shipboard under poor handling conditions, they invite more microorganisms to grow on it. These microorganisms can be examplified by the species of Achromonobacter, Bacillus, Micrococcus, Pseudomonas, etc.

8.1.4 Microorganisms of Processed Food Products:

The quality and quantity microorganisms associated with processed food including baked and fermented ones depends upon the ingredients used and processing methods. Microorganisms present in flour, sugar, fat, milk, egg, water, colours, manhandling, instruments, etc. may contaminate the baked food products.

Spore forming bacteria may escape destruction and become responsible for ropiness in baked bread during baking process. The ropiness of the bread is caused by *Bacillus subtilis* or *Bacillus licheniformis*. Further, the baked products

are subject to contamination by moulds such as *Mucor, Rhizopus, Aspergillus*, etc.

The fermented foods like pickles get contaminated by microorganisms through air, man, and equipment's. Most of these organisms do not multiply as the reduction of the medium is considerably acid. Some yeasts and yeast-like forms such as Torula, Oidium, etc. which are acid-tolerant, establish in these foods on

8.2 Dairy Products

Here is a list of top seven products of milk:- 1. Yoghurt 2. Kefir 3. Koumiss 4. Butter Milk 5. Butter 6. Cheese 7. Rennin.

1. Yoghurt:

Yoghurt is derived from a Turkish word 'Jugurt' which is the most popular fermented milk in the world now-a-days. It is made from milk, skimmed milk or flavoured milk. For the preparation of yoghurt, the milk should be free from contamination. The solid content (not fat) should be between 11-15% which can be obtained by adding skim or whole milk powder in fresh milk that normally contains 8% solids.

The product can be further improved by adding small amount of modified gums which bind water and impart thickening to the product. At this stage, the size of the fat particles in the milk should be around 2µm because this improves the milk's viscosity, product's stability and milk appear form. The milk is then heated at 80-90°C for 30 min., starter culture is added to it.

Heating improves the milk by inactivating immunoglobulins, remove excessive oxygen to produce microaerophilic environment which supports the growth of starter culture. Besides, heating also induce the interactions between whey or serum proteins and casein which increase yoghurt viscosity.

The milk is now cooled to 40-43°C so as to allow fermentation using starter organisms such as *S. salivarius* sub sp. *thermophilus* and Lb. delbruckii sub sp. bulgaricus together at a level of 2% by volume (106-107 cfu/ ml).

It is to be carried out for about 4h during which lactose is converted into lactic acid, pH decreases to a level of 6.3-6.5 to 4.6-4.7. The flavour in yoghurt is due to acetaldehyde which should be present at 23-41 mg/kg.

Both the organisms are lacking in alcohol dehydrogenase, and produce acetaldehyde from glucose portion of lactose via pyruvate and due to the action of threonine aldolase. Diacetyl, an important flavour giving agent is sometimes present in yoghurt. Finally, after completion of fermentation, the yoghurt is cooled to 15-20°C before adding fruity flavoured agents. It is then cooled to 5°C which helps in keeping upto 3 weeks.

Microbial pathogens or contaminants generally do not occur in yoghurt due to high acidity and low pH. But sometimes, it is spoiled by acidoduric microbe such as molds and yeasts.

Yeasts, particularly lactose fermenters Kluyveromyces fragilis and Saccharomyces cerevisae are particularly important but the yeast-like fungus, Geotricum and molds such as Mucor, Rhizopus, Aspergillus, Alternaria and Penicillium also spoil yoghurt. A good yoghurt should contain not more than 10 yeast g-1 with almost complete absence of coliform and molds.

2. Kefir: Kefir is in-fact, fermented milk, produced by a mixed lactic acid bacteria and alcoholic yeast. The microflora responsible is not spread uniformly throughout the milk but is supplemented as discrete kefir 'grains'.

The kefiran, i.e. large layers of polysaccharide material folds upon to produce a cauliflower like florets produce kefir. The outer smooth layer contains lactobacilli while the inner, rough side contains yeast and lactic acid bacteria. The capsular homo-fermentative Lactobacillus kefiranolaciens produces kefiran.

Lactobacillus kefir contributes the required effer-vescence in the product. Several yeasts such as Candida kefir, Saccharomyces cerevisiae and S.exiques have been observed, the latter utilize galactose preferentially glucose.

For commercial production of kefir, milk is homogenized and heated at 85- 95°C for 3-10 minutes. It is then cooled to 22°C before addition of kefir grains at a size of 5%. The fermentation should be carried out for 8-12 h. The product contains 0.8% acidity, 0.01% to 1.0% ethanol, carbon dioxide, acetaldehyde, and diacetyl etc.

3. Koumiss:

Koumiss is pro-duced from mare's milk which is greyish white drink, produced mostly in East European countries. It has L. delbruckii sub sp. bulgaricus and a number of lac-tose fermenting yeast responsible to pro-duce koumiss.

These are spread through-out the koumiss. The lower fat content and higher carbohydrate level in the milk is suitable for koumiss production.

Saccharomyces Cerevisiae, an yeast involved in the Production of Fermented Milk

4. Butter Milk:

It is a liquid substance which is separated from cream during churning process. A mixture of starting cultures such as Lactobacillus lactis sub sp. diacetylactis and Leuconostoc mesenteroides sub sp. cremoris produce butter milk.

Since citrate me-tabolism can provide the pyruvate, lead to the accumulation of diacetyl, hence fresh milk should contain citrate for the production of butter milk. If not, the milk is supplemented with 0.1-0.2% sodium citrate to ensure good flavour development. For the production, milk is pasteurized, homogenized and fer-mented at 22°C for 12-16 h. The final product contains 0.7-0.9% lactic acid. *Streptococcus*, Lactic Acid producing Bacterium, is responsible for Separation of Butter Fat

5. Butter:

Lactic acid producing bacteria are responsible for subsequent separation of butter fat in the churning process. These organisms (Uuconostoc citrovorum, Streptococcus cremoris or S. lactis) produce a small amount of acetoin which is spontaneously oxidized to diacetyl.

This substance gives butter and similar products with their buttery flavour and aroma. When the pH reaches about 4.3, it ceases growth, but its enzymes attack the citrates in the milk and produce diacetyl. But neither S. ceremoris nor Leuconostoc alone can produce the desired result in commercial practice.

6. Cheese:

There are about 2000 varieties of cheese made from mammalian milk. Cheese is thought to have originated in south western Asia some 8000 years ago. The Romans encouraged technical improvements and stimulated the development of new varieties during their invasions in Europe between 60 B.C. and A.D. 300. The cheese name is derived from Latin name caseus.

There are two groups of cheese, fresh cheese and ripened cheese. The fresh cheese are made up of milk coagulated by acid or high heat e.g. cottage cheese, while ripened cheese are made through lactic acid bacterial fermentation and coagulated by an enzyme preparation. The curd is removed and salted and whey is separated. The salted curd is held in controlled environment.

During this process, various physical and chemical changes occur to give a characteristic flavour and texture. Just as the variety of grape influences the flavour and bouquet of wine, so the mammalian origin of milk influences the flavour and aroma of a natural ripened cheese.

There are mainly three categories of cheeses:

(a) Soft cheese,

(b) Hard cheese, and

(c) Semisoft cheese.

The soft cheeses are ripened by the enzymes from yeast and other fungi that grow on the surface, hard cheese ripened by lactic acid bacteria which grows throughout the cheese, die, autolyze and release hydrolytic enzymes. The semisoft cheeses are ripened by proteolytic and lipolytic organisms which soften the curd and give it flavour.

Some common cheeses in international market

7. Rennin (Milk-coagulating Enzymes):

The milk coagulation enzyme, rennet, has its importance in cheese production. Certain commonly used cultures which have an ability to convert lactose to lactic acid and lower the pH, are Streptococcus lactis, S. cremoris, S. thermophilus, Lacto-bacillus Helveticas and L. bulgaricus. These organisms bring the desired curd structure and flavour with minimum gas produc-tion.

Rennet is an extract from the fourth stomach of 3 to 4 week old calves which have been fed on milk. The enzyme so purified, called ren-nin, chymase or chymosin.

Rennin production of microbial origin is now widely recommended due to good coagulation of casein without hydrolysis, good odour and struc-ture of cheese, nontoxic, low pro-tein denaturation in order to prevent the development of bitter taste during ripening process and low lipase activity, to check the rancidity development.

There are several genera of fungi reported to produce rennin. These are species of *Aspergillus, Candida, Coriolus, Rhizopus, Mucor, Penicillium, Torulopsis etc. Mucor pusillus, M. miehei* and *Endothecia parasiticus* are widely used.The enzyme is an acid protease, which is stable at pH 4.0- 5.5 with a molecular weight of 34000-37500 Dalton at 50°C temperature.

Fermentation:

A culture consists of soy meal (3%), glucose (1%), skim milk (1%), NaNO3 (0.3%), K2HPO4, (0.05%), MgSO4.7H2O (0.025%) at pH 6 litre-1 is mixed, autoclaved and fermentation takes 48 hours at 28°C. The extracellular enzyme is concentrated after separating the mycelium. Recently cDNA from calf protein has been cloned in E. coli resulting into the microbial production of calf renin enzyme.

9. Microbes in biological warfare

Biological and toxin weapons are either microorganisms like virus, bacteria or fungi, or toxic substances produced by living organisms that are produced and released deliberately to cause disease and death in humans, animals or plants. The act of bioterrorism can range from a simple hoax to the actual use of these biological weapons, also referred to as agents. Several nations have or are seeking to acquire biological warfare agents, and there are concerns that terrorist groups or individuals may acquire the technologies and expertise to use these destructive agents. Biological agents may be used for an isolated assassination, as well as to cause incapacitation or death to thousands. If the environment is contaminated, a long-term threat to the population could be created.

Biological agents like anthrax, botulinum toxin and plague can pose a difficult public health challenge causing large numbers of deaths in a short amount of time. Biological agents which are capable of secondary transmission can lead to epidemics. An attack involving a biological agent may mimic a natural event, which may complicate the public health assessment and response. In case of war and conflict, high-threat pathogens laboratories can be targeted, which might lead to serious public health consequences. Biological weapons form a subset of a larger class of weapons sometimes referred to as unconventional weapons or weapons of mass destruction, which also includes chemical, nuclear and radiological weapons. The use of biological agents is a serious concern, and the risk of using these agents in a terrorist attack is thought to be increasing.

9.1 How Are Biological Agents Delivered and Detected

Although there are more than 1,200 biological agents that could be used to cause illness or death, relatively few possess the necessary characteristics to make them ideal candidates for biological warfare or terrorism agents. The ideal biological agents are relatively easy to acquire, process, and use. Only small amounts (on the order of pounds and often less) would be needed to kill or incapacitate hundreds of thousands of people in a metropolitan area. Biological warfare agents are easy to hide and difficult to detect or protect against. They are invisible, odorless, tasteless, and can be spread silently.

9.2 Delivery

Biological warfare agents can be disseminated in various ways.Through the air by aerosol sprays: To be an effective biological weapon, airborne germs must be dispersed as fine particles. To be infected, a person must breathe a sufficient quantity of particles into the lungs to cause illness.

Used in explosives (artillery, missiles, detonated bombs): The use of an explosive device to deliver and spread biological agents is not as effective as the delivery by aerosol. This is because agents tend to be destroyed by the blast, typically leaving less than 5% of the agent capable of causing disease.

Put into food or water: Contamination of a city's water supplies requires an unrealistically large amount of an agent as well as an introduction into the water after it passes through a regional treatment facility.

Absorbed through or injected into the skin: This method might be ideal for assassination but is not likely to be used to cause mass casualties.

9.3 Biological warfare agents

Biological warfare agents differ greatly in the type of organism or toxin used in a weapons system, lethality, length of incubation, infectiousness, stability, and ability to be treated with current vaccines and medicines. There are five different categories of biological agents that could be weaponized and used in warfare or terrorism. These include:

Bacteria—single-cell organisms that cause diseases such as anthrax, brucellosis, tularemia, and plague.

Rickettsiae—microorganisms that resemble bacteria but differ in that they are intracellular parasites that reproduce inside cells. Typhus and Q fever are examples of diseases caused by rickettsia organisms.

Viruses—intracellular parasites, about 1/100 the size of bacteria, that can be weaponized to cause diseases such as Venezuelan equine encephalitis.

Fungi—pathogens that can be weaponized for use against crops to cause such diseases as rice blast, cereal rust, wheat smut, and potato blight.

10. Microbial leaching (copper, uranium)

Soil microorganisms are very closely involved as catalytic agents in many geological processes. These include mineral formation, mineral degradation, sedimentation and geochemical cycling. In recent years, a new discipline of mineral science namely bio-hydrometallurgy or microbial mining (mining with microbes) is rapidly growing. Broadly speaking, bio-hydrometallurgy deals with the application of biotechnology in mining industry. In fact, microorganisms can be successfully used for the extraction of metals (e.g., copper, zinc, cobalt, lead, uranium) from low grade ores. Mining with microbes is both economical and environmental friendly.

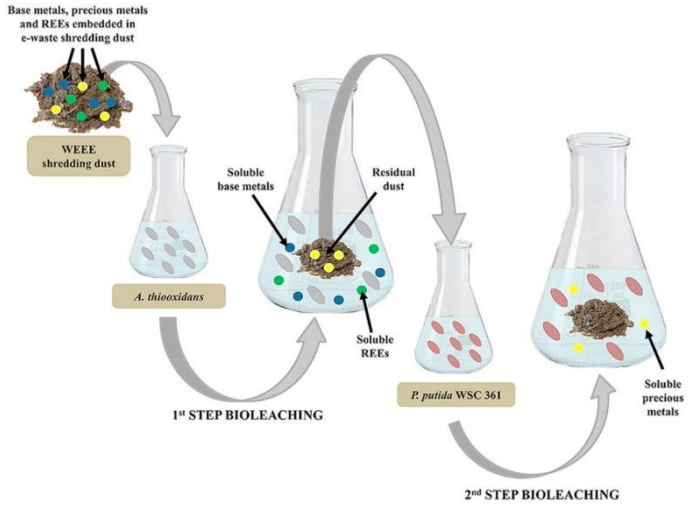


Fig: Bioleaching process.

The term metal is used to any substance that is hard, possessing silvery lusture, and is a good conductor of heat and electricity. Some of the metals, however, are relatively soft, malleable and ductile e.g., sulfur. An ore is a naturally occurring solid mineral aggregate from which one or more minerals can be recovered by processing. Majority of microorganisms can interact with metals.

The metals can be recovered by the microorganisms by two processes.

1. Bioleaching or microbial leaching: This broadly involves the extraction or solubilization of minerals from the ores by microorganisms.

2. Bio-sorption: It deals with the microbial cell surface adsorption of metals from the mine wastes or dilute mixtures.

10.1 Bioleaching:

In microbial leaching (bioleaching), metals can be extracted from large quantities of low grade ores. Although recovery of metals (e.g. copper) from the drainage water of mines has been known for centuries, the involvement of

microbes in this process was recognized about 40 years ago.

The bacteria which are naturally associated with the rocks can lead to bioleaching by one of the following ways.

1. Direct action of bacteria on the ore to extract metal.
2. Bacteria produce certain substances such as sulfuric acid and ferric iron which extract the metal (indirect action).

In practice, both the methods may work together for efficient recovery of metals.

10.2 Organisms for bioleaching:

The most commonly used microorganisms for bioleaching are Thiobacillus ferrooxidans and Thiobacillus thiooxidans. Thiobacillus ferrooxidans is a rod-shaped, motile, non-spore forming, Gram-negative bacterium. It derives energy for growth from the oxidation of iron or sulfur. This bacterium is capable of oxidising ferrous iron (Fe^{2+}) to ferric form (Fe^{3+}), and converting sulfur (soluble or insoluble sulfides, thiosulfate, elemental sulfur) to sulfate (SO^{2-}_4). Thiobacillus thiooxidans is comparable with T. ferrooxidams, and grows mostly on sulfur compounds.

Several studies indicate that the two bacteria *T. ferrooxidans* and *T. thiooxidans*, when put together, work synergistically and improve the extraction of metals from the ores. Besides the above two bacteria, there are other microorganisms involved in the process of bioleaching. A selected few of them are briefly described below.

Sulfolobus acidocaldarius and *S. brierlevi* are thermophilic and acidophilic bacteria which can grow in acidic hot springs (>60°C). These bacteria can be used to extract copper and molybdenum respectively from chalcopyrite ($CuFeS_2$) and molybdenite (MoS_2).

A combination of two bacteria *Leptospirillum ferrooxidans* and *Thiobacillus organoparpus* can effectively degrade pyrite (FeS_2) and chalcopyrite ($CuFeS_2$). The individual organisms alone are of no use in extracting metals.

Pseudomonas aeruginosa can be employed in mining low grade uranium (0.02%) ore. This organism has been shown to accumulate about 100 mg uranium per one liter solution in less than ten seconds. Another organism, *Rhizopus arrhizus* is also effective for extracting uranium from waste water.

Certain fungi have also found use in bioleaching. Thus, *Aspergillus niger* can extract copper and nickel while *Aspergillus oryzae* is used for extracting gold. Among the various microorganisms, *T. ferrooxidans* and *T. thiooxidans* are the most widely used in bioleaching. The utilization of many of the other organisms is still at the experimental stage.

10.3 Mechanism of bioleaching:

The mechanism of bioleaching is rather complex and not well understood. The chemical transformation of metals by microorganisms may occur by direct or indirect bioleaching.

10.3.1 Direct bioleaching:

In this process, there is a direct enzymatic attack on the minerals (which are susceptible to oxidation) by the microorganisms. For instance, certain bacteria (e.g., *T. ferrooxidans*) can transfer electrons (coupled with ATP production) from iron or sulfur to oxygen. That is these organisms can obtain energy from the oxidation of Fe^{2+} to Fe^{3+} or from the oxidation of sulfur and reduced sulfur compounds to sulfate as illustrated below.

$$4FeSO_4 + 2H_2SO_4 + O_2 \rightarrow 2Fe_2(SO_4)_3 + 2H_2O$$

$$2S^{\circ} + 3O_2 + 2H_2O \rightarrow 2H_2SO_4$$

$$2FeS_2 + 7O_2 + 2H_2O$$

As is evident from the third reaction given above, iron is extracted in the soluble form the iron ore pyrite (FeS_2).

10.3.2 Indirect bioleaching:

In this indirect method, the bacteria produce strong oxidizing agents such as ferric iron and sulfuric acid on oxidation of soluble iron or soluble sulfur respectively. Ferric iron or sulfuric acid, being powerful oxidizing agents react with metals and extract them. For indirect bioleaching, acidic environment is absolutely essential in order to keep ferric iron and other metals in solution. It is possible to continuously maintain acidic environment by the oxidation of iron, sulfur, metal sulfides or by dissolution of carbonate ions.

10.4 Commercial Process of Bioleaching:

The naturally occurring mineral leaching is very slow. The microbial bioleaching process can be optimized by creating ideal conditions— temperature, pH, and nutrient, O2 and CO2 supply etc.

The desired microorganisms with nutrients, acid etc., are pumped into the ore bed. The microorganisms grow and produce more acid. The extracted leach liquor is processed for the metal recovery. The leach liquor can be recycled again and again for further metal extraction.

10.5 Microbial Bioleaching

In commercial bioleaching, three methods are commonly used-slope leaching, heap leaching and in situ leaching

Commercial Bioleaching Processes

Slope leaching: The ore is finally ground and dumped in large piles down a mountainside. This ore is then subjected to continuous sprinkling of water containing the desired microorganism (T. ferrooxidans). The water collected at the bottom is used for metal extraction. The water can be recycled for regeneration of bacteria.

Heap leaching: In this case, the ore is arranged in large heaps and subjected to treatments as in slope leaching.

In situ leaching: The ore, in its original natural place is subjected to leaching. Water containing the microorganisms is pumped through drilled passages. In most cases, the permeability of rock is increased by subsurface blasting of the rock. As the acidic water seeps through the rock, it collects at the bottom which is used for metal extraction. This water can be recycled and reused.

Selected examples of microbial bioleaching are briefly described below:

10.5.1 Bioleaching of Copper:

Copper ores (chalcopyrite, covellite and chalcocite) are mostly composed of other metals, besides copper. For instance, chalcopyrite mainly contains 26% copper, 26% iron, 33% sulfur and 2.5% zinc.

Bioleaching of copper ore (chalcopyrite) is widely used in many countries. This is carried out by the microorganism Thiobacillus ferrooxidans which oxidizes insoluble chalcopyrite (CuFeS2) and converts it into soluble copper sulfate (CuSO4). Sulfuric acid, a byproduct formed in this reaction, maintains acidic environment (low pH) required for growth of the microorganisms.

Copper leaching is usually carried out by heap and in situ process (details given above). As the copper-containing solution (i.e., copper in the dissolved state) comes out, copper can be precipitated and the water is recycled, after adjusting the pH to around 2.

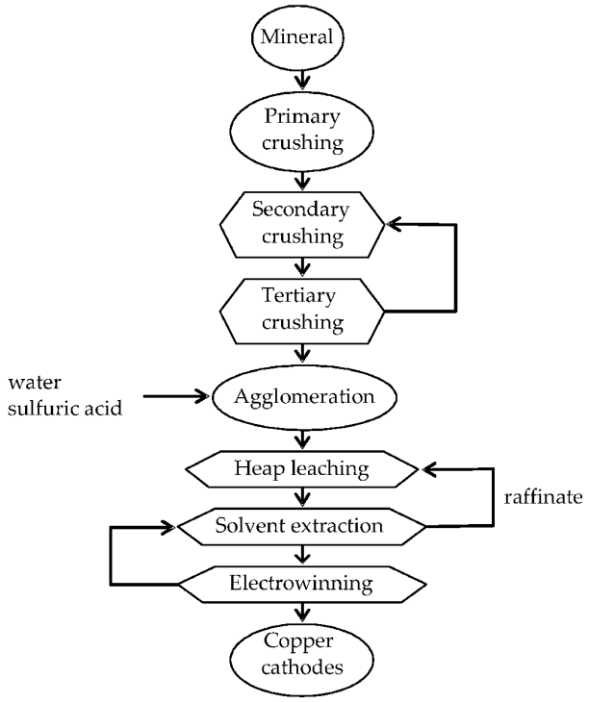


Fig: Bioleaching of Copper.

Extraction of copper by bioleaching is very common since the technique is efficient, besides being economical. It is estimated that about 5% of the world's copper production is obtained via microbial leaching. In the USA alone, at least 10% of the copper is produced by bioleaching process.

10.5.2 Bioleaching of Uranium:

Bioleaching is the method of choice for the large-scale production uranium from its ores. Uranium bioleaching is widely used in India, USA, Canada and several other countries. It is possible to recover uranium from low grade ores (0.01 to 0.5% uranium) and low grade nuclear wastes.

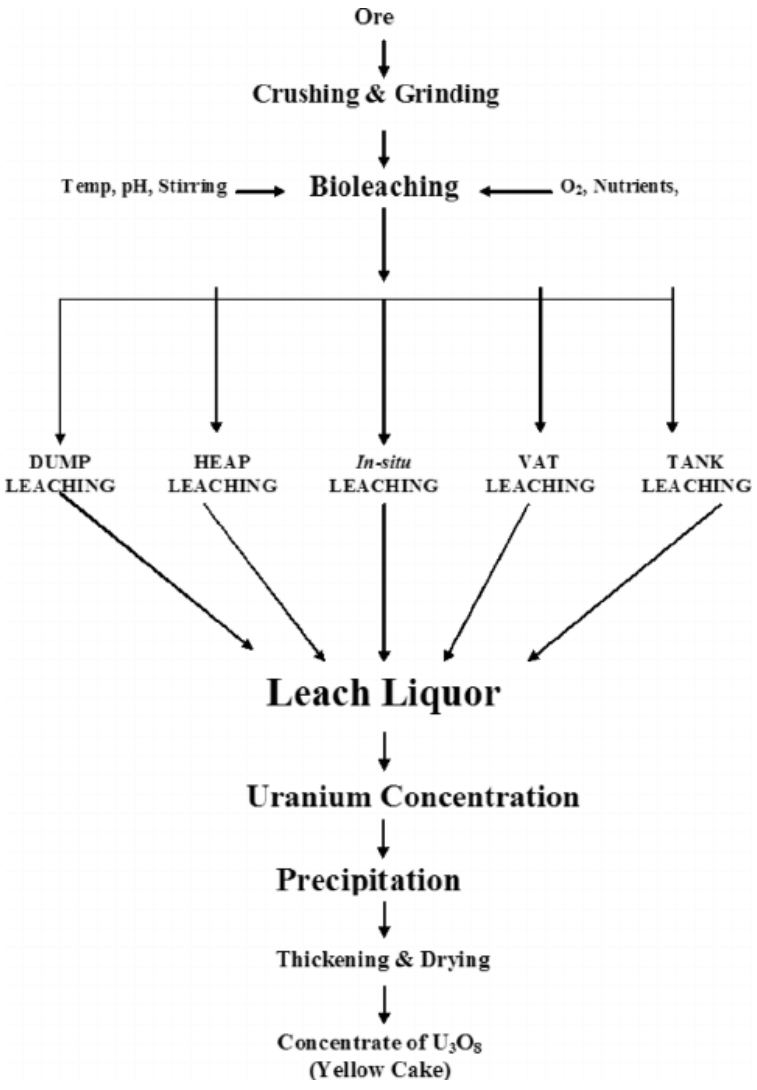


Fig: Bioleaching of Uranium.

In situ bioleaching technique is commonly used for extracting uranium. In the technique employed, the insoluble tetravalent uranium is oxidized (in the presence of hot H2SO4/Fe3+ solution) to soluble hexavalent uranium sulfate.

$$UO2 + Fe2(SO4)3 \rightarrow UO2SO4 + 2FeSO4$$

Bioleaching of uranium is an indirect process since the microbial action is on the iron oxidant, and not directly on the uranium. The organism *Thiobacillus ferrooxidans* is capable of producing sulfuric acid and ferric sulfate from the pyrite (FeS_2) within the uranium ore.

For optimal extraction of uranium by bioleaching, the ideal conditions are temperature 45-50°C, pH 1.5-3.5, and CO_2

around 0.2% of the incoming air.

The soluble form of uranium from the leach liquor can be extracted into organic solvents (e.g., tributyl phosphate) which can be precipitated and then recovered.

The Heap leaching process is sometimes preferred instead of the in situ technique. This is because the recovery of uranium is much higher with heap leaching.

10.6 Advantages of Bioleaching:

When compared to conventional mining techniques, bioleaching offers several advantages. Some of them are listed below.

1. Bioleaching can recover metals from low-grade ores in a cost-effective manner.
2. It can be successfully employed for concentrating metals from wastes or dilute mixtures.
3. Bioleaching is environmentally friendly since it does not cause any pollution (which is the case with conventional mining techniques).
4. It can be used to produce refined and expensive metals which otherwise may not be possible.
5. Bioleaching is a simple process with low-cost technology.
6. It is ideally suited for developing countries.

The major limitation or disadvantage of bioleaching is the slowness of the biological process. This problem can, however, be solved by undertaking in-depth research to make the process faster, besides increasing the efficiency.

11. Role in biosorption, biotransformation of xenobiotics

❖ A **xenobiotic** is a chemical substance found within an organism that is not naturally produced or expected to be present within the organism. It can also cover substances that are present in much higher concentrations than are usual. Natural compounds can also become xenobiotics if they are taken up by another organism, such as the uptake of natural human hormones by fish found downstream of sewage treatment plant outfalls, or the chemical defenses produced by some organisms as protection against predators.

✓ The term **xenobiotics**, however, is very often used in the context of pollutants such as dioxins and polychlorinated biphenyls and their effect on the biota, because xenobiotics are understood as substances foreign to an entire biological system, i.e. artificial substances, which did not exist in nature before their synthesis by humans.

Xenobiotics may be grouped as carcinogens, drugs, environmental pollutants, food additives, hydrocarbons, and pesticides.

Biotransformation of xenobiotics

- Biotransformation is the process by which a xenobiotics is chemically altered by the body. The liver is the principal, but not the only, site of xenobiotics biotransformation. The liver has enzymes that facilitate chemical reactions such as oxidation, reduction, and hydrolysis of xenobiotics.
- *Biotransformation* is the metabolic conversion of endogenous and xenobiotic chemicals to more water-soluble compounds.
- Xenobiotic biotransformation is accomplished by a limited number of enzymes with broad substrate specificities.
- Phase I reactions involve hydrolysis, reduction, and oxidation. These reactions expose or introduce a functional group (—OH, —NH_2, —SH, or —COOH), and usually result in only a small increase in hydrophilicity.
- Phase II biotransformation reactions include glucuronidation, sulfonation (more commonly called sulfation), acetylation, methylation, and conjugation with glutathione (mercapturic acid synthesis), which usually result in increased hydrophilicity and elimination.

General overview-

• Biotransformation is the metabolic conversion of endogenous and xenobiotic chemicals to more water-soluble compounds. Generally, the physical properties of a xenobiotic are changed from those favoring absorption

(lipophilicity) to those favoring excretion in urine or feces (hydrophilicity). An exception to this general rule is the elimination of volatile compounds by exhalation.

• Chemical modification of a xenobiotic by biotransformation may alter its biological effects. Some drugs undergo biotransformation to active metabolites that exert their pharmacodynamic or toxic effect. In most cases, however, biotransformation terminates the pharmacologic effects of a drug and lessens the toxicity of xenobiotics.

Basic properties of xenobiotic biotransforming enzymes-

 Xenobiotic biotransformation is accomplished by a limited number of enzymes with broad substrate specificities.

 The synthesis of some of these enzymes is triggered by the xenobiotic (by the process of enzyme induction), but in most cases the enzymes are expressed constitutively (i.e., synthesized in the absence of an external stimulus). Although the synthesis of steroid hormones is catalyzed by cytochrome P450 enzymes in steroidogenic tissues, this family of enzymes in the liver converts steroid hormones into water-soluble metabolites to be excreted.

 The structure (i.e., amino acid sequences) of a biotransforming enzyme may differ among individuals, which can lead to differences in rates of xenobiotic biotransformation. The study of the causes, prevalence, and impact of heritable differences in xenobiotic biotransforming enzymes is known as pharmacogenetics.

Reactions catalyzed by xenobiotic biotransforming enzyme(general overview)-

The reactions catalyzed (by the xenobiotic biotransforming enzyme) can generally be divided into four categories-

(1) Hydrolysis
(2) Reduction
(3) Oxidation and
(4) Conjugation.

❖ Hydrolysis, reduction, and oxidation reactions expose or introduce a functional group (—OH, —NH_2, —SH, or —COOH), and usually result in only a small increase in hydrophilicity.

❖ Conjugation biotransformation reactions include glucuronidation, sulfonation (more commonly called sulfation), acetylation, methylation, conjugation with glutathione (mercapturic acid synthesis), and conjugation with amino acids (such as glycine, taurine, and glutamic acid). This leads to large increase in xenobiotic hydrophilicity and thus promoting the excretion of foreign chemicals(Xenobiotics

Distribution of the enzymes-

❖ Xenobiotic biotransforming enzymes are widely distributed throughout the body and are present in several subcellular compartments. In vertebrates, the liver is the richest source of enzymes catalyzing biotransformation reactions.

❖ The xenobiotic biotransforming enzymes are also located in the skin, lung, nasal mucosa, kidney, eye, gastrointestinal tract, as well as numerous other tissues.

❖ Intestinal microflora plays an important role in the biotransformation of certain xenobiotics.

❖ Biotransformation enzymes are located primarily in the endoplasmic reticulum (microsomes) or the soluble fraction of the cytoplasm (cytosol), with lesser amounts in mitochondria, nuclei, and lysosomes.

Enzymes involved during biotransformation (Enzymes catalyzing different reactions during biotransformation)-

Hydrolysis

Different enzymes catalyzing hydrolysis reactions-

I. Carboxylesterases, Cholinesterases, and Paraoxonase
II. Alkaline phosphatase
III. Peptidases
IV. Epoxide Hydrolase

Reduction

Certain metals and xenobiotics containing an aldehyde, ketone, disulfide, sulfoxide, quinone, *N*-oxide, alkene, azo, or nitro group are often reduced in vivo. The reaction may proceed enzymatically or nonenzymatically by interaction with reducing agents, such as the reduced forms of glutathione, FAD, FMN, and NADP. Likewise, enzymes, such as alcohol dehydrogenase (ADH), aldehyde oxidase, and cytochrome P450, can catalyze both reductive and oxidative reactions depending on the substrate and the conditions. These are possible types of reduction reactions can be taken place -

- Azo- and Nitro-Reduction
- Carbonyl Reduction
- Disulfide Reduction
- Sulfoxide and N-Oxide Reduction
- Quinone Reduction

❖ Dehalogenation-Reductive dehalogenation, Oxidative dehalogenation , Double dehalogenation reactions can take place.

Oxidation

- Alcohol Dehydrogenase
- Aldehyde Dehydrogenase
- Dihydrodiol Dehydrogenase
- Molybdenum Hydroxylases
- Xanthine Oxidoreductase
- Aldehyde Oxidase
- Monoamine Oxidase
- Peroxidase-Dependent Cooxidation
- Flavin Monooxygenases
- Cytochrome P450

(Cytochrome P450 catalyzes the following types of oxidation reactions-

1. hydroxylation of an aliphatic or aromatic carbon;
2. epoxidation of a double bond;
3. heteroatom (*S*-, *N*-, and *I*-) oxygenation and *N*-hydroxylation;
4. heteroatom (*O*-, *S*-, *N*-, and *Si*-) dealkylation;
5. oxidative group transfer;
6. cleavage of esters;
7. dehydrogenation etc.)

Conjugation-

Conjugation reactions include glucuronidation, sulfonation (more commonly called sulfation), acetylation, methylation, conjugation with glutathione (mercapturic acid synthesis), and conjugation with amino acids (such as glycine, taurine, and glutamic acid). The co-substrates for these reactions react with functional groups that are either present on the xenobiotic or are introduced or exposed during oxidation, reduction, or hydrolysis reactions. With the exception of methylation and acetylation, conjugations result in a large increase in xenobiotic hydrophilicity, which greatly facilitates excretion of foreign chemicals.

- Glucuronidation
- Sulfonation
- Methylation
- Acetylation

➢ Xenobiotics can be conjugated with amino acids(Amino acid conjugation) or with glutathione (Glutathione conjugation).

➢ Conjugation of xenobiotics with glutathione includes an enormous array of electrophilic xenobiotics, or xenobiotics that can be biotransformed to electrophiles.

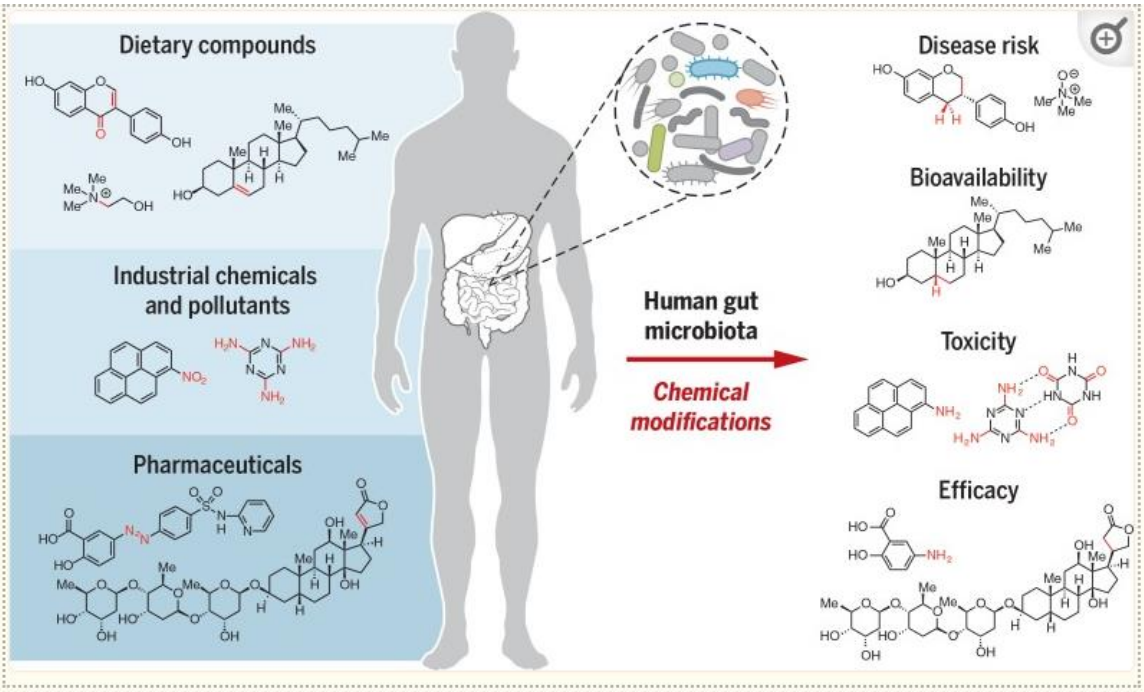
➢ Glutathione conjugates formed in the liver can be effluxed into bile and blood, and they can be converted to mercapturic acids in the kidney and excreted in urine.

➢ In some cases, conjugation with glutathione enhances the toxicity of a xenobiotic. Glutathione conjugates of various compounds can activate xenobiotics to become toxic by releasing a toxic metabolite, being inherently toxic itself, or being degraded to a toxic metabolite.

Role of microbes in biotransforming xenobiotics-

Chemical transformation of xenobiotics by the gut microbiota

- The human gut microbiota makes key contributions to the metabolism of ingested compounds (xenobiotics), transforming hundreds of dietary components, industrial chemicals, and pharmaceuticals into metabolites with altered activities, toxicities, and lifetimes within the body. The chemistry of gut microbial xenobiotic metabolism is often distinct from that of host enzymes.
- Humans ingest various small molecules that are foreign to the human body (xenobiotics), including dietary components, environmental chemicals, and pharmaceuticals. Microorganisms that inhabit our gastrointestinal tract (the human gut microbiota) can directly alter the chemical structures of such compounds, thus modifying their lifetimes, bioavailabilities, and biological effects.
- The majority of human microbiota-xenobiotic interactions occur within the GI tract .Role of gut microbiota in the transformation of xenobiotics is very important



Human gut microbes metabolize xenobiotics. Themicroorganisms that inhabit the human gut alter the chemical structures of ingested compounds, including dietarycomponents, industrial chemicals, and drugs.These changes affect xenobiotic toxicity, biological activity, and bioavailability.The gutmicrobialenzymesresponsibleformanyofthesetransformationsarepoorlyunderstood.Me,methyl.

- Many of the enzyme classes associated with xenobiotic metabolism (such as hydrolases, lyases, oxidoreductases, and transferases) are widely distributed among sequenced gut microorganisms.

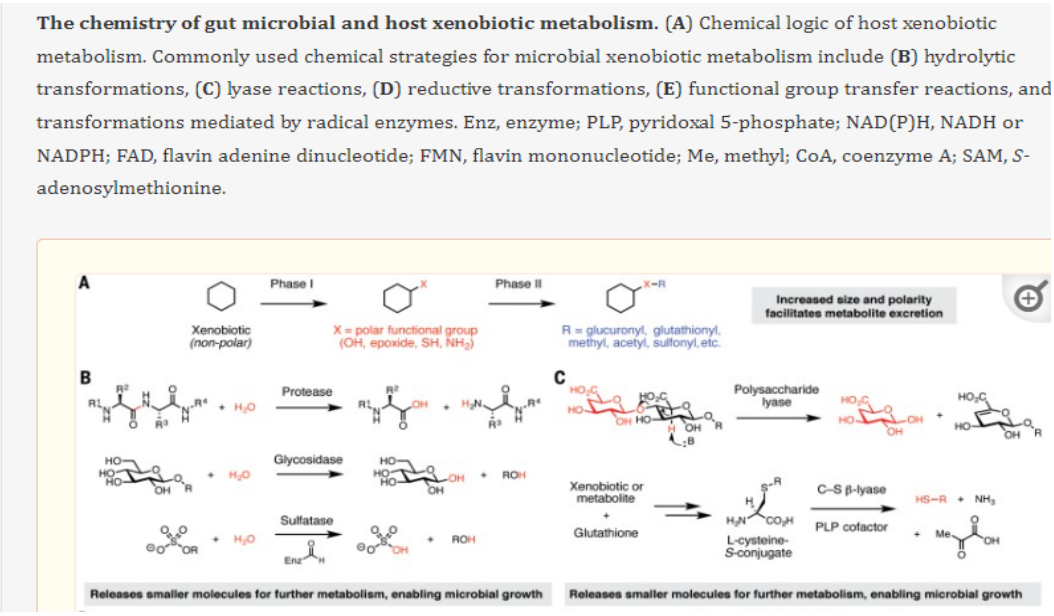
Microbial biotransformations or microbial biotechnology are gaining importance and extensively utilized to generate metabolites in bulk amounts with more specificity.

Microbial cells are ideal choice for biotransformation due to below reasons-

- High surface to volume ratio.
- Higher growth rate of microbial cells.
- Higher rate of metabolism in microbes leads to efficient transformation.
- Easy to maintain sterile condition.

Microbial biotransformation is widely used in the transformation of various pollutants or a large variety of compounds such as hydrocarbons, pharmaceutical substances and metals.

- Transformation of antibiotics, pesticides, pollutants, petroleum hydrocarbons using microbes is now an emerging area of research.



The chemistry of gut microbial and host xenobiotic metabolism. (**A**) Chemical logic of host xenobiotic metabolism. Commonly used chemical strategies for microbial xenobiotic metabolism include (**B**) hydrolytic transformations, (**C**) lyase reactions, (**D**) reductive transformations, (**E**) functional group transfer reactions, and transformations mediated by radical enzymes. Enz, enzyme; PLP, pyridoxal 5-phosphate; NAD(P)H, NADH or NADPH; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; Me, methyl; CoA, coenzyme A; SAM, *S*-adenosylmethionine.

Role of microbes in biosorption of xenobiotics

- Physico-chemical mechanisms of removal, which may be encompassed by the general term "biosorption", include adsorption, ion exchange and entrapment .
- In living cells, biosorption can be directly and indirectly influenced by metabolism
- Biosorption is a technique that can be used for the removal of pollutants from waters, especially those that are not easily biodegradable such as metals and dyes. A variety of biomaterials are known to bind these pollutants. The biosorption abilities of bacterial biomass towards dyes and metal ions are elucidated here.
- The properties of the cell wall constituents, such as peptidoglycan, and the role of functional groups, such as carboxyl, amine and phosphonate have their biosorption potentials.
- Metal components and dyes can be considered as xenobiotics and several bacterial species are reported to have metal and dye biosorption properties.

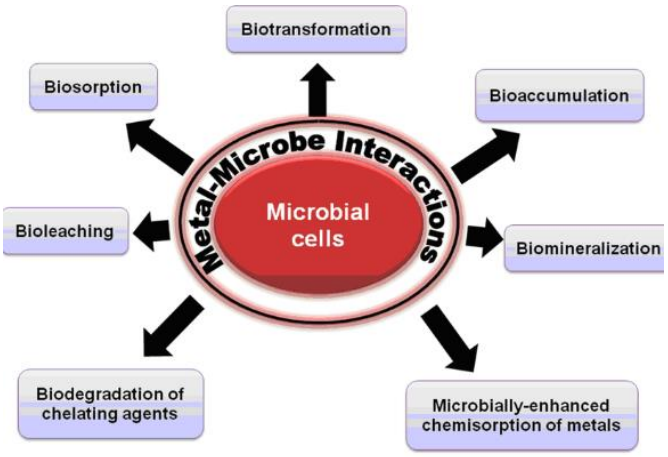
Metal	Organism
Lead	*Bacillus* sp. (ATS-1)
	Corynebacterium glutamicum
	Enterobacter sp. J1
	Pseudomonas aeruginosa PU21
	Pseudomonas aeruginosa PU21 [b]
	Pseudomonas putida
	Pseudomonas putida
	Streptomyces rimosus [a]
	Streptoverticillium cinnamoneum [a]
Mercury	*Bacillus* sp.
Nickel	*Bacillus thuringiensis*
	Streptomyces rimosus [a]
Palladium	*Desulfovibrio desulfuricans*
	Desulfovibrio fructosivorans
	Desulfovibrio vulgaris
Platinum	*Desulfovibrio desulfuricans*
	Desulfovibrio fructosivorans
	Desulfovibrio vulgaris
Thorium	*Arthrobacter nicotianae* IAM 12342
	Bacillus licheniformis IAM 111054
	Bacillus megaterium IAM 1166
	Bacillus subtilis IAM 1026
	Corynebacterium equi IAM 1038
	Corynebacterium glutamicum IAM 12435
	Micrococcus luteus IAM 1056
	Nocardia erythropolis IAM 1399
	Zoogloea ramigera IAM 12136
Uranium	*Arthrobacter nicotianae* IAM 12342
	Bacillus licheniformis IAM 111054
	Bacillus megaterium IAM 1166
	Bacillus subtilis IAM 1026
	Corynebacterium equi IAM 1038
	Corynebacterium glutamicum IAM 12435
	Micrococcus luteus IAM 1056
	Nocardia erythropolis IAM 1399
	Zoogloea ramigera IAM 12136
Zinc	*Aphanothece halophytica*
	Pseudomonas putida
	Pseudomonas putida CZ1
	Streptomyces rimosus
	Streptomyces rimosus [a]
	Streptoverticillium cinnamoneum [a]
	Thiobacillus ferrooxidans [a]
	Thiobacillus ferrooxidans [a]

Fig: Metal absorption my microbes

Mechanism of Biosorption

- The bacterial cell wall is the first component that comes into contact with metal ions/dyes, where the solutes can be deposited on the surface or within the cell wall structure.
- Since the mode bisorption is extracellular, the chemical functional groups of the cell wall also play vital roles in biosorption. Due to the nature of the cellular components, several functional groups are present on the bacterial cell wall, including carboxyl, phosphonate, amine and hydroxyl group.
- As they are negatively charged and abundantly available, carboxyl groups actively participate in the binding of metal cations. Several dye molecules, which exist as dye cations in solutions, are also attracted towards carboxyl and other negatively charged functional groups.(It is reported that carboxyl groups of the cell wall peptidoglycan of *Streptomyces pilosus* were responsible for the binding of copper).
- Amine groups are also very effective for removing metal ions, as it not only chelates cationic metal ions, but also adsorbs anionic metal species or dyes via electrostatic interaction or hydrogen bonding(It is reported that amine groups of *Corynebacterium glutamicum* were responsible for the binding of reactive dye anions via electrostatic attraction)

Characteristics of bacterial surface reveals the potential binding sites on bacterial surface during biosorption. The nature of the binding sites and their involvement during biosorption can be approximately evaluated using FT-IR analysis. To analyze the morphology of the cell surface before and after biosorption, SEM micrographs are often used.



Fig; Potentiality of Microbes in various aspects including Biosorption & Biotransformation

12. Microorganisms in the recovery of precious metals

The metals can be recovered by the microorganisms by two processes: (1) **Bioleaching** : This broadly involves the extraction or solubilization of minerals from the ores by the microorganisms. and (2) **Bio-Sorption**: It deals with the microbial cell surface adsorption of metals from the mine wastes or dilute mixtures.

12.1 Bioleaching:

The bacteria which are naturally associated with the rocks can lead to bioleaching by one of the following ways.

1. Direct action of bacteria on the ore to extract metal.
2. Bacteria produce certain substances such as sulfuric acid and ferric iron which extract the metal (indirect action).

The most commonly used microorganisms for bioleaching are *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*. *Thiobacillus ferrooxidans* is a rod-shaped, motile, non-spore forming, Gram-negative bacterium. It derives energy for growth from the oxidation of iron or sulfur. This bacterium is capable of oxidising ferrous iron (Fe^{2+}) to ferric form (Fe^{3+}), and converting sulfur (soluble or insoluble sulfides, thiosulfate, elemental sulfur) to sulfate ($SO4^2$). *Thiobacillus thiooxidans* is comparable with *T. ferrooxidams*, and grows mostly on sulfur compounds.Several studies indicate that the two *bacteria T. ferrooxidans* and *T. thiooxidans*, when put together, work synergistically and improve the extraction of metals from the ores. Besides the above two bacteria, there are other microorganisms involved in the process of bioleaching.

Certain fungi have also found use in bioleaching. Thus, *Aspergillus niger* can extract copper and nickel while *Aspergillus oryzae* is used for extracting gold.

12.1.1 Mechanism of bioleaching: The mechanism of bioleaching is rather complex and not well understood. The chemical transformation of metals by microorganisms may occur by direct or indirect bioleaching.

Direct bioleaching:

In this process, there is a direct enzymatic attack on the minerals (which are susceptible to oxidation) by the microorganisms. For instance, certain bacteria (e.g., *T. ferrooxidans*) can transfer electrons (coupled with ATP production) from iron or sulfur to oxygen. That is these organisms can obtain energy from the oxidation of Fe^{2+} to Fe^{3+} or from the oxidation of sulfur and reduced sulfur compounds to sulphate.

$$4FeSO_4 + 2H_2SO_4 + O_2 \rightarrow 2Fe_2(SO4)_3 + 2H_2O$$

$$2S° + 3O_2 + 2H_2O \rightarrow 2H_2SO_4$$

$$2FeS_2 + 7O_2 + 2H_2O$$

As is evident from the third reaction given above, iron is extracted in the soluble form the iron ore pyrite (FeS_2).

Indirect bioleaching:

In this indirect method, the bacteria produce strong oxidizing agents such as ferric iron and sulfuric acid on oxidation of soluble iron or soluble sulfur respectively. Ferric iron or sulfuric acid, being powerful oxidizing agents react with metals and extract them. For indirect bioleaching, acidic environment is absolutely essential in order to keep ferric iron and other metals in solution. It is possible to continuously maintain acidic environment by the oxidation of iron, sulfur, metal sulfides or by dissolution of carbonate ions.

12.1.2 Advantages of Bioleaching:

When compared to conventional mining techniques, bioleaching offers several advantages. Some of them are listed below.

1. Bioleaching can recover metals from low grade ores in a cost-effective manner.
2. It can be successfully employed for concentrating metals from wastes or dilute mixtures.
3. Bioleaching is environmental friendly, since it does not cause any pollution (which is the case with conventional mining techniques).
4. It can be used to produce refined and expensive metals which otherwise may not be possible.
5. Bioleaching is a simple process with low cost technology.
6. It is ideally suited for the developing countries.

The major limitation or disadvantage of bioleaching is the slowness of the biological process. This problem can, however, be solved by undertaking an in depth research to make the process faster, besides increasing the efficiency.

12.2 Bio Sorption

Bio sorption primarily deals with the microbial cell surface adsorption of metals from the mine wastes or dilute mixtures. The microorganisms can be used as bio sorbents or bio accumulators of metals. The process of bio sorption performs two important functions.

1. Removal of toxic metals from the industrial effluents.
2. Recovery of valuable but toxic metals.

Both the above processes are concerned with a reduction in environmental poisoning/pollution.

A wide range of microorganisms (bacteria, algae, yeasts, moulds) are employed in bio sorption. In fact, some workers have developed bio sorbent-based granules for waste water/industrial effluent treatment, and metal recovery.

In general, the microbial cell membranes are negatively charged due to the presence of carboxyl (COO–), hydroxyl (OH–) phosphoryl (PO3-4) and sulfhydryl (HS–) groups. This enables the positively charged metal ions (from solutions) to be adsorbed on to the microbial surfaces.

The different groups of microorganisms used in bio sorption processes are briefly described below.

12.2.1 Bacteria:

Several bacteria and actinomycetes adsorb and accumulate metals such as mercury, cadmium, lead, zinc, nickel, cobalt and uranium. For example, Rhodospirullum sp can accumulate Cd, Pb and Hg. Bacillus circulans can adsorb metals such as Cu, Cd, Co, and Zn. By use of electron microscopy, deposition of metals on the bacterial cell walls was recorded. It appears that the cell wall composition plays a key role in the metal adsorption.

12.2.2 Fungi:

There is a large scale production of fungal biomass in many fermentation industries. This biomass can be utilized for metal bio sorption from industrial effluents. Immobilized fungal biomass is more effective in bio sorption due to increased density, mechanical strength and resistance to chemical environment. Further, immobilized biomass can be reused after suitable processing.

The fungus *Rhizopus arrhizus* can adsorb several metallic cations e.g., uranium, thorium. *Pencillium lapidorum*, *P. spimuiosum* are useful for the bio sorption of metals such as Hg, Zn, Pb, Cu. Several fungi were tried with some degree of success to selectively adsorb uranium e.g.,*Aspergillus niger*, A. oryzae, *Mucor haemalis*, *Penicillium chrysogenum*.

Edible mushrooms were also found to adsorb certain metals. For instance, fruit bodies of *Agaricus bisporus* can take up mercury while *Pleurotus sajor-caju* can adsorb lead and cadmium. Many yeasts, commonly used in fermentation industries, are capable of adsorbing and accumulating metals. For instance, *Saccharomyces cerevisae* and *Sporobolomyces salmonicolour* can respectively adsorb mercury and zinc.

12.2.3 Algae:

Several species of algae (fresh water or marine) can serve as bio accumulators of metals. For instance, *Chlorella vulgaris* and *C. regularis* can accumulate certain metals like Pb, Hg, Cu, Mo and U. The green algae *Hydrodictyon reticulatum* adsorbs and accumulates high quantities of Pb, Fe and Mn. Some workers are in fact trying to use marine algae (e.g., *Luminaria*, *Ulva*, *Codium* sp) as bioaccumulators to reduce the metal pollution in rivers.

12.2.4 Higher plants in control of metal pollution:

Besides the microorganisms described above, there are some higher aquatic plants (i.e., aquatic macrophytes) that can accumulate potential toxic wastes including many metals. Water hyacinth (Eichornia crassipes), duck weeds (Spirodel sp), water lettuce (Pistia stratiotes) and certain ferns (Salvinia sp) are important in the control of metal pollution.

12.2.5 Advantages of biosorption:

1. Cheaper production of biomass (bacteria or fungi)
2. Use of biomass for removal of heavy metals.
3. Multiple heavy metals uptake at a time.
4. Treatment of large volumes of wastewater.
5. No need for chemical additions as highly selective for uptake and removal of specific metals.

13. Microbes in composting:

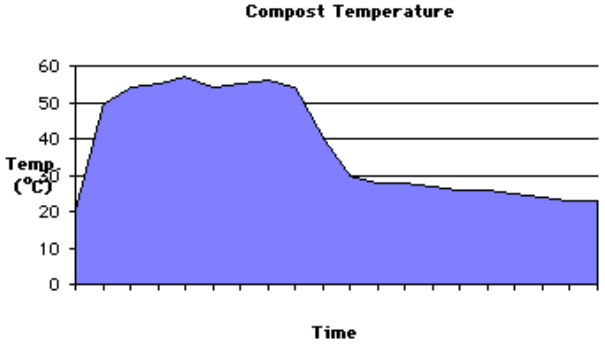
Compost is defined a biological process that transforms raw organic materials into a nutrient rich, biologically-stable

soil additive suitable for plant and crop use.

According to Biddlestone (1973) composting is the decomposition of heterogenous organic matter by a mixed microbial population in a moist warm aerobic environment. Incomplete microbial degradation of organic waste, where the microbial processes vary from aerobic to anaerobic form are stated as compost.

13.1 The Phases of Composting

In the process of composting, microorganisms break down organic matter and produce carbon dioxide, water, heat, and humus, the relatively stable organic end product. Under optimal conditions, composting proceeds through three phases: 1) the mesophilic, or moderate-temperature phase, which lasts for a couple of days, 2) the thermophilic, or high-temperature phase, which can last from a few days to several months, and finally, 3) a several-month cooling and maturation phase.



Different communities of microorganisms predominate during the various composting phases. Initial decomposition is carried out by mesophilic microorganisms, which rapidly break down the soluble, readily degradable compounds. The heat they produce causes the compost temperature to rapidly rise.

As the temperature rises above about 40°C, the mesophilic microorganisms become less competitive and are replaced by others that are thermophilic, or heat-loving. At temperatures of 55°C and above, many microorganisms that are human or plant pathogens are destroyed. Because temperatures over about 65°C kill many forms of microbes and limit the rate of decomposition, compost managers use aeration and mixing to keep the temperature below this point.

During the thermophilic phase, high temperatures accelerate the breakdown of proteins, fats, and complex carboydrates like cellulose and hemicellulose, the major structural molecules in plants. As the supply of these high-energy compounds becomes exhausted, the compost temperature gradually decreases and mesophilic microorganisms once again take over for the final phase of "curing" or maturation of the remaining organic matter.

13.2 Factors Affecting Composting

(a) Microorganisms:

The selection of suitable microbes depends on the type of composting process i.e. aerobic or anaerobic, type of raw material, etc. The efficient cellulolytic cultures, such as species of *Aspergillus*, *Trichoderma*, *Penicillium* and *Trichurus* accelerate composting for efficient recycling of dry crop wastes with high C:N ratio and reduce the composting period by about 1 month.

Enrichment of partially composted crop wastes can be achieved by *Azotobacter* and phosphate solubilizers to improve the nitrogen as compared with controls.

Moreover, the presence of a mixture of anaerobic forms of microorganisms in dung or biogas slurry proved potent in making compost. Actually, the compost carries agriculturally useful microorganisms which aid in the improvement of soil fertility.Actinomycetes form long, thread-like branched filaments that look like gray spider webs stretching through compost. These filaments are most commonly seen toward the end of the composting process, in the outer 10 to 15 centimeters of the pile. Sometimes they appear as circular colonies that gradually expand in diameter.Fungal species are numerous during both mesophilic and thermophilic phases of composting. Most fungi live in the outer layer of compost when temperatures are high. Compost molds are strict aerobes that grow both as unseen filaments

and as gray or white fuzzy colonies on the compost surface.Rotifers are microscopic multicellular organisms also found in films of water in the compost. They feed on organic matter and also ingest bacteria and fungi.

(b) Soil:

The soil can be defined as a natural medium for plant growth composed of minerals, organic materials and living organisms. The biological activities and mi-crobial metabolism in the soil contribute to its texture and fertility.

(c) Organic Matter:

The amount of organic matter present in any soil determines its natural suitability for plant cultivation. The value of com-post has not only in its N P K content but also in the sub-stantial quantities of humus which are essential for main-tenance of soil organic matter and fertility levels in tropical and sub-tropical soils.

13.3 Roles of Compost:

Composting is one of the oldest solid waste treatment methods known to man. Composting reduces the soluble nitrogen contents of agricultural wastes. It also results in phosphorus compounds becoming bound up in new microbial cells so that run-off can be avoided. Higher crop yields have also been claimed for composted versus directly applied animal manures.

14 Microbes in biopesticide formulation

Biopesticides are certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals. The biopesticides are derived in three major categories: biochemical, microbial and bioprotectors incorporated into the plant. They are used to control and combat diseases that occur in the vegetal crops or against pests. In the category of biopesticides are those substances that are naturally found and that fight pests through non-toxic mechanisms.Microbial pesticides are formulated from microorganisms, such as bacteria or fungi. They kill different larvae of specific insects or interfere with weeds growth in agricultural fields.

14.1 Advantages of using biopesticides

1. Biopesticides are usually inherently less toxic than conventional pesticides.
2. Biopesticides generally affect only the target pest and closely related organisms, in contrast to broad spectrum, conventional pesticides that may affect organisms as different as birds, insects and mammals.
3. Biopesticides often are effective in very small quantities and often decompose quickly, resulting in lower exposures and largely avoiding the pollution problems caused by conventional pesticides.
4. When used as a component of Integrated Pest Management (IPM) programs, biopesticides can greatly reduce the use of conventional pesticides, while crop yields remain high.

The following points highlight the three main types of bio-pesticides. The types are: 1. Bio-Insecticides 2. Bio-Nematicides 3.Bio-Herbicides.

14.2 Bio-Insecticides

Several bacterial pathogens of different insects are being used as insecticides. These are *Bacillus, Clostridium, Pseudomonas, Enterobacter, Proteus, Serratia* etc. Out of these, *Bacillus thuringiensis* has been used extensively. It shows insecticidal activity against larvae of Lepidoptera. *B. thuringiensis* var. *israelensis* (BTI) is highly active against larvae of mosquito vec-tors of malaria. Unlike DDT, the pathogen is environmentally safe in use and mosquito does not show any resistance against the bacterium. In India, it has been found that 0.4% thuricide is more effective than malathione, endrine and DDT to control insect pests of crucifer, lac and sugar-cane.With the help of recombinant DNA techno-logy, the gene having insecticidal properties of *Bacillus thuringiensis* has been transferred to the crops plans like tomato in 1987, with the help of bacterium, *Agrobacterium tumifaciens*.

Different entomogenous fungi have been used to protect different crops from insect dam-age. Species of the different genera like *Aschersonia, Beauveria, Coelomomyces, Entomophthora, Hirsutella* etc., are in common use.

Viruses are also very much effectively used as bioinsecticide. There are three major groups of viruses that can infect different insects.List if some viruses, registered trade names in parenthesis and target insects are given below:

(a) Nuclear polyhedrosis viruses (NPV)-Tobacco budworm and Cotton bollworm.

(b) Granulosis viruses (GV) of Baculoviridae-Insects of different food crops like Codling moths.

(c) Cytoplasmic polyhedrosis viruses (CPV) of Reoviridae-Pine caterpillar.

14.3 Bio-Nematicides

Different fungi are known to act as nematicide. Fungi of different genera like Arthrobotrys, Dactylella, Dactylaria and Monacrosporium are used to control different members of genera like Heterodera, Meloidogyne and Rotylenchulus, cause diseases of different crop plants.

The fungi damage nematode in four different ways:

i) By haustoria:

Fungi penetrate haustoria in the body of the nematode, digest the cell contents and draw the nutrients.

ii) Catching by loop:

The fungal mycelium forms loops at intervals. As a nematode passes through the loop, it constricts and thus the nematode is trapped.

iii) Production of adhesive hyphae:

The fungal mycelium produces some adhe-sive branch which may stick with the body of nematode on accidental con-tact.

iv) Formation of hyphal mesh:

The myce-lium forms mesh-like cobweb and is able to catch nematodes.

The other groups of fungi are commonly present in the soil and can act as nematicides. These are *Verticillium chlamydosporium, Paecilomyces lilacinus and Dactylella oviparasitica* — they often attack nematodes as well as their eggs**.**

14.4 Bio-Herbicides

Fungi are found to be much more suitable as herbicide than bacteria and viruses. Fungus like Phragmidium violacerum is used to control European blackberry in Chile and Puccinia chondrillina to control rush skeleton weed in Australia. Others, like Phytophthora palmivora, has been developed as herbicide at commercial level.

The name of the registered product, pro-ducing fungi in parenthesis and the target weeds are:

i. DE VINE (Phytophthora palmivora) — Milk-weed vine in USA,

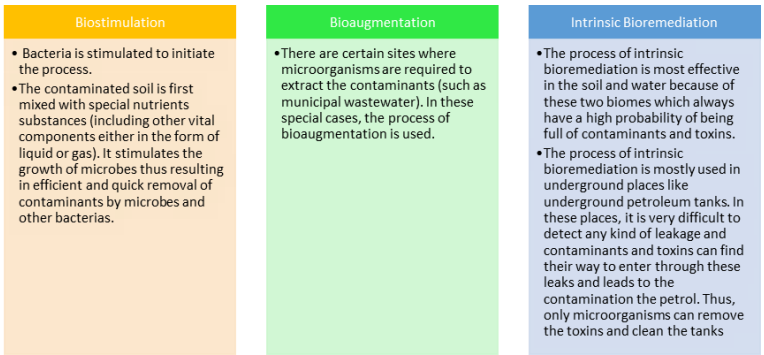
ii. LUBOA (Colletotrichum gloeosporiodes f. sp. cuscutae) — Dodders in China,

iii. BIOMAL (Colletotrichum gloeosporioides f. sp. malvae) — Round-leaved mallow in Canada and USA

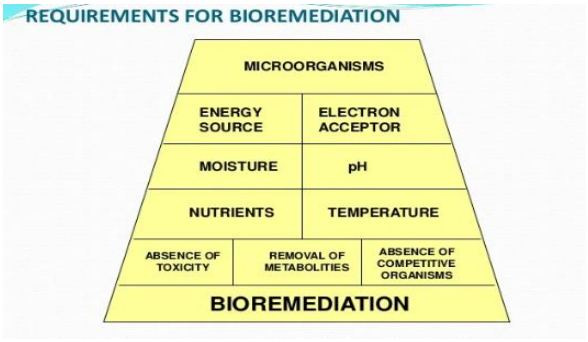
15. Microbes in bioremidiation

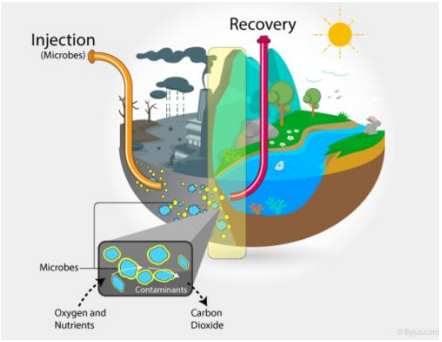
Bioremediation is a biotechnical process, which abates or cleans up contamination. It is a type of waste management technique which involves the use of organisms to remove or utilize the pollutants from a polluted area. Microorganisms like Bacteria and Fungi are the main role player when it comes to executing the process of Bioremediation. Bacteria are the most crucial microbes in this process as they break down the waste into nutrients and organic matter.

Types of bioremidiation-



Requirements





Process of bioremidiation:

 Bioremediation is a biological mechanism of recycling wastes in to another form that can be used and reused by other organisms. Nowadays, microorganisms are essential for a key alternative solution to overcome challenges of environmental pollution.

 Microorganisms are survive in all place on the biosphere and the nutritional capacity of microorganisms is completely varied, so it is used in the bioremediation of environmental pollutants.

 Bioremediation is highly involved in degradation, eradication, immobilization, or detoxification diverse chemical wastes and physical hazardous materials from the surrounding through the all-inclusive and action of microorganisms. The main principle is degrading and transforming pollutants such as hydrocarbons, oil, heavy metal, pesticides, dye etc.

 There are two types of factors (biotic and abiotic conditions) that determine rate of degradation.

 For bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products. As bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate.

 Heavy metals cannot be destroyed biologically but only can be transformed from one oxidation state or organic complex to another. Bacteria are also efficient in heavy metals bioremediation.

 Microorganisms uptake heavy metals actively (bioaccumulation) and/or passively (adsorption). Microbial methylation plays an important role in heavy metals bioremediation(because methylated compounds are frequently volatile). As for example, Mercury [Hg (II)] can be biomethylated by a number of different bacterial species such as Alcaligenes faecalis, Bacillus pumilus, Bacillus sp., Pseudomonas aeruginosa and Brevibacterium iodinium to gaseous methyl mercury.

 Biodegradation is very fruitful and attractive option to remediating, cleaning, managing and recovering technique for solving polluted environment through microbial activity.

 Bioremediation using microbes is more acceptable and mainly relies on the enzymes produced by them and takes part in the metabolic pathways. These microbes attack the pollutants and degrade them completely or convert them into less harmful products. The successful application of systems biology (SB) and metabolic engineering (ME) approaches in different fields of life sciences makes it attractive for environmental scientists to use these approaches for bioremediation.

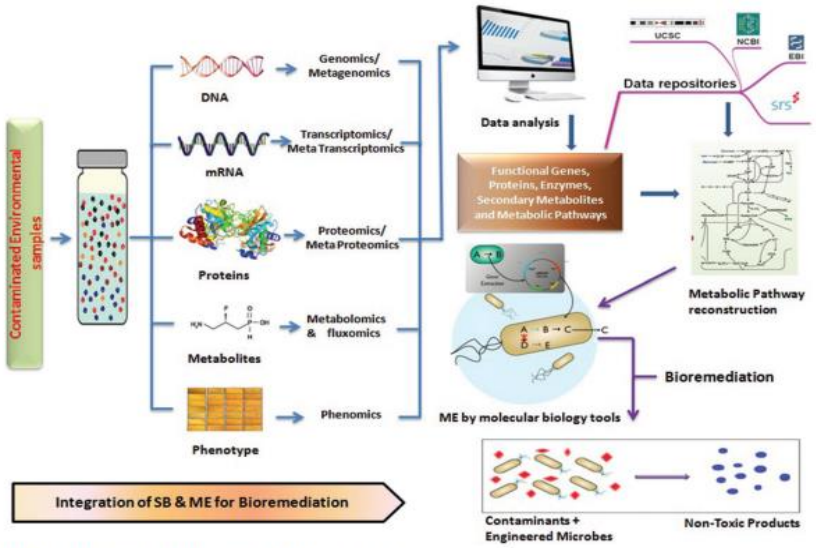


Figure 1. Integrated SB and ME approaches for bioremediation.

Fig.: - Integrated System Biology and Metabolic Engineering approaches for Bioremediation: conversion of toxic to non toxic compounds using engineered microbes

15.1 Microorganisms in oil bioremediation-

Bioremediation of petroleum contaminated environments is a process in which the biological pathways within microorganisms or plants are used to degrade or sequester toxic hydrocarbons, heavy metals, and other volatile organic compounds found within fossil fuels. Oil spills happen frequently at varying degrees along with all aspects of the petroleum supply chain, presenting a complex array of issues for both environmental and public health. While traditional cleanup methods such as chemical or manual containment and removal often result in rapid results, bioremediation is less labor-intensive, expensive, and averts chemical or mechanical damage. The efficiency and effectiveness of bioremediation efforts are based on maintaining ideal conditions, such as pH, RED-OX potential, temperature, moisture, oxygen abundance, nutrient availability, soil composition, and pollutant structure, for the desired organism or biological pathway to facilitate reactions. Three main types of bioremediation used for petroleum spills include microbial remediation, phytoremediation, and mycoremediation. Bioremediation has been implemented in various notable oil spills including the 1989 Exxon Valdez incident where the application of fertilizer on affected shoreline increased rates of biodegradation.

Microorganisms	Compound
Fusariumsp.	oil
Alcaligenes odorans, Bacillus subtilis, Corynebacterium propinquum, Pseudomonas aeruginosa	oil
Bacillus cereus A	diesel oil
Aspergillus niger, Candida glabrata, Candida krusei and Saccharomyces cerevisiae	crude oil
B. brevis, P. aeruginosa KH6, B. licheniformis and *B. sphaericus*	crude oil
Pseudomonas aeruginosa, P. putida, Arthobacter sp and Bacillus sp	diesel oil
Pseudomonas cepacia, Bacillus cereus, Bacillus coagulans, Citrobacter koseri and Serratia ficaria	diesel oil, crude oil

15.2 Microorganisms in bioremediation of different toxic compounds

Due to the rapid industrial growth, the environment and public health are threatened by the huge amount of toxic pollutants that have accumulated in the environment. Therefore, maintaining and protecting the environment from toxic pollutants has become a great challenge for mankind over the past few decades. Recently, various strategies have been intensively exploited to protect the environment by preventing the dispersion of toxic pollutants into it. For example, physicochemical methods such as electrochemical treatments, excavation, ion exchange, precipitation, reverse osmosis, evaporation, and sorption have been developed for the removal of toxic substances. However, many of these techniques are not yet commonly applied to the actual treatment of contamination due to critical drawbacks such as high cost and secondary contamination possibly associated with them. As an alternative, microbial bioremediation has attracted much attention as a promising technology that can overcome the shortcomings of the currently used physicochemical methods. Specifically, extremophilic microorganisms offer the most suitable approach for the treatment of toxic pollutants because not only can they detoxify toxic pollutants through microbial cellular metabolism but also they can withstand extremely harsh conditions. Herein, we focus on recent trends in bioremediation processes for the treatment of toxic pollutants such as inorganic heavy metals, harmful organic substances, and radioactive elements using extremophilic microorganisms and on the perspectives of this approach in public health.

Microorganisms	Approaches	Toxic compounds
Brevibacterium epidermidis EZ-K02	Genomics	Benzoate, *p*-hydroxybenzoate, acetophenone, catechol, gentisate, arsenic, cobalt, and cadmium
Microbacterium oleivorans	Genomics	Nitroacetate and nitriloacetate
Irpex lacteus	Genomics	Aromatic dye
Bacillus subtilis HUK15	Genomics	Hexachlorocyclohexane (HCH)
Anaeromyxobacter sp. Fw109-5	Genomics	Nitrate and uranium
Bacillus, Caprothermobacter, Rhodobacter, Pseudomonas, Achromobacter, Desulfitobacter, Desulfosporosinus, T78, Methanobacterium, Methanosaeta	Metagenomics	Sulfate-reducing, CO_2-assimilating, hydrocarbon-rich petroleum refinery waste
Proteobacteria and *Firmicutes*	Metagenomics	Degradation of fatty acids, chloroalkanes, and chloroalkanes
Naegleria, Vorticella, Arabidopsis, Asarum and *Populus*	Metagenomics analysis of South African petroleum-contaminated water aquifer sites	Hydrocarbons
Koribacter, Acidomicrobium, Bradyrhizobiu, Burkholderia, Solibacter, Singulisphaera, and Desulfomonile	Metagenomic analysis	Organophosphorus-containing pesticides
Pseudomonas, Rhodococcus, Bordetella, Chromobacter and Variovorax	Metagenomic analysis of soil sample	Polychlorinated biphenyls (biphenyl, benzoate, and protocatechuate)
Thiobacillus sp.	Metagenomics	Thiocyanate (SCN^-)
Bacillus, Pseudaxanthomonas sp. and *Alcanivorax* sp.	Metagenomics	Total petroleum hydrocarbons
Aspergillus niger asemoA, Talaromyces purpurogenusasemoF, Trichoderma harzianum asemoJ, and Aspergillus flavus asemoM	Transcriptomics study showed the presence of *lig, mnp, cbh,* and *lcc* gene encoding lignin peroxidase, manganese peroxidase, catalase, and laccase	Degradation of hydrocarbons
Acinetobacter venetianus RAG-1	Transcriptomics	Alkanol (dodecanol)
Dehalococcoides mccartyi	Comparative Genomics and Transcriptomics	Organohalides
Actinobacteria, Rhodococcus, and Mycobacterium	Metatranscriptomics	Hydrocarbons, gasoline, and diesel oil additives
Pseudomonas aeruginosa san ai	Proteomics	Cadmium
Penicillium oxalicum	Proteomics	Polycyclic aromatic hydrocarbons (anthracene)
Sphingomonas sp. GY2B	Proteomics	Phenanthrene
Miscanthus sinensis	Proteomics	Antimony (SB)
Microbial sediment sample	Metaproteomics and Metabolomics	Crude oil hydrocarbons
Rhizobiales, Burkholderiales and *Actinomycetales*	Metaproteomic	Polycyclic aromatic hydrocarbon
Pseudomonas putida	Metabolomics	Antibiotics and aromatic hydrocarbons
Pseudomonas putida KT2440	Fluxomics	Hydrocarbons
Rhodococcus aetherivorans BCP1 *and Rhodococcus opacus* R7	Phenomics	Antimicrobial compounds
Pseudomonas stutzeri 5190	Phenomics	Anthropogenic compounds

15.3 Microorganisms in dye bioremediation-

Microorganisms are widely distributed in the biosphere because their metabolic capacity is very impressive and they can easily grow in a wide range of environmental conditions. The nutritional versatility of microorganisms can also be exploited for the biodegradation of contaminants. This type of process is called bioremediation. It is continued through the ability of certain microorganisms to convert, modify, and use toxic pollutants to obtain the production of energy and biomass in the process.The quality of life on Earth is inextricably linked to the global quality of the

environment. Unfortunately, advances in science, technology, and industry have led to large amounts of pollutants, ranging from dirty wastewater to nuclear waste, being extracted or introduced into the ecosystem, which is a serious problem for the survival of humanity itself on Earth.

Microorganisms	Compound
B. subtilis strain NAP1, NAP2, NAP4	oil-based based paints
Myrothecium roridum IM 6482	industrial dyes
Pycnoporus sanguineous, Phanerochaete chrysosporium and Trametes trogii	industrial dyes
Penicillium ochrochloron	industrial dyes
Micrococcus luteus, Listeria denitrificans and Nocardia atlantica	Textile Azo Dyes
Bacillus spp. ETL-2012, *Pseudomonas aeruginosa, Bacillus pumilus* HKG212	Textile Dye (Remazol Black B), Sulfonated di-azo dye Reactive Red HE8B, RNB dye
Exiguobacterium indicum, Exiguobacterium aurantiacums, Bacillus cereus and Acinetobacter baumanii	azo dyes effluents
Bacillus firmus, Bacillus macerans, Staphylococcus aureus and Klebsiella oxytoca	vat dyes, Textile effluents

15.4 Microorganisms for metal bioremediation

Microorganisms adapt to and resist heavy metals in highly contaminated areas. Extra-cellular polymeric substances present on the biomass cell wall can attach to heavy metals by mechanisms like proton exchange or micro-precipitation of metals. Biomass surfaces have a negative charge because of the presence of carboxyl, amino, phosphoryl, and sulfo groups as potential ion exchange sites and metal sinks. The process of bioremediation takes place through various mechanisms like redox process, adsorption, complexation, ion-exchange, precipitation, and electrostatic attraction.

Microorganisms may initiate metal mobilization/immobilization by redox reactions; and hence, impact bioremediation processes. Heavy metals like Fe, As, Cr, and Hg undergo oxidation and reduction cycles. Bioremediation is facilitated by converting an element from its insoluble and stationary form in sediments into its mobile and soluble phase. Mobilization can also have deleterious impacts when toxic metal ions are redistributed and released from their solid phase from sediments into the solution phase. This increases their bioavailability and heavy metals can reach microbial metabolic systems. The bacteria reduces Hg(II) to the elemental and more volatile form of Hg. Microbial reduction can also enhance the solubility of ions like Fe(III) and As(V) by reducing them to Fe(II) and As(III), respectively, and can facilitate leaching from soil. Studies have reported bacteria from different natural aquifers which can transform As. Pokhrel and Viraraghavan employed Aspergillus niger to remove As(V) and As(III). Heavy metal biomethylation is an important process in soil and water and may modify toxicity, volatility, and mobility of heavy metals. It also serves as an important means of detoxification as volatile methylated species can be removed from cells. Dimethylmercury and alkyl arsines, the methylated products of Hg and As, respectively, are volatile and evaporate and are lost from soil. The organic matter fraction of soil serves as the methyl donor. Yet another indirect mechanism of metal mobilization involves the microbial decomposition of organic matter, which accelerates the release of these ions. Schizophyllum commune has been found to release heavy metals along with dissolved organic matter. Excretion of metabolites like carboxylic acids and amino acids by microbes is an important mechanism of chelating metal ions.

Microorganisms	Compound
Saccharomyces cerevisiae	Heavy metals, lead, mercury and nickel
Cunninghamella elegans	Heavy metals
*Pseudomonas fluorescens*and *Pseudomonas aeruginosa*	Fe 2+, Zn2+, Pb2+, Mn2+ and Cu2
*Lysinibacillus sphaericus*CBAM5	cobalt, copper, chromium and lead
Microbacterium profundi strain Shh49T	Fe
Aspergillus versicolor, A. fumigatus, Paecilomyces sp., *Paecilomyces* sp., *Terichoderma* sp., *Microsporum* sp., *Cladosporium* sp.	cadmium
Geobacter spp.	Fe (III), U (VI)
Bacillus safensis (JX126862) strain (PB-5 and RSA-4)	Cadmium
Pseudomonas aeruginosa, *Aeromonas* sp.	U, Cu, Ni, Cr
*Aerococcus*sp., *Rhodopseudomonas palustris*	Pb, Cr, Cd

15.5 Microrganisms used for the removal of pesticides-

Biological remediation or bioremediation is an attractive technology that results in the complete conversion of organic compounds into less harmful end products such as CO2 and H2O. It is considered low-cost and environmentally friendly compared to physical or chemical methods for removing contaminants. There are basically three types of bioremediation with microorganisms: remediation through improved natural attenuation (taking advantage of the natural capacities of the microorganisms present in the matrix); bioaugmentation (introduction of nonnative and/or genetically modified microorganisms); and biostimulation (addition of electron acceptors or nutrients). Biodegradation may be referred to as the complete mineralization of organic contaminants into carbon dioxide, water, inorganic compounds, and cell proteins or the transformation of complex organic contaminants into other simpler organic compounds by biological agents such as microorganism. Many indigenous microorganisms in water and soil are capable of degrading hydrocarbon contaminant.. Bioagents such as bacteria, fungi, and archaea are the main microbes that can decontaminate a site. The microbes that have a resistant gene for a pesticide pollutant can survive in that contaminated site. They can then utilize the pesticide as a food source, break it down as a source of carbon, and increase the biomass. They do not store or collect the pesticide, so this helps in decontaminating the site. Bioremediation is a biotechnological method that can ensure the maintenance of environmental balance and stability.

The microbial degradation of pesticides leads to eith

Microorganisms	Compound
Bacillus, Staphylococcus	Endosulfan
Enterobacter	Chlorpyrifos
Pseudomonas putida, Acinetobacter sp., Arthrobacter sp.	Ridomil MZ 68 MG, Fitoraz WP 76, Decis 2.5 EC, malation
Acenetobactor sp., Pseudomonas sp., Enterobacter sp. and Photobacterium sp.	chlorpyrifos and methyl parathion

16. Microbes in biopolymer production

- Biopolymers have occupied a major position in the field of biotechnology They are biocompatible and eco-friendly in nature.
- Biopolymers are a heterogeneous, amenable group of materials synthesized from biological origin or from any basic building units such as amino acids, lipids, and sugars.
- Microbial biopolymers such as polyesters, polyamides, and polysaccharides are successfully produced from pure cultures, mutants selected from the laboratory, or genetically modified organisms.
- The biopolymers range from viscous fluids to bioplastics. The molecular mass and composition of the biopolymer decide its physical properties .
- Biopolymer from bacteria has wide range of applications in biomedical and other industries. They possess varying biological functions with distinct properties.

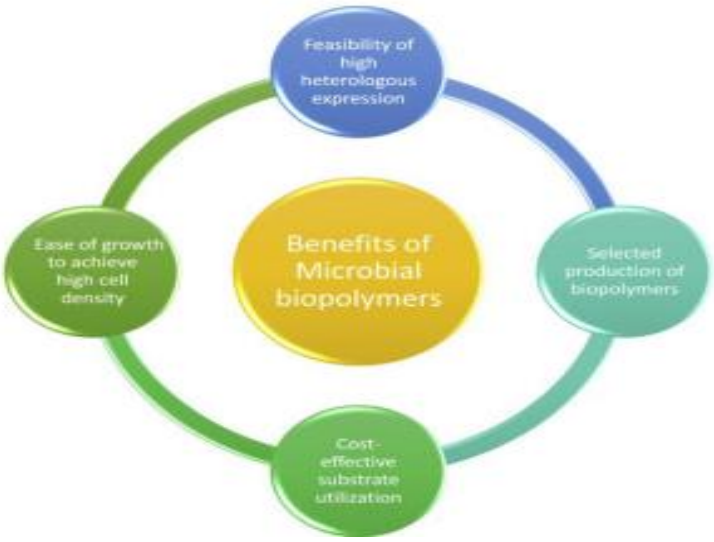


Fig: Benefits of Microbial Biopolymers

- A huge variety of biopolymers, such as polysaccharides, polyesters, and polyamides, are naturally produced by microorganisms.
- Use of microorganisms for the production of the most important biopolymers and polymer precursors, the biosynthetic pathways, physical properties and industrial production processes and the genetic and metabolic engineering of microorganisms for biopolymer production is now becoming an emerging topic.
- Biosynthesis of xanthan, alginate, cellulose, cyanophycin, poly (gamma-glutamic acid), levan , glucan,hyaluronic acid, organic acids, oligosaccharides and polysaccharides, polyhydroxyalkanoates etc. can be elucidated in context of biopolymer synthesis by microorganisms-

16.1 Xanthan Biosynthesis by Xanthomonas Bacteria-

 Plant-pathogenic bacteria of the genus Xanthomonas are able to produce the acidic exopolysaccharide xanthan gum.

 Xanthomonas campestris is a bacterial species capable of producing xanthan gum; it is preferred for genetic modifications. Corn syrup and molasses are widely used feedstocks for the large-scale production of xanthan from Xanthomonas campestris through fermentation technique. Upon polymerization, the xanthan gum is exuded from the bacteria and precipitated out by alcohol precipitation with subsequent removal of the bacterial biomass.

 Because of its physical properties, it is widely used as a viscosifer , thickener, emulsifier or stabilizer in both food and non-food industries.

 Xanthan consists of pentasaccharide repeat units composed of D-glucosyl, D-mannosyl, and D-glucuronyl

acid residues in a molar ratio of 2:2:1 and variable proportions of O-acetyl and pyruvyl residues.

 The xanthan polymer has a branched structure with a cellulose-like backbone. Synthesis originates from glucose as substrate for synthesis of the sugar nucleotides precursors UDP-glucose, UDP-glucuronate, and GDP-mannose that are required for building the pentasaccharide repeat unit. This links the synthesis of xanthan to the central carbohydrate metabolism. The repeat units are built up at undecaprenylphosphate lipid carriers that are anchored in the cytoplasmic membrane. Specific glycosyltransferases sequentially transfer the sugar moieties of the nucleotide sugar xanthan precursors to the lipid carriers. Acetyl and pyruvyl residues are added as non-carbohydrate decorations. Mature repeat units are polymerized and exported in a way resembling the Wzy-dependent polysaccharide synthesis mechanism of Enterobacteriaceae.

16.2 Microbial Production of Alginate

 Alginate is the main representative of a family of polysaccharides that neither show branching nor repeating blocks or unit patterns and this property distinguishes it from to other polymers like xanthan or dextran.

 Although only consisting of two different components, β-D-mannuronic acid and its C5-epimer α-L-guluronic acid, its unique and random structural pattern has attracted a lot of scientific and commercial interest over the past decade.

 The polymer alginate is devoid of branching patters or monomeric repeats like any other polymeric compounds. It is made of two unique elements: as a-L-guluronic acid (C5- epimer) and b-D-mannuronic acid. It is generally produced from brown algae. Azotobacter and Pseudomonas also possess the biosynthetic pathway of producing alginate.

 Two bacterial genera Pseudomonas and Azotobacter, which played a major role in the unravelling of its biosynthesis pathway.

 The pathway involves the generation of the cytosolic precursor GDP-mannuronic acid its polymerization to poly-mannuronic acid while traversing the cytoplasmic membrane. In the bacterial periplasm it can undergo enzymatic modification in form of acetylation or epimerization before the polymer is finally exported through the outer membrane and released into the environment

 Traditionally, the self-assembly processes of algal alginates were mainly used in biotechnology for encapsulation purposes but given the option of fine-tuning its material properties, bacterial alginates are more and more considered for the production of micro- or nanostructures suitable for medical applications.

16.3 Bacterial Cellulose Production

 Gluconacetobacter xylinus (formerly Acetobacter xylinum) has for technical reasons been a favorite model system used for studies of the biochemistry and genetics of cellulose biosynthesis, even though the vast majority of the polymer is produced by green land-plants. Structurally cellulose is a simple polysaccharide, in that it consists only of one type of sugar (glucose), and the units are linearly arranged and linked together by β-1,4 linkages only.

16.4 Cyanophycin: Biosynthesis

 The polyamide molecule cyanophycin consists of a backbone made of poly (aspartic acid) and the amine group of arginine residues associated with the -COOH group of aspartic acid.

 synthesis, large-scale production, biodegradation and potential applications of the polyamide cyanophycin (CGP), which is a non-ribosomally synthesized compound consisting of a poly (aspartic acid) backbone with arginine residues linked to the carboxyl group of each aspartate by their amino group can be elucidated in this aspect.

 In its natural host microorganisms, CGP functions as a storage polymer for nitrogen, carbon and energy. The key enzyme of CGP synthesis is represented by the CGP synthetase (CphA)..

16.5 Poly(gamma-glutamic acid)

 Biosynthesis of poly-γ-glutamic acid (γ-PGA) can be performed by various strains of Bacillus.

 There are many potential applications of γ-PGA as a thickener, cryoprotectant, drug carrier, biological adhesive, flocculant, or heavy metal absorbent with biodegradability in the fields of food, cosmetics, medicine, water treatments and environment also.

16.6 Microbial Hyaluronic Acid Biosynthesis

 Hyaluronic acid (HA) is a biopolymer with valuable applications in the pharmaceutical and cosmetic industry. Currently, HA is produced commercially by either extraction from animal tissues (i.e. rooster comb)or bacterial fermentation.

 Increased concerns over the contamination of animal derived products with infectious agents have made bacterial fermentation a more desirable production system to meet future demands. The high viscosity of HA dictates low titres of 5-10 g/L, a level readily achieved through batch fermentation of Group C streptococci.

 Metabolic engineering and the recent advance in omics technologies are providing new opportunities. Heterologous hosts such as B. subtilis, L. lactis, and E. coli. have been successfully engineered to produce HA and may prove more amenable to engineering high molecular weight Hyaluronic acid.

16.7 Levan

 Levan, a homopolysaccharide which is composed of D-fructofuranosyl residues joined by 2,6 with multiple branches by 2,1 linkages has great potential as a functional biopolymer in foods, feeds, cosmetics, and the pharmaceutical and chemical industries.

 Levan can be used as food or a feed additive with prebiotic and hypocholesterolemic effects.

 Levan is also shown to exert excellent cell-proliferating, skin moisturizing, and skin irritation-alleviating effects as a blending component in cosmetics.

 Levan derivatives such as sulfated, phosphated, or acetylated levans are asserted to be anti-AIDS agents.

 Apart from these levan is used as a coating material in drug delivery formulations.

16.8 Microbial Exopolysaccharides

 Microorganisms synthesize a wide spectrum of multifunctional polysaccharides including intracellular polysaccharides, structural polysaccharides and extracellular polysaccharides or exopolysaccharides (EPS).

 Exopolysaccharides generally constitute of monosaccharides and some non-carbohydrate substituents (such as acetate, pyruvate, succinate, and phosphate).

 Having a wide diversity in composition, exopolysaccharides have found multifarious applications in various food and pharmaceutical industries.

16.9 Polyhydroxyalkanoates

 Polyhydroxyalkanoates (PHAs) are organic polyesters composed of (R)-3-hydroxy fatty acids which are synthesized by most bacteria as a carbon and energy storage material in times of unbalanced nutrient availability.

 They are deposited intracellularly as insoluble spherical inclusions called PHA granules which consist of a polyester core surrounded by a phospholipid layer with attached proteins. One of these proteins is the PHA synthase, the key enzyme of PHA biosynthesis, which catalyzes polyester formation from different (R)-3-hydroxyacyl-CoA precursors.

 The PHA synthase remains covalently attached to the polyester and thus to the PHA granule; other granule-associated proteins are involved in depolymerization, regulation or structural stabilization.

16.10 Dextran

- The dextran is the generic name given to the diverse family of microbial-derived polysaccharides. It is produced by a polymerization reaction in the outer cell by the enzyme dextran sucrase.
- It is the fuel source of bacteria and yeast, which is made up of the monomers of simple sugar.
- Dextran is commercially produced from Leuconostoc mesenteroides and sucrose is used as the major feedstock for its enzymatic conversion or for the fermentation process.
- It can be produced mainly through enzymatic filtration or in industrial large-scale fermenter systems. Enzymatic filtration is highly preferred over the other method because of its uniformity in the product's quality and owing to increased product yield.

16.11 Pullulan

- Aureobasidium pullulans and different species of yeast are capable of producing water-soluble

polysaccharides in the outer cell. The linear polymer of pullulan consists of monomers of three glucose molecules.

- This biopolymer is readily biodegradable and is highly resistant to heat.
- The versatile nature of pullulan makes it useful for various applications. In industry, pullulan is selectively preferred as a plastic material that mimics the properties of polyvinyl chloride and polystyrene in terms of its transparency, hardness, strength, luster , and toughness.

16.12 Glucans

- Glucans are the polymers made up of the monomers of glucose molecules
- The diverse glucan family includes pullulan, cellulose, and yeast glucan.
- Saccharomyces cerevisiae is the major source of glucan molecules because it is the main cell wall component of the yeast (12%-14% of its cell dry weight).
- It is also found in wide varieties of microorganisms such as lichen, fungi, and bacteria, as well as in higher plants.
- Glucan has wide applications in the medical and food industries.

16.13 Gellan

- Four molecules of sugar monomeric repeats (glucose-glucuronic acid glucose-rhamnose) make up the gellan polymer.
- The plant-derived bacterium Pseudomonas elodea produces this gellan polymer.
- The production of gellan is similar to the fermentation process of xanthan polymers.
- It also occupies a major position in the food industry, such as in icings, frostings, jellies, and jams.

17. Microbes in single cell proteins & single cell oil

17.1 Single cell protein (SCP)

Single cell protein refers to the crude, a refined or edible protein extracted from pure microbial cultures (monoculture) as dead or dried cell biomass. They can be used as a protein supplement for both humans and animals. Microorganism like algae, fungi, yeast, and bacteria have very high protein content in their biomass. Common source for SCP are represented by waste and raw material (starch, fruit, fruit waste, molasses etc.), combustible waste and/or byproducts (natural gas, petroleum byproducts, ethanol, methanol, biomass, etc.).

17.1.1 Name of some microbes used as SCP - Microbes which are used as SCP listed below – *Methylococcus capsulatus, Methylomonas methanica, Methylovibrio soehngenii, Spirullina, Candida utilis, Fusarium graminearum, Methylobactor, Arthrobactor, Bacillus, Pseudomonas, Vibrio* etc.

17.1.2 Average composition of the main microorganisms (% dry weight)

	Fungi	Yeast	Algae	Bacteria
Potein	30-45	45-55	40-60	50-65
Fat	2-8	2-6	7-20	1.5-3.0
Nucleic acids	7-10	6-12	3-8	8-12

17.1.3 Application of SCP

- Provides instant energy.
- It is extremely good for healthy eyes and skin.
- Provides the best protein supplemented food for undernourished children
- Serve as a good source of vitamins, amino acids, minerals, crude fibres.
- Control obesity, lower blood sugar level in diabetic patient, reducing body weight, cholesterol and stress.
- As it serve as excellent and convenient source of proteins and others nutrients, it is widely used for feeding cattle, birds, fishes.

Limitation of using SCP –

 The nucleic acid content of microbial biomass is very high (3-8% in algae; 8-12% in bacteria; 6-12% in yeast). This highly hazardous, since humans have a limited capacity to degrade nucleic acid.

 There is a possibility of contamination of pathogenic microorganism in the SCP.

 The digestion of microbial cells is rather slow. This is frequently associated with indigestion and allergic reaction.

17.2 Microbes in single cell oil

Single cell oils (SCOs) are intracellular storage lipids comprising of triacyglycerols (TAGs). SCOs are produced by oleaginous microorganisms which are able to accumulate between 20% and up to 80% lipid per dry biomass in the stationary growth phase under nutrient limitations, e.g., nitrogen or phosphor, with simultaneous excess of carbon source. Microbial SCOs seems to be intriguing substitutes for crude, plant, and fish oil. Microbial lipid production is independent from season, climate, and location.

17.2.1 Name of some microbes used as SCO

The genus *Mortierella* are excellent producers of SCO with high amounts of PUFAs . The genus Mortierella can be divided into two subgenera Mortierella (*M. alpina, M. hyalina, M. elongata*) and *Micromucor* (*M. ramanniana, M. isabellina, M. vinaces*), varying in their composition of SCO . High amounts of ARA up to 70% are produced by *M. alpina*. The genera *M. hyalina* and *M. elongata* produce up to 23% ARA and tend to have higher concentrations of oleic acid.

The most prominent DHA producer amongst microalgae is the heterotrophic dinoflaggelate *Crypthecodinium cohnii* containing more than 50% (w/w) DHA of total fatty acids. Other significant DHA producers are green microalgae of the genus *Schizochytrium.*

17.2.3 Application of SCO

- Microbial oil has potential for application in biodiesel production because of their similarity to plant's oil in composition and structure of fatty acid.
- SCO can be used form any valuable chemicals with applications not only for nutrition but also for fuels and are therefore an ideal basis for a bio-based economy.

18. Microbial Enhanced Oil Recovery (MEOR)

Crude oil is the major source of energy worldwide being exploited as a source of economy, including Oman. As the price of crude oil increases and crude oil reserves collapse, exploitation of oil resources in mature reservoirs is essential for meeting future energy demands. As conventional recovery methods currently used have become less efficient for the needs, there is a continuous demand of developing a new technology which helps in the upgradation of heavy crude oil. Microbial enhanced oil recovery (MEOR) is an important tertiary oil recovery method which is cost-effective and eco-friendly technology to drive the residual oil trapped in the reservoirs. The potential of microorganisms to degrade heavy crude oil to reduce viscosity is considered to be very effective in MEOR.

MEOR is a tertiary oil recovery technique. Recovering oil usually requires three stages. At the primary recovery only 12% to 15% of the oil in the well is recovered without the need to introduce other substances into the well. The oil well is then flooded with water or other substances to drive out an additional oil (15% to 20%) from the well which is known as the secondary recovery. Tertiary recovery is the last phase which is accomplished through several different methods, including MEOR, for the additional extraction of trapped oil from the well. In principle, the process of MEOR results in some beneficial effects such as formation of stable oil-water emulsions reduced interfacial tension and clogging the high permeable zones. In in situ MEOR method, bacteria inoculated with water in to the well will progress into high-permeability zones at first. Then at a later stage they will grow and occlude those zones due to their size and the negative charge on their cell surface. This scenario helps to increase the sweep efficiency, and thus a more efficient oil recovery can be achieved.

Microorganisms can synthesize useful products by fermenting low-cost substrates or raw materials. Therefore, MEOR can substitute chemical enhanced oil recovery (CEOR), which is a very pricey technology. In MEOR, the chosen microbial strains are used to synthesize compounds analogous to those used in CEOR processes which are

very expensive, to increase the recovery of oil from depleted and marginal reservoirs. Furthermore, microbial products are biodegradable and have low toxicity. Microbial technologies are becoming approved universally as lucrative and eco-friendly approaches to improve oil production.

4. Fungi: General & unique characters, nutrition, thallus structure, spores, basic idea of different groups; Mushrooms: basidiocarp, ascocarp, macroscopic & microscopic features, cultivation procedure of edible mushrooms; beneficial & harmful fungi.

Fungus (pl. fungi) is a Latin word which means mushrooms. Fungi are nucleated, spore bearing, achlorophyllous organisms which generally reproduce sexually and asexually, and whose usually filamentous branched somatic structures are typically surrounded by cell walls containing cellulose or chitin, or both (Alexopoulos, 1952).

In simpler words it may also be defined as "non-green, nucleated thallophytes". The common examples of fungi are the yeasts, molds, mushrooms, polypore's, puff balls, rusts and smuts. The branch of botany that deals with the study of fungi is known as mycology (Gr. mykes = mushroom + logos = discourse) and the person knowing fungi is known as mycologist.

The Italian botanist Pier' Antonio Micheli deserves the honor of being called 'Founder of the science of mycology' because he was the first person to give somatic description of fungi in his book Nova plant-arum Genera published in 1729. Anton De Bary (1831-1888) is called the 'father of modern mycology'. At present about 5100 genera and more than 50,000 species of fungi are known.

Characteristics of Fungi:

1. Fungi are cosmopolitan in distribution i.e., they can grow in any place where life is possible.
2. They are heterotrophic in nature due to the absence of chlorophyll. On the basis of their mode of nutrition, they may be parasite, saprophyte or symbionts.
3. The plant body may be unicellular (*Synchytrium, Saccharomyces*) or filamentous (*Mucor, Aspergillus*). The filament is known as hypha (plural, hyphae) and its entangled mass is known as mycelium.
4. The hypha may be aseptate i.e., coenocytic (without septa and containing many nuclei) or septate. The septate mycelium in its cell may contain only one (monokaryotic), two (dikaryotic) or more nuclei.
5. The septa between the cell may have different types of pores: micropore (*Geotrichum*), simple pore (most of the Ascomycotina and Deuteromycotina) or dolipore (Basidiomycotina, except rusts and smuts).
6. The cells are surrounded by distinct cell wall (except slime molds), composed of fungal cellulose i.e., chitin; but in some lower fungi (members of Oomycetes), the cell wall is composed of celluloseor glucan.
7. The cells generally contain colourless proto¬plasm due to absence of chlorophyll, contai¬ning nucleus, mitochondria, endoplasmic reti¬culum, ribosomes, vesicle, microbodies, etc.
8. The cells are haploid, dikaryotic or diploid. The diploid phase is ephemeral (short-lived).
9. In lower fungi like Mastigomycotina, the reproductive cells (zoospores and gametes) may be uni- or biflagellate, having whiplash and/or tinsel type of flagella. But in higher fungi like Zygomycotina, Ascomycotina, Basidiomycotina and Deuteromycotina, motile cells never form at any stage.
10. In response to functional need, the fungal mycelia are modified into different types such as: Plectenchyma, Stroma, Rhizo- morph, Sclerotium, Hyphal trap, Appresorium, Haustorium, etc.
11. The unicellular fungi, where entire plant body becomes converted into reproductive unit, are known as holocarpic fungi (e.g., *Synchytrium*). However, in many others, only a part of the mycelial plant body is converted into reproductive unit, thus they are called eucarpic fungi (e.g., *Pythium, Phytophthora*).
12. They reproduce by three means: Vegetative, asexual and sexual.

(a) Vegetative reproduction takes place by fragmentation (*Mucor, Penicillium, Fusarium*), budding

(*Saccharomyces, Ustilago*) and fission (*Saccharomyces).*

(b) Asexual reproduction takes place by different types of spores. These are zoospores (*Synchytrium*), conidia (*Pythium, Aspergillus*), oidia (*Rhizopus*), chlamydospore (*Fusarium*), etc. The spores may be unicellular (*Aspegillus*) or multicellular (*Alternaria*).

Classification of Fungi:

Taxonomy has a dual purpose first to name an organism according to some internationally accepted system and then to indicate the relationship of the particular organism with other living organisms.

The classification of fungi is still in a state of flux. A stable or ideal scheme is yet to be proposed. The grouping or categories used in the classification of fungi are as follows:

Kingdom
Division
Class
Order
Family
Genus
Species

The kingdom is the largest of the categories and includes many divisions: each division may include many classes and so on down to the species which is the unit of classification. Each of these categories may be divided into subgroups, subdivisions, subclasses, suborders, if necessary. Species are sometimes broke-down into varieties, biological strains and physiological or cultured races.

In accordance with the recommendations of the committee on International rules of Botanical Nomenclature:

(a) The name of divisions of fungi should end in—mycota.

(b) The name of subdivisions should end in—mycotina.

(c) The name of classes should end in—mycetes.

(d) The name of subclasses should end in—mycetideae.

(e) The name of orders should end in—ales.

(f) The name of families should end in a suffix—aceae.

Genera and species have no standard endings. The name of an organism is binomial. It is composed toparts—the first is noun designating the genus in which the organism has been classified, and the second is often an adjective describing the noun which denotes the species. The first letter of each generic name is always a capital.

Classification of Fungi by Ainsworth G. C. (1966, 71, 73):

Ainsworth G. C. (1966, 71, 73) proposed a more natural system of classification of fungi. This classification is based on morphology, especially of reproductive structure. He includes fungi along with slime molds under the kingdom Mycota.

Based on the presence or absence of Plasmodium and pseudoplasmodium; the kingdom Mycota is further divided into two divisions:

Myxomycota i.e., slime molds and Eumycota or true fungi. Divisions are subsequently divided into subdivision, class, subclass, order, family and then to genus. According to his classification, division ends in mycota, subdivision in mycotina, class in mycetes, subclass in mycetidae order in ales and family in aceae.

A schematic outline of Ainsworth's (1973) classification is given:

Kingdom: Mycota

Important features:

i. Free-living, parasitic or mutualistic symbionts, devoid of chlorophyll.

ii. Cell wall composition is very variable, majority contain chitin and glucan.

iii. Reserve food materials are oil, mannitol and glycogen.

iv. Except some unicellular members, majority are filamentous.

A. Division. Myxomycota:

Wall-less organisms possess either a Plasmodium (a mass of naked multinucleate protoplasm having amoeboid movement) or a pseudoplasmodium (an aggregation of separate amoeboid cells). Both areof slimy consistency, hence slime molds.

1. Class. Acrasiomycetes (cellular slime molds)
2. Class. Hydromyxomycetes (net slime molds)
3. Class. Myxomycetes (true slime molds)
4. Class. Plasmodiophoromycetes (endo- parasitic slime molds).

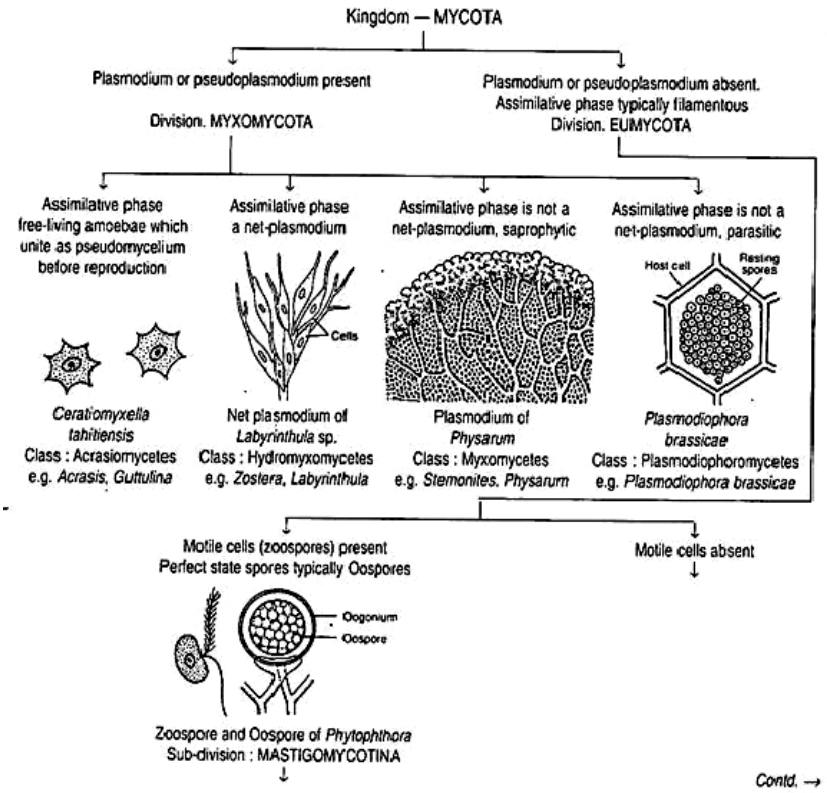
B. Division Eumycota (True fungi, all with walls):

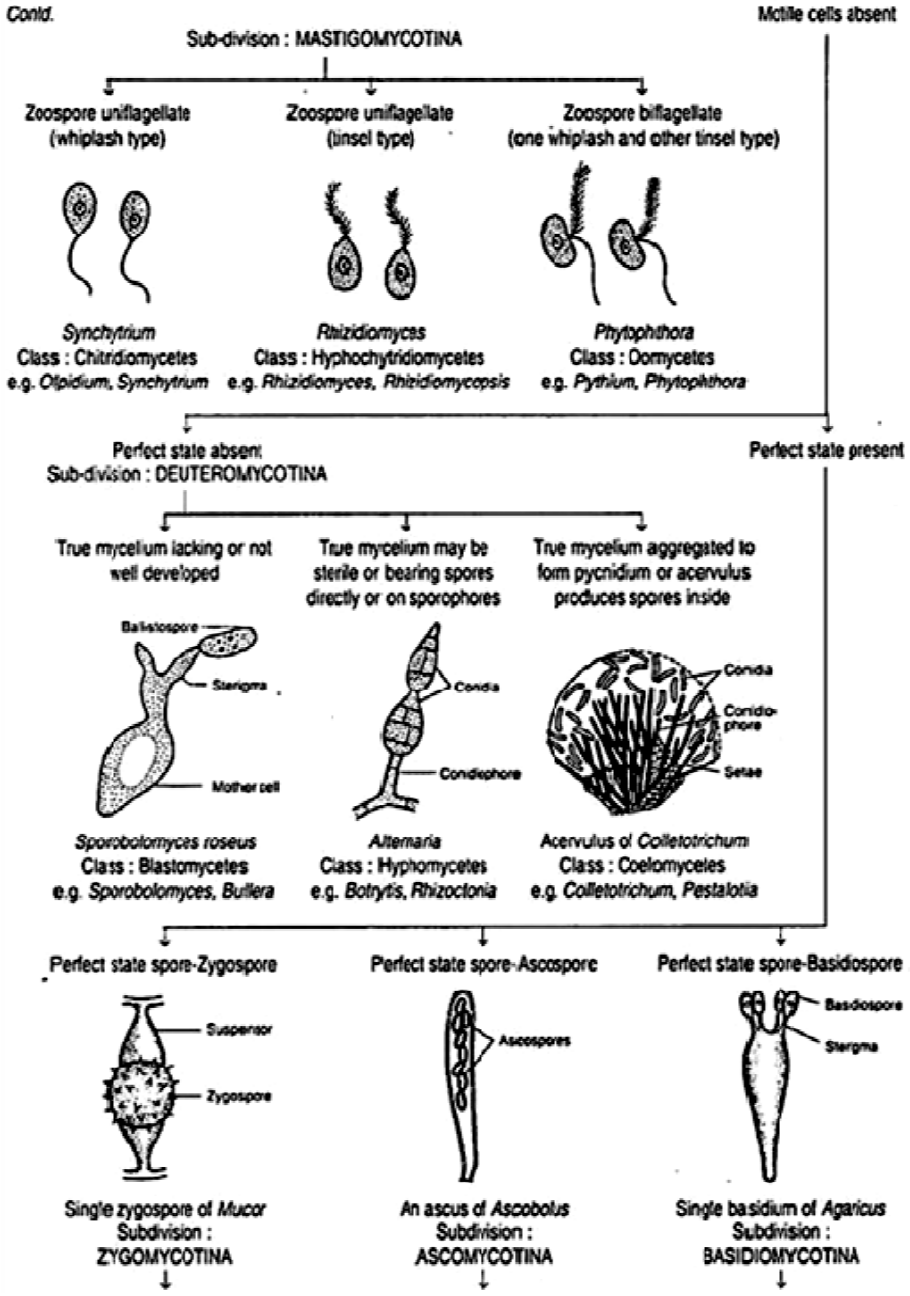
a. Subdivision Mastigomycotina (motile cells – zoospores present, perfect state spore-oospore).

1. Class. Chitridiomycetes (unicellular, zoospore with single whiplash flagellum).
2. Class. Hyphochytridiomycetes (unicellular, zoospore with single tinsel flagellum).
3. Class. Oomycetes (aseptate mycelium, zoospores with two flagella).

b. Subdivision. Zygomycotina (mycelium aseptate, perfect state spore-zygospore).
1. Class. Zygomycetes (mycelium immersed in the host tissue).
2. Class. Trichomycetes (mycelium not immersed in the host tissue).
c. Subdivision. Ascomycotina (yeasts or septate mycelium, perfect state spore- ascospores formed in ascus, usually within ascocarp).
1. Class. Hemiascomycetes (no ascocarp, asci naked).
2. Class. Loculoascomycetes (fruit body an ascostroma, asci bitunicate i.e., 2-walled).
3. Class. Plectomycetes (fruit body cleistothecium, asci unitunicate i.e., 1-walled).
4. Class. Laboulbeniomycetes (fruit body perithecium, asci unitunicate, exoparasite of arthopods).
5. Class. Pyrenomycetes (fruit body perithecium, asci unitunicate, not parasitic on arthopods.
6. Class. Discomycetes (fruit body apothecium, asci unitunicate).
d. Subdivision. Basidiomycotina (yeast or septate mycelium, perfect state spore – basidiospore formed on a basidium).
1. Class. Teliomycetes. Basidiocarp lacking, teliospores grouped in sori or scattered within the host tissue, parasitic on vascular plant.
2. Class. Hymenomycetes. Basidio- carp present. Hymenium is completely or partly exposed at maturity. Basidiospore ballistospores.
3. Class. Casteromycetes. Basidiocarp present. Hymenium enclosed in basidiocarp. Basidiospore not ballistospores.
e. Subdivision. Deuteromycotina or Fungi imperfecti. Yeast or septate mycelium. Perfect state unknown.
1. Class. Blastomycetes. Budding (Yeast or Yeast like) cells with or without pseudomycelium. True mycelium lacking or not well-developed.
2. Class. Hyphomycetes. Mycelia sterile or bearing asexual spore directly or on conidiophore, in various aggregation.
3. Class. Coelomycetes. Mycelial; asexual spore formed in pycnidium or acervulus.

Schematic representation of the outline with figure, the classification of G.C. Ainsworth (1973) is given:





4. Salient Features of Important Classes of Fungi:

The important classes of true fungi are the Chytridiomycetes, Oomycetes, Zygomycetes, A comycei Basidiomycetes and Deuteromycetes (according to Alexopoulos, 1962).

Their respective salient features as follows:

Class—Chytridiomycetes:

1. The members of this class are usually aquatic but some live in moist soil.

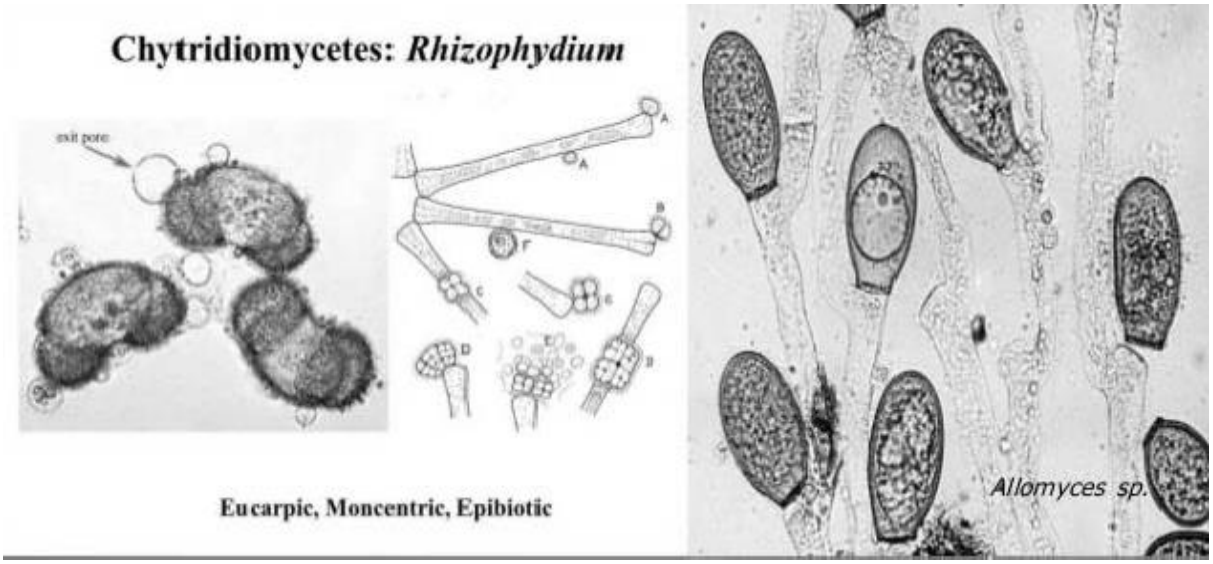
2. Most of the members are parasitic on algae. However, some members have also parasitized small water animals and seed plants.

3. The thallus is coenocytic, holocarpic or eucarpic or filamentous.

4. The cell wall is made up of chitin (a polymer of N-acetyl glucosamine).

5. All the members of this class produce motile cells (zoospores or gametes), each equipped with a single posterior, whiplash flagellum.

6. Some members of this class (e.g., *Allomyces*) exhibit a true alternation of generation.



7. The important member of this class is *Synchytriun endobioticum*. It causes the important disease Black wart of potato.

Class—Oomycetes:

1. The members of this class are characterized by oogamous sexual reproduction.

2. The members of this group exhibit progressive evolution from aquatic to land habitat. Some members of this class are aquatic, others are terrestrial and still others live in association with terrestrial seed plants.

3. Biologically the members are aquatic saprophytes or parasites. Some members are terrestrial facultative to obligate parasites.

4. The thallus may be unicellular or filamentous. The filamentous forms are coenocytic.

5. The chief component of the cell wall is cellulose.

6. This class is an assemblage of unique fungi in possessing a diploid thallus and meiosis occurs before the formation of gametes.

7. Asexual reproduction takes place by bi-flagellated zoospores. The zoospores are kidney shaped or pear shaped having two flagella. One of the flagellum is of tinsel type and the other of whiplash type.

8. Important diseases caused by the members of the class are mildews, blights (late blight of potato caused by Phytophthora infestans) and white rust (white rust of crucifers caused by Albugo Candida) of crop plants.

Class—Zygomycetes:

1. This class includes about 70 genera and 450 species. The members are terrestrial in this class. They live in soil, on dung or on decaying plant and animal matter.

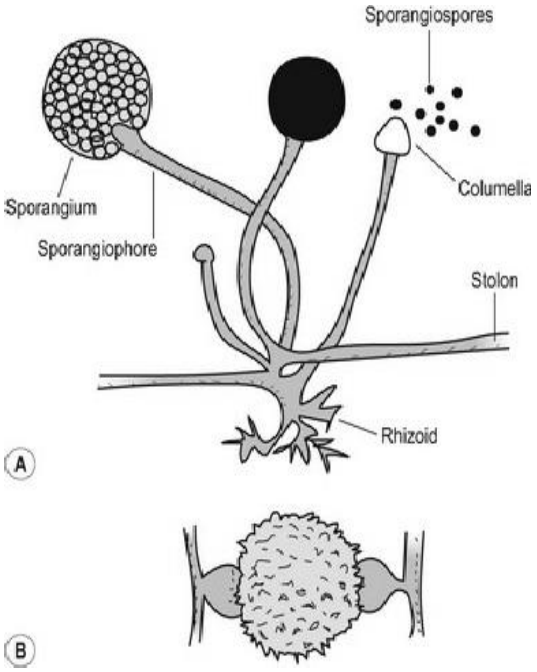
2. Biologically the members are saprophytic but some are parasitic on plants, insects and soil animals (e.g., Amoeba and nematodes). Some members attack the human body causing the disease mucormycosis.

3. The thallus is coencocytic and filamentous.

4. The cell walls are chiefly composed of fungal chitin, cellulose may also be present along with it.

5. The most characteristic feature of zygomycetes, is the complete absence of motile (flagellate) sexual or asexual cells.

6. Asexual reproduction takes place by means of non-motile sporangiospores produced in large number within terminal sporangia.



7. Chlamydospores are present.

8. Sexual reproduction takes place by gametangial copulation. The gametangia of equal or unequal size unite to form the resting spore called zygospore.

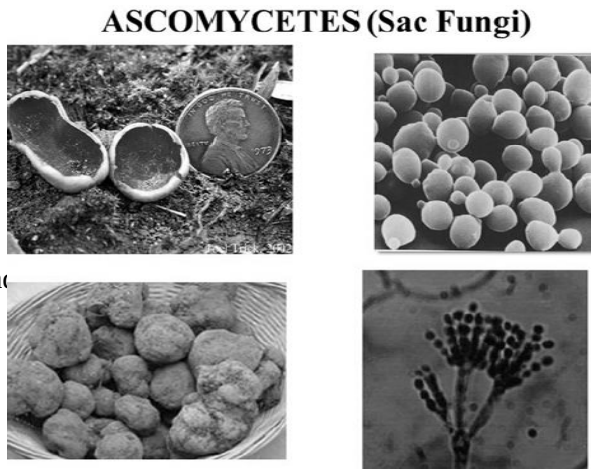
9. Zygospore on germination produces a hypha which bears a terminal sporangium.

10. Economically the members of this class are very important. They are employed in industry to produce organic acids like oxalic acid, lactic acid and fumaric acid.

Class—Ascomycetes:

1. Generally the members of this class are terrestrial. Some, however, are marine.

2. Terrestrial members are saprophytic as well as parasitic. The saprophytic members grow on soil rich in humus, decaying vegetable or animal matter, on dung, food stuffs and rotting logs. Parasitic members attack both plants and animals including man.



3. The thallus may be unicellular (e.g., Yeast) while others are filamentous having septate mycelium with uninucleate or multinucleatecells.

4. Component of cell wall is chitin.

5. Asexual reproduction takes place by fission, budding, fragmentation, arthrospores, chlamydospores or conidia, according to species and environmental conditions.

6. Commonly called as sac fungi on account of the presence a sac-like structure termed as the ascus. This ascus is the product of sexual reproduction.

7. The ascus contains a definite number of spores called the ascospores.

8. Motile phase is completely absent in the sexual and asexual reproduction.

9. Origin of dikaryophase takes place in life cycle.

10. Members are economically important. They are employed in agriculture, medicines and various industries.

11. Important diseases caused by the members of this class are apple scab, powdery mildew of grapes and peaches etc.

Class—Basidiomycetes:

1. The members of this class are characterized by the exogenous production of spores termed as basidiospores.

2. Generally the members of this class are terrestrial. Some, however, grow on logs and treestumps.

3. Biologically the members are parasites (rusts and smuts) as well as saprophytes.

4. The vegetative mycelium is well developed, septate and is of three different types—primary, secondary and tertiary.

5. Septal pore in this class is complex. It is dolipore type.

6. Clamp connections are present.

7. Motile cells are absent in life cycle.

8. Asexual reproduction is by conidia and chlamydospores.

9. Sex organs are absent in this class but monokaryotic hyphal cells or oidia etc. act as sexual cells.

10. The characteristic reproductive organ of this class is ba sidium. In it both karyogamy and meiosis take place.

11. Some members cause very serious diseases of plants like rusts and smuts (e.g., Puccinia graminis tritici causes black rust of wheat; Ustilago tritici causes loose smut of wheat).

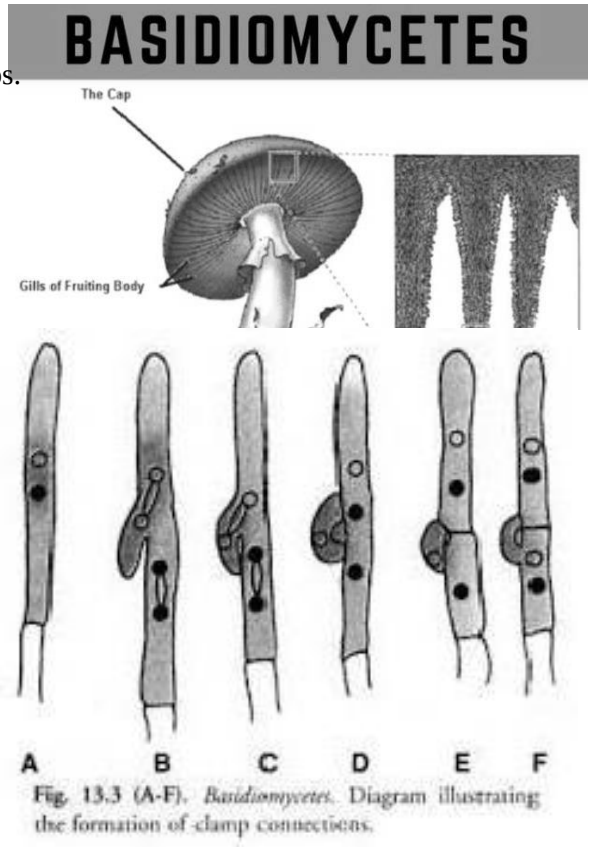
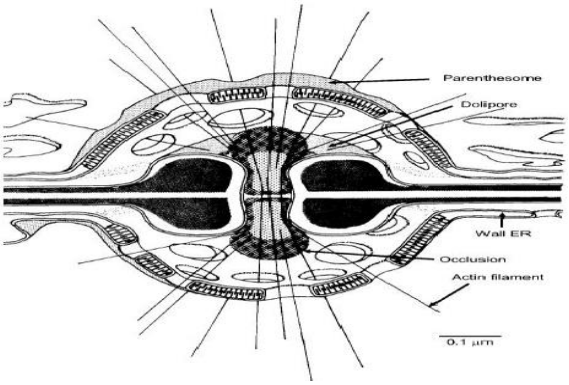


Fig. 13.3 (A-F). *Basidiomycetes*. Diagram illustrating the formation of clamp connections.

Dolipore/parenthesome septum

Perforated (homobasidiomycetes)

Imperforated (heterobasidiomycetes)



Functions:

1. To secure the integrity of hyphal cell;

2. To maintain intercellular communication and transfer of cell organelles, except nuclei;

3. Repair of hyphal damage - pore rapidly plugged by electron dense material in the compartment of a hypha adjacent to the damaged segment.

Class—Deuteromycetes:

1. This class includes only those members of fungi in which asexual or imperfect stage is known. The sexual or the perfect stage is unknown.

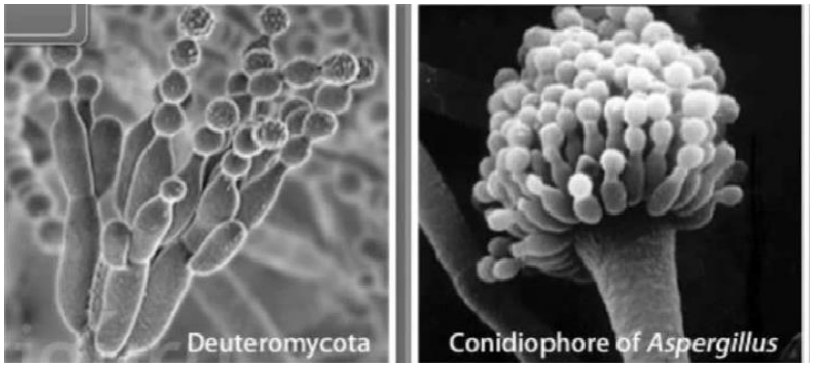
2. Biologically the members are saprophytes as well as parasites.

3. The vegetative mycelium is septate and profusely branched.

4. Some of the members of this class resemble with members of Ascomycetes and a few resemble with Basidiomycetes in structure and reproduction.

5. Parasexual cycle (sometimes nuclear fusion occurs followed by reduction division) has been observed in some members of this class.

6. Many members cause serious diseases of plants e.g., early blight of potato caused by *Alternaria solani* and red rot of sugarcane caused by *Colletotrichum falcatum*.



a) The Cell Wall of the Fungal Cell:

Except slime molds (Myxomycetes), the fungal cell consists of a rigid cell wall and cell organelles. However, composition of cell wall of different fungal groups differs. Chemical analysis of cell wall reveals that it contains 80-90% polysaccharides, and remaining proteins and lipids.

Chitin (a polymer of N-acetyl glucosamine), cellulose (a polymer of D-glucose) or other glucans are present in cell walls in the form of fibrils forming layers. In most of the fungi the cell wall lacks cellulose (except Oomycetes) usually chitin and cellulose are found together e.g. Ceratocystis and Rhizidiomyces contain a form of chitin called fungus cellulose. It is similar to the chitin of insects.

The microfibril layers run parallel to the surface. Several non-fibril materials are also associated with micro-fibrils. Though chitin is the most usual component yet cellulose is present in cell walls of Oomycetes along with glucans. An amino acid and hydroxy-protein are present in the cell wall of Oomycetes along with cellulose.

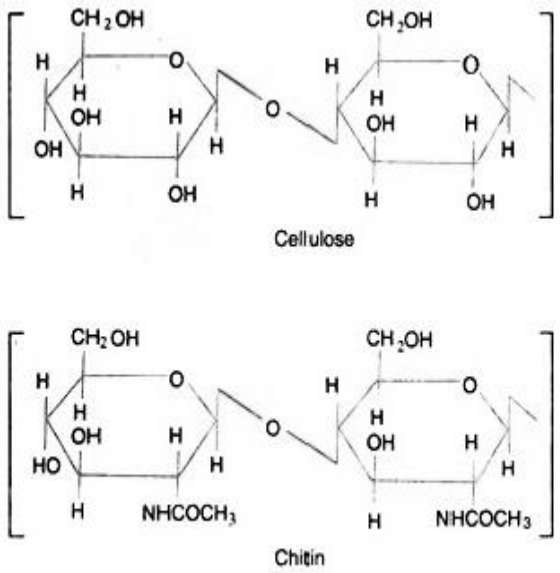


Fig. 4.36 : Structural formula representing the units of cellulose and chitin.

Several other substances have also been found to be associated together in cell walls and cell wall components such as proteins enzymes, etc. In Peronospora in Phytophthora and and Saprolegnia true cellulose is present, but Pythium cellulose is totally absent and glucans predominates in their walls. In the cell wall of some fungi the presence of chitin has been reported.

The basic constituents of cell walls of Zygomycetes, Ascomycetes and Basidiomycetes are chitin. But in yeasts and some Hemiascomycetidae chitin is absent. Micro-fibrils of mannans and β-glucan constitute their cell wall. Various chemical substances found in cell walls seem to be correlated with fungal taxonomy.

Table 4.5 : Taxonomy of fungal cell walls.

Categories	*Taxonomic groups*	*Features*
Cellulose-glycogen	Acrasiales	Pseudoplasmodia
Cellulose-glucan	Oomycetes	Biflagellate zoospores
Cellulose-chitin	Hypochytridiomycetes	Anteriorly uniflagellate zoospores
Chitosan-chitin	Zygomycetes	Zygospores
Chitin-glucan	Chytridiomycetes	Posteriorly unitlagallate zoospore
	Ascomycetes	Septate hyphae, ascospores
	Basidiomycetes	Septate hyphae, basidiospores
	Deuteromycetes	Septate hyphae
Mannan-glucan	Saccharomycetaceae	Yeast cells, ascospore
	Cryptococcaceae	Yeast cells
Mannan- chitin	Sporobolomycetaceae	Yeast cells, ballistospores
	Rhodotorulaceae	Yeast (carotenoid pigment)
Polygalacturosamine-galactan	Trichomycetes	Heterogenous group

(b) The Protoplast in the Fungal Cell:

The living substance of the cell within the cell wall is the protoplast. It lacks the chloroplasts but is differentiated into the other usual cell parts such as plasma or cell membrane, vacuolated cytoplasm, cell organelles and one or more nuclei.

Cell Membrane:

It is a delicate, extremely thin, living membrane which closely invests the protoplast. The cell or plasma membrane is pressed against the cell or hyphal wall except for occasional invaginations in some regions. The Invagination is either in the form of an infolded convoluted pocket or a pouch enclosing granular or vesicular material.

Moore and Mc Lear (1961) named it lomasome. Actually the plasma membrane is the surface layer of the protoplast altered to perform special functions. It is differentially permeable and shows a typical tripartite structure under the electron microscope. There is an electron dense layer on either side of the less dense central region.

Cytoplasm:

Within the plasma membrane is the colorless cytoplasm in which sap-filled vacuoles may occur. In young hyphae and hyphal tips, the cytoplasm appears rather uniform and homogeneous. Immersed in the cytoplasm are structures known as the organelles and inclusions.

The organelles are living structures, each with a specific function. The inclusions are dead, have no specific function and thus are not essential to cell survival.

Amongst the cell organelles are included the endoplasmic reticulum, mitochondria, ribosomes, Golgi

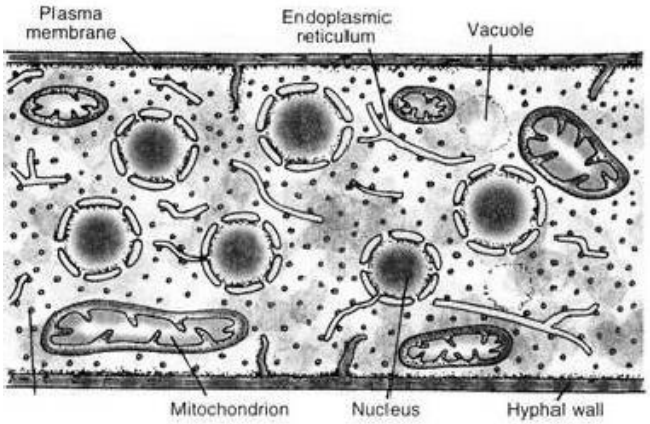


Fig. 1.9. *Fungi*. Fine structure of a hypha near the growing tip of *Mucor* based on an electron micrograph.

apparatus and vacuoles. Lomasomes which are membranous structures lying between the cell walland plasma membrane are common. Examples of inclusions are the stored foods (glycogen, and oil drops) pigments and secretory granules.

(i) Endoplasmic Reticulum:

The presence of endoplasmic reticulum in the fungal cytoplasm has been demonstrated by the use of electron micro-scope. It is composed of a system of membranes or microtubular structures usually beset with small granules which by some scientists are likened to the ribosomes. In many fungi, the endoplasmic reticulum is highly vesicular. Usually it is loose and more irregular than in the cells of green plants.

(ii) Mitochondria:

The cytoplasm contains small, usually spherical bodies known as the mitochondria. Each mitochondrion is enveloped by a double membrane. The inner membrane is infolded to form the cristae which are in the form of parallel flat plates or irregular tubules.

The cristae contain the same fluid that fills the space between the two membranes. The mitochondria function as the power house of the cell. There is no fundamental difference between the mitochondria of fungi and those of green plants. However, Hawker (1965) holds that the cristae of fungal mitochondria are fewer, flatter and more irregular than those of the green plants.

(iii) Golgi Apparatus (Dictyosomes):

With the exception of Oomycetes there is less certainty of the occurrence of structures similar to those of the golgi apparatus (dictyosomes) m fungi. Moore and Muhlethaler (1963) reported a golgi apparatus consisting of three flattened sacs surrounded by many bubble-like structures in Saccharomyces cells.

(iv) Vacuole:

The cytoplasm of young hyphae or fungal cells and hyphal tips lacks vacuoles. They appear further back or in the old cells. With age, they enlarge and show a tendency to coalesce and ultimately reduce the cytoplasm to thin lining layer immediately within the cell wall.

(v) Inclusions:

The cytoplasm contains various kinds of inclusions. Examples of stored foods are lipid globules, granules of glycogen, oils and the carbohydrate trehalose, proteinaceous material and volutin. The glycogen may occur in vacuoles.

There are no starch grains. Of the pigments, the fungi lack chlorophyll. Carotenoids are often conspicuous by their presence and may occur throughout the cytoplasm or concentrated in the lipid granules or distributed in the cell wall. The cytoplasm, in addition, secretes several kinds .of ferments, enzymes and organic acids.

Nucleus:

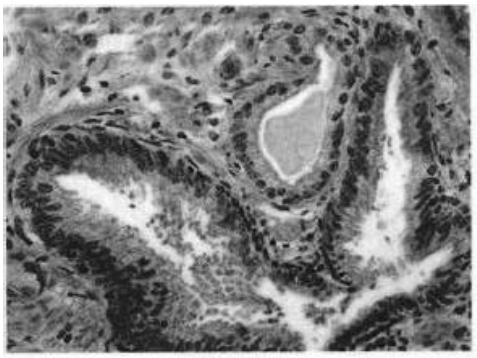
The cytoplasm in the individual cells contains one, two ormore globose or ellipsoid nuclei which in the somatic portion are small and usually range from or 3µ in diameter. They cannot be seen without special techniques.

Structurally the nucleus consists of:

(i) A central, dense body with a clear area around it.

(ii) Chromatin strands, and

(iii) The whole structure surrounded by a definite nuclear, membrane.



Nucleolus.

The central body takes heavy iron haematoxylin stain and is usually Feulgen-negative. In electron micrographs, it appears as an amorphous or granular mass. Mycologists usually designate it as the nucleolus. Bakerspigel (1960) stated that it contains RNA. During nuclear division, the chromatin strands become organised into chromosomes which are extremely small and difficult to count.

Under the electron microscope, the nuclear membrane is seen to consist of inner and outer layers of electron dense material and the middle one of electron transparent substance. The nuclear membrane has pores. At certain points, the nuclear membrane is continuous with the endoplasmic reticulum.

Hyphal Forms:

In response to functional need, the fungal mycelia are modified into different types of struture:

1. Plectenchyma:

When the component hyphae completely interwoven to form a compact thick tissue, it is called plectenchyma.

It is of two types:

Prosenchyma or prosoplectenchyma, where the hyphae remain more or less parallel to each other,retain their individuality and do not fuse and pseudoparenchyma or para-plectenchyma, where the hyphae are com-pletely fused to each other, form a compact mass and lose their individuality. In cross-section the whole mass looks like paren-chyma of angio plant.

2. Sclerotia (sing. Sclerotium):

It is a compact structure of different shapes and sizes, formed by the aggregation of mycelia. It may be round to elongated pod-like, very minute dots to large ball-like structure weighing approximat

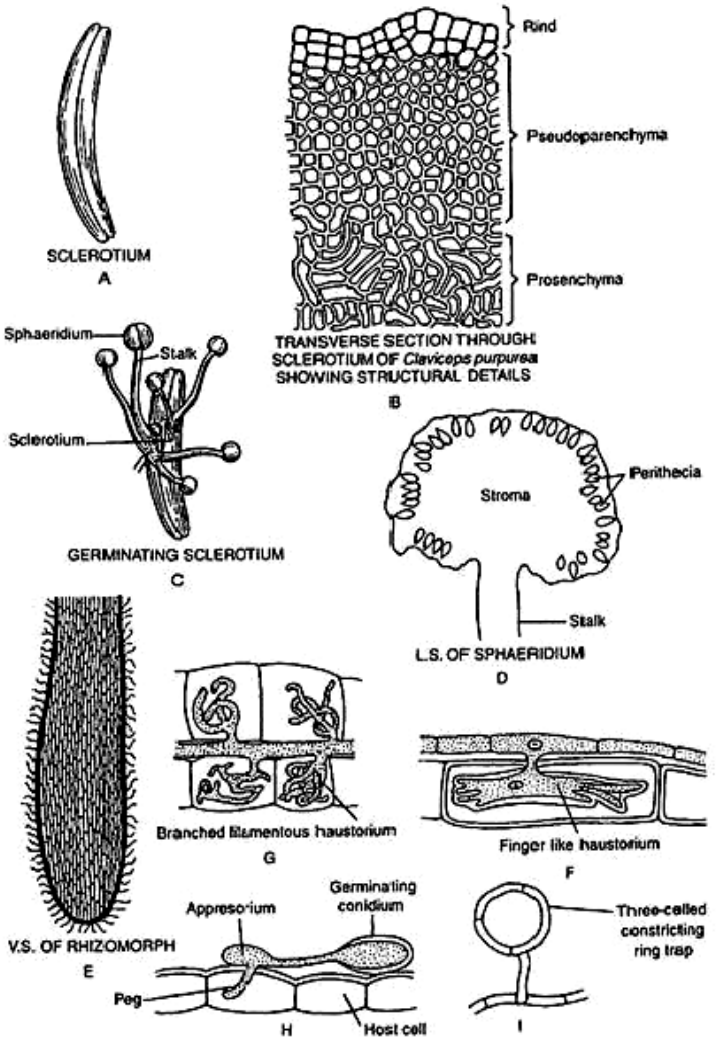


Fig. 4.6 : Different type of modifications of the hyphal structure : A-D. *Claviceps purpurea*, E. *Armillariella mellea* (= *Armillaria mellea*), F. Haustorium of *Erysiphae graminis* in epidermal cell of oat in longitudinal section, G. Haustoria of *Peronospora calotheca* in stem cells of *Asperula odorata*, H. Appresorium of *Erysiphe graminis tritici* on wheat, and I. Hyphal trap of *Monocresporium*

3. Rhizomorph:

In this case, hyphae are aggre-gated longitudinally in varying degree of complexity, where the hyphae lose their indi-viduality and the whole structure behaves as an organised unit. They can withstand adverse environmental conditions and after few years, they can start growing.

4. Haustoria (sing. Haustorium):

These are intracellular outgrowth of the mycelium that grows intercellularly for absorption of nutri-ent from host cells. They are of various shapes and sizes, like simple, knob-like, coiled, branched, etc. They penetrate the cell wall and generally do not rupture the cell membrane during absorption.

5. Appresoria (sing. Appresorium):

It is the swollen tip of germ tube or mycelium of plant pathogenic fungi which helps the myce-lium to adhere to the surface of the host and also helps in penetration.

6. Hyphal trap:

Certain fungi develop sticky hypha or hyphal loops to catch predators like nematode, protozoa, small animals etc., known as hyphal trap. The fungi of this kind is known as Predaceous fungi.

7. Stroma:

It is a solid body of various shapes and sizes, formed by the compact aggrega-tion of mycelium. Reproductive structures and fruit bodies are developed inside the stroma.

Reproduction in Fungi:

In unicellular fungi (*Synchitrium, Saccharomyces*), entire vegetative cell is transformed into a reproductive unit, called **Holocarpic**. However, in others (*Pythium, Penicillium, Helminthosporium*), only a part of the vegetative body forms reproductive unit and the rest portion remains as vegetative, called **Eucarpic**.

The fungi reproduces by all the three means:

1. Vegetative Reproduction:

It takes place by the following ways:

(a) Fragmentation:

It is common in filamen-tous fungi (*Rhizopus, Alternaria, Fusarium*) where the hyphae break up into two or more fragments due to some external force and each one develops into a new individual.

(b) Budding:

It takes place in unicellular fungi (*Saccharomyces, Schizosaccharomyces*). A small outgrowth, the bud emerges out from the parent cell. Nucleus divides into two and one passes to the bud. The bud is then separated by partition wall, but continues its growth.

(c) Fission:

Normally unicellular fungi (*Saccharomyces, Schizosaccharomyces*) reproduce by this method, where the vegetative cell elongates, and divides into two daughter cells of equal size by simple constriction in the middle with simultaneous nuclear division.

2. Asexual Reproduction:

It takes place by means of several types of spore generally form during favourable condition. The spores may be unicellular (*Penicillium, Aspergillus*) or multicellular (*Fusarium, Helminthosporium*).

Some of the spores are:

(a) Zoospore:

The zoospores may be uni- or biflagellate, generally pear-shaped, produced in sporangium, e.g., *Synchytrium, Phytophthora*.

(b) Conidia:

These are exogenously pro-duced non-motile spores develop by constriction at the end of specialised hyphal branches, called conidiophores. They may produce singly (*Phytophthora, Pythium*) or in chain (*Penicillium, Aspergillus*).

(c) Oidia:

In some fungi (*Mucor mucedo*), the hyphal tips often divide by trans-verse wall into large number of small segments, may remain in chain or becomes free from each other, these are known as oidia. The oidia on germina-tion develop into new plants.

(d) Chlamydospore:

The chlamydospores are thick walled round to oval in outline, coloured brown or black. They produce either terminally or in intercalary at some intervals throughout the length of hyphae, e.g., *Fusarium.*

(e) Sporangiospores:

These are globose, multinucleate, non-motile aplanospores, formed inside the sporangium. The sporangiospore germinates by producing germ tube. Later on, it develops pro-fusely branched mycelium.

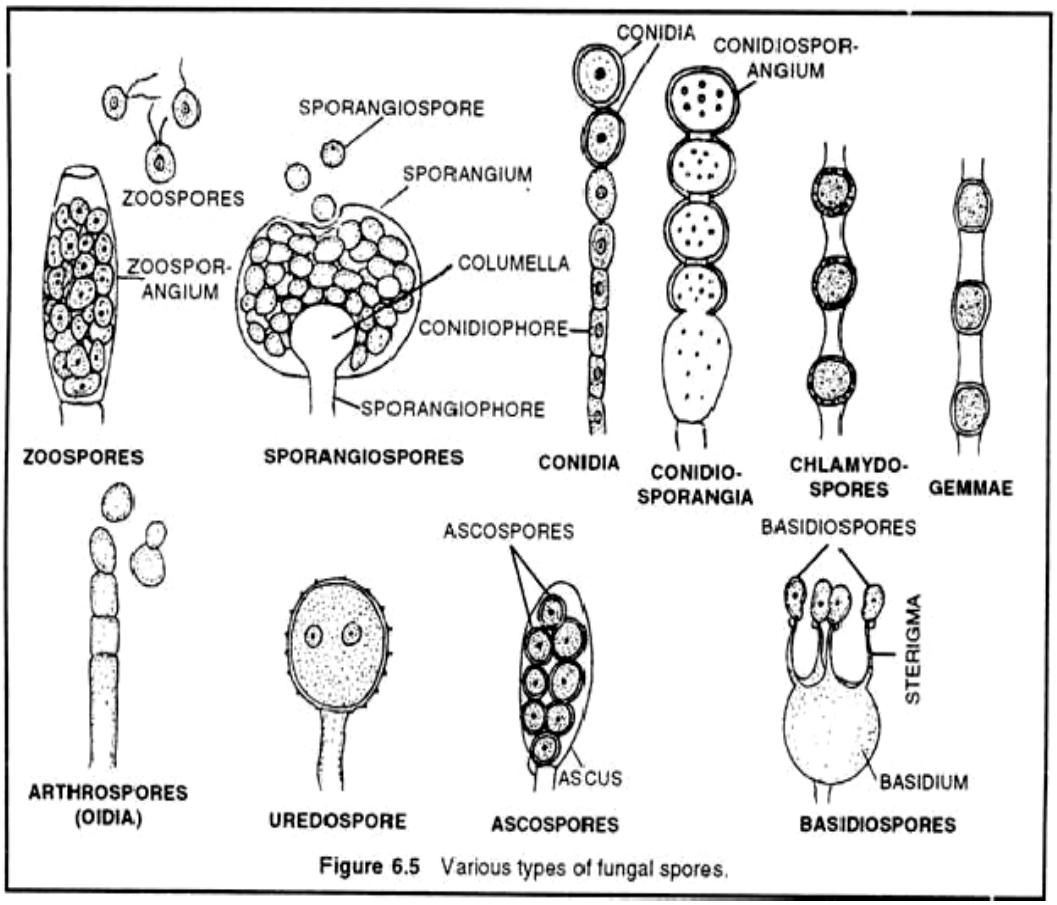


Figure 6.5 Various types of fungal spores.

Sexual Reproduction:

It is the process of union between two compatible nuclei. The nuclei in some members are contributed by two well-organized gametes.

The whole process of sexual reproduction consists of three phases, in the sequence of plasmogamy, karyogany and meosis:

(i) Plasmogamy:

It involves the union of two protoplasts, brings two haploid nuclei close together in the same cell.

(ii) Karyogamy:

It involves the fusion of two haploid nuclei brought together during plasmogamy. This results in the forma-tion of diploid nucleus i.e., zygote, which is ephemeral (short-lived).

(iii) Meiosis:

It follows karyogamy and reduce the number of chromosome from diploid zygote nucleus to original haploid number in the daughter nuclei.

The plasmogamy i.e., the first phase of sexual reproduction, differs in different fungi.

The different methods of plasmogamy are:

(a) Planogametic Copulation:

Planogametes are motile gametes. This process involves the fusion of two gametes, where either one or both are motile.

Depending on the structure and nature of gametes, it is of three types:

Isogamy, Anisogamy and Oogamy:

(i) Isogamy:

The uniting gametes are morphologically similar, but physio-logically different. This process is common in primitive unicellular fungi, e.g., *Synchytrium*.

(ii) Anisogamy:

Both the uniting gametes are morphologically simi-lar, but different physiologically and in size. The smaller one is more active, considered as male and the larger less active one as female, e.g., *Allomyces*.

(iii) Oogamy:

Both the uniting gametes are morphologically and physio-logically different. The male gamate is smaller and motile, and the female gamete is larger and non-motile, e.g., *Monoblepharis*.

(b) Gametangial Contact:

The uniting gametes are present in different gametangium, thus the male and female gametangia are known as antheridium and Oogonium (Ascogonium in Asc-mycotina), respectively. The gametes are never released from gametangium. Both the gametangia come in close contact and transfer male gamete to the egg through fertilization tube. The gametangia do not lose their identity, e.g., *Ascobolus, Pythium*.

(c) Gametangial Copulation:

The process involves the fusion of the entire content of the uniting gametangia.

Such fusion occurs in the following two ways:

Various Methods of Plasmogamy in Fungi

(i) The two gametangia fuse by the dissolution of their common wall resulting into the formation of a sin-gle cell in which content of both the gametangia mix with each other and their morphological identity are completely lost, e.g., *Rhizopus, Mucor*.

(ii) The entire thallus acts as game-tangium. Both the gametangia come in close contact and the male gametangium transfer its entire content to the female gametangium through the pore developed in con-tact area e.g., *Rhizophidium, Polyphagus*.

(d) Spermatisation:

Certain fungi produce many unicellular non-motile, male cells, the spermatia. The spermatia are brought in contact by agents like wind, water and insect either to the trichogyne of the ascogonium or to somatic hyphae or even to special receptive hyphae. The wall at the point of contact dis-solves and content of spermatia passes to the female organ, e.g., *Puccinia, Podospora*.

(e) Somatogamy:

In many higher fungi belonging to Asomycotina and Basidiomycotina, the development of gametes and gametangia are complete-ly lacking. In such fungi, somatic hyphae anastomose with each other to bring together the compatible nuclei. It is regarded as a reduced and efficient form of sexuality, designated as somatogamy, e.g., *Polyporus, Agaricus, Morchella*.

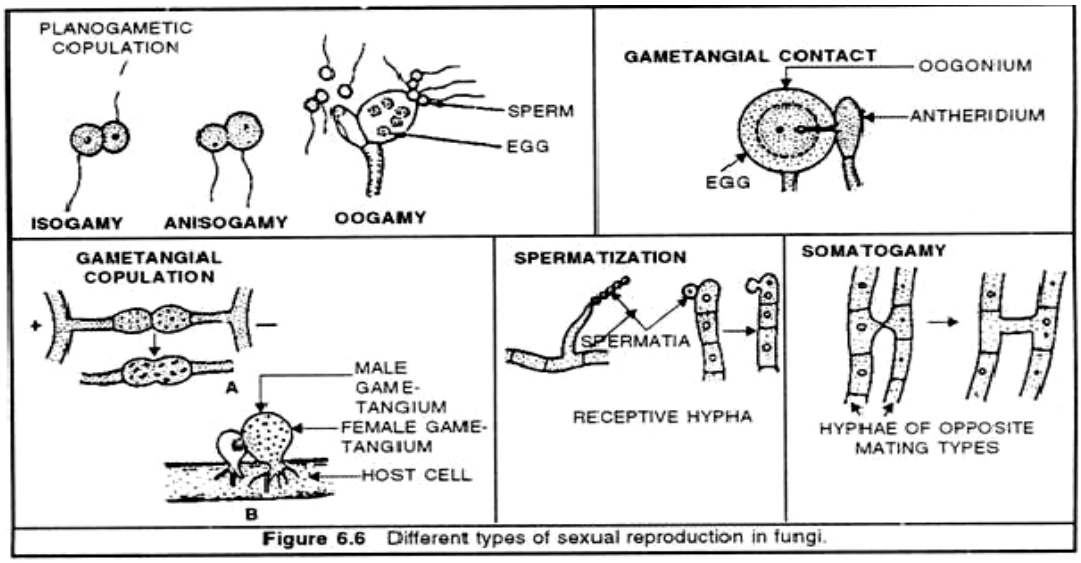


Figure 6.6 Different types of sexual reproduction in fungi.

Heterothallism

The term Heterothallism was first used by an American geneticist A.F. Blakeslee in 1904 when he observed that zygospores could develop in some spp. only when two mycelia of different strains were allowed to come in contact with each other.

According to Blakeslee (1904) Heterothallic condition is **"essentially similar to that in dioecious plants and animals and although in this case the two complimentary individuals which are**

needed for sexual reproduction are in general not so conspicuously differentiated morphologically as in higher forms, such a morphological difference is often distinctly visible."

He concluded that the Zygospore formation is a sexual process. In homothallic species, the mycelium is bisexual while the mycelium in heterothallic species is unisexual, (+) and (-) strains represent the two different sexes.

Heterothallism may therefore be defined as the condition in which Zygospore formation takes place only when mycelia arising from asexual spores of two genetically different mating types (+) and (-), are allowed to interact.

On the other hand the condition, in which one individual originating from a single asexual spore is capable of forming zygospores independently, is known as Homothallism.

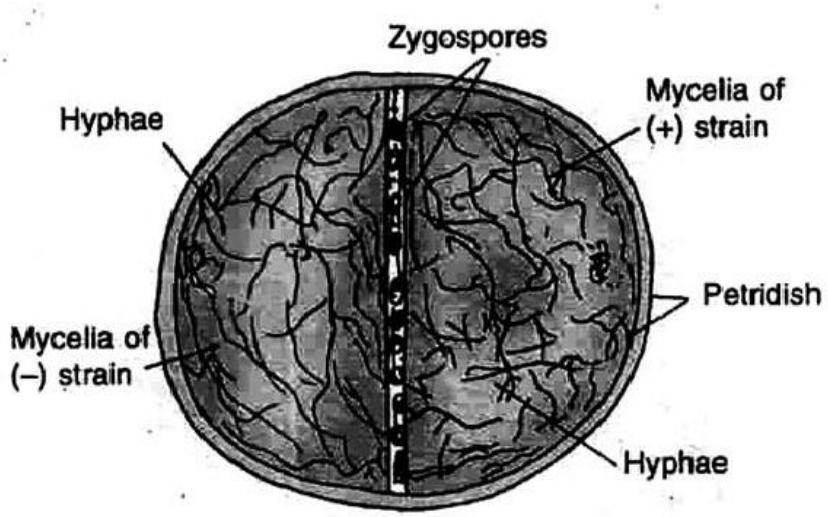


Fig. 17.2 *Mucor hiemalis*. Zygospore formation indicating Heterothallism.

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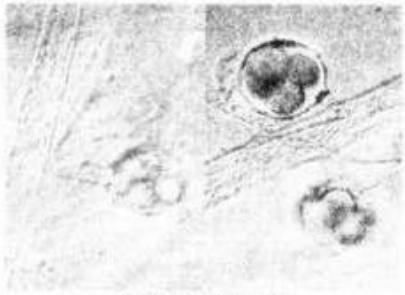
Morphological heterothallism:

Morphological heterothallism may be defined as the condition when morphologically different maleand female sex organs are produced in two closely associated mycelia.

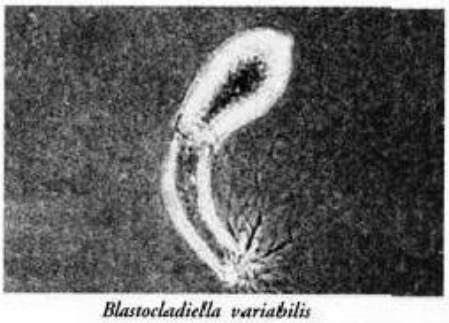
The two sex organs or gametes are so morphologically different that it is easier to term one of them as male and the other as female-examples of such type of morphological heterothallic fungi are: Achlya ambisexualis, A. bisexualis, Blastocladiella variabilis, Dictyuchus monosporus, Phytophthora palmivora and Peronospora parasitica (photographs given below).

However, in Blastocladiella variabilis the male and female gametangia are morphologically distinct,the male being smaller than the female.

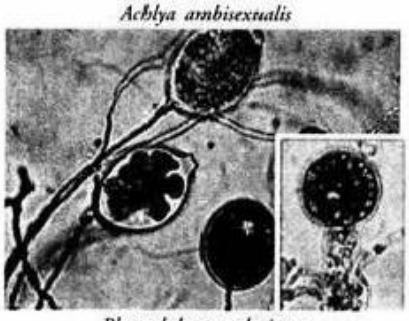
Whitehouse (1949) also used the term haplodioecious for morphologically heterothallic species of fungi.



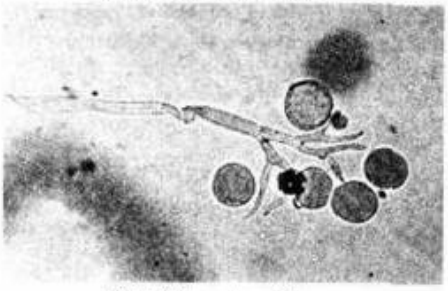
Achlya ambisexualis



Blastocladiella variabilis



Phytophthora palmivora



Phytophthora parasitica

Physiological Heterothallism:

In physiological heterothallism, the interacting thalli differ in mating type or incompatibility, irrespective of the presence or absence of the sex organs or gametes. This means that sexual reproduction takes place by two morphologically similar but physiologically different hyphae in physiological heterothallism. The gametangia as well as gametes do not show morphological differentiation but physiologicallythey behave differently.

Physiological heterothallism may be of two types:

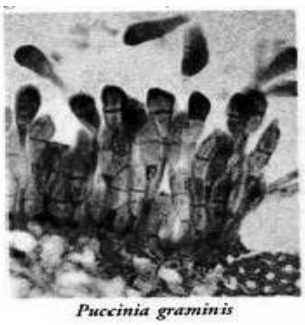
(i) Two Allelomorphs or Two-Allele Heterothallism:

When nuclei of both the mating types are different in genetic characters, this type of Heterothallism is known as Two-Allele heterothallism. In these types compatibility is governed by a pair of Alleles represented by A and a located at single same locus of the chromosome.

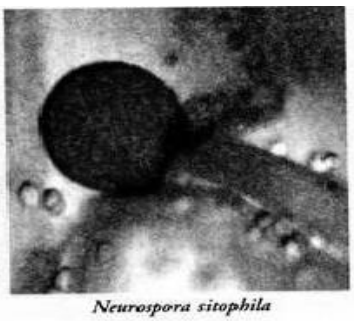
Due to the dominance of A over a, A is represented by (+) and a by (-). At the time of meiosis, separation of the chromatids take place. Half of the haploid spores thus have (+) and the other half (-) allele.

The spores bearing (+) allele will produce (+) mycelia and the spores with (-) allele will give rise to(-) mycelia. The mycelia of (+) and (+) and (-) and (-) are self-sterile or self-incompatible. Thus, two complimentary mating types (+) and (-) are essential for sexual reproduction.

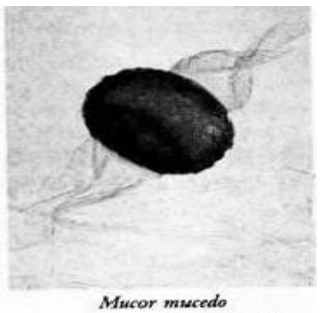
Two-Allele heterothallism has been reported in several fungi of like Ascobolus magnificus, Puccinia graminis, Neurospora sitophila Mu mucedo, Ustilago kolleri etc. (photographs given below)



Puccinia graminis



Neurospora sitophila



Mucor mucedo

(ii) Multiple Allelomorph or Multiple Allele Heterothallism:

In this type of heterothallism, more than two (multiple) alleles determine the sexual compatibility. These may be located at one (bipolar) or two (tetrapolar) loci.

Because of the larger number of alleles involved in this type of heterothallism, chances of mating of compatible strains increase.

As stated above, the multiple allele heterothallism may be of two types:

(a) Bipolar Multiple-allele heterothallism

(b) Tetrapolar multiple-allele heterothallism.

(a) Bipolar Multiple-Allele Heterothallism:

This type of heterothallism is controlled by multiple alleles at a single locus, instead of a pair of Alleles. For example, if the locus is named as L, the multiples alleles will be designated as L_1, L_2, L_3, L_4—Ln and these are present on the single locus L.

The meiotic division will give rise .to thalli which may be of several mating types, generally equal to the number of alleles. The thallus containing the allele L_1 can mate with a thallus of any mating type except L_1.

Similarly L_2 can mate with any thallus except that containing L_2 allele and so on. In this type of heterothallism, incompatibility factors are more commonly involved. Bipolar multiple allele heterothallism is characteristic of Basidiomycetes except rusts and smuts

	L_1	L_2	L_3	L_4
L_1	N	C	C	C
L_2	C	N	C	C
L_3	C	C	N	C
L_4	C	C	C	N

Fig. 17.3. Bipolar multiple allele heterothallism. C-compatible, N-non compatible

(b) Tetrapolar Multiple Allele heterothallism:

This type of heterothallism is characteristic of Basidiomycetes except rusts. In this type of heterothallism, which is very similar to bipolar multiple allele heterothallism, compatibility is determined by two loci.

Multiple allele—the compatible factor is present on two loci L_1 and $L_2$of two Chromatids of a chromosome. At the time of meiotic division, both the loci are separated with chromatids.

It is estimated that at least 100 alleles are present on each locus. In Schizophyllum commune, 122, alleles of factor A and 61 of B have been identified in the laboratory.

According to rough estimates, the number of alleles may be even more, about 350-450 of L_1, and 65of L_2. Any two mating types, which differ in allele present on L_1 and L_2 are compatible.

If the allele composition of mating type is A_1 B_1, it would be compatible with any other type of allele composition except A_1 B_1. But the mating type with allele composition is not fully compatible with allele composition A_2 B_2 or A_2 B_2.

Figure 17.4 fully explains type of heterothallism which has been reported in Ustilago maydis and Comprinus firmaterius.

	A_1B_1	A_1B_2	A_2B_1	A_2B_2
A_1B_1	N	NFC	NFC	C
A_1B_2	NFC	N	C	NFC
A_2B_2	NFC	C	N	NFC
A_2B_2	C	NFC	NFC	N

Fig. 17.4. Tetrapolar multiple allele heterothallism. N-non compatible, NFC—Not fully Compatible, C-Compatible.

This type of heterothallism encourages out-breeding. Whereas in bipolar multiple Allele heterothallism, the out-breeding is 25%, in tetrapolar, it is 100%. This may be due to enormous increase in the number of possible mating types of thalli.

According to Garrett (1963), **"heterothallism promotes the out-breeding and therefore subserves the same end as the sexual process, which it renders most efficient. Hetrothallism is not thesame as sex, it is refinement super imposed upon it."**

Parasexual Cycle:

In some fungi, true sexual cycle comprising of nuclear fusion and meiosis is absent. Th se fungi derive the benefits of sexuality through a cycle known as Parasexual Cycle.

The Parasexual Cycle is defined as a cycle in which plasmogamy, karyogamy and meiosis (haploidisation) take place but not at a specified time or at specified points in the life-cycle of an organism.

Generally parasexual cycle occurs in those fungi in which true sexual cycle does not take p ace. The members of class Deuteromycetes (Deuteromycotina) in which sexual cycle does not occur, exhibit parasexual cycle generally.

Parasexual cycle was first discovered by Pontecarvo and Roper of University of Glasgow in 1952 in Aspergillus nidulans, the imperfect stage of Emericella nidulans.

Since then parasexual cycle has been discovered not only in several members of Deuteromycetes but also in fungi belonging to Ascomycetes and Basidiomycetes.

Steps Involved in Parasexual Cycle:

According to Pontecarvo (1958), parasexual cycle in A. nidulans involves the following steps:

(i) Formation of heterokaryotic mycelium

(ii) Fusion between two nuclei (Karyogamy)

(a) Fusion between like nuclei

(b) Fusion between unlike nuclei

(iii) Multiplication of diploid nuclei

(iv) Occasional Mitotic crossing over.

(v) Sorting out of diploid nuclei

(v) Occasional haploidisation of diploid nuclei, and

(vii) Sorting of new haploid strains.

A brief account of these steps are being presented below:

Thus, after the parasexual cycle has operated for some time, the mycelium may contain the following types of nuclei:

(a) Haploid nuclei like those of both the parents,

(b) Haploid nuclei with various new genetic recombinations,

(c) Several types of diploid homozygous nuclei,and

(d) Several types of diploid hetero

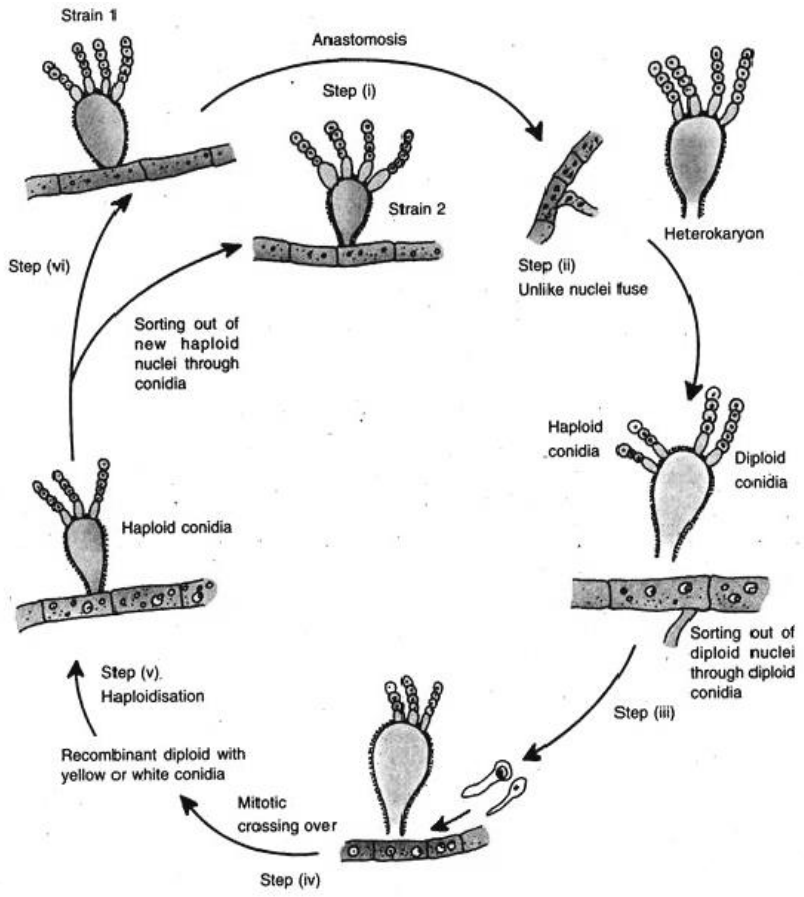


Fig. 17.10. Pontecarvo's (1958) idea of Parasexual cycle

Significance of Parasexual Cycle:

Parasexual cycle is of importance in industrial processes. Several fungi which are used i various industrial processes belong to fungi imperfecti or Deuteromycetes and in these fungi only parasexual cycle operates.

New and better strains of these fungi are obtained by mutation through parasexual cycle. The strainsof desirable characters can be developed through mitotic recombinations.

Parasexuality can also be applied in the analysis of genetic and physiological processes of perfect and imperfect fungi. Parasexual cycle has also been successfully employed in genetic control of pathogenicity and host-range in several species of Fusarium.

Economic importance of fungi:-

1. Role of Fungi in Medicine:

Some fungi produce substances which help to cure diseases caused by the pathogenic microorganisms. These substances are called the **antibiotics**.

The term antibiotic, therefore, denotes an organic substance, produced by a microorganism, which inhibits the growth of certain other microorganisms. The most important antibiotics are produced by the moulds, actinomycetes or bacteria.

They are used to combat the evil effects of pathogenic bacteria and viruses. The use of antibiotics is not limited to disease treatment.

The discovery of antibiotic agents as drugs is comparatively a recent history. The role of fungi m producing antibiotic substances was first established by Sir Alexander Fleming in 1929.

He extracted the great antibiotic drug **Penicillin** from *Penicillium notatum*. It was the first antibioticto be widely used. Penicillin is an organic substance lethal to microbes. It is far more effective than ordinary drugs and germicides.

It has no adverse effect on human protoplasm but kills bacteria especially gram-positive type. Penicillin is now produced on a commercial scale all over the world including India from theimproved strains of *P. notatum and P. chrysogenum*.

Streptomycin is obtained from *Streptomyces griseus*. It is of great value in medicine. It destroys many organisms which are not killed by penicillin particularly the gram-negative organisms. A numbers of antibiotics have also been extracted from *Aspergillus* cultures.

However, these have not been proved so effective as penicillin. Some of the actinomycetes which are not considered to be true filamentous bacteria are the sources of many antibiotics such as chloromycetin, aureomycin, terramycin, etc.

They inhibit the growth of many pathogenic bacteria and are also used successfully in the treatment ofvarious virus diseases. Many animal and human diseases which do not respond readily to other antibiotics are effectively cured by aureomycin.

Griseofulvin which is recovered from mycelium of *Penicillium griseofulvum* and many other species has antifungal properties. It acts on the hyphae by interfering with wall formation Consequently the hyphal tips curl and cease to grow.

Claviceps purpurea produces sclerotia in the ovaries of the flowers of grasses such as rye The sclerotium is called the ergot of rye. Ergot is used in veterinary and human medicine.

It contains a mixture of alkaloids which cause rapid and powerful contractions of the uterus. The medicine is thus used to control bleeding during child birth. Ergot is highly poisonous. A derivative of ergot known by the name of **lysergic acid (LSD)** is used in experimental psychiatry.

2. Role of Fungi in Industry:

The industrial uses of fungi are many and varied. In fact the fungi form the basis of many important industries. There are a number of industrial processes in which the biochemical activities of certain fungi are harnessed to good account.

A brief sketch of some of the most important of these processes is given below:

(i) Alcoholic fermentation:

In brewing or wine making industry alcohol is the important product. The other by-product which iscarbon dioxide was formerly allowed to escape as a useless thing.

Now carbon dioxide is also considered a valuable by-product. It is collected, solidified and sold as“dry ice”. In the baking or bread- making industry CO2 is the useful product.

It serves two purposes:

(i) Causes the dough to rise.
(ii) Makes the bread light.
The other by-product, which is alcohol, is incidental. The yeasts secrete the enzyme complex called zymase which brings about conversion of sugar into alcohol. Many excellent yeast strains are now available.
The yeasts lack diastase. So they cannot break starch into sugar. There are a number of fungipopularly known as the moulds. They secrete a whole range of enzymes and thus bring about fermentation of complex carbohydrates.
In producing industrial alcohol moulds are employed as starters to bring about scarification of the starch. At the second stage yeast is employed to act on the sugar.
M, rouxii and some species of *Rhizopus*. *Aspergillus flavus* is used in the production of African native beer.

(ii) Enzyme preparations:

Takamine on the basis of his intensive study of the enzymes produced by *Aspergillus flavus-oryzae* series has introduced in the market a few products of high enzymic activity. These are Digestin, Polyzime, Taka diastase, etc. They are used for dextrinization of starch and desiring of textiles.
Cultures of Aspergillus niger and A. oryzae on trays of moist, sterile bran yield a well-known amylase which contains two starch splitting components.
Invertase is extracted from Saccharoymces cerevisiae. It has many industrial uses. It hydrolyses sucrose to a mixture of glucose and fructose.

(iii) Preparation of organic acids:

Oxalic acid is the fermentation product of *Aspergillus niger*. Citric acid is made by mould fermentation. Many species of *Penicillium* are used for the purpose. The acid is produced on a commercial scale and is cheaper than the acid made from the citrus fruits.
The gluconic acid is prepared from sugars. The moulds chiefly employed for this purpose are some species of Penicillium and Aspergillus.
The gallic acid as the fermentation product of an extract of tannin by Aspergillus gallomyces.

(iv) Gibberellins:

These are plant hormones produced by the fungus *Gibberella fujikuroi* which cause a disease of rice accompanied by abnormal elongation. Gibberellin is used to accelerate growth of several horticultural crops.

(v) Cheese Industry:

Certain fungi popularly known as the cheese moulds play an important role in the refining of cheese. They give cheese a characteristic texture and flavour.
The two chief kinds of mould refined cheese are:
(a) Camembert and Brie types. They are soft.
(b) Roquefort Gorgonzola and Stilton types. They are green or blue veined cheese. The moulds concerned are *Penicillium camemberti* and *P. caseicolum* in the first type and P. *roqueforti* in the second type.

(vi) Manufacture of Proteins:

As a supplement to the normal diet, some fungi particularly the yeasts are employed to synthesize proteins. The yeast (*Saccharomyces cerevisiae* and *Candida utilis*) contain high percentage of protein of great nutritive value.

(vii) Vitamins:

The yeasts, are the best source of vitamin B complex. A number of preparations of high potency have been made from the dried yeast or yeast extracts and sold in the market.
A number of moulds and yeasts are utilised in the synthesis of Ergosterol which contains Vitamin D. Riboflavin—another vitamin useful both in human and animal food—is obtained from a filamentous yeast, *Ashby gossypii*.

1. Role of Fungi in Agriculture:

The fungi play both a negative and a positive role in agriculture.

A. Negative Role: They have a negative value because they are the causative agents of different diseases of our crop, fruit and other economic plants. These fungal diseases take a heavy toll and cause tremendous economic losses.

The modest estimate is that about 30 thousand different diseases (including bacterial and virus) attack the economic plants grown for food or commercial purposes.

The more important of these diseases are:

(i) Damping off disease:

The seedlings of almost every type of plant grown as a commercial crop such as tomatoes, com, cotton, mustard, peas, beans, tobacco, spinach, etc., are prone to this disease. It is caused by a species of *Pythium.*

(ii) The potato blight:

(Late blight of potatoes) is another destructive crop disease. It does a great damage to the potato tubers. A heavy attack of this disease in Ireland in 1845 destroyed the entire potato crop and caused so severe a famine that over a million Irish people migrated to U.S.A. Besides potatoes, it infects egg plants, tomatoes, etc.

(iii) Downy mildews of grapes:

It ruins the vine yards and thus causes heavy losses to the crop. When the disease was first introduced into France from U.S.A, it caused a havoc to the vine yards.

(iv) Ergot disease of rye:

It is an important disease of a cereal crop—rye. It results in the formation of poisonous sclerotia in the rye kernel. It is called ergot of rye. Ergot is highly poisonous to man. Ergot poisoning causes hallucinations, insanity and finally death.

(v) Apple scab:

It is a serious disease of the apple crop. It lowers the quality as well as quantity of the fruit.

(viii) Red rot disease of sugarcane:

It is a serious disease of sugarcane whose incidence has increased during the last few years, particularly in the northern parts of the country.

(ix) Rust diseases:

They attack our cereal crops and forest timber. Some of them such as black stem rust, yellow rust andorange rust are a serious threat to our wheat crop.

They affect the agricultural economy of our country seriously. The farmer and the Agriculture department must wage a constant war against them.

TABLE II

List of some common disease of human beings caused by fungi

	Name of the disease	Pathogen
1.	Aspergillosis	*Apsergillus flavus, A. niger*
2.	Blastomycosis	*Blastomyces dermatidis*
3.	Otomycosis	*Aspergillus fumigatus*
4.	Neuritis	*Mucor pusillus*
5.	Onychomycosis	*Trichophyton purpureum*
6.	Candidiasis	*Candida albicans*
7.	Histoplasmosis	*Histoplasma capsulatum*
8.	Geotrichosis	*Geotrichum candidum*
9.	Chromomycosis	*Cladosporium immitis*
10.	Allergy	Spores of *Aspergillus, Chaeotomium etc*
11.	Dermatomycosis	*Trichoderma viride*

TABLE 1

List of some important plant diseases caused by fungi

	Name of the disease	Pathogen
1.	Club root of Crucifers	*Plasmodiophora brassicae*
2.	Wart disease of potato	*Synchytrium endobioticum*
3.	Stem Rot of Papaya	*Pythium aphanidermatum*
4.	Damping off of seedlings	*Pythium sp.*
5.	Late blight of potato	*Phytophthora infestans*
6.	White rust of crucifers	*Albugo candida*
7.	Downy mildew of peas	*Peronospora pisi*
8.	Green ear disease of Bajra	*Sclerospora graminicola*
9.	Powdery mildew of peas	*Erysiphe polygoni*
10.	Powdery mildew of wheat	*Erysiphe graminis*
11.	Leaf curl of peaches	*Taphrina deformans*
12.	Stem gall of coriander	*Protomyces macrosporous*
13.	Ergot disease of rye	*Claviceps purpurea*
14.	Rust of wheat	*Puccinia graminis*
15.	Rust of pea	*Uromyces pisi*
16.	Rust of gram	*Uromyces ciceris-arieteni*
17.	Rust of Linseed	*Melampsora lini*
18.	Covered smut of barley	*Ustilago hordei*
19.	Loose smut of wheat	*Ustilago nuda*
20.	Bajra smut	*Tolyposporium penicillariae*
21.	Grain smut of Jowar	*Sphacelotheca sorghi*
22.	Bunt of wheat	*Tilletia tritici*
23.	Early blight of potato	*Alternaria solani*
24.	Wilt of pigeon pea	*Fusarium oxysporum*
25.	Red rot of sugarcane	*Colletotrichum falcatum*
26.	Tikka disease of groundnuts	*Cercospora personata*
27.	Stripe disease of barley	*Helminthosporium graminieum*

(a) Destruction of timber:

Several fungi such as *Polyporus, Serpula lacrymans, Fusarium negundi, Coniophora cerebella, Lentinus lapidens and Penicillium divaricatum* cause destruction of valuable timbers by reducing the mechanical strength of the wood.

(b) Destruction of textiles:

Several fungi are able to grow on cotton and woolen textiles causing their destruction. These include spp. of *Alternaria, Penicillum, Aspergillus, Mucor and Fusarium.* Spp. of Stachybotrys causes destruction of cotton in storage. *Chaetomium globosum* is reported to cause greatest damage to textiles.

(c) Destruction of Paper:

Paper pulp wood is destroyed by the growth of Polyporus adustus, Polystictus hirsutus etc. several fungi such as species of *Chaetomium, Aspergillus, Stachybotrys, Alternaria, Fusarium, Dematium, Mucor, Cladosporium* etc. cause extensive damage to paper of books, newspapers and paper industry.

B. Positive Role of Fungi:

Some soil fungi are beneficial to agriculture because they maintain the fertility of the soil. Some saprophytic fungi particularly in acid soils where bacterial activity is at its minimum cause decay and decomposition of dead bodies of plants and their wastes taking up the complex organic compounds (cellulose and lignin) by secreting enzymes.

The enzymes convert the fatty carbohydrate and nitrogenous constituents into simpler compounds such as carbon dioxide, water, ammonia, hydrogen sulphide, etc.

Some of these return to the soil to form humus and the rest of the air from where they can again be used as raw material for food synthesis. There are fungi in the soil which produce more ammonia from proteins than the ammonifying bacteria.

They also bring about rot, decay and decomposition of animal and plant remains releasing plant nutrients in a form available to green plants as food. The soil fungi utilize many inorganic salts.

These are prevented from being lost from the soil by leaching. Some fungi form mycorrhizal association with the roots of certain plants and help them in their nutrition.

Such plants will grow satisfactorily only when the mycelium of the appropriate fungal partner is present in the soil. The fertile soil contains twice as much living fungus cell material as the material from bacteria and other soil microorganisms.

Giberrrelin produced by *Gibberella fujikuroi* is used as growth hormone accelerating plant growth. Many insect pests can be controlled by the growth of fungi such as *Empusa sepulchrasis,Metarrhizium anisopliae, Cordyceps melothac* etc.

Some common fungal inhabitants of the soil help to combat diseases caused by soil borne fungi. *Trichoderma lignorum* and *Gliocladium fimbriatum* are found in damp soils. They have an inhibitory effect on the growth of the mycelium of *Pythium*.

They serve to suppress fungi causing the damping off disease of the seedlings and thereby influence favourably the growth of crops.

Drechsler (1937) reported that there are some predacious fungi in the soil. They trap and destroy the nematodes (eel worms). Some species of these predatory soil fungi form loops on the mycelium (A). These loops act as nooses.

They tighten and strangle the nematodes as they try to pass through (B). The mycelium later sends outspecial hyphae (C) to absorb nutrition from the captive. Some

predatory soil fungi produce conidia which are sticky.

As the nematodes pass through the soil the stickly conidia stick to their bodies. There the conidia germinate to produce hyphae which penetrate into the tissues of the host and absorb nourishment.

At the National Botanical Research Institute, Lucknow and at several other national institutes, fungi are being tested as biopesticides especially as nematicides and as fungicides.

An important fungus being used as nematicide is Beauveria bassiana against borers, thrips, and aphids. *Trichoderma viride* and *T. harzianum* are other examples which are used against a large number of soil-borne pathogens.

2. Role of Fungi as Food and as Food Producers:

Many species of fungi are edible, about 2000 species of them have been reported from all over the world. Of these, about 200 are said to occur in the Western Himalayas.

Many edible fungi are of great economic value as food. They are regarded as delicacies of the table. There are said to be over 200 species of edible fungi.

The fructifications of some fungi such as the field mushroom *Agaricus campestris* (dhingri), *Podaxon podaxis* (Khumb), the honey coloured mushrooms, the fairy ring mushrooms, the puff balls (*Lycoperdon* and *Clavatia*), morels (*Morchella, guchhi*), and truffles are edible.

The content of available food in them is not high but they supply vitamins and are valuable as appetisers. Yeasts and some filamentous fungi are valuable sources of vitamins of the B-complex.

A few of the mushrooms are fatally poisonous, some cause only discomfort. To the former category belong *Amanita*.

The fungi are also important as producers of foodstuffs. Certain species of *Penicillium* are active in the refining of certain kind of cheeses. Some fungi, such as red bread mold, *Neurospora sitophila* and others, complete their sexual life cycle in a few days and thus make ideal organisms for the study of the laws of heredity.

The slime molds (*Physarum polycephalum*) are now widely used in research. P. polycephalum has proved an excellent experimental organism for the study of DNA synthesis, meiotic cycle and the mechanism of protoplasmic streaming.

In tropical conditions, many fungi such as *Mucor sp., Penicillium, Neurospora,*

Fusarium, Aspergillus

etc., grow on meat causing sufficient spoilage.

Aflatoxins the most potent carcinogenic agent-are produced by Aspergillus flavus, A. fumigatus, A. parasiticus and Penicillium islandicum on dried foods and groundnut meal.

Aflatoxins are reported to bind with DNA and prevent its transcription arresting protein synthesis. These are responsible for liver cancer in animals and human beings.

Mushroom toxins are produced by several poisonous mushrooms. These cause diarrhoea vomiting, liver damage, complete unconsciousness etc. Mushroom toxins are commonly produced by *Amanita phalloides*, spp. of Helvella and some species of Inocybe.

Ergot toxins produced by *Claviceps purpurea* contain poisonous alkaloids like ergotamine, ergometrimine, ergocrystinine, ergocrystinine and ergonovin. These cause diarrhoea, abdominal pain, vomiting and psychiatric disturbances.

Fungi as Test Organism:

Fungi form very good research material for genetical studies and other biological processes Genus *Neurospora* has become very good material for genetic studies while Physarum polycephalum is used to study steps in DNA synthesis, morphogenesis and mitotic cycle.

To detect the presence and quantity of vitamin B in given sample, Neurospora crassa is commonly used. Similarly *Aspergillus niger* is used for detection of trace elements like zinc, nickel and copper even when they are present in very minute quantities.

Cultivation Procedure of Mushrooms:

It is the technique to develop the fruit bodies of edible fungi. About a dozen fungi are culti-vated in 100 countries with an annual produc-tion of 2.2 million tonnes. The common four genera are *Agaricus, Lentinus, Volvariella* and *Pleurotus*.

Together, they yield a major share of the total production. Out of many, *Agaricus brunnescens* (syn. *A. bisporus*), white button yields 56%; *Lentinus edodes*, shiitake yields 14%; *Volvariella volvacea*, paddy straw yields 8% and *Pleurotus* spp., oyster 7.7%.

The cultivation procedure of some of the mushrooms is given:

A. Cultivation of *Agaricus brunnescens* (Syn. *A. bisporus*):

The Agaricus brunnescens (syn. A. bisporus) is commonly known as white button mushroom (Fig. 4.107, 4.108). It contributes a major share in the mushroom production of the world. It is a temperate mushroom and can grow well in temperate conditions. Optimum temperature, optimum moisture, proper ventilation and good quality of spawn are very essential prerequisites for mushroom growth.

These are:

a. The optimum temperature for the mycelial growth is 24°C, while it is 14-18°C for the formation and develop-ment of fruit body.

b. Optimum moisture requires nearly at the saturation point. However, direct application of excess water in bed is harmful for the growing crop.

c. Proper ventilation is essential to remove toxic gases by the introduction of ade-quate fresh air.

d. Good quality of spawn i.e., the spawn should be prepared from the tissue of single fruit body and its productive capacity should be good enough.

The cultivation procedure is:

1. Production of spawn,
2. Preparation of compost,
3. Filling of trays with compost,
4. Spawning i.e., inoculation of compost,
5. Watering of inoculated compost filled trays,
6. Casing,
7. Harvesting of mushrooms (fruit bodies), and
8. Storage of mushrooms.

1. Production of Spawn:

The spawn (seed of mushroom) is a pure cul-ture of the mycelia grown on a special medium. Themedium is prepared by the grains of wheat, rye, sorghum or bajra along with some ingredients.

The preparation of spawn mainly consists of three steps:

a. Preparation of substrate,
b. Inoculation of substrate, and
c. Incubation of inoculated substrate for spawn production.

Preparation of Substrate Take 900 gms of grains (wheat or sorghum) in 600-900 ml of water in a container and boil for 15-20 minutes, After boiling, decant the excess water and allow the grains to surface drying by spreading on polythene sheet in shade for a few hours.

The grains are then mixed with chemicals like 2% calcium sulphate (gypsum) and 0.5% calcium carbonate (chalk) on

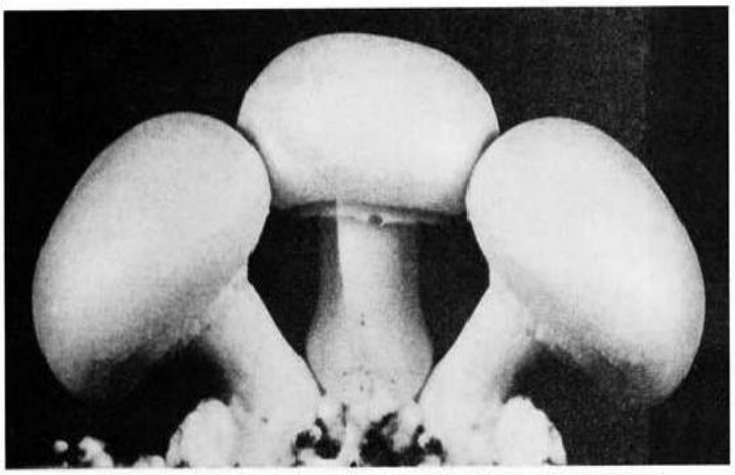


Fig. 4.108 : Mature fruit bodies of *Agaricus brunnescens* (syn. *A. bisporus*) on mushroom bed
[Courtesy : Dr. Sumi Datta]

dry weight basis and adjust the pH of the grain at 7-7.8. About 300-350 gms grains were then filled in milk bottles/ polypropylene bags.

Place a ring of tin (3.5 cm height and 3 cm diametre) towards the inner side of the open-end of polypropylene bag, tighten it with rubber band and then push the margin of the bag towards the inner side and thus a mouth is prepared.

Plug the mouth of the bottle and/or polypropylene bag with non-absorbent cotton. Then cover the mouth with brown paper and tighten it with rubber band. Sterilise the substrate by autoclave at 15lb pressure for 30 minutes for 2 consecutive days. Kept the sterilised substrate in open air to cool down near to room temperature, thus making the substrate ready for inoculation.

Inoculation of Substrate:

The substrate is then inoculated with the mycelial culture (developed earlier, either in Potato Dextrose Agar i.e., PDA or Yeast Potato Dextrose Agar i.e., YPDA or Malt extract Agar and Rice bran decoction medium).

Incubation:

Incubate the inoculated container at 20-25°C in dark for 3 weeks. Shake the contai-ner after a few days, when the mycelial growth becomes visible on the grain.

Storage of Spawn:

Store the spawn at 0-4°C in a refrigerator for a maximum period of 6 months, if it is not nee-ded immediately.

The spawn can be purchased from any spawn-growing centre. (The spawn is also available in"National Centre for Mushroom Research and Training (NCMRT)", Chambaghat, Solan 173 213, Himachal Pradesh, India

2. Preparation of Compost:

The compost used in the cultivation is of two types:

Natural and Synthetic:

i. Natural Compost:

The natural compost is prepared by mixing barley or wheat straw with fresh and pure horse dung (not with the dung of other animal). Mixed, rain wet or old dung is not sui-table for the preparation of compost. Commonly 100 kg of dung is mixed with 33 kg of straw. The mixture is then stacked a metre high heap.

The heap of mixture should be kept under shade in open air. After 3-4 days, the heap was turned (to release ammonia) and stacked again. The turning process is repeated 4-5 times at an interval of 5-6 days. During this process, gypsum ($CaSO_4.2H_2O$) is added @ 25 kg/tonne (1,000 kg) dung. Finally, 40 ml nemagon is sprayed and added to the mixture. The compost was then filled in the tray of 100 x 50 x 15 cm size.

ii. Synthetic Compost:

The ingredients required for the synthetic compost are:

(a) Chopped wheat straw (3-6 cm size) 300 kg
(b) Wheat bran 30 kg
(c) Calcium ammonium nitrate or Ammonium sulphate 6 kg
(d) Urea 4 kg
(e) Potash 1.5 kg
(f) Calcium sulphate (gypsum) 30 kg
(g) Sawdust 10 kg

Wet the sawdust with water by spraying and mix half of the ingredient, except wheat straw and gypsum. Next day, spread the wheat straw on the cement floor and wet it thoroughly by spraying with water. The sawdust-chemical mix-ture is then mixed thoroughly with wetted wheat straw. This mixture is then stacked under shade into a metre high heap and covered with poly-thene sheet.

After 5 days, the stack is scraped and rest half ingredient is thoroughly mixed with it and the entire mixture is then stacked again. This process is repeated six times. Calcium sulphate is added in the 3rd and 4th turning.

Normally the compost becomes ready to cultivate after 6th turning, but 2 or more turning may begiven if the smell of ammonia is yet there in the compost. During last turning, insecticide like malathion (10 ml dissolve in 5 I water) is added to the pre-pared compost. The prepared compost will be brown or dark brown in colour and is sufficient enough to fill 25 trays of 100 x 50 x 15 cm size.

3. Filling of Trays with Compost:

Mix 3 kg of calcium carbonate with the compost prepared earlier. Fill the wooden trays with compost and compress fairly by using a wooden board (1 2 cm x 25 cm), so that a space of about 3 cm deep is left on the top of the tray.

4. Spawning i.e., Inoculation of Compost:

Spread the spawn on the surface of compost and then cover by a thin layer of compost. Little pressure with the fingers is given to make good contact of spawn with compost. Finally the trays are covered with old newspaper. The trays are arranged one after the other in vertical stacks in such a way that sufficient aeration between the trays is maintained.

5. Watering of Inoculated Compost Filled Trays:

Sprinkled water to be given on newspaper to maintain humidity. Water should be applied twice a day or less depending on the availability of moisture. The room temperature should be maintained between 24°C and 25°C for 12-15 days for the good growth of mycelium on the compost. The mycelium appears in the form of white cottony growth on the surface of bed.

6. Casing:

The process of covering the mycelial mat on compost, surface is made with a thin layer of soil mixed with different substances.

The casing can be done with different types of mixture like:

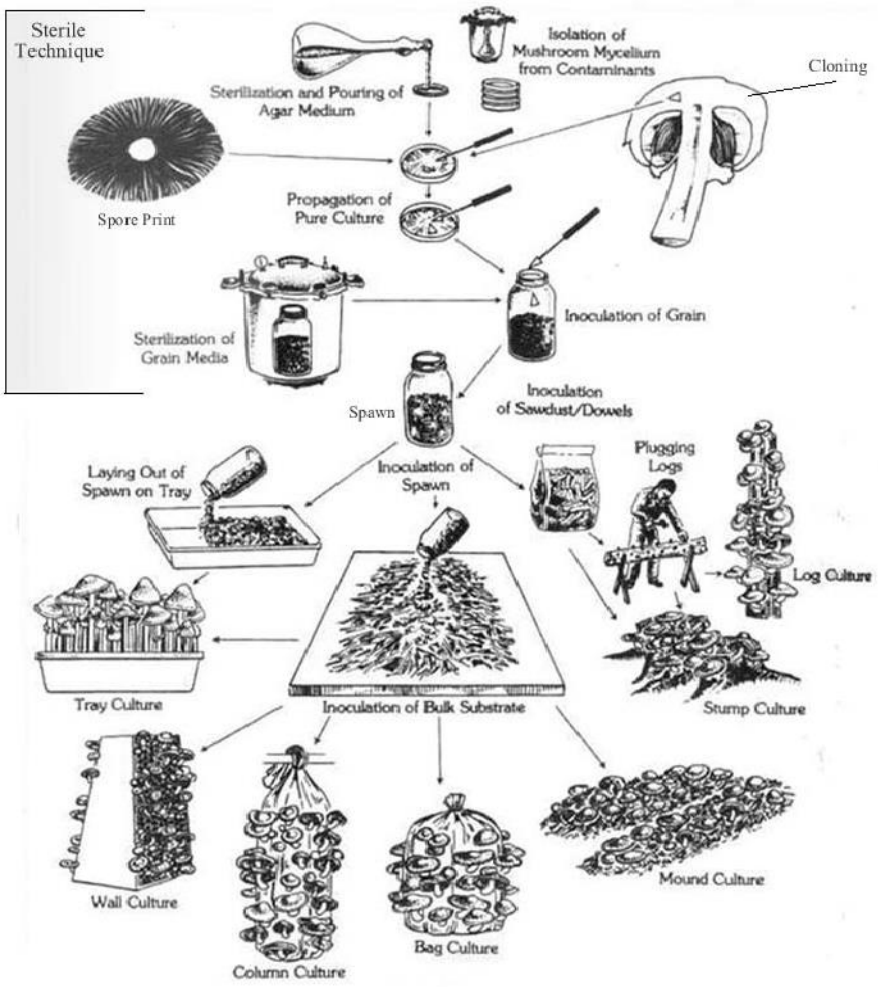
i. Soil : Sand : : 1 : 1;
ii. Well-rotten cow dung: light soil: : 3 : 1 ;
iii. Spent compost: Sand: Slaked lime : : 4:1:1 etc.

Casing soil should be sterilised either by chemicals like methyl bromide, formalin etc. or by heating at 70-75°C temperature for 6 hours to kill the inhabiting fungi, nematodes, insects etc.

The fruit bodies of mushroom are expected to appear after 5-20 days of casing. After casing, the room temperature should be maintained between 14-18°C for the good growth of the fruit body. The fruit bodies attain the size of button stage from pinhead within 7-8 days. Next crop appears at an interval of8-10 days.

7. Harvesting of Mushrooms i.e., Fruit Bodies:

When the cap of the fruit body is tight with its stalk, the fruit bodies are harvested. The fruit bodies are harvested by twisting and uprooting, after holding the basal region of stalk with fingers. Thelower part of the stalk is cut out where the compost remains attached.



8. Storage of Mushrooms:

The fruit bodies may be stored at 4°C for a few days, if it is not consumed or marketed immediately.

B. Cultivation of Paddy Straw Mushroom (*Volvariella Volv cea*):

The paddy straw mushroom is also called tropical, straw or Chinese mushroom (Fig. 4.109). In West Bengal, it is called as 'Poal chatu'. The genus Volvariella belongs to the family Pluteaceae under the order Agaricales of Basidiomycotina.

The common edible species under this genus are *V. volvacea, V. diplasia* and *K esculenta*; those are grown commercially in different countries like Burma (Myanmar), China, Philippines, Malaya, India etc.

In addition to paddy straw, other substrates like water hyacinth, cotton waste, banana leaves, sawdust, sugarcane thrash (bagasse) etc., are used as substrate due to the presence cellulose, hemicellulose and lignin.

In India, the cultivation of this mushroom was first initiated in Coimbatore, Tamil Nadu, and now it ispopular in different tropical parts due to the requirement of temperature ranges between 30-45°C.

The process of cultivation of straw mush-room is as follows:

1. Requirements,
2. Preparation of spawn,
3. Cultivation procedure,
4. Harvesting of fruit bodies, and
5. Preservation of fruit bodies.

1. Requirements:

i. Spawn of V. volvacea (600-800 gms grain spawn/bed),
ii. Bricks,
iii. Bamboo frame (1 m x 1 m),
iv. Small water tank,
v. Paddy straw (preferably from aman variety), apx. 36 kg,
vi. Loose straw 5-6 kg,
vii. Powder of Gram or Arhar seeds 200-250 gm,
viii. Thermometer (0-100°C scale), and ix. White polythene sheet.

2. Preparation of Spawn:

The spawn can be prepared following the same procedure as adopted in Agaricus brun-nescens (seepage 395). But in addition to grains of wheat or sorghum, the rice straw can also be used as substrate.

3. Cultivation Procedure:

Fresh paddy straw, not more than one year old and preferably from the Aman variety, should be collected from farmer or from any store. 24 straw bundles of about 1.5 kg each along with some loose straw are immersed completely in a water-filled tank by putting some weight on the bundles for about 12-15 hours. Then take out the straw bundles from the tank and keep them in stack on cement floor to drain off excess water.

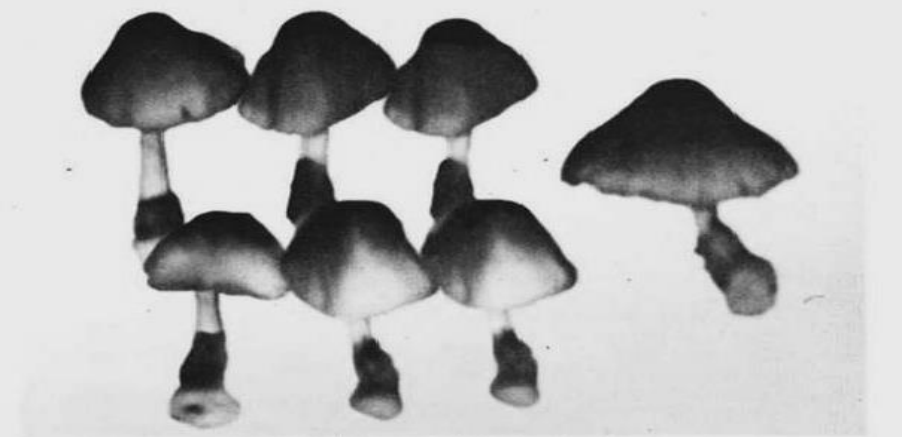


Fig. 4.109 : Mature fruit bodies of *Volvariella volvacea*

4. Preparation of Bed and Spawning:

One square bed of 1 m x 1 m x 1 m or 1 m x 0.75 m x 1 m is prepared with pre-soaked straw, keeping the butt ends (basal region) at one side, placed close to each other and arranged length-wise on a bamboo frame, supported on 4 pillars made of bricks. Same number of soaked straw bundles are placed on the previous one by keeping the butt ends in o posite direction.

Inoculate the bed with spawn. The bids of spawn are placed about 8-10 cm inside the margin, maintaining a space of about 5 cm from each other. About 160-200 grams spawn is required for each layer. Powders of Cram or Arhar seeds of about 50 gms or more are spread along the line of spawning.

Second and third layers are arranged and inoculated in a similar process, but 2nd layer is placed at right angle to the 1st layer and the third layer is like the 1st layer. The spawn and seed powder on the 2nd layer will be given like the 1st layer, but on the 3rd layer those will be dis-tributed uniformly throughout the bed.

Finally, cover the top layer with loose straw. Loosely bind the bed with rope made of wheat straw at the three regions, one in the middle and one on each side. Press the bed with the help of woo-denboard to release the internal air and thus the spawn get compressed with the wet straw bun-dles. Coverthe bed with

polythene sheet.

Watering should be done once or twice with the help of micro-sprayer. The temperature of the bed should remain 30-35°C after spawning and it should not go below 30°C during the growing season. The relative humidity should be between 80-90%.

Polythene sheet should be removed after 7-10 days of spawning for the appearance of button of the mushroom. After that the buttons quickly develop into fruit bodies.

The straw once used in the mushroom culti-vation can be used again. The bed should be pre-pared under shade away from direct sunlight and rain and also in well-aerated condition, but wind should not blow very fast.

5. Harvesting of Mushroom:

The fruit bodies are harvested by gentle twisting when the volva is about to rupture or is just ruptured. The production continues for 25- 30 days, but in two phases. The total production per bed is approximately 3 kg. The production of second phase is comparatively less.

6. Preservation:

The fruit bodies are consumed fresh or can be preserved by drying or in refrigerator for 27- 48 hours. Drying can be done either in the sun or in oven at 50-60°C temperature.

C. Cultivation of Oyster Mushroom (*Pleurotus*):

Species of *Pleurotus* are commonly called Oyster mushroom or Dhingri or Wood fungus (Fig. 4.110). It is the fourth important mushroom in the world ranking with an annual production of about 15,000 tones. It grows commercially in Japan, Taiwan, Italy, France, Thailand, Philippines and India, out of which the first three are the leading countries in its production.

The genus *Pleurotus* contains more than 50 species, of which *P. flabellatus, P. ostreatus, P. sajor- caju, P. sapidus, P. fossulatus, P. cornu- copieae, P. sapathulatus* and *P. florida* have been cultivated in India.

The process of cultivation of Oyster mushroom is as follows:

1. Requirements,
2. Preparation of spawn,
3. Cultivation procedure,
4. Harvesting of fruit bodies,
5. Preservation of fruit bodies.

Requirements:

i. Spawn of *Pleurotus* – 60 gms (30 gms grain spawn/kg of paddy straw),

ii. Chopped and dry paddy straw (1-2 cm) -2 kg,

iii. Gunny or polythene bag (40″ x 24″) – 1 piece.

iv. Horse gram powder – 50 gms (25 gm.s/kg),

v. Thick polythene sheet (5 ft x 5 ft) – 1 piece,

vi. Polythene bags (30″ x 18″) – 2 piece, and

vii. Water sprayer. Preparation of spawn

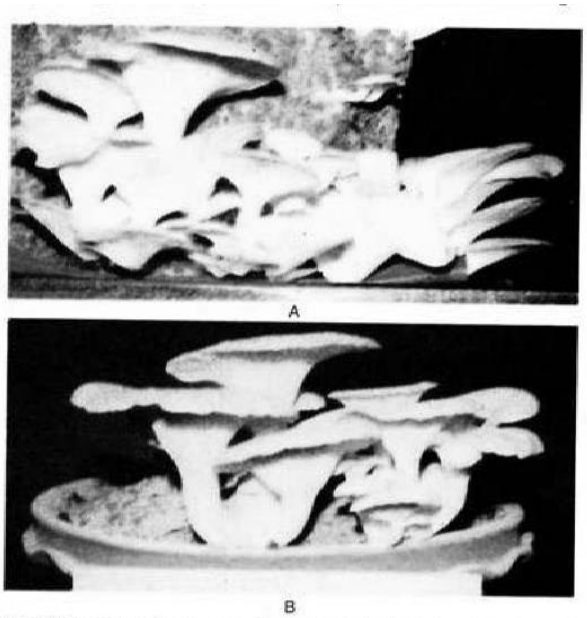


Fig. 4.110 : Fruit bodies of *Pleurotus sajor-caju* on mushroom bed : A. Mushroom bed prepared in polythene bag and B. Mushroom bed prepared on earthern pot

The spawn can be prepared as per method adopted in Agaricus brunnescens (see page 395). The spawn should not be older than 1 month.

Cultivation Procedure:

Take two kg of chopped straw (preferably of Aman variety) in a gunny or incised polythene bag and tighten the mouth with rope. Immerse the bag completely in water (90 I water contai-ning 7 gm Bavistin, a fungicide along with 125 ml formaldehyde) filled tank by putting some weight on it for approximately 12-15 hours.

Then take out the straw bags from the tank and keep the straw pieces in a wicker basket or a scuttle (Beng. Jhuri). Put more water in the wet straw to remove dirt, rags etc. Wait for one or more hours to drain off excess water.

The wet straw pieces are then kept on polythene sheet and mixed with powder of Horse gram (20-25 gms/kg) and spawn (30 gms/kg) and if possible 10 gms ferti-liser of IFCO or P.P.L (10:26: 26) maybe added.

Take 2 polythene bags (appx. 30″ x 18″) and make 6-12 holes at the lower side of each bag. Then the entire mixture is put equally inside the two bags. Keep the filled bags on a bench or table in room at 21-30°C and 65-80% humidi-ty, with sufficient light and ventilation for 15-16 days, for spawn running.

Spray water on bed twice a day by micro-sprayer. After 15-16 days, the straw pieces are covered with the mycelium and form a solid cylindrical mass. Remove the polythene bag and keep the mass on the same polythene bag in the same place. The compact mass should be watered 4-8 times throughout the day with the micro-sprayer.

The young fruit bodies will be developed after 3-4 days (i.e., 18-20 days of spawning) from all sides of the bed.

Within 2-3 days, the fruit bodies attain the size of harvesting. After harvesting the straw- mycelium mass again put inside the bag and tighten the-mouth with rope. Keep it for 7 days and then again remove the mass from polythene bag and keep the polythene bag as before.

Next crop of mushroom will be available within 7 days i.e., approximately 36 days after starting. Repeat the process again and the third crop will be available in 50 days. During cropping period light should be provided for 15-20 minutes/day for better yield.

Harvesting of Mushroom:

The fruit bodies are harvested by gentle twisting after holding the base of the fruit bodies with fingers.The fruit bodies can be harvested generally 3 times i.e., at 22, 36 and 50 days and the total production will be 2 kg. Afterwards the bed should be destroyed.

Preservation:

After harvesting, the fresh mushrooms can be sold in the market or they can be dried in sun (for three consecutive days) or in oven at 65°C. The cultivation of Pleurotus can also be done on earthen tray or tub.

Some Poisonous Mushrooms:

1. *Amanita phalloides* (death cap): Toxic principles are α and β-amanitin, and phalloidin.
2. *A. virosa* (destroying angles),
3. *A. verna* (fool's cap),
4. *A. muscaria* (fly-agaric) etc.

Diseases of Mushrooms:

Like higher plants, mushrooms also suffer from different diseases caused by fungi, bacteria and viruses.

5. Algae: Understanding algae as a plant group; Societal issues involving algae.

Understanding algae as a plant group

Algae is a name given to large, extremely diverse, related, distantly related or even unrelated groups of mostly aquatic photosynthetic organisms though not restricted to being aquatic or even exclusively photosynthetic.The extent of diversity in terms of size, morphology, metabolism, modes of nutrition, life cycle patterns, and ecology makes the term algae more of a concept rather than being a taxonomic unit. They range in size from being microscopic to extremely large entities that can reach >200 ft in length.They contain varied arrays of photosynthetic pigments compared to those in plants and their cells have featuresnot consistent with common features ofother plant and animal groups.In addition to their ecological roles as oxygen producers and food base for all aquatic life,algae are economically important as sources of crude oil, food, fodder, fertilizer and a number of bioactive compounds that have pharmaceutical and industrial applications useful for mankind.

Algae are defined as organisms that photosynthesize but lack the specialized multicellular reproductive structure of plants. Structurally, algae lack true roots, stems, and leaves-features that they share with othernonvascular thalloids (e.g., mosses, liverworts and hornworts).

The algae are not closely related in an evolutionary sense, and the phylogeny of the groupsvery different from each other making them a polyphyletic group in true sense. Specific groups of algae share features with protozoa and fungi and,indeed some algae appear to have a closer evolutionary relationship with the protozoa or fungi than they do with other algae. In terms of light harvesting pigments and the consequential photosynthesis, it is found thatthe light reactions of many algae differ from those of land plants because some of them use different pigments to harvest light.Since the amount of light absorbed depends upon the pigment composition and concentration found in the alga,potentially some algae can convert more light energy of the wavelength into the chemical energy via photosynthesis.All algae use chlorophyll a to capture photosynthetically active light. Chlorophytes or the green algae and Euglenophytes use chl. b, dinoflagellates and cryptomonads use chlorophyllides, Dinophyceae and Chromophytes use an array of carotenoids.Phycobiliproteins which are either blue (phycocyanin) or red (phycoerythrins) are found in the Blue Green Algae, Rhodophytes of the red algae and Crytomonads.At one time it was believed that algae with specialized green-absorbing accessory pigments(carotenoids)outcompeted green algae in deeper water. Some algae,however,grow as well as others in deep water.The explanation of this paradox is that the cell structure of the deep-water green algae is designed to capture virtually all light,green or otherwise.Evolutionary changes in cell structure can also compensate for the absence of these pigments.

In terms of nutrient storage, the storage product of the green algae is usually starch.Cryptophyceae members also store starch and these are stored outside the chloroplast but within the surrounding membranes of the chloroplast ER.Most Dinophyceae store starch outside their chloroplast,often as a cap over a bulging pyrenoid.Storage product of red algae is floridean starch which is more highly branched than amylopectin.Chrysolaminarin is a storage product of diatoms and yellow green algae,laminarin in Phaeophytes,leucosinin Chrysophytes,and paramylon in Euglenophytes form the major storage products of different algal groups.

Reproduction inalgae is of varied types keeping in mind the varied nature of the algal groups. Many algae regenerate by sexual reproduction,involving male and female gametes, some by asexual means, and some using both ways.Asexual means of reproduction involve ordinary cell division to fragmentation,or in many cases by formation of varied kinds of spores.

The algal members exhibit varied types of life cycles.Four main patterns of life cycles are observed in algal groups. All four patterns exhibit an alternation of generations, implying that there are distinct haploid and diploid stages. The haploid stage is referred to as the gametophyte, and the diploid as the sporophyte.The four main types of life cycles in algae include the haplontic, diplontic, diplohaplontic and triphasic life cycles.

In the haplontic life cycle the only diploid part of the cycle is the zygote. The algae exhibiting a haplontic life cycle possesses gametophytic plant body . Here, the diploid zygote produces four meiospores through meiotic division. After germination, these four meiospores become haploid gametophyte plants.Eg. *Spirogyra* and *Chlamydomonas*

In diplontic life cycle only the gametes are haploid. These algae possess sporophyticplant body, and sex organs

occurring on the sporophyte produce gametes. The predominant stage in this type of life cycle is the multicellular, diploid plant body.Gametes are produced by meiosis in the sporophyte, and the zygote is formed immediately after fertilization. Eg. *Fucus* and *Sargassum*.

In the diplohaplontic life cycle, the haploid and diploid stages are equally prominent. Both stages produce vegetative plants, which differ only in function and chromosome number.The diploid sporophyte reproduces asexually, while the haploid gametophyte reproduces through a sexual process. An alternation of generations is accomplished through the fusion of gametes and meiosis in sporophytes.There are two distinct types of diplohaplontic life cycles. The first is called isomorphic, and the second is referred to as heteromorphic.

In an isomorphic diplohaplontic life cycle, the gametophytic and sporophytic plants are morphologically similar. Both stages in this type of life cycle are free living and are independent ofeach other. Eg. *Cladohora* and *Ulva*

In the heteromorphic diplohaplontic life cycle, the gametophytic and sporophytic plants are morphologically different. In most of the cases, the sporophytic plant is complex, while the gametophyte is relatively simple. Eg. *Laminaria*

The triphasic life cycle of algae is unique because instead of two generations, there are three separate generations. There are two types of triphasic life cycles of algae: haplobiontic and diplobiontic.In the haplobiontic life cycle there are two haploid generations and one diploid generation kind, while in the diplobiontic type of life cycle there are two diploid generations and one haploid generation .

Societal issues involving algae:

Algae, as primary producers, are not only ecologically important, but have an immense effect on human society. The members of the group have economically been used as food and medicines for centuries. Various species of algae are consumed not only food but also leads to production of agar, carrageenans, and alginates. These extracts derived from algae are used in numerous food, pharmaceutical, cosmetic, and industrial applications.

Source of Commercially Important Bio-Molecules

- An important use of algae lies in extraction of important bioactive compounds like antibiotics, algicides, toxins, pharmaceutically active compounds & plant growth regulators.
- Antibiotics obtained from a wide range of algae show great chemical diversity in the forms of fatty acids, bromophenols, tannins, terpenoids, polysaccharides and alcohols. Similar observations are made for neurotoxic & hepatotoxic compounds

Vitamin, Protein, Carotenoids, PUFA

- Algae are good sources of vitamins & vitamin precursors, most notably ascorbic acid, riboflavin and tocopherols.
- Protein levels of *Spirulina* is as high as in soyabean ranging from 50% to 70% of the algal dry weight.
- Micro-algae produce a wide variety of carotenoids, with over 40 carotene &xanthophylls isolated & characterized. Carotenoids have a combined market size of US$ 1billion.

- Important Algal groups that contain vlcPUFAs include diatoms, chrysophytes, cryptophytes and dinoflagellates.

Gelling Agents – Alginates, Carrageenan & Agar

- Macro-algae have long been used for the production of phycocolloids such as alginates, carrageenans or agars.
  - Carrageenan mainly obtained from *Eucheuma&Kappaphycus*
  - Alginate from *Laminaria, Macrocystis,Ascophyllum*& others
  - Agar from Mainly *Gelidium*&*Gracilaria*

Bio-fuels

- Most promising venture in algal biomass utilization in recent times has been in the production of bio-fuels.
- Use of micro-algae for bioenergy generation for biodiesel, biomethane&biohydrogen or combined applications for biofuels production are much researched topics.

Bio-Fertilizers

- Microalgae have been shown to efficiently recycle N:P:K nutrients from wastewater streams and stabilize soil aggregates;
- Microalgae can be grown in environments that cannot support traditional land-based crops and therefore do not displace those crops;

Other societal impacts include their role in bloom formation vis-à-vis, toxin production and also their utility in fighting climate change. Toxins released by some types of algae can cause severe ecological problems when they are present in large quantities (blooms). High nutrient levels and warm temperatures often result in favourable conditions for algae blooms to form. These blooms can be identified as floating mats of decaying, bad-smelling and gelatinous scum. There is much still to be learned about algal toxins in lakes, rivers and bays. Their presence tells us that a waterbody is most likely stressed by high levels of nutrients such as nitrogen and phosphorus, that risks to the recreational uses of the waterbody and to human and animal health are possible and that related problems, such as low dissolved oxygen due to the decomposition of algal blooms, are likely to also occur.Although there are relatively few documented cases of severe human health effects, exposure to algal toxins may produce allergic reactions such as skin rashes, eye irritations, respiratory symptoms and in some cases gastroenteritis, liver and kidney failure or death. The most likely pathway to exposure for humans is through accidental ingestion or inhalation during recreational activities in lakes, rivers, oceans and bays.

The algae are efficiently capable of removing carbon-di-oxide from atmosphere and can be utilized in a number of ways to reduce carbon in the atmosphere. Trees and algae sequester carbon dioxide naturally. Trees consume CO_2 as part of their photosynthesis and release oxygen back into the air. Algae replicates the same process but since it multiplies on a faster rate ,therefore they sequester atmospheric carbondioxide much easily.Algae can consume more carbon dioxide than trees because it can cover more surface area, grow faster, and be more easily produced by bioreactors, given its relative size. Bioreactors can produce large amounts of algae and optimize its growth (and related sequestration) cycle in a way that is easier than trees and takes the overgrowth of algae,

dehydrates it, and ultimately puts it to use as fuel or biomass. Thus, algae is an increasingly viable alternative for many costly climate-change solutions.

6. Bryophyte, Pteridophyte & Gymnosperm

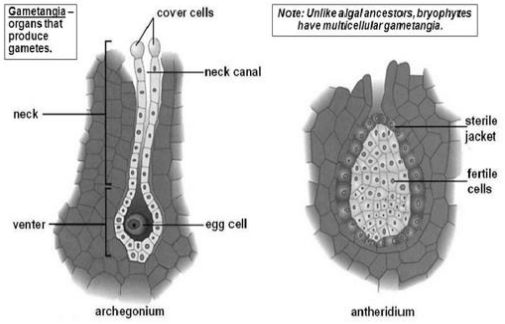
Bryophytes:

Bryophyta (Gr. Bryon = mass; phyton = plant), a division of kingdom Plantae comprises of mosses, Hornworts and Liverworts. They are groups of green plants which occupy a position between the thallophytes (Algae) and the vascular cryptogams (Pteridophytes).

Bryophytes produce embryos but lack seeds and vascular tissues. They are the most simple and primitive group of Embryophyta. They are said to be the first land plants or non-vascular land plants (Atracheata). Presence of swimming antherozoids is an evidence of their aquatic ancestory.

General Characteristics of Bryophytes:

1. The life cycle of bryophytes is distinctly differentiated into gametophytic and sporophytic phases (heteromorphic).
2. The gametophytic phase is predominant and ecologically persistent, i.e., green, indepen-dent andlong-lived.
3. The sporophytic phase is very short-lived, and completely dependent upon the gametophyte.
4. Unlike most of the higher plants, bryophytes are not found as single individuals but in groups of individuals which have charac-teristic features depending on their family, genus or species.
5. The gametophytic plant body is either thalloid or differentiated into the erect axis (stem) and lateral appendages (leaves).
6. Roots are absent in bryophytes. The rhizoids perform the function of roots. They are either unicellular and unbranched or multicellular and branched.
7. They never form xylem tissue, the special lignin-containing water-conducting tissue that is found in the sporophytes of all vas-cular plants. However, the plant body is made up of parenchymatous cells only.
8. They reproduce by vegetative and sexual methods. Asexual reproduction is comple-tely absent in bryophytes.
9. Vegetative propagation takes place by some special structures, like gemmae, tubers, protonema, cladia, innovation, etc.
10. Sexual reproduction is only of



oogamous type. They produce large, multicellular sex organs forreproduction.

Bryophytes are unisexual, either homothallic (monoecious) or heterothallic (dioecious).

11. The male sex organs, called antheridia, are stalked, globose or ovoid with one celled thick jacket surrounding androgonial mother cells.

12. The female sex organs, called archegonia, are vase-shaped or flask-shaped structure ha v ing the basal swollen venter containing a ventral canal cell and an egg, and the upper elongated neck containing neck canal cells. Both the venter and neck are surrounded by the sterile jacket.

13. The sperms are motile and biflagellate having two whiplash flagellae.

14. Bryophytes require water for sperm dispersal and subsequent fertilisation.

15. The sperms move short distances in the water film and ultimately reach the open necks of the archegonia. The slimy mucilage secretions in the archegonial neck help to pull the sperm downwardto the egg.

16. The zygote does not pass any resting phase. Embryonic growth of the sporophyte begins within the venter of the archegonium soon after fertilisation.

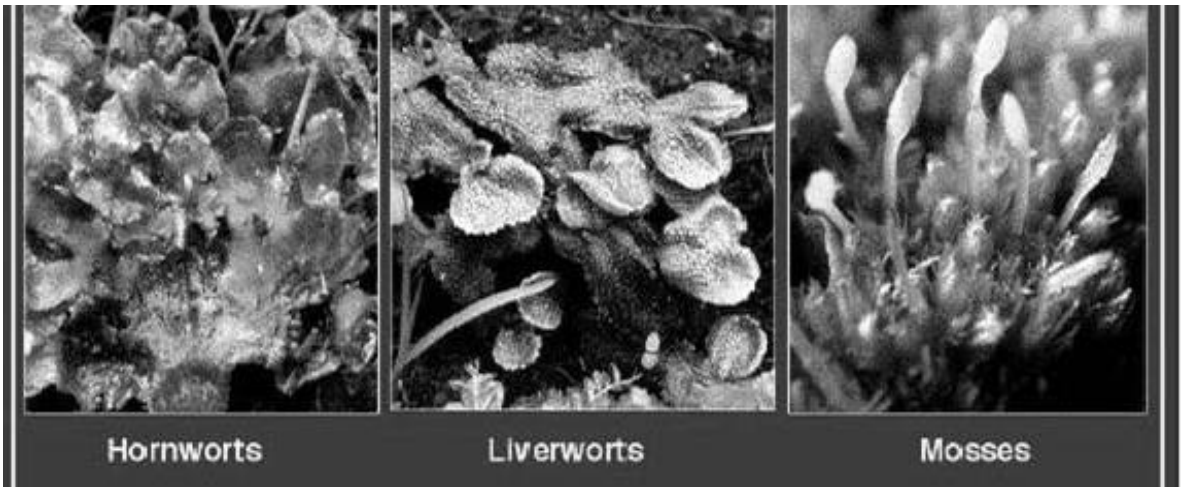
17. The embryo follows exoscopic mode of development. In this development, the zygote first divides transversely to form an outer epibasal cell and an inner hypobasal cell and the embryo develops from the epibasal cell. Thus, the shoot forming apical cell is directed outwards, i.e. towards the neck of the archegonium.

18. The sporophyte consists of only bulbous foot, with or without an unbranched seta and a single terminal sporangium Sometimes the sporophyte is represented only by a capsule (*Riccia*). The capsulehas a protective cove-ring called calyptra which is a part of gametophyte.

As the Sporophyte of bryophytes is monosporangiate (containing a single sporan-gium), they are called sporogonium.

19. Bryophytes are homosporous — isospores (spores are identical morphologically and physiologically) are produced from the sporogenous cells of the capsule.

20 The spore after germination either produces a filamentous germ tube that gives rise to a young gametophyte (*Riccia, Marchantia*) or produces a protonema which bears leafy budsultimately form the adult gametophytic shoot that will



Bryophyte classification:

The term Bryophyta was first introduced by **Braun (1864)**, however, he included algae, fungi, lichensand mosses in this group.

The rank of division Bryophyta to this well-defined group of plants was first given by Schimper (1879).

Eichler (1883) was the first to divide Bryophyta into two groups:

Group I. Hepaticae

Group II. Musci.

Proskauer (1957) however changed the name Anthoceropsida to Anthoerotopsida and thus, the Bryophyta may be classified as follows:

Division

Bryophyta

Class 1.

Hepaticopsida (Hepaticae)

Class 2.

Anthocerotopsida (Anthocerotae)

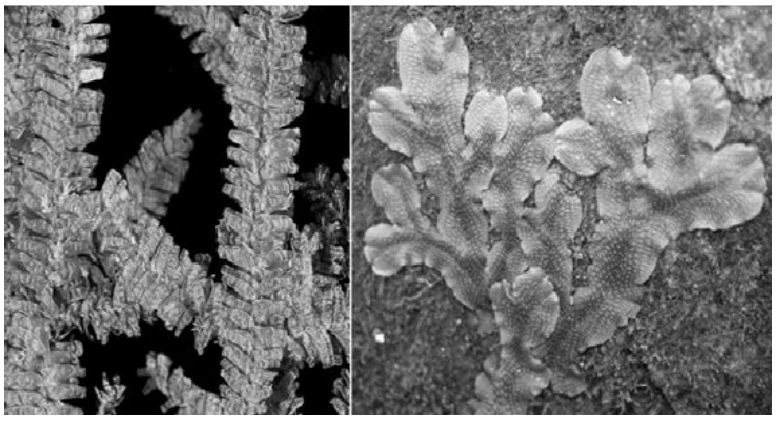
Class 3.

Bryopsida (Musci)

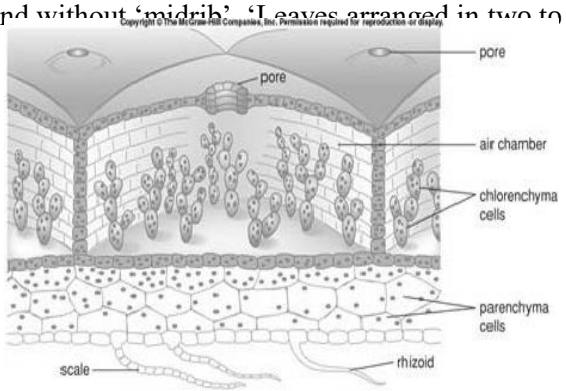
Hepaticopsida:

1. This class includes about 280 genera and 9500 species.

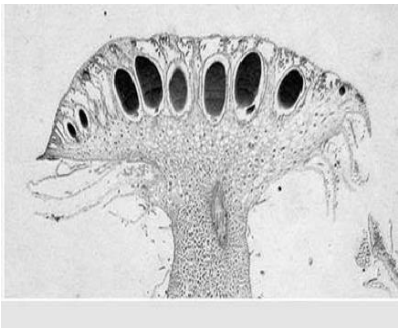
2. The name of this class is derived from a latin word Hepatica which means liver. Hence members of this class are commonly known as liverworts.



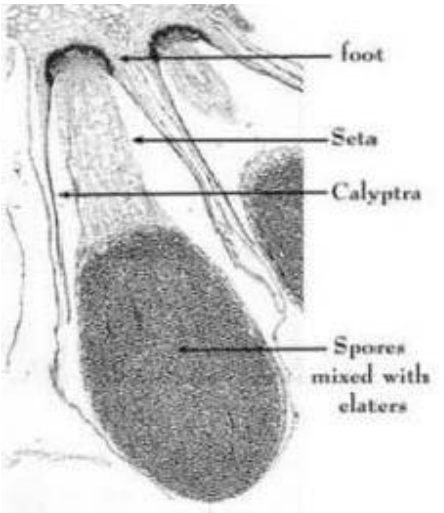
3. Plant body is gametophytic and the gametophyte is either thalloid or foliose. Thalloid forms are prostrate, lobed, dorsiventral and d chotomously branched.
4. In foliose forms, 'leaves' are entire, lobed or divided and without 'midrib'. 'Leaves arranged in two to three rows on the axis.



5. Rhizoids are unicellular and branched.
6. Photosynthetic cells contain many chloroplasts.
7. Pyrenoids are absent.
8. Sex organs are borne dorsally or apically, superficial or embedded in gametophytic tissue
9. Members may be monoecious or dioecious.
10. Sporophyte is either simple or represented by capsule o nly (e.g., *Riccia*) or may differentiated into foot, seta and capsule (e.g., *Marchantia*).



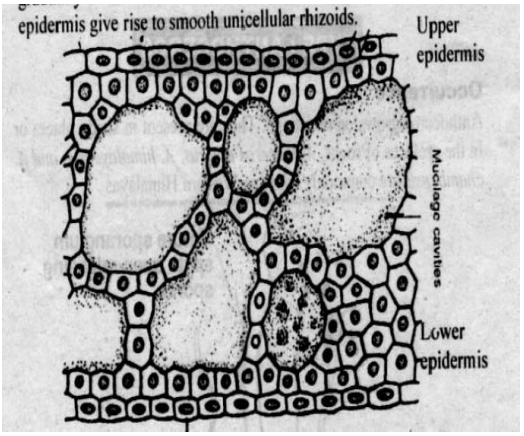
11. Archesporiuin is endothecial in origin.
12. Sporogenous tissue either for ms only spores (e.g., *Riccia*) or is differentiated into sterile elater mother cells and fertile spore mother cells.
13. Columella is absent in the capsule.
14. Elaters are unicellular, hygroscopic with spiral thickenings.
15. Capsule wall is one to several layers thick and without stomata.
16. Dehiscence of the capsule is irregular or in definite number of valves.
17. Spores on germination form the gametophytic plant body.
18. Plants show heteroinorphic alternation of generation.



Anthocerotopsida:

1. This class is represented by about 6 genera and 300 species.
2. Plant body is flat, dorsiventral, thalloid,

gametophytic and variously lobed.



3. Smooth walled rhizoids are present.
4. Tuberculated rhizoids and scales are absent.
5. Internally the thallus is not differentiated into zones.
6. All cells are alike.
7. Air chambers or air pores are absent.
8. Each cell has a single chloroplast and each chloroplast contains a single pyrenoid.
9. Mucilage cavities open on the ventral surface byslime pores.
10. Sex organs are embedded in the thallus.
11. Antheridia develop either singly or in groups in closed cavities called antheridial chambers.The sporophyte is differentiated into foot, an intermediate zone or meristematic zone and capsule.

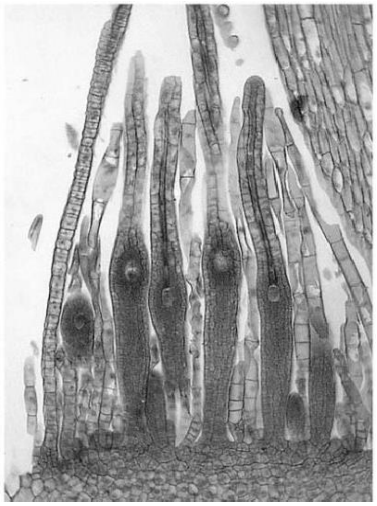
12. Due to the presence of the meristematic zone, the sporophyte shows indeterminate growth i.e., it continues to grow indefinitely.
13. Archesporium is amphithecial in origin.
14. Sporogenous tissue forms the fertile spores and sterile elaters. Elaters do not have spiral thickenings and are known as pseudo elaters.
15. Capsule wall is four to six layered thick and epidermis has the stomata.
16. Capsule matures from apex to base and usually dehisce by two valves.

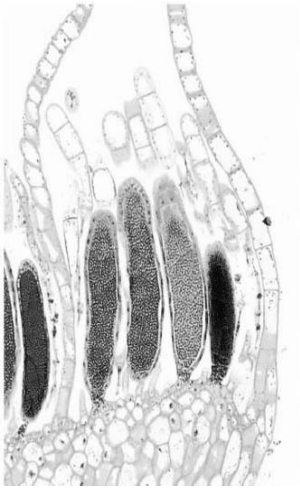
Musci or Bryopsida:

1. It is the largest class in Bryophyta and includes about 700 genera and 14,000 species.
2. The main plant body is gametophytic and can be differentiated into two stages-juvenile stage and leafy stage or gametophore.
3. Juvenile stage is represented by green, filamentous branched structures called protonema. It develops from the germination of the spore.

4. Gametophores are erect leafy branches which develop on the protonema.

5. Gametophores can be branched or un-branched and can be differentiated into three parts-rhizoids, ‘stem’ and ‘leaves’.





6. Branches arise below the ‘leaves’.

7. ‘Leaves’ are with midrib, un-lobed and arranged spirally in three to eight rows on the axis or

8. Rhizoids are multicellular, filamentous, oblique branched withsepta.

9. The axis is differentiated into central conducting strand enclosed by cortex.

10. Sex organs borne apically in the groups on main ‘stem’ or a branch.

11. The sporophyte is green in early stages and can be differentiated into foot, seta and capsule.

12. The seta is usually elongated and rigid.

13. Columella is usually present and endothecial inorigin.

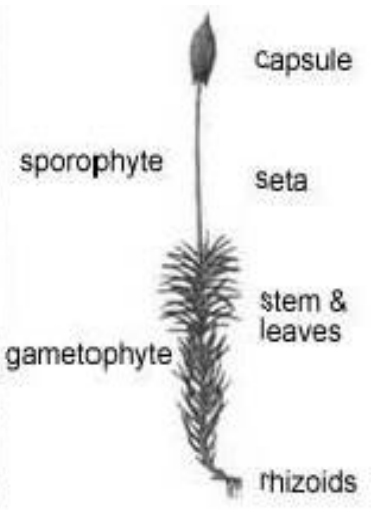
14. Archesporiurn (spore forming tissue is differentiated only in spores.

1. Elaters are absent.

15. Dehiscence of capsule takes place by separation of lid or operculum.

16. Peristome helps in the dispersal of spores.

17. Spores on germination produce the protonema



Vegetative Reproduction in Bryophytes:

The following are the common methods of vegetative propagation noted among the bryophytes:

1. Fragmentation or Progressive Death and Decay of the Older Parts:

Propagation through this process involves progressive death and decay of the older parts of the thallusand consequent separation of the younger parts at the point of dichotomy of the thallus.

These separated parts, called fragments, develop to form new mature thallus. This is the common method of vegetative reproduction found in liver-worts (e.g. *Riccia, Marchantia, Plagiochasma*), hornworts (e.g. *Anthoceros*) and in some mosses (e.g. *Sphagnum*).

2. By Tubers:

The tubers are round struc-tures formed on the thaiIi under unfavourable conditions like drought. They remain unaffected and lie dormant while the rest of the thallus gets dried. On the return of favourable environmental conditions, the tubers resume growth and develop into new thalli.

Tubers are frequently produced in species of Riccia (*R. discolor, R. vesicata, R. billardieri*), Anthoceros (*Anthoceros pearsoni, A. himalayensis, A. laevis*), *Asterella angustata*, and *Fossombronia himalayensis*.

3. By Gemmae:

Gemmae are special propagative organ with definite form. They are quite abundant in the members of liverworts, but less common in hornworts and mosses. They are of different shapes, stalked or sessile and may develop on different parts of the parent plant.

4. By the Formation of Adventitious Branches:

In some thailoid liverworts and horn-worts adventitious branches develop usually from the underside of the midrib of the thallus. These adventitious branches on detachment from the parent plants develop into independent plants (e.g., *Riccia fluitans, Marchantia, Targionia, Reboulia, Asterella,*

Sphaerocarpos, Anthoceros, etc.). This process, in fact, achievesthe luxuriant growth of *Riccia fluitans* rapidly covering the whole pond surface.

5. By Cladia:

Cladia are small detachable branches which serve the purpose of vegetative reproduction. These may develop either from the individual cells of leaves or stems and are known as leaf cladia (*Bryopteris fruticulosa, Frullania fragilifolia*) or stem cladia (e.g., *Leptolejeunia* sp., *Drepanolejeunea* sp., etc.) as per their mode of origin.

6. By the Formation of Innovations:

The formation of innovations is frequently noticed in *Sphagnum*. In this case, one of the divergent branches in each node develops more strongly and becomes dominant and erect like the main axis and is called innovation. During humification process innovation is separated from the main axis due to the progressive death and decay of the lower basal parts of the main axis and eventually establishes as an independent plantbody.

7. By Protonema:

The green, filamentous protonema which develops from the germination of spore is known as primary protonema. The primary protonema may break into small pieces — either accidentally or due to the death of some cells in between. Each of these fragments bearing buds is capable of forming a new plant (e.g., *Funaria*).

The protonema that develops from any part of the plant other than the spores are called secondary protonema. They may develop from the rhizoids of gametophore (e.g., *Funaria* sp.), from leafy gametophore (e.g., *Funaria, Sphagnum*), from primary protonema (Sphagnum), from wounded portion of the leafy shoot (e.g., *Funaria*).

Additional buds develop via formation of secondary protonema, thus increasing the number of buds derived from a single spore.

8. Persistent Apices:

In many thalloid bryophytes (e.g., *Anthoceros, Cyathodium, Athalamia* etc.) and in some creeping mosses, all parts of the thallus except the growing apex becomes dry during the dry summer season. These apical parts remain dormant during dry season. These apices, on return of favourable environmental condition (rainy season), become active and form new plants.

Ecological Importance of Bryophytes:

Bryophytes are important constituents of the ecosystem in temperate and tropical forests that have significant ecological importance. Bryo-phytes are important stabiliser of substrata that later become suitable for higher plants colonisa-tion. Extensive bryophyte mats are significant in the water balance of the forest.

They are capable of absorbing water and nutrients directly through the surface. They prevent soil erosion

as they have trample-resistant structure and high regenerative capacity. Some bryophytes provide suitable substrata for the biological fixation of N2 in association with blue green algae.

The recent increase in atmospheric pollution has revealed the bryophytes as “bioindicators” of pollution and accumulators of heavy metals.

The ability of bryophytes to grow on open and nutrient poor areas and their tolerance to desic-cation can be exploited in successful stabilisa-tion of soil on road sides and open areas. Bryophytes also harbour a number of inverte-brates and provide them shelter, food and a place for deposition of eggs.

The details are as:

1. Bryophytes and Plant Succession:

Among the bryophytes, the mosses are consi-dered to be the most potent forms in successional process. They colonise over the nutrient-poor sites where no other plant can survive. After death and decay, they form humus, in other way increasing soil fertility.

Thus, the accumulated organic matters become suitable for the micro-organisms. The microorganism increases the nutrient availability and makes the site suitable for growth of higher plants. The important species under this category are Cephalozia media, Isopterygium elegans, Lepidozia septans, Pellia epiphylia and *Tetrapis pellucida*.

2. Bryophytes and Animal Association:

Bryophytes possess several attributes viz., incon-spicuous forms, relative abundance in the com-munity, ability to survive in extreme environ-mental conditions and water absorbing and retention capacity, which affect the distribution and abundance of dependent animals and microorganisms.

(i) Bryophytes and Animal Succession:

While bryophytes participate in the early stages of plant succession,’ their associated animals form similar stages of faunal succession. For example, moss cushions developing on rock faces are first colonised by rhizopods, rotifers, nematodes and ciliates.

As dead material form under the cushions, rotifers and tardigrades become abundant and arthropods begin to appear. As a thicker decomposition layer is formed, the composition of the fauna becomes similar to that of the soil fauna.

(ii) Shelter:

Water retention is a unique feature that makes bryophyte community an attractive habitat for many invertebrates. Bryophytes provide food and nesting materials for small mammals and invertebrates. Indirectly, they serve as a matrix for a variety of interactions between organisms. Insects are the most richly represented on bryophytes.

Many protozoa, rotifera, nematodes, earthworms, molluscs, arthropods like spiders, millipeds, centipeds and various crustaceans are found in bryophyte com-munities. Large pores of Sphagnum leaves facili-tate

the entrance of water and allow unicellular animals to enter the leaf cells and live inside them.

(iii) Food:

Many invertebrates feed on bryophytes. Orthopterans, beetles, moth and caterpillars bite and chew whereas bugs, aphids and mites suck out the contents of moss cells.

(iv) Ovipositor and Pupation:

The animals which feed on bryophytes also ovideposit their eggs there. Snails and slugs are frequently depositing their eggs upon the gametophores. Water beetles appear to live preferentially among mosses and spend their dormancy period.

Many insects associated with bryophytes deposit their eggs there, and -the larval stages often browse on the gametophores. Pupation of the water beetle takes place within a small cell.

(v) Camouflage:

Some insects have morphologies, surface patterns or appendages that permit them to blend in with their bryophyte habitat. A few insects paste the parts of game-tophores on their wings and thus camouflage themselves against predation.

Camouflage may be used by larvae that construct their cases from blades of *Fontinalis, Hygrophynum, Anomobryu*m and *Plagiochila*.

3. Bryophytes and Cyanobacteria:

In natural association, cyanobacteria typically grow in association with bryophytes. Nitrogen is often a limiting nutrient for plant growth. Even small contributions from biological nitrogen fixation may, therefore, be important to the ecosystem.

Some mosses, hornworts and liverworts provide suitable habitats for the biological fixation of nitrogen in association with cyanobacteria (e.g., *Nostoc*). The ability of cyanobacteria to fix atmospheric nitrogen allow a few bryophytes to grow in areas that are naturally low in nitrogen and serve as fertiliser to soil.

4. Bryophytes as Ion-Exchanger:

The cell walls of *Sphagnum* function as ion-exchanger. They rapidly absorb cations, such as calcium and magnesium, supplied by rain water, and in exchange^ release hydrogen ions into the water. Rydrogeri ions make the soil acidic (pH 3-4). Therefore, Sphagnum creates as well as main-tains a nutrient-poor, acidic environment that fosters their own growth, but is mostly intolerable to other plants.

5. Bryophytes Maintain Water Balance in the Forests:

In forests, especially in the montane tropics, bryophytes (especially *Sphagnum*) absorb huge quantities of water and maintain humidity over dry periods, thus preventing rapid run-off and flooding. Without bryophytes, rain-forest would be merely wet and mountain rocks would be barren.

The huge bryophyte mats in the forest floor slow down and delay run-off during rain. It has been predicted that the excessive flooding in India is at least partly due to loss of bryophyte covers.

6. Bryophytes Conserve Soil and Prevent Soil Erosion:

On bare and disturbed soil bryophytes are primary pioneers and they have the ability to stabilise soils. The soils in semi-arid regions are held in place by crusts predominantly composed of bryophytes, thus preventing the soil from blowing away. They also prevent soil erosion by slowing down and delaying run-off during rain.

When clay-rich soil has been laid bare due to landslides or road making, the first colonisation and subsequent stabilisation are substantially by bryophytes. The soil surface rapidly becomes bound together by rhizoid pro-duction followed by rapid branching of prostrate stems, thus preventing further soil erosion.

7. Bryophytes as Pollution Indicator:

The investigations with bryophytes in relation to different pollutants prove their potential as bioindicators of pollution. Due to their habitat diversity, structural simplicity, totipotency and rapid rate of multiplication bryophytes appear to be ideal organisms for pollution studies both under field and laboratory conditions.

Phytosociological and eco-physiological studies indi-cate that the decline and absence of mosses — especially epiphytic ones — in urban-industrial areas is a phenomenon primarily induced by air pollution caused by different gaseous and parti-culate pollutants. These plants can be reliable indicators and also monitor the air pollution.

Some bryophytes are very sensitive to pollution and show visible symptoms of injury even in the presence of minute quantities of pollutants. Such plants serve as good bio-indicators of the nature and degree of pollution. Some bryophytes have the capacity to absorb and retain pollutants in quantities much higher than those absorbed by other plant groups present in the same habitat.

Their efficient absorbing capacity is due to the absence of cuticle, presence of single cell thick lamina and larger surface area as compared to the volume. These plants, therefore, act as effective sink of pollutants and prevent their recycling for a considerable period of time.

Economic importance of Bryophytes:

1. Protection from soil erosion:

Bryophytes, especially mosses, form dense mats over the soil and prevent soil erosion by running water.

2. Soil formation:

Mosses are an important link in plant succession on rocky areas. They take part in binding soil in rock crevices formed by lichens. Growth of Sphagnum ultimately fills ponds and lakes with soil.

3. Water retention:

Sphagnum can retain 18-26 times more water than its weight. Hence, used by gardeners to protect desiccation of the seedling during transportation and used as nursery beds.

4. Peat:

It is a dark spongy fossilized matter of Sphagnum. Peat is dried and cut as cakes for use as fuel. Peat used as good manure. It overcomes soil alkalinity and increases its water retention as well as aeration. On distillation and fermentation yield many chemicals.

5. As food:

Mosses are good source of animal food in rocky and snowclad areas.

6. Medicinal uses:

Decoction of Polytrichum commune is used to remove kidney and gall bladder stones. Decoction prepared by boiling Sphagnum in water for treatment of eye diseases. Marchantia polymorpha has been used to cure pulmonary tuberculosis.

7. Other uses:

Bryophytes arc used as packing material for fragile goods, glass wares etc. Some bryophytes act as indicator plants. For example, Tortell tortusa grow well on soil rich in lime.

Pteridophyta:

Pteridophytes are considered as first land vascular plants on Earth which appeared in the Late Silurian (~400 million years ago) as evinced by the discovery of fossil *Cooksonia*, an earliest plant with conducting system. They flourished during Devonian to Carboniferous (Late Palaeozoic) with varied forms like arborescent lycopods, giant horsetails, tree ferns and thus the Late Palaeozoic age is straightforwardly considered as "Age of Pteridophyta".

Today, this plant group is represented approximately by 13,600 named species of ferns and lycophytes, mostly dominated by ferns (more than 10,000 species) and reached at second highest position among land plant groups after angiosperm. However, the statures of most of the present-day forms are herbaceous instead of arborescent as was in ancient ages.

Pteridophytes, especially the ferns, are not only worthy in forming a balanced ecosystem, but they attract common people for their exquisite lacy foliage and so are valued as horticultural plants since long. The plants are also explored worldwide as natural resources for food, fibre, handicraft, construction material, bio-fertilizer, medicine etc. for human benefit. Ferns and lycophytes have even been used by tribal people in many countries since ancient ages as ethno-medicine and in traditional rituals and ceremonies for spiritual purposes. Recently researchers are paying attention to utilize various terrestrial and aquatic ferns for heavy metal accumulation from the environment by proving their potential in phytoremediation. The existing knowledge from many places across the world gradually enriches the understanding that easy growing common ferns have abilities to absorb unamenable soil pollutants particularly heavy metals/metalloids. As such, the multifaceted usages of ferns and

lycophytes have bound people for considering this group of plants as unequivocal component in their life.

Habitat

- Generally, grow in tropic, mesic forest
- Also occur in temperate region
- Even they grow in alpine region (*Lycopodium*)
- May grow along the mangrove region at sea side (*Acrostichumaureum*)
- Some ferns grow in desert region
- Some grow at road side in polluted area

Habit

- Generally, herb and shruby (e.g. *Oleandra*)
- Tree fern (e.g. *Cyathea*, *Dicksonia*)
- Climbers (e.g. *Lygodium*)

General Characters

- The pteridophytes are vascular plants (plants with xylem and phloem) that produce neither flower nor seeds. Instead, they reproduce and disperse only via spores.
- Plants characterised by a very distinct alteration of generations with independent inconspicuous and sort-lived gametophyte (sexual) and conspicuous and dominant sporophyte (asexual) stages (Fig. 1).

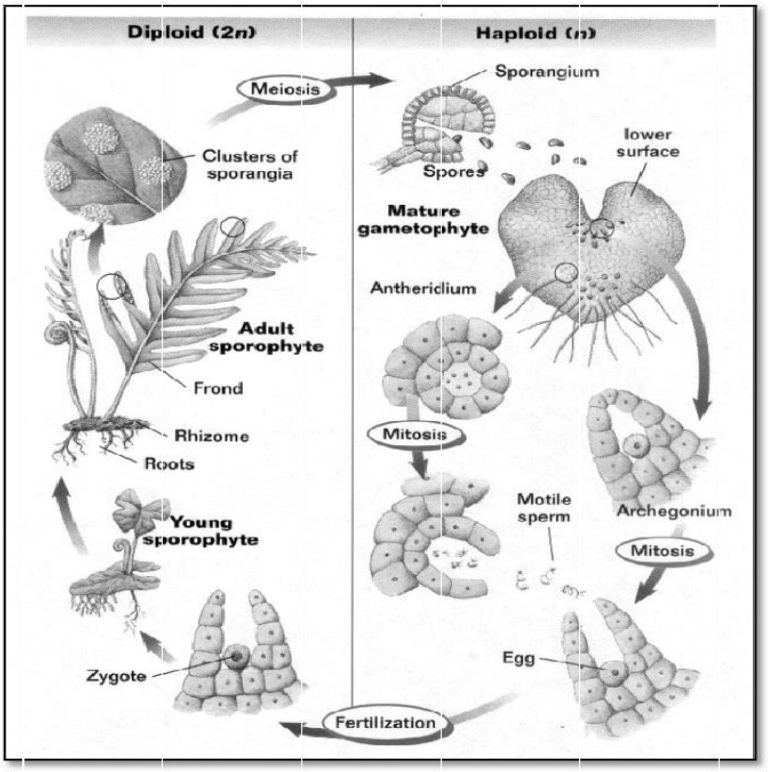


Fig. 1: Alteration of generations with sexual and asexual stages
(Source: https://www.bioexplorer.net/plant-life-cycle.html/)

- Sporophyte vascular herbs, shrubs (*Oleandra*), some with limited secondary thickenings (*Botrychium*, *Isoetes* root), mostly terrestrial, rupestral (lithophytes) or epiphytic, climbers (*Lygodium*), sometime floating, submerged or emergent aquatic; mostly soft or delicate, sometimes harsh or stout; rarely arborescent (*Cyathea*, *Dicksonia*) (Fig. 2).

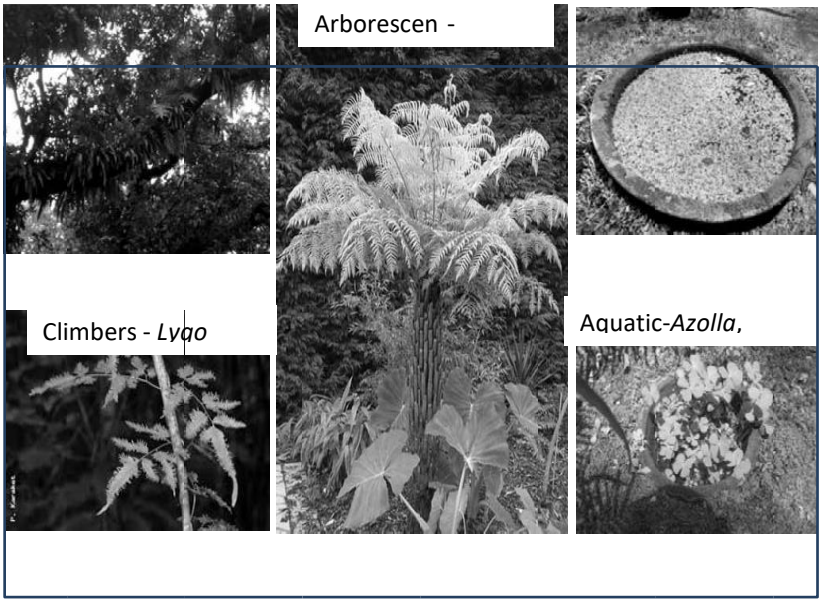


Fig. 2: Diversity of Pteridophytes

- Sporophytic plants with leaves, axes and roots (the latter missing in Psilotaceae) and well developed conducting tissue.
- Stem often rhizomatous, radial or dorsiventral, erect, prostrate, climbing, or subterranean; often with scales and/or hairs; stele simple to complex i.e. protostelic, solenostelic, dictyostelic and polystelic.
- Leaves either small, simple and bract-like or linear with a simple vein, straight in bud, or a broad frond with branched or divided veins (Fig. 3a), simple to several times pinnately divided, conduplicate or mostly circinate in bud (Fig. 3b); bearing sporangia (Fig. 3).

Fig. 3a: Microphyll and megaphyll

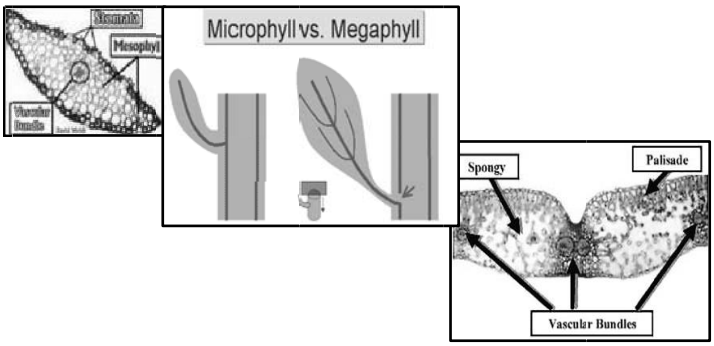


Fig.3b: Circinate vernation

- Leaf stalk or stipe is mostly present; stipules are mostly lacking.
- Sporangia thick or thin walled, homosporous or heterosporous, mostly grouped in sori or fused in synangia, or present as special structures viz., spike or sporocarp or solitary in axils or sporophylls which may be grouped into strobili (Fig. 4); sori naked or often protected by an indusium.
- Spores monolete or trilete; germinating to form an avascular autotrophic or mycotrophic prothallus (gametophyte stage) bearing flagellated male gamates (antherozoids) in antheridia and/or female gamates (egg cells) in flask shaped archegonia
- Gamete transfer and fertilization by water, producing a new plant (sporophyte stage) which obliterates the prothallus.

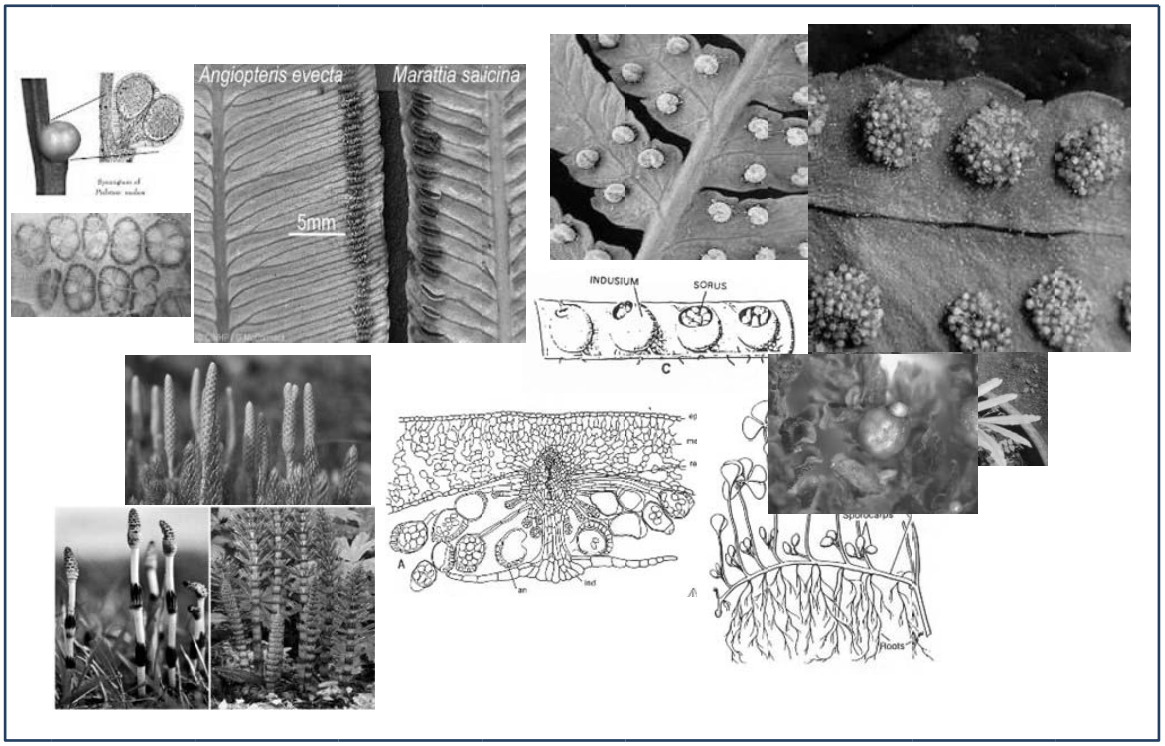


Fig 4: Diversity in reproductive structures

Fern ally

Fern ally is a general term covering a somewhat diverse group of vascular plants that are not flowering plants and not true ferns. Like ferns, these plants disperse by shedding spores to initiate an alternation of generations (*Lycopodium, Selaginella*)

True Ferns

True Ferns are vascular plants differing from lycophytes by having true leaves (megaphylls). They differ from seed plants (gymnosperms and angiosperms) in their mode of reproduction—lacking flowers and seeds.

Classification:

Based on morpho-anatomical characters, numerous classification schemes *(Bower 1928, Eames 1936, Tippo 1942, Holtumm 1949, Smith 1955, Bold 1957, Zimmermann 1959, Mehra 1961, Sporne 1962, Nayar 1970, Pichi Sermolli 1970, Kato 1983, Gifford & Foster 1989 etc.)* have been proposed for pteridophytes

PTERIDOPHYTES [based on Sporne 1962]				
CLASS	**ORDER**	**CLASS**	**SUB CLASS**	**ORDER**
PSILOPSIDA	Rhyniales	PTEROPSIDA	Primofilices	Cladoxylales
	Zosterophyllales			Coenopteridales
	Trimerophytales		Eusporangiatae	Marattiales
PSILOTOPSIDA	Psilotales			Ophioglossales
LYCOPSIDA	Protolepidodendrales		Osmundidae	Osmundales
	Lycopodiales		Leptosporangiatae	Filicales
	Lepidodendrales			Marsileales
	Isoetales			Salviniales
	Selaginellales	PROGYMNOSPERMOPSIDA		Aneurophytales
SPHENOPSIDA	Hyeniales			Protopityales
	Sphenophyllales			Archaeopteridales
	Calamitales			
	Equisetales			

Bower (1928) recognized 12 families, and divided Polypodiaceae into 15 subfamilies. His classification was based on the structure and development of the sorus. He recognized 3 groups – Simplices. Gradatae, Mixtae

Simplices	***Gradatae***	***Mixtae***
Coenopteridaceae	Hymenophyllaceae	Polypodiaceae (divided into seven tribes)
Marattiaceae	Cyatheceae	
Osmundaceae	Protocyatheaceae	

Gleicheniaceae	Plagiogyriaceae
Schizaeaceae	Dixoniaceae
Matoniaceae	Loxosoniaceae

Ching (1940) divided Polypodiaceae into 33 families

Earlier classifications were based primarily on:

- External morphology of the shoot
- Apical meristem and its further segmentation
- Architecture and venation
- Vascular anatomy of stem and leaf
- Dermal appendages
- Position and structure of the sorus
- Indusial protection
- Characters of the sporangium
- Nature and form of sporangia
- Spore output per sporangium
- Mode of spore germination & type of prothallus
- Features of gametophyte
- Position and structure of sex organs
- Cytology
- Palynology

Cytological parameter is based on chromosome base numbers

- Chromosome base numbers have been used for classification purposes
- Commonly the base number is uniform for a genus or family, or it ranges around a given number
- More rarely, the number varies drastically in some genera: as in *Thelypteris*, which has x numbers ranging from 27 to 36

Recent Classification is based on molecular systematic studies in addition to morphological data.

- Smith *et al.* (2006)
- Rothfels *et al.* (2012)
- Christenhusz and Chase (2014)

Classification of Smith *et al.* 2006

This classification was based on both morphological and molecular data that was mainly obtained from

- Six chloroplast markers *(rbcl, atpA, atpB, accl, 16S rDNA, ITS)*
- One nuclear gene *(18S rDNA)*
- Three mitochondrial genes *(atp1, nad2, nad5)*

This classification divides ferns into four Monophyletic classes; 11 monophyletic orders, 37 families of which 32 are monophyletic.

The classes are- Psilotopsida, Equisetopsida, Marattiopsida ,Polypodiopsida

The complete classification scheme proposed by Smith et al. (2006)-Table 1a, b

Table 1a: Complete classification scheme proposed by Smith et al. (2006)

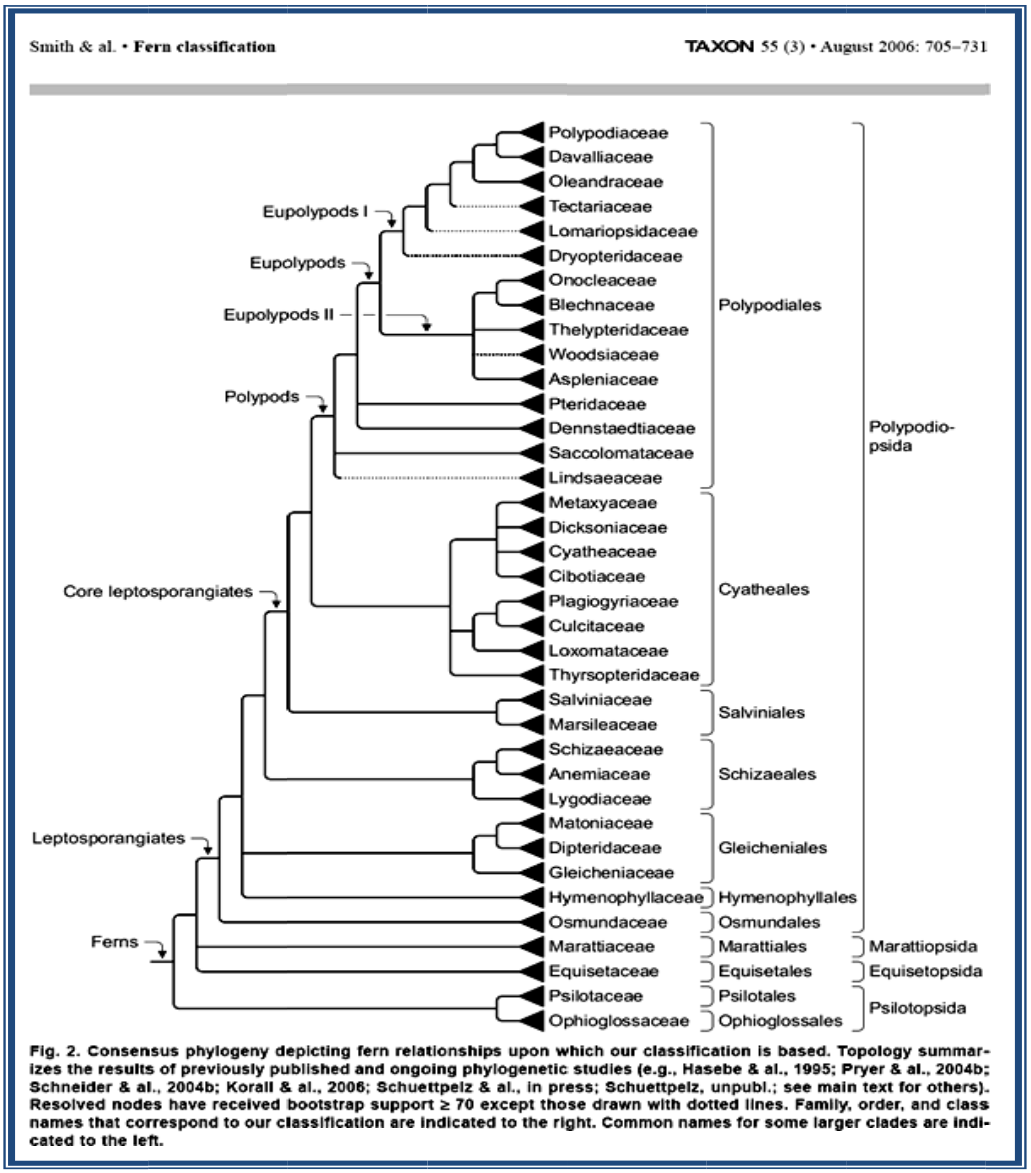


Fig. 2. Consensus phylogeny depicting fern relationships upon which our classification is based. Topology summarizes the results of previously published and ongoing phylogenetic studies (e.g., Hasebe & al., 1995; Pryer & al., 2004b; Schneider & al., 2004b; Korall & al., 2006; Schuettpelz & al., in press; Schuettpelz, unpubl.; see main text for others). Resolved nodes have received bootstrap support ≥ 70 except those drawn with dotted lines. Family, order, and class names that correspond to our classification are indicated to the right. Common names for some larger clades are indicated to the left.

CLASS	ORDERS
Polypodiopsida	Polypodiales
	Cyatheales
	Salviniales
	Schizaeales
	Gleicheniales
	Hymenophyllales
	Osmundales
Marattiopsida	Marattiales
Equisetopsida	Equisetales
Psilotopsida	Psilotales
	Ophioglossales

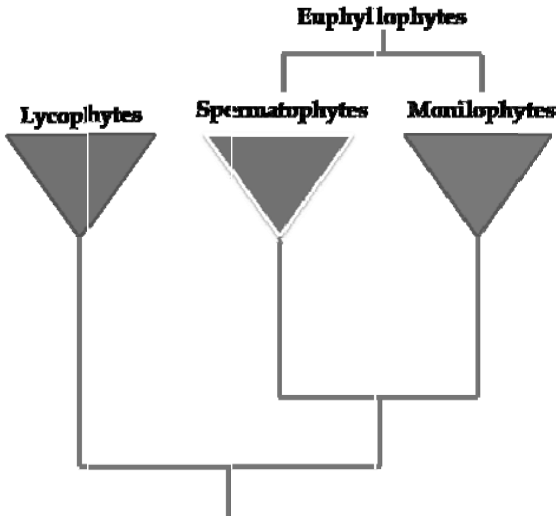


Table 1b: Classification scheme proposed by Smith et al. (2006) showing the orders

Christenhusz and Chase (2014) proposed classification based on molecular analysis

They have aimed for a stable family and generic classification of ferns, and the current understanding of relationship of ferns and Lycopod clades.

- They are of the view that due to its vague circumscription and now evident non-monophyly, the term fern ally should be avoided.
- They also recommend that the term eusporangiate should be dispensed with because this includes ferns that are not leptosporangiate, and just describes the pleisomorphic state and the taxa included do not form a clade.

- The term ‘moniliophytes’ is unclear and has never been published as a formal taxon and more over its etymology is obscure.
- The leptospoangiate ferns are a natural group forming the majority of extant ferns

A comparison in two systems that are based on molecular data & morphology

	Smith et al. 2006	**Christenhusz & Chase 2014**
Families	37	38
Sub families	0	16

Terms to be dispensed with: Eusporangiate and fernally

- Although Sphenophytes share a common ancestors with fern there exact placement within fern is uncertain
- Ferns in the past include Equisetaceae & Psilotaceae
- Sphenophytes, Psilotophytes, Marattiophytes could be treated as independent lineages

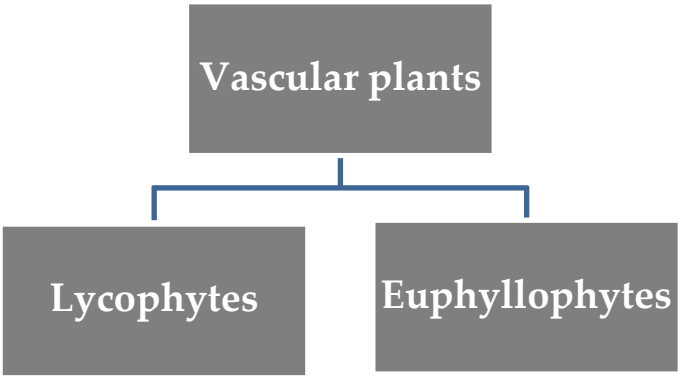
A revised family level classification for Eupolypod II Ferns (a clade of ferns in the order Polypodiales, under the class Polypodiopsida-Polypodiidae: Polypodiales) by Rothfels et al. 2012

- A family level classification for the Eupolypod II clade of leptosporangiate ferns (comprising over 2500 species), has been given on the plea that these were not well understood at the time of publication of the fern classification by Smith et al, 2006
- The composition and particularly the relationship among the major clades of this group have historically been controversial and challenged phylogenetic resolution until very recently
- Their classification reflects the current available data, largely derived from published molecular phylogenetic studies
- They provide circumscription for each family, which summarize their morphological, geographical, and ecological characters, as well as a dichotomous key to the eupolypod II families

By the pteridophytic classification it is assumed that vascular plans have a basal dichotomy separating:

Lycophytes: vascular plants having lycophylls or leaves without leaf gaps (microphylls), vein single medium, meristem intercalary

Euphyllophytes: vascular plants having euphylls or leaves with an associated leaf gap in the vascular stele (megaphylls), veins branched, and with marginal or apical meristem



This dichotomy occurred in the early – mid Devonian ca. 400 million years ago

Monilophytes (ferns) are characterized by-

- Lateral origin of the root in the endodermis
- mesarh protoxylem in shoot
- A pseudo- endospore
- Plasmodial tapetum
- Sperm cell with 30-1000 flagella

Euphyllophytes (or "Eu-phyllo-phyte" – true leaved plant): it includes most living plants except bryophytes and lycophytes

Features: Plant with upright, 3-dimensional branching

Stems: Upright growth of stems with lateral branches (pseudo-monopodial growth)

Branching in 360^0 (spiral branching off a main axis)

Leaves: True leaves in most derived forms ancestral members are leafless

Roots: True roots

Reproductive structures: Ancestral member spore-bearing; derived members are seed bearing

Geologic age: 420 my (Silurian Period) to Present

Lycophytes: include *Selaginella, Lycopodium, Isoetes*

- They are the oldest living vascular plants
- Reproduce through spores
- Have a single vascular vein through their leaves

There are about 1,000 species of Lycophytes worldwide

Evolution form homospory to heterospory

- The evolution of homospory to heterospory – in which a megaspore develops into a female gametophyte that includes one or more egg cells
- and a microspore develops into a male gametophyte that includes sperm – is among the most important transitions in the evolution of plants, with profound effects on plant reproduction

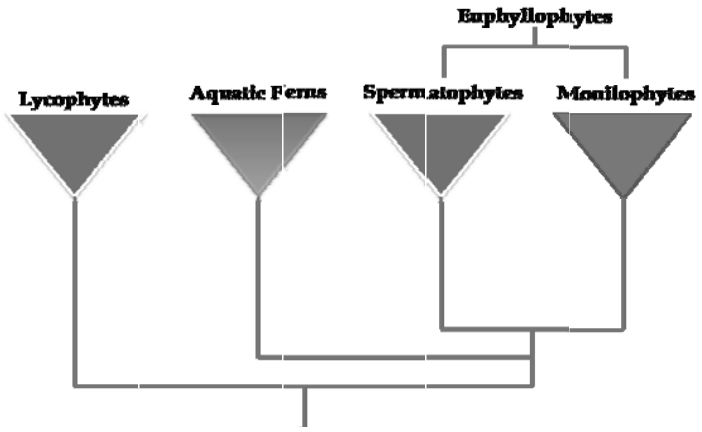
and the life cycle.

- However, the nuclear genome of a homosporous vascular plant has yet to be sequenced

Divorce the heterosporous ferns from the homosporous extant ferns:

The aquatic ferns at first sight do not physically resemble other homosporous ferns

- They are heterosporous; remaining extant ferns are homosporous
- Their gametophytes are endosporic and entirely different
- Morphology of the sporophyte is different
- Habit and habitat are different
- Aquatic ferns are quite different and should be treated as separate from the rest of the ferns and perhaps be treated on an equal par as the Psilotaceae, Equisetaceae etc.
- Chromosome number in Marsileaceae is rather low compared to the homosporous ferns



A suggestion given by Khullar 2015

ORDINAL FEATURES

Lycopodiales

- Living and extinct plants
- Sporophyte with primary growth only, no vascular cambium
- Leaves eligulate
- Majority have definite strobili

Selaginellales

- Living & extinct plants with primary growth only, no vascular cambium
- Microphylls with ligule
- Definite strobili found
- Heterosporous
- Gametophytes endosporic

- Sperms biflagellate in living members

Isoetales

- Living and extinct plants
- Sporophyte with cormlike stems
- Sc. Growth present in root
- Ligulate microphylls
- Heterosporous
- Endosporic gametophytes
- Sperms multiflagellate in living members

Ophioglossales

- Species mostly terrestrial (a few epiphytic), temperate and boreal, but a few pantropical.
- Vernation nodding (not circinate)
- Rhizomes and petioles fleshy
- Root hairs lacking
- Aerophores absent
- Fertile leaves each with a single sporophore arising at the base of, or along, the trophophore stalk, or at the base of the trophophore blade (several sporophores per blade in *Cheiroglossa*)
- Sporangia large, with walls two cells thick, lacking an annulus
- Spores globose-tetrahedral, trilete, many (>1000) per sporangium
- Gametophytes subterranean, non-photosynthetic, mycorrhizal; $x = 45$ (46)

Psilotales

- Roots absent
- Stems bearing reduced, un-veined or single veined
- Euphylls
- Sporangia large, with walls two cells thick, lacking an annulus;
- Two or three sporangia fused to form a synangium, seemingly borne on the adaxial side of a forked leaf
- Spores reniform, monolete, many (>1000) per sporangium;
- Gametophytes subterranean (*Psilotum*), non-photosynthetic, mycorrhizal; $x = 52$.

Equisetales

The spermatozoids of *Equisetum* share several important features with other ferns that support their inclusion in this clade (Renzaglia & al., 2000). Kato (1983) adduced additional morphological characters, including root characters, supporting a relationship between horsetails and ferns.

- Stems whorled, lacunate

- Leaves whorled, connate
- Sporangia with helical secondary wall thickenings (Bateman, 1991), borne on peltate sporangiophores that collectively comprise strobili
- Sporangia large, lacking an annulus, many (>1000) per sporangium
- Spores green, with circular aperture and four paddle-like, coiled elaters
- Gametophytes green, surficial; $x = 108$.

Marattiales

- Roots large, fleshy, with polyarch xylem
- Root hairs septate
- Roots, stems, and leaves with mucilage canals
- Rhizomes fleshy, short, upright or creeping, with a polycyclic dictyostele
- Vernation circinate
- Leaves large, fleshy, 1–3-pinnate (rarely simple in *Danaea*, or 3–5-foliate in *Christensenia*) with enlarged, fleshy, starchy stipules at the base and swollen pulvinae along petioles and rachises (and sometimes other axes)
- Petiole and stem xylem polycyclic
- Stems and blades bearing scales pneumathodes (lenticels) scattered all around petioles and/or rachises
- Sporangia free or in round or elongate synangia (fused sporangia), lacking an annulus, enclosing 1000–7000 spores
- Spores usually bilateral or ellipsoid, monolete
- Gametophytes green, surficial; $x = 40$ (39)

Osmundales

- Temperate and tropical
- Stem anatomy distinctive, an ectophloic siphonostele, with a ring of discrete xylem strands, these often conduplicate or twice conduplicate in cross-section
- Stipules at bases of petioles
- Leaves dimorphic or with fertile portions dissimilar to sterile
- Sporangia large, with 128–512 spores, opening by an apical slit, annulus lateral
- Spores green, sub-globose, trilete
- Gametophytes large, green, cordate, surficial; $x = 22$.

Hymenophyllales

- Terrestrial and epiphytic; pantropical and south-temperate, but gametophytes survive in north-temperate regions as far north as Alaska.
- Rhizomes slender, creeping, wiry, or sometimes erect and stouter, protostelic

- Vernation circinate
- Blades one cell thick between veins (a few exceptions)
- Stomata lacking
- Cuticles lacking or highly reduced
- Scales usually lacking on blades, indument sometimes of hairs
- Sori marginal, indusia conical (campanulate), tubular, or clam-shaped (bivalvate), with receptacles, usually elongate, protruding from the involucres
- Sporangia maturing graduately in basipetal fashion, each with an uninterrupted, oblique annulus;
- Spores green, globose, trilete;
- Gametophytes filamentous or ribbon-like, often reproducing by fragmentation or production of gemmae; x = 11, 12, 18, 28, 32, 33, 34, 36, and perhaps others.

Gliecheniales

- Root steles with 3–5 protoxylem poles
- Antheridia with 6–12 narrow, twisted or curved cells in walls

Schizaeales

- Fertile-sterile leaf blade differentiation
- Absence of well-defined sori
- Sporangia each with a transverse, subapical, continuous annulus

Salviniales

- Fertile-sterile leaf blade differentiation
- Veins anastomosing
- Aerenchyma tissue often present in roots, shoots, and petioles
- Annulus absent
- Plants heterosporous
- Spores with endosporous germination
- Monomegaspory
- Gametophytes reduced.

Cyatheales

- Some of the species have trunk-like stems but others have creeping rhizomes
- Some have only hairs on the stems and blades, others have scales
- Sori are abaxial or marginal, either indusiate or exindusiate
- Spores are globose or tetrahedral-globose, with a trilete scar
- Gametophytes green, cordate

Polypodiales

- Indusia laterally or centrally attached (indusia lost in many lineages)
- Sporangial stalks 1–3 cells thick, often long
- Sporangial maturation mixed
- Sporangia each with a vertical annulus interrupted by the stalk and stomium
- Gametophytes green, usually cordate (sometimes ribbon shaped in some epiphytes), surficial

Reproduction in Pteridophytes:

(i) Reproduction takes place by means of spores which are produced inside sporangia.

(ii) The development of the sporangium may be leptosporangiate (sporangium originates from a single cell) or eusporangiate (sporangium develops from a group of cells).

(iii) Sporangia may be borne either on stem or leaves. On the stem they may be terminal (e.g., *Rhynia*) or lateral (e.g., *Lycopodium*). On the leaves (sporophylls) they may be ventral, marginal (*Pteris, Adiantum*) or dorsal (e.g., Polypodiceae). In *Equisetum* the sporangia are borne on special structures called sporangiophores which constitute a cone. In *Marsilea, Azolla, Salvinia* sporangia are produced in sporocarps.

(iv) Spores on germination give rise to multicellular gametophytic bodies called prothalli (sing. prothallus).

(v) In homosporous Pteridophytes prothalli are monoecious (antheridia and archegonia develop on the same prothallus). In heterosporous species prothalli are always dioecious. Microspores on germination give rise to male prothalli and megaspores to the female prothalli.

(vi) Antheridia and archegonia are developed on prothalli.

(vii) Antheridium is surrounded by a single layered sterile jacket.

(viii) Archegonium consists of four vertical rows of neck cells, 1-2 neck canal cells, ventral canal cell and egg.

(ix) Antherozoids are unicellular, biflagellate (e.g., Selaginella) or multiflagellate (e.g., Equisetum and ferns) and motile.

(x) Antherozoids are attracted towards the neck of the archegonium chemotactically by certain substances like malic acid) present in the mucilaginous substance formed by the degeneration of neck canal cells and venter canal cell.

(xi)Water is essential for fertilization (zooidogamous). Therefore, Pteridophytes are also known as amphibians of the plant kingdom.

(xii) Fertilization results in the formation of zygote or oospore, which ultimately develops into well-developed sporophyte.

(xiii) The fertilized egg divides transversely or vertically. Another cross wall forms a quadrant stage producing stem, leaf, foot and root.

(xiv) Plants show heteromorphic alternation of generation. The main plant body is sporophytic and forms a dominant phase in the life cycle.

Economic Importance of Pteridophytes:

The economic importance of Pteridophytes is not well-documented, because due attention has not been given towards their use in human welfare. However, there are many reports on their uses, especially as food plants, medicinal plants and horticultural plants.

Some of the aspects of economic importance of pteridophytes are given:

i. Pteridophytes Used as Food:

The young leaf tips of ferns, the circinate ptyxis or the chroziers are used as vegetable. The young fronds of Ampelopteris prolifera are sold in the market as 'dheki shaak' in India and Bangladesh. The croziers of Matteuccia struthiopters as canned or frozen are served as spring vegetable in USA and Canada. Leaves of *Marsilea*, commonly called 'shushni', are used as vegetable.

The rhizome of many ferns such as *Pteris*, rich in starch, is used as food.

The corm (modified stem) of Isoetes is used as food by pigs, ducks and other animals.

ii. Pteridophytes Used as Fodder:

Dry fronds of many ferns form the livestock for catties. The quadrifid lamina of *Marsilea* resembles a clover (*Trifolium*) has been used as fodder for animals as a substitute for clover.

iii. Pteridophytes Used as Medicine:

The spores of *Lycopodium* have been wide-ly used in pharmacy as protective dusting pow-der for tender skin and also as water-repellants. The foliages of Lycopodium are used as tincture, powder, ointment and cream as a stomachic and diuretic. The foliage decoction is used in home-opathy to treatdiarrhoea, bladder irritability, eczema, rheumatism, constipation and inflammation of liver.

Equisetum is rich in silicic acid and silicates. Potassium, aluminium and manganese, along with fifteen types of flavonoid compounds, have been reported from Equisetum. The flavonoids andsaponins are assumed to cause the diuretic effect. The silicon is believed to exert connective tissue- strengthening and anti-arthritic action.

Several ferns have been used as herbal medicine. An oil (5% Filmaron and 5-8% Filicic acid) extracted from the rhizome of Aspidium is used as a vermifuge, especially against tape-worm. The decoction of Asplenium is used for cough and a good hair wash. The expectorant of *Polypodium* is used as a mild laxative, while the tonic is used for dyspepsia, loss of appetite and hepatic problem.

The root decoction of *Osmunda regalis* is used for treatment of jaun-dice. The ointment made from itsroot

is used for application to wound. The extraction of Osmanda vulgaris, commonly known as 'Green oil charity', is used as remedy for wounds. The chemically active principal 'Marsiline' isolated from *Marsilea* is found to be very effective against sedative and anti-convulsant principal.

The rhizome and frond bases of *Dryopteris* have been used to determine the origin and pathways of dispersed pathogenic insects like corn ear- worm. The preparation of Ophioglossum vulga-tum as 'Green oil charity' is also used as remedy for wounds.

iv. Pteridophytes Used as Horticultural Plants:

Many species of pteridophytes are cultivated for their aesthetic value. Many variants and cultivars of Psilotum have been brought in culti-vation in nurseries and greenhouses in the nick-name of 'whisk fern'.

Some epiphytic species of Lycopodium (e.g., *L. phlegmaria, L. lucidulum*) are aesthetically more valued and can be grown on hanging baskets.

Several species of Selaginella are used as a ground cover in an undisturbed area because of their decent foliage and colour. *Selaginella willdenovii, S. uncinata*, etc., are grown in gar-dens for their decent blue colour. 5. lepidophyl-la, S. bryopteris, etc., are sold as dried under the name 'resurrection plants' which rejuvenate on contact with water.

Several ferns such as *Angiopteris, Asplenium, Marattia, Microsorium, Nephrolepis, Phymatodes*, etc., have aesthetic values for their beautiful habit, graceful shape of the leaves, and beautiful soral arrangement. Thus, these characte-ristics make them horticulturally important plants.

v. Pteridophytes Used as Biofertiliser:

Azolla is a free-floating water fern which can multiply very quickly through vegetative propagation. There are hundreds of moss-like leaves harbouring live colonies of dinitrogen fixer Cyanobacterium — *Anabaena azollae*.

The relationship between the alga and Azolla is sym-biotic where the alga provides nitrogen to the plant. Thus, Azolla in full bloom in the water-logged rice fields may serve as a green manure. Rice farmers of our country are using Azolla as biofertiliser for the better production of their crops.

vi. Pteridophytes Used as Indicator Plants:

Like angiosperms, pteridophytes are being used as indicator plants.

Equisetum accumulates minerals, especially gold, in their stem. The rate of accumulation even reaches up to 4.5 ounce per ton. Equi-setum may be referred to as gold indicator plants which help in searching a region for gold ore deposits. Similarly, Asplenium adulterinum is an indicator of nickel and Actinopteris australis is a cobalt indicator plant. Thus, these plants are found to be valuable in prospecting for new ore deposits.

vii. Pteridophytes Used for Various Purposes:

There are various applications of pterido-phytes:

The stem of *Equisetum* was used for polishing wood in ancient times and to clean utensils.

The roots and stems of *Osmunda* are used to make beds for growing orchids. Water boiled with *Lycopodium clavatum* is used for dyeing the woollen clothes which becomes blue when dipped in a bath of Brazil wood.

The powder of *Lycopodium* is highly inflammable and is used in pyrotechny and for artificial lighting. Thus, *Lycopodium* powder finds its wide use in demonstration of artificial lighting on the stage, because it disperses easily in the air and only a small quantity is needed to produce an explosion.

Some of the pteridophyte members are con-sidered to be the obnoxious weeds. *Pteridium aquilinum* is a carcinogenic plant which can rapidly invade the open forest lands, thus elimi-nating the other plants of the forest floor. The free-floating water fern, Salvinia, quickly propa-gates vegetatively, and thus occupy the entire water surface of lakes, ponds and irrigation reser-voirs preventing free flow of water.

Gymnosperms:

The term gymnosperms (gymnos = naked; sperma = seed) was introduced by Theophrastus in 300 BC to describe plants with unprotected seeds. According to Goebal, gymnosperms are phanerogams without ovary.

The phanerogams or Spermatophyta (sperm = seed; phyton = plant) or seed plants are those plants which reproduce by means of seeds, not spores. Gymnosperms are the vascular plants where seeds are not enclosed within an ovary (opposite to an angiosperm or flowering plants where seeds are enclosed by mature ovaries or fruits).

In these plants the ovules are borne naked or the surface of the megasporophylls, which are often arranged in the cones. Fossil records indicate that the gymnosperms must have evolved approximately 300 million years ago from non-seed producing ancestors of the extinct division of Progymnospermophyta which were fern like in appearance (form a bridge between pteridophytes and angiosperms).

Gymnosperms were dominant plants over the earth's surface during the jurassic and cretaceous periods of mesozoic era. At present about 83 genera and approximately 790 species of living gymnosperms are distributed throughout temperate, tropical and arctic regions of the world.

External Features of Gymnosperms:

1. Gymnosperms are predominantly woody plants, represented by trees, shrubs or rarely climbers.
2. They are usually xerophytic, some of them are deciduous while others are evergreen. *Sequoia sempervirens* (California or Coast red wood) is probably the tallest living tree reaching a height nearly 112 m and attaining a growth of 15 m. Smallest gymnosperm is *Zamia pygmaea*.

It is 25 cm tall. *Taxodium maxicanum* has a trunk with the enormous diameter of 17 meter. The bristlecone pines (three species of pines i.e., *P. aristata, P. longaeva* and *P. balfouriana*) are thought to

reach an age greater than that of any other single organism known, upto nearly 5000 years.

3. Plant body is sporophytic and can be differentiated into root, stem and leaves.

4. Generally the plants possess well developed tap root system. In some gymnosperms the roots show symbiotic relationship e.g., coralloid roots of *Cycas* with algae and mycorrhizal roots of *Pinus* with fungi.

5. Stem is erect, woody and branched (unbranched in *Cycas* and tuberous in *Zamia*). Presence of leaf scars on the stem is the characteristic feature of gymnosperms.

6. The arrangement of the leaves on the stem may be spiral or cyclic. They may be of one kind (monomorphic) or two kinds (dimorphic, foliage leaves and scale leaves). Foliage leaves are green, simple, may be small (microphyllous e.g., *Pinus*) or large (megaphyllous e.g., *Cycas*). Their main function is photosynthesis. Scale leaves are present around the reproductive structures and apex. They are mainly protective in nature.

7.

Classification of Gymnosperms:

In older times gymnosperms were kept among angiosperms. It was Robert Brown (1827) who first of all recognised these plants due to presence of naked ovules and placed them in a distinct group called gymnosperms. Bentham and Hooker (1862-83) in their 'Genera Planterum' placed this group in between dicotyledonae and monocotyledonae.

The classification of gymnosperms is quite controversial because several genera and a few orders like the cordiatales and cycadeoidales are known only in fossil state. Attempts have, however, been made from time to time to classify them. Some of the important classifications are as follows:

Van Tieghem (1898) treated gymnosperms as one of the two divisions of Spermatophyta and further divided it as follows:

Classification Proposed by Sporne (1974):

Sporne (1974) adopted classification of Pilger and Melchior (1954) and recognized following three classes and nine orders:

1. Cycadopsida: Pteridospermales, Bennettitales, Pentoxylales and Cycadales
2. Coniferopsida: Cordaitales, Coniferales, Taxales and Ginkgoales
3. Gnetopsida: Gnetales

Cycadales:

(i) Cycadales include 11 living genera and more than 100 species of cycads.

(ii) Cycads resembles with the pteridophytes.

(iii) The members are woody sporophytes which appear palm-like.

(iv) All are dioecious,

(v) Young leaves show circinate vernation.

Examples: *Cycas, Microcycas, Zamia pygmaea* (smallest gymnosperm), *Chigua, Stangeria* etc.

Ginkgoales:

(i) Ginkgoales include only one living member i.e. *Ginkgo biloba* (Maiden hair tree) and is the oldest living seed plants persisted with little change till now.

(ii) Leaves are leathery, fan- shaped and deciduous

(iii) Male strobili catkin-like,

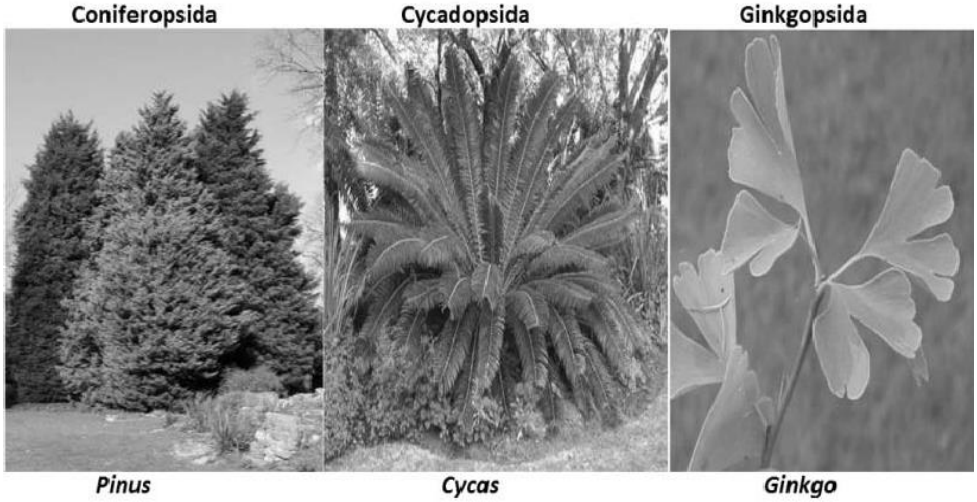
(iv) Wood pycnoxylic.

(v) Ovules arise in groups (2-10).

(vi) Endosperm has beak like protuberance called tent pole.

Coniferales:

(i) It is the largest order of living gymnosperm.



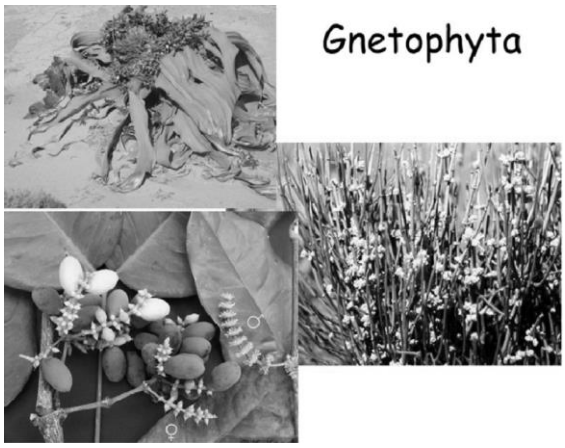
(ii) All members are perennial with ex-current (conical) appearance,

(iii) Leaves dimorphic i.e. foliage andscale leaves present,

(iv) Wood is pycnoxylic.

(v) Pollens and seeds are winged,

(vi) They are dominant forest- makers in the colder region of earth due to xerophytic nature, evergreen nature, presence of mycorrhizas, scale leaves, resins to plug injury, enzymes activity even at – 35° C.



Examples:

Pinus, Sequoiea (Red wood tree, the largest gymnosperm, 366 ft.), *Taxodium, Juniperus, Saxegothea, Araucaria* (commonly called Puzzle)

Gnetales:

(i) A small but highly evolved group of gymnosperms represented by 3 genera i.e. *Ephedra, Gnetum* and *Welwitschia*.

(ii) They are the ancestors of Angiosperms (flowering plants),

(iii) Unlike other members, gnetales have vessels in xylem.

(iv) Flowers arranged in compound strobili or inflorescence,

(v) Embryo with 2 Cotyledons.

Economic Importance of Gymnosperms:

1. Ornamental value:

A number of gymnosperms are grown as ornamental plants, e.g., *Cycas, Araucaria, Thuja* etc.

2. Food Value:

i. 'Sago' starch obtained from pith and cortex of stem of *C. revolute, C. rumphi* etc.

ii. 'Seed starch' obtained from seeds of *Cycas rumphii*, *Dioon edule* etc. It is prepared into flour and cooked before eating.

iii. Seeds of *Pinus gerardiana* (chilgoza) are edible.

iv. 'Kaffir bread' prepared from the stem pith of Encephalartos.

v. Young leaves of Cycas cooked as vegetables.

3. Medicinal value:

i. Ephedrine (alkaloid) extracted from *Ephedra* used in treating asthma, cough, cold, bronchitis etc.

ii. Tincture of *Ephedra* is a cardiac stimulant.

iii. The juice extracted from young leaves of *Cycas revoluta* is used for curing blood vomiting and flatulence.

4. Industrial Use:

i. **Gum**-*Cycas* gum used as adhesive, antidote for snake bites and using malignant ulcers.

ii. **Tannins** – Tannins extracted from bark of *Araucaria, Pinus, Sequoia* etc. used in leather industry.

iii. **Canada balsam** – It is turpentine obtained from *Abies balsamea* and used as a mounting medium in biological preparations.

iv. **Amber (fossil resin)** – obtained from *Pinus succinifera*. Wood of *Pinus* is used for doors, poles, beams, railway wagon flooring etc.

v. Plywood prepared from *Podocarpus*.

vi. Papers like newsprints, writing and printing papers are being prepared from the wood pulp of *Pinus, Picea,Abies, Gnetum* etc.

vii. The leaves of cycads are used for preparing baskets, mats, hats, brooms etc.

viii. The fibres obtained from the leaves of *Cycas* and Macrozamia are used for stuffing pillows and making mattresses.

5. Source of oils:

i. Oils extracted from seeds of *C. revoluta, Macrozamia reidlei, Pinus cembra* and *Cephalotaxus drupacea* are used as edible oils.

ii. Red cedar wood oil extracted from the heart wood of *Juniperus virginiana* is used for cleaning microscopic preparations and for oil immersion lenses.

iii. Oils obtained from *Cedrus deodara, Ciyptomeria japonica* and *Cupressus serm-perivirens* are used in preparations of perfumes.

7. Plant and Medicine:

Introduction, source and medicinal uses of the following plant-derived pharmaceutical compounds - (artemisinin, aspirin, atropine, campothecin, cannabadiol, ephedrine, digoxin, diosgenin, galanthamine, L-dopa, morphine, codeine, quinine, colchicine, vincristine, vinblastine, podophyllotoxin, taxol); Importance of phytopharmaceuticals; Classification of plant secondary metabolites; Exploration of secondary metabolites in therapeutics.

Introduction

The term "**medicinal plant**" includes various types of plants used in herbalism ("herbology" or "herbal medicine"). It is the use of plants for medicinal purposes, and the study of such uses.

The word "**herb**" has been derived from the Latin word, *"**herba**"* and an old French word *"**herbe**"*. Nowadays, herb refers to any part of the plant like fruit, seed, stem, bark, flower, leaf, stigma or a root, as well as a non-woody plant. Earlier, the term "herb" was only applied to non-woody plants, including those that come from trees and shrubs. These medicinal plants are also used as food, flavonoids, medicine or perfume and also in certain spiritual activities.

Plants have been used for medicinal purposes long before prehistoric period. Ancient Unani manuscripts Egyptian papyrus and Chinese writings described the use of herbs. Evidence exist that Unani Hakims, Indian Vaids and European and Mediterranean cultures were using herbs for over 4000 years as medicine. Indigenous cultures such as Rome, Egypt, Iran, Africa and America used herbs in their healing rituals, while other developed traditional medical systems such as Unani, Ayurveda and Chinese Medicine in which herbal therapies were used systematically.

Traditional systems of medicine continue to be widely practised on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments.

Among ancient civilisations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. About 8,000 herbal remedies have been codified in AYUSH systems in INDIA. Ayurveda, Unani, Siddha and Folk (tribal) medicines are the major systems of indigenous medicines. Among these systems, Ayurveda and Unani Medicine are most developed and widely practised in India.

Recently, WHO (World Health Organization) estimated that 80 percent of people worldwide rely on herbal medicines for some aspect of their primary health care needs. **According to WHO, around 21,000 plant species have the potential for being used as medicinal plants.**

As per data available over three-quarters of the world population relies mainly on plants and plant extracts for their health care needs. More than 30% of the entire plant species, at one time or other was used for medicinal purposes. It has been estimated, that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as India and China, the contribution is as much as 80%. Thus, the economic importance of medicinal plants is much more to countries such as India than to rest of the world. These countries provide two third of the plants used in modern system of medicine and the health care system of rural population depend on indigenous systems of medicine.

Treatment with medicinal plants is considered very safe as there is no or minimal side effects. These remedies are in sync with nature, which is the biggest advantage. The golden fact is that, use of herbal treatments is independent of any age groups and the sexes.

The ancient scholars only believed that herbs are only solutions to cure a number of health related problems and diseases. They conducted thorough study about the same, experimented to arrive at accurate conclusions about the efficacy of different herbs that have medicinal value. Most of the drugs, thus formulated, are free of side effects or reactions. This is the reason why herbal treatment is growing in popularity across the globe. These herbs that have medicinal quality provide rational means for the treatment of many internal diseases, which are otherwise considered difficult to cure.

Medicinal plants such as ***Aloe***, ***Tulsi***, ***Neem***, ***Turmeric*** and ***Ginger*** cure several common ailments. These are considered as home remedies in many parts of the country. It is known fact that lots of consumers are using Basil (*Tulsi*) for making medicines, black tea and other activities in their day to day life.

In several parts of the world many herbs are used to honor their kings showing it as a symbol of luck. Now, after finding the role of herbs in medicine, lots of consumers started the plantation of tulsi and other medicinal plants in their home gardens.

Medicinal plants are considered as rich resources of ingredients which can be used in drug development either pharmacopoeial, non-pharmacopoeial or synthetic drugs. A part from that, these plants play a critical role in the development of human cultures around the whole world. Moreover, some plants are considered as important source of nutrition and as a result of that they are recommended for their therapeutic values. Some of these plants include ginger, green tea, walnuts, aloe, pepper and turmeric etc. Some plants and their derivatives are considered as important source for active ingredients which are used in aspirin and toothpaste etc.

Apart from the medicinal uses, herbs are also used in natural dye, pest control, food, perfume, tea and so on. In many countries different kinds of medicinal plants/ herbs are used to keep ants, flies, mice and flee away from homes and offices. Nowadays medicinal herbs are important sources for pharmaceutical manufacturing.

Recipes for the treatment of common ailments such as diarrhoea, constipation, hypertension, low sperm count, dysentery and weak penile erection, piles, coated tongue, menstrual disorders, bronchial asthma, leucorrhoea and fevers are given by the traditional medicine practitioners very effectively.

Over the past two decades, there has been a tremendous increase in the use of herbal medicine; however, there is still a significant lack of research data in this field. **Therefore since 1999, WHO has published three volumes of the WHO monographs on selected medicinal plants.**

Sources and Use of some plant-derived pharmaceutical products

Artemisinin ($C_{15}H_{22}O_5$)

Source- *Artemisia annua*

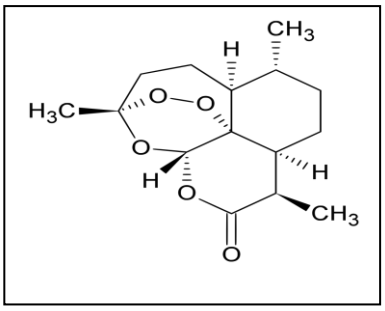
Uses:

1. The World Health Organization (WHO) recommends artemisinin or one of its derivatives — typically in combination with a longer-lasting partner drug — as frontline therapy for all cases of malaria.

2. For uncomplicated malaria, the WHO recommends three days of oral treatment with any of five artemisinin-based combination therapies (ACTs): artemether/lumefantrine, artesunate/amodiaquine (ASAQ), artesunate/mefloquine, dihydroartemisinin/piperaquine, or artesunate/sulfadoxine/pyrimethamine. In each of these combinations, the artemisinin derivative rapidly kills the parasites, but is itself rapidly cleared from the body. The longer-lived partner drug kills the remaining parasites and provides some lingering protection from reinfection.

3. For severe malaria, the WHO recommends intravenous or intramuscular treatment with the artemisinin derivative artesunate for at least 24 hours.

4. Artemisinins are not used for malaria prevention because of the extremely short activity (half-life) of the drug. To be effective, it would have to be administered multiple times each day.



Aspirin ($C_9H_8O_4$)

Source- Bark of *Salix alba* (Willow tree)

Uses:

1. Aspirin is used in the treatment of a number of conditions, including fever, pain, rheumatic fever, and inflammatory conditions, such as rheumatoid arthritis, pericarditis, and Kawasaki disease.

2. Lower doses of aspirin have also been shown to reduce the risk of death from a heart attack, or the risk of stroke in people who are at high risk or who have cardiovascular disease, but not in elderly people who are otherwise healthy.

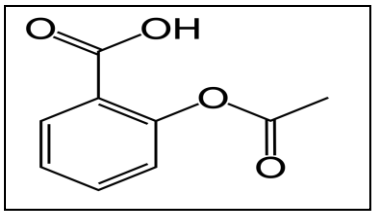
3. There is some evidence that aspirin is effective at preventing colorectal cancer, though the mechanisms of this effect are unclear.

4. In the United States, the selective initiation of low-dose aspirin, based on an individualised assessment, has been deemed reasonable for the primary prevention of cardiovascular disease in people aged between 40 and 59 who have a 10% or greater risk of developing cardiovascular disease over the next 10 years and are not at an increased risk of bleeding.

5. Aspirin is a first-line treatment for the fever and joint-pain symptoms of acute rheumatic fever.

6. Along with rheumatic fever, Kawasaki disease remains one of the few indications for aspirin use in children in spite of a lack of high quality evidence for its effectiveness.

7. Low-dose aspirin supplementation has moderate benefits when used for prevention of pre-eclampsia. This benefit is greater when started in early pregnancy.



Atropine ($C_{17}H_{23}NO_3$)

Source- *Datura stramonium* (Jimson weed)

Uses:

1. Topical atropine is used as a cycloplegic, to temporarily paralyze the accommodation reflex, and as a mydriatic, to dilate the pupils.

2. Injections of atropine are used in the treatment of symptomatic or unstable bradycardia.

3. Atropine was previously included in international resuscitation guidelines for use in cardiac arrest associated with asystole.

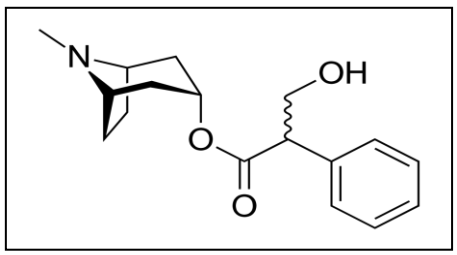
4. Atropine is also useful in treating second-degree heart block Mobitz type 1 (Wenckebach block), and also third-degree heart block with a high Purkinje or AV-nodal escape rhythm. It is usually not effective in second-degree heart block Mobitz type 2, and in third-degree heart block with a low Purkinje or ventricular escape rhythm.

5. Atropine has also been used in an effort to prevent a low heart rate during intubation of children; however, evidence does not support this use.

6. Atropine's actions on the parasympathetic nervous system inhibit salivary and mucus glands. The drug may also inhibit sweating via the sympathetic nervous system. This can be useful in treating hyperhidrosis, and can prevent the death rattle of dying patients.

7. Atropine or diphenhydramine can be used to treat muscarine intoxication.

8. Atropine has been observed to prevent or treat irinotecan induced acute diarrhea.

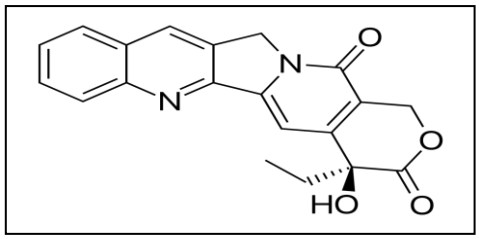


Campothecin ($C_{20}H_{16}N_2O_4$)

Source- Bark and stem of *Camptotheca acuminata (*Happy tree)

Uses:

1. It has been used clinically more recently in China for the treatment of gastrointestinal tumors.
2. It is used in cancer today, topotecan, irinotecan, belotecan, and trastuzumab deruxtecan.
3. Camptothecin, an quinolone alkaloid, is used as a chemotherapeutic agent in the treatment of leukemia.

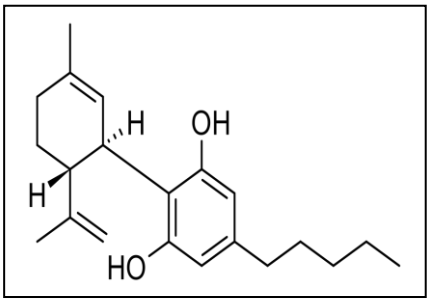


Cannabidiol ($C_{21}H_{30}O_2$)

Source- *Cannabis sativa*

Uses:

1. In the European Union, cannabidiol (Epidyolex) is indicated for use as adjunctive therapy of seizures associated with Lennox Gastaut syndrome (LGS) or Dravet syndrome (DS), in conjunction with clobazam, for people two years of age and older.
2. It is used for seizure disorder (epilepsy).
3. It is also used for anxiety, pain, a muscle disorder called dystonia, Parkinson disease, Crohn disease, and many other conditions, but there is no good scientific evidence to support these uses.



Ephedrine ($C_{10}H_{15}NO$)

Source- *Ephedra*

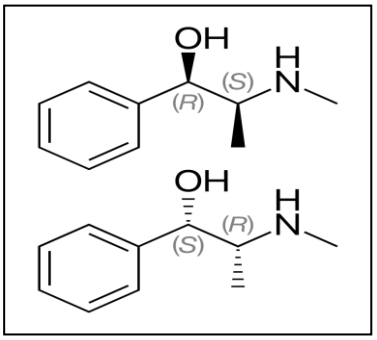
Uses:

1. Both ephedrine and pseudoephedrine increase blood pressure and act as bronchodilators, with pseudoephedrine having considerably less effect.

2. Ephedrine may decrease motion sickness, but it has mainly been used to decrease the sedating effects of other medications used for motion sickness.

3. Ephedrine is also found to have quick and long-lasting responsiveness in congenital myasthenic syndrome in early childhood and also even in the adults with a novel COLQ mutation.

4. Ephedrine also decreases gastric emptying. Methylxanthines such as caffeine and theophylline have a synergistic effect with ephedrine with respect to weight loss.



Digoxin ($C_{41}H_{64}O_{14}$)

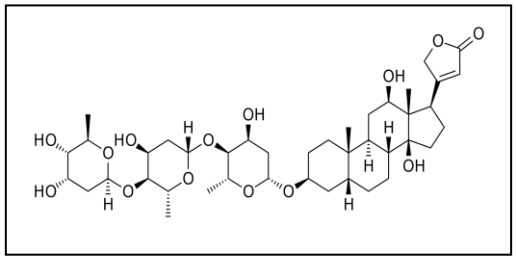
Source- *Digitalis lanata*

Uses:

1. The most common indications for digoxin are atrial fibrillation and atrial flutter with rapid ventricular response, though beta blockers and/or calcium channel blockers may be preferred in some patients, such as those without heart failure or hemodynamic instability.

2. Currently, the recommendation for heart failure is a triple therapy of ACE inhibitor, beta blocker and mineralocorticoid antagonists. Digoxin is a third-line therapy.

3. Digoxin is also used intrafetally or amniotically during abortions in the late second trimester and third trimester of pregnancy. It typically causes fetal demise (measured by cessation of cardiac activity) within hours of administration.

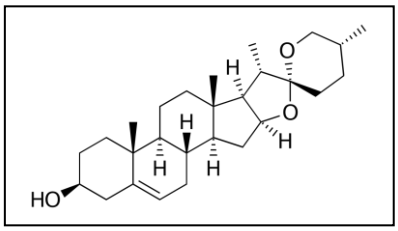


Diosgenin ($C_{27}H_{42}O_3$)

Source- Stem of *Dioscorea*

Uses:

1. Diosgenin or wild yam is often promoted as a “natural alterative” to estrogen therapy.
2. It is also used in vaginal dryness in older women, PMS (premenstrual syndrome), menstrual cramps, weak bones (osteoporosis).
3. It is used severely for increasing energy and sexual drive in men and women.
4. It is used in breast enlargement.

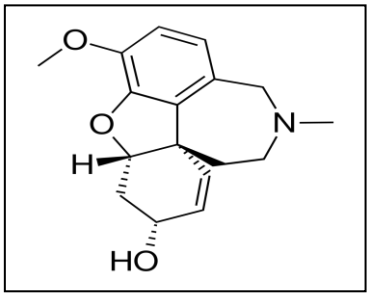


Galanthamine($C_{17}H_{21}NO_3$)

Source- Flower of *Galanthus nivalis* (Common snowdrop), *Galanthus caucasicus* (Caucasian snowdrop), *Galanthus woronowii* (Voronov's snowdrop)

Uses:

1. Galantamine is indicated for the treatment of mild to moderate vascular dementia.
2. In the United States, it is approved by the Food and Drug Administration as safe and effective for the treatment of mild to moderate dementia.
3. As with other cholinesterase inhibitors, galantamine may not be effective for treating mild cognitive impairment.
4. Galantamine is used to treat the symptoms of Alzheimer's disease (AD; a brain disease that slowly destroys the memory and the ability to think, learn, communicate and handle daily activities). Galantamine is in a class of medications called acetylcholinesterase inhibitors.



L-Dopa ($C_9H_{11}NO_4$)

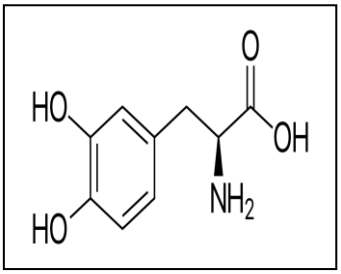
Source- *Mucuna pruriens* (velvet bean), and *Vicia faba* (broad bean)

Uses

1. L-DOPA is used to increase dopamine concentrations in the treatment of Parkinson's disease, Parkinsonism,

dopamine-responsive dystonia and Parkinson-plus syndrome.

2. It is efficacious for the short term treatment of restless leg syndrome.



Morphine ($C_{17}H_{19}NO_3$)

Source- *Papaver somniferum*

Uses

1. Morphine is used primarily to treat both acute and chronic severe pain. Its duration of analgesia is about three to seven hours.

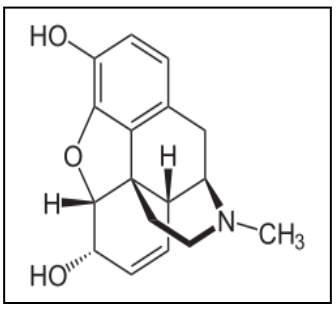
2. It is used for pain due to myocardial infarction and for labor pains. However, concerns exist that morphine may increase mortality in the event of non ST elevation myocardial infarction.

3. Morphine has also traditionally been used in the treatment of acute pulmonary edema.

4. Morphine is effective in relieving cancer pain.

5. Morphine is beneficial in reducing the symptom of shortness of breath due to both cancer and noncancer causes.

6. Morphine is also available as a slow-release formulation for opiate substitution therapy (OST) in Austria, Germany, Bulgaria, Slovenia, and Canada for persons with opioid addiction who cannot tolerate either methadone or buprenorphine.



Codeine ($C_{18}H_{21}NO_3$)

Source- Sap of *Papaver somniferum*

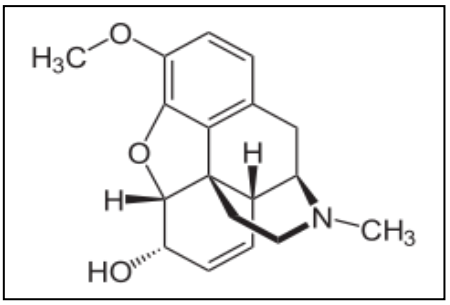
Uses:

1. Codeine is used to treat mild to moderate pain. It is commonly used to treat post-surgical dental pain.

2. Weak evidence indicates that it is useful in cancer pain, but it may have increased adverse effects, especially constipation, compared to other opioids. The American Academy of Pediatrics does not recommend its use in children due to side effects.

3. Codeine is used to relieve coughing. In Europe, it is not recommended as a cough medicine in those under 12 years of age. Some tentative evidence shows it can reduce a chronic cough in adults.

4. It is used to treat diarrhea and diarrhea-predominant irritable bowel syndrome, although loperamide (which is available without a prescription for milder diarrhea), diphenoxylate, paregoric, or even laudanum are more frequently used to treat severe diarrhea.



Quinine ($C_{20}H_{24}N_2O_2$)

Source- Bark of Cinchona

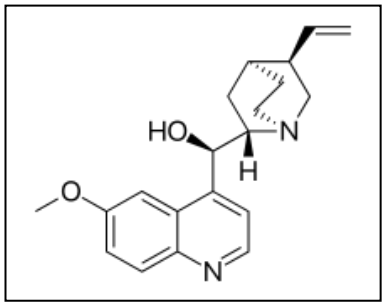
Uses:

1. Quinine is no longer recommended by the World Health Organization (WHO) as a first-line treatment for malaria, because there are other substances that are equally effective with fewer side effects. They recommend that it be used only when artemisinins are not available.

2. Quinine is also used to treat lupus and arthritis.

3. Quinine was frequently prescribed as an off-label treatment for leg cramps at night.

4. Quinine can also act as a competitive inhibitor of monoamine oxidase (MAO), an enzyme that removes neurotransmitters from the brain.



Colchicine ($C_{22}H_{25}NO_6$)

Source- *Colchicum autumnale*

Uses:

1. Colchicine is a medicine for treating inflammation and pain.

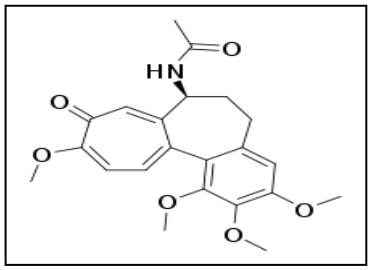
2. Colchicine is also used as an anti-inflammatory agent for long-term treatment of Behçet's disease.

3. Colchicine is effective for prevention of atrial fibrillation after cardiac surgery

4. Long-term (prophylactic) regimens of oral colchicine are absolutely contraindicated in people with

advanced kidney failure (including those on dialysis).

5. It is also used in the treatment of familial Mediterranean fever, in which it reduces attacks and the long-term risk of amyloidosis.



Vincristine ($C_{46}H_{56}N_4O_{10}$)

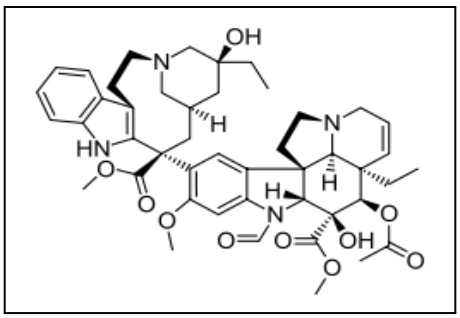
Source- *Catharanthus roseus*

Uses:

1. Vincristine is delivered via intravenous infusion for use in various types of chemotherapy regimens. Its main uses are in non-Hodgkin's lymphoma as part of the chemotherapy regimen CHOP, Hodgkin's lymphoma as part of MOPP, COPP, BEACOPP, or the less popular Stanford V chemotherapy regimen in acute lymphoblastic leukemia (ALL).

2. It is used for nephroblastoma.

3. It is also used to induce remission in ALL with dexamethasone and L-Asparaginase, and in combination with prednisone to treat childhood leukemia. Vincristine is occasionally used as an immunosuppressant, for example, in treating thrombotic thrombocytopenic purpura (TTP) or chronic idiopathic thrombocytopenic purpura (ITP).



Vinblastine ($C_{46}H_{58}N_4O_9$)

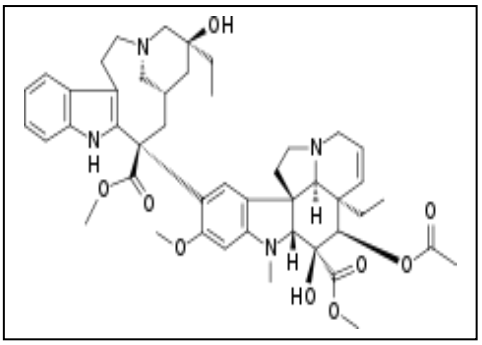
Source- *Catharanthus roseus*

Uses:

1. Vinblastine is a component of a number of chemotherapy regimens.

2. It is used in Hodgkin lymphoma.

3. It is also used to treat histiocytosis according to the established protocols of the Histiocytosis Association.




Podophyllotoxin ($C_{22}H_{22}O_8$)

Source- rhizomes of *Podophyllum*

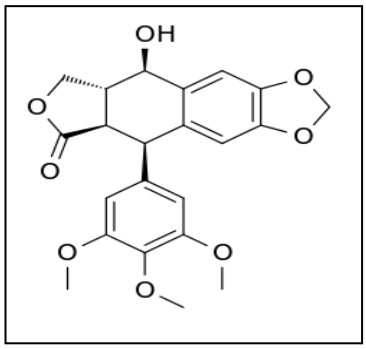
Uses:

1. Podophyllotoxin possesses a large number of medical applications, as it is able to stop replication of both cellular and viral DNA by binding necessary enzymes.

2.Podophyllotoxin and its derivatives are used as cathartic, purgative, antiviral agent, vesicant, antihelminthic, and antitumor agents.

3. Podophyllotoxin derived antitumor agents include etoposide and teniposide. These drugs have been successfully used in therapy against numerous cancers including testicular, breast, pancreatic, lung, stomach, and ovarian cancers.

4. Podophyllotoxin cream is commonly prescribed as a potent topical antiviral.




Taxol ($C_{47}H_{51}NO_{14}$)

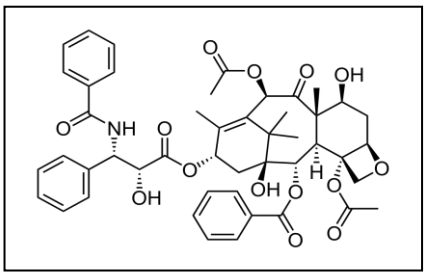
Source- *Taxus brevifolia*

Uses:

1. Taxol is approved in the UK for ovarian, breast, lung, bladder, prostate, melanoma, esophageal, and other types of solid tumor cancers as well as Kaposi's sarcoma.

2. It is used for non-small-cell lung cancer in patients unsuitable for curative treatment, and in first-line and second-line treatment of ovarian cancer.

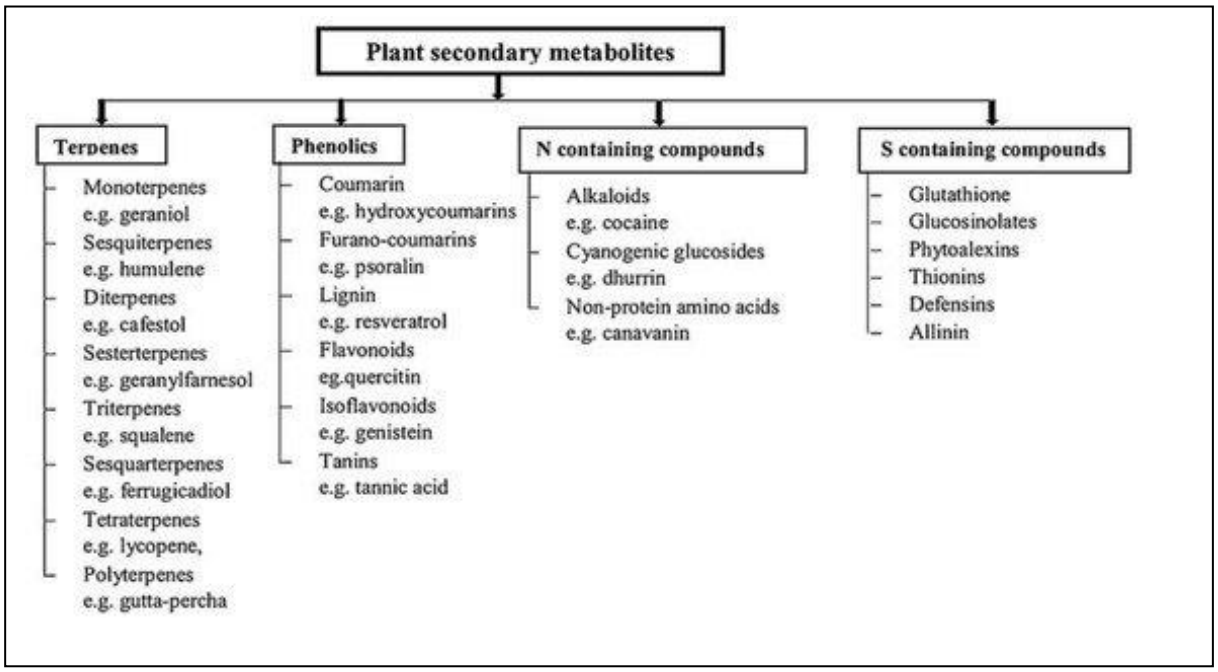
3. It is approved in the United States for the treatment of breast, pancreatic, ovarian, Kaposi's sarcoma and non-small-cell lung cancers.



Importance of Phytopharmaceuticals

Phytopharmaceutical drug is defined as purified and standardized fraction with defined minimum four bio-active or phytochemical compounds (qualitatively and quantitatively assessed) of an extract of a medicinal plant or its part, for internal or external use of human beings or animals for diagnosis, treatment. Many of these substances have anti-inflammatory, anti-allergic, antioxidant, antimicrobial, antispasmodic, anti-ageing, antidiabetic, and many other therapeutic effects. Phytopharmaceuticals can be added to or enhanced in traditional foods through genetic engineering.

Classification of plant secondary metabolites



Exploration of plant secondary metabolites in therapeutics

Plants are an essential source for discovering novel medical compounds for drug development, and secondary metabolites are sources of medicines from plants. Secondary metabolites include alkaloids, flavonoids, terpenoids, tannins, coumarins, quinones, carotenoids, and steroids. Each year, several new secondary metabolites are extracted from plants, providing a source of possibilities to investigate against malignant

illnesses, despite certain natural chemicals having distinct anticancer activities according to their physicochemical features. Secondary metabolites found in plants are frequently great leads for therapeutic development. However, changes in the molecular structure of these compounds are improving their anticancer activity and selectivity and their absorption, distribution, metabolism, and excretion capacities while minimizing their toxicity and side effects. In this section, we will discuss the most significant breakthroughs in the field of plant secondary metabolites, some of which are currently in clinical use and others that are in clinical trials as anticancer drugs. This study gives an up-to-date and thorough summary of secondary plant metabolites and their antioxidant, antibacterial, and anticancer effects. Furthermore, antioxidant and antibacterial, and anticancer effects of secondary metabolites are addressed. As a result, this article will serve as a thorough, quick reference for people interested in secondary metabolite antioxidants, anticancer, and antibacterial properties. Plants are essential in pharmacological research and drug development, not only when bioactive substances are used as therapeutic agents directly, but also as starting materials for drug production or as models for pharmacologically active molecules. Secondary metabolites differ depending on the plant species. Secondary metabolites are molecules produced by plants that remain unknown in their roles in growth, photosynthesis, reproduction, and other primary processes. Secondary compounds are widely employed in plants, primarily in Asia. Secondary metabolites boost human immunity because pharmaceuticals are mainly based on plant components. Secondary compounds in plants can serve as medicinal for humans. Several criteria have been considered to classify secondary metabolites, including chemical structure, composition, solubility, and biosynthetic pathway.

8. Plant Tissue Culture: Concept ofcellular totipotency; culture media; organogenesis; somatic embryogenesis; haploid plant production and micropropagation.

Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition. It is widely used to produce clones of a plant in a method known as micropropagation. Different techniques in plant tissue culture may offer certain advantages over traditional methods of propagation, including:

- The production of exact copies of plants that produce particularly good flowers, fruits, or have other desirable traits.
- To quickly produce mature plants.
- The production of multiples of plants in the absence of seeds or necessary pollinators to produce seeds.
- The regeneration of whole plants from plant cells that have been genetically modified.

- The production of plants in sterile containers that allows them to be moved with greatly reduced chances of transmitting diseases, pests, and pathogens.
- The production of plants from seeds that otherwise have very low chances of germinating and growing, i.e. orchids and *Nepenthes*.
- To clean particular plants of viral and other infections and to quickly multiply these plants as 'cleaned stock' for horticulture and agriculture.
- Reproduce recalcitrant plants required for land restoration
- Storage of genetic plant material to safeguard native plant species.

Plant tissue culture relies on the fact that many plant cells have the ability to regenerate a whole plant (Cellular totipotency). Single cells, plant cells without cell walls (protoplasts), pieces of leaves, stems or roots can often be used to generate a new plant on culture media given the required nutrients and plant hormones.

Applications of Plant Tissue culture:

Plant tissue culture is used widely in the plant sciences, forestry, and in horticulture. Applications include:

- The commercial production of plants used as potting, landscape, and florist subjects, which uses meristem and shoot culture to produce large numbers of identical individuals.
- To conserve rare or endangered plant species.
- A plant breeder may use tissue culture to screen cells rather than plants for advantageous characters, e.g. herbicide resistance/tolerance.
- Large-scale growth of plant cells in liquid culture in bioreactors for production of valuable compounds, like plant-derived secondary metabolites and recombinant proteins used as biopharmaceuticals.
- To cross distantly related species by protoplast fusion and regeneration of the novel hybrid.
- To rapidly study the molecular basis for physiological, biochemical, and reproductive mechanisms in plants, for example in vitro selection for stress tolerant plants.
- To cross-pollinate distantly related species and then tissue culture the resulting embryo which would otherwise normally die (Embryo Rescue).
- For chromosome doubling and induction of polyploidy, for example doubled haploids, tetraploids, and other forms of polyploids. This is usually achieved by application of antimitotic agents such as colchicine or oryzalin.
- As a tissue for transformation, followed by either short-term testing of genetic constructs or regeneration of transgenic plants.
- Certain techniques such as meristem tip culture can be used to produce clean plant material from virused stock, such as sugarcane, potatoes and many species of soft fruit.
- Production of identical sterile hybrid species can be obtained.
- Large scale production of artificial seeds through somatic embryogenesis.

Cellular totipotency:

In the preceding units of this course you have read that innumerable cells which constitute the body of a higher plant or animal and containing identical genetic material can be traced to a single cell-the zygote. During development cells undergo diverse structural and functional specialisation depending upon their position in the body. Leaf cells bear chloroplasts and act as the site of photosynthesis. The colourless root hairs perform the function of absorbing nutrients and water from the soil and some other cells become part of the colourful petals. Normally fully differentiated cells do not revert back to a meristematic: state, which suggests that the cells have undergone a permanent change. In earlier sections of this unit you have read that the regenerative capacity is retained by all living cells of a plant. Several horticultural plants regenerate whole plant from root, leafiand stem cuttings. Highly differentiated and mature cells such as those of pith and cortex and highly specialised cells as those of microspores and endosperm, retain full potential to give rise to full plants under suitable culture conditions. G. Haberlandt was the first to test this idea experimentally. This endowment called "cellular totipotency" is unique to plants. Animal cells possibly because of their higher degree of specialisation do not exhibit totipotency. Whole plant regeneration from cultured cells may occur in one of the two pathways: i) shoot bud differentiation, (organogenesis) and ii) embryo formation (Embryogenesis). The Embryos are bipolar structures with no organic connection with the parent tissue and can germinate directly into a complete plant. On the other hand, shoots are monopolar. They need to be removed from the parent tissue and rooted to establish a plantlet. Often the same tissue can be induced to form shoots or embryos by manipulating the components of the culture conditions.

Culture Medium

In nature green plants are capable of synthesising organic compounds necessary for their growth and development from the mineral nutrients and they can absorb water from the soil and obtain C02 from the atmosphere. However, in tissue cultures the normal biosynthetic potentiality of the cells is weakened, therefore, it is necessary to provide all the essential organic and inorganic nutrients (including sucrose) and growth regulators, particularly an auxin and a cytokinin.

Nutritional requirements for optimal growth of tissue cultures may vary with the source (plant). They are also affected by the age of the explant and the stage of development. For example very young embryos require a more elaboratecomponent of the medium as compared to mature embryos. Similarly culture requirements of single cells are more complex than shoots.

Composition: A standard plant tissue culture medium (Basal medium) contains all the essential macroelements (Carbon~hydrogeonx, Oxygen nitrogen, phosphorus, sulphar, calcium,potassium and magnesium) and iron and microelements(iron, manganese, copper, zinc, boron and molybdenum) but the concentration of their salts in different formulations vary. In addition some vitamins and sucrose (2-3%)
are universal constituents of plant tissue culture media.

Composition of Murashige and Skoog 's (MS) basal medium, widely used in plant tissue culture studies (1960)

Besides the nutrients, one or more plant growth regulators (PGR7S) are generally required for supporting good growth of the cultured material. The PGR'S most widely used in tissue culture media are auxins (2,4-D, IAA, NAA, IBA) and cytokinins (BAP, Klnetin). The PGR7s are particularly important for the growth of Callus tissues and organogenicl embryogenic differentiation. Usually the medium is gelled with 0.8% bacteriological agar.

The composition of Ms medium (Murashige and Skoog's) medium found satisfactory for a wide range of monocotyledonous and dicotyledons species.

Major salts (macronutrients) per litre

- Ammonium nitrate (NH_4NO_3) **1650 mg/l**
- Calcium chloride ($CaCl_2 \cdot 2H_2O$) **440 mg/l**
- Magnesium sulfate ($MgSO_4 \cdot 7H_2O$) **370 mg/l**
- Monopotassium phosphate (KH_2PO_4) **170 mg/l**
- Potassium nitrate (KNO_3) **1900 mg/l**.

Minor salts (micronutrients) per litre

- Boric acid (H_3BO_3) **6. 2 mg/l**
- Cobalt chloride ($CoCl_2 \cdot 6H_2O$) **0.025 mg/l**
- Ferrous sulfate ($FeSO_4 \cdot 7H_2O$) **27.8 mg/l**
- Manganese(II) sulfate ($MnSO_4 \cdot 4H_2O$) **22.3 mg/l**
- Potassium iodide (KI) **0.83 mg/l**
- Sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$) **0.25 mg/l**
- Zinc sulfate ($ZnSO_4 \cdot 7H_2O$) **8.6 mg/l**
- Ethylenediaminetetraacetic acid ferric sodium (FeNaEDTA) 36.70 mg/l
- Copper sulfate ($CuSO_4 \cdot 5H_2O$) **0.025 mg/l**

Vitamins and organic compounds per litre

- Myo-Inositol **100 mg/l**
- Nicotinic Acid **0.5 mg/l**
- Pyridoxine · HCl **0.5 mg/l**
- Thiamine · HCl **0.1 mg/l**
- Glycine **2 mg/l**
- Tryptone **1 g/l** (optional)
- Indole Acetic Acid **1-30 mg/l** (optional)

- Kinetin **0.04-10 mg/l** (optional)

Preparation:

Now that you are familiar with the constituents the preparation of medium is quite simple. Several plant tissue culture media are now available commercially in the form of dry powders, containing all ing~edientes xcept growth regulators, sucrose and agar. They are very convenient to prapare media for routine maintenance of cultures. Generally concentrated stock solutions of major inorganic= nutrients (200 times concentrated expressed as 20 x) micro inorganic nutrients (200 x concentrated) iron (200 x concentrated) and organic nutrients except sucrose are prepared and stored in a refrigeratsr at 4" c. Separate stock solutions are prepared and stored for each growth regulator by dissolving it in a minimal quantity of appropriate solvent and adjusting the final volume with distilled water.

A general protocol for media preparation is as follows:

1. Prepare stock solutions one day before the medium is to be made.

2. Weigh the required quantities of agar and sucrose and dissolove them in water (about 314th the final volume of the medium) by heating in a-waterbath or autoclaving at low pressure.

3. Pipette the required volumes of each of the stock solutions into the above solution kept on a stirrer.

4. Make up the final volume of the medium by addition of distilled water.

5. Adjust the pH to 5.8 with 0.1-0.5 N NaoH or Hcl.

Organogenesis

Organogenesis refers to the differentiation of organs such as roots, shoots or flowers. Shoot bud differentiation may occur directly from the explant or from the callus. The stimulus for organogenesis may come from the medium, from the endogenous compounds produced by the cultured tissue or substances carried over from the original explant. Organogenesis is chemically controlled by growth regulators. Skoog while working with tobacco pith callus, observed that the addition of an auxin Indole Acetic Acid (IAA) enhanced formation of roots and suppressed shoot differentiation. He further observed that adenine sulphate, (Cytokinin) reversed the inhibition of auxin and promoted the formation of shoots. You should know that:

1) Organogenesis is contolled by a balance between cytokinin and auxin concentration i.e. it is their relative rather than the absolute concentration that determines the nature of differentiation.

2. A relatively high auxin: Cytokinin ration induces root formation, whereas a high cytokinin: auxin ratio favours shoot bud differentiation.

3. Differential response to exogenously applied growth regulators may be due to differences in the endogenous levels of the hormones within the tissue.

4. Organogenesis is a complex process. Whereas in the cultured tissues of many species organogeiiesis can be demonstrated in this pattern, some plants, notably the monocots, are exceptions. Plant tissues respond

differently to exogenously applied PGR's because of differences in the levels of endogenous Plant Growth Regulators (Fig. 11.5 A,B).

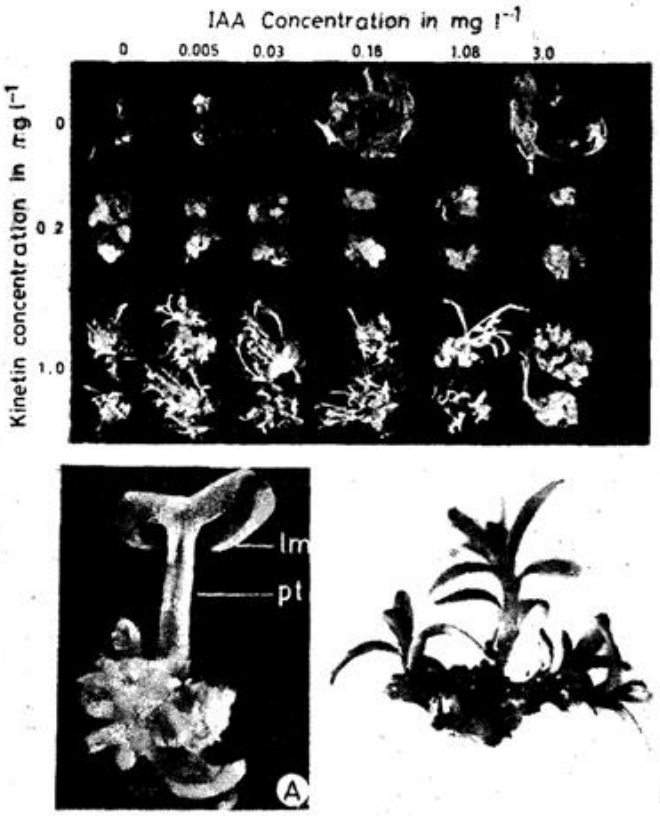


Fig. 11.5: Organogenesis in tobacco callus cultures as affected by IAA and kinetin at different concentrations, individually and in various combinations. Note that root formation has occurred only in the absence of kinetin (and in the presence of 0.18-3.0 mg/l of IAA) (A) and shoot differentiation in the presence of kinetin, particularly with 0.005-0.18 mg/l (B).

Somatic Embryogenesis

The process of embryo development is called embryogenesis. It is not the monopoly of the egg to form an embryo. Any cell of the female gametophyte (Embryo sac) or even of the sporophytic tissues around the embryo sac may give

rise to an embryo. Thus we can say that 'The phenomenon of embryogenesis is not necessarily confined to the reproductive cycle". In this subsection we will discuss – some examples of "embeos formed in culture", also referred to as "somatic -embryos".

The first observation of somatic embryos were made ***Dacus Carota.*** Other plants in which the phenomenon has been studied in some detail are ***Ranunculus scleratus, citrus and coflea spp.***

In ***Rarrunculus scleratus*** somatic as well as various floral tissues, including anthers proliferated to form callus which, after limited unorganised growth differentiated several embryos. These embryos germinated in situ and a fresh crop of embryos appeared on the surface of the seedling. The embryos were derived from

individual epidermal ells of the hypocotyl.(Fig. 11.6) Citrus is commonly cited as an example of natural polyembryony.

In the preceding units of this course (Block I) you have read about polyembryony and parthenocarpy. The nucellus cultures of monoembryonate as well as polyembryonate cultures of citrus could be promoted if malt is added to the basal.

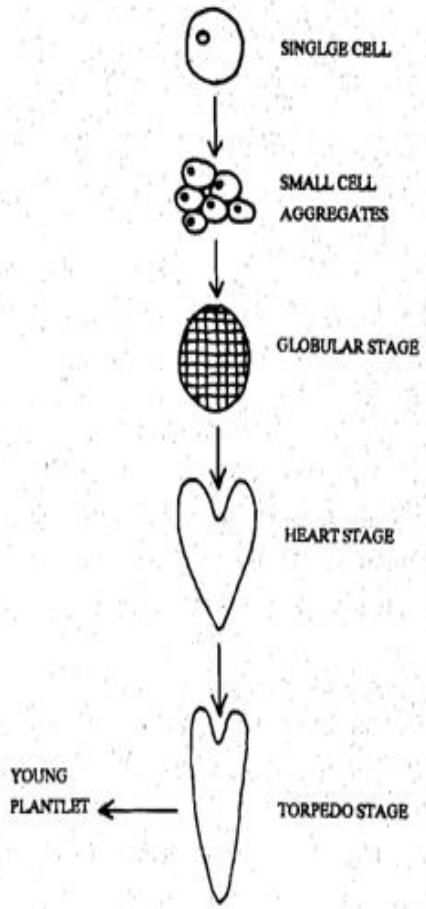


Fig. 11.7: Stages of somatic embryogenesis. Following repeated cell divisions, cell aggregates progressively develop and pass through globular, heart, and torpedo stages before ultimately forming plantlets.

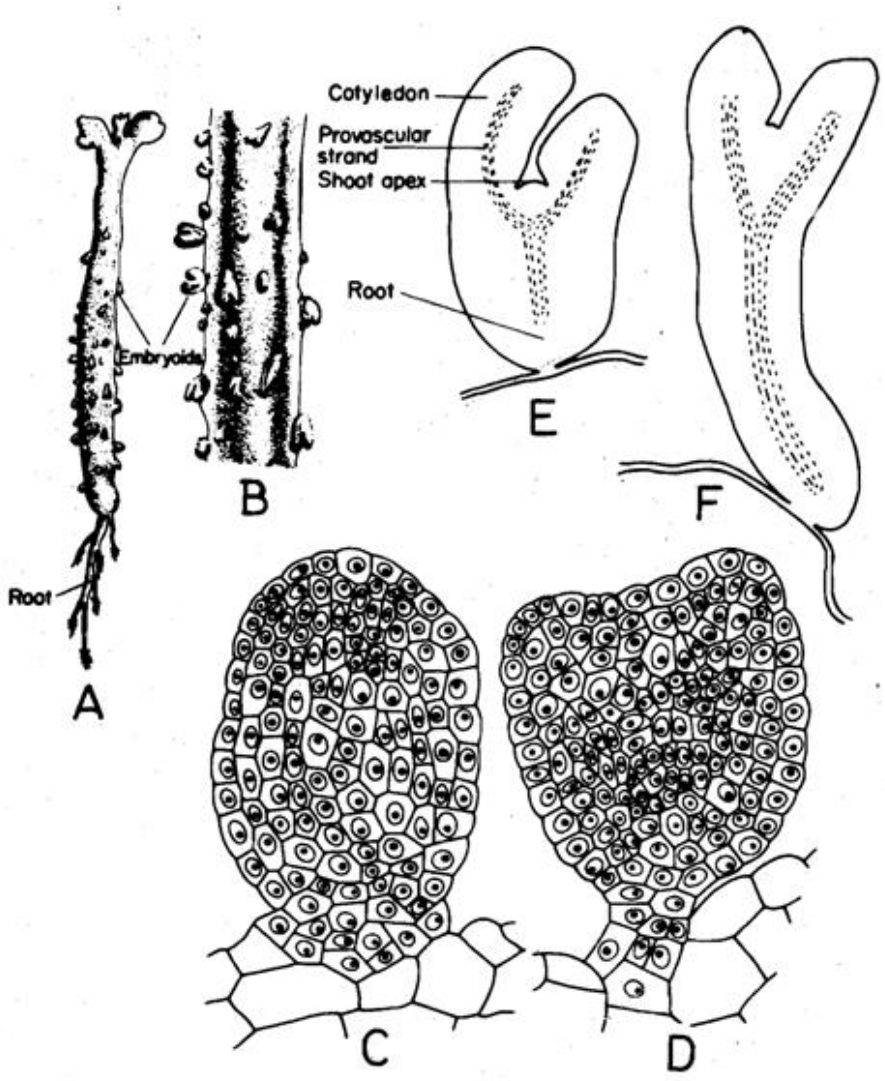


Fig. 11.6: Stages in the differentiation of somatic embryos from stem epidermal cells of Ranunculus. A One-month old seedling bearing embryos all over the stem. B. A portion of the seedling in A enlarged to show several embryos hanging from the surface of the stem. C-F. Sectional views of globular (C), heart shape (D), and cotyledonary (E,F) embryos differentiated from epidermal cells of the stem.

Somatic Hybridization and Cybridization

In the early 1970's an altogether new approach to raise hybrids which could not be produced through the conventional method of hybridization was proposed. It is involves the fusion of somatic cells and regeneration of plants from the fusion products (somatic hybridization).

Plant cells are bounded by a rigid cellulose wall and are cemented together by a pectin-rich matrix to forni tissues. An essential step in fusion of plant cells is to bring together the plasma membrane by degrading the cellulosic wall. Thus, the first step in sonlatic hybridization is tlie isolation of plant protoplasts (spherical naked cells which have bcen stripped off their cell wall). (Fig. 11.8A)

Of the several kinds of materials tested for protoplast isolation mesophyll (leaf Parenchyma) and rapidly fast growing cell cultures have been most useful. Several potent and fairly purified enzymes (e.g., Pectolyase y-

23, Onozuka R-10, Macerozyme R-10, Driesalase) obtained from fungi are available. These can convert plant tissues into a large number of protoplasts. In practice, small pieces of leaves or cells from actively growing cell cultures are iiicubated in a mixture of a cellulase and a niacerozyme, at 30°c, in dark, for 3-12 hr (the duration of treatment varies with the tissue) to digest the cell wall and middle lamella respectively.

The enqme solution also contains a suitable osmoticuni (Sucrose or mannitol) as the freshly isolated protoplasts may burst or shrink. After incubation the protoplast are cleaned by repeated washing in salt solution or culture medium. The protoplasts are directly cultured as single cells or used in fusion experiments. Freshly isolated protoplasts are also useful for genetic transformation as they behave like animal cells. They can rcadily take up macromolecules, such as purified DNA.

The protoplasts are cultured either in liquid medium or on agar plates. The protoplasts readily regenerate a cellulose cell wall, and under suitable culture conditions, the cells undergo divisions to form a totipotent callus. Complete plants have been regenerated from isolated protoplasts of several plant such as rice, cotton, potato, tomato and mustard.

The freshly isolated protoplasts readily fuse (Fig. 11.8 b,c) with each other when brought in intimate contact, irrespective of their taxonomic relationship. Several chemical substances tliat facilitate fusion of protoplasts (fusogens) have been used.

Of these the high niolecular weight (1,500-1,600) polyethylene glycol (PEG) applied in tlie presence of high pH (8-10) and high cat+ has been niost effective. In recent years electrofusion of protoplast as become popular because of the control, efficiency and versatility of this method.

A highly significant application of protoplast fusion is the production of asynimetric hybrids by partlal genome transfer from an irradiated donor protoplast to an acceptor protoplast and the selective transfer of cytoplasmic genes. Many important agronomic traits, such as herbicide resistance and cytoplasniic male sterility, are often controlled by extra nuclear genes. Selective transfer of cytoplasmic traits is achieved by (lie fusion of norilia1 protoplasts of the recipient parent' with the donor's protoplasts in which the nucleus has been rendered lnactlve by irradiation or with its enucleated sub protoplasts or non protoplasts. Such hybrids are called Cybrids. Medgyesy et al. (1 980) transferred streptomycin resistance (controlled by chloroplast DNA) from ***Nicotina tabacum*** to ***N. sylvestris*** by fusing iodoacetate treated. Non-dividing protoplasts of streptomycin-resistant *N.* ***tabaccum*** with nornal protoplasts of streptomycin sensitivity ***N .sylvestris.***

Alloplasniic male sterile lines of ***Brassica napas*** and ***Brassica oleracea*** produced by substituting their cytoplasm by the ogura cytoplasm of male sterile ***Raphanus sativus*** could not be until used for hybrid seed production because of their yellowing of leaves at low temperature (Jourdan et al., 1985). By fusing the protoplasts of these lines with those neither containing nor iiacl ytoplasm of respective species, rcsearclicrs working with pelletier (1983), Robertson (1985) and Menzel (1987) replaced the sensitive chloroplasts by insensitive ones. The new alloplasm clines retained the uselul male sterility while acquiring functional chloroplasts.

Fusion of dissimilar protoplasts (from different parents) results in tlie formation of heterokaryons. After the fusion treatment the fusion mixture contains, besides heterokaryons, the unfused protoplasts and homokaryons (the fusion product of siniilar protoplasts from the parents). It has been possible in some cases to isolate the heterokaryons mechanically, using micropipettes or by using a cell sorting machine.

Generally, however, a suitable selection pressure is applied which permits the growth of only hybrid cells by selectively suppressing the division of the other types of cells. The nuclei in a heterokaryon fuse to form a hybrid cell. The latter may divide and produce a callus mass and eventually whole plants thus may be differentated.

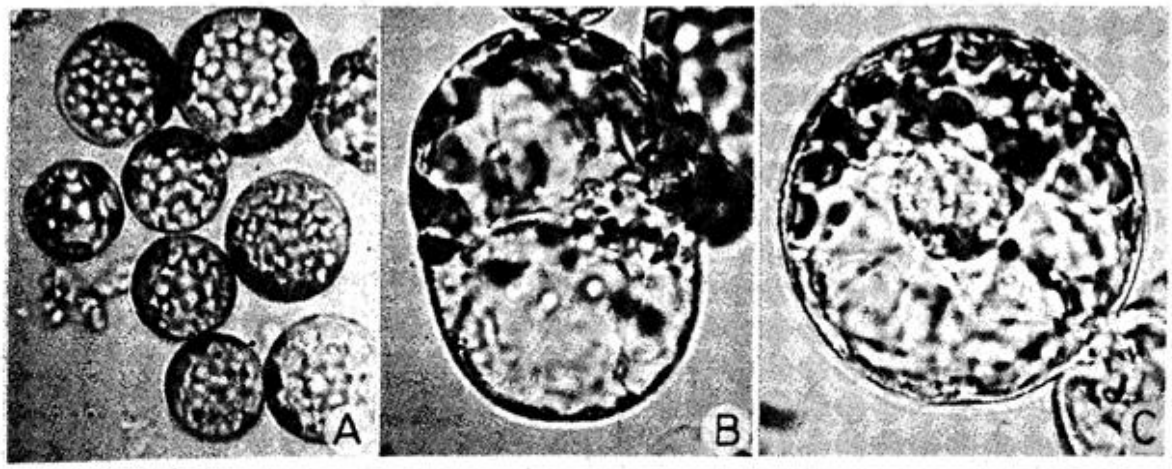


Fig. 11.8: A. Freshly isolated protoplasts of white clover. B,C. Stages in the fusion of a nonchlorophyllous protoplast from suspension culture of *Petunia hybrida* with a green mesophyll protoplast of *P. parodii*. First the fusion body apears dumbbell shaped which eventually becomes spherical. Even in C the chloroplasts are confined to one side of the heterkaryon. With the mixing of cytoplasm of the two protoplasts the chloroplasts would become evenly distributed.

Several interspecific and intergeneric somatic hybrids have been produced. Fusion between potato and tomato created 'Pomato' (Melchers et al. 1978) and fusion between Arabidopsis and Brassica resulted in Arabidobrassica. However, such distant hybrids are generally sterile and do not produce viable seeds. Therefore, it has been now realised that somatic hybridization is likely to be successful only when closely related but sexually incompatible parents are involved. For example, Solanum breviciense, a wild species, is resistant to potato leaf roll virus (PLRV) and potato virus y (PVY) but it can not be directly crossed with Solanum tuberosum (potato). Some of the somatic hybrids between these two species, showing resistance to PLRV and PVY, are cross compatible with S. tuberosum allowing introgression of virus resistance gene in potato cultivars.

Haploid Plant Production

The higher plants are normally diploid, with two sets of chromosomes in their somatic cells. Their haploids (with dne set of chromosomes) arise in nature by parthenogenesis due to malfunction in the normal sexual process. However, such events are extremely rare and unpredictable.

In 1964, two Indian scientists, Guha and Maheshwari, observed that in cultured anthers of Datura innoxia some of the microspores, instead of following the normal gametophytic mode of development, formed sporophytes (Androgenic plants). As expected, those sporophytes were haploid (Guha and Maheshwari, 1966). This report caused much excitement because of the considerable importance of haploids in genetics and plant breeding. To-date androgenic haploids of over 200 species, including many major crop plants (Cereals, tomato and potato), have been raised through anther and/or isolated pollen culture (Fig. 11.9).

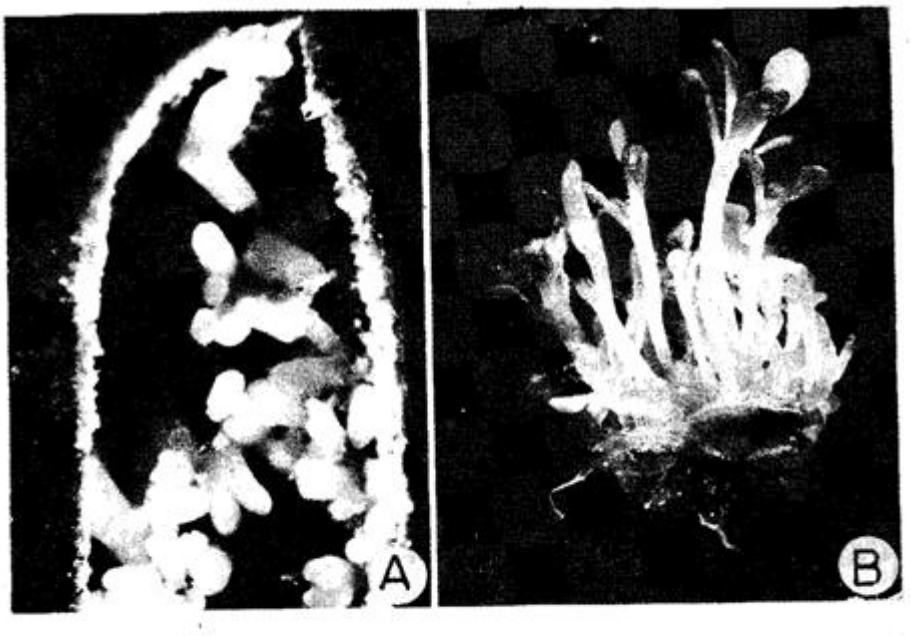


Fig. 11.9: Androgenesis in anther cultures of tobacco. A. A burst anther showing numerous pollen embryos. B. The pollen embryos have germinated.

In practice, anthers at the late uninucleate stage of microspore development are excised from surface-sterilised buds and cultured on a nutrient medium. Often a low temperature (4-5°c) shock during initial 2-3 days of culture enhances the androgenic response. However, In ***Brassica*** species treatment with higher temperature (30-35°C) has proved beneficial. Under inductive conditions the microspores undergo repeated divisions to form multicellular structures. Depending on the plant and the culture medium, such structures directly develop into an embryo or form a callus from which plants are regenerated via organogenesis or embryogenesis (Fig. 11.10).

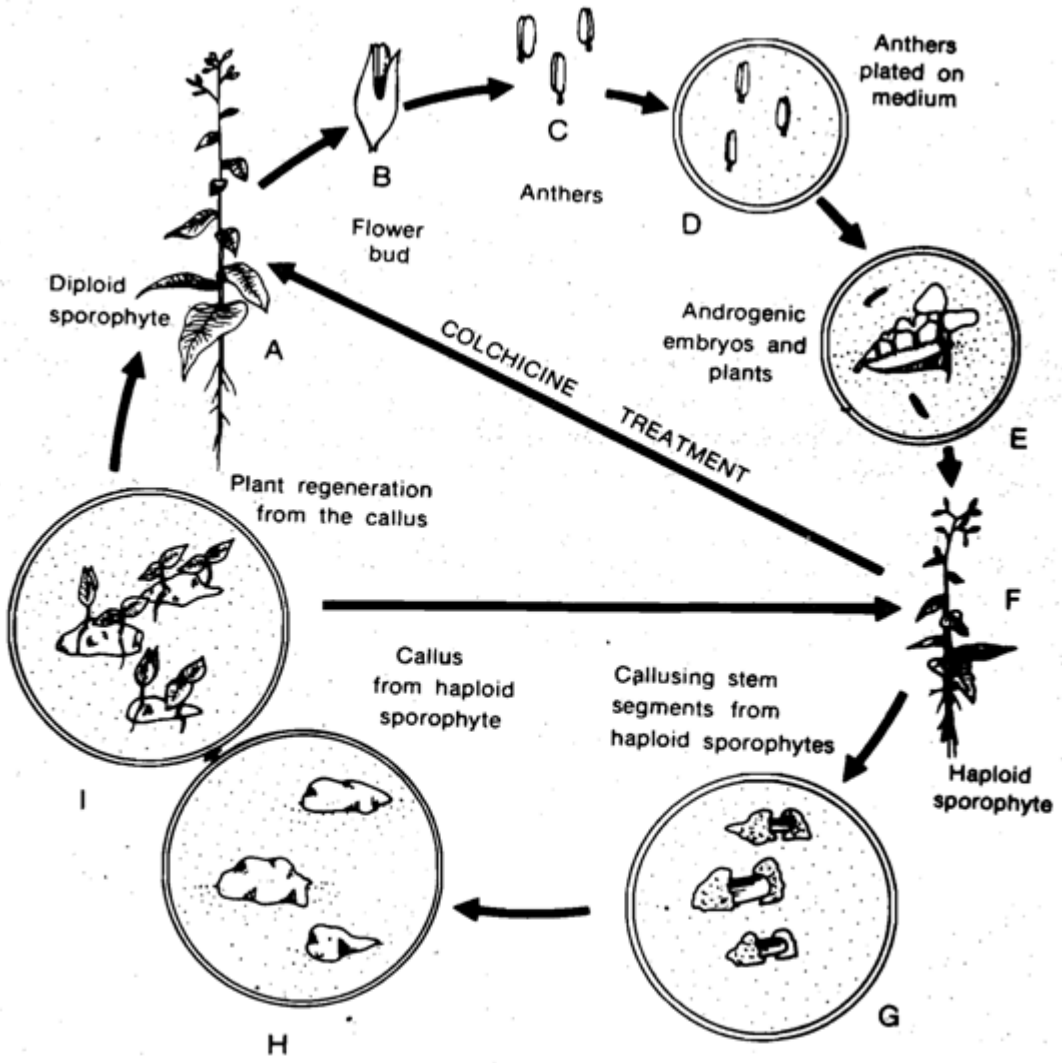


Fig. 11.10: A sumary diagram of haploid production by anther culture and their diploidization to raise homozygous diploid plants.

Androgenic haploids of some species, such as wheat, mustard and tobacco, can also be raised through isolated microspore/pollen culture (Pollen cultures). It must be realized that in spite of serious efforts androgenic haploids have not been possible in many other economically important plants.

In-vitro gynogenesis is another approach to produce haploids (Yang and Zhou, 1990). In this technique unfertilized ovules are cultured on media which stimulate the egg (parthenogenesis) or any other haploid cell of the embryo sac (apogamy) to undergo embryogenic development without fertilization. Invitro gynogenesis, Fust observed in *Hordeum vulgare* by San Noeum (1967), has now been reported in at least 16 species. This technique of haploid production is especially useful in plants in which the androgenic response is unsatisfactory, a large proportion of pollen plants are non-haploids or albinos, as in many cereals.

Haploids are extremely important **m** genetics and plant breeding. In haploids it is possible to detect recessive mutants which do not express themselves in diploid state due to the presence of the dominant allele. In cross pollinated plants and F1, hybrids, with high degree of heterozygosity, the fixation of a particular trait through

the conventional method of backcrossing takes 7-8 years. By anther or pollen culture this can be achieved in a single generation. Regeneration of plants from pollen grains also permits the screening of gametic variations at sporophytic level and selecting useful variants (gametoclodal variation). The Chinese scientists have developed and released about 20 new improved varieties of wheat and 61 varieties of rice through anther culture.

Micropropagation:

Micropropagation or **tissue culture** is the practice of rapidly multiplying plant stock material to produce many progeny plants, using modern plant tissue culture methods.

Micropropagation is used to multiply a wide variety of plants, such as those that have been genetically modified or bred through conventional plant breeding methods. It is also used to provide a sufficient number of plantlets for planting from seedless plants, plants that do not respond well to vegetative reproduction or where micropropagation is the cheaper means of propagating (e.g. *Orchids*.) Cornell University Botanist Frederick Campion Steward discovered and pioneered micropropagation and plant tissue culture in the late 1950s and early 1960s.

Steps:

In short, steps of micropropagation can be divided into **4 stages**, viz:

1. **Selection of mother plant**
2. **Multiplication**
3. **Rooting and acclimatizing**
4. **Transfer new plant to soil**

1. Selection of mother plant

Micropropagation begins with the selection of plant material to be propagated. The plant tissues are removed from an intact plant in a sterile condition. Clean stock materials that are free of viruses and fungi are important in the production of the healthiest plants. Once the plant material is chosen for culture, the collection of explant(s) begins and is dependent on the type of tissue to be used; including stem tips, anthers, petals, pollen and other plant tissues. The explant material is then surface sterilized, usually in multiple courses of bleach and alcohol washes, and finally rinsed in sterilized water. This small portion of plant tissue, sometimes only a single cell, is placed on a growth medium, typically containing Macro and micro nutrients, water, sucrose as an energy source and one or more plant growth regulators (plant hormones). Usually the medium is thickened with a gelling agent, such as agar, to create a gel which supports the explant during growth. Some plants are easily grown on simple media, but others require more complicated media for successful growth; the plant tissue grows and differentiates into new tissues depending on the medium. For example, media containing cytokinin are used to create branched shoots from plant buds.

2. Multiplication

Multiplication is the taking of tissue samples produced during the first stage and increasing their number. Following the successful introduction and growth of plant tissue, the establishment stage is followed by multiplication. Through repeated cycles of this process, a single explant sample may be increased from one to hundreds and thousands of plants. Depending on the type of tissue grown, multiplication can involve different methods and media. If the plant material grown is callus tissue, it can be placed in a blender and cut into smaller pieces and recultured on the same type of culture medium to grow more callus tissue. If the tissue is grown as small plants called plantlets, hormones are often added that cause the plantlets to produce many small offshoots. After the formation of multiple shoots, these shoots are transferred to rooting medium with a high auxin\cytokinin ratio. After the development of roots, plantlets can be used for hardening.

3. Pre-transplant

This stage involves treating the plantlets/shoots produced to encourage root growth and "hardening." It is performed *in vitro*, or in a sterile "test tube" environment.

"Hardening" refers to the preparation of the plants for a natural growth environment. Until this stage, the plantlets have been grown in "ideal" conditions, designed to encourage rapid growth. Due to the controlled nature of their maturation, the plantlets often do not have fully functional dermal coverings. This causes them to be highly susceptible to disease and inefficient in their use of water and energy. In vitro conditions are high in humidity, and plants grown under these conditions often do not form a working cuticle and stomata that keep the plant from drying out. When taken out of culture, the plantlets need time to adjust to more natural environmental conditions. Hardening typically involves slowly weaning the plantlets from a high-humidity; low light, warm environment to what would be considered a normal growth environment for the species in question.

4. Transfer from culture

In the final stage of plant micropropagation, the plantlets are removed from the plant media and transferred to soil or (more commonly) potting compost for continued growth by conventional methods. This stage is often combined with the "pretransplant" stage.

Methods:

Meristem culture: In Meristem culture, the meristem and a few subtending leaf primordia are placed into a suitable growing media, where they are induced to form new meristem. These meristems are then divided and further grown and multiplied. To produce plantlets the meristems are taken of from their proliferation medium and put on a regeneration medium. When an elongated rooted plantlet is produced after some weeks, it can be transferred to the soil. A disease free plant can be produced by this method. Experimental result also suggests that this technique can be successfully utilized for rapid multiplication of various plant species, e.g. Coconut, Strawberry and Sugarcane.

Callus culture:

A callus is mass of undifferentiated parenchymatous cells. When a living plant tissue is placed in an artificial growing medium with other conditions favorable, callus is formed. The growth of callus varies

with the homogenous levels of auxin and Cytokinin and can be manipulated by endogenous supply of these growth regulators in the culture medium. The callus growth and its organogenesis or embryogenesis can be referred into three different stages.

- Stage I: Rapid production of callus after placing the explants in culture medium
- Stage II: The callus is transferred to other medium containing growth regulators for the induction of adventitious organs.
- Stage III: The new plantlet is then exposed gradually to the environmental condition.

Embryo culture:

Main article: Embryo rescue

In embryo culture, the embryo is excised and placed into a culture medium with proper nutrient in aseptic condition. To obtain a quick and optimum growth into plantlets, it is transferred to soil. It is particularly important for the production of interspecific and intergeneric hybrids and to overcome the embryo.

Protoplast culture:

In protoplast culture, the plant cell can be isolated with the help of wall degrading enzymes and growth in a suitable culture medium in a controlled condition for regeneration of plantlets. Under suitable conditions the protoplast develops a cell wall followed by an increase in cell division and differentiation and grows into a new plant. The protoplast are first cultured in liquid medium at 25 to 28°C with a light intensity of 100 to 500 lux or in dark and after undergoing substantial cell division, they are transferred into solid medium congenial or morphogenesis in many horticultural crops respond well to protoplast culture.

Advantages:

Micropropagation has a number of advantages over traditional plant propagation techniques:

- The main advantage of micropropagation is the production of many plants that are clones of each other.
- Micropropagation can be used to produce disease-free plants.
- It can have an extraordinarily high fecundity rate, producing thousands of propagules while conventional techniques might only produce a fraction of this number.
- It is the only viable method of regenerating genetically modified cells or cells after protoplast fusion.
- It is useful in multiplying plants which produce seeds in uneconomical amounts, or when plants are sterile and do not produce viable seeds or when seed cannot be stored.
- Micropropagation often produces more robust plants, leading to accelerated growth compared to similar plants produced by conventional methods - like seeds or cuttings.
- Some plants with very small seeds, including most orchids, are most reliably grown from seed in sterile culture.
- A greater number of plants can be produced per square meter and the propagules can be stored longer and in a smaller area.

Disadvantages:

Micropropagation is not always the perfect means of multiplying plants. Conditions that limit its use include:

- Labour may make up 50%-69% of operating costs.
- A monoculture is produced after micropropagation, leading to a lack of overall disease resilience, as all progeny plants may be vulnerable to the same infections.
- An infected plant sample can produce infected progeny. This is uncommon as the stock plants are carefully screened and vetted to prevent culturing plants infected with virus or fungus.
- Not all plants can be successfully tissue cultured, often because the proper medium for growth is not known or the plants produce secondary metabolic chemicals that stunt or kill the explant.
- Sometimes plants or cultivars do not come true to type after being tissue cultured. This is often dependent on the type of explant material utilized during the initiation phase or the result of the age of the cell or propagule line.
- Some plants are very difficult to disinfect of fungal organisms.
- The major limitation in the use of micropropagation for many plants is the cost of production; for many plants the use of seeds, which are normally disease free and produced in good numbers, readily produce plants in good numbers at a lower cost. For this reason, many plant breeders do not utilize micropropagation because the cost is prohibitive. Other breeders use it to produce stock plants that are then used for seed multiplication.
- Mechanisation of the process could reduce labour costs, but has proven difficult to achieve, despite active attempts to develop technological solutions.

9. Genetically Modified Crops: Recombinant DNA technology and its use in crop improvement.

Genetically modified crops (GMCs, GM crops, or biotech crops) are defined as crops whose genomes have been altered in ways that do not occur naturally. Although the definition of GMCs includes organisms that have been genetically modified by selective breeding, the most commonly used definition refers to organisms modified through genetic engineering or recombinant DNA technologies. Genetic engineering allows one or more genes to be cloned and transferred from one organism to another—either between individuals of the same species or between those of unrelated species. It also allows an organism's endogenous genes to be altered in ways that lead to enhanced or reduced expression levels. When genes are transferred between unrelated species, the resulting organism is called **transgenic**. The term **cisgenic** is sometimes used to describe gene transfers within a species. In contrast, the term **biotechnology** is a more general one, encompassing a wide range of methods that manipulate organisms or their components—such as isolating enzymes or producing wine, cheese, or yogurt. Genetic modification of plants or animals is one

aspect of biotechnology.

Examples in food crops include resistance to certain pests, diseases, or environmental conditions, reduction of spoilage, or resistance to chemical treatments (e.g. resistance to an herbicide), or improving the nutrient profile of the crop. Examples in non-food crops include production of pharmaceutical agents, biofuels, and other industrially useful goods, as well as for bioremediation.

Farmers have widely adopted GM technology. It is the fastest adopted crop technology in the world. Acreage increased from 1.7 million hectares in 1996 to 185.1 million hectares in 2016, some 12% of global cropland. As of 2016, major crop (soybean, maize, canola and cotton) traits consist of herbicide tolerance (95.9 million hectares) insect resistance (25.2 million hectares), or both (58.5 million hectares). In 2015, 53.6 million ha of GM maize were under cultivation (almost 1/3 of the maize crop). GM maize outperformed its predecessors: yield was 5.6 to 24.5% higher with less mycotoxins (−28.8%), fumonisin (−30.6%) and thricotecens (−36.5%). Non-target organisms were unaffected, except for Braconidae, represented by a parasitoid of European corn borer, the target of Lepidoptera active Bt maize. Biogeochemical parameters such as lignin content did not vary, while biomass decomposition was higher.

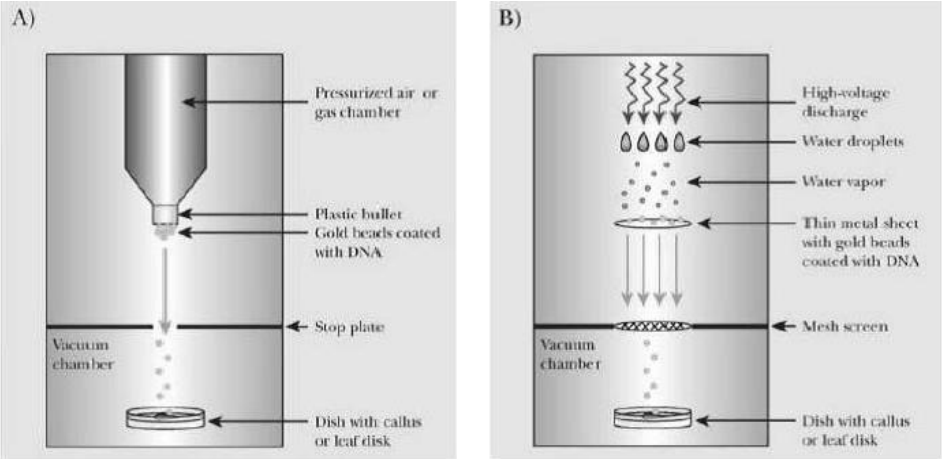
There is a scientific consensus that currently available food derived from GM crops poses no greater risk to human health than conventional food, but that each GM food needs to be tested on a case-by- case basis before introduction. Nonetheless, members of the public are much less likely than scientists to perceive GM foods as safe. The legal and regulatory status of GM foods varies by country, with some nations banning or restricting them, and others permitting them with widely differing degrees of regulation.

However, opponents have objected to GM crops on grounds including environmental impacts, food safety, whether GM crops are needed to address food needs, whether they are sufficiently accessible to farmers in developing countries and concerns over subjecting crops to intellectual property law. Safety concerns led 38 countries, including 19 in Europe, to officially prohibit their cultivation.

Methods:

Genetically engineered crops have genes added or removed using genetic engineering techniques, originally including **gene guns**, **electroporation**, **microinjection** and ***Agrobacterium***. More recently, **CRISPR** and **TALEN** offered much more precise and convenient editing techniques.

Gene guns (also known as biolistics) target genes into plant cells. It is the most common method. DNA is bound to tiny particles of gold or tungsten which is subsequently shot into plant tissue or single plant cells under high pressure. The accelerated particles penetrate both the cell wall and membranes. The DNA separates from the metal and is integrated into plant DNA inside the nucleus. This method has been applied successfully for many cultivated crops, especially monocots like wheat or maize, for which transformation using *Agrobacterium tumefaciens* has been less successful. The major disadvantage of this procedure is that serious damage can be done to the cellular tissue.



***Agrobacterium tumefaciens*-mediated transformation** is another common technique. *Agrobacteria* are natural plant parasites. Their natural ability to transfer genes provides another engineering method.To create a suitable environment for themselves, these *Agrobacteria* insert their genes into plant hosts, resulting in a proliferation of modified plant cells near the soil level (crown gall). The genetic information for tumor growth is encoded on a mobile circular DNA fragment (plasmid). When in *Agrobacterium* infects a plant, it transfers this T-DNA to a random site in the plant genome. When used in genetic engineering the bacterial T-DNA is removed from the bacterial plasmid and replaced with the desired foreign gene. The bacterium is a vector, enabling transportation of foreign genes into plants. This method works especially well for dicotyledonous plants like potatoes, tomatoes, and tobacco. *Agrobacteria* infection is less successful in crops like wheat and maize

Ti plamid:

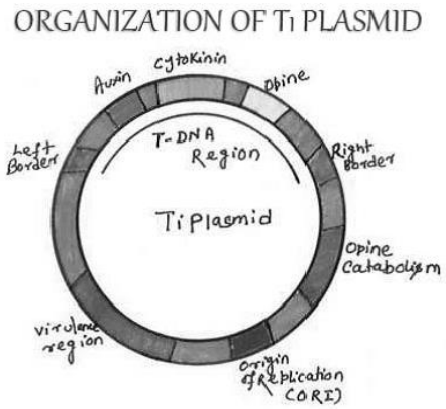
< The size of Ti plasmid is approx. 200 kb.

< The Ti plasmid has three important regions:

(i) T-DNA region

(ii) Virulence region

(iii) Opine catabolism region



There is ori region that is responsible for the origin of DNA replication.

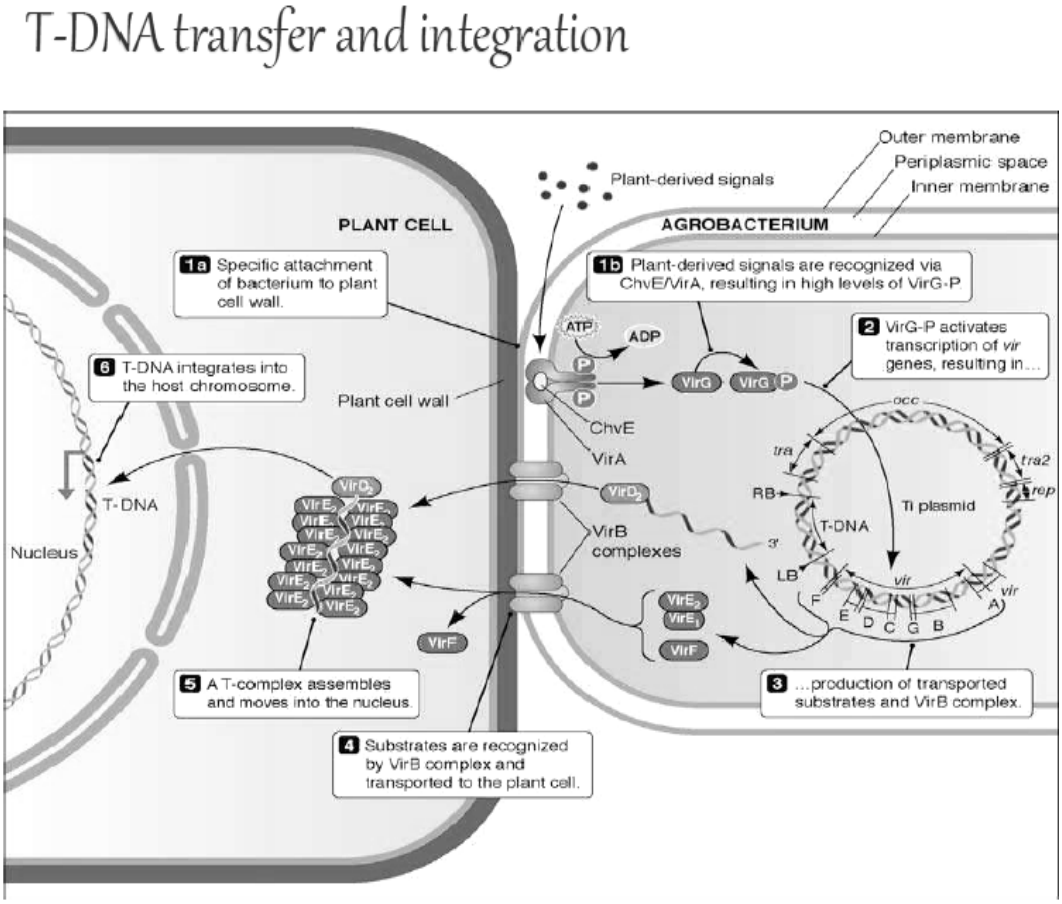


Fig: Diagrammatic representation of T-DNA transfer &its integration into host plant cell genome

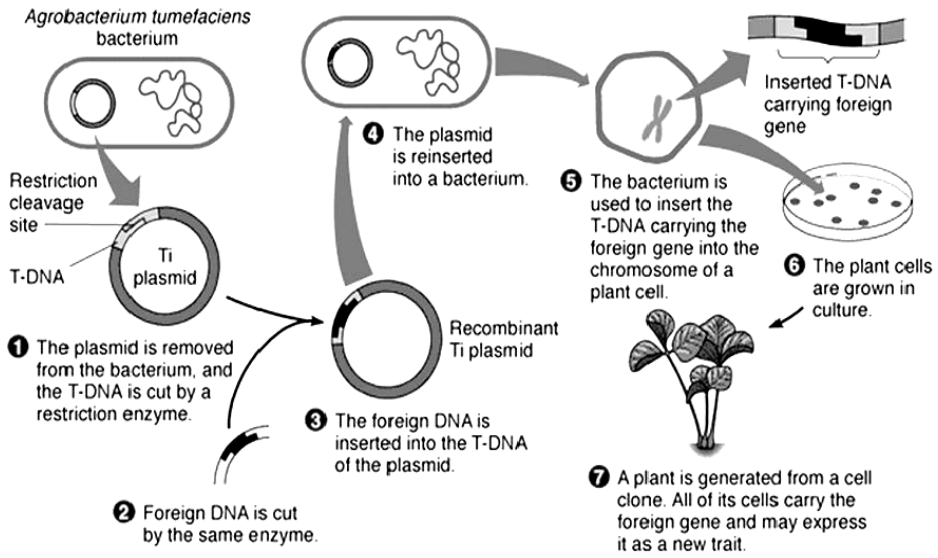
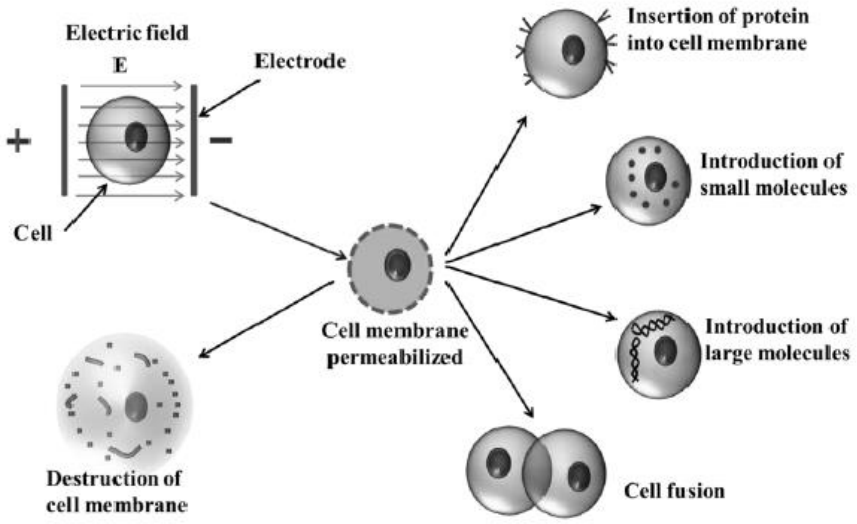


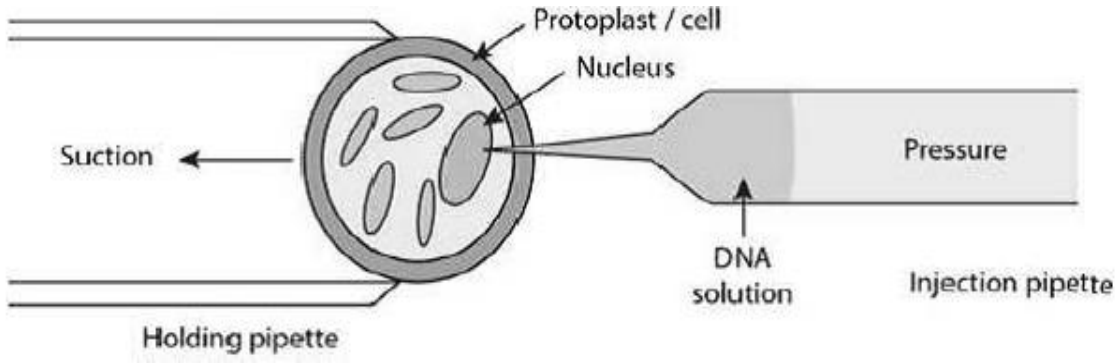
Fig.1: *Agrobacterium*-mediated genetic transformation.

Electroporation is used when the plant tissue does not contain cell walls. Electroporation involves the creation of pores in the cell membrane using electric pulse of high field strength. If DNA ispresent in the buffer solution at sufficient concentration, it will be taken up through these pores.



Microinjection is a direct physical method involving the mechanical insertion of the desirable DNA into a target cell.

- The technique of microinjection involves the transfer of the gene through a micropipette intothe cytoplasm or nucleus of a plant cell or protoplast.
- The most significant use of this is the introduction of DNA into the oocyte and the eggs of animals, either the transient expression analysis or to generate transgenic animals.
- The major limitations of microinjection are that it is slow, expensive, and has to be performedby trained and skilled personnel.



Types of modifications –

Transgenic

Transgenic plants have genes inserted into them that are derived from another species. The inserted genes can come from species within the same kingdom (plant to plant), or between kingdoms (for example, bacteria to plant). In many cases the inserted DNA has to be modified slightly in order to be correctly and efficiently expressed in the host organism.

Transgenic plants are used to express proteins, like the cry toxins from *B. thuringiensis*, herbicide-resistant genes, antibodies etc.

Cisgenic

Cisgenic plants are made using genes found within the same species or a closely related one, where conventional plant breeding can occur. Some breeders and scientists argue that cisgenic modification is useful for plants that are difficult to crossbreed by conventional means (such as potatoes), and that plants in the cisgenic category should not require the same regulatory scrutiny as transgenics.

Subgenic

Genetically modified plants can also be developed using **gene knockdown** or **gene knockout** to alter the genetic makeup of a plant without incorporating genes from other plants. In 2014, Chineseresearcher Gao Caixia filed patents on the creation of a strain of wheat that is resistant to powderymildew. The strain lacks genes that encode proteins that repress defenses against the mildew. The

researchers deleted all three copies of the genes from wheat's hexaploid genome. Gao used the TALENs and CRISPR gene editing tools without adding or changing any other genes.

Multiple trait integration

With multiple trait integration, several new traits may be integrated into a new crop.

Herbicide Resistant GM crops:

Weed infestations destroy about 10 percent of crops worldwide. To combat weeds, farmers often apply herbicides before seeding a crop and between rows after the crops are growing. As the most efficient broad-spectrum herbicides also kill crop plants, herbicide use may be difficult and limited. Farmers also use tillage to control weeds however; tillage damages soil structure and increases erosion.

A good or an ideal herbicide is expected to possess the following characteristics:

i. Capable of killing weeds without affecting crop plants.
ii. Not toxic to animals and microorganisms.
iii. Rapidly translocated within the target plant.
iv. Rapidly degraded in the soil.

Glyphosate Resistance:

Glyphosate, is a glycine derivative. It acts as a broad-spectrum herbicide and is effective against 76 of the world's worst78 weeds. Glyphosate is less toxic to animals and is rapidly degraded by microorganisms. In addition, it has a short half-life. The American chemical company Monsanto markets glyphosate as **Round up**.

Mechanism of action of glyphosate:

Glyphosate is rapidly transported to the growing points of plants. It is capable of killing the plants even at a low concentration. Glyphosate acts as a competitive inhibitor of the enzyme 5- enoylpyruvylshikimate 3-phosphate synthase (EPSPS). This is a key enzyme in shikimic acid pathway that results in the formation of aromatic amino acids (tryptophan, phenylalanine and tyrosine), phenols and certain secondary metabolites.

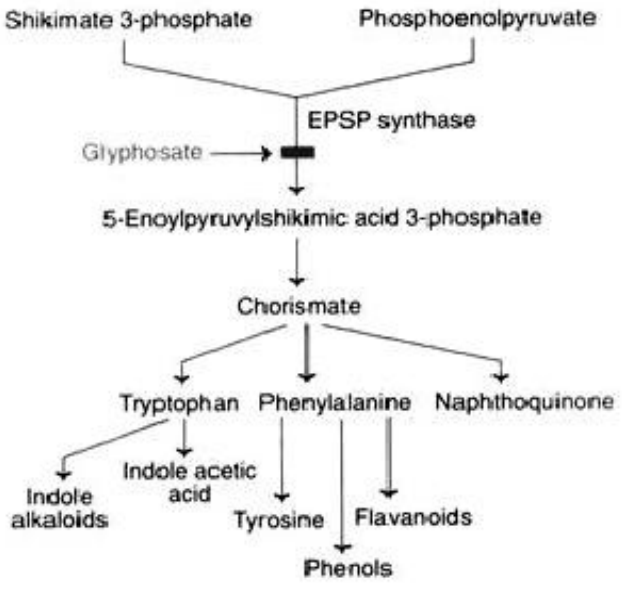


Fig. 50.5 : Shikimate pathway indicating the action of the herbicide glyphosate (EPSP synthase-5-Enolylpyruvylshikimate 3-phosphate synthase)

The enzyme EPSPS catalyses the synthesis of 5-enoylpyruvylshikimate 3-phosphate from shikimate 3-phosphate and phosphoenoylpyruvate. Glyphosate has some structural similarly with the substrate phosphoenol pyruvate. Consequently, glyphosate binds more tightly with EPSPS and blocks the normal shikimic acid pathway. Thus, the herbicide glyphosate inhibits the biosynthesis of aromatic amino acids and other important products.

This results in inhibition of protein biosynthesis (due to lack of aromatic amino acids). As a consequence, cell division and plant growth are blocked. Further, the plant growth regulator indole acetic acid (an auxin) is also produced from tryptophan. The net result of glyphosate is the death of the plants. Glyphosate is toxic to microorganisms as they also possess shikimate pathway.

Glyphosate is non-toxic to animals (including humans), since they do not possess shikimate pathway.

Strategies for glyphosate resistance:

There are three distinct strategies to provide glyphosphate resistance to plants:

1. Overexpression of crop plant EPSPS gene:

An overexpressing gene of EPSPS was detected in *Petunia*. This expression was found to be due to gene amplification rather than an increased expression of the gene. EPSPS gene from *Petunia* was isolated and introduced into other plants. The increased synthesis of EPSPS (by about 40 fold) in transgenic plants provides resistance to glyphosate. These plants can tolerate glyphosate at a dose of 2-4 times higher than that required to kill wild-type plants.

2. Use of mutant EPSPS genes:

An EPSPS mutant gene that conferred resistance to glyphosate was first detected in the bacterium Salmonella typhimurium. It was found that a single base substitution (C to 7) resulted in the change of an amino acid from proline to serine in EPSPS. This modified enzyme cannot bind to glyphosate, and thus provides resistance.

The mutant EPSPS gene was introduced into tobacco plants using *Agrobacterium* Ti plasmid vectors. The transgene produced high quantities of the enzyme EPSPS. However, the transformed tobacco plants provided only marginal resistance to glyphosate. The reason for this was not immediately identified.

3. Detoxification of glyphosate:

The soil microorganisms possess the enzyme glyphosate oxidase that converts glyphosate to glyoxylate and aminomethylphosponic acid. The gene encoding glyphosate oxidase has been isolated from a soil organism Ochrobactrum anthropi. With suitable modifications, this gene was introduced into crop plants e.g. oilseed rape. The transgenic plants were found to exhibit very good glyphosate resistance in the field.

Use of a combined strategy:

More efficient resistance of plants against glyphosate can be provided by employing a combined strategy. Thus, resistant (i.e. mutant) EPSPS gene in combination with glyphosate oxidase gene are used. By this approach, there occurs glyphosate resistance (due to mutant EPSPS gene) as well as its detoxification (due to glyphosate oxidase gene).

Phosphinothricin Resistance:

Phosphinothricin (or glufosinate) is also a broad spectrum herbicide like glyphosate. Phosphinothricin is more effective against broad-leafed weeds but least effective against perennials.

Phosphinothricin-a natural herbicide:

Phosphinothricin is an unusual herbicide, being a derivative of a natural product namely bialaphos.

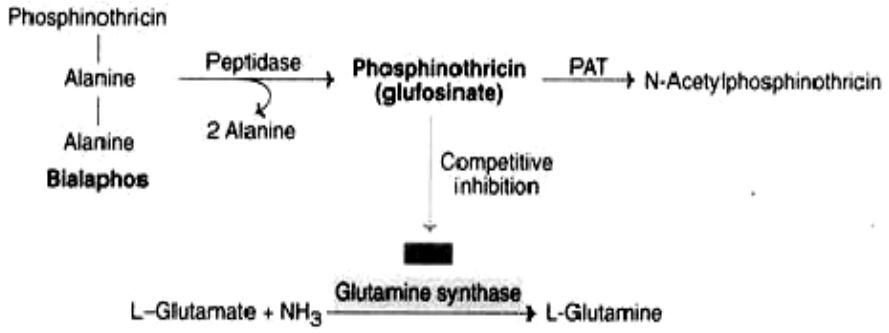


Fig. 50.7 : The formation, mode of action and detoxification of phosphinothricin (PAT-Phosphinothricin acetyl transferase).

Certain species of Streptomyces produce bialaphos which is a combination of phosphinothricin bound to two alanine residues, forming a tripeptide. By the action of a peptidase, bialaphos is converted to active phosphinothricin.

Mechanism of action of phosphinothricin:

Phosphinothricin acts as a competitive inhibitor of the enzyme glutamine synthase. This is possible since phosphinothricin has some structural similarity with the substrate glutamate. As a consequence of the inhibition of glutamine synthase, ammonia accumulates and kills the plant cells. Further, disturbance in glutamine synthesis also inhibits photosynthesis. Thus, the herbicidal activity ofphosphinothricin is due to the combined effects of ammonia toxicity and inhibition of photosynthesis.

Strategy for phosphinothricin resistance:

The natural detoxifying mechanism of phosphinothricin observed in Streptomyces sp has prompted scientists to develop resistant plants against this herbicide. The enzyme phosphinothricin acetyltransferase (of *Streptomyces* sp) acetylates phosphinothricin, and thus inactivates the herbicide.

The gene responsible for coding phosphinothricin acetyl transferase (bar gene) has been identified in *Streptomyces hygroscopicus*. Some success has been reported in developing transgenic maize and oilseed rape by introducing bar gene. These plants were found to provide resistance tophosphinothricin.

Bromoxynil

Tobacco plants have been engineered to be resistant to the herbicide bromoxynil.

Glufosinate

Crops have been commercialized that are resistant to the herbicide glufosinate, as well. Crops engineered for resistance to multiple herbicides to allow farmers to use a mixed group of two, three, or four different chemicals are under development to combat growing herbicide resistance.

2,4-D

In October 2014 the US EPA registered Dow's Enlist Duo maize, which is genetically modified to be resistant to both glyphosate and 2,4-D, in six states. Inserting a bacterial aryloxyalkanoate dioxygenase gene, aad1 makes the corn resistant to 2,4-D. The USDA had approved maize and soybeans with the mutation in September 2014.

Insect (Pest) Resistance GM crops:

It is estimated that about 15% of the world's crop yield is lost to insects or pests. The damage to cropsis mainly caused by insect larvae and to some extent adult insects.

The majority of the insects that damage crops belong to the following orders (with examples):

i. Lepidoptera (bollworms).
ii. Coleoptera (beetles).
iii. Orthoptera (grasshoppers).
iv. Homoptera (aphids).

Till some time ago, chemical pesticides are the only means of pest control. Transgenic plants with insect resistance transgenes have been developed. About 40 genes obtained from microorganisms of higher plants and animals have been used to provide insect resistance in crop plants.

Resistance Genes from Microorganisms:

***Bacillus thuringiensis* (Bt) toxin:**

Bacillus thuringiensis was first discovered by Ishiwaki in 1901, although its commercial importance was ignored until 1951. *B. thuringiensis* is a Gram negative, soil bacterium. This bacterium produces a parasporal crystalline proteinous toxin with insecticidal activity. The protein produced by *B. thuringiensis* is referred to as insecticidal crystalline protein (ICP). ICPs are among the endotoxins produced by sporulating bacteria, and were originally classified as δ-endotoxins (to distinguish them from other classes of α-, β- and γ-endotoxins).

Bt toxin genes:

Several strains of B. thuringiensis producing a wide range of crystal (cry) proteins have been identified. Further, the structure of cry genes and their corresponding toxin (δ-endotoxin) products have been characterized. The cry genes are classified into a large number of distinct families (about 40) designated as cry 1…… cry 40, based on their size and sequence similarities. And within each family, there may be sub-families. Thus, the total number of genes producing Bt toxins (Cry proteins) is more than 100.

There are differences in the structure of different Cry proteins, besides certain sequence similarities. The molecular weights of Cry proteins may be either large (~130 KDa) or small (~70KDa). Despite the differences in the Cry proteins, they share a common active core of three domains.

Mode of action of Cry proteins:

Most of the Bt toxins (Cry proteins) are active against Lepidopteran larvae, while some of them are specific against Dipteran and Coleopteran insects. The pro-toxin of Cry I toxin group has a molecular mass of 130 kilo Daltons (130 KDa).

When this parasporal crystal is ingested by the target insect, the pro-toxin gets activated within its gut by a combination of alkaline pH (7.5 to 8.5) and proteolytic enzymes. This results in the conversion of pro-toxin into an active toxin with a molecular weight of 68 KDa. The active form of toxin protein gets itself inserted into the membrane of the gut epithelial cells of the insect. This result in the formation of ion channels through which there occurs an excessive loss of cellular ATP. As a consequence, cellular metabolism ceases, insect stops feeding, and becomes dehydrated and finally dies.

Some workers in the recent years suggest that the Bt toxin opens cation-selective pores in the membranes, leading to the inflow of cations into the cells that causes osmotic lysis and destruction of epithelial cells (and finally the death of insect larvae). The Bt toxin is not toxic to humans and animals since the conversion of pro-toxin to toxin requires alkaline pH and specific proteases.

The usage Bt is commonly used for a transgenic crop with a cry gene e.g. Bt cotton. In the same way, Cry

proteins are also referred to as Bt proteins. It may also be stated here that the authors use four different names for the same group of proteins-*δ*-endotoxin, insecticidal crystal protein (ICP), Cry andnow Bt.

Bt-based genetic transformation of plants:

It has been possible to genetically modify (GM) plants by inserting Bt genes and provide pestresistance to these transformed plants. For an effective pest resistance, the bacterial gene in transgenic plants must possess high level expression. This obviously means that the transgene transcription should be under the effective control of promoter and terminator sequences. The early attempts to express cry 1A and cry 3A proteins under the control of CaMV 35S or Agrobacterium T-DNA promoters resulted in a very low expression in tobacco, tomato and potato plants.

In March 1995, the first Bt crop deregulated in the U.S. were seven lines of Colorado Potato Beetle Resistant Bt Potato by Monsanto. Since then, many more Bt crops have been deregulated, engineered to produce a variety of different Bt proteins from various subspecies of Bt. Bt crops include:

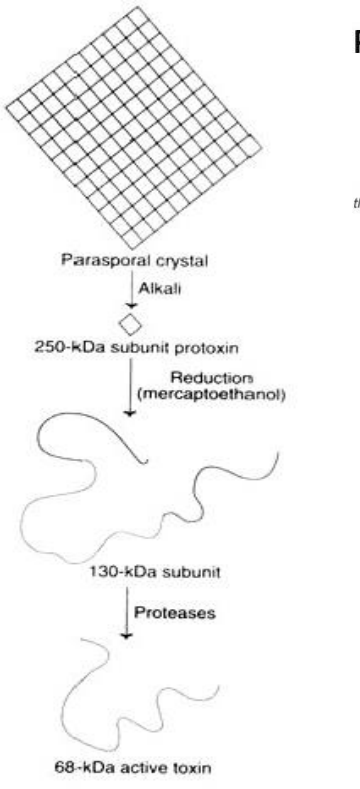
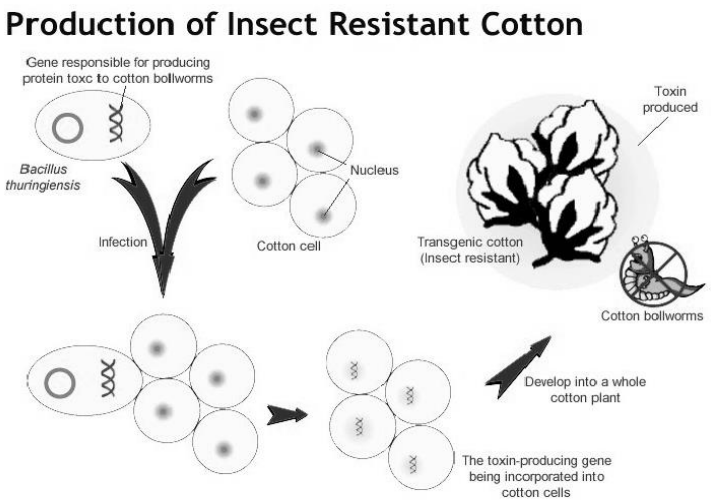


***Fig. 50.2 : A diagrammatic representation of the formation of active toxin from the parasporal crystal of* Bacillus thuringiensis.**



Corn:

European Corn Borer Resistant Corn (first deregulated in the U.S. in May 1995)Corn Rootworm Resistant Corn (first deregulated in the U.S. in October 2002)

Cotton:

Lepidopteran Resistant Cotton (first deregulated in the U.S. in June 1995)

Potato:

Colorado Potato Beetle Resistant Bt Potato (first deregulated in the U.S. in March 1995) Potato Tuber Moth Resistant Bt Potato (being developed in South Africa)

Soybean:

Bt Soybean (first deregulated in the U.S. in October 2011, not yet sold commercially)

Tomato:

Lepidopteran Resistant Tomato (first deregulated in the U.S. in March 1998, not yetsold commercially)

Golden Rice —The Provitamin A Enriched Rice:

About one-third of the world's population is dependent on rice as staple food. The milled rice that is usually consumed is almost deficit in P-carotene, the pro-vitamin A. As such, vitamin A deficiency (causing night blindness) is major nutritional disorder world over, particularly in people subsisting on rice.

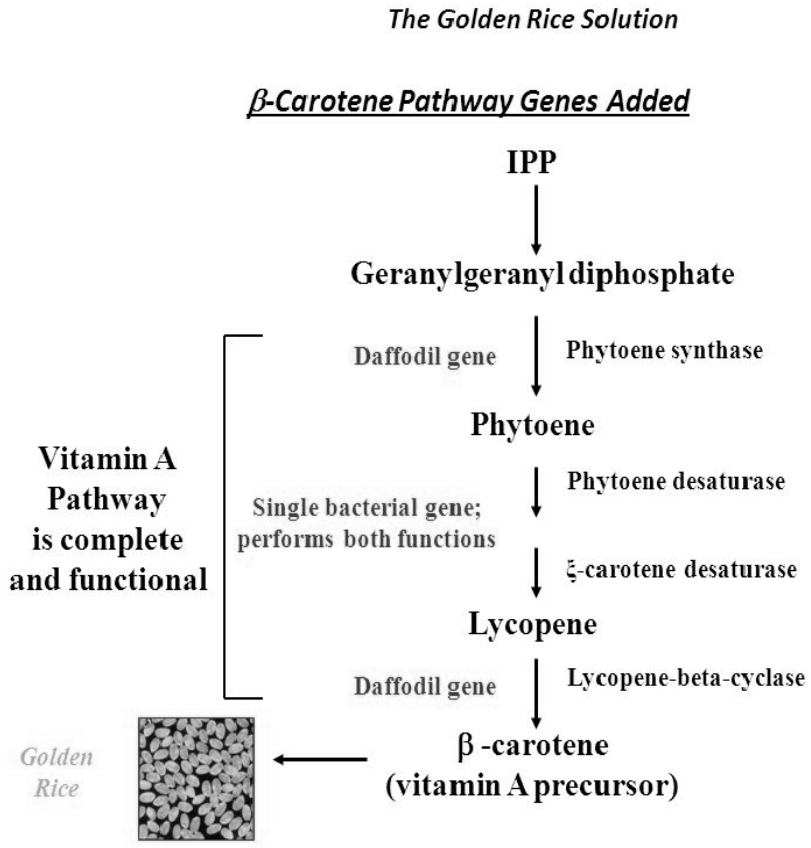
To overcome vitamin A deficiency, it was proposed to genetically manipulate rice to produce β-carotene, in the rice endosperm. The presence of β-carotene in the rice gives a characteristic yellow/orange colour, hence the pro-vitamin A-enriched rice are appropriately considered as Golden Rice.

The genetic manipulation to produce Golden Rice required the introduction of three genes encoding the enzymes phytoene synthase, carotene desaturase and lycopene β-cyclase. It took about 7 years to insert three genes for developing Golden Rice.

Golden Rice has met almost all the objections raised by the opponents of GM foods. However, many people are still against the large scale production of Golden Rice, as this will open door to the entry of many other GM foods.

Another argument put forth against the consumption of Golden Rice is that it can supply only about 20% of daily requirement of vitamin A. But the proponents justify that since rice is a part of a mixed diet consumed (along with many other foods), the contribution of pro-vitamin A through Golden Rice is quite substantial.

Recently (in 2004), a group of British scientists have developed an improved version of Golden Rice. The new strain, Golden Rice 2 contains more than 20 times the amount of pro-vitamin A than its predecessor. It is claimed that a daily consumption of 70 g rice can meet the recommended dietary allowance for vitamin A.



Genetically modified tomato:

Flavr Savr is a genetically modified tomato, was the first commercially grown genetically engineered food to be granted a license for human consumption. It was produced by Californian company Calgene 1992. Calgene introduced a gene in plant which synthesize a complementary mRNA to PG gene and inhibiting the synthesis of PG enzyme. On May 21, 1994, the genetically engineered Flavr Savr tomato was introduced.

Fruit ripening is an active process characterized by increased respiration accompanied by a rapid increase in ethylene synthesis. As the chlorophyll gets degraded, the green color of fruit disappears and a red pigment, lycopene is synthesized. The fruit gets softened as a result of the activity of cell wall degrading enzymes namely polygalacturonase (PG) and methyl esterase. The phyto hormone ethylene production is linked to fruit ripening as the same is known to trigger the ripening effect. The breakdown of starch to sugars and accumulation of large number of secondary products improves the flavor, taste and smell of the fruits.

Genes involved in tomatoes ripening:

i. **pTOM5** encodes for phytoene synthase which promote lycopene synthesis that gives red coloration
ii. **pTOM6** gene encodes for polygalacturonase. This enzyme degrades the cell wall, resulting in fruit softening.

iii. **pTOM** gene encodes for ACC oxidase. This enzyme catalyzes the ethylene formation that triggers the fruit ripening.

Development of flavr savr tomato

Softening of fruits is largely due to degradation of cell wall (pectin) by enzyme polygalacturonase(PG). The gene encoding PG has been isolated and cloned (pTOM6).

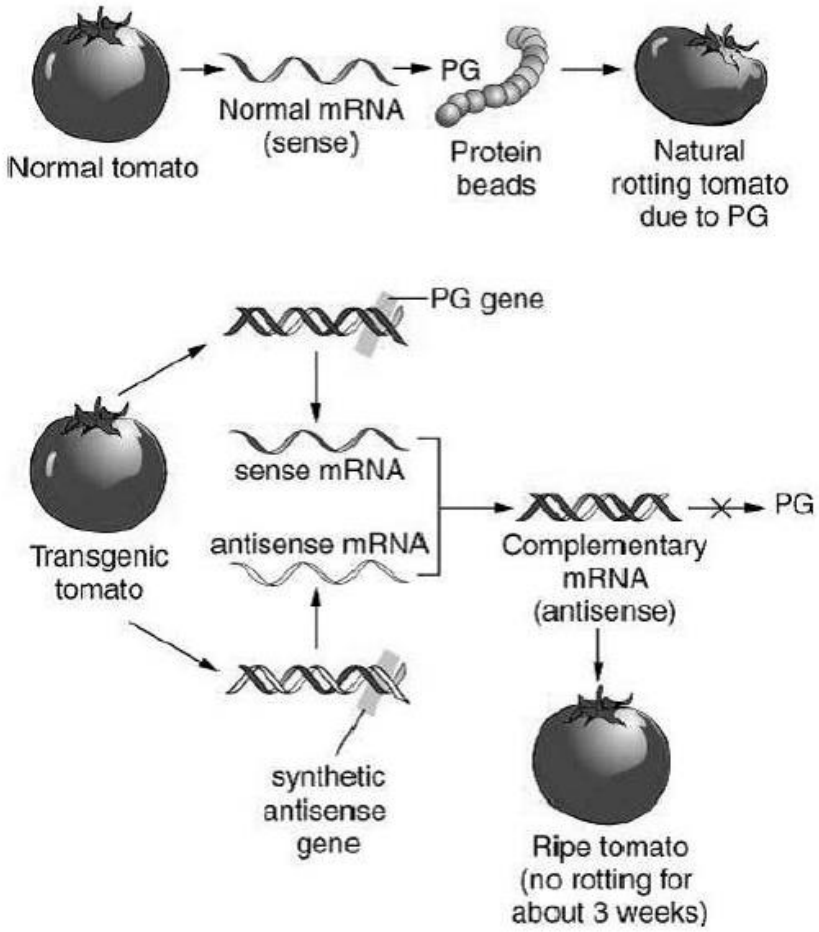
Procedure involves: -

1. Isolation of DNA from tomato plant that encodes the enzyme polygalacturonase (PG).
2. Transfer of PG gene to a vector bacteria and production of complementary DNA(cDNA) molecules.
3. Introduction of cDNA into a fresh tomato plant to produce transgenic plant.

Mechanism of pg antisense RNA approach

In normal plants, PG gene encodes a normal or sense mRNA that produce the enzyme PG and it isactively involved in fruit ripening.

- The cDNA of PG encodes for antisense mRNA, which is complementary to sense mRNA.
- The hybridization between sense and antisense mRNA render the sense mRNA ineffective.
- Consequently, no polygalacturonase is produced hence fruit ripening is delayed.



Advantages:

1. Slower ripen rate
2. Ripen longer on vine
3. Fully developed flavors
4. Increase the shelf life

The rise and fall of Flavr Savr Tomato:

The genetically engineered tomato, known as Flavr Savr (pronounced flavour saver) by employing PCantisense RNA was approved by U.S. Food and Drug Administration on 18th May 1994. The FDA ruled that Flavr Savr tomatoes are as safe as tomatoes that are bred by conventional means, and therefore no special labeling is required. The new tomato could be shipped without refrigeration too far off places, as it was capable of resisting rot for more than three weeks (double the time of a conventional tomato).

Although Flavr Savr was launched with a great fanfare in 1995, it did not fulfill the expectation for the following reasons:

i. Transgenic tomatoes could not be grown properly in different parts of U.S.A.
ii. The yield of tomatoes was low.
iii. The cost of Flavr Savr was high.

It is argued that the company that developed Flavr Savr, in its overenthusiasm to become the first Biotech Company to market a bioengineered food had not taken adequate care in developing the transgenic plant. And unfortunately, within a year after its entry, Flavr Savr was withdrawn, and it is now almost forgotten.

Virus resistance

Papaya, potatoes, and squash have been engineered to resist viral pathogens such as **cucumber mosaic virus** which, despite its name, infects a wide variety of plants. Virus resistant papaya were developed in response to a papaya ringspot virus (PRV) outbreak in Hawaii in the late 1990s. They incorporate PRV DNA. By 2010, 80% of Hawaiian papaya plants were genetically modified.

Potatoes were engineered for resistance to **potato leaf roll virus** and **Potato virus Y** in 1998. Poor sales led to their market withdrawal after three years.

Yellow squash that were resistant to at first two, then three viruses were developed, beginning in the 1990s. The viruses are watermelon, cucumber and zucchini/courgette yellow mosaic. Squash was the second GM crop to be approved by US regulators.

Edible oils

Some **GM soybeans** offer improved oil profiles for processing. *Camelina sativa* has been modified to produce plants that accumulate high levels of oils similar to fish oils.

Toxin reduction

A genetically modified *Cassava* under development offers lower **cyanogen glucosides** and enhanced protein and other nutrients (called BioCassava).

In November 2014, the USDA approved a potato that prevents bruising and produces less acrylamide when fried. They do not employ genes from non-potato species. The trait was added to the Russet Burbank, Ranger Russet and Atlantic varieties.

Stress resistance

Plants have been engineered to tolerate non-biological stressors, such as drought, frost, and high soil salinity. In 2011, Monsanto's DroughtGard maize became the first drought-resistant GM crop to receive US marketing approval.

Drought resistance occurs by modifying the plant's genes responsible for the mechanism known as the crassulacean acid metabolism (CAM), which allows the plants to survive despite low water levels.

This holds promise for water-heavy crops such as rice, wheat, soybeans and poplar to accelerate their adaptation to water-limited environments. Several salinity tolerance mechanisms have been identified in salt-tolerant crops. For example, rice, canola and tomato crops have been genetically modified to increase their tolerance to salt stress.

Drugs

In 2012, the FDA approved the first plant-produced pharmaceutical, a treatment for **Gaucher's Disease**. Tobacco plants have been modified to produce therapeutic antibodies.

Biofuel

Algae is under development for use in **biofuels**. Researchers in Singapore were working on **GM *Jatropha*** for biofuel production. Syngenta has USDA approval to market a maize trademarked Enogen that has been genetically modified to convert its starch to sugar for ethanol. Some trees have been genetically modified to either have less lignin, or to express lignin with chemically labile bonds. Lignin is the critical limiting factor when using wood to make bio-ethanol because lignin limits the accessibility of cellulose microfibrils to depolymerization by enzymes. Besides with trees, the chemically labile lignin bonds are also very useful for cereal crops such as maize, barley, and oats.

Materials

Companies and labs are working on plants that can be used to make bioplastics. Potatoes that produce industrially useful starches have been developed as well. Oilseed can be modified to produce fatty acids for detergents, substitute fuels and petrochemicals.

Bioremediation

Scientists at the University of York developed a weed (*Arabidopsis thaliana*) that contains genes frombacteria that could clean TNT and RDX-explosive soil contaminants in 2011. 16 million hectares in the US (1.5% of the total surface) are estimated to be contaminated with TNT and RDX. However *A. thaliana* was not tough enough

for use on military test grounds. Modifications in 2016 included switchgrass and bentgrass.

Genetically modified plants have been used for bioremediation of contaminated soils. Mercury, selenium and organic pollutants such as polychlorinated biphenyls (PCBs).

Marine environments are especially vulnerable since pollution such as oil spills are not containable. In addition to anthropogenic pollution, millions of tons of petroleum annually enter the marine environment from natural seepages. Despite its toxicity, a considerable fraction of petroleum oil entering marine systems is eliminated by the hydrocarbon-degrading activities of microbial communities. Particularly successful is a recently discovered group of specialists, the so-called hydrocarbonoclastic bacteria (HCCB) that may offer useful genes.

Advantages of GM crops:

There is a need to produce inexpensive, safe and nutritious foods to help feed the world's growing population.

Genetic modification may provide:

- Better quality food.
- Higher nutritional yields.
- Inexpensive and nutritious food, like carrots with more antioxidants.
- Foods with a greater shelf life, like tomatoes that taste better and last longer.
- Food with medicinal benefits, such as edible vaccines - for example, bananas with bacterial or rotavirus antigens.
- Crops and produce that require less chemical application, such as herbicide resistant canola.

Disadvantages of GM crops:

Food regulatory authorities require that GM foods receive individual pre-market safety assessments. Also, the principle of 'substantial equivalence' is used. This means that an existing food is compared with its genetically modified counterpart to find any differences between the existing food and the new product. The assessment investigates:

- Toxicity (using similar methods to those used for conventional foods).
- Tendency to provoke any allergic reaction.
- Stability of the inserted gene.
- Whether there is any nutritional deficit or change in the GM food.
- Any other unintended effects of the gene insertion.

Economic concerns:

Bringing a GM food to market is a lengthy and costly process, and of course agri-biotech companies wish to

ensure a profitable return on their investment. Many new plant genetic engineering technologies and GM plants have been patented, and patent infringement is a big concern of agribusiness. Yet consumer advocates are worried that patenting these new plant varieties will raisethe price of seeds so high that small farmers and third world countries will

not be able to afford seeds for GM crops, thus widening the gap between the wealthy and the poor. One way to combat possible patent infringement is to introduce a "suicide gene" into GM plants. These plants would be viable for only one growing season and would produce sterile seeds that do notgerminate. Farmers would need to buy a fresh supply of seeds each year. However, this would be financially disastrous for farmers in third world countries who cannot afford to buy seed each year and traditionally set aside a portion of their harvest to plant in the next growing season.

10. Plants in Forensic Investigation:

Introduction:

"Forensic" comes from the *Latin* word "*forensis*" meaning forum. During the time of the Romans, a criminal charge meant presenting the case before the public. Both the person accused of the crime & the accuser would give speeches based on their side of the story.

Forensic botany is a marriage of many disciplines and results ultimately in their application tomatters of law. The botanical aspects of forensic botany include plant anatomy, plant growth and behavior, plant reproductive cycles and population dynamics, and plant classification schemes (morphological and genetic) for species identification. The forensic aspects require an understanding of what is necessary for botanical evidence to be accepted as evidence in our judicial system.Forensics requires recognition of pertinent evidence at a crime scene, appropriate collection and preservation of evidentiary material, maintenance of a chain of custody, an understanding of scientific testing methods, validation of new forensic techniques, and admissibility criteria for court.

1. Application of botany in law enforcement i.e. scientific use of plant materials to solve crimes
2. Examples of plant life or plant remains that can be used as evidences are wood, seed, fruit, leaf, twig, trichrome, pollen, spore, algal cell etc.
3. Plant allow forensic botanists to identify things such as what season the crime took place or geographical location, whether or not a body has been moved following a murder, and how long a body has been buried if it was buried
4. These forms of physical evidences can be sometimes traced to an individual suspect

5. The scientific methods utilized in forensic botany, and these methods range from simple techniques (e.g., light microscopy) to more technical molecular biology techniques (e.g.,DNA sequencing).
6. Plants have been used as evidence in criminal cases for kidnapping, child abuse, hit-and-run motor vehicle accidents, drug enforcement, homicide, sexual and physical assault, the establishment of time of death, and verification of an alibi. In addition, new applications are under development to use plant material in forensics as "tracers" to aid in the identification of missing persons, to track drug distribution patterns, and to link bodies to primary crime scene locations after they have been dumped at secondary sites.

Unique roles of forensic scientists:

The individual with the best argument would determine the outcome of the case.

- Assist in recognition and collection of physical evidence
- Document and maintain chain-of-custody
- Analyze and evaluate the evidence using a variety of scientific approaches
- Interact with the legal system
    - Assist attorneys (and often law enforcement personnel)
    - Testify in Court

What botanical evidences can do?

- Determine the circumstances and cause of death
- Estimate time frames in relations to the death
- Establish where the death could have taken place
- Determine if there were multiple crime scenes
- Prove or disprove an alibi
- Solve crimes by matching crime scene evidence to suspect
- Identify illegal products from endangered species

Advantages of using plant sample:

- Plant evidence is long lasting, which means that plant parts to remain identifiable for a very long periods of time
- Plant cell wall is made of some chemical compounds which are nearly indestructible and do not decay quickly
- Pollen grains and spores also have walls that are made of decay resistant material- sporopollenin
- Ecological and molecular restraints of various plant species allow a forensic botanist to narrow down the possibilities of where a crime was committed, when it was committed and who

committed the crime

Plant in our society

To understand the widespread application and potential utility of plants in forensics, we discuss a few brief examples of plant usage in human society. As these examples are presented, consider the number of plant-based items that may be found on your person, among your private possessions, and in your home and workplace — and consider, one day, that they may be useful as critical trace evidence.

1. **Food**

Apples are generally considered to be a wholesome, healthful addition to the daily diet as a good source of vitamins and fiber. In actuality, this concept was promoted by the apple industry in response to the renouncement of apples by Carry Nation as part of the Prohibition Act. The Women's Christian Temperance Union was opposed to apples because they were, in part, responsible for alcohol use on the frontier. In the early 1900s, the apple industry began promoting the healthful benefits of apples, and today, we have many apple cultivars to choose from at our markets. Interestingly, burglars often sample fruit and other foods in the homes they are invading while pilfering goods.

2. **Fiber**

One prevalent clothing fiber in our society is cotton. Cotton comes from the elongated epidermal hairson the seeds of the *Gossypium hirsutum* plant. Prior to mechanized harvesting of cotton fibers, flax (*Linum usitatissimum*) was the most common fiber plant for the textile industry. Flax fibers, unlike cotton, are sclerenchyma fibers from the stem of the flax plant. Flax fibers are commonly woven into linen, and certain cultivars are used to produce cigarette papers and linseed oil. As trace evidence, the source of clothing, carpet fibers, rope, twine, and threads can be useful for associating a victim to a suspect or individuals back to a primary crime scene for an investigative lead.

3. **Medicine**

Herbal remedies and folklore investigations to identify active chemical components have long been part of human culture and have played an important role in the discovery of useful medicinal compounds (e.g., aspirin). In addition, well-preserved stomach contents from ancient human remains have yielded insight into rituals involving herbs and food. For example, in 1984, a peat cutter near Manchester, England, discovered a well-preserved human leg and called in the police to investigate. The body of Lindow Man was recovered, and radiocarbon dated to approximately A.D. 50–100, and he was determined to be a member of the Celtic tribes.

4. **Beauty**

Plants are key components in many herbal shampoos, soaps, cosmetics, and perfumes. Not only are botanicals used in human cosmetics and have appealing scents, but they beautify our environment as well. The *Dahlia* flower, for example, has an unusual history. Originating from Mexico and called *Cocoxochitl* by the Aztec Indians, it was discovered and seeds were sent to a French priest studying botany in Madrid,

Spain.

5. Recreation

The American obsession with green lawns can be visualized in the numerous golf courses, city parks, and extensive front and backyards that are ingrained parts of suburban life. Prior to the Civil War, few Americans had lawns. However, in the 1950s, the term "lawn" was used in reference to a portion of land kept closely mown in front or around a house. In the 1950s, turfgrass breeding programs gave rise to several new grass varieties that offered improved heat, drought, and disease resistance. In the 1970s and '80s, lawn specialists began recommending blends of grasses (e.g., fescue, bluegrass, perennial ryegrass) rather than a monoculture of a single grass species. Today, American homeownersnspend enormous capital and energy on achieving a perfectly groomed, green lawn as a setting for their homes. In fact, an entire lawn-care industry has developed around this particular aspect of suburban homes. Grass samples may be one of the most abundanttypes of botanical evidence found at crime scenes simply due to the American obsession with the lawn.

6. Law Enforcement

Plants may be present as biological evidence in many ways:

• Seeds caught and carried in a pant cuff

• Grass stains on a dress after a sexual assault

• Plant leaves and stems snagged and carried in a vehicle's undercarriage, grill, wheel wells, hood, ortrunk

• Stomach contents with vegetable matter to aid in verification of an alibi

• Use of pollen to date the burial of skeletal remains in a mass grave

All of these examples and more can assist the forensic community in associating a person to an object, a person to a crime scene, or a suspect to a victim. "Every criminal leaves a 'trace' (evidence)" is a phrase with some accuracy. That "trace" may very well be biological plant material.

Analysis of samplesPlant Anatomy

The study of the internal structure of plants is called plant anatomy. When a stem, root, or leaf is dissected, the cells can be arranged in specific patterns that may be useful for classification and identification. For example, the internal arrangement of cells in the root structure of a dicotyledonous (the first true leaves of a seedling occur in pairs) versus a monocotyledous (the first true leaf is a single leaf) plant is characteristic.

Analysis of anatomical samples:

Cell shape and orientation of certain structures within a cell can be helpful in classification of a species. In order to learn about plant anatomy and specific plant structures within the plant body plan,it is important to take a practical approach. In the following sections we describe examples of in expensive laboratory exercises that can be performed as training for forensic botanists.

Plant Anatomical Features used in Forensic Botany

The Vascular Cambium

- Lateral meristematic region
- Division of cells here produces secondary xylem and secondary phloem tissues
- Diameter expansion forces tangential elongation of phloem cells
- Epidermis and primary phloem layers eventually fall off
- Dead phloem cells compose the outer bark

Two Cell Types

Fusiform Initials - divide to produce new xylem or phloem cells that have longitudinally elongatedshapes

Ray initials - short, rounded cells that divide to produce new xylem or phloem ray cells

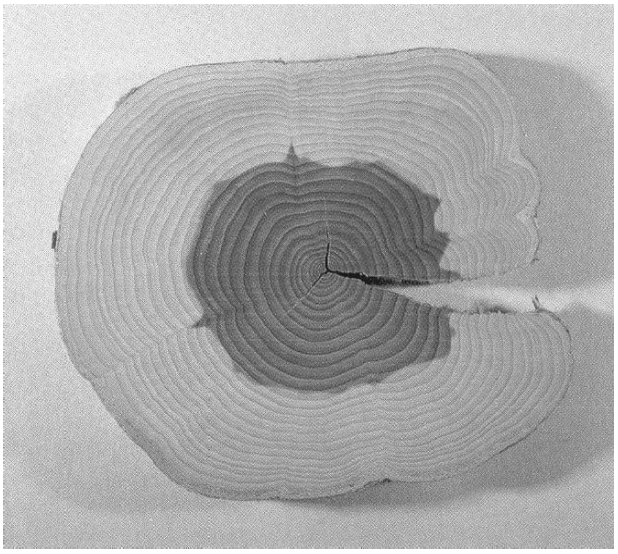
Cambium: the growing (generative) layer between the xylem and phloem.

Xylem: principle strengthening and water conducting tissue of the stem, roots, and branches.

Phloem: inner bark, principal function to distribute manufactured foodstuffs.

Bark: dead, outer tissue that protects the cambium from the external environment and exposure to pathogens and physical injury.

Vessel: the composite, tube-like structure found in hardwoods from the fusion of cells in a longitudinal column.



Fibre: an elongated cell with pointed ends and a thick or infrequently thin wall.

Rays: ribbon-shaped tissue extending in a radial direction across the grain of the wood.

Sapwood and Heartwood:

In mature trees, the xylem has both living and dead cells.

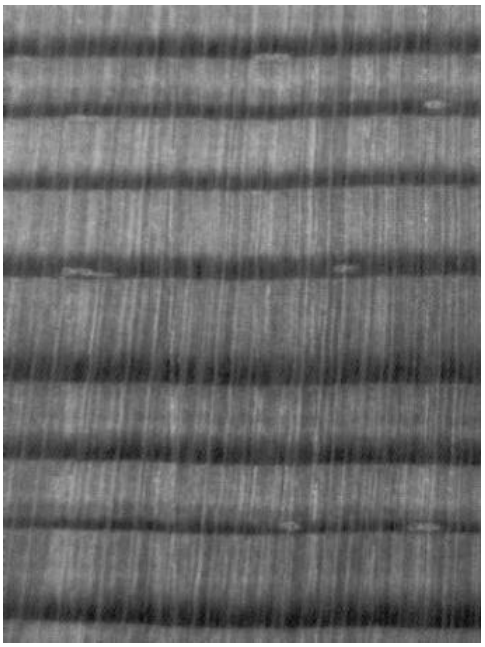
Sapwood contains the only living cells in the xylem (not all sapwood cells are alive either) and has a conductive function.

Heartwood is composed of dead cells and lends mechanical support only.

Growth rings:

Mark annual growth boundaries in trees grown in temperate climates

Often composed of 2 distinct segments



Early wood (spring wood) Late wood (summer wood)

Early wood and latewood cells have different characteristics

Cell Differences within Growth Rings

Earlywood

- Large Radial diameter cells
- Lower density than latewood

Latewood

- Smaller radial diameter cells
- Thicker cell walls

Irregularities in Annual Ring Formation

False rings

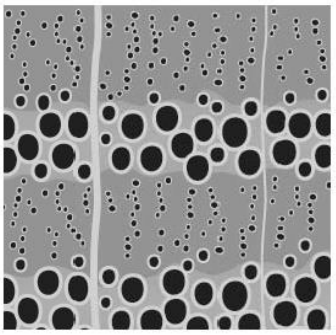
- Growth interrupted by environment (e.g. defoliation)
- Slow growth may cause formation of latewood type cells

Discontinuous rings

- Cambium was dormant in one region
- One-sided crowns, suppressed, or over mature trees

Trees grown in tropical environments

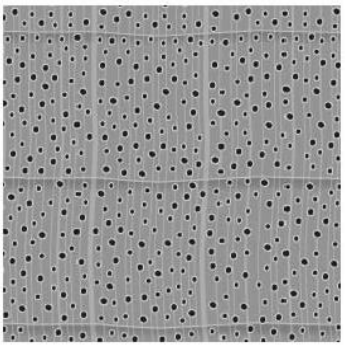
- Almost continual growth can limit occurrence of rings
- In some climates, stopping and restarting of growth can give more than one growth increment in a year



Ring-porous: The largest pores are in the early wood while those in the late wood are more evenly distributed and uniform in size. These woods typically have distinct figures and patterns, and the uneven uptake of stain (the large pores soak up more color).

Semi- ring/diffuse porous- Pores are large in the early wood and

smaller toward the latewood, but without the distinct zoning, as seen in ring-porous woods.



Ring porous wood

Diffuse porous- Pores are distributed fairly evenly across the early wood and latewood. Most domestic diffuse porous woods have relatively small-diameter pores, but some tropical woods of this type(e.g. mahogany) have rather large pores.

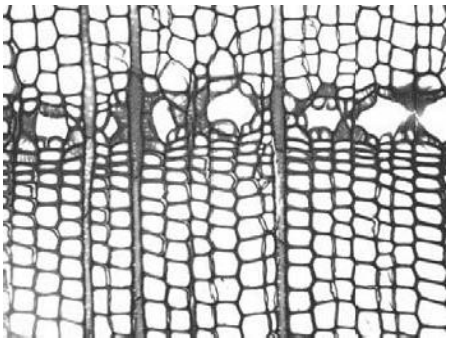
Non porous- Softwoods (gymnospermous wood) don't have vessel cells. However, different softwoods have different growth-ring characteristics. In white pine, the rings are non-distinct, and stain uptake is fairly even, as in diffuse porous woods. In yellow pine, where the rings are clearly visible, stain uptake in early wood is more pronounced than in latewood, as in ring-porous woods**.**

Diffuse porous wood

Criteria for identification…...

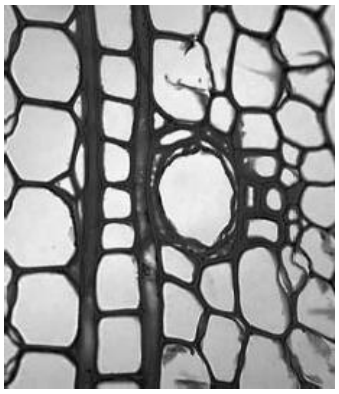
- Structure of tracheid walls and xylem rays
- Resin canals
- Pitting in rays
- Crystals

Resin canals:



Traumatic resin canals (produced by injury)

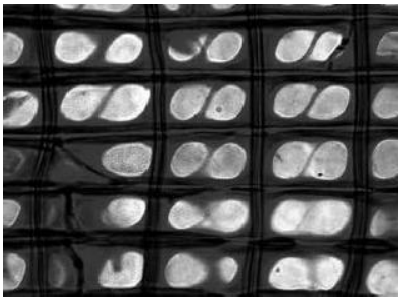
Resin canals with thin-walled epithelial cells



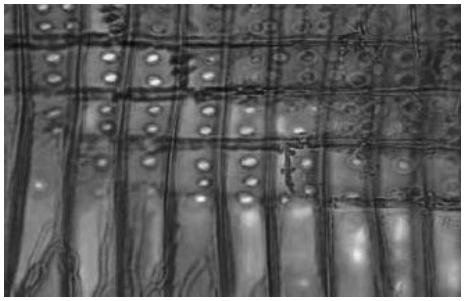
Resin canals with thick walled epithelial cells

The wood formed immediately after wounding will contain traumatic resin canals. Wounding may be caused by freezing, fires, or mechanical damage. Traumatic resin canals do not have as regular ashape as the normal resin canals, and are formed by some species that normally do not have resin canals.

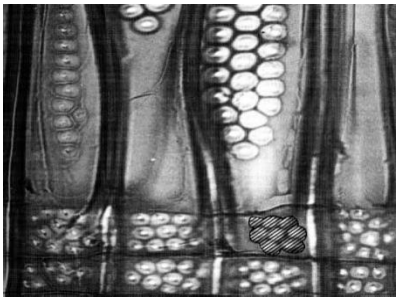
Pits:



Large window-like pits(large pinoid pits-occupying practically the entire cross-field)



Cupressoid pits (The pit aperture is approx. the same size as the pit border)



Araucaroid (pits 2 or more rows alternating pits side by side)

Crystals:

In ordinary cells

- In chambered cells
- In idioblasts
- Raphides/ druses
- Oil/ mucilage cells

Since 1930s: became increasingly more common in forensic applications (Lane et al., 1990). Cell wall: particularly important for two reasons:

◊it is not easily digested by most organisms and therefore persists when other plant features are destroyed (Bock and Norris, 1997)

◊ the size, shape, and pattern of cell walls is often taxon-specific (Lane et al., 1990).

Unique cell types: sclereids, trichomes- are useful in identifying botanical material (Lane et al.,1990).

Tissue organization of different plant parts (Willey and Heilman, 1987).Wood characteristics (secondary tissue organization)

Roots: Frequently found with exposed or shallowly buried bodies

Plant roots, like their above-ground counterparts, exhibit annual growth rings that can be useful in pinning down the post-mortem interval, or at least the time since the body came to be at the locationwhere it was found.

Three ways in which the roots can be used to date the remains or otherwise characterize the burialsite:

(1) **Examine root development after it has been damaged.** When a grave is dug or the ground otherwise disturbed, roots can be damaged but still continue growing. If the meristematic zone is damaged, no secondary xylem cells can be produced, leaving a permanent lesion. The number of growth rings laid down after

the lesion indicates the number of years since the damage occurred.

(2) **Examine roots in direct contact with the remains.** Roots in contact with the bones or personal effects of the deceased can be cross-sectioned at the point of contact and the annual rings counted, establishing a minimum time frame for time since death. The contact must be penetrative, i.e. the roots must be growing through clothing or bones, in order for the interpretation tobe valid.

(3) **Examine branch growth.** Annual longitudinal growth of the roots, in addition to radial growth, can be estimated, and a time frame determined from the length of a root from its point of contact with the remains to its distal end.

Stomach contents:

Characteristic cell types from food plants can be used to identify a victim's last meal (Bock and Norris, 1997). Knowledge about which can be useful in determining the victim's whereabouts or actions prior to death

Some of these cell types include (Dickison, 2000):

Sclereids (pears)

Starch grains (potatoes and other tubers)Raphide crystals (pineapple)

Druse crystals (citrus, beets, spinach) Silica bodies (cereal grasses and bamboos)

Time since death can be approximated by the state of digestion of the stomach contents

It normally takes at least a couple of hours for food to pass from the stomach to the small intestine

A meal still largely in the stomach implies death shortly after eating, while an empty or nearly-empty stomach suggests a longer time period between eating and death (Batten, 1995).

However, there are numerous mitigating factors to take into account:

- the extent to which the food had been chewed
- the amount of fat and protein present
- physical activity undertaken by the victim prior to death
- mood of the victim
- physiological variation from person to person.

All these factors affect the rate at which food passes through the digestive tract.

A Case Study:

In a case where a young woman had been stabbed to death, witnesses reported that she had eaten herlast meal at a particular fast food restaurant.

Provenance

Her stomach contents did not match the limited menu of the restaurant, leading investigators concluded that she had eaten at some point after being seen in the restaurant.

The investigation led to the apprehension of a man whom the victim knew, and with whom she had shared her actual final meal (Dickison, 2000).

Tree ring analysis (Dendrochronology) is a common technique for dating masterworks by European

painters, many of which were painted directly on wood.

Given that the samples are in good condition, analysts can pinpoint the exact year when the tree, fromwhich the wood for the painting was taken, was cut down.

Case Study

A Peter Paul Reubens painting originally dated 1616 was shown to be at least 10 years younger, and a painted wall panel recovered from a house in Switzerland in the 1970s was determined to have been painted on spruce harvested in 1497 (Schweingruber, 1988).

Dendrochronology techniques are useful in determining the provenance of wooden art objectsand musical instruments

In one case, two violins forming part of an inheritance were claimed to have been made by Antonio Stradivari.

The sounding-boards of the instruments were x-rayed and compared to standard curves for sprucefrom the Alpine region of northern Italy, where Stradivarius is known to have worked.

The oldest rings from the samples dated to 1902 and 1894 respectively for the two violins. Furthermore, these oldest rings were not the outermost rings of the wood from which the violins wereconstructed.

Allowing for a period of seasoning before the wood could be used to make the instruments, analyses showed that the violins could not have been made before 1910.

Given that Stradivari did his best work at the turn of the 17th century, the instruments were deemed to be fakes (Schweingruber, 1988).

The Kidnapping of Charles Lindbergh, Jr. (March 01, 1932)

A critical piece of evidence in the case was a crude homemade wooden ladder left at the scene. Xylotomist Arthur Koehler of the United States Forest Service undertook a meticulous examination of the ladder and when the case finally came to trial four years later, offered the first botanical testimony ever to be heard and accepted in American courts.

The ladder had been constructed in three sections, presumably for ease of transport Koehler identified each side rail and rung with a number and identified each piece to species. Through careful examination of the characteristic milling marks left on each piece and comparisons with local mills, he was able to trace all

components of the ladder back to their respective retail sources.

He also noted distinctive marks left on the wood by a dull, nicked hand plane. Of particular interest was rail #16, a piece of low- grade pine which had four distinctive square nail holes. It was also relatively unweathered.

The low grade of the wood, the nail holes, and its unweathered condition suggested that particular piece of wood had been

removed from some interior construction. Without a suspect however, progress on the case was slow. In September of 1934, some of the notes used to pay the ransom were used at a gas station by Bruno Hauptmann, a carpenter who lived in the Bronx, New York City. He was arrested when $14 600 of the ransom money was found in his garage.

Upon searching the attic for more ransom money, police noticed that one of the floorboards was eight feet shorter than the others. The square nail holes in rail 16 lined up exactly with holes in one of the attic floor beams, and the annual ring pattern of rail 16 matched that of the short floorboard. A hand plane recovered from Hauptmann's garage was indeed dull and damaged, and made marks identical to those on the ladder and on a homemade shelf in the Hauptmann garage.

Hauptmann was convicted of kidnapping and murder and was executed on April 3rd, 1936.

Pollen Analysis:

- Pollen and spores are chemically extracted from samples
- To identify pollen and spores, specialists can use a compound light microscope, a scanning electronic microscope, reference collections that may consist of photos and illustrations or perhaps even actual dried specimens arranged systematically (herbariums).
- Pollen and spore evidence that has been collected, analyzed, and interpreted can be presented in court.
- These “fingerprints” can be used to confirm certain aspects of a crime.

How to collect pollen and spore?

- During an investigation, control samples must be collected as well as evidence samples.
- Samples must be collected wearing gloves and with clean tools (such as brushes and cellophane tape) and placed in sterile containers, which then must be sealed and labeled with care.

- Sampling instruments must be cleaned after each use, or new ones must be used.
- Collected evidence must be secured, and the chain of custody must be maintained.

Pollen Fingerprint

Pollen fingerprint is the number and type of pollen grains found in a geographic area at a particular time of year.

Four (4) essential parts-

(1) number of pollen grains
(2) type of pollen grains
(3) found in a certain area
(4) at a particular time

What it does?

Pollen fingerprint can link a piece of evidence to a particular place and time.

Advantages of pollen analysis in forensic botany:

Since both pollen and spores have resistant structures, they at times can help to determine such things as

Whether a victim/suspect was moved or not

Where is the crime's location, whether it occurred in a city or in the village In

which season it may have occurred

How do you think pollen collected here differs from pollen in Darjeeling?

How would pollen collected in the summer differ from pollen collected in the winter?

How should you analyze the pollen?

What instruments or techniques should you use?

Srebrenica massacre or Srebrenica genocide (part of the Bosnian War)

Some of the more than 6,10 graveston s at the Srebrenica-Potočari Memorial and Cemeteryfor t e Victims of the 1995 massacre	
Location	**S ebrenica, Bosnia and erzegovina**
Date	**1 –22 July 1995**
Target	**Bosniak men and boys**
Attack type	**Military assault, mass murder, ethnic cleansing, genocide**
Deaths	**8,373**
Perpetrators	**Army of Republika Srpska** **S orpions paramilitary group**

In July 1995 a massacre of civilians followed the burial in seven mass graves. Three months later the bodies were exhumed and transported to a number of new burial sites in an attempt to conceal evidence of the massacre and to deflect blame.

Could palynology help in relating the secondary burial sites with the original primary burial sites and thereby more closely link the massacre to known or suspected perpetrators

Case study:

"Murder on the Danube" case

The first time police used pollen to solve a crime was in Austria in 1959. A forensic scientist studying the mud on a murder suspect's boot found what turned out to be a 20-million-year-old pollengrain from a hickory tree. That species no longer grew in Austria then. But investigators were able to locate a Miocene sediment outcrop on the Danube River, from which such a pollen grain could have become recycled into the environment.

"We know you killed him," they told the murder suspect, in the best police procedural fashion, "and we know where." Then they took him to the outcrop. The suspect was so unnerved that he led them straight to the victim's grave.

Re-exhumation was commenced by the United Nations International Criminal Tribunal for the former Yugoslavia (ICTY) in 1997.

The objective of the palynological and associated soil analyses was to determine the environmental profile of the original burial sites and to try and find a connection with the secondary sites where different environmental profiles existed. These analyses were done independently of all other forensic investigations being undertaken at the same time to ensure credibility. Five of the original sites and 19

secondary sites were investigated in detail. Analyses indicated that the original mass graves each hada different geological and botanical profile which easily separated each site. Samples from all sites were taken from the fill of the graves close to and from varying distances away from bodies or body parts and from sediment surrounding the mass graves. Over 240 comparator samples were collected from various sources to determine the background pollen profile of each site, the local vegetation was recorded and abundance of major species determined. Results showed that sediments and associated spores and pollen from the original mass graves had indeed been transferred along with the bodies to the numerous secondary burial sites and that even some botanical evidence at the primary burial sites pointed to the original execution site or sites. Pollen found at the original burial sites consisted of cultivated grasses (cereals including wheat and maize), wild grasses (Poaceae), pines (*Pinus*), spruces (*Picea*), sedges (Cyperaceae), beeches (*Fagus*) and walnut (*Juglans*). Various combinations of these pollen types, plus many others, were subsequently found in exotic material sampled from within the graves at the secondary burial sites, proving a link between the original and subsequent burial sites. The accuracy of the evidence provided by the pollen was confirmed by other types of forensic evidence and presented in court. The investigation showed the importance of being able to differentiate between imported and local fill used at grave scenes. This was probably the first time that environmental profiling was used systematically in a war crimes investigation.

An example of the use of forensic palynology in assessing an alibi.

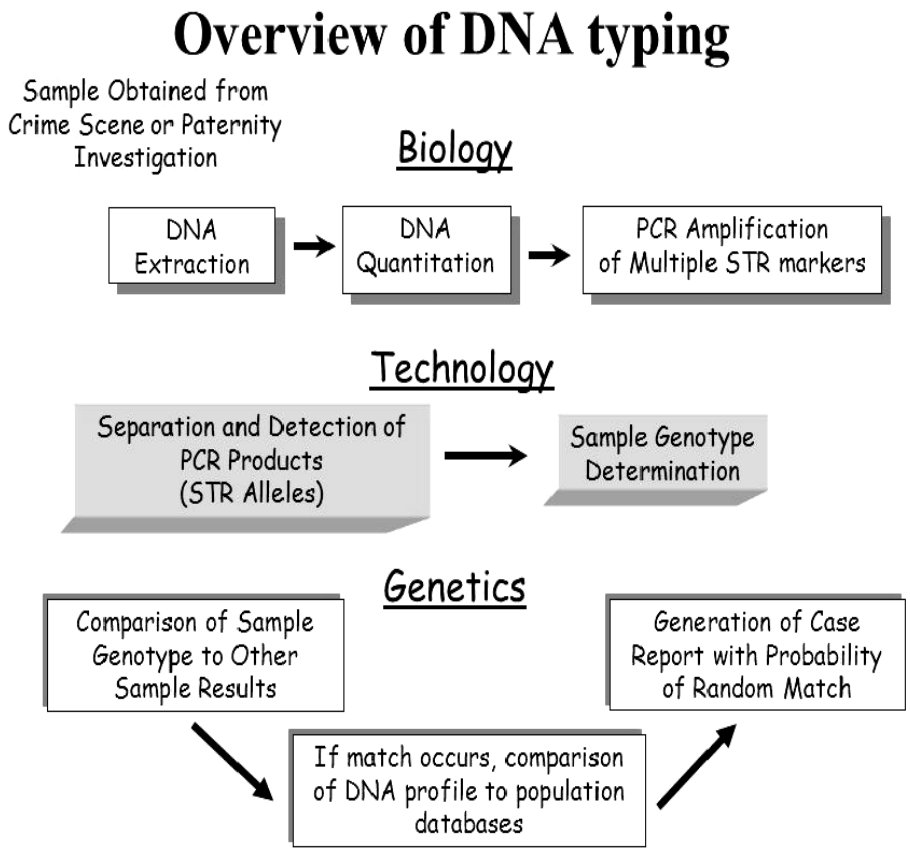
A man was found shot in the back on Mount Holdsworth in the Tararua Ranges north of Wellington, the capital city of New Zealand. Police investigations pinpointed one individual who had been seen in the area, knew, and had the means and motive to kill the victim. His alibi was that an eyewitness was mistaken as he never had been in the area and the jacket he was reported to have been wearing had been purchased in The Netherlands and brought to Wellington, where it never had left the city. Furthermore the distinctive board shorts that he was reported to have been wearing had been purchased in a small coastal New Zealand town after the victim had been murdered. Pollen of *Nothofagus menziesii*, a mountain plant, on the clothing suggested that the alibi was untrue and thatthe clothing had been in mountains in the vicinity of Mount Holdsworth or a similar mountain scene where *Nothofagus menziesii* was growing.

General DNA Typing Considerations

Many different types of biological evidence are commonly submitted to forensic science laboratories for examination. Initially, evidence that was suitable for DNA analysis was limited to human biological substances that contain nucleated cells. This limitation has been overcome in the last 5years with the implementation of mitochondrial DNA sequencing, and plant and animal DNA testing in the forensic arena. The quantity of DNA that can be extracted from common biological sources varies greatly with evidentiary samples. Note that, in practice, crime scenes samples may contain considerably less usable DNA depending on environmental conditions. DNA has been isolated from other sources such asgastric fluids and fecal stains. However, it can be difficult to generate a DNA profile from these

sources due to significant degradation.

Four factors affect the ability to obtain a DNA profile. The first issue is sample quantity. The sensitivity of PCR-based DNA typing methods is noteworthy but still limited. The second concern is sample degradation. Prolonged exposure of even a large bloodstain to the environment or to bacterial contamination can degrade the DNA and render it unsuitable for further analysis. The thirdconsideration is sample purity. While most DNA typing methods are robust, dirt, grease, some dyes in fabrics, and so forth can seriously compromise the DNA typing process. Environmental insults will not change DNA type "A" into type "B," but they can adversely affect the ability of the scientist to obtain a complete DNA profile from the sample. The last issue is the ratio of major contributor to the minor contributor(s) from DNA mixtures. Both DNA profiles can readily be detected with, for



example, 1:1 and 3:1 DNA mixtures. However, with mixture ratios such as 25:1 or 50:1, the quantity of the major DNA species prevents the detection of the minor source.

Plant DNA Typing Considerations

For DNA analysis of botanical samples, many of the same considerations for human identity testing still apply. The four factors (quantity, quality, purity, and mixture ratios) may all play a role in determining whether further testing should be performed or whether an interpretable DNA profile can be obtained from a plant sample. Two factors need to be considered for obtaining a sufficient quantity of plant DNA for profiling: size of the plant fragment and ability of the analyst to mechanically break

the plant cell wall for the sufficient release of nuclear DNA contents. The size of a plant fragment can seldom be enhanced other than to initially collect as much sample as possible from the crime scene. However, new technologies are aiding in the DNA extraction process for improving DNA yield from plant samples. Traditionally, plant cells have been disrupted by the mechanical pressure of grinding by hand in a mortar and pestle with the addition of liquid nitrogen to increase the fragility of the cell wall. Some useful equipment for breaking plant cell walls may be a mechanical homogenizer (consisting of a rotating blade with a serrated tip) or the addition of metallic beads prior to high-speed oscillation of samples. In addition, several companies manufacture commercial plant DNA extraction kits that minimize the number of centrifugation steps and maximize DNA yields. The use of some of these commercial kits also seems to improve the final purity of the plant DNA so that the PCR amplification process is not inhibited by the presence of secondary plant metabolites (e.g., tannins, resins, phenolic compounds). Finally, care in collection and preservation of botanical evidence as well as the development of plant species-specific probes and PCR primer sets in the future will address mixture interpretation issues.

Preparation of Genomic DNA from Plant Tissue

Although many commercially available plant DNA extraction kits have recently become available, there are some very reliable traditional chemical extractions for plant DNA purification. The following protocol is one example of such a procedure.

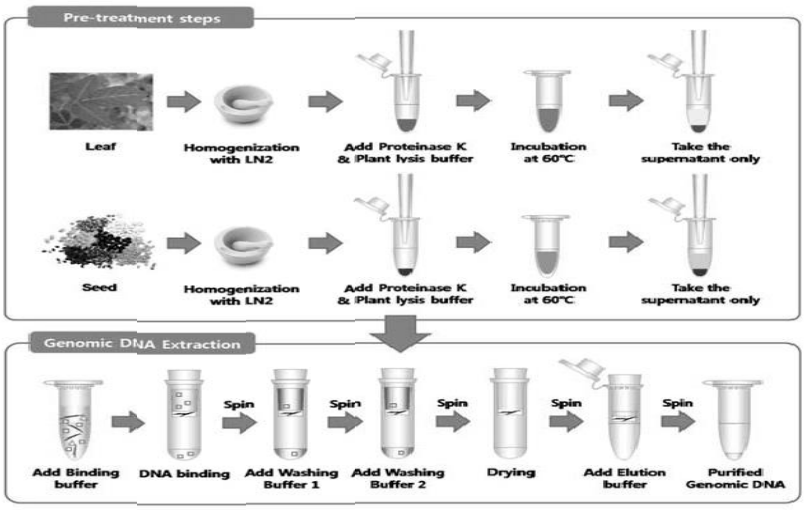
Materials:

i. Cold, sterile waterLiquid nitrogen
ii. Extraction buffer (100 mM Tris-Cl, pH 8; 100 mM EDTA; 250 mM NaCl; 100 μg/mL proteinase K)10% (wt/vol) N-lauroylsarcosine
iii. Isopropanol
iv. TE buffer (10 mM Tris-Cl, pH 8; 1 mM EDTA)
v. Cesium chloride (CsCl)
vi. 10 mg/mL ethidium bromide
vii. CsCL-saturated isopropanol Ethanol
viii. 3M sodium acetate, pH 5.2
ix. Beckman JA-14, JA-20, JA-21, and Vti80 rotors

Procedure:

- Harvest 10–50 g of fresh plant tissue.
- Rinse with cold, sterile water; dry with tissues; and freeze with liquid nitrogen. Mechanically grind with a mortar and pestle.

- Transfer frozen powder to a 250-mL centrifuge bottle and immediately add 5 mL of extraction buffer per gram of starting fresh plant tissue; gentle mix. Add 10% N lauroylsarcosine to a final concentration of 1%; incubate 2 h at 55°C.
- Centrifuge for 10 min in a JA-14 rotor at 6000 rpm chilled to 4°C. Save the supernatant and repeat this step if debris is still present.
- Add 0.6 volumes of isopropanol and mix. If no visible precipitate forms, place at –20°C for 30 min. Centrifuge for 15 min in a JA-14 rotor at 8000 rpm at 4°C. Discard the supernatant.
- Resuspend the pellet in 9 mL TE buffer, add 9.7 g of solid CsCl, mix, and incubate 30 min on ice. Centrifuge for 10 min in a JA-20 rotor at 8000 rpm at 4°C and save the supernatant.
- Add 0.5 mL of 10 mg/mL ethidium bromide and incubate 30 min on ice.
- Centrifuge 10 min in a JA-20 rotor at 8000 rpm at 4°C. Transfer supernatant to two 5-mL ultracentrifuge tubes and seal.
- Centrifuge in a Vti80 rotor at 20°C for 4 h at 80,000 rpm. Collect the band of DNA using a 15-G needle and syringe.
- Remove any residual ethidium bromide by repeated extraction of the DNA band with CsCl-saturated isopropanol.
- Add 2 volumes of water and 6 volumes of 100% ethanol, mix, and incubate for 1 h at –20°C. Centrifuge for 10 min in a JA-20 or JA-21 rotor at 8000 rpm at 4°C.
- Resuspend the pellet in TE buffer and precipitate again by adding 1/10 volume of 3M sodium acetate and 2 volumes of 100% ethanol. Repeat the centrifugation step. Air-dry the pellet by inverting the tube over a paper towel for 15 min, then resuspend the pellet in the TE buffer. The expected plant DNA yield should be 104 μg of 50 Kb high molecular-weight DNA per gram of starting plant tissue.



Classic forensic botany cases: Case histories by using Plant anatomy and systematic, Palynology, Plant ecology, Limnology, Plant Molecular Biology and DNA, Drug enforcementand DNA.

Forensic botany is defined as the use of plant evidence in court. It is subdivided into several botanical subspecialties, including plant anatomy (the study of cellular features), plant systematics (taxonomy and species identification), palynology (the study of pollen), plant ecology (plant succession patterns), and limnology (the study of freshwater ecology). In the past decade, molecular biology and the use of DNA methods have been important tools to further the research of these disciplines.

Plant Anatomy and Systematics

Plant systematics is a broad discipline that includes the study of evolutionary relationships between plant species and taxonomy (the identification of plant species). Species identification is a typical first step in analyzing botanical evidence for casework. Plant anatomy uses features such as leaf morphology and tree growth ring patterns to aid in species identification and in performing physical matches of evidence, respectively. The kidnapping and death of Charles Lindbergh's young son in 1932 was the first modern-era case to use such botanical evidence in court. A wooden ladder was usedto gain access to the second-story nursery to kidnap Lindbergh's son.

Arthur Koehler, a wood identification expert for the Forest Products Laboratory of the U.S. Forest Service in Wisconsin, was able to provide critical evidence against Bruno Richard Hauptman, who was later convicted of the crime.

Koehler had an excellent academic record and had provided evidence in several cases prior to the famous Lindbergh trial. His testimony is noteworthy since the use of scientific experts in the mid-1930s was generally limited to fingerprints, handwriting, bullet comparisons, and analyses of stomach contents. Koehler first identified the four tree species used to construct the ladder as yellow pine, ponderosa pine, Douglas fir, and birch, via microscopic analysis of wood-grain patterns. Next, Koehler analyzed the tool marks left on the wood from both the commercial planing mill and the hand plane used by Hauptman during the construction of the ladder. Koehler used oblique light in a darkened room to observe the plane patterns left on the wood. Amazingly, he was able to trace the wood by the mill plane marks to a shipment of yellow pine delivered to the National Lumber and Millwork Company in Bronx, New York. The hand-plane marks on the ladder exactly matched those made by a hand plane found in Hauptman's possession. Finally, Koehler compared the annual growth rings and knot patterns on rail 16 of the ladder to a section of wood in Hauptman's attic. The patternof knots and growth rings on rail 16 exactly matched the exposed end of wood.

Palynology

Forensic palynology refers to the use of pollen in criminal investigations. The major plant groups identified as pollen sources include flowering plants, conifers, and ferns. Ferns technically produce spores instead of pollen but are included in pollen types. Pollen is microscopic and not visually obvious trace evidence during crime scene collection, but is retained on clothing, embedded in carpets, and pervasive in soil. Pollen grain morphology can be used to identify a plant genus and often the species.

Crime scenes that are restricted to a few square meters, such as a rape scene or the entry point of a burglary, are good choices for pollen evidence.

Localized areas have a specific pollen distribution pattern representing the combination of plant species found in the surrounding vegetation. Common pollen types from plants that use wind for distribution (e.g., grass, bracken spores) will be less useful than pollen from uncommon, poorly distributed species (e.g., flax, willow). Insect-distributed pollen is typically deposited within a few feet of the source plant. Pollen analysis consists of species identification and an estimation of the percentage that each plant species represents in an evidentiary sample. A similar pollen composition from shoeprints and from the shoes that made the prints indicates a strong match correlation.

Pollen evidence collected from a burglary entrance and a suspect's shoes, for example, could providea linkage in a case. A case that exemplifies the use of pollen in criminal casework is described by Horrocks et al. In Auckland, New Zealand, a prostitute alleged that the defendant had raped her in an alleyway approximately seven meters from his car after failing to pay her in advance for her services. The defendant claimed that he had never been more than one meter away from the car and had not entered the alleyway. Furthermore, he claimed that he had not had sex with the victim and the soil on his clothing was from the driveway area. An examination of the crime scene and the evidence showed no footprints and no seminal fluid stains. A soil sample was collected from the defendant's clothing, the disturbed area of ground in the alleyway, and from the driveway area near the defendant's car. All the soil samples were prepared for pollen analysis by deflocculation with potassium hydroxide, acetylation to remove cellulose and organic matter, and a silicate removal step using hydrofluoric acid. Samples were bleached to remove additional organic matter and analyzed under a microscope for pollen identification and counting. The types of pollens were similar between the two locations, but the amounts of each type were different in each sample. The alleyway contained 76% *Coprosma* (an evergreen shrub) pollen, but the driveway sample contained only 8%.The defendant's clothing contained approximately 80% *Coprosma* and only small amounts of other pollen species. These results support the victim's account of the sexual assault taking place in the alleyway. Pollen analysis has also been utilized to establish time of death.

In Magdeburg, Germany, a mass grave containing 32 male skeletons was discovered in February of 1994. The identities of both the victims and the murderers were unknown. Two hypotheses were proposed: (1) the victims were killed in the spring of 1945 by the Gestapo at the end of World War II, or (2) the victims were Soviet soldiers killed by the secret police after the German Democratic Republic revolt in June of 1953. The ability to differentiate between the spring and summer was critical to solving the case. Pollen analysis was performed on 21 skulls. Seven of the skull nasal cavities contained high amounts of pollen from plantain, lime tree, and rye. All of these plant species release pollen during the months of June and July. Pollen analysis supported the hypothesis that the remains were of Soviet soldiers killed by the Soviet secret police after the June 1953 revolt.

Plant Ecology

Plant ecology involves studying **the growth patterns of vegetation in areas that have been disturbed**. These patterns and the vegetative (non-flowering) portion of plants can be useful in estimating time of death.

- For example, when a body is discovered lying on top of a weed plant with a broken top, useful information can be obtained to define **time windows for when the death occurred**. A certain amount of shading will eventually kill a plant, so if the weed plant is **lacking chlorophyll, a minimum amount of time must have alreadyelapsed**.
- If **new shoots are present at the base of the plant**, this may establish a **second time window**. Agricultural research on many plant species has defined the time for new shoot initiation after the top of a plant has been removed.
- The **length of the new shoot** can sometimes establish a **third time window**.

In one case, the brain cavity of a skull was filled with plant roots. The anatomy and developmental stage of the roots indicated that the plant was approximately one year old, and the plant was putatively identified as *Ranunculus ficaria* L (buttercup family). The predictable stages of plant development were useful in estimating the time that the skeletal remains had been in their present location. The investigators were able to determine that the skeleton had been there for at least one year; however, a maximum time could not be established. The plant could have developed secondarily sometime after the body had lain in its present location, so a maximum time estimate was not possible.

Limnology

Limnology is the study of freshwater ecology and can be applied to a subset of forensic cases. In particular, aquatic plants (e.g., algae, diatoms) have been useful to link suspects to a crime scene or to establish that drowning occurred in freshwater

Limnology is the study of freshwater ecology

and can be applied to a subset of forensic cases. In particular, aquatic plants (e.g., algae, diatoms) have been useful to link suspects to a crime scene or to establish that drowning occurred in freshwater

In 1942 Incze demonstrated that, during drowning, diatoms could enter the systemic circulation via the lungs. Their presence can be demonstrated in tissues like liver, brain and bone marrow.

Properties for which diatom is used as clue:

a: Diatom populations vary seasonally in lakes, rivers, and ponds.

- In early spring, diatom populations expand in freshwater.
- Following this expansion, the live diatoms decline but a large number of dead diatoms remain in summer water.
- In the fall, a second diatom expansion occurs and then progressively declines through the winter months.

b: Each species has a characteristic shape and refractive pattern from the silica in the cell wall which can be used for identification.

c: Diatoms do not occur naturally in the body.

When a person drowns in freshwater, diatoms are taken in along with water into the lungs. The diatoms are dispersed to the internal organs of the body.

In a study of 771 cases, the diatom test was positive for 28% of presumed freshwater drowning cases but was rarely positive for domestic water drowning. The low rate of diatoms observed in domestic drowning could be traced back to cleaning agents containing crushed diatoms for abrasives.

In 1991, two young boys were brutally attacked by teenage assailants while fishing at a suburban pond in Connecticut. The boys were held at knifepoint, bound with duct tape, and savagely beaten and dragged into the pond to drown. One boy managed to get free, save himself, and rescue his friend. After many hours of criminal investigation, three suspects were apprehended. To link the suspects to the crime scene, investigators seized the sedimentencrusted sneakers of both the victims and the assailants and analyzed them for algal and diatom species. microscopic analysis of samples from each pair of sneakers plus reference samples from the pond showed the same species and distribution pattern of each species. These results supported the position that the samples all originated from a common freshwater location.

Plant Molecular Biology and DNA

The previously discussed cases have relied on traditional botanical methods for species identification. In the age of DNA analysis, forensic botany is using molecular biology to aid in criminal and civil investigations. The first criminal case to gain legal acceptance using plant DNA typing was a homicide that occurred in 1992 in Arizona's Maricopa County. A woman's body was found under a paloverde tree in the Arizona desert. Near the body was a beeper eventually traced to a suspect, Mark Bogan. A few seed pods from a paloverde tree were found in the back of Bogan's truck. Officials wanted to know if DNA could match those seed pods to the tree where the body was discovered. Dr. Timothy Helentjaris from the University of Arizona used a technique called randomly amplified polymorphic DNA (RAPD) analysis to generate a band pattern from the evidence in question. He also surveyed a small population of other paloverde trees to determine if the band patterns were unique to each individual. His convincing testimony on plant evidence helped convict Mark Bogan of murder. RAPD marker analysis has also been utilized in civil court cases to identify patent infringements. In Italy, RAPD analysis of a patented strawberry variety "Marmolada" helped settle a lawsuit involving the unauthorized commercialization of the plant.

Molecular methods can be used to identify a plant species from minute leaf fragments and pollen grains. Forensic botanists have utilized DNA technology because often botanical trace evidence does not contain the necessary morphological or histological features that would allow one to identify a plant at the genus or species level. This is particularly true for fragmented and deteriorated plant material.

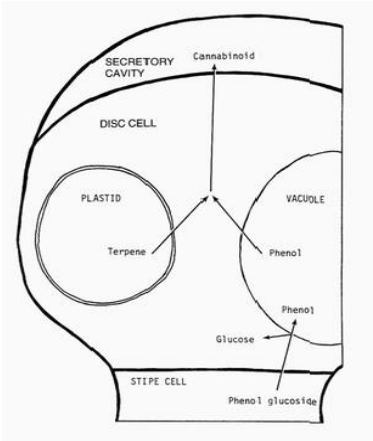
The Bode Technology Group Inc. (Dr. Robert Bever; Springfield, VA) is developing and utilizing molecular methods to analyze botanical trace evidence. This type of analysis is a valuable tool for potentially linking an individual to a crime scene or physical evidence to a geographic location. One useful application for the molecular analysis of botanical trace evidence is the identification of a geographic region where a kidnapped individual may be located. Based on flowering times and the plant species represented in the trace pollen evidence found with a ransom note, a geographic region may be identified and would provide the police with an investigative lead. Plant systematists have characterized many loci that are useful for the identification of plants, including several nuclear (18S, ITS1, ITS2) and chloroplast (rbcL, atpB, ndhF) genes.

Bode Technology Group has identified a DNA extraction, cloning, and sequencing procedure to identify plants using some of those genes. Using these methods, they have identified numerous species of plants from physical evidence. These include species of algae, evergreens, and many flowering herbs, shrubs, and trees. Many plants have a limited geographic distribution or grow in specific habitats. Some of these locations will be general areas, such as roadsides or areas of new construction. Other locations will be more specific, like the Mohave Desert or southern Florida, for plant species that have a severely restricted geographic range. Linking botanical trace evidence to a geographic region could provide law enforcement and investigators with valuable information.

Drug Enforcement and DNA:

Often in drug seizures, identification of the seized substance is a problem, especially if the plant material is fragmented and dried. A variety of methods are currently employed to identify *Cannabis sativa* L. (marijuana).

- Marijuana can be identified by **classical botanical characterization**, especially if the **type of cystolith** found in an individual's vehicle back to a plant from a growing area near the suspect'shome, for example.
- The Connecticut State Forensic Science Laboratory is developing a **molecular strategy for creating unique band patterns from marijuana samples, which uses a technique called amplified fragment length polymorphism (AFLP) analysis.**



AFLP analysis is based on the selective PCR amplification of restriction fragments from a total digest of plant DNA to generate a fluorescent band pattern. Validation of the AFLP technique on marijuana samples and the construction of a marijuana AFLP database for comparative purposes were developed at the Connecticut State Forensic Science Laboratory.

Cannabis has special trichomes. The resin within these trichomes has encouraged our ancestors to work with this plant for millennia, spreading the seeds world wide. **Cannabis resin contains strong smelling medicinal compounds that reflect in the sun to call extra attention**. Trichomes, the resin gland heads, protect the cannabis plant from insects and predation by being sticky and intoxicating. Trichomes also protect growing seeds from the sun and wind by reflecting solar radiation and creatinga physical barrier.

The Cannabinoids and Terpenoids in Resin:

Cannabis resin includes: cannabinoids (THC, CBD, CBN, THC-V, over 90 discovered so far), terpenoids (smell and "high" modulation), and plant waxes/oils. One group of cannabinoids, the THCgroup, is primarily responsible for the psychoactive properties of cannabis.

Forensic botany uses plant DNA to trace crimes

Date: **February 29, 2016**

Source: **Sam Houston State University**

Summary: **The field of forensic botany is being advanced with the publication of two recent studies**

that use marijuana DNA to link drug supplies and pollen DNA to aid in forensic investigations. Publication in International Journal of Legal Medicine

A test was developed to individualize samples of marijuana that could be used to link drugs across cases. The study examined 11 cases containing a total of 199 samples from U.S. Customs and Border Protection, which included four matching genotypes indicating drugs came from the same source. "The use of a DNA-based method for identification will allow federal law enforcement agencies (e.g., U.S. Customs and Border Protection (CBP) and Drug Enforcement Administration (DEA)) to form links between cases involving the cross-border trafficking of *Cannabis*," said Dr. David Gangitano, one of the authors on the study.

Publication in Science and Justice

Researchers found that pine pollen could provide a viable source of DNA for criminal investigations. Pine pollen remains viable for DNA testing for at least two weeks on cotton clothing and can help linka suspect or victim to a location. The study examined a new collection device, a high-throughput method for DNA extraction and amplification, and a newly-developed system for genotyping.

This study has shown that pollen can be a stable source of forensic DNA evidence, as a proof-of-principle, and that may persist on cotton clothing for at least 14 days of wear. This method can be applied in forensic cases where pollen grains larger than 10 μm (e.g., from herbs or trees) may be transferred to clothing (worn by suspect or victim) by primary contact.

11. Intellectual Property Rights

Definition

Intellectual property Right (IPR) is a term used for various legal entitlements which attach to certain types of information, ideas, or other intangibles in their expressed form. The holder of this legal entitlement is generally entitled to exercise various exclusive rights in relation to the subject matter of the Intellectual Property. The term intellectual property reflects the idea that this subject matter is the product of the mind or the intellect, and that Intellectual Property rights may be protected at law in the same way as any other form of property. Intellectual property laws vary from jurisdiction to jurisdiction, such that the acquisition, registration or enforcement of IP rights must be pursued or obtained separately in each territory of interest.

Intellectual Property

Intellectual property is an intangible creation of the human mind, usually expressed or translated into a tangible form that is assigned certain rights of property. Examples of intellectual property include an

author's copyright on a book or article, a distinctive logo design representing a soft drink company and its products, unique design elements of a web site, or a patent on the process to manufacture chewing gum.

Intellectual Property Rights

Intellectual property rights (IPR) can be defined as the rights given to people over the creation of their minds. They usually give the creator an exclusive right over the use of his/her creations for a certain period of time.

Intellectual property (IP) refers to creations of the mind: inventions, literary and artistic works, and symbols, names, images, and designs used in commerce.

Differentiating between Intellectual Property and Physical Property

Property can be of two types: Physical property and Intellectual property. Physical property exists in physical shape as tangible products whereas intellectual property is intangible. IP is created by the human mind, physical property on the other hand includes wealth which have physical existence like buildings, land, metal wealth and cash. Furthermore, physical property can be both private or public in nature but IP is essentially private, i.e., it belongs to a person or legal entity (companies, institutions). Physical property has limits of attainment but intellectual property is limitless and can be created in a never-ending way.

Types of Intellectual Property Rights

One can broadly classify the various forms of IPRs into two categories:

• IPRs that stimulate inventive and creative activities (patents, utility models, industrial designs, copyright, plant breeders' rights and layout designs for integrated circuits) and

• IPRs that offer information to consumers (trademarks and geographical indications).

IPRs in both categories seek to address certain failures of private markets to provide for an efficient allocation of resources. IP is divided into two categories for ease of understanding:

1. Industrial Property

2. Copyright

Industrial property, which includes inventions (patents), trademarks, industrial designs, and geographic indications of source; and

Copyright, which includes literary and artistic works such as novels, poems and plays, films, musical works, artistic works such as drawings, paintings, photographs and sculptures, and architectural designs. Rights related to copyright include those of performing artists in their performances, producers of phonograms in their recordings, and those of broadcasters in their radio and television programs

Intellectual property shall include the right relating to:

i. Literary, artistic and scientific works;

ii. Performance of performing artists;

iii. Inventions in all fields of human endeavour;

iv. Scientific discoveries;

v. Industrial designs;

vi. Trademarks, service marks and etc;

vii. Protection against unfair competition.

Rights protected under Intellectual Property

The different types of Intellectual Property Rights are:

i. Patents

ii. Copyrights

iii. Trademarks

iv. Industrial designs

v. Protection of Integrated Circuits layout design

vi. Geographical indications of goods

vii. Biological diversity

viii. Plant varieties and farmers rights

ix. Undisclosed information

Importance of IPR

With the increasing domain of knowledge economy, IP is also assuming much greater importance each day. Since the Trade Related aspects of Intellectual Property Rights (TRIPS) was agreed upon under the aegis of the World Trade Organization (WTO) IP has gained a rightful importance in the eyes of policy makers, academicians, researchers and business executives. The importance lies in:

Exclusive rights in use of IP is the biggest initiative that motivates the creation of intellectual property. Artists, Inventors, Innovators are encouraged and motivated in a big way to go for creative pursuits. They realize that they can get financial benefits from their creations, their creations will remain protected through legal instruments and they can further carry on their creative pursuits.

Intellectual property is the primary means of wealth creation in the society. Goods and services created through IP benefit the whole society at large. It can lead to cost saving, better production skills and methods and protect natural and physical resources from getting depleted in an unsustainable way.

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International Conventions

The most important Conventions and Treaties at International level include:

1. The Paris Convention for the Protection of Industrial Property

2. The Berne Convention for the Protection of Literary and Artistic Works

3. The WIPO Copyright Treaty (WCT)

4. The Patent Cooperation Treaty (PCT)

5. Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure

6. The Madrid Agreement Concerning the International Registration of Marks and the Protocol Relating to the Madrid Agreement

7. The Hague Agreement Concerning the International Deposit of Industrial Designs
8. The Trademark Law Treaty (TLT)
9. The Patent Law Treaty (PLT)
10. Treaties on Classification
11. Special Conventions in the Field of Related Rights: The International Convention for the Protection of Performers, Producers of Phonograms and Broadcasting Organizations ("the Rome Convention")
12. The WIPO Performances and Phonograms Treaty (WPPT)
13. The International Convention for the Protection of New Varieties of Plants
14. The Agreement on Trade-Related Aspects of Intellectual Property Rights ("TRIPS") and WIPO-WTO Cooperation
15. Progressive Development of International Intellectual Property Law

World Intellectual Property Organisation

The World Intellectual Property Organization (WIPO) is one of the 17 specialized agencies of the United Nations, located in Geneva, Switzerland. The Organization has External Offices at Rio de Janeiro in Brazil, Tokyo in Japan, Singapore and New York.

The **mission of WIPO** is to promote innovation and creativity for the economic, social and cultural development of all countries, through a balanced and effective international intellectual property system.

The origin of WIPO goes back to 1883 and 1886 when the Paris Convention for the Protection of Industrial Property and the Berne Convention for the Protection of Literary and Artistic Works, respectively, were concluded. Both Conventions provided for the establishment of an international bureau. The two bureaus were united in 1893 and, in 1970, were replaced by the World Intellectual Property Organization, by virtue of the WIPO Convention.

The WIPO Convention, the constituent instrument of the World Intellectual Property Organization (WIPO), was signed at Stockholm on July 14, 1967, entered into force in 1970 and was amended in 1979. WIPO is an intergovernmental organization that became in 1974 one of the specialized agencies of the United Nations system of organizations.

WIPO currently has 185 Member States, and 68 intergovernmental organizations (IGOs) and International non-governmental organizations (NGOs) and 63 National NGOs that are accredited as observers at WIPO meetings.

The core tasks of WIPO are:

- working with Member States to support a balanced evolution of international IP law
- administering treaties

- assisting governments and organizations in developing the policies, structures and skillsneeded to harness the potential of IP for economic development
- servicing global registration systems for trademarks, industrial designs and appellations of origin and a global filing system for patents
- delivering arbitration, mediation and other dispute resolution services
- promoting respect for IP
- providing a forum for informed debate and for the sharing of IP knowledge
- identifying IP-based solutions that can help confront global challenges and maximize the benefits of the IP system for all

How WIPO works?

WIPO's Member States determine the strategic direction and activities of the Organization. They meet in the Assemblies, committees and working groups. The WIPO Secretariat, or International Bureau, is based in Geneva. WIPO staff, drawn from more than 90 countries, includes experts in diverse areas of IP law and practice, as well as specialists in public policy, economics, administration and IT. The respective divisions of the Secretariat are responsible for coordinating the meetings of Member States and implementing their decisions; for administering the international IP registration systems; for developing and executing the programs designed to achieve WIPO's goals; and for providing a repository of IP expertise to assist its members.

It has to present a program and a budget every 2 years about performance measures, budget planning for all the events of the organization which requires member state approval. t is a self financing organization and its funds are majorly used for organizing events.

WIPO Arbitration and Mediation Centre was created in 1994 for the settlement of international commercial disputes arising between private parties located in Geneva, Switzerland and it has an office in Singapore

WIPO's Goals

The strategic goals defined in WIPO's revised Program and Budget for 2008/09 are:

- A balanced evolution of the international normative framework for IP
- Provision of premier global IP services
- Facilitating the use of IP for development
- Coordination and development of global IP infrastructure
- World reference source for IP information and analysis
- International cooperation on building respect for IP
- Addressing IP in relation to global policy issues

- A responsive communications interface between WIPO, its Member States and all stakeholders
- An efficient administrative and financial support structure to enable WIPO to deliver its programs

Indian Scenario

History of Copyright Law in India

Modern copyright law developed in India gradually, in a span of more than 150 years. Copyright law entered India in 1847 through an enactment during the East India Company's regime. According to the 1847 enactment, the term of copyright was for the lifetime of the author plus seven years post- mortem. But in no case could the total term of copyright exceed a period of forty-two years. The government could grant a compulsory licence to publish a book if the owner of copyright, upon the death of the author, refused to allow its publication. The act of infringement comprised in a person's unauthorized printing of a copyright work for (or as a part of attempt of) "sale hire, or exportation", or "for selling, publishing or exposing to sale or hire".

Suit or action for infringement was to be instituted in the "highest local court exercising original civil jurisdiction." The Act provided specifically that under a contract of service copyright in "any encyclopedia, review, magazine, periodical work or work published in a series of books or parts" shall vest in the "proprietor, projector, publisher or conductor." Infringing copies were deemed to be copies of the proprietor of copyrighted work. Importantly, unlike today, copyright in a work was notautomatic. Registration of copyright with the Home Office was mandatory for the enforcement of rights under the Act. However, the Act also specifically reserved the subsistence of copyright in the author, and his right to sue for its infringement to the extent available in law other than the 1847 Act. At the time of its introduction in India, copyright law had already been under development in Britain for over a century and the provisions of the 1847 enactment reflected the learnings from deliberations during this period.

In 1914, the then Indian legislature enacted a new Copyright Act which merely extended most portions of the United Kingdom Copyright Act of 1911 to India. It did, however, make a few minor modifications. First, it introduced criminal sanctions for copyright infringement (sections 7 to 12).

Second, it modified the scope of the term of copyright; under section 4 the "sole right" of the author to "produce, reproduce, perform or publish a translation of the work shall subsist only for a period of ten years from the date of the first publication of the work." The author, however, retained her "sole rights" if within the period of ten years she published or authorised publication of her work a translation in any language in respect of that language. The 1914 Act was continued with minor adaptations and modifications till the 1957 Act was brought into force on 24th January, 1958.

History of Patent Law in India

The first legislation in India relating to patents was the Act VI of 1856. The objective of this legislation was to encourage inventions of new and useful manufactures and to induce inventors to disclose secret of their inventions. The Act was subsequently repealed by Act IX of 1857 since it had been enacted without the approval of the sovereign. Fresh legislation for granting 'exclusive privileges' was introduced in 1859 as Act XV of 1859. This legislation contained certain modifications of the earlier legislation, namely, grant of exclusive privileges to useful inventions only and extension of priority period from 6 to 12 months. The Act excluded importers from the definition of inventor. The 1856 Act was based on the United Kingdom Act of 1852 with certain departures including allowing assignees to make application in India and also taking prior public use or publication in India or United Kingdom for the purpose of ascertaining novelty.

The Act of 1859 provided protection for invention only and not for designs whereas United Kingdom had been protecting designs from 1842 onwards. To remove this lacuna, the 'Patterns and Designs Protection Act' (Act XIII) was passed in 1872. This Act amended the 1859 Act to include any new and original pattern or design or the application of such pattern to any substance or article of manufacture within the meaning of 'new manufacture'. The Act XV of 1859 was further amended in 1883 by XVI of 1883 to introduce a provision to protect novelty of the invention, which prior to making application for their protection were disclosed in the Exhibitions of India. A grace period of 6 months was provided for filing such applications after the date of the opening of such Exhibition.

In 1888, new legislation was introduced to consolidate and amend the law relating to invention and designs in conformity with the amendments made in the UK law.

In 1911, the Indian Patents and Designs Act, 1911, (Act II of 1911) was brought in replacing all the previous legislations on patents and designs. This Act brought patent administration under the management of Controller of Patents for the first time. This Act was amended in 1920 to provide for entering into reciprocal arrangements with UK and other countries for securing priority. In 1930, further amendments were made to incorporate, *inter-alia*, provisions relating to grant of secret patents, patent of addition, use of invention by Government, powers of the Controller to rectifyregister of patent and increase of term of the patent from 14 years to 16 years. In 1945, another amendment was made to provide for filing of provisional specification and submission of complete specification within nine months. After Independence, it was felt that the Indian Patents & Designs Act, 1911 was not fulfilling its objective. It was found desirable to enact comprehensive patent law owing to substantial changes in political and economic conditions in the country. Accordingly, the Government of India constituted a committee under the Chairmanship of Justice (Dr.) Bakshi Tek Chand, a retired Judge of Lahore High Court, in 1949, to review the patent law in India in order to ensure that the patent system is conducive to the national interest.

The Committee submitted its interim report on 4th August, 1949 with recommendations for prevention of misuse or abuse of patent right in India and for amendments to sections 22, 23 & 23A ofthe Patents & Designs Act, 1911 on the lines of the United Kingdom Acts of 1919 and 1949.

Based on the recommendations of the Committee, the 1911 Act was amended in 1950 (Act XXXII of 1950) in relation to working of inventions and compulsory licence/revocation. In 1952, an amendment as made to provide compulsory licence in relation to patents in respect of food and medicines, insecticide, germicide or fungicide and a process for producing substance or any invention relating to surgical or curative devices, through Act LXX of 1952. The compulsory licence was also available on notification by the Central Government. Based on the recommendations of the Committee, a bill was introduced in the Parliament in 1953 (Bill No.59 of 1953). However, the bill lapsed on dissolution of the Lok Sabha.

In 1957, the Government of India appointed Justice N. Rajagopala Ayyangar Committee to examine the question of revision of the Patent Law and advise government accordingly. The report of the Committee, which comprised of two parts, was submitted in September, 1959. The first part dealt with general aspects of the patent law and the second part gave detailed note on the several clauses of the lapsed bill of 1953. The first part also dealt with evils of the patent system and solution with recommendations in regard to the law. The committee recommended retention of the patent system, despite its shortcomings. This report recommended major changes in the law which formed the basis of the introduction of the Patents Bill, 1965. This bill was introduced in the Lok Sabha on 21st September, 1965, which, however, lapsed.

In 1967, an amended bill was introduced which was referred to a Joint Parliamentary Committee and on the final recommendation of the Committee, the Patents Act, 1970 was passed. This Act repealed and replaced the 1911 Act so far as the patents law was concerned. However, the 1911 Act continued to be applicable to designs. Most of the provisions of the 1970 Act were brought into force on 20th April, 1972 with the publication of the Patents Rules, 1972.

This Act remained in force for about 24 years till December 1994 without any change. An ordinance effecting certain changes in the Act was issued on 31st December 1994, which ceased to operate after six months. Subsequently, another ordinance was issued in 1999. This ordinance was later replaced by the Patents (Amendment) Act, 1999 that was brought into force retrospectively from 1st January, 1995. The amended Act provided for filing of applications for product patents in the areas of drugs, pharmaceuticals and agro chemicals though such patents were not allowed. However, such applications were to be examined only after 31st December, 2004. Meanwhile, the applicants could be allowed Exclusive Marketing Rights (EMRs) to sell or distribute these products in India, subject to fulfillment of certain conditions.

The second amendment to the 1970 Act was made through the Patents (Amendment) Act, 2002 (Act 38 0f 2002). This Act came into force on 20th May, 2003 with the introduction of the new Patents Rules, 2003 by replacing the earlier Patents Rules, 1972.

The third amendment to the Patents Act, 1970 was introduced through the Patents (Amendment) Ordinance, 2004 with effect from 1st January, 2005. This Ordinance was later replaced by the Patents (Amendment) Act, 2005 (Act 15 Of 2005) on 4th April, 2005 which was brought into force from 1st January, 2005.

Intellectual Property Trends – India

During 2009-10, 34,287 patent applications were filed, 6069 examined and 6168 patents granted. The number of applications filed by the Indian applicants was 7044. Out of the applications filed by the Indian applicants, Maharashtra accounted for the maximum number followed by Delhi, Tamil Nadu, Karnataka, Andhra Pradesh, West Bengal etc.

During 2009-10, 6092 design applications were filed, 6266 examined and 6025 registered. Thenumber of applications filed by the Indian applicants was 4267. The number of registered designs in force at the end of 2009-10 was 39008.

During 2009-10, 1,41,943 trademark applications were filed, 25875 examined and 67,490 registered. The number of applications filed by the Indian applicants was 1,34,403. The total number of registered trademarks as of 31st March, 2010 is 8,22,825.

During 2009-10, 40 Geographical indications applications were filed and 14 were registered. A total of 120 Geographical Indications have been registered till 31st March, 2010.

During 2009-10, 142 applications were received for access of bio-resources for research/commercial use, transfer of research results, intellectual property rights and third party transfer. Totally, 13 agreements have been signed. So far, 11 patents have been granted on the applications cleared by NBA. The NBA has also received a royalty amount of Rs.37.89 lakhs from the applicants who have exported bio-resources.

In 2010-11, a total of 642 applications representing 28 crops were received by the Authority for seeking plant variety protection under the Act. The applications belong to new (395), extant (216), farmers' varieties (30) and essentially derived variety (1) categories.

Initiatives of Government of India towards protection of IPR

1. The Government has brought out A Handbook of Copyright Law to create awareness of copyright laws amongst the stakeholders, enforcement agencies, professional users like the scientific and academic communities and members of the public.

2. National Police Academy, Hyderabad and National Academy of Customs, Excise and Narcotics conducted several training programs on copyright laws for the police and customs officers.

3. The Department of Education, Ministry of Human Resource Development, Government of India has initiated several measures in the past for strengthening the enforcement of copyrights that include constitution of a Copyright Enforcement Advisory Council (CEAC), creation of separate cells in state police headquarters, encouraging setting up of collective administration societies and organization of seminars and workshops to create greater awareness of copyright laws among the enforcement personnel and the general public.

4. Special cells for copyright enforcement have so far been set up in 23 States and Union Territories,

i.e. Andhra Pradesh, Assam, Andaman & Nicobar Islands, Chandigarh, Dadra & Nagar Haveli, Daman & Diu, Delhi, Goa, Gujarat, Haryana, Himachal Pradesh, Jammu & Kashmir, Karnataka, Kerala, Madhya Pradesh, Meghalaya, Orissa, Pondicherry, Punjab, Sikkim, Tamil Nadu, Tripura and West Bengal.

5. The Government also initiates a number of seminars/workshops on copyright issues. The participants in these seminars include enforcement personnel as well as representatives of industry organizations.

Copyright:

Copyright is the set of exclusive rights granted to the author or creator of an original work, including the right to copy, distribute and adapt the work. Copyright lasts for a certain time period after which the work is said to enter the public domain. Copyright gives protection for the expression of an idea and not for the idea itself. For example, many authors write textbooks on physics covering various aspects like mechanics, heat, optics etc. Even though these topics are covered in several books by different authors, each author will have a copyright on the book written by him / her, provided the book is not a copy of some other book published earlier.

Content and Substance

According to Section 14 of the Act, "copyright" means the exclusive right subject to the provisions of this Act, to do or authorise the doing of any of the following acts in respect of a

work or any substantial part thereof, namely:-

(a) in the case of a literary, dramatic or musical work, not being a computer programme, -

i. to reproduce the work in any material form including the storing of it in any medium by electronicmeans;

ii. to issue copies of the work to the public not being copies already in circulation;

iii. to perform the work in public, or communicate it to the public;

iv. to make any cinematograph film or sound recording in respect of the work;

v. to make any translation of the work;

vi. to make any adaptation of the work;

vii. to do, in relation to a translation or an adaptation of the work, any of the acts specified in relationto the work in sub-clauses (i) to (vi);

(b) in the case of a computer programme,-

i. to do any of the acts specified in clause (a);

ii. to sell or give on commercial rental or offer for sale or for commercial rental any copy of thecomputer programme:

Provided that such commercial rental does not apply in respect of computer programmes where theprogramme itself is not the essential object of the rental.

(c) in the case of an artistic work,-

i. to reproduce the work in any material form including depiction in three dimensions of a twodimensional work or in two dimensions of a three dimensional work;

ii. to communicate the work to the public;

iii. to issue copies of the work to the public not being copies already in circulation;

iv. to include the work in any cinematograph film;

v. to make any adaptation of the work;

vi. to do in relation to an adaptation of the work any of the acts specified in relation to the work in sub-clauses (i) to (iv);

(d) In the case of cinematograph film, -

i. to make a copy of the film, including a photograph of any image forming part thereof;

ii. to sell or give on hire, or offer for sale or hire, any copy of the film, regardless of whether suchcopy has been sold or given on hire on earlier occasions;

iii. to communicate the film to the public;

(e) In the case of sound recording, -

i. to make any other sound recording embodying it;

ii. to sell or give on hire, or offer for sale or hire, any copy of the sound recording regardless ofwhether such copy has been sold or given on hire on earlier occasions;

iii. to communicate the sound recording to the public.

iv. Explanation : For the purposes of this section, a copy which has been sold once shall be deemed to be a copy already in circulation.

Period and Assignment of Copyright

Sec.18 of the Copyright Act, 1957 deals with assignment of copyright. The owner of the copyright in an existing work or the prospective owner of the copyright in a future work may assign to any person the copyright either wholly or partially and either generally or subject to limitations and either for the whole term of the copyright or any part thereof.

The mode of assignment should be in the following manner:

- Assignment should be given in writing and signed by the assignor or by his duly authorized agent.
- The assignment should indentify the work and specify the rights assigned and the duration and territorial extent of such assignment.
- The assignment should also specify the amount of royalty payable, if any, to the author or his legal heirs during the currency of the assignment and the assignment may be subject to revision, extension or termination on terms mutually agreed upon by the parties.
- Where the assignee does not exercise the rights assigned to him within a period of one year from the date of assignment, the assignment in respect of such rights will be deemed to have lapsed after the expiry of the said period unless otherwise specified in the assignment.

The period of assignment will be deemed to be 5 years from the date of assignment unless specifically mentioned. If the territorial extent of assignment of the rights is not specified, it will be presumed to extend within India.

Infringement and Remedies

Some of the commonly known acts involving infringement of copyright: Making infringing copies for sale or hire or selling or letting them for hire;

Permitting any place for the performance of works in public where such performance constitutes infringement of copyright;

Distributing infringing copies for the purpose of trade or to such an extent so as to affect prejudiciallythe interest of the owner of copyright ;

Public exhibition of infringing copies by way of trade; andImportation of infringing copies into India.

Civil Remedies for Copyright Infringement

A copyright owner can take legal action against any person who infringes the copyright in the work.The copyright owner is entitled to remedies by way of injunctions, damages and accounts.

Penalties

Any person who knowingly infringes or abets the infringement of the copyright in any work commits criminal offence under Section 63 of the Copyright Act. The minimum punishment for infringementof copyright is imprisonment for six months with the minimum fine of Rs. 50,000/-. In the case of a second and subsequent conviction the minimum punishment is imprisonment for one year and fine of Rs. one lakh.

Purpose, Policy and Meaning of Patent

Patent is a grant for an invention by the Government to the inventor in exchange for full disclosure of the invention. A patent is an exclusive right granted by law to applicants / assignees

to make use of and exploit their inventions for a limited period of time (generally 20 years from filing). The patent holder has the legal right to exclude others from commercially exploiting his invention for the duration of this period. In return for exclusive rights, the applicant is obliged to disclose the invention to the public in a manner that enables others, skilled in the art, to replicate the invention. The patent system is designed to balance the interests of applicants / assignees (exclusive rights) and the interests of society (disclosure of invention).

Meaning of 'Invention' under Patent Law

Sec.2(1)(J) - Invention" means a new product or process involving an inventive step and capable of industrial application

What is meant by 'New"?

The invention to be patented must not be published in India or elsewhere, or in prior public knowledge or prior public use with in India or claimed before in any specification in India A featureof an invention that involves technical advance as compared to the existing knowledge or have economic significance or both and makes the invention not obvious to a person skilled in the art.

What can be patented?

Any invention concerning with composition, construction or manufacture of a substance, of an article or of an apparatus or an industrial type of process.

What cannot be patented?

Inventions falling within Section 20(1) of the Atomic Energy Act, 1962

Who are the beneficiaries of the patent grant?

1. The inventor is secure from competition and can exploit the invention for his gain.

2. For the public the invention becomes public knowledge. The technology is freely available after expiry of patent and cheaper and better products become available.

Objectives and Patentability

What is meant by patentable invention?

A new product or process, involving an inventive step and capable of being made or used in an industry. It means the invention to be patentable should be technical in nature and should meet the following criteria –

- **Novelty:** The matter disclosed in the specification is not published in India or elsewhere before the date of filing of the patent application in India.
- **Inventive Step:** The invention is not obvious to a person skilled in the art in the light of the prior publication/knowledge/ document.
- **Industrially applicable:** Invention should possess utility, so that it can be made or used in an industry.

What is not an 'Invention'?

According to Sec 3 of the Patent Act, 1970

- Frivolous inventions
- Inventions contrary to well established natural laws
- Commercial exploitation or primary use of inventions, which is contrary to public order or morality
- which causes serious prejudice to health or human, animal, plant life or to theenvironment
- Mere Discovery of a Scientific Principle or
- Formulation of an Abstract Theory or
- Discovery of any living thing or
- Discovery of non–living substance occurring in nature
- Mere discovery of any new property or new use for a known substance or of themere use of a known process, machine or apparatus, unless such known processresults in a new product or employs at least one new reactant.
- Substance obtained by mere admixture resulting only in the aggregation of the properties of the components thereof or a process for producing such substance
- Mere arrangement or re-arrangement or duplication of known devices, each functioning independently of one another in a known way
- Method of Agriculture or Horticulture
- Any process for medicinal, surgical, curative, prophylactic, diagnostic, therapeutic or other treatment of human beings or a similar treatment of animals to render them free of disease or to increase their economic value orthat of their products
- Plants & animals in whole or any part thereof other than micro- organisms, but including seeds, varieties an d species and essentially biological process for production or propagation of plants & animals
- mathematical method or
- business method or
- algorithms or
- computer programme per se
- A literary,dramatic, musical or artistic work or any other aesthetic creation including cinematographic work and television productions
- Presentation of information
- Topography of integrated circuits.
- Inventions which are Traditional Knowledge or an aggregation or duplication ofknown properties

of traditionally known component or components

Procedure:

Application is required to be filed according to the territorial limits where the applicant or the first mentioned applicant in case of joint applicants, for a patent normally resides or has domicile or has a place of business or the place from where the invention actually originated. If the applicant for the patent or party in a proceeding having no business place or domicile in India, the appropriate office will be according to the address for service in India given by the applicant or party in a proceeding . The appropriate office once decided in respect of any proceedings under the Act shall not ordinarily be changed. The four patent offices are located at Kolkata, Mumbai, Delhi & Chennai.

Publication:

All the applications for patent, except the applications prejudicial to the defence of India or abandoned due to non-filing of complete specification within 12 months after filing the provisional or withdrawn within 15 months of filing the application, are published in the Patent Office Journal just after 18 months from the date of filing of the application or the date of priority whichever is earlier. The publication includes the particulars of the date of the application, application number, name and address of the applicant along with the abstract. The applications for patent are not open for public inspection before publication. After the date of publication of the application, as stated above, the complete specification along with provisional and drawing, if any, abstract , application on any formor on plain paper and any correspondence between the office and applicant may be inspected at the appropriate office by making a written request to the Controller in the prescribed manner and on the payment of prescribed fee.

Request for examination:

An application for patent will not be examined if no request is made by the applicant or by any other interested person in Form-18 with prescribed fee of Rs.2,500/- or Rs.10,000/- for natural person and other than natural person respectively, within a period of 48 months from the date of priority of the application or from the date of filing of the application, whichever is earlier.

Where no request for examination of the application for patent has been filed within the prescribed period, the aforesaid application will be treated as withdrawn and, thereafter, application cannot be revived.

Examination:

Application for patent, where request has been made by the applicant or by any other interested person, will be taken up for examination, according to the serial number of the requests received on Form 18. A First Examination Report (FER) stating the objections/requirements is communicated to the applicant or his agent according to the address for service ordinarily within six (06) months from the date of request for examination or date of publication whichever is later. Application or complete

specification should be amended in order to meet the objections/requirements within a period of 12 months from the date of First Examination Report (FER). No further extension of time is available in this regard. If all the objections are not complied with within the period of 12 months, the application shall be deemed to have been abandoned. When all the requirements are met the patent is granted, after 6 months from the date of publication, the letter patent is issued, entry is made in the register of patents and it is notified in the Patent Office, Journal.

Withdrawal of patent application:

The application for patent can be withdrawn at least 3 (Three) months before the first publication which will be 18 (Eighteen) months from the date of filing or date of priority whichever is earlier. The application can also be withdrawn at any time before the grant of the patent. The application withdrawn after the date of publication cannot be filed again as it is already laid open for public inspection. However, application withdrawn before the publication can be filed again provided it is not opened to public otherwise

Opposition proceedings to grant of patents

Where an application for a patent has been published but a patent has not been granted, any person may, in writing represent by way of opposition to the Controller against the grant of any Patent. The representation shall be filed at the appropriate office and shall include a statement and evidence, if any, in support of the representation and a request for hearing if so desired.

Grant of Patent

When all the requirements are met or in case of opposition under section 25(1), if the opposition is decided in favour of the applicant, the patent is granted, after 6 months from the date of publication under section 11 A, the letter patent is issued, entry is made in the register of patents and it is notified in the Patent Office, Journal, thereafter opening the application, specification and other related documents for public inspection on payment of prescribed fee.

Term and Date of Patent

Term of every patent will be 20 years from the date of filing of patent application, irrespective of whether it is filed with provisional or complete specification. Date of patent is the date on which the application for patent is filed. The term of patent in case of International applications filed under the Patent Cooperation Treaty designating India, will be 20 years from the International filing date accorded under the Patent Cooperation Treaty. A patent will have cease to effect on the expiration of the period prescribed for the payment of any renewal fee, if that fee is not paid within the prescribed period.

Rights and Obligations of Patent Holder

Where a patent covers a product, the grant of patent gives the patentee the exclusive right to prevent others from performing, without authorisation, the act of making, using, offering for sale, selling or

importing that product for the above purpose. Where a patent covers a process, the patentee has the exclusive right to exclude others from performing, without his authorisation, the act of using that process, using and offering for sale, selling or importing for those purposes, the product obtained directly by that process in India. Where a patent is granted to two or more persons, each of those persons will be entitled to an equal undivided share in the patent unless there is an agreement to the contrary.

Infringement and Remedies

Infringement of a patent consists of the unauthorized making, importing, using, offering for sale or selling any patented invention within the India.

Remedies against infringement of a patented invention

1. Interlocutory Injunction
2. A patent owner at the start of a trial can request for an interim injunction to restrain the defendant from committing the acts complained of until the hearing of the action or further orders. Permanent injunction is given based on the merits of the case at the end of the trial.
3. Relief of damages: An award of damages focuses on the losses sustained by the claimant. A patent owner is entitled to the relief of damages as compensation to the patentee and not punishment to the infringer.
4. Account of profits: Account of profits focuses on the profits made by the defendant, without reference to the damage suffered by the claimant at the hands of the defendant. The purpose of the account is to prevent the unjust enrichment of the defendant by the use of the claimant's invention.The patent owner may also opt for the account of profits where he has to prove use of invention andthe amount of profit derived from such illegal use.

Penalties

1. Contravention of secrecy provisions relating to certain inventions (Sec.118) - If any person fails to comply with any directions given under section 35 or makes or causes to be made an application in contravention of section 39 he shall be punishable with imprisonment up to 2 years or with fine orwith both. (Section 35 deals with secrecy directions relating to inventions relevant for defence purposes and Section 39 deals with residents not to apply for patents outside India without prior permission.
2. Falsification of entries in register etc (Sec.119) - If any person makes, or causes to be made, a false entry in any register kept under this Act, he shall be punishable with imprisonment for a term that mayextend to 2 years or with fine or with both.
3. Unauthorized claim of patent rights (Sec.120) - If any person falsely represents that any article soldby him is patented in India or is the subject of an application for a patent in India, he will be punishable with fine that may extend to Rs.1,00,000. The use of words 'patent',

4. Patented', 'Patent applied for', 'Patent pending', 'Patent registered' without mentioning the name of the country means they are patented in India or patent applied for in India.
5. Wrongful use of words, "patent office" (Sec.121) - If any person uses on his place of business or any document issued by him or otherwise the words “patent office” or any other words which reasonably lead to the belief that his place of business is, or is officially connected with, the patent office, he will be punishable with imprisonment for a term that may extend to 6 months, or with fine, or with both.
6. Refusal or failure to supply information (Sec.122) - If any person refuses or fails to furnish information as required under section 100(5) and 146 he shall be punishable with fine, which may go up to Rs 10,00,000/-. If he furnishes false information knowingly he shall be punishable with imprisonment that may extend to 6 months or with fine or with both.
7. Practice by non-registered patent agents (Sec.123) - Any person practicing as patent agent without registering is liable to be punished with a fine of Rs 1,00,000/- in the first offence and Rs.5,00,000/- for subsequent offence.
8. Deals with offences by companies (Sec.124) - When offence is committed by a company as well asevery person in charge of and responsible to the company for the conducts of its business at the timeof the commission of the offence will be deemed to be guilty and will be liable to be preceded against and punished accordingly. Provided that nothing contained in this sub-section shall render any such person liable to any punishment if he proves that the offence was committed without his knowledge or that he exercised all due diligence to prevent the commission of such offence.

Protection of Plant Varieties & Farmers’ Rights

Meaning and Content

A plant variety represents a more precisely defined group of plants, selected from within a species, with a common set of characteristics.

The Protection of Plant Varieties and Farmers' Right Act, 2001 has been enacted to provide for the establishment of an effective system for protection of plant varieties, the rights of farmers and plant breeders and to encourage the development of new varieties of plants.

The objectives of the Act are:

- to establish an effective system for protection of plant varieties, the rights of farmers and plant breeders and to encourage the development of new varieties of plants;
- to recognize and protect the rights of the farmers in respect of their contribution made at any time in conserving, improving and making available plant genetic resources for the development of new plant varieties;
- to protect plant breeders’ rights to stimulate investment for research and development both in

- the public and private sector for development of new plant varieties;
- to facilitate the growth of seed industry in the country that will ensure the availability of high qualityseeds and planting material to the farmers.

Procedure

- ✓ The application for protection under the Act can be made by any of the following persons:
- ✓ Any person claiming to be the breeder of the variety;
- ✓ Any successor of the breeder of the variety;
- ✓ Any person being the assignee or the breeder of the variety in respect of the right to make suchapplication;
- ✓ Any farmer or group of farmers or community of farmers claiming to be breeder of the variety;
- ✓ Any person authorized to apply on behalf of farmers; or
- ✓ Any university or publicly funded agricultural institution claiming to be breeder of the variety.

Criteria for registration of new variety

Novelty - A new variety is deemed to be novel if, at the date of filing of the application for registration for protection, the propagating and harvested material of such variety has not been sold or otherwise disposed of by or with the consent of its breeder or his successor for the purposes of exploitation of such variety for a certain period of time before the date of filing of the application. For sale or disposal of a new variety in India, this time period is earlier than one year. Outside of India, in the case of trees and vines, the time period is earlier than six years. In any other case in India, it is earlier than four years.

Distinctiveness - A new variety is deemed distinct if it is clearly distinguishable by at least one essential characteristic from any other variety whose existence is a matter of common knowledge in any country at the time of filing of the application.

Uniformity - A new variety is deemed uniform if subject to the variation that may be expected from the particular features of its propagation it is sufficiently uniform in its essential characteristics.

Stability - A new variety is deemed stable if it's essential characteristics remain unchanged after repeated propagation or, in case of a particular cycle of propagation, at the end of each such cycle.

Period of Validity

The duration of protection of registered varieties is different for different crops which are asbelow:

1. For trees and vines - 18 years.
2. For other crops - 15 years.
3. For extant varieties - 15 years from the date of notification of that variety by the Central Government under section 5 of the Seeds Act, 1966.

Biodiversity and EnvironmentDocumentation

India has been a party to the Convention on Biological Diversity since 5th June 1992 and ratified the Convention on 18th February 1994. The Convention on Biological Diversity is one of the most broadly subscribed international environmental treaties in the world. Opened for signature at the Earth Summit in Rio de Janeiro Brazil in 1992, it currently has 189 Parties - 188 States and the European Community - who have committed themselves to its three main goals: the conservation of biodiversity, sustainable use of its components and the equitable sharing of the benefits arising out of the utilization of genetic resources. The Secretariat of the Convention is located in Montreal, Canada.

India is also a signatory to Cartagena Protocol on Biosafety signed on 23rd January 2001 and ratified on 11th September 2003.

IPR issues in biodiversity conservation

India enacted The Biological Diversity Act, 2002 and The Biological Diversity Rules, 2004 to fulfill its commitments in the Convention on Biological Diversity and in the Cartagena Protocol on Biosafety. Various states have also enacted state specific Biological diversity rules namely – Kerala Biological Diversity Rules, 2005; Sikkim State Biological Diversity Rules, 2006; Nagaland Biological Diversity Rules, 2010; Rajasthan Biological Diversity Rules, 2010; A.P. State Biological Diversity Rules, 2009, West Bengal Biological Diversity Rules, 2005; U.P. State Biodiversity Rules, 2010; Maharashtra Biological Diversity Rules, 2008 etc. The Biodiversity Act - 2002 primarily addresses access to genetic resources and associated knowledge by foreign individuals, institutions or companies, to ensure equitable sharing of benefits arising out of the use of these resources and knowledge to the country and the people.

The Act has specific provisions about ownership of intellectual property rights associated with exploitation of biodiversity. Industries have to obtain prior consent of the National Biodiversity Authority before exploring the biodiversity in India. In the event of R&D based on exploitation of biodiversity and associated local knowledge, there is a provision for sharing of benefits of such work with the local community. No direct flow of funds is expected to the community. Instead the Union Government will reach the benefits through State Governments to the community.

The Biological diversity Act of 2002 contains 65 sections under 12 chapters while the Biological Diversity Rules of 2004 consists of 24 rules and one schedule. According to Section 2(b) of the Biological Diversity Act, 2002 “Biological Diversity means the variability among living organisms from all sources and the ecological complexes of which they are part and includes diversity within species or between species and of eco-systems..

Access to Plant Genetic Resources and Benefit Sharing

Chapter II of the Biological Diversity Act, 2000 lays down certain regulations with reference to access to Biological Diversity. The following regulations have been placed in Section 3 to 7 of the

said act. Section 3 of the above referred act, requires the following categories of persons to seek previous permission of the National Biodiversity Authority, to obtain any biological resource occurring in India or knowledge associated thereto for research or commercial utilization or for bio- survey and bio-utilization –

- A person who is not a citizen of India.
- A citizen of India who is a non-resident as per section 2(30) of the Income-tax Act, 1961.
- A body corporate, association, organization –o Not incorporated or registered in India; or

o Incorporated or registered in India under any law for the time being in force which has any non-Indian participation in its share capital or management.

Rule 14 of the Biological Diversity Rules, 2004 mentions the procedure for access to biological resources and associated traditional knowledge.

Section 4 of the above referred act requires that every person shall seek the previous approval of the national bio-diversity authority before transferring the results of any research related to any biological recourses occurring in, or obtained from India to any person as referred to in section 3 above. Rule 17 of the Biological Diversity Rules, 2004 mentions the procedure for seeking approval for transferring results of research.

Bioprospecting & Biopiracy

Biodiversity prospecting is the exploration, extraction and screening of biological diversity and indigenous knowledge for commercially valuable genetic and biochemical resources. While it is true that biodiversity prospecting does not always involve the use of indigenous knowledge, it is clear that valuable chemical compounds derived from plants, animals and micro-organisms are more easily identified and are of greatest commercial value when collected with indigenous knowledge and/or found in territories traditionally inhabited by indigenous peoples.

Between 1956 and 1976 the U.S. National Cancer Institute screened over 35,000 plants and animals for anti-cancer compounds. The program was terminated in 1981 because of its failure to identify a greater number of new anti-cancer agents. A retrospective study conducted on the project concluded that the success rate in finding valuable species could have been doubled if medicinal folk knowledge had been the only information used to target species. Similarly in another instance scientists found that 86 percent of the plants used by Samoan healers displayed significant biological activity when tested in the laboratory.

Biopiracy can be defined as the stealing of knowledge from traditional and indigenous communities or individuals. The term can also be used to suggest a breach of a contractual agreement on the access and use of traditional knowledge to the detriment of the provider and bioprospecting without the consent of the local communities. The Action Group on Erosion, Technology and Concentration [ETC

group, Canada (former RAFI)] defines it as "the appropriation of the knowledge and genetic resources of farming and indigenous communities by individuals or institutions seeking exclusive monopoly control (usually patents or plant breeders' rights) over these resources and knowledge".

There is a distinct difference between biopiracy and bioprospecting. The term 'biopiracy' describes the unauthorized and uncompensated taking and use of biological resources. In contrast, bioprospecting refers to the search for valuable active chemical compounds in nature, and involves accessing natural resources through legal means, securing prior informed consent from the custodians of the relevant natural resources and promoting equitable benefit sharing agreements with appropriate parties. Biopiracy deprives not only the custodians of biological resources but also the country concerned.

The modus operandi of the MNCs has been to collect the plant varieties and their germplasms from poor countries in order to cross- breed them with other varieties, and claim that they had invented something novel, non-obvious and of practical use (which are the requirements for acquiring patent rights), and then to patent them in their own countries or in any other country of their choice. Thus even though India is rich in biodiversity and has a rich biodiversity related intellectual heritage, biopiracy directs this wealth away from India and denies us our rights to use our resources and knowledge, for our needs and our economic benefits.

IPR in environmental sustainability

Creating a world that is sustainable for future generations requires transitions towards novel technologies which are environmentally friendly and socially sustainable. While intellectual property rights (IPR) can incentivise technological innovations, the debate on positive and negative effects of IPR over the last decades has identified a number of issues that hamper the diffusion of innovation. Amongst these issues are the increase of costs for negotiating licensing deals when necessary IPRs are distributed amongst several owners, and deterrent effects of the uncertainty whether existing IPRs are infringed and whether the owners are going to sue. Accordingly, increasing IPR protection is discussed as hindering market competition and for its negative impacts on costs of new technologies, most importantly those of high societal. Whereas the list of theoretical issues associated with IPR and diffusion of sustainable technologies is long, we lack empirical evidence about the extent of these potential problems.

IP issues in Biotechnology

Biotechnology is generally defined as "the application of science and technology to living organisms, as well parts, products and models thereof, to alter living or non-living materials for the production of knowledge, goods and services"1 . This definition is deliberately broad and covers all modern biotechnology but also many other traditional or borderline activities. Taken all together, theseactivities belong to what is commonly called "Life Sciences". Biotechnology is a field where technology advances rapidly but returns on investments may be slow. For this reason, it is important for public

research organisations and enterprises to protect the innovation that they generate with Intellectual Property Rights (IPR), which provide a basis for return on investment in research and development, by granting monopoly rights for a certain period of time to their owners.

Biotechnology is usually subdivided into three sectors that may overlap, namely:

Healthcare biotechnology or red biotechnology which plays an important role< in drug discovery (insulin, erythropoietin, etc.) and today is improving outcomes for patients and addressing unmet medical needs for the future;

Agriculture biotechnology or green biotechnology that is used to enhance< plants in order to improve their resistance to disease, tolerance for herbicides or difficult environment conditions, or to achieve higher yields with less inputs (water, fertilizers, etc.);

Industrial biotechnology or white technology, representing the "third wave" in< biotechnology, because it follows innovation in the health and agricultural areas; this sector encompasses the application of biotechnology-based tools to traditional industrial processes ("bioprocessing") and the manufacturing of bio-based products (biofuels, bio-plastics and bio-based chemicals). In this technology enzymes and/or micro-organisms, such as fungi, yeast, bacteria (also referred as "biocatalysts"), are used to make intermediate and endproducts more efficiently, reduce environmental impacts of processes and products and/or enable the creation of new products from renewable resources.

It is obvious that management of IP and IPR is a multidimensional task and calls for many different actions and strategies which need to be aligned with national laws and international treaties and practices. It is no longer driven purely by a national perspective. IP and its associated rights are seriously influenced by the market needs, market response, cost involved in translating IP into commercial venture and so on. In other words, trade and commerce considerations are important in the management of IPR. Different forms of IPR demand different treatment, handling, planning, and strategies and engagement of persons with different domain knowledge such as science, engineering, medicines, law, finance, marketing, and economics. Each industry should evolve its own IP policies, management style, strategies, etc. depending on its area of specialty. Pharmaceutical industry currently has an evolving IP strategy. Since there exists the increased possibility that some IPR are invalid, antitrust law, therefore, needs to step in to ensure that invalid rights are not being unlawfully asserted to establish and maintain illegitimate, albeit limited, monopolies within the pharmaceutical industry. Still many things remain to be resolved in this context.

More than any other technological area, drugs and pharmaceuticals match the description of globalization and need to have a strong IP system most closely. Knowing that the cost of introducinga new drug into the market may cost a company anywhere between $ 300 million to $1000 million along with all the associated risks at the developmental stage, no company will like to risk its IP becoming a public property without adequate returns. Creating, obtaining, protecting, and managingIP must become a corporate activity in the

same manner as the raising of resources and funds. The knowledge revolution, which we are sure to witness, will demand a special pedestal for IP and treatment in the overall decision-making process.

Competition in the global pharmaceutical industry is driven by scientific knowledge rather than manufacturing know-how and a company's success will be largely dependent on its R&D efforts. Therefore, investments in R&D in the drug industry are very high as a percentage of total sales; reports suggest that it could be as much as 15% of the sale. One of the key issues in this industry is the management of innovative risks while one strives to gain a competitive advantage over rival organizations. There is high cost attached to the risk of failure in pharmaceutical R&D with the development of potential medicines that are unable to meet the stringent safety standards, being terminated, sometimes after many years of investment. For those medicines that do clear development hurdles, it takes about 8-10 years from the date when the compound was first synthesized. As product patents emerge as the main tools for protecting IP, the drug companies will have to shift their focus of R&D from development of new processes for producing known drugs towards development of a new drug molecule and new chemical entity (NCE). During the 1980s, after a period of successfully treating many diseases of short-term duration, the R&D focus shifted to long duration (chronic) diseases. While looking for the global market, one has to ensure that requirements different regulatoryauthorities must be satisfied.

It is understood that the documents to be submitted to regulatory authorities have almost tripled in the last ten years. In addition, regulatory authorities now take much longer to approve a new drug. Consequently, the period of patent protection is reduced, resulting in the need of putting in extra efforts to earn enough profits. The situation may be more severe in the case of drugs developed through the biotechnology route especially those involving utilization of genes. It is likely that the industrialized world would soon start canvassing for longer protection for drugs. It is also possiblethat many governments would exercise more and more price control to meet public goals. This would on one hand emphasize the need for reduced cost of drug development, production, and marketing, and on the other hand, necessitate planning for lower profit margins so as to recover costs over a longer period. It is thus obvious that the drug industry has to wade through many conflicting requirements. Many different strategies have been evolved during the last 10 to 15 years for cost containment and trade advantage. Some of these are out sourcing of R&D activity, forming R&D partnerships and establishing strategic alliances.

12. Let’s sum up

- Plants are multicellular and mostly photosynthetic organisms which found essentially everywhere, both in water and on land. The aquatic plants include red, brown and green algae and the land plants include mosses, ferns, gymnosperms and angiosperms.

- The microorganisms have always played an essential role in the biosphere with fermented foods and beverages, plant and animal diseases and nutrient cycling foremost.
- Mushrooms are the fruit bodies of edible fungi, commonly belonging to Basidiomycotina (*Agaricus campestris, A. brunnescens, Pleurotus sajor-caju, Volvariella volvacea* etc.) and rarely to Ascomycotina (*Morchella conica, M. esculenta*).
- Most remediation activity still makes use of conventional methods such as excavation and reburial, capping, and soil washing and burning. However, newly emerging biological cleanup methods, such as phytoremediation, are often simpler in design and cheaper toimplement.
- Stress is usually defined as an external factor that exerts a disadvantageous influence on the plant. This chapter will concern itself with environmental or abiotic factors that produce stress in plants, although biotic factors such as weeds, pathogens, and insect predation can alsoproduce stress.
- Genetically modified crops are defined as crops whose genomes have been altered in waysthat do not occur naturally. Genetically engineered crops have genes added or removed using genetic engineering techniques, originally including gene guns, electroporation, microinjection and *Agrobacterium.*
- Plants are a vital component of biodiversity and healthy ecosystems. They provide a range of ecosystem services, from production of oxygen and removal of atmospheric carbon dioxide emissions, creation and stabilization of soil, protection of watersheds and provision of natural resources including food, fibre, fuel, shelter and medicine.
- Conservation of biodiversity is protection, upliftment and scientific management of biodiversity so as to maintain it at its threshold level and derive sustainable benefits for the present and future generation.
- The forensic aspects require an understanding of what is necessary for botanical evidence tobe accepted as evidence in our judicial system. Forensics requires recognition of pertinent evidence at a crime scene, appropriate collection and preservation of evidentiary material, maintenance of a chain of custody, an understanding of scientific testing methods, validation of new forensic techniques, and admissibility criteria for court.
- Intellectual property Right (IPR) is a term used for various legal entitlements which attach to certain types of information, ideas, or other intangibles in their expressed form. The holder of this legal entitlement is generally entitled to exercise various exclusive rights in relation to the subject matter of the Intellectual Property.

13. Suggested Readings

1. Bhattacharya K., Hait G. & Kumar A. K. (2011) AText Book of Botany Vol I New CentralBook Agency
2. Bhattacharya K., Hait G. & Kumar A. K. (2011) AText Book of Botany Vol II New CentralBook Agency
3. Ganguli,H.C., Das, K.S.K. & Dutta, C.T. College Botany, Vol. I, latest Ed., New CentralBook Agency
4. Ganguli,H.C. and Kar, A.K. College Botany, Vol. II, latest Ed., New Central Book Agency
5. Mukherjee, S. College Botany, Vol. III, latest Ed., New Central Book Agency
6. Lee, R. E. (2008). Phycology.Cambridge University Press.
7. Shaw, A. Jonathan and Goffinet Bernard, Bryophyte Biology, 2009, Cambridge universityPress
8. Gifford, E. M. and Foster, A. S. (1998). Morphology & Evolution of Vascular Plants (3rded.), Freeman and Co.
9. Dubey, H.C. An Introduction to Fungi (2nd ed.), 1990, Vikas Publishing House 23
10. Coyle. H. M. Forensic_Botany-Principles and Applications to Criminal Casework CRCPRESS Boca Raton London New York Washington, D.C.
11. Taiz, L., & Zeiger, E. Plant Physiology (4th ed.), 2006, Sinauer Associates, Inc. Publishers
12. http://www.biologydiscussion.com/
13. https://en.wikipedia.org/

14. Assignments

1. What is IPR?
2. Give two example of angiosperic plant.
3. Define GMO
4. What are the strategies of ex-situ conservation.
5. Mention two case study associated with plant.
6. Describe cultivation processes of mushroom.
7. What is Golden rice?
8. Write a short note on ecological roles of bryophytes
9. Name two pteridophytes with secondary thickenings.
10. Mention the difference between dictyostelic and solenostelic structures in pteridophytes.
11. What are the differences between fern ally and true fern?
12. Depict the classification system of fern by Sporne (1962) and Smith *et al.* (2006) by highlighting the differences between two systems
13. Distiguish between heterothallism and paraseuality.
14. What is dolipore septum?
15. How Drug enforcement help to solve forensic cases
16. What is typing? Give an overview of DNA typing.
17. Mention the role of pollen in case study. What are the advantages?
18. Name two DNA markers and mention its role in forensic botany.
19. How Diatoms help to solve forensic cases.
20. Distinguish ring porous wood and diffuse porous wood.

21. What botanical evidence can do?
22. How botanical samples are collected?
23. Describe impact of water stress on plants life .
24. Explain structure and function of HSPs in plant.
25. What is chilling injury? How do you overcome it.
26. What is hydroactive closure?
27. Explain the range of thallus structure of algae.
28. Write economic importance of jute.
29. Write the botanical characters of cotton?
30. Give the botanical name of any two fibre yielding plants.
31. Give the botanical name of any two vegetable oil yielding plants
32. What is the origin of center of wheat?
33. Write the botanical name of any two millets.
34. Name two medicinally important plant with their uses.

All the materials are self writing and collected from ebook,journals and websites.