POST GRADUATE DEGREE PROGRAMME
(CBCS)
IN
BOTANY

SEMESTER - III

Course: BOHCT 3.1

(Principles of Plant Pathology & Crop Protection)

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING
UNIVERSITY OF KALYANI
KALYANI – 741 235,
WEST BENGAL

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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 UGC Regulations, 2017 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.) Sankar Kumar Ghosh, 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.

Prof Manas Mohan Adhikary
Director
Directorate of Open and Distance Learning
University of Kalyani

SYLLABUS
COURSE – BOHCT3.1
Principles of Plant Pathology & Crop Protection

Course	Group	Details Contents Structure		Study hour
BOHCT3.1	**Principles of Plant Pathology & Crop Protection**	**Unit 1. Historical aspects**	Historical and developmental aspects of plant pathology	1
		Unit 2. Inoculum	Production, liberation and dispersal of inoculum, inoculum potential; factors affecting inoculum potential.	1
		Unit 3. Host-pathogen interaction	Host-pathogen interaction: penetration and disease development, role of cell-wall degrading enzymes and toxins, recognition mechanism and signal transduction during plant-pathogen interaction.	1
		Unit 4. Defense mechanisms of plants	Defense mechanisms of plants against infection: Pre existing structural and biochemical defense, induced structural and biochemical defense, hypersensitive reaction, phytoalexins, PR proteins, systemic acquired resistance, induced systemic resistance; Gene for gene hypothesis; concept of horizontal and vertical resistance.	1
		Unit 5. Physiological and molecular changes in diseased plants.	Physiological (photosynthesis, respiration, translocation of water and nutrients) and molecular (protein and nucleic acid) changes in diseased plants.	1
		Unit 6. Predisposition and disease epidemics	Predisposition, survival of pathogen in nature and its spread, disease epidemics.	1
		Unit 7. plant disease control	Principles of plant disease control: exclusion, eradication, protection and therapy.	1
		Unit 8. Strategies of plant disease management	Strategies of plant disease management with special emphasis on cultural and biological management; plant quarantine; integrated pest management.	1
		Unit 9. Fungicides	Fungicides: types and uses	1
		Unit 10. Fungal diseases-I	Fungal diseases-I: wart and early blight of potato, downy mildew of grapes, powdery mildew of rose, white rust of crucifers, peach leaf curl, linseed rust	1

Course	Group	Details Contents Structure		Study hour
BOHCT3.1		**Unit 11. Fungal diseases-II**	Fungal diseases-II: smut and red rot of sugarcane, Tikka disease of groundnut, Panama disease (*Fusarium* wilt) of banana, blast and sheath blight of paddy.	1
		Unit 12. Disease caused by Bacteria, Virus, Nematode and Mycoplasma	Bacterial diseases: leaf blight of paddy, Moko disease (bacterial wilt) of banana Viral diseases: Tungro viral disease of paddy, mosaic of potato Disease caused by nematode: root knot of tomato Disease caused by mycoplasma-like organism: little leaf of brinjal	1

Content

COURSE – BOHCT 3.1

Principles of Plant Pathology & Crop Protection

Hard Core Theory Paper **Credits: 3**

Content Structure

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1. Introduction

2. Objectives

3. Historical and developmental aspects of plant pathology

4. Production, liberation and dispersal of inoculum, inoculum potential; factors affecting inoculum potential

5. Host-pathogen interaction: penetration and disease development, role of cell-wall degrading enzymes and toxins, recognition mechanism and signal transduction during plant-pathogen interaction

6. Defense mechanisms of plants against infection: Pre-existing structural and biochemical defense, induced structural and biochemical defense, hypersensitive reaction, phytoalexins, PR proteins, systemic acquired resistance, induced systemic resistance; Gene for gene hypothesis; concept of horizontal and vertical resistance

7. Physiological (photosynthesis, respiration, translocation of water and nutrients) and molecular (protein and nucleic acid) changes in diseased plants.

8. Predisposition, survival of pathogen in nature and its spread, disease epidemics

9. Principles of plant disease control: exclusion, eradication, protection and therapy

10. Strategies of plant disease management with special emphasis on cultural and biological management; plant quarantine; integrated pest management

11. Fungicides: types and uses.

12. Study of some plant diseases with reference to symptoms, etiology and control measures:

i. Fungal diseases: wart and early blight of potato, downy mildew of grapes, powdery mildew of rose, white rust of crucifers, peach leaf curl, linseed rust, smut and red rot of sugarcane, Tikka disease of groundnut, Panama disease (*Fusarium* wilt) of banana, blast and sheath blight of paddy.

ii. Bacterial diseases: leaf blight of paddy, Moko disease (bacterial wilt) of banana

iii. Viral diseases: Tungro viral disease of paddy, mosaic of potato

iv. Disease caused by nematode: root knot of tomato

v. Disease caused by mycoplasma-like organism: little leaf of brinjal

13. Suggested Reading

14. Assignment

1. Introduction

Plant pathology (also **phytopathology**) is the scientific study of diseases in plants caused by pathogens (infectious organisms) and environmental conditions (physiological factors). Organisms that cause infectious disease include fungi, oomycetes, bacteria, viruses, viroids, virus-like organisms, phytoplasmas, protozoa, nematodes and parasitic plants. Not included are ectoparasites like insects, mites, vertebrate, or other pests that affect plant health by consumption of plant tissues. Plant pathology also involves the study of pathogen identification, disease etiology, disease cycles, economic impact, plant disease epidemiology, plant disease resistance, how plant diseases affect humans and animals, pathosystem genetics, and management of plant diseases.

Crop protection is the science and practice of managing plant diseases, weeds and other pests (both vertebrate and invertebrate)that damage agricultural crops and forestry. Agricultural crops include field crops (maize, wheat, rice, etc.), vegetable crops (potatoes, cabbages, etc.) and fruits. The crops in field are exposed to many factor. The crop plants may be damaged by insects, birds, rodents, bacteria, etc. Crop protection encompasses:

- Pesticide-based approaches such as herbicides, insecticides and fungicides
- Biological pest control approaches such as cover crops, trap crops and beetle banks
- Barrier-based approaches such as agrotextiles and bird netting
- Animal psychology-based approaches such as bird scarers
- Biotechnology-based approaches such as plant breeding and genetic modification.

2. Objectives

After completion of the module you will be able to:

- provide an overview of fungi systematics and theories explaining its origin.
- apply this knowledge in understanding the evolutionary significance of fungi and use it as a basis for understanding the evolutionary pathways to other plant groups.

 describe the general characteristics of important groups of algae especially the phytoplankton and explain their ecology, role in environment and in human welfare;

 apply the knowledge and skills acquired to identify various fungi species.

3. Historical and developmental aspects of plant pathology

Plant diseases find mention in the earliest religious literature Rigveda, Atharvanaveda (1500-500 B.C.), the Artha Shashtra of Kautilya (321-186 B.C.). The book "Vraksha Ayurveda" written by Surapal in ancient India contained information on plant diseases. Plant diseases like rust, smut, downy mildew, powdery mildew and blight were mentioned in the Bible. The Greek Philosopher, Theophrastus (370-286 B.C.) was the first to study and write about the diseases of trees, cereals and legumes. In his book 'Enquiry into plants' Theophrastus has recorded his observations, imaginations and experiences but they were not based on any experiments. In 1729, Italian botanist P. A. Micheli proposed that fungi came from spores; father of Mycology. Up to the 18th Century it was believed that microorganisms and their spores formed through spontaneous generation and were the result rather than the cause of disease. 1775 - Tillet showed that he could increase the number of wheat plants developing covered smut by dusting wheat kernels before planting with smut dust/smut spores. 1821 - E. M. Fries published Systema Mycologicum for naming of fungi; he was named as Linnaeus of Mycology. 1821 - Robertson of England stated that sulphur is effective against peach mildew.

Golden Era (1800 – 1900)- Devastating epidemic, the Late blight (1845) in Ireland dramatized the effect of plant diseases on human suffering besides stimulated interest in their cause and control.

1845 - Irish Potato famine due to *Phytophthora infestans* caused starvation of million and immigration of 1.5 million people.

1858 - J. G. Kuhn published first textbook in Plant Pathology – The Diseases of Cultivated Crops, their Causes and their Control

1861 -Anton de Bary (Germany) worked out the life cycle of potato late blight and first to prove experimentally *Phytophthora infestans* is the cause of potato late blight. He proved that fungi are causes but not the results of diseases. He is the Father of Modern Plant Pathology.

1918 - E. J. Butler published book on Fungi and Disease in Plants; he made exhaustive study on Indian fungi and the diseases caused by them. He is called as the Father of Modern Plant Pathology in India; He joined as the first Director of Imperial Bureau of Mycology (Commonwealth Mycological Institute, CMI) now CAB International Mycological Institute in Kew, England in 1920. He began the journal Review of Applied Mycology; with S.G. Jones he wrote, 'Plant Pathology' in 1949

1948 -B. B. Mundkur started Indian Phytopathological Society with its journal Indian Phytopathology. He has written a book ‘Fungi and Plant Diseases’ in 1949, which is the second, book in plant pathology in India.

1951-57 -E. A. Gaümann was one of the first to investigate the physiology of the wilts caused by

Fusarium spp. He put forth the involvement of toxin (toxin theory) in wilt diseases

1961 - Ross – Coined the term SAR, resistance that developed in the untreated portion of TMV inoculated plants

1966 -van Schmeling and Marshall Kulka were the first to find out systemic fungicides (oxathiin compounds – carboxin and oxycarboxin).

1970 -S. D. Garrett investigated the management of root diseases and he is the pioneer worker in the field of biological control.

1972 – G. Rangaswami wrote a book on Diseases of Crop Plants in India. 1985 - Van Loon – Isolated first pathogenesis related proteins (PR protein).
1980s onward - Advent of Molecular Plant Pathology – detailed studies on plant-pathogen interaction, host-pathogen recognition, signalling pathways undertaken.

4. Production, liberation and dispersal of inoculum, inoculum potential; factors affecting inoculum potential

In every infectious disease a series of more or less distinct events occurs in succession and leads to the development and perpetuation of the disease and the pathogen. This chain of events is called a **disease cycle**. A disease cycle sometimes corresponds fairly closely to the **life cycle** of the pathogen, but it refers primarily to the appearance, development, and perpetuation of the disease as a function of the pathogen rather than to the pathogen itself. The disease cycle involves changes in the plant and its symptoms as well as those in the pathogen and spans periods within a growing season and from one growing season to the next. The primary events in a disease cycle are inoculation, penetration, establishment of infection, colonization (invasion), growth and reproduction of the pathogen, dissemination of the pathogen, and survival of the pathogen in the absence of the host, i.e., overwintering or over summering of the pathogen. In some diseases there may be several **infection cycles** within one disease cycle.

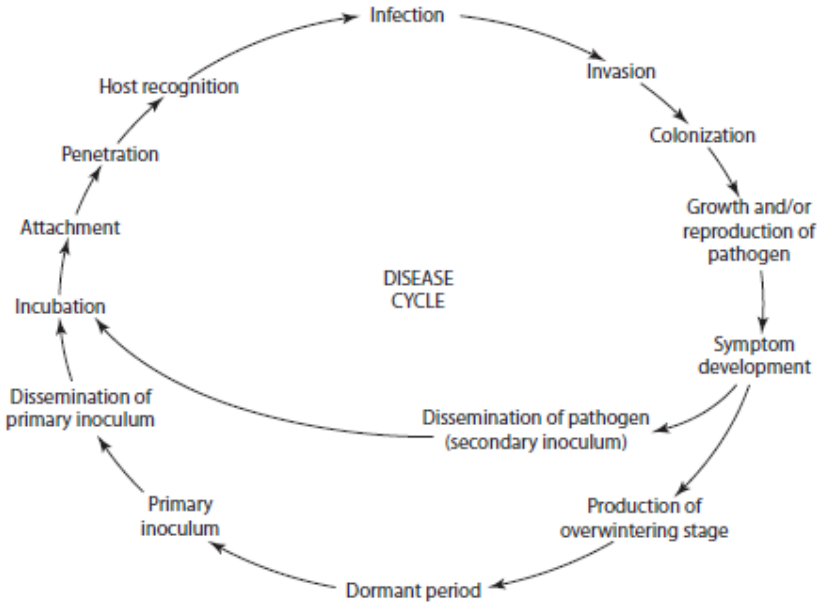


FIGURE 2-2 Stages in development of a disease cycle.

Inoculation:
Inoculation is the initial contact of a pathogen with a site of plant where infection is possible. The pathogen(s) that lands on or is otherwise brought into contact with the plant is called the **inoculum**. The inoculum is any part of the pathogen that can initiate infection. Thus, in fungi the inoculum may be spores (Figs. 2-3A–2-3C), **sclerotia** (i.e., a compact mass of mycelium), or fragments of mycelium. In bacteria, mollicutes, protozoa, viruses, and viroids, the inoculum is always whole individuals of bacteria (Fig. 2-3D), mollicutes, protozoa, viruses, and viroids, respectively. In nematodes, the inoculum may be adult nematodes, nematode juveniles, or eggs. In parasitic higher plants, the inoculum may be plant fragments or seeds. The inoculum may consist of a single individual of a pathogen, e.g., one spore or one multicellular sclerotium, or of millions of individuals of a pathogen, e.g., bacteria carried in a drop of water. One unit of inoculum of any pathogen is called a **propagule**.

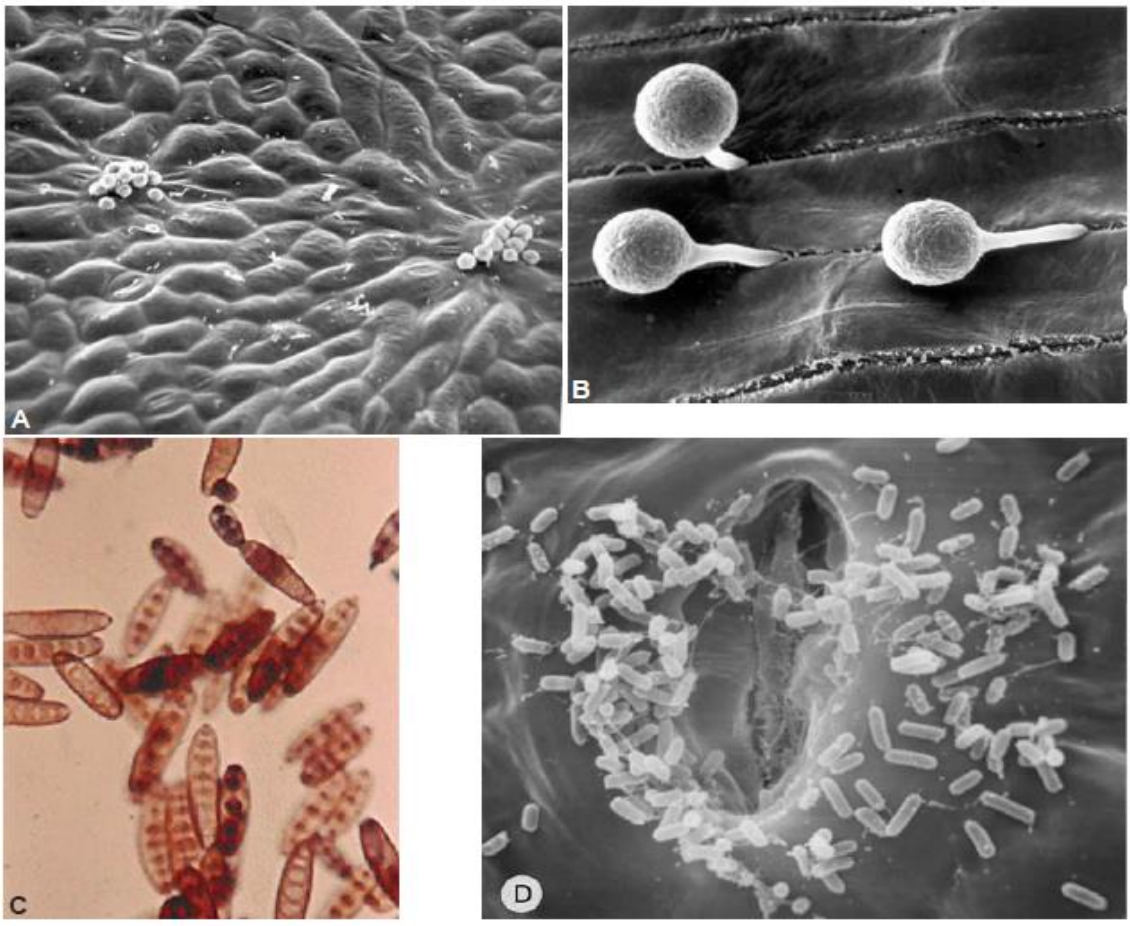


FIGURE 2-3 Types of inoculum and ways in which some pathogens enter a host plant. (A) Two groups of zoospores of the grape downy mildew oomycete have gathered over two leaf stomata. (B) Encysted zoospores of the soybean root rot pathogen *Phytophthora sojae* germinating and penetrating the root. (C) Mitospores (conidia) of a fungus that causes a corn leaf spot disease. (D) Bacteria of *Pseudomonas syringae* that causes bacterial spot and canker of stone fruits are seen in and surrounding a stoma of a cherry leaf. [Photographs courtesy of (A) D. J. Royle, (B) C. W. Mims and K. Enkerli, University of Georgia, and (D) E. L. Mansvelt, Stellenbosch, South Africa.]

Types of Inoculum

An inoculum that survives dormant in the winter or summer and causes the original infections in the spring or in the autumn is called a **primary inoculum**, and the infections it causes are called **primary infections**. An inoculum produced from primary infections is called a **secondary inoculum** and it, in turn, causes **secondary infections**. Generally, the more abundant the primary inoculum and the closer it is to the crop, the more severe the disease and the losses that result.

Sources of Inoculum

In some fungal and bacterial diseases of perennial plants, such as shrubs and trees, the inoculum is produced on the branches, trunks, or roots of the plants. The inoculum sometimes is present right in the plant debris or soil in the field where the crop is grown; other times it comes into the field with the seed, transplants, tubers, or other propagative organs or it may come from sources outside the field. Outside sources of inoculum may be nearby plants or fields or fields many miles away. In many plant diseases, especially those of annual crops, the inoculum survives in perennial weeds or alternate hosts, and every season it is carried from them to the annual and other plants. Fungi, bacteria, parasitic higher plants, and nematodes either produce their inoculum on the surface of infected plants or their inoculum reaches the plant surface when the infected tissue breaks down. Viruses, viroids, mollicutes, fastidious bacteria, and protozoa produce their inoculum within the plants; such an inoculum almost never reaches the plant surface in nature and, therefore, it can be transmitted from one plant to another almost entirely by some kind of vector, such as an insect.

Landing or Arrival of Inoculum:

The inoculum of most pathogens is carried to host plants passively by wind, water, and insects. An airborne inoculum usually gets out of the air and onto the plant surface not just by gravity but by being washed out by rain. Only a tiny fraction of the potential inoculum produced actually lands on susceptible host plants; the bulk of the produced inoculum lands on things that cannot become infected. Some types of inoculum in the soil, e.g., zoospores and nematodes, may be attracted to the host plant by such substances as sugars and amino acids diffusing out of the plant roots. Vector transmitted pathogens are usually carried to their host plants with an extremely high efficiency.

Prepenetration Phenomena

Attachment of Pathogen to Host

Pathogens such as mollicutes, fastidious bacteria, protozoa, and most viruses are placed directly into cells of plants by their vectors and, in most cases, they are probably immediately surrounded by cytoplasm, cytoplasmic membranes, and cell walls. However, almost all fungi, bacteria, and parasitic higher plants are first brought into contact with the external surface of plant organs.

Before they can penetrate and colonize the host, they must first become attached to the host surface (Figs. 2-3–2-6). Attachment takes place through the adhesion of spores, bacteria, and seeds through adhesive materials that vary significantly in composition and in the environmental factors they need to become adhesive. Disruption of adhesion by nontoxic synthetic compounds results in failure of the spores to infect leaves. In any case, the act of attachment often seems necessary for the subsequent transmission of signals for germ tube extension and production of infection structure. It is now clear that many proteins of the fungal cell wall, in addition to their structural role, play an important role in the adhesion of fungi, as well as in the host-surface perception by the fungus.

Spore Germination and Perception of the Host Surface

It is not clear what exactly triggers spore germination, but stimulation by the contact with the host surface, hydration and absorption of low molecular weight ionic material from the host surface, and availability of nutrients play a role. Spores also have mechanisms that prevent their germination until they sense such stimulations or when there are too many spores in their vicinity.

Once the stimulation for germination has been received by the spore, the latter mobilizes its stored food reserves, such as lipids, polyoles, and carbohydrates, and directs them toward the rapid synthesis of cell membrane and cell wall toward the germ tube formation and extension (Figs. 2-4 and 2-5). The germ tube is a specialized structure distinct from the fungal mycelium, often growing for a very short distance before it differentiates into an appressorium.

The germ tube is also the structure and site that perceives the host surface and, if it does not receive the appropriate external stimuli, the germ tube remains undifferentiated and, when the nutrients are exhausted, it stops growing. When appropriate physical and chemical signals, such as surface hardness, hydrophobicity, surface topography, and plant signals, are present, germ tube extension and differentiation take place.

The perception of signals from plant surfaces by pathogenic fungi (Fig. 2-6) seems to be the result of signaling pathways mediated by cyclic adenosine monophosphate (cAMP) and mitogen-activated protein kinase (MAPK), which have been implicated in regulating the development of infection-related phenomena in many different fungi. In response to a signal from the host plant, e.g., the presence of a hydrophobic plant surface, which transmits a cue for appressorium formation, the fungus perceives the extracellular signal and its transmission via the plasma membrane and, as a first step, it accumulates intracellular signaling molecules and induces a phosphorylation cascade. In some fungi, the receptor of the signal is a protein in the plasma membrane of the fungal spore. Signaling pathways for infection-related development are also achieved through mitogen-activated protein kinases (MAPKs) and their upstream regulatory kinases.

All of these together comprise a functional unit that transmits input signals from the periphery of the cell to the cell nucleus to elicit the expression of appropriate genes. A MAP kinase, K1 or P1, regulates appressorium formation in response to a signal from the plant surface but it is also required for invasive growth or viability in its host plant.

After attachment of the propagule to the host surface, as spores and seeds germinate, germ tubes also produce mucilaginous materials that allow them to adhere to the cuticular surface of the host, either along their entire length or only at the tip of the germ tube. In regions of contact with the germ tube, the structure of the host cuticle and cell walls often appears altered, presumably as a result of degradative enzymes contained in the mucilaginous sheath.

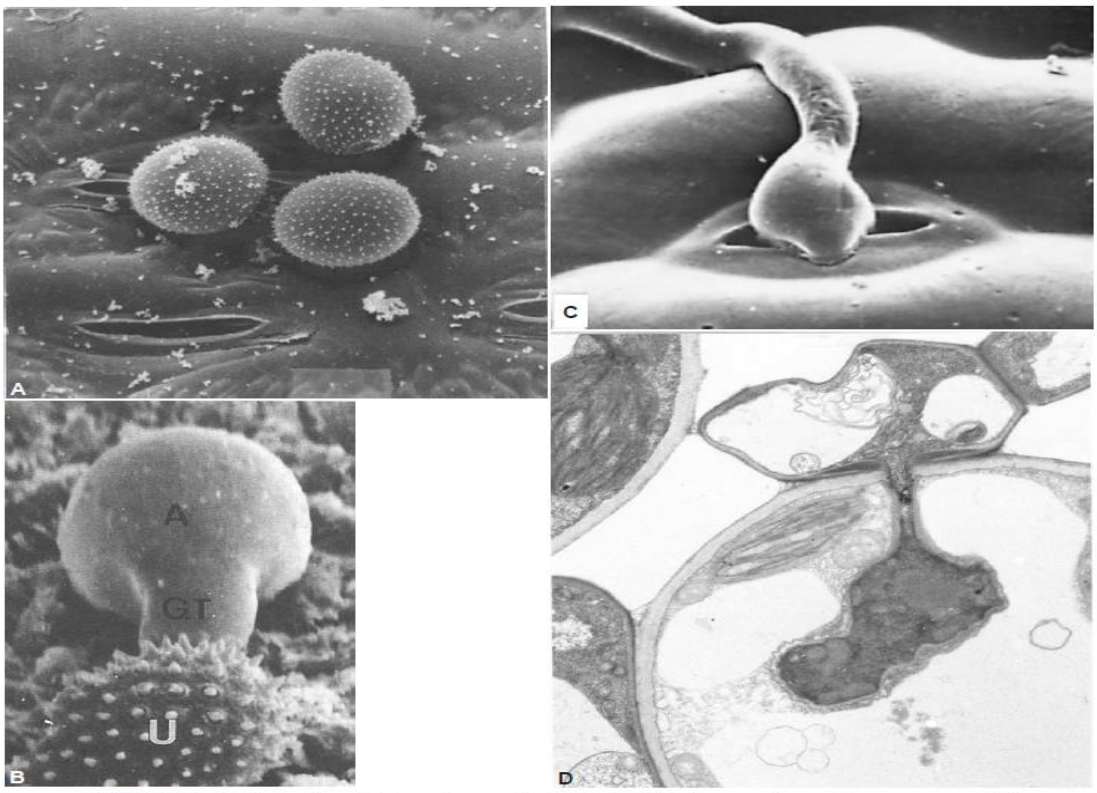


FIGURE 2-4 Methods of germination and penetration by fungi. (A) Uredospores of a rust fungus on a grass leaf next to open stomata. (B) A rust uredospore (U) that has germinated and produced a dome-like appressorium. (C) Uredospore germination, germ tube elongation, and appressorium penetration through a stoma. (D) A haustorium of a rust fungus inside a host cell. (E) A spore of the apple black rot fungus that has germinated directly into mycelium. (F) Two multicellular conidia of *Alternaria* sp. (G) A germinating conidium of *Alternaria* with a germ tube covered with extracellular material. [Photographs courtesy of (A) Plant Pathology Department, University of Florida, (B and C) W. K. Wynn and (D) C. W. Mims, University of Georgia, (E) J. Rytter and J. W. Travis, Pennslyvania State University, (F and G) Mims *et al.* (1997). *Can. J. Bot.* 75, 252–260.]

Appressorium Formation and Maturation

Once appressoria are formed, they adhere tightly to the leaf surface (Figs. 2-4 and 2-9). Subsequently, appressoria secrete extracellular enzymes, generate physical force, or both to bring about penetration of the cuticle by the fungus. Appressoria must be attached to the host plant surface strongly enough to withstand the invasive physical force applied by the fungus and to resist the chemical action of the enzymes secreted by the fungus.

Appressoria of some fungi contain lipids, polysaccharides, and proteins. Fungi that produce melanin pigmented appressoria produce a narrow penetration hypha from the base of the appressorium and use primarily physical force to puncture the plant cuticle with that hypha. Mobilization of spore-stored products to the developing appressorium and glycerol biosynthesis in it is regulated by the cAMP signalling pathway, whereas the initial movement of lipid and glycogen reserves to the developing appressorium was also found to be regulated by the K1 MAP. This indicates that the maturation of appressoria and their specific biochemical activity are intimately associated with genetic control of the initial development of appressoria.

The production of penetration hyphae by appressoria, or directly from germ tubes, is not well understood at the genetic level. Production of the penetration peg requires the localization of actin to the hyphal tip and rapid biosynthesis of the cell wall as the hypha grows through the cuticle and the layers of the epidermal cell walls. Production of penetration hyphae appears to be regulated by a MAP kinase pathway.

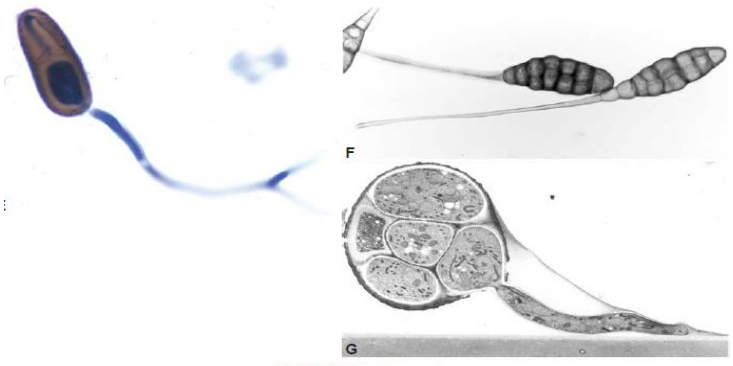


FIGURE 2-4 (*Continued*)

Recognition between Host and Pathogen

It is still unclear how pathogens recognize their hosts and vice versa. It is assumed that when a pathogen comes in contact with a host cell, an early event takes place that triggers a fairly rapid response in each organism that either allows or impedes further growth of the pathogen and development of disease. The nature of the "early event" is not known with certainty in any host–parasite combination, but it may be one of many biochemical substances, structures, and pathways.

These may include specific host signal compounds or structures, or specific pathogen elicitor molecules, and either of them may induce specific actions or formation of specific products by the other organism (Fig. 2-6).

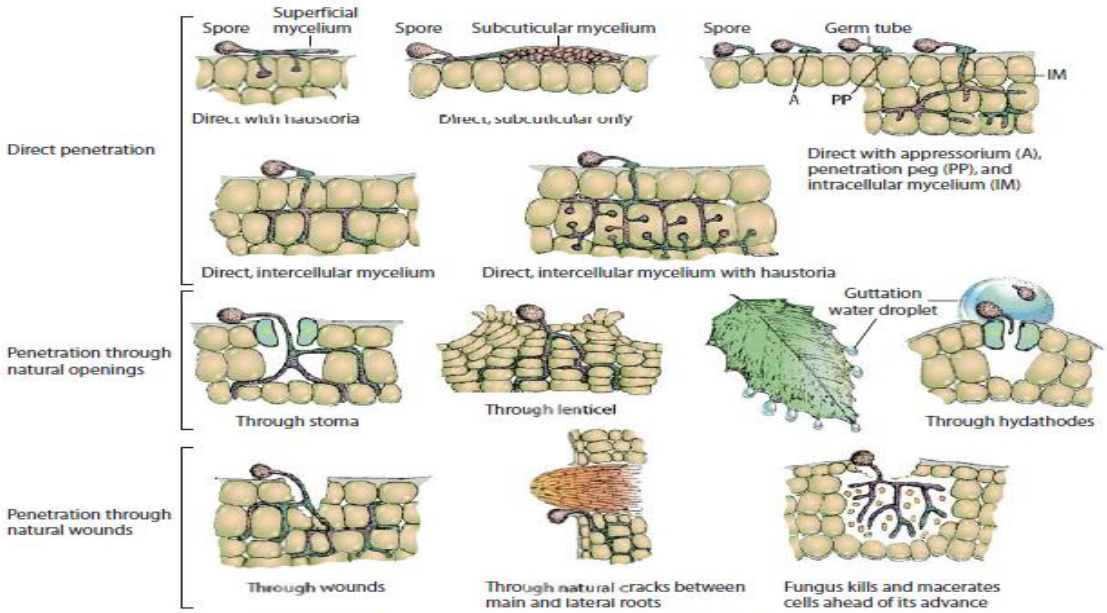


FIGURE 2-5 Methods of penetration and invasion by fungi.

Germination of Spores and Seeds

Almost all pathogens in their vegetative state are capable of initiating infection immediately. Fungal spores and seeds of parasitic higher plants, however, must first germinate (Figs. 2-4 and 2-5). Spores germinate by producing a typical mycelium (Figs. 2-4E and 2-4G) that infects and grows into host plants or they produce a short germ tube that produces a specialized infectious structure, the haustorium (Figs. 2-4B–2-4D).

In order to germinate, spores require a favourable temperature and also moisture in the form of rain, dew, or a film of water on the plant surface or at least high relative humidity. The moist conditions must last long enough for the pathogen to penetrate or else it desiccates and dies. Most spores can germinate immediately after their maturation and release, but others (so-called resting spores) require a dormancy period of varying duration before they can germinate. When a spore germinates it produces a germ tube, i.e., the first part of the mycelium,that can penetrate the host plant. Some fungal spores germinate by producing other spores, e.g., sporangia produce zoospores and teliospores produce basidiospores. Spore germination is often favoured by nutrients diffusing from the plant surface; the more nutrients (sugars and amino acids) exuded from the plant, the more spores germinate and the faster they germinate. In some cases, spore germination of a certain pathogen is stimulated only by exudates of plants susceptible to that particular pathogen. In other cases, spore germination may be inhibited to a lesser or greater extent by materials released into the surrounding water by the plant, by substances contained within the spores themselves, especially when the spores are highly concentrated ("quorum sensing"), and by saprophytic microflora present on or near the plant surface.

Penetration

Pathogens penetrate plant surfaces by direct penetration of cell walls, through natural openings, or through

wounds (Figs. 2-3–2-5). Some fungi penetrate tissues in only one of these ways, others in more than one. Bacteria enter plants mostly through wounds, less frequently through natural openings, and never directly through unbroken cell walls (Fig. 2-5). Viruses, viroids, mollicutes, fastidious bacteria, and protozoa enter
through wounds made by vectors, although some viruses and viroids may also enter through wounds made by tools and other means. Parasitic higher plants enter their hosts by direct penetration. Nematodes enter plants by direct penetration and, sometimes, through natural openings (Fig. 2-10).

Penetration does not always lead to infection. Many organisms actually penetrate cells of plants that are not susceptible to these organisms and that do not become diseased; these organisms cannot proceed beyond the stage of penetration and die without producing disease.

Infection

Infection is the process by which pathogens establish contact with susceptible cells or tissues of the host and
procure nutrients from them. Following infection, pathogens grow, multiply, or both within the plant tissues and invade and colonize the plant to a lesser or greater extent. Growth and/or reproduction of the pathogen (colonization) in or on infected tissues are actually two concurrent substages of disease development (Fig. 2-2).

Successful infections result in the appearance of symptoms, i.e., discolored, malformed, or necrotic areas on the host plant. Some infections, however, remain latent, i.e., they do not produce symptoms right away but at a later time when the environmental conditions or the stage of maturity of the plant become more favorable.

All the visible and otherwise detectable changes in the infected plants make up the **symptoms** of the disease.

Symptoms may change continuously from the moment of their appearance until the entire plant dies or they may develop up to a point and then remain more or less unchanged for the rest of the growing season. Symptoms may appear as soon as 2 to 4 days after inoculation, as happens in some localized viral diseases of herbaceous plants, or as late as 2 to 3 years after inoculation, as in the case of some viral, mollicute, and other diseases of trees. In most plant diseases, however, symptoms appear from a few days to a few weeks after inoculation.

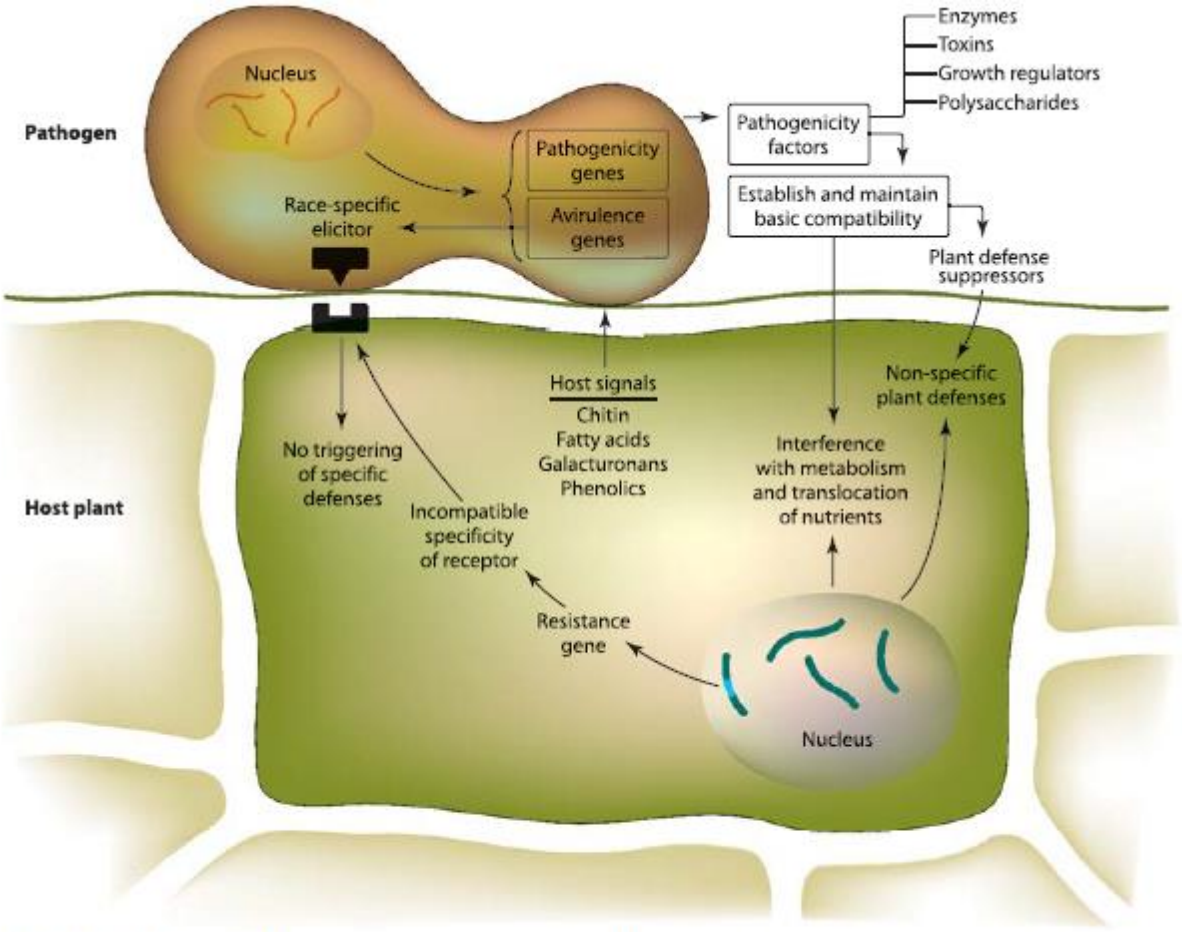


FIGURE 2-6 Establishment of infection in a compatible reaction between a pathogen and its host plant.

Invasion

Various pathogens invade hosts in different ways and to different extents (Figs. 2-4, 2-5, 2-9, and 2-12). Some fungi, such as those causing apple scab and black spot of rose, produce mycelium that grows only in the area between the cuticle and the epidermis (subcuticular colonization) (Fig. 2-11A); others, such as those causing powdery mildews, produce mycelium only on the surface of the plant (Fig. 2-11B) but send haustoria into the epidermal cells. Most fungi spread into all the tissues of the plant organs (leaves, stems, and roots) they infect, either by growing directly through the cells as an **intracellular mycelium** or by growing between the cells as an **intercellular mycelium** (Figs. 2-11C and 2-11D). Fungi that cause vascular wilts invade the xylem vessels of plants (Fig. 2-11E). Bacteria invade tissues intercellulary, although when parts of the cell walls dissolve, bacteria also grow intracellularly. Bacteria causing vascular wilts, like the vascular wilt fungi, invade the xylem vessels (Fig. 2-11E).

Most nematodes invade tissues intercellularly, but some can invade intracellularly as well (Fig. 2-12). Many nematodes do not invade cells or tissues at all but feed by piercing epidermal cells with their stylets.

Viruses, viroids, mollicutes, fastidious bacteria, and protozoa invade tissues by moving from cell to cell intracellularly. Viruses and viroids invade all types of living plant cells, mollicutes and protozoa invade phloem sieve tubes and perhaps a few adjacent phloem parenchyma cells, and most fastidious bacteria invade xylem vessels and a few invade only phloem sieve tubes.

Many infections caused by fungi, bacteria, nematodes, viruses, and parasitic higher plants are local, i.e., they involve a single cell, a few cells, or a small area of the plant. These infections may remain localized throughout the growing season or they may enlarge slightly or very slowly. Other infections enlarge more or less rapidly and may involve an entire plant organ (flower, fruit, leaf), a large part of the plant (a branch), or the entire plant.

Infections caused by fastidious xylem- or phloem inhabiting bacteria, mollicutes, and protozoa and natural infections caused by viruses and viroids are **systemic**, i.e., the pathogen, from one initial point in a plant, spreads and invades most or all susceptible cells and tissues throughout the plant.

Growth and Reproduction of the Pathogen (Colonization)

Individual fungi and parasitic higher plants generally invade and infect tissues by growing on or into them from one initial point of inoculation. Most of these pathogens, whether inducing a small lesion, a large infected area, or a general necrosis of the plant, continue to grow and branch out within the infected host indefinitely so that the same pathogen individual spreads into more and more plant tissues until the spread of the infection is stopped or the plant is dead. In some fungal infections, however, while younger hyphae continue to grow into new healthy tissues, older ones in the already infected areas die out and disappear so that a diseased plant may have several points where separate units of the mycelium are active. Also, fungi causing vascular wilts often invade plants by producing and releasing spores within the vessels, and as the spores are carried in the sap stream they invade vessels far away from the mycelium, germinate there, and produce a mycelium, which invades more vessels.

All other pathogens, namely bacteria, mollicutes, viruses, viroids, nematodes, and protozoa, do not increase much, if at all, in size with time, as their size and shape remain relatively unchanged throughout their existence. These pathogens invade and infect new tissues within the plant by reproducing at a rapid rate and increasing their numbers tremendously in the infected tissues. The rate of reproduction varies considerably among the various kinds of pathogens, but in all types, one or a few pathogens can produce tremendous numbers of individuals within one growing season. Some fungi produce spores more or less continuously (Fig. 2-14), whereas others produce them in successive crops. In either case, several thousand to several hundreds of thousands of spores may be produced per square centimeter of infected tissue. Even small specialized sporophores can produce millions of spores, and the number of spores produced per diseased plant is often in the billions or trillions (Fig. 2-14). The number of spores produced in an acre of heavily infected plants, therefore, is generally astronomical, and enough spores are released to land on every conceivable surface in the field and the surrounding areas, enough to easily inoculate with a heavy inoculum every plant in the area.

Dissemination of the Pathogen

A few pathogens, such as nematodes, oomycetes, zoosporic fungi, and bacteria, can move short distances on their own power and thus can move from one host to another one very close to it. Fungal hyphae can grow between tissues in contact and sometimes through the soil toward nearby roots for a few to many centimeters.

Both of these means of dissemination, however, are quite limited, especially in the case of zoospores and bacteria. The spores of some fungi are expelled forcibly from the sporophore or sporocarp by a squirting or puffing action that

results in the successive or simultaneous discharge of spores up to a centimeter or so above the sporophore. The seeds of some parasitic plants are also expelled forcibly and may arch over distances of several meters.
Almost all dissemination of pathogens responsible for plant disease outbreaks, and even for disease occurrences of minor economic importance, is carried out passively by such agents as air and insects (Figs. 2-13– 2-15). To a lesser extent, water, certain other animals, and humans may be involved (Fig. 2-15).

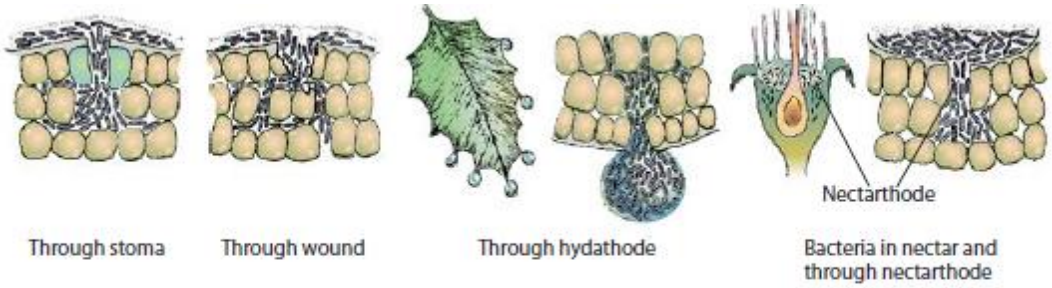


FIGURE 2-7 Methods of penetration and invasion by bacteria.

Dissemination by Air

Spores of most oomycetes and most fungi and the seeds of most parasitic plants are disseminated by air currents that carry them as inert particles to various distances.
Air currents pick up spores and seeds off the sporophores (Figs. 2-13A–2-13E, 2-14, and 2-16) or while they are being expelled forcibly or are falling at maturity. Depending on the air turbulence and velocity, air currents may carry the spores upward or horizontally in a way similar to that of particles contained in smoke.
While airborne, some of the spores may touch wet surfaces and get trapped; when air movement stops or when it rains, the rest of the spores land or are "washed out" from the air and are brought down by the raindrops.
Most of the spores, of course, land on anything but a susceptible host plant. Also, the spores of many fungi are actually too delicate to survive a long trip through the air and are therefore successfully disseminated through the air for only a few hundred or a few thousand meters. The spores of other fungi, however, particularly those of the cereal rusts, are very hardy and occur commonly at all levels and at high altitudes (several thousand meters) above infected fields. Spores of these fungi are often carried over distances of several kilometers, even hundreds of kilometers, and in favourable weather may cause widespread epidemics. Some fungi can spread into new areas quite rapidly and may cause severe epidemics over large areas, including entire continents, within a few years. This happened, for example, in the airborne pathogens of sugar cane smut in the Americas (Fig. 2-18) and of barley stripe rust in South America (Fig. 2-15).
Air dissemination of other pathogens occurs rather infrequently and only under special conditions, or indirectly. For example, bacteria causing fire blight of apple and pear produce fine strands of dried bacterial exudate containing bacteria, and these strands may be broken off and disseminated by wind. Bacteria and nematodes present in the soil may be blown away along with plant debris or soil particles in the dust. Wind also helps in the dissemination of bacteria, fungal spores, and nematodes by blowing away rain splash droplets containing these pathogens, and wind carries away insects that may contain or are smeared with viruses, bacteria, mollicutes, protozoa, or fungal spores. Finally, wind causes adjacent plants or plant parts to rub against one another, which may help the spread by contact of bacteria, fungi, some viruses and viroids, and possibly some nematodes.

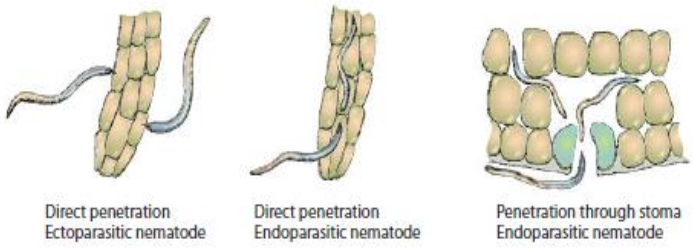


FIGURE 2-10 Methods of penetration and invasion by nematodes.

Dissemination by Water

Water is important in disseminating pathogens in three ways. (1) Bacteria, nematodes, and spores and mycelial fragments of fungi present in the soil are disseminated by rain or irrigation water that moves on the surface or through

the soil. (2) All bacteria and the spores of many fungi are exuded in a sticky liquid (Figs. 2-16A, 2-16B, and 2-16D) and depend on rain or (overhead) irrigation water, which either washes them downward or splashes them in all directions, for their dissemination (3) Raindrops or drops from overhead irrigation pick up the fungal spores and any bacteria present in the air and wash them downward, where some of them may land on susceptible plants. Although water is less important than air in the long-distance transport of pathogens, the water dissemination of pathogens is more efficient for nearby infections, as the pathogens land on an already wet surface and can move or germinate immediately.

Dissemination by Insects, Mites, Nematodes, and Other Vectors

Insects, particularly aphids, leafhoppers, and whiteflies, are by far the most important vectors of viruses, whereas leafhoppers are the main vectors of mollicutes, fastidious bacteria, and protozoa. Each one of these pathogens is transmitted, internally, by only one or a few species of insects during feeding and movement of the insect vectors from plant to plant. Specific insects also transmit certain fungal, bacterial, and nematode pathogens, such as the fungus causing Dutch elm disease, the bacterial wilt of cucurbits, and the pine wilt nematode. In all diseases in which the pathogen is carried internally or externally by one or a few specific vectors, dissemination of the pathogen depends, to a large extent or entirely, on that vector. In many diseases, however, such as bacterial soft rots, fungal fruit rots, anthracnoses, and ergot, insects become smeared with various kinds of bacteria or sticky fungal spores as they move among plants. The insects carry these pathogens externally from plant to plant and deposit them on the plant surface or in the wounds they make on the plants during feeding. In such diseases, dissemination of the pathogen is facilitated by but is not dependent on the vector.

Insects may disseminate pathogens over short or longdistances, depending on the kind of insect, the insect–pathogen association, and the prevailing weather conditions, particularly wind. A few species of mites and nematodes can transmit internally several viruses from plant to plant. In addition, mites and nematodes probably carry externally bacteria and sticky fungal spores with which they become smeared as they move on infected plant surfaces.

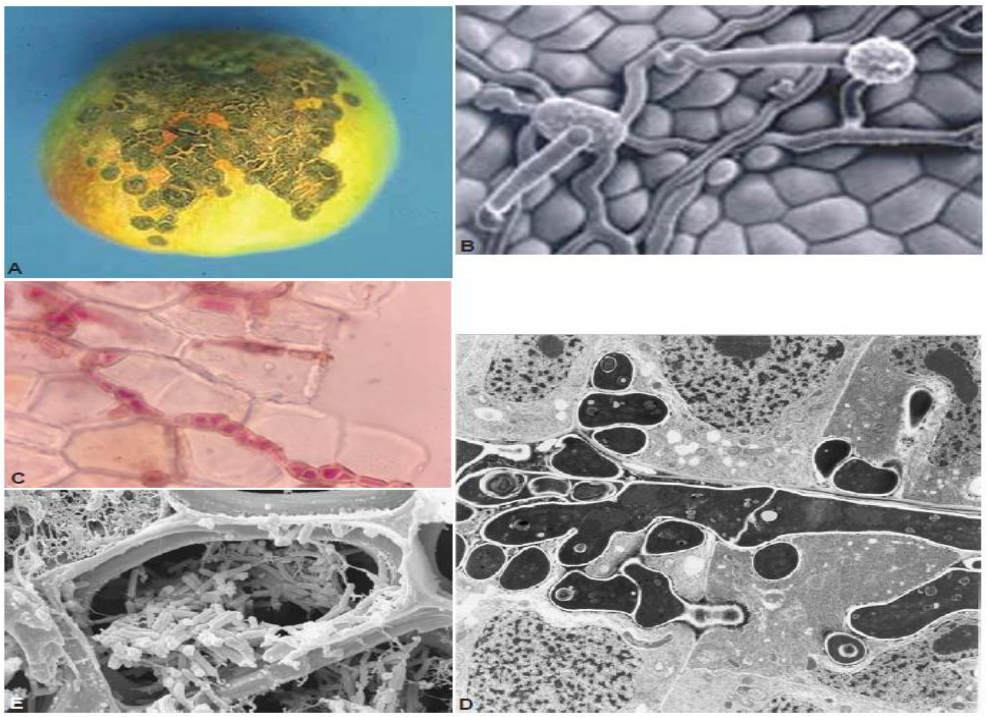


FIGURE 2-11 Types of invasion of pathogens in infected plants. (A) In apple scab disease, the pathogenic fungus grows only between the cuticle and the epidermal cells of leaves and fruit. (B) In powdery mildews the fungal mycelium grows only on the surface of host plants, but sends haustoria into the epidermal cells. (C) In many diseases the fungal mycelium (stained red here) grows only intercellularly (between the cells). (D) Hyphae of the smut fungus *Ustilago* in an infected leaf. (E) In bacterial vascular diseases, bacteria grow in and may clog the xylem vessels. [Photographs courtesy of (A) University of Oregon, (B) G. Celio, APS, (D) Mims *et al.* (1992). *Intern. J. Plant Sci.* **153**, 289–300, and (E) E. Alves, Federal University of Lavras, Brazil.]

Almost all animals, small and large, that move among plants and touch the plants along the way can disseminate pathogens such as fungal spores, bacteria, seeds of parasitic plants, nematodes, and perhaps some viruses and viroids.

Most of these pathogens adhere to the feet or the body of the animals, but some may be carried in contaminated mouthparts.

Finally, some plant pathogens, e.g., the zoospores of some fungi and certain parasitic plants, can transmit viruses as they move from one plant to another (zoospores) or as they grow and form a bridge between two plants (dodder).

Dissemination by Pollen, Seed, Transplants, Budwood and Nursery Stock

Some viruses are carried in the pollen of plants infected with these viruses and, when virus-carrying pollen pollinates a healthy plant, the virus may infect not only the seed produced from such pollination, which will then grow into a virus-infected plant, it may also infect the plant that was pollinated with the virus-carrying pollen.

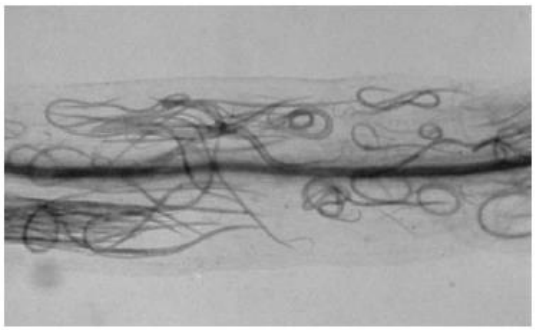


FIGURE 2-12 Alfalfa shoot invaded by plant parasitic nematodes (*Ditylenchus dipsaci*). (Photograph courtesy of J. Santo.)

Many pathogens are present on or in seeds, transplants, bud wood, or nursery stock and are disseminated by them as the latter are transported to other fields or are sold and transported to other areas near and far. Dissemination of pathogens through seed, transplants, and so on is of great practical importance because it introduces the pathogen along with the plant at the beginning of the growth season and enables the pathogen to multiply and be disseminated by all the other means of spread discussed. It is also important because it brings pathogens into new areas where they may have never existed before.

Dissemination by Humans

Human beings disseminate all kinds of pathogens over short and long distances in a variety of ways. Within a field, humans disseminate some pathogens, such as tobacco mosaic virus, through the successive handling of diseased and healthy plants. Other pathogens are disseminated through tools, such as pruning shears, contaminated when used on diseased plants (e.g., pear infected with fire blight bacteria), and then carried to healthy plants. Humans also disseminate pathogens by transporting contaminated soil on their feet or equipment, using contaminated containers, and using infected transplants, seed, nursery stock, and budwood as mentioned previously. Finally, humans disseminate pathogens by importing new varieties into an area that may carry pathogens that have gone undetected, by traveling throughout the world, and by importing food or other items that may carry harmful plant pathogens.

Examples of the role of humans as a vector of pathogens can be seen in the introduction into the United States of the fungi causing Dutch elm disease and white pine blister rust and of the citrus canker bacterium, in the introduction in Europe of the powdery and downy mildews of grape, and, more recently, in the rapid spread of sorghum ergot almost throughout the world (Fig. 2-20).

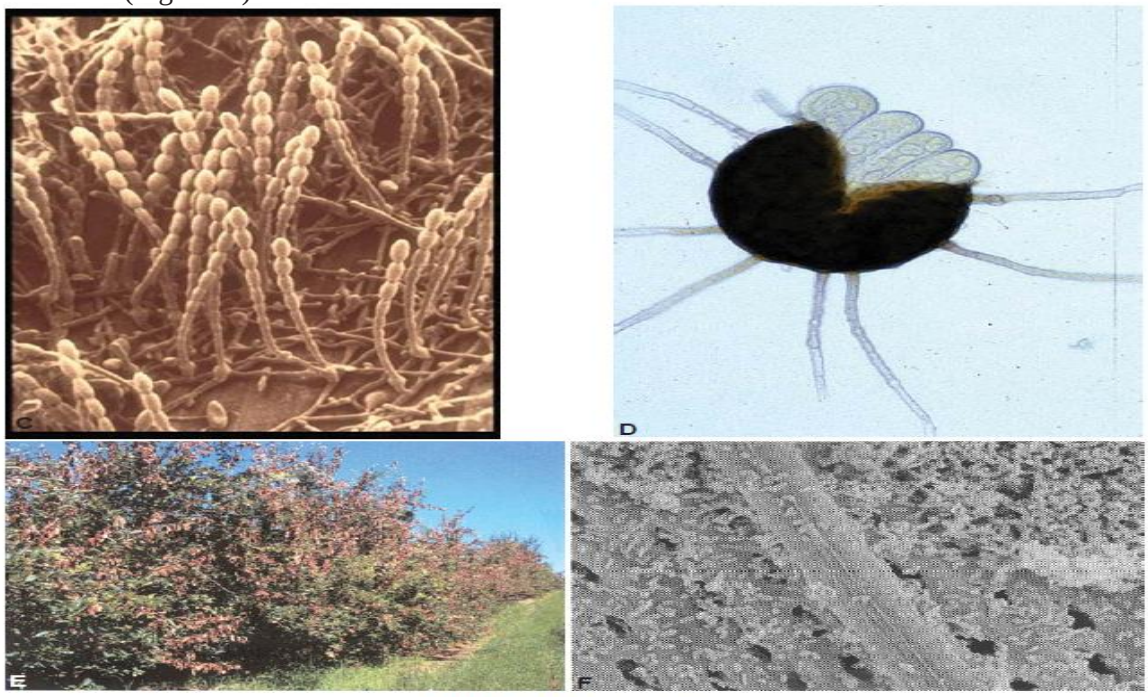


FIGURE 2-13 (*Continued*)

5. Host-pathogen interaction: penetration and disease development, role of cell-wall degrading enzymes and toxins, recognition mechanism and signal transduction during plant-pathogen interaction

The host-pathogen interaction is defined as how microbes or viruses sustain themselves within host organisms on a molecular, cellular, organismal or population level. This term is most commonly used to refer to disease-causing microorganisms although they may not cause illness in all hosts. Because of this, the definition has been expanded to how known pathogens survive within their host, whether they cause disease or not.

On the molecular and cellular level, microbes can infect the host and divide rapidly, causing disease by being there and causing a homeostatic imbalance in the body, or by secreting toxins which cause symptoms to appear. Viruses can also infect the host with virulent DNA, which can affect normal cell processes (transcription, translation, etc.), protein folding, or evading the immune response.

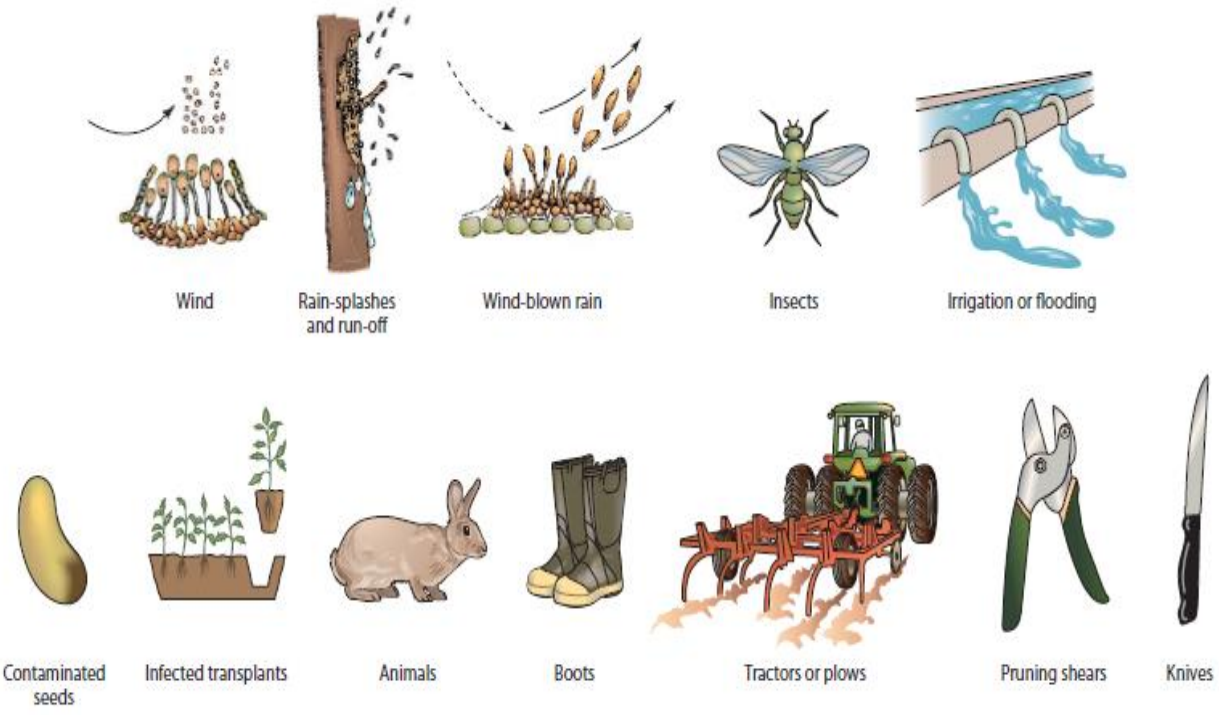


FIGURE 2-15 Means of dissemination of fungi and bacteria.

Penetration and disease development:

The following points highlight the three main stages of host pathogen relationship. The stages are: 1. Pre-Penetration Stage 2. Penetration Stage 3. Post-Penetration Stage.

1. Pre-Penetration Stage:

During pre-penetration stage the pathogen (inoculum) on arrival on the host surface interacts sharply with the surrounding environment and host itself. The environment which is an aggregate of all external conditions including temperature, moisture (relative humidity), light and the competing microorganisms; affects the life and form of the pathogen of the inoculum. For example, the development and abstraction of conidia are

favored by high air temperature and humidity in downy mildew.
Whereas in powdery mildew both the number of spores and their germination are greater in bright sunlight. In cereal rusts uredospore's germinate at low temperature, but the infectionprocess is delayed at this temperature. Again soil pH plays a very vital role in the growth of bacterial plant pathogen in the rhizosphere (area of soil immediately surrounding the roots).
Whether a pathogen will survive and grow on the host surface also depends on its behavior with the exudates of, the host surface and the microbial population present on it. The exudates of the host surface may encourage or inhibit the growth of the pathogen. The root exudates mainly sugars and amino acids are nutrients for the growth of fungi and bacteria.
But root exudates like hydrocyanic acid, various organic acids and antibiotics are antifungal and antibacterial. For example, spores of *Rhizopus arrhizus* germinate only in presence of proline (amino acid) present in the rhizosphere region; whereas exudates of root of onion varieties inhibit spore germination of *Colletotrichum circinaus*.
Leaves also exude substances which may go in favor or against the growth of the pathogen. The glands of leaf hairs of gram contain malic acid which is antifungal and arrests the growth of *Uromyces ciceris arietini*.
Protocatechuic acid, an exudate of onion skin is also antifungal. The pathogen has to neutralize these exudates or has to be resistant to them for survival. Besides these, the rhizosphere region contains microbial population which is antagonistic to the growth of the pathogen. As such, the pathogen has to overcome the above barriers during pre-penetration stage before it can survive for host penetration.

2. Penetration Stage:

The success of host penetration leading to disease development is a very compli•cated process which is a combined effect of various factors like:

i) The nature and behavior of the pathogen including its multiplication capacity,
ii) Favorable physical conditions, and
iii) Host susceptibility.

Of all these factors, the factor nature and behavior of the pathogen is the most important one which controls the overall disease development. The nature and behavior of pathogen encompass the inoculum potential of the pathogen. The inoculum potential is again a measure of the biological energy available for the colonization of a host.
It is a function of:

i) Inoculums density which refers to the number of viable propagules per unit area of leaf or stem or per unit volume of soil;
ii) The nutrients available to the infectious units that allow them to germinate or grow;
iii) The environment (temperature range of 15 to 25°C, moisture content 70 per cent, and relative humidity 90 to 95 per cent);
iv) The virulence (aggressiveness) or genetic capacity of the pathogen to cause disease; and
v) The susceptibility of the host.

Besides these, the physiological state of the host may have an effect on the ability of the pathogen to attack it or on the extent to which a pathogen may harm it. The concept that encompasses this phenomenon is termed predisposition.

Factors involved in predisposition are:

(i) Age of the host;
(ii) Environmental conditions to which the pathogen has been exposed, i.e., light, humidity, soil environment, and temperature;
(iii) Infection by other pathogens; and
(iv) Presence of chemicals, i.e., pesticides, herbicides.

Generally adverse conditions predispose a plant to greater susceptibility to attack by a pathogen. Temperature predisposition phenomenon in nature is extremely variable depending on the nature of host and pathogen. In most bacterial and fungal diseases, free moisture is necessary for pathogen development.
Most known cases of predisposition usually result in increased susceptibility of plants that were genetically

resistant. A few cases are known in which a predisposing treatment has greatly increased resistance of plants that were genetically susceptible.

Certain fungal pathogens exhibit specificity in the part of host tissue infection. For example, some of them may remain restricted in the cortical tissue only through•out the entire period of attack and cause damage to it. Again others remain restricted in the vascular tissue only. Whereas, still others do not attack plant unless its heart wood is developed and the pathogen remains confined there causing damage.

Host penetration takes place:

i) Through natural openings,
ii) Through wounds,
iii) By direct penetration of surface cells causing tissue disintegration, and
iv) Through specific parts or organs.

Both bacteria and viruses enter the host tissue mainly through wounds. Whereas, the fungal pathogens gain entrance in the host through natural openings, wounds and by direct penetration through cuticle and outer wall of the surface cells, or root hairs, or through specific parts or organs of the host.

i) Entry through natural openings:

Both bacteria and fungi gain entrance into the host through natural openings such as: stomata, lenticels, hydathodes, nec•taries, leaf scars, stigma, etc. This is a process in which the pathogens have an easy access to the host, except in cases where sub-stomatal hairs may cause resistance against the host entry.

ii) Entry through wounds:

Wounds caused due to natural calamities (storm, fire, etc.); during field operations; by insects, by accidental breaking of parts or otherwise; offer easy passages of pathogens in the host. But so far as viruses are concerned, the host entry is only through wounds.

iii) Entry by direct penetration of surface cells:

The entry of pathogen by direct penetration of the outer wall of the host surface cells is rather a difficult process for which the pathogen usually requires high moisture or free water supply. It is even more difficult in leaves with waxy covering on their surface which allows water to rim off freely.

Fungal pathogens penetrate into host either by boring through the outer wall of the surface cell or penetration is effected by pressure and sometimes due to chemical softening or solubilizing of the barrier caused by the solvent action of enzymes secreted by the infecting organ.

After the hypha made contact with a suitable host surface, some growth in close contact with it takes place. This is followed by the development of an aspersorium or increase in diameter of hypha serving as adherent area from which develops penetration tube.

The penetration tube penetrates through the cuticle at a point softened by enzymatic action and followed by mechanical pressure. In some cases germ lube produced by spore germination passes down between the racial wall of the adjoining cells without actually entering the cells.

This papilla is penetrated by the penetration hypha which enters the host cell and ultimately develops into a haustorium.

Bacteria are mostly weak parasites which cannot employ force to effect pene•tration. Their penetration is effected by chemical action. The plant parasitic nematodes pierce the host surface with spears or stylet.

The entire process of Direct Penetration, however, depends on:

a) The nature of cell wall layers,
b) The potentialities of the enzyme system of the pathogen, and
c) The potential force the pathogen can exert.

Role of cell wall degrading enzyme and toxins:

The main components of the host 'cell wall are pectin, cellulose, hemicellulose, lignin and small quantity of protein. The three main softening or solubilizing enzymes found in fungi are: pectolytic, cellulolytic, and lignolytic. The outer layer of host plant cell may have a layer of wax which is followed by cuticle impregnated with wax.

The cutin gradually decreases with the depth of the epidermis and is replaced by pectin which occurs as a homogeneous layer in some host plant. Subsequently in the secondary wall of the cell pectin is replaced by cellulose. Cellulose layers contain appreciable amounts of protein.

No pathogen secretes enzymes that would degrade wax. Only mechanical entry can enable a pathogen to

breach this layer. Cutin layer is penetrated either by pres•sure (mechanically exerting force) or by the action of degrading enzymes: cutinase, cutin esterase, and carboxyl cutin esterase. Cutinase breaks cutin into fatty acid and hydroxyfatty acid.

Pectic substances which form basic material of middle lamella, primary and secondary walls are degraded by pectinolytic enzymes.

The pectinolytic enzymes act against pectin and pectic substances in many steps:

a) The enzymes pectin methyl esterases (PME) hydrolyse the pectic substances into methanol and pectic acid;
b) The enzymes pectin glycosidases, polygalacturonases (PG) and polymethyl galacturo- nases (PMG) degrade the pectic acid and methylated chains of pectin. Besides these, wall modifying enzyme (WME) modifies the pectic material for subsequent degradation.

The degradation of pectic substances provides nutrients for many fungal pathogens and due to weakening of the cell wall facilitates inter- and intra-cellular invasion by hyphae. Pectic enzymes are produced both constitutively and adaptively. High C/N ratio of the substrate favors increased mycelial growth and low enzyme synthesis whereas lower ratios give poorer growth but increased enzyme synthesis.

Cellulose forms the structural framework of cell walls. Cellulolytic enzymes act upon the cellulose and break it to simple compounds and make the way for the easy penetration of the pathogen into the host cell.

According to unienzyme theory, complete degradation of cllulose into glucose units is by a single enzyme: Cellulose Cellulose Cellobiose Cellobiase Glucose. Whereas, the multienzyme theory explains cellulose degradation in a series of steps through two groups of enzymes.

One group of enzymes loosens the cellulose fibrils of the crystalline area by hydrolytic mechanism. The other group of enzymes penetrates the cellulose lattice and causes hydrolytic cleavage of hydrogen bondage.

The degradation of hemicelluloses by the enzymes hemicellulases produces simpler sugars. In vascular wilt diseases the molecules released by cellulose degradation can cause plugging of the vessels.

The most complex chemical compound in the plant cell wall is lignin which occurs chiefly in the matrix surrounding the cellulose fibrils. It is one of the most structurally complex biopolymers whose degradation is caused by polyphenol oxidases produced by wood-rotting fungi. But fungi cannot utilize the degradation products.

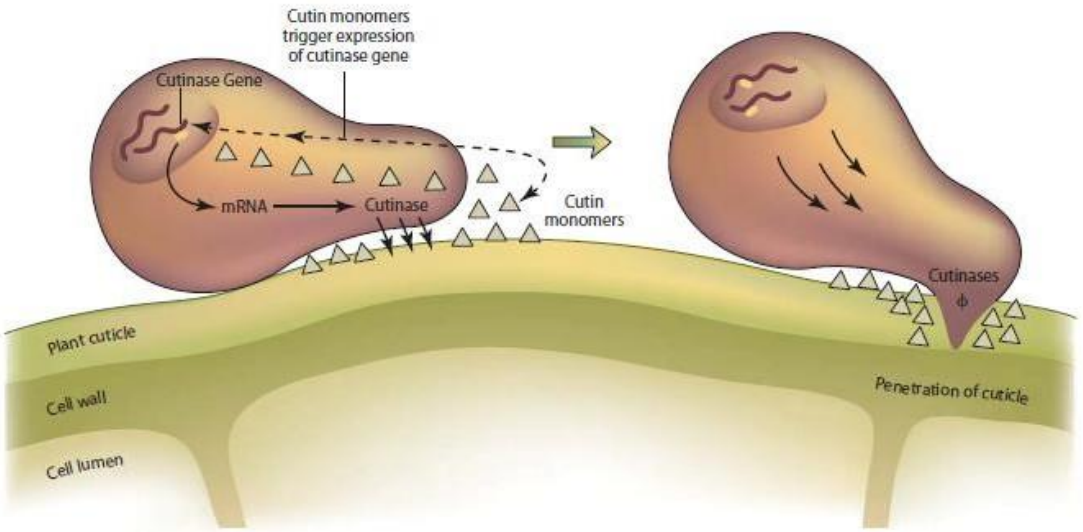


FIGURE 5-5 Diagrammatic representation of cuticle penetration by a germinating fungus spore. Constitutive cutinase releases a few cutin monomers from the plant cuticle. These trigger expression of the cutinase genes of the fungus, leading to the production of more cutinase(s), which macerates the cuticle and allows penetration by the fungus.

Toxins That Affect a Wide Range of Host Plants

Living plant cells are complex systems in which many interdependent biochemical reactions are taking place concurrently or in a well-defined succession. These reactions result in the intricate and well-organized

processes essential for life. Disturbance of any of these metabolic reactions causes disruption of the physiological processes that sustain the plant and leads to the development of disease. Among the factors inducing such disturbances are substances that are produced by plant pathogenic microorganisms and are called toxins. Toxins act directly on living host protoplasts, seriously damaging or killing the cells of the plant. Some toxins act as general protoplasmic poisons and affect many species of plants representing different families. Others are toxic to only a few plant species or varieties and are completely harmless to others. Many toxins exist in multiple forms that have different potency. Fungi and bacteria may produce toxins in infected plants as well as in culture medium. Toxins, however, are extremely poisonous substances and are effective in very low concentrations. Some are unstable or react quickly and are bound tightly to specific sites within the plant cell.

Toxins injure host cells either by affecting the permeability of the cell membrane (Fig. 5-2) or by inactivating or inhibiting enzymes and subsequently interrupting the corresponding enzymatic reactions. Certain toxins act as antimetabolites and induce a deficiency for an essential growth factor.

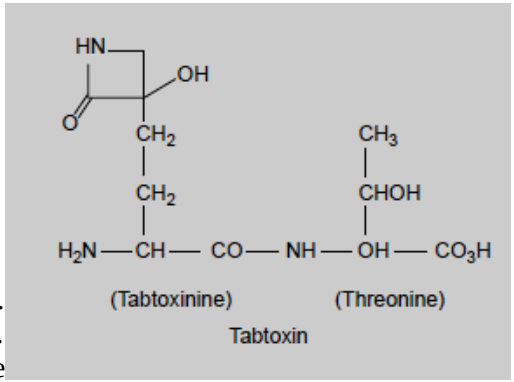
Toxins That Affect a Wide Range of Host Plants

Several toxic substances produced by phytopathogenic microorganisms have been shown to produce all or part of the disease syndrome not only on the host plant, but also on other species of plants that are not normally attacked by the pathogen in nature. Such toxins, called nonhost-specific or nonhost-selective toxins. These toxins increase the severity of disease caused by a pathogen, i.e., they affect the virulence of the pathogen, but are not essential for the pathogen to cause disease, i.e., they do not determine the pathogenicity of the pathogen. Several of these toxins, e.g., tabtoxin and phaseolotoxin, inhibit normal host enzymes, thereby leading to increases in toxic substrates or to depletion of needed compounds. Several toxins affect the cellular transport system, especially H+/K+ exchange at the cell membrane. Some, e.g., tagetitoxin, act as inhibitors of transcription in cell organelles, such as the chloroplasts. Others, e.g., cercosporin, act as photosensitizing agents, causing the peroxidation of membrane lipids.

Several toxic substances produced by phytopathogenic microorganisms have been shown to produce all or part of the disease syndrome not only on the host plant, but also on other species of plants that are not normally attacked by the pathogen in nature. Such toxins, called **nonhost- specific or nonhost-selective toxins**.

Tabtoxin

Tabtoxin is produced by the bacterium *Pseudomonas syringae*; pv. tabaci, which causes the wildfire disease of tobacco; by strains of pv. tabaci occurring on other hosts such as bean and soybean; and by other pathovars (subspecies) of *P. syringae*, such as those occurring on oats, maize, and coffee. Toxin-producing strains cause necrotic spots on leaves, with each spot surrounded by a yellow halo.



Phaseolotoxin

Phaseolotoxin is produced by the bacterium *Pseudomonas syringae* pv. phaseolicola, the cause of halo blight of bean and some other legumes. Phaseolotoxin is a modified ornithine–alanine– arginine tripeptide carrying a phosphosulfinyl group.

Tentoxin

Tentoxin is produced by the fungus *Alternaria alternate* (previously called *A. tenuis*), which causes spots and chlorosis in plants of many species. Tentoxin is a cyclic tetrapeptide that binds to and inactivates a protein (chloroplast-coupling factor) involved in energy transfer into chloroplasts. The toxin also inhibits the light-dependent phosphorylation of ADP to ATP.

Cercosporin

Cercosporin is produced by the fungus *Cercospora* and by several other fungi. It causes damaging leaf spot and blight diseases of many crop plants, such as Cercospora leaf spot of zinnia and gray leaf spot of corn.

Host-Specific or Host-Selective Toxins

A host-specific or host-selective toxin is a substance produced by a pathogenic microorganism that, at physiological concentrations, is toxic only to the hosts of that pathogen and shows little or no toxicity against nonsusceptible plants.

Victorin, or HV Toxin

Victorin, or Hv-toxin, is produced by the fungus *Cochliobolus* (*Helminthosporium*) *victoriae*. This fungus appeared in 1945 after the introduction and widespread use of the oat variety Victoria and its derivatives, all of which contained the gene Vb for resistance to crown rust disease. *C. victoriae* infects the basal portions of susceptible oat plants and produces a toxin that is carried to the leaves, causes a leaf blight, and destroys the entire plant.

T Toxin [*Cochliobolus* (*Helminthosporium*) heterostrophus Race T Toxin]

T toxin is produced by race T of *C. heterostrophus* (*Bipolaris maydis*), the cause of southern corn leaf blight. Race T, indistinguishable from all other *C. heterostrophus* races except for its ability to produce the T toxin, appeared in the United States in 1968. By 1970, it had spread throughout the corn belt, attacking only corn that had the Texas male-sterile (Tms) cytoplasm. T toxin is a mixture of linear, long (35 to 45 carbon) polyketols.

HC Toxin

Race 1 of *Cochliobolus* (*Helminthosporium*) *carbonum* (*Bipolaris zeicola*) causes northern leaf spot and ear rot disease in maize. It also produces the hostspecific HC toxin, which is toxic only on specific maize lines.

Other Nonhost-Specific Toxins

Numerous other nonhost-specific toxic substances have been isolated from cultures of pathogenic fungi and bacteria and have been implicated as contributing factors in the development of the disease caused by the pathogen. Among such toxins produced by fungi are fumaric acid, produced by *Rhizopus* spp. in almond hull rot disease; oxalic acid, produced by *Sclerotium* and *Sclerotinia* spp. in various plants they infect and by *Cryphonectria parasitica*, the cause of chestnut blight; alternaric acid, alternariol, and zinniol produced by *Alternaria* spp. in leaf spot diseases of various plants; ceratoulmin, produced by *Ophiostoma ulmi* in Dutch elm disease; fusicoccin, produced by *Fusicoccum amygdale* in the twig blight disease of almond and peach trees; ophiobolins, produced by several *Cochliobolus* spp. in diseases of grain crops; pyricularin, produced by *Pyricularia grisea* in rice blast disease; fusaric acid and lycomarasmin, produced by *Fusarium oxysporum* in tomato wilt; and many others.

Host-Specific or Host-Selective Toxins

A **host-specific** or **host-selective** toxin is a substance produced by a pathogenic microorganism that, at physiological concentrations, is toxic only to the hosts of that pathogen and shows little or no toxicity against non susceptible plants. Most host-specific toxins must be present for the producing microorganism to be able to cause disease. So far, host-specific toxins have been shown to be produced only by certain fungi (*Cochliobolus*, *Alternaria*, *Periconia*, *Phyllosticta*, *Corynespora*, and *Hypoxylon*), although certain bacterial polysaccharides from *Pseudomonas* and *Xanthomonas* have been reported to be host specific.

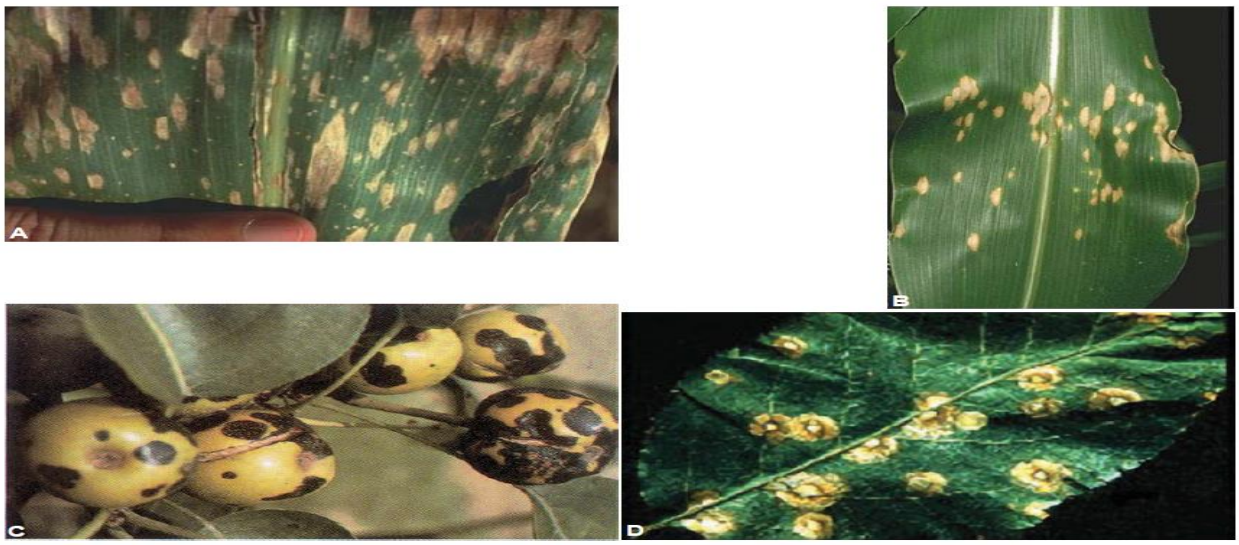


FIGURE 5-15 Symptoms caused by host-selective toxins. (A) Southern corn leaf blight symptoms caused by two race T of the fungus *Cochliobolus (Helminthosporium) heterostrophus* and its toxin, T toxin, on a corn plant containing Texas male-sterile cytoplasm. (B) Northern corn leaf spot symptoms caused by the fungus *Cochliobolus carbonum* and its toxin, HC toxin, on corn. (C) Fruit spots on Japanese pear caused by one of the strains of the fungus *Alternaria alternata* and its toxin, AK toxin. (D) Leaf spots caused by the AM toxin produced by another strain of the fungus *A. alternata* and its toxin, AM toxin, on apple leaves. [Photographs courtesy of (A) C. Martinson and (B) G. Munkvold, Iowa State University, (C) T. Sakuma, and (D) J. W. Travis, Pennsylvania State University.]

Entry through specific parts or organs:

In such cases the pathogens exhibit specificity of host parts or organs (stem, leaf, root hairs, floral parts, coleoptile, buds, etc.) during their entry in the host.

Some examples are:

Synchytrium endobioticun infects and remains confined in the epidermal cells of potato tuber, *Erysiphe graminis* infects the epidermal cells of leaves of cereals and grasses, *Ustilago nuda* causes infection only in the flowers of wheat, *Claviceps purpurea* infects the ovaries of rye flower, certain wood rotting fungi are – *Merulius lacrimans* and *Fomes annosus*; soil inhabiting pathogens inter through root hairs – *Plasmodiophora brassicae*.

Post-Penetration Stage:

Usually with the success of the penetration process, post-penetration is successful. But the entry of the pathogen in the host tissue may not always ensure immediate infection leading to disease development. The process may be delayed or there may be failure for various reasons.

The delay may be in cases where the pathogen has incubation period and infection is established only after the expiry of the incubation period. The success of post-penetration process depends largely upon competition of pathogen for nutrition, and production of enzymes and toxic substances and their effects on host metabolic activities.

Again due to toxic effect of host cytoplasm, the pathogen may fail to establish biological relationship with the host. Host-pathogen interaction may also result hypersensitivity of the host tissue, whereby rapid death of the affected cells prevents the further spreading of the pathogen due to shortage of nutrition.

But in most of the plant diseases, host infection is followed by invasion, a condition when a pathogen grows rapidly in the host tissue. For example, bacteria invade host tissues intracellular and destroy them. Whereas, fugal mycelia invade inter- or intracellular but may or may not cause destruction immediately after invasion. Viruses always invade host tissues intracellular.

They multiply in the living host cells by directing them to manufacture viral nucleic acid and viral protein, their movement from cell to cell is through plasmodesmata. Again fungal hyphae and spores, and bacterial cells may move through vascular tissues once they gain entrance in them. Successful host invasion of the pathogen is invariably associated with disease syndromes of various types of varying degrees.

After penetrating the host cell walls, the pathogen comes in contact with the host cytoplasm from which it gets its required nutrition. In response to the activities of the pathogen, the host metabolic processes (osmoregulation, respiration, photosynthesis, etc.) get upset.

Signal transduction:

Perception of pathogen presence

Plant defense signaling is activated by the pathogen-detecting receptors that are described in an above section. The activated receptors frequently elicit reactive oxygen and nitric oxide production, calcium, potassium and proton ion fluxes, altered levels of salicylic acid and other hormones and activation of MAP kinases and other specific protein kinases. These events in turn typically lead to the modification of proteins that control gene transcription, and the activation of defense-associated gene expression.

Transcription factors and the hormone response

Numerous genes and/or proteins as well as other molecules have been identified that mediate plant defense signal transduction. Cytoskeleton and vesicle trafficking dynamics help to orient plant defense responses toward the point of pathogen attack.

Mechanisms of transcription factors and hormones

Plant immune system activity is regulated in part by signaling hormones such as: Salicylic acid , Jasmonic acid, Ethylene. There can be substantial cross-talk among these pathways

Regulation by degradation:

As with many signal transduction pathways, plant gene expression during immune responses can be regulated by degradation. This often occurs when hormone binding to hormone receptors stimulates ubiquitin-associated degradation of repressor proteins that block expression of certain genes. The net result is hormone-activated gene expression. Examples:

Auxin: binds to receptors that then recruit and degrade repressors of transcriptional activators that stimulate auxin-specific gene expression.

Jasmonic acid: similar to auxin, except with jasmonate receptors impacting jasmonate-response signaling mediators such as JAZ proteins.
Gibberellic acid: Gibberellin causes receptor conformational changes and binding and degradation of Della proteins.
Ethylene: Inhibitory phosphorylation of the EIN2 ethylene response activator is blocked by ethylene binding. When this phosphorylation is reduced, EIN2 protein is cleaved and a portion of the protein moves to the nucleus to activate ethylene-response gene expression.

Ubiquitin and E3 signaling

Ubiquitination plays a central role in cell signaling that regulates processes including protein degradation and immunological response. Although one of the main functions of ubiquitin is to target proteins for destruction, it is also useful in signaling pathways, hormone release, apoptosis and translocation of materials throughout the cell. Ubiquitination is a component of several immune responses. Without ubiquitin's proper functioning, the invasion of pathogens and other harmful molecules would increase dramatically due to weakened immune defenses.

E3 signaling

The E3 Ubiquitin ligase enzyme is a main component that provides specificity in protein degradation pathways, including immune signaling pathways. The E3 enzyme components can be grouped by which domains they contain and include several types. These include the Ring and U-box single subunit, HECT, and CRLs. Plant signaling pathways including immune responses are controlled by several feedback pathways, which often include negative feedback; and they are be regulated by De-ubiquitination enzymes, degradation of transcription factors and the degradation of negative regulators of transcription.

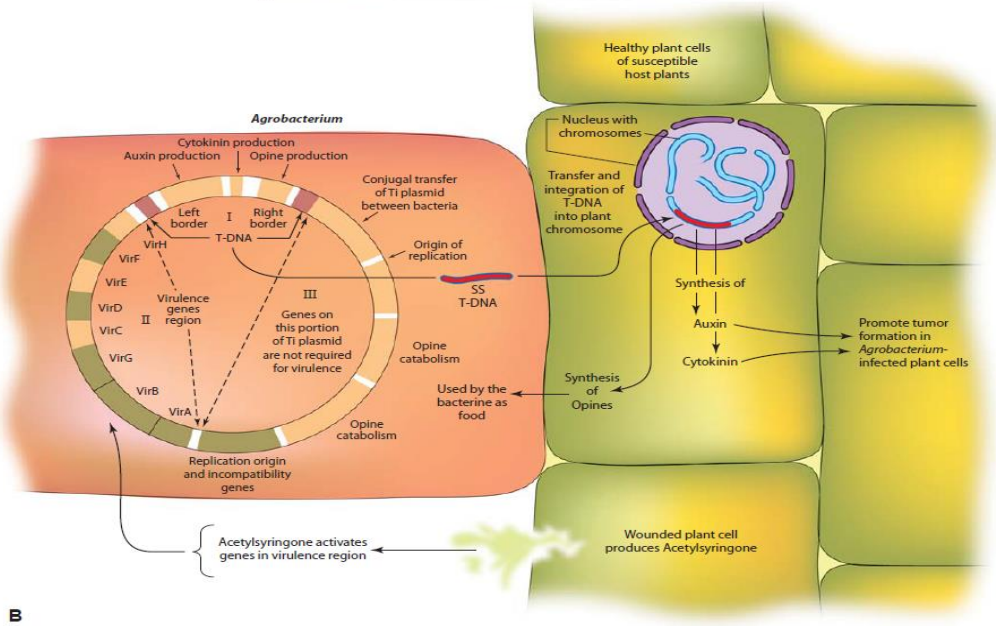


FIGURE 5-17 (A) External and cross-sectional view of crown gall on a rose stem caused by the bacterium *Agrobacterium tumefaciens*. (B) Schematic representation of the structure of Ti plasmid of the bacterium and of the transfer, integration, and expression of T-DNA in an infected plant that results in the production of crown gall tumors. Genes A, B, D, and G are needed for tumor formation on any susceptible plant species. Genes C, E, F, and H affect the host plant range and/or the size of tumors caused by the bacterium. The functions of the proteins of virulence genes are as follows: A, receptor of wound signal; B, codes for proteins that form membrane pores; C, enhances transfer of T-DNA; D, codes for proteins that nick T-DNA at its borders, help transport T-DNA across membranes, and carry signal compounds to the nucleus; E, protects T-DNA from nuclease enzymes and also carries nuclear localization signals; F, may increase host range of tumor induction; G, activates other virulence genes; H, protects the bacterium from toxic plant compounds. The entire diagram presents a simplified scheme of interaction of gene products of host cells and T-DNA that lead to the production of a gall. [Photograph (A) courtesy of Oregon State University.]

6. Defense mechanisms of plants against infection: Pre-existing structural and biochemical defense, induced structural and biochemical defense, hypersensitive reaction, phytoalexins, PR proteins, systemic acquired resistance, induced systemic resistance; Gene for gene hypothesis; concept of horizontal and vertical resistance

In general, plants defend themselves against pathogens by a combination of weapons from two arsenals:
(1) structural characteristics that act as physical barriers and inhibit the pathogen from gaining entrance and spreading through the plant and **(2)** biochemical reactions that take place in the cells and tissues of the plant and produce substances that are either toxic to the pathogen or create conditions that inhibit growth of the pathogen in the plant. The combinations of structural characteristics and biochemical reactions employed in the defense of plants are different in different host–pathogen systems. In addition, even within the same host and pathogen, the combinations vary with the age of the plant, the kind of plant organ and tissue attacked, the nutritional condition of the plant, and the weather conditions.

I. Structural Defense:
In plants some structures are already present to defend the attack while in others, the structures to defend the host develops after the infection. In this way, structural defense can be characterised as (A) Preexisting defense structures and (B) Defense structures developed after the attack of the pathogen.

A. Preexisting Defense Structures:

(i) Cuticular Wax:
Wax-mixtures of long chain aliphatic compounds get deposited on the cuticular surface of some plants. Deposition of wax on the cuticular surface is thought to play a defensive role by forming a hydrophobic surface where water is repelled.
As a result, the pathogen does not get sufficient water to germinate or multiply. In addition, a negative charge usually develops on the leaf surface due to the presence of fatty acids – the main component of cuticle. The negative charge prevents/reduces the chance of infection by many pathogens.

(ii) Cuticle Thickness:
The thickness of cuticle is most important for those which try to enter the host through the leaf surface. The cuticle thickness obstructs the path of pathogen. In addition, a thick cuticle checks the exit of the pathogen from inside the host, thus reducing the secondary infection.

(iii) Structure of Epidermal Cell Walls:
Tough and thick outer walls of epidermal cells may directly prevent the entry of the pathogen completely or make the entry difficult. The presence or absence of lignin and silicic acid in the cell walls may show variation in resistance to penetration of the pathogen. Most outer walls of epidermal cells of rice plants are lignified and are seldom penetrated by blast disease of rice pathogen. In resistant varieties of potato tubers (resistant to *Pythium debaryanum*) the epidermal cells contain higher fibre content than the susceptible ones.

(iv) Structure of Natural openings:
Structure of natural openings like stomata lenticels etc. also decide the fate of the entry of the pathogen. In Szincum variety of citrus, the stomata are small and possess very narrow openings surrounded by broad lipped raised structures which prevent entry of water drops containing citrus canker bacterium.
In the same way, the size and internal structures of lenticels may play a defensive role against the pathogens. Varieties having small lenticels in the apple fruits prevent the entry of the pathogen while those having large openings easily allow the pathogen to enter.
Nectaries provide openings in the epidermis and may play a defensive role due to high osmotic concentration of the nectar. In resistant varieties of apple, presence of abundant hairs in the nectaries acts as a defense mechanism while susceptible varieties are devoid of abundant hairs.

Internal Defense Structures:
There are many preexisting internal defense structures inside the plant that prevent the entry of pathogen beyond these structures. In some plants, cell walls of certain tissues become thick and tough due to environmental conditions and this makes the advance of the pathogen quite difficult.
In case of stems of cereal crops, vascular bundles or extended areas of sclerenchyma cells checks the progress of rust

pathogen. Leaf veins effectively obstruct the spread of pathogen like the angular leaf spot pathogen.

B. Defense Structures Developed after the Attack of the Pathogen:

After the pathogen has successfully managed to overcome the preexisting defense mechanisms of the host, it invades the cells and tissues of the host.

In order to check the further invasion by the pathogen, the host plants develop some structures/mechanisms which may be defense reactions in the cytoplasm, cell wall defense structures, defense structures developed by the tissues and ultimately the death of the invaded cell i.e. necrosis. These will be briefly discussed here.

(i) Defense Reactions in the Cytoplasm:

The cytoplasm of the invaded cell surrounds the hyphae of the pathogen and the nucleus of the host cell gets stretched to break into two. In some host cells, the cytoplasm and the nucleus of the infected cells enlarge.

The cytoplasm becomes granular and dense and develops granular particles. These result in the disintegration of the pathogen mycelium and thus the invasion stops. Such cytoplasmic defence mechanisms can be seen in weak pathogens like Annillaria and some mycorrhizal fungi.

(ii) Cell Wall Defense Structures:

Cell wall defense structures are of limited help to the host. These include morphological changes in the cell wall of the host.

Three types of cell wall defense structures are generally observed:

(i) Cell walls thicken in response to the pathogen by producing a cellulose material, thus preventing the entry of the pathogen

(ii) The outer layer of cell walls of the parenchyma cells in contact with invading bacterial cells produce an amorphous fibrillar material that traps the bacteria thus preventing them to multiply a

(iii) Callose papillae get deposited on the inner layers of the cell walls due to invasion by fungal pathogens.In raw cases, the hyphal tips of the infecting fungal pathogen penetrating the cell wall and thereafter growing into the cell lumen get enveloped by callose material that, later become infused with phenolics forming a sheath around the hyphae.

(iii) Defense Structures Developed by the Tissues:

The following four developments take place in the tissues after penetration:

(a) Gum Deposition:

Plants produce a variety of gummy substances around lesions or spots as a result of infection. These gummy substances inhibit the progress of the pathogen. The gummy substances are commonly produced in stone fruits.

(b) Abcission Layers:

Abscission layers are usually formed to separate the ripe fruits and old leaves from the plant. But in some stone fruit trees, these layers develop in their young leaves in response to infection by several fungi, bacteria or viruses. An abscission layer is a gap formed between two circular layers of cells surrounding the point of infection.

This gap is created by the dissolution of one or two layers of the middle lamella, one or two layers of cells surrounding the infected loci resulting in the infected locus becoming unsupported, shrivels, dies and falls down along with the pathogen. Abscission layer formation protects the healthy leaf tissue from the attack of the pathogen.

Different defence structures developed after infection

C. Tyloses:

Tyloses are out growths of protoplasts of adjacent live parenchyma cells protruding into xylem vessels through pits under stress or in response to attack by the vascular pathogens. Their development blocks the Xylem vessels, obstructing the flow of water and resulting in the development of wilt symptoms.

However, tyloses are formed in some resistant plants ahead of infection and the prevent the plant from being attacked.

D. Formation of Layers:

Some pathogens like certain bacteria, some fungi and even some viruses and nematodes stimulate the host to form multilayered cork cells in response to infection, these develop as a result of stimulation of host cells by substances secreted by thus, pathogen.

These layers inhibit the further invasion by the pathogen and also block the flow of toxic substances secreted by the pathogen. Cork layers also stop the flow of nutrients of the host thus also depriving the pathogen of the nutrients.

Examples of cork layer formation as a result of infection are: soft not of potato caused by *Rhizopus* sp., potato tuber disease caused by *Rhizoctonia* sp., Scab of potato caused by *Streptomyces scabies* and necrotic lesions on tobacco

caused by tobacco mosaic virus.

IV. Necrosis or Hypersensitive Type of Defense:

Necrosis or hypersensitive type of defense is another defense mechanism adopted by some pathogens like *Synchytrium endobioticum* causing wart disease of potato, *Phytophthora infestans* causing late blight disease of potato and *Pyricularia oryzae* causing blast of rice etc.

In such diseases, the host nucleus moves toward the pathogen when the latter comes in contact with the protoplasm of the host. The nucleus soon disintegrates into brown granules which first accumulate around the pathogen, later dispersing throughout the host cytoplasm.

Soon the cell membrane swells and finally the cell burst and dies. These cause the pathogen nucleus to disintegrate into a homogenous mass and its cytoplasm dense. As a result, the pathogen fails to grow beyond the necrotic or dead cells and the further growth of the pathogen is stopped.

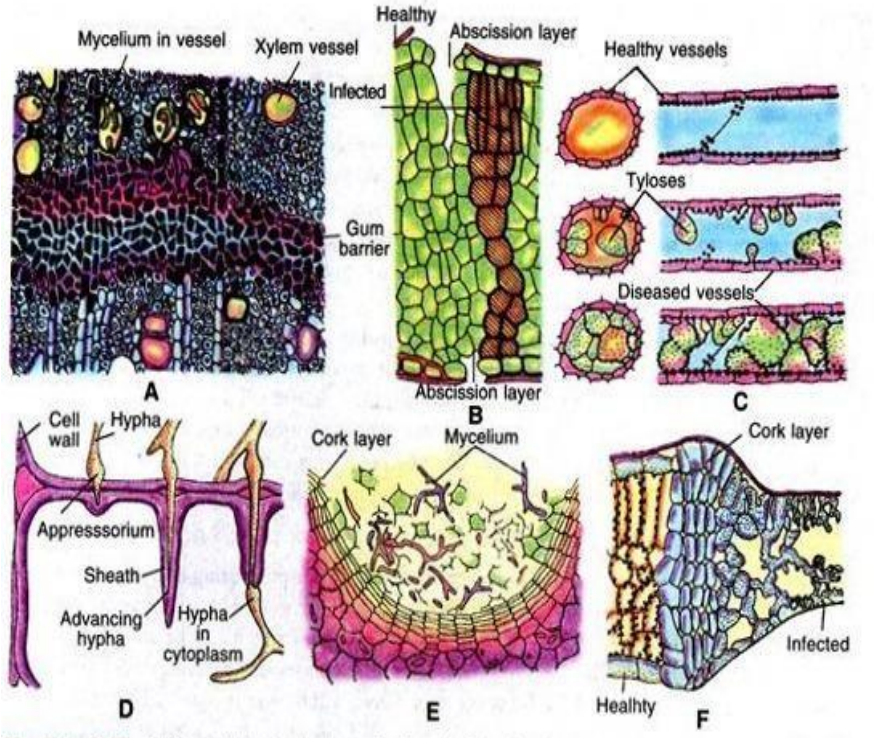


Fig. 21.1. (A-F). Different defence structures developed after infection : (A) Gum deposition in cells ; (B) Abscission layer ; (C) Tyloses ; (D) Formation of sheath around developing hyphae (E, F)./ Development of cork layer in tuber (E) and leaf (F).

II. Biochemical Defense:

Although structural defense mechanisms do prevent the attack of the pathogen, the defense mechanism also includes the chemical substances produced in the plant cells before or after the infection.

It has now been established that biochemical defense mechanisms play more important role than the structural defense mechanisms. This has been supplemented by the fact that many pathogens entering non host plants naturally or artificially inoculated fail to cause infections in absence of any structural barriers. This does suggest that chemical defense mechanisms rather than structural mechanisms are responsible for resistance in plants against certain pathogens.

(A) Preexisting Biochemical Defense:

(i) Inhibitors Released in the Prepenetration Stage:

Plant generally exudes organic substance through above ground parts (phyllosphere) and roots (rhizosphere). Some of the compounds released by some plants are known to have an inhibitory effect on certain pathogens during the prepenetration stage.

For example fungistatic chemicals released by tomato and sugar beet prevent the germination of Botrytis and *Cercospora*. Presence of phenolics like protocatechuic acid and catechol in scales of red onion variety inhibit

the germination of conidia of *Colletotrichum circinans* on the surface of red onion.
Inhibitors present in high concentrations in the plant cells also play an important role in defense of plants. Presence of several phenolics, tannins and some fatty acid like compounds such as dienes in cells of young fruits, leaves or seeds afford them resistance to Botrytis.
The tubers of resistance vars of potato against potato scab disease contain higher concentrations of chlorogenic acid around the lenticels and tubers than the susceptible vars. Several other compounds like saponin tomatin in tomato and avinacin in oats have antifungal activity. Some enzymes like glucanases and chitinases present in cells of some plants may break down the cell wall components of pathogens.

(ii) Lack of nutrients essential for the pathogen is another preexisting biochemical defense mechanism.

Plant varieties or secies which do not produce any of the chemicals essential for the growth of pathogen may act as resistant variety.
For example, a substance present in seedling varieties susceptible to *Rhizoctonia* initiates hyphae cushion formation from which the fungus sends penetration hyphae inside the host plants. When this substance is not present, hyphal cushions are not formed and the infection does not occur.

(iii) Absence of Common Antigen in Host plant:

It is now clear that the presence of a common protein (antigen) in both the pathogen and host determines diseases occurrence in the host. But if the antigen is present in the host and absent in the host or vice-versa, it makes the host resistant to the pathogen. For example, varieties of linseed which have an antigen common to their pathogen are susceptible to the disease rust of linseed caused by *Melampsora lini*. In contrast, the absence of antigen in linseed varieties but occurring in the pathogen are resistant to the pathogen. Another example is leaf spot disease of cotton caused by *Xanthomonas campestris* pv. malvacearum.

FIGURE 6-2 Onion smudge, caused by the fungus *Colletotrichum circinans*, develops on white onions but not on colored ones, which, in addition to the red or yellow pigment, also contain the phenolics protocatechuic acid and catechol, both of which are toxic to the fungus. (Photograph courtesy of G. W. Simone.)

(B) Post-Infection-Biochemical Defense Mechanism:

In order to sight infections caused by pathogens or injuries caused by any other means, the plant cells and tissues produce by synthesis many substances (chemicals) which inhibit the growth of causal organism.
These substances are generally produced around the site of infection or injury with the main aim at overcoming the problem.
Some such important chemicals are described below:

(i) Phenolic Compounds:

These are the most common compounds produced by plants in response to injury or infection. The synthesis of phenolic compounds takes place either through "acetic acid pathway" or "Shikimic acid pathway".
Some common phenolic compounds toxic to pathogens are chlorgenic acid, caffeic acid and ferulic acid. These phenolic compounds are produced at a much faster rate in resistant varieties than in susceptible varieties.
Probably that the combined effect of all phenolics present is responsible for inhibiting the growth of the infection.

(ii) Phytoalexins:

Phytoalexins are toxic antimicrobial substances synthesized 'de novo' in the plants in response to injury, infectious agents or their products and physiological stimuli. The term phytoalexin was first used by the two phytopathologists Muller and Borger (1940) for fungi static compounds produced by plants in response to mechanical or chemical injury or infection.

All phytoalexins are lipophilic compounds and were first detected after a study of late blight of potato caused by Phytophthora infestans. Phytoalexins are believed to be synthesized in living cells but surprisingly necrosis follows very quickly.

According to Bill (1981), peak concentration of phytoalexins almost always coincides with necrosis. Although the exact mechanism of production of phytoalexin has not been properly understood, it is considered that a metabolite of the host plant interacts with specific receptor on the pathogen's membrane resulting in the secretion of "phytoalexin elicitor" which enters the host plant cells and stimulates the phytoalexin synthesis.

Phytoalexins are considered to stop the growth of pathogens by altering the plasma membrane and inhibiting the oxidative phosphorylation.

Phytoalexins have been identified in a wide variety of species of plants such as Soyabean, Potato, sweet potato, barley, carrot, cotton etc. are being investigated. Some common phytoalexins are Ipomeamarone, Orchinol, Pistatin, Phaseolin, Medicarpin, Rishitin, Isocoumarin, 'Gossypol' Cicerin, Glyceolin, Capisidiol etc.

The following Table gives a list of phytoalexins, chemical nature the host and the pathogens in response to which these are produced:

	Phytoalexin	*Host*	*Pathogen*	*Chemical Nature of the phytoalexin*
1.	Ipomeamarone	*Ipomoea batata*	*Ceratocystis fimbriata*	Furanosesquiterepene ketone.
2.	Orchinol	*Orchid malitaris*	*Rhizoctonia repens*	Phenanthrene
3.	Pistatin	*Pisum sativum*	*Ascochyta pisi*	Kievitone
4.	Phaseolin	*Phaseolus* sp.	*Monilia fruticola*	
5.	Medicaripin	*Medicago lupulina*	*Colletotrichum phomoides*	Dimethyl-homoptero-carpin
6.	Rishitin	*Solanum tuberosum* var. *rishiri*	*Phytophthora infestans*	Nonsequiter-pene
7.	Isocoumarin	*Daucus carrota*	*Fusarium oxysporum f. lycopersici* *Rhizopus solonifer*	–
8.	*Cicerin*	*Cicer arietinum*	*Ascochyta rabie*	
9.	Gossypol	*Gossypium* sp.	*Verticillium alboatrum*	
10.	Capisidiol	*Capsicum fruitescens*	*Peronospora trifolium*	
11.	Wyerone acid	Broad bean	*Botrytis fabae*	

(iii) Substances Produced in Host to Resist Enzymes Produced by Pathogen:

Some hosts produce chemicals which neutralize the enzymes produced by pathogen, thus defending the host. Therefore these substances help plants to defend themselves from the attack of the pathogen.

In bean plants, infection with *Rhizoctonia solani* causes necrosis. In resistant bean varieties, the entry of pathogen causes the separation of methyl group from methylated pectic substances and forms polyvalent cations of pectic salts which contain calcium.

The calcium ions accumulate in infected as well as neighbouring healthy tissues and because of the calcium accumulation, the pathogen fails to disintegrate middle lamella by its polygalacturonase enzymes. These are known to dissolve the middle lamella of healthy tissue in susceptible varieties.

(iv) Detoxification of Pathogen Toxins and Enzymes:

In some cases, the plants produce chemicals which deactivate the toxins produced by the pathogens. For example, *Pyricularia oryzae* which causes blast disease of rice produces Picolinic acid and pyricularin as toxins.

Although resistant varieties convert these toxins into N-methyl picolininic acid pyrecularin into other compounds, the susceptible varieties do get affected by these toxins. Similarly in case of cotton and tomato wilts, the toxin fusaric acid produced by the pathogen gets converted into non- toxic N-methyl-fusaric acid amide in resistant varieties. As in case of detoxification of toxins, the toxic enzymes produced by the pathogen is deactivated by phenolic compounds or their oxidation products. Some varieties of cider apple are resistant to brown not disease caused by S*clereotinia fructigena*. It may be because of the resistant varieties producing pheolic oxidation products which inactivate the pectinolytic enzymes produced by the pathogen.

(v) Biochemical Alterations:

It has been observed that infection of the host by the pathogen brings about biochemical changes in the host which may prove toxic to the pathogenic microorganisms and cause resistance to the pathogen. Production of certain new enzymes and other compounds are synthesized and accumulated in higher concentration. This may also add to the resistance of the plant by being toxic to pathogenic microorganisms.

Induced structural defences:

Despite the preformed superficial or internal defense structures of host plants, most pathogens manage to penetrate their hosts through wounds and natural openings and to produce various degrees of infection. Even after the pathogen has penetrated the preformed defense structures, however, plants usually respond by forming one or more types of structures that are more or less successful in defending the plant from further pathogen invasion. Some of the defense structures formed involve the cytoplasm of the cells under attack, and the process is called **cytoplasmic defense reaction**; others involve the walls of invaded cells and are called **cell wall defense structures**; and still others involve tissues ahead of the pathogen (deeper into the plant) and are called **histological defense structures**. Finally, the death of the invaded cell may protect the plant from further invasion. This is called the **necrotic** or **hypersensitive defense reaction** and is discussed here briefly

Cytoplasmic Defense Reaction:

In a few cases of slowly growing, weakly pathogenic fungi, such as weakly pathogenic *Armillaria* strains and the mycorrhizal fungi, that induce chronic diseases or nearly symbiotic conditions, the plant cell cytoplasm surrounds the clump of hyphae and the plant cell nucleus is stretched to the point where it breaks in two.

In some cells, the cytoplasmic reaction is overcome and the protoplast disappears while fungal growth increases. In some of the invaded cells, however, the cytoplasm and nucleus enlarge. The cytoplasm becomes granular and dense, and various particles or structures appear in it. Finally, the mycelium of the pathogen disintegrates and the invasion stops.

Cell Wall Defense Structures

Cell wall defense structures involve morphological changes in the cell wall or changes derived from the cell wall of the cell being invaded by the pathogen. The effectiveness of these structures as defense mechanisms seems to be rather limited, however. Three main types of such structures have been observed in plant diseases.

(1) The outer layer of the cell wall of parenchyma cells coming in contact with incompatible bacteria swells and produces an amorphous, fibrillar material that surrounds and traps the bacteria and prevents them from multiplying.

(2) Cell walls thicken in response to several pathogens by producing what appears to be a cellulosic material. phenolic substances that are cross-linked and further increase its resistance to penetration. (3) Callose **papillae** are deposited on the inner side of cell walls in response to invasion by fungal pathogens. In some cases, hyphal tips of fungi penetrating a cell wall and growing into the cell lumen are enveloped by cellulosic (callose) materials that later become infused with phenolic substances and form a sheath or lignituber around the hypha (Fig. 6-4).

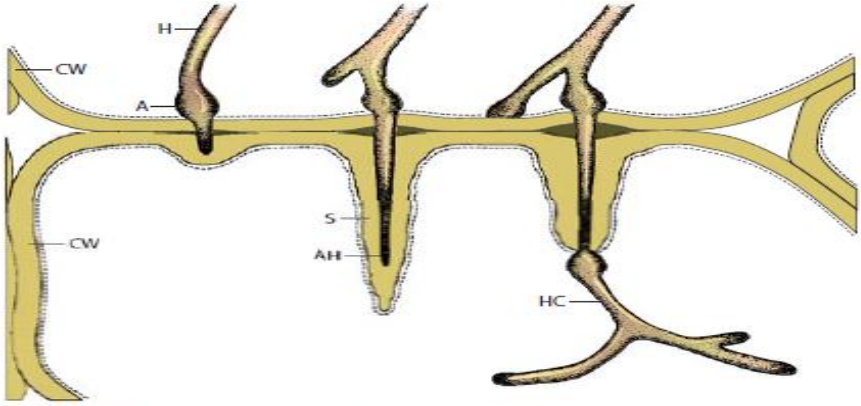


FIGURE 6-4 Formation of a sheath around a hypha (H) penetrating a cell wall (CW). A, appressorium; AH, advancing hypha still enclosed in sheath; HC, hypha in cytoplasm; S, sheath.

Histological Defense Structures Formation of Cork Layers

Infection by fungi or bacteria, and even by some viruses and nematodes, frequently induces plants to form several layers of cork cells beyond the point of infection (Figs. 6-5 and 6-6), apparently as a result of stimulation of the host cells by substances secreted by the pathogen. The cork layers inhibit further invasion by the pathogen beyond the initial lesion and also block the spread of any toxic substances that the pathogen may secrete. Furthermore, cork layers stop the flow of nutrients and water from the healthy to the infected area and deprive the pathogen of nourishment.

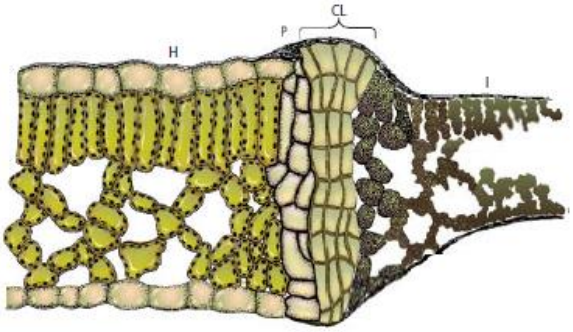


FIGURE 6-5 Formation of a cork layer (CL) between infected (I) and healthy (H) areas of leaf. P, phellogen. [After Cunningham (1928). *Phytopathology* 18, 717–751.]

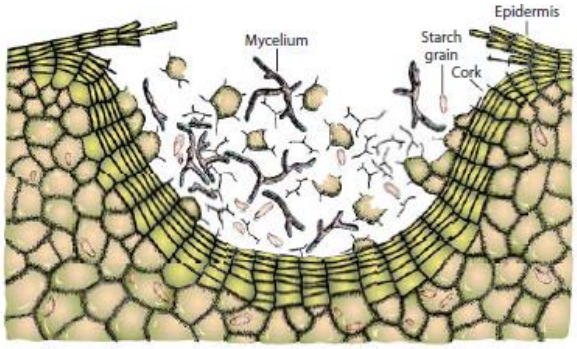
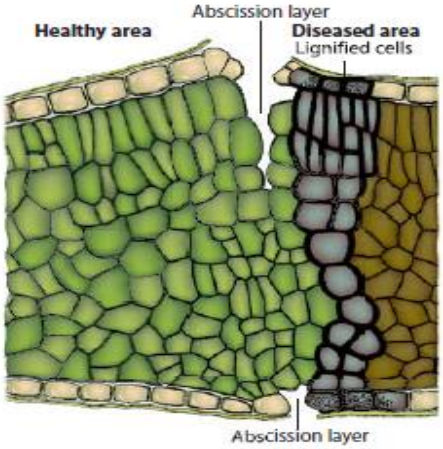


FIGURE 6-6 Formation of a cork layer on a potato tuber following infection with *Rhizoctonia*. [After Ramsey (1917). *J. Agric. Res.* 9, 421–426.]

Formation of Abscission Layers

Abscission layers are formed on young, active leaves of stone fruit trees after infection by any of several fungi, bacteria, or viruses. An abscission layer consists of a gap formed between two circular layers of leaf cells surrounding the locus of infection. Upon infection, the middle lamella between these two layers of cells is dissolved throughout the thickness of the leaf, completely cutting off the central area of the infection from the rest of the leaf (Fig. 6-7).



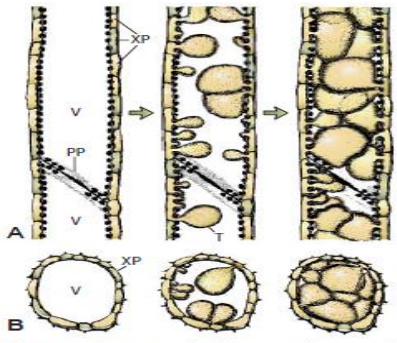


FIGURE 6-8 Development of tyloses in xylem vessels. Longitudinal (A) and cross section (B) views of healthy vessels (left) and of vessels with tyloses. Vessels at right are completely clogged with tyloses. PP, perforation plate; V, xylem vessel; XP, xylem parenchyma cell; T, tylosis.

Formation of Tyloses

Tyloses form in xylem vessels of most plants under various conditions of stress and during invasion by most of the xylem-invading pathogens. Tyloses are overgrowths of the protoplast of adjacent living parenchymatous cells, which protrude into xylem vessels through pits (Fig. 6-8).

Deposition of Gums

Various types of gums are produced by many plants around lesions after infection by pathogens or injury.

Gum secretion is most common in stone fruit trees but occurs in most plants. The defensive role of gums stems from the fact that they are deposited quickly in the intercellular spaces and within the cells surrounding the locus of infection, thus forming an impenetrable barrier that completely encloses the pathogen. The pathogen then becomes isolated, starves, and sooner or later dies.

Necrotic Structural Defense Reaction:

Defense through the Hypersensitive Response

The hypersensitive response is considered a biochemical rather than a structural defense mechanism but is described here briefly because some of the cellular responses that accompany it can be seen with the naked eye or with the microscope. In many host–pathogen combinations, as soon as the pathogen establishes contact with the cell, the nucleus moves toward the invading pathogen and soon disintegrates. At the same time, brown, resin-like granules form in the cytoplasm, first around the point of penetration of the pathogen and then throughout the cytoplasm. As the browning discoloration of the plant cell cytoplasm continues and death sets in, the invading hypha begins to degenerate (Fig. 6-9). In most cases the hypha does not grow out of such cells, and further invasion is stopped. In bacterial infections of leaves, the hypersensitive response results in the destruction of all cellular membranes of cells in contact with bacteria, which is followed by desiccation and necrosis of the leaf tissues invaded by the bacteria.

Although it is not quite clear whether the HR is the cause or the consequence of resistance, this type of necrotic defense is quite common, particularly in diseases caused by obligate fungal parasites and by viruses (Fig. 6-10A), bacteria (Fig. 6-10B), and nematodes.

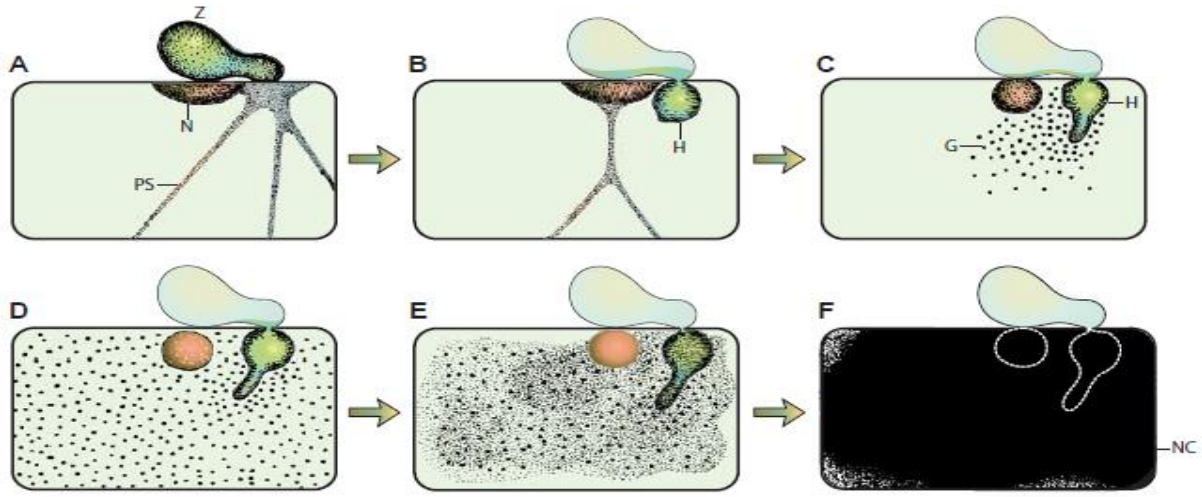


FIGURE 6-9 Stages in the development of the necrotic defense reaction in a cell of a very resistant potato variety infected by *Phytophthora infestans*. N, nucleus; PS, protoplasmic strands; Z, zoospore; H, hypha; G, granular material; NC, necrotic cell. [After Tomiyama (1956). *Ann. Phytopathol. Soc. Jpn.* **21**, 54–62.]

Induced biochemical defenses:

Induced Biochemical Nonhost Resistance As mentioned earlier, nonhost resistance is the resistance that keeps a plant protected from pathogens that are, through evolution, incompatible with that host.

Although the nature of nonhost resistance is unknown, for a pathogen it can be as big a gap to bridge as the difference between the features of a potato plant and an oak tree, or as close as the difference between the features of potato and tomato, or barley and wheat.

It appears, however, that in some plant/pathogen interactions of taxonomically unrelated plants (e.g., potato and oak or oak and wheat), nonhost resistance is controlled by constitutive defenses and/or defenses induced by nonspecific stimuli in a nonspecific manner.
Such defenses include physical topography and the structures present on the plant, the presence of toxic or the absence of essential compounds, and so on.

Induced Biochemical Defenses in Quantitative (Partial, Polygenic, General, or Horizontal) Resistance

In quantitative (partial, polygenic, multigenic, general, field, durable, or horizontal) resistance, plants depend on the action of numerous genes, expressed constitutively or upon attack by a pathogen (induced resistance).
These genes provide the plants with defensive structures or toxic substances that slow down or stop the advance of the pathogen into the host tissues and reduce the damage caused by the pathogen. Quantitative resistance is particularly common in diseases caused by nonbiotrophic pathogens. Quantitative resistance may vary considerably, in some cases being specific against some of the strains of a pathogen, in others being effective against all strains of a pathogen, or providing resistance against more than one pathogen. Genes for quantitative resistance are present and provide a basal level of resistance to all plants against all pathogens regardless of whether the plant also carries major (or R) genes against a particular pathogen.

Function of Gene Products in Quantitative Resistance

Unlike most major (or R) genes involved in monogenic resistance, which appear to code for components that help the host recognize the pathogen and to subsequently express the hypersensitive response, genes for quantitative resistance seem to be involved directly in the expression or production of some sort of structural or biochemical defense. Quantitative resistance defenses are basically the same ones that follow the hypersensitive response in monogenic resistance; in quantitative resistance, however, defenses generally do not follow a hypersensitive response and cell death because the latter do not usually occur in quantitative resistance. Genes involved in quantitative resistance are present in the same areas of plant chromosomes that contain the genes involved in defense responses, such as the production of phenylalanine ammonia lyase, hydroxyproline-rich glycoproteins, and pathogenesis–related proteins.

Pathogenesis-Related Proteins:

Pathogenesis-related proteins, often called PR proteins, are a structurally diverse group of plant proteins that are toxic to invading fungal pathogens. They are widely distributed in plants in trace amounts, but are produced in much greater concentration following pathogen attack or stress. PR proteins exist in plant cells intracellularly and also in the intercellular spaces, particularly in the cell walls of different tissues. Varying types of PR proteins have been isolated from each of several crop plants. Different plant organs, e.g., leaves, seeds, and roots, may produce different sets of PR proteins. Different PR proteins appear to be expressed differentially in their hosts in the field when temperatures become stressful, low or high, for extended periods. The several groups of PR proteins have been classified according to their function, serological relationship, amino acid sequence, molecular weight, and certain other properties. PR proteins are either extremely acidic or extremely basic and therefore are highly soluble and reactive. At least 14 families of PR proteins are recognized. The better known PR proteins are PR1 proteins (antioomycete and antifungal), PR2 (b-1,3- glucanases), PR3 (chitinases), PR4 proteins (antifungal), PR6 (proteinase inhibitors), thaumatine-like proteins, defensins, thionins, lysozymes, osmotinlike proteins, lipoxygenases, cysteine-rich proteins, glycine-rich proteins, proteinases, chitosanases, and peroxidases. There are often numerous isoforms of each PR protein in various host plants. Although healthy plants may contain trace amounts of several PR proteins, attack by pathogens, treatment with elicitors, wounding, or stress induce transcription of a battery of genes that code for PR proteins.

Systemic acquired resistance:

Induced resistance is at first localized around the point of plant necrosis caused by infection by the pathogen or by the chemical, and it is then called local acquired resistance. Subsequently, resistance spreads systemically and develops in distal, untreated parts of the plant and is called systemic acquired resistance. It is known now that several chemical compounds, e.g., salicylic acid, arachidonic acid, and 2,6-dichloroisonicotinic acid, may induce localized and systemic resistance in plants at levels not causing tissue necrosis. Jasmonic acid is another type of compound, derived primarily from oxidation of fatty acids, that leads to systemic acquired resistance, often in cooperation with salicylic acid and ethylene, leading to the production of defensins. Probenazole, a synthetic chemical used in Asia for the control of rice blast disease caused by the fungus *Magnaporthe grisea*, has been shown to act upstream from the salicylic acid transcribing gene and, thereby, causing accumulation of salicylic acid. Probenazole induces systemic acquired

resistance in rice against rice blast, in tomato against the bacterial pathogen *P. syringae* pv. tabaci, and in tobacco against the tobacco mosaic virus.

Systemic acquired resistance acts nonspecifically throughout the plant and reduces the severity of disease caused by all classes of pathogens, including normally virulent ones. It has been observed in many dicot and monocot plants, but has been studied most in cucurbits, solanaceous plants, legumes, and gramineous plants following infection with appropriate fungi, bacteria, and viruses. Systemic acquired resistance is certainly produced in plants following expression of the hypersensitive response.

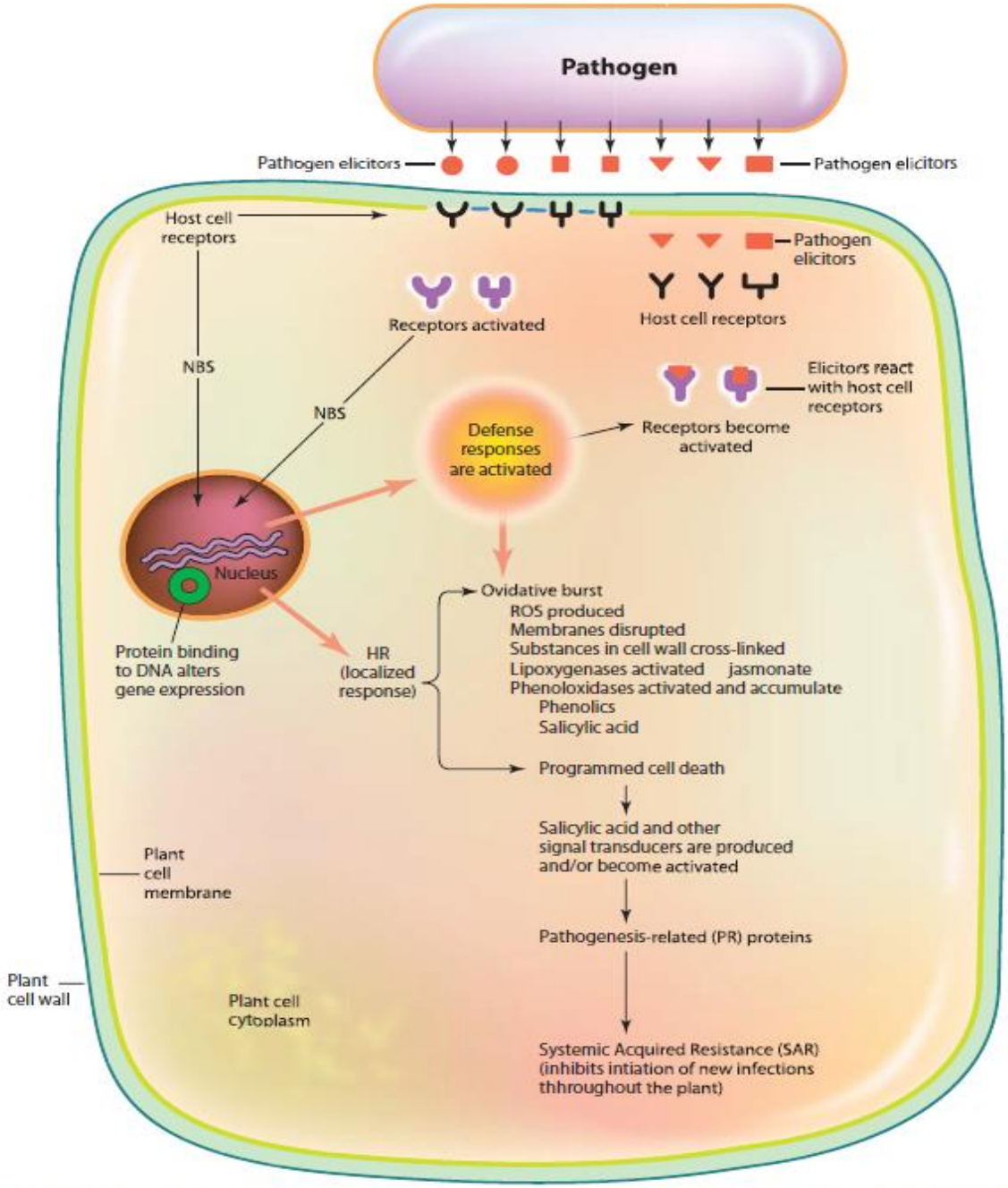


FIGURE 6-11 Diagram of the hypothetical steps in the hypersensitive response defense of plants following interaction of an elicitor molecule produced by a pathogen avirulence gene with a receptor molecule produced by the matching host R gene.

Gene for gene hypothesis:

For each gene conditioning rust reaction in the host there is a specific gene conditioning pathogenicity in the parasite. For each resistance gene in the host there is a corresponding gene for avirulence in the pathogen conferring resistance and viceversa. Flor (1946, 47) showed correlation between inheritance of pathogenicity and resistance to linseed rust caused by *Melampsora lini* which is now commonly known as gene -for –gene hypothesis. that "for each gene conditioning rust reaction in the host there is a specific gene conditioning pathogenicity in the parasite.

The concept has been applied with varying degree of proof to other host pathogen combinations including viruses, bacteria, fungi, nematodes, insects and a flowering plant (Orobanche).

RR = homozygous resistant ; Rr = heterozygous resistant; rr = homozygous susceptible
AVR AVR = homozygous avirulent; AVR avr = heterozygous avirulent; avr avr = homozygous virulent

		HOST GENOTYPE	
		RR or Rr	rr
PATHOGEN GENOTYPE	AVR avr or AVR AVR	Disease-resistant	Susceptible to disease
	avr avr	Susceptible to disease	Susceptible to disease

Gene-for-gene hypothesis Multifactor Interactions

Avirulence/virulence	Resistance/susceptibility			
	R1 R2	r1 R2	R1r2	r1r2
A1A2	-	-	-	+
a1A2	-	-	+	+
A1a2	-	+	-	+
a1a2	+	+	+	+

where, – = Resistance
+ = Susceptible

(Agrios 2007)

Resistance genes

Classes of resistance gene

There are several different classes of R Genes. The major classes are the NBS-LRR genes and the cell surface pattern recognition receptors (PRR). The protein products of the NBS-LRR Rgenes contain a nucleotide binding site (NBS) and a leucine rich repeat (LRR). The protein products of the PRRs contain extracellular, juxtamembrane, transmembrane and intracellular non-RD kinase domains.

Within the NBS-LRR class of R genes are two subclasses:

One subclass has an amino-terminal Toll/Interleukin 1 receptor homology region (TIR). This includes the *N* resistance gene of tobacco against tobacco mosaic virus (TMV).
The other subclass does not contain a TIR and instead has a leucine zipper region at its amino terminal.
The protein products encoded by this class of resistance gene are located within the plant cell cytoplasm.
The PRR class of R genes includes the rice XA21 resistance gene that recognizes the ax21 peptide and the Arabidopsis FLS2 peptide that recognizes the flg22 peptide from flagellin here are other classes of R genes, such as the extracellular LRR class of R genes; examples include rice Xa21D for resistance against *Xanthomonas* and the *cf* genes of tomato that confer resistance against *Cladosporium fulvum.*
The *Pseudomonas* tomato resistance gene (Pto) belongs to a class of its own. It encodes a er/Thr kinase but has no LRR. It requires the presence of a linked NBS-LRR gene, *prf*, for activity.

Specificity of resistance genes

R gene specificity (recognising certain Avr gene products) is believed to be conferred by the leucine rich repeats. LRRs are multiple, serial repeats of a motif of roughly 24 amino acids in length, with leucines or other hydrophobic residues at regular intervals. Some may also contain regularly spaced prolines and arginines.
LRRs are involved in protein-protein interactions, and the greatest variation amongst resistance genes occurs in the LRR domain. LRR swapping experiments between resistance genes in flax rust resulted in the specificity of the resistance gene for the avirulence gene changing.

Recessive resistance genes

Most resistance genes are autosomal dominant but there are some, most notably the *mlo* gene in barley, in which monogenic resistance is conferred by recessive alleles. *mlo*protects barley against nearly all pathovars of *powdery mildew.*

Avirulence genes

The term "avirulence gene" remains useful as a broad term that indicates a gene that encodes any determinant of the specificity of the interaction with the host. Thus, this term can encompass some conserved microbial signatures (also called pathogen or microbe associated molecular patterns (PAMPs or MAMPs)) and pathogen effectors (e.g. bacterial type III effectors and oomycete effectors) as well as any genes that control variation in the activity of those molecules.
There is no common structure between avirulence gene products. Because there would be no evolutionary advantage to a pathogen keeping a protein that only serves to have it recognised by the plant, it is believed that the products of Avr genes play an important role in virulence in genetically susceptible hosts.
Example: AvrPto is a small triple-helix protein that, like several other effectors, is targeted to the plasma membrane by N-myristoylation. AvrPto is an inhibitor of PRR kinase domains. PRRs signal plants to induce immunity when PAMPs are detected. The ability to target receptor kinases is required for the virulence function of AvrPto in plants. However, Pto is a resistant gene that can detect AvrPto and induce immunity as well. AvrPto is an ancient effector that is conserved in many *P. syringae* strains, whereas Pto R gene is only found in a few wild tomato species. This suggests recent evolution of the Pto R gene and the pressure to evolve to target AvrPto, turning a virulence effector to an avirulence effector.
Unlike the MAMP or PAMP class of avr genes that are recognized by the host PRRs, the targets of bacterial effector avr proteins appear to be proteins involved in plant innate immunity signaling, as homologues of Avr genes in animal pathogens have been shown to do this. For example, the AvrBs3 family of proteins possess DNA binding domains, nuclear localisation signals and acidic activation domains and are believed to function by altering host cell transcription.

Biotrophy and gene for gene systems:

All the parasites in which gene for gene relationship has been proved are essentially biotrophic or biotrophs at least for some time after start of infection.
(*Xanthomonas campestris* pv. *malvacearum*, *Phytophthora infestans*, *Venturia inaequalis* (Vander Plank, 1978).
The genes-for-gene systems thus involve biotrophy.
But the converse is not necessarily true. For example, *Plamodiophora brassicae* , the cause of club root of crucifers, is biotrophic but no evidence has yet been presented in the literature to suggest that host-pathogen interaction in them is based on a gene for gene systems.
According to Van der Plank (1978), specificity in gene for gene relationships lies in susceptibility.

Table. The diagonal check for specificity in a gene-for gene relationship

Pathogen	Plant				
	R_1R_1[b]	R_2R_2	R_3R_3	R_4R_4	R_5R_5
v_1v_1	S	R	R	R	R
v_2v_2	R	S	R	R	R
v_3v_3	R	R	S	R	R
v_4v_4	R	R	R	S	R
v_5v_5	R	R	R	R	S

a. Plant reaction when resistance gene R_1, R_2, R_3, R_4, R_5 at
interact with virulence genes v_1, v_2, v_3, v_4, v_5 at five loci
pathogen

b. Resistance is assumed to be dominant and RR can be r
by Rr. Virulence is assumed to be recessive. However, re
resistance and dominant virulence are also known.

R= resistant S= susceptible

- He explains it with the help of interactions of five host and five pathogens attacking them specifically.
- Suppose there are five host varieties with five ddifferent R genes; R1, R2, R3 ----------R5. A

plant with resistance gene R1 is attacked by a pathogen having virulence gene v1 and not to pathogen without this particular resistance gene irrespective of how many the virulence genes it may have.

Vander Plank (1978) elaborated protein for proteins hypothesis as a biochemical explanation of gene for gene interaction.

The protein for protein hypothesis states that in gene for gene diseases the mutual recognition of host and pathogen is not by the genes themselves but by their coded proteins.

Vander Plank (1978) hypothesized that in susceptibility the pathogen excretes a protein (virulence for product) into the host cell which copolymenizes with a complementary host protein (resistance gene product). This co-polymerization interferes with one auto regulation of the host gene that codes for the protein and by so doing turns the gene on to produce more protein.

In resistance, the protein specified by the gene for avirulence in the pathogen and excreted into the host does not polymerize with the protein coded for by the gene for resistance. It is not recognized by the host at all.

From a practical point of view, gene for gene relationship can be used to study the following:

1. The source of pathogenic variability in pathogens
2. The mutability of resistance and virulence genes
3. Why host resistance is expressed under one set of conditions and not others
4. Prediction of putative genotypes
5. Race nomenclature
6. Genetic dissection of complex loci
7. Cataloguing and storing of R genes in the form of plant seeds or cuttings and V genes in the form of pathogen strains
8. Management and deployment of resistance genes in space and tome
9. Detection of linkage and allelic relationship
10. Geographic distribution of R and V genes

11. Synthesis of multilines and multigene cultivars.

Vertifolia effect:

The vertifolia effect was discovered by Van der Plank (1963) who named it after a potato cultivar of this name, in which the effect was very pronounced. The vertifolia effect is a loss of horizontal resistance which occurs during breeding for vertical resistance. Its meaning was later extended to include the loss of horizontal resistance that occurs during breeding under the protection of pesticides.

The level of horizontal resistance can only be assessed by the level of parasitism. Clearly, if there is no parasitism because of a functioning vertical resistance, or a pesticide, the level of horizontal resistance cannot be assessed. Because individual plants with a high level of horizontal resistance are rather rare in a mixed screening population, the chances are that individuals with a relatively low level of horizontal resistance will then be selected on the basis of their other attributes. The loss is usually quite small in a single breeding cycle but, after many cycles, it can become very serious indeed.

The prime example of the vertifolia effect is the loss of horizontal resistance to potato blight (*Phytophthora infestans*) that has continued ever since both the discovery of Bordeaux mixture in the late nineteenth century, and the discovery of vertical resistance in the twentieth century. A loss of horizontal resistance to cotton pests has continued ever since the discovery of DDT in the 1940s.

The vertifolia effect is a very modern phenomenon. Its overall consequences are seen in the high levels of horizontal resistance in heritage cultivars, when they are compared to modern cultivars. This is the main reason why heritage cultivars are so valued by organic farmers. One of the main objectives of most amateur plant breeders will be to restore the horizontal resistances that were lost to the vertifolia effect.

Immunization of plants against pathogens:

Defense through Plantibodies:

In humans and animals, defenses against pathogens are often activated by natural or artificial immunization, i.e., by a subminimal natural infection with the pathogen or by an artificial injection of pathogen proteins and other antigenic substances. Both events result in the production of antibodies against the pathogen and, thereby, in subsequent prolonged protection (immunity) of the human or animal from infection by any later attacks of the pathogen.

Plants, of course, do not have an immune system like that of humans and animals, i.e., they do not produce antibodies. In the early 1990s, however, transgenic plants were produced that were genetically engineered to incorporate in their genome, and to express, foreign genes, such as mouse genes that produce antibodies against certain plant pathogens. Such antibodies, encoded by animal genes but produced in and by the plant, are called plantibodies. It has already been shown that transgenic plants producing plantibodies against coat proteins of viruses, e.g., *artichoke mottle crinkle virus*, to which they are susceptible, can defend themselves and show some resistance to infection by these viruses. It is expected that, in the future, this type of plant immunization will yield dividends by expressing animal antibody genes in plants that will produce antibodies directed against specific essential proteins of the pathogen, such as viral coat proteins and replicase or movement proteins, and fungal and bacterial enzymes of attack.

Systemic acquired resistance:

Induction of Plant Defenses by Artificial Inoculation with Microbes or by Treatment with Chemicals:

Induced resistance is at first localized around the point of plant necrosis caused by infection by the pathogen or by the chemical, and it is then called local acquired resistance (Fig. 6-22A). Subsequently, resistance spreads systemically and develops in distal, untreated parts of the plant and is called systemic acquired resistance (Fig. 6-22B). It is known now that several chemical compounds, e.g., salicylic acid, arachidonic acid, and 2,6-dichloroisonicotinic acid, may induce localized and systemic resistance in plants at levels not causing tissue necrosis. Jasmonic acid is another type of compound, derived primarily from oxidation of fatty acids, that leads to systemic acquired resistance, often in cooperation with salicylic acid and ethylene, leading to the production of defensins. Probenazole, a synthetic chemical used in Asia for the control of rice blast disease caused by the fungus *Magnaporthe grisea*, has been shown to act upstream from the salicylic acid transcribing gene and, thereby, causing accumulation of salicylic acid. Probenazole induces systemic acquired resistance in rice against rice blast, in tomato against the bacterial pathogen *P. syringae* pv. *tabaci*, and in tobacco against the *tobacco mosaic virus*.

Similarly, riboflavin was shown to induce systemic acquired resistance but it activates it in a distinct manner not involving salicylic acid. Salicylic acid and isonicotinic acid are true SAR activators because not only do they induce

resistance to the same spectrum of pathogens and induce expression of the same genes as pathogens, but these chemicals have no antimicrobial activity.

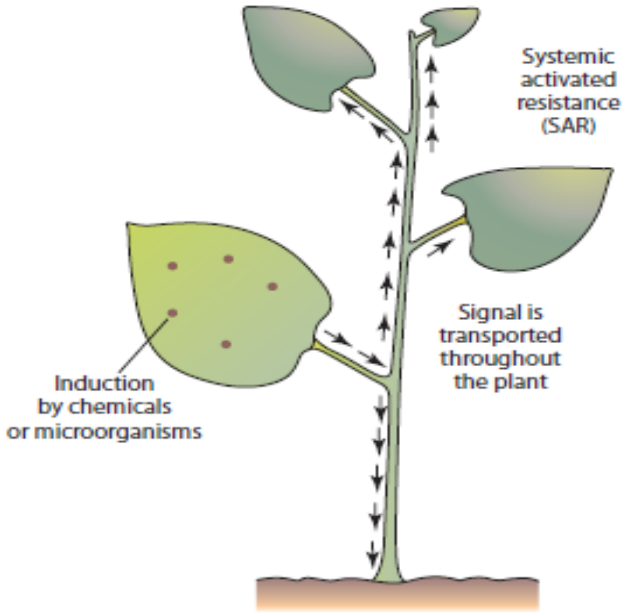


FIGURE 6-24 Principle of systemic activated (or acquired) resistance. A leaf treated with certain chemicals or with pathogens causing necrotic lesions produces a signal compound(s) that is transported systemically throughout the plant and activates its defense mechanisms, making the entire plant resistant to subsequent infections.

R Gene Resistance, Race-Specific, Monogenic, or Vertical Resistance:
Many plant varieties are quite resistant to some races of a pathogen while they are susceptible to other races of the same pathogen. In other words, depending on the race of the pathogen used to infect a variety, the variety may appear strongly resistant to one pathogen race and susceptible to another race (**race specific)** under a variety of environmental conditions. Such resistance differentiates clearly between races of a pathogen, as it is effective against specific races of the pathogen and ineffective against others (Figs. 4-9 and 4-10). Such resistance is sometimes called strong, major, race-specific, qualitative, or differential resistance, but it was more commonly referred to in the past as **vertical resistance**.

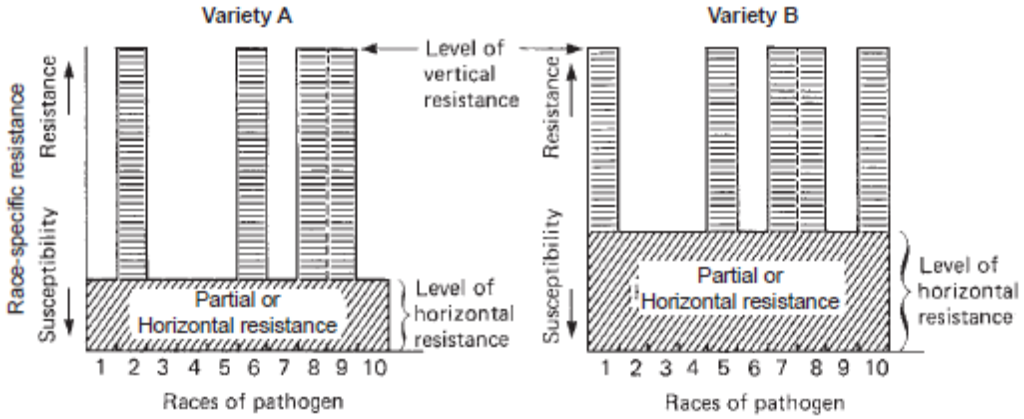


FIGURE 4-9 Levels of horizontal and vertical resistance of two plant varieties toward 10 races of a pathogen. [After Vanderplank (1984).]

Race-specific resistance is always controlled by one or a few genes (thereby the names **monogenic** or **oligogenic resistance**). These genes, referred to as R genes, control a major step in the recognition of the pathogen by the host plant and therefore play a major role in the expression of resistance. In the presence of race-specific resistance, the host and pathogen appear incompatible. The host may respond with a hypersensitive reaction, may appear immune, or may inhibit pathogen reproduction. Often, race-specific resistance inhibits the initial establishment of pathogens that arrive at a field from host plants that lack, or have different, major genes for resistance. Race-specific resistance inhibits the development of epidemics by limiting the initial inoculum or by limiting reproduction after infection.

Complete resistance may be provided by a single resistance gene. Often, it is desirable to combine, or **pyramid**, more than one resistance gene (R1R2, R1R3, R1R2R3) in the same plant, which then is resistant to all the pathogen races to which each of the genes provides resistance. A plant species may have as many as 20 to 40 resistance genes against a particular pathogen, although each variety may have only one or a few of these genes. For example, wheat has 20 to 40 genes for resistance against the leaf rust fungus *Puccinia recondita*, barley has a similar number of genes against the powdery mildew fungus *Erysiphe graminis hordei*, and cotton has almost as many against the bacterium *Xanthomonas campestris* pv. *malvacearum*. Each gene for resistance, such as R2, makes the plant resistant to all the races of the pathogen that contain the corresponding gene for avirulence. This pathogen race and its avirulence gene (A2), however, are detected because the pathogen attacks plants that lack the particular gene for resistance (R2).

Whether partial or race specific, true resistance is generally controlled by genes located in the plant chromosomes in the cell nucleus. There are, however, several plant diseases in which resistance is controlled by genetic material contained in the cytoplasm of the cell.

Such resistance is sometimes referred to as **cytoplasmic resistance**. The two best-known cases of cytoplasmic resistance occur in the southern corn leaf blight caused by *Bipolaris (Helminthosporium) maydis* and the yellow leaf blight caused by *Phyllosticta maydis*. Resistance in these is conferred by the lack of a gene in mitochondria of normal cytoplasm of various types of corn that encodes a receptor for the host-specific toxin produced by each pathogen. The presence of such a gene in mitochondria of Texas male-sterile cytoplasm makes all corn lines with Texas male-sterile cytoplasm susceptible to these pathogens.

Varieties with race-specific (monogenic or oligogenic) resistance generally show complete resistance to a specific pathogen under most environmental conditions, but a single or a few mutations in the pathogen may

produce a new race that may infect the previously resistant variety. On the contrary varieties with partial (polygenic) resistance are less stable and may vary in their reaction to the pathogen under different environmental conditions, but a pathogen will have to undergo many more mutations to completely break down the resistance of the host. As a rule, a combination of major and minor genes for resistance against a pathogen is the most desirable makeup for any plant variety.

Horizontal resistance	**Vertical resistance**
Controlled by many genes (Poly-/ multigene resistance)	Controlled by one or a few genes (Monogenic or oligogenic)
Each gene plays a minor role	The gene plays a major role in expression resistance
Non-differential types of Resistance (Non- specific)	Differentiates between races of a pathogen. (host variety resistant to one race but not other race)
Affected by environmental conditions. Does not provide complete protection but slows down the disease spread.	Less affected by environmental conditions. Provide complete resistance
Quantitative in nature	Qualitative in nature

Varieties with partial resistance though less stable and may vary in their reaction to the pathogen under different environmental conditions, but a pathogen will have to undergo several mutations to completely break down the resistance of the host.	In plant varieties with vertical resistance, resistance may break down if there is a single or a few mutations in the pathogen producing a new race that may infect the previously resistant variety.

7. Physiological (photosynthesis, respiration, translocation of water and nutrients) and molecular (protein and nucleic acid) changes in diseased plants

A. Effect on photosynthesis:

In disease symptoms such as cause chlorosis, necrotic lesions, reduced growth, mosaic, leaf spot, blight, and other kinds of diseases in which there is destruction of leaf tissue, e.g., cereal rusts, viral mosaics, yellowing and stunting diseases etc. photosynthesis is affected. Photosynthesis is also reduced in defoliations which causes decrease in the photosynthetic surface of the plant. Plant pathogens reduce photosynthesis, especially in the late stages of diseases, by affecting the chloroplasts and causing their degeneration. Photosynthesis is reduced because the toxins, such as tentoxin and tabtoxin, which inhibit some of the enzymes that are involved directly or indirectly in photosynthesis.

In leaf spot, blight, and other kinds of diseases in which there is destruction of leaf tissue, e.g., in cereal rusts and fungal leaf spots (Figs. 3-1A–3-1C), bacterial leaf spots (Fig. 3-1D), viral mosaics (Fig. 3-1E) and yellowing and stunting diseases (Fig. 3-1F), or in defoliations, photosynthesis is reduced because the photosynthetic surface of the plant is lessened. Even in other diseases, however, plant pathogens reduce photosynthesis, especially in the late stages of diseases, by affecting the chloroplasts and causing their degeneration. The overall chlorophyll content of leaves in many fungal and bacterial diseases is reduced, but the photosynthetic activity of the remaining chlorophyll seems to remain unaffected. In some fungal and bacterial diseases, photosynthesis is reduced because the toxins, such as tentoxin and tabtoxin, produced by these pathogens inhibit light chlorophyll some of the enzymes that are involved directly or indirectly in photosynthesis. In plants infected by many vascular pathogens, stomata remain partially closed, chlorophyll is reduced, and photosynthesis stops even before the plant eventually wilts.

Most virus, mollicute, and nematode diseases also induce varying degrees of chlorosis and stunting. In the majority of such diseases, the photosynthesis of infected plants is reduced greatly. In advanced stages of disease, the rate of photosynthesis is no more than one-fourth the normal rate.

A. Effect of pathogens on host plant respiration

Respiration is the process by which cells, through the enzymatically controlled oxidation (burning) of the energy-rich carbohydrates and fatty acids, liberate energy in a form that can be utilized for the performance of various cellular processes. Plant cells carry out respiration in, basically, two steps. The first step involves the degradation of glucose to pyruvate and is carried out, either in the presence or in the absence of oxygen, by enzymes found in the ground cytoplasm of the cells. The production of pyruvate from glucose follows either the glycolytic pathway, otherwise known as glycolysis, or, to a lesser extent, the pentose pathway.

The second step, regardless of the pathway, involves the degradation of pyruvate, however produced, to CO2 and water. This is accomplished by a series of reactions known as the Krebs cycle, which is accompanied by the so-called terminal oxidation and is carried out in the mitochondria only in the presence of oxygen. The energy produced through respiration is utilized by the plant for all types of cellular work, such as accumulation and mobilization of compounds, synthesis of proteins, activation of enzymes, cell growth and division, defense reactions, and a host of other processes.

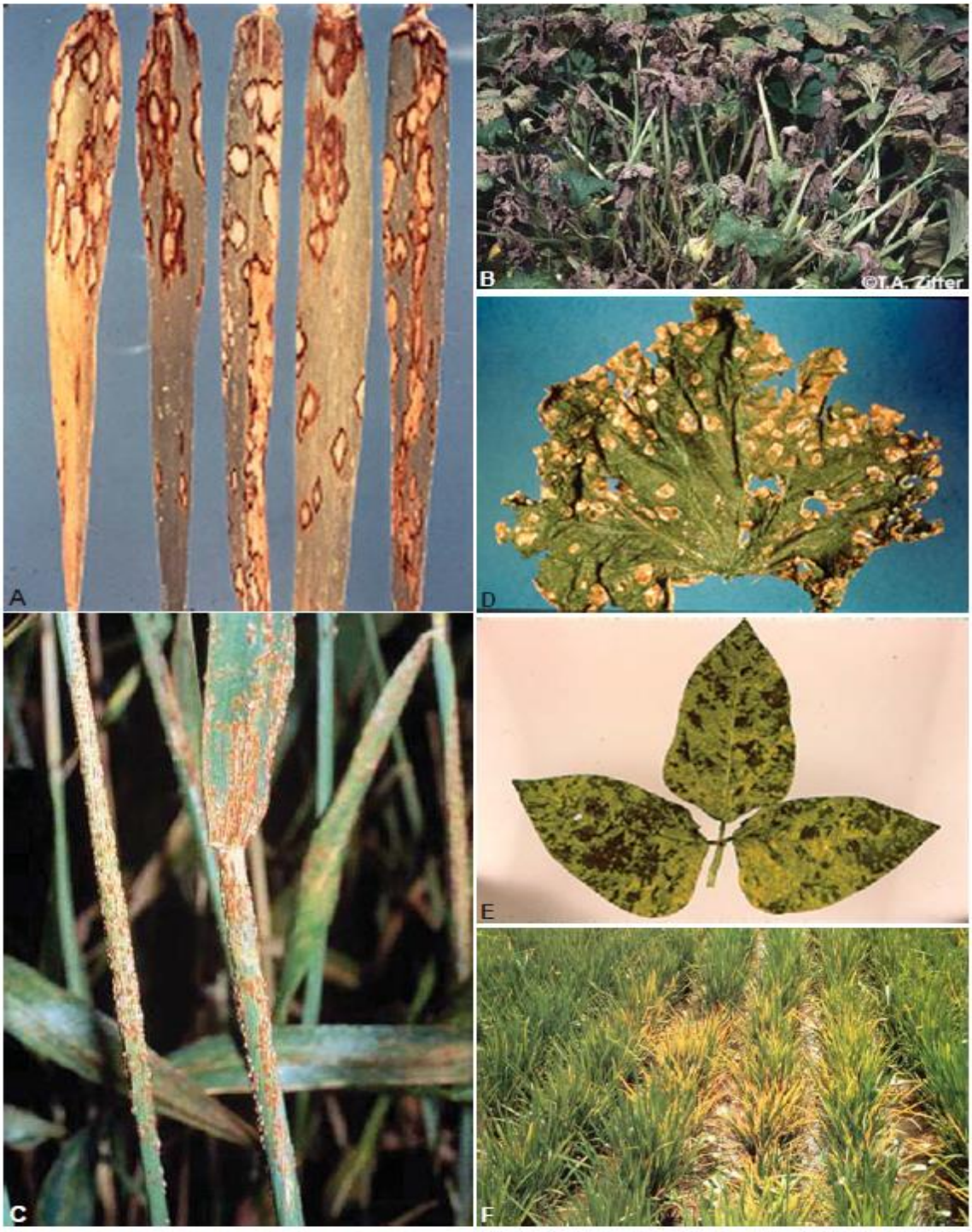


FIGURE 3-1 Ways in which pathogens reduce photosynthetic area and, thereby, photosynthesis in plants. (A) Spots on barley leaves caused by the fungus *Rhynchosporium sp.* (B) Nearly complete destruction of pumpkin leaves infected heavily with the downy mildew oomycete *Pseudoperonospora cubensis*. (C) Countless tiny lesions on stems and leaves of wheat plant infected with the stem rust fungus *Puccinia graminis f.sp. tritici*. is. (D) Angular leaf spots on cucumber leaf caused by the bacterium *Pseudomonas lacrymans*. (E) Reduced chlorophyll in yellowish areas of virus-infected plants, such as cowpea infected with *cowpea chlorotic mottle virus* or (F) by stunting and yellowing of rice plants infected with the *rice tungro virus*. [Photographs courtesy of (A) Plant Pathology Department, University of Florida, (B) T. A. Zitter, Cornell University (C) I. Evans and (D) R. J. Howard, W.C.P.D., and (F) H. Hibino.]

The complexity of respiration, the number of enzymes involved in respiration, its occurrence in every single cell, and its far-reaching effects on the functions and existence of the cell make it easy to understand why the respiration of plant tissues is one of the first functions to be affected when plants are infected by pathogens. Several changes in the metabolism of the diseased plant accompany the increase in respiration after infection.

Thus, the activity or concentration of several enzymes of the respiratory pathways seems to be increased. The accumulation and oxidation of phenolic compounds, many of which are associated with defense mechanisms in plants, are also greater during increased respiration. Increased respiration in diseased plants is also accompanied by an increased activation of the pentose pathway, which is the main source of phenolic compounds. Increased respiration is sometimes accompanied by considerably more fermentation than that observed in healthy plants, probably as a result of an accelerated need for energy in the diseased plant under conditions in which normal aerobic respiration cannot provide sufficient energy.

B. Effect on translocation of water & nutrients

In plant diseases in which the pathogen infects the leaves, transpiration is usually increased. This is the result of destruction of at least part of the protection afforded the leaf by the cuticle, an increase in the permeability of leaf cells, and the dysfunction of stomata. In diseases such as rusts, in which numerous pustules form and break up the epidermis (Figs. 3-6A and 3-6B), in most leaf spots (Fig. 3-6E), in which the cuticle, epidermis, and all the other tissues, including xylem, may be destroyed in the infected areas, in the powdery mildews, in which a large proportion of the epidermal cells are invaded by the fungus (Fig. 3-6C), and in apple scab (Fig. 3-6D), in which the fungus grows between the cuticle and the epidermis—in all these examples, the destruction of a considerable portion of the cuticle and epidermis results in an uncontrolled loss of water from the affected areas. If water absorption and translocation cannot keep up with the excessive loss of water, loss of turgor and wilting of leaves follow. The suction forces of excessively transpiring leaves are increased abnormally and may lead to collapse or dysfunction of underlying vessels through the production of tyloses and gums.

Interference with Translocation of Organic Nutrients through the Phloem

Organic nutrients produced in leaf cells through photosynthesis move through plasmodesmata into adjoining phloem elements. From there they move down the phloem sieve tubes (Fig. 3-7) and eventually, again through plasmodesmata, into the protoplasm of living nonphotosynthetic cells, where they are utilized, or into storage organs, where they are stored. Thus, in both cases, the nutrients are removed from "circulation."

Plant pathogens may interfere with the movement of organic nutrients from the leaf cells to the phloem, with their translocation through the phloem elements, or, possibly, with their movement from the phloem into the cells that will utilize them. Obligate fungal parasites, such as rust and mildew fungi, cause an accumulation of photosynthetic products, as well as inorganic nutrients, in the areas invaded by the pathogen. In these diseases, the infected areas are characterized by reduced photosynthesis and increased respiration. However, the synthesis of starch and other compounds, as well as dry weight, is increased temporarily in the infected areas, indicating translocation of organic nutrients from uninfected areas of the leaves or from healthy leaves toward the infected areas.

In stem diseases of woody plants in which cankers develop (Figs. 3-8A–3-8C), the pathogen attacks and remains confined to the bark for a considerable time.

During that time the pathogen attacks and may destroy the phloem elements in that area, thereby interfering with the downward translocation of nutrients.

FIGURE 3-2 Examples of reduction of water absorption by plants. (A) Destruction of roots of young seedlings by the damping-off oomycete *Pythium sp*. (B) Roots and stems of pepper plants killed by *Phytophthora sp*. (C) Wheat roots at different stages of destruction by the take-all fungus *Gaeumannomyces tritici*. (D) Infection of crown and roots of corn plant with the fungus *Fusarium*. (E) Numerous galls caused by the bacterium *Agrobacterium tumefaciens* on roots of a cherry tree. (F) Root knot galls caused by the nematode *Meloidogyne sp*. on roots of a cantaloupe plant. [Photographs courtesy of (A) Plant Pathology Department, University of Florida, (B) K. Pernezny, University of Florida, (C) W. McFadden, W.C.P.D., (D) Plant Pathology Department, Iowa State University, (E) Oregon State University, and (F) B. D. Bruton, USDA, Lane, Oklahoma.]

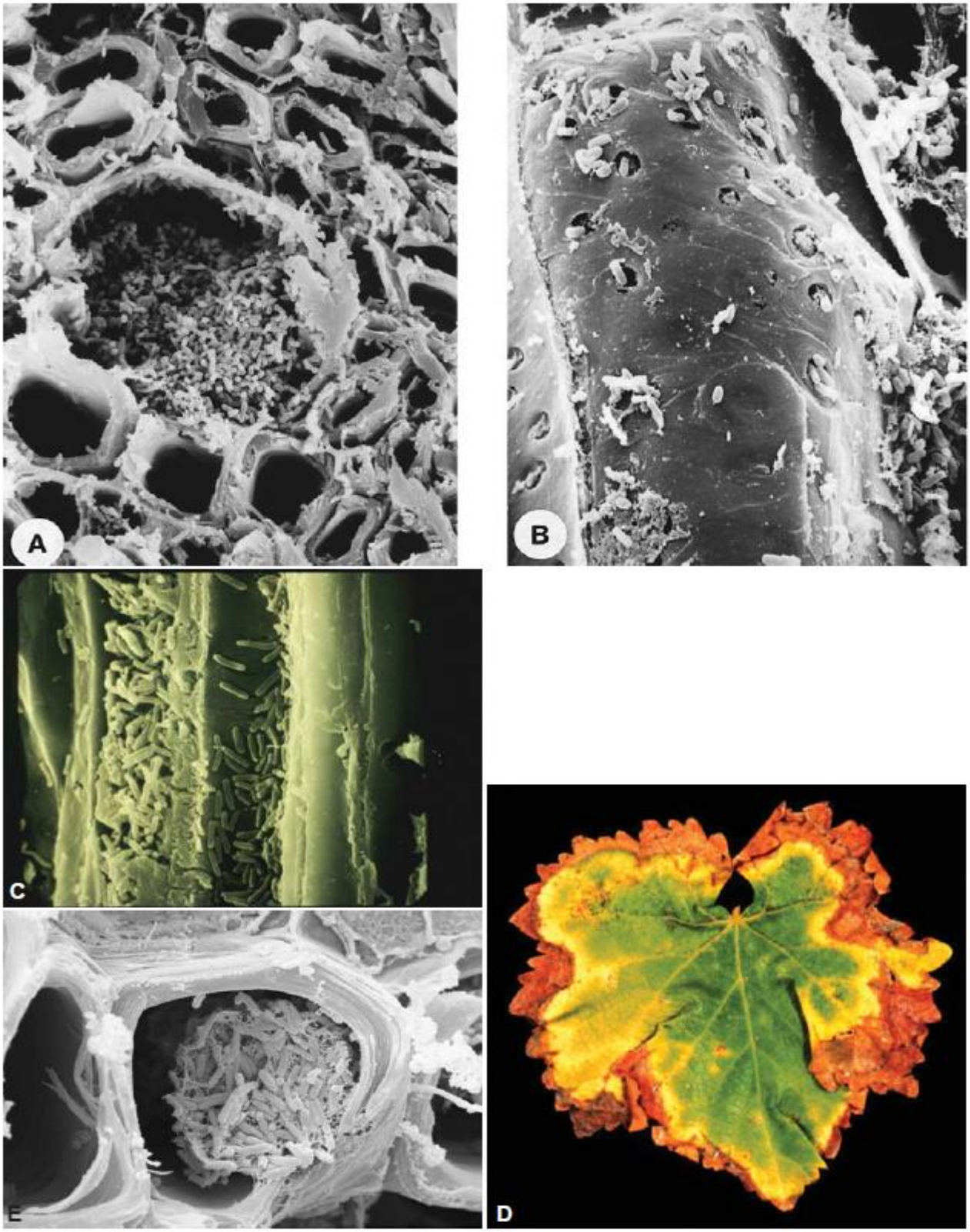


FIGURE 3-4 (A) *Pseudomonas* bacteria clogging a xylem vessel of a young plant shoot. (B) Bacteria moving from one vessel to another and to adjacent parenchyma cells through xylem pits. (C) Bacteria of the xylem-inhabiting *Xylella fastidiosa* in a vessel of a grape plant. (D) Marginal scorching of a grape leaf from a plant infected with *X. fastidiosa*, the cause of Pierce's disease of grape. (E) *Xylella* bacteria in a cross section of a xylem vessel of an infected grape leaf. [Photographs courtesy of (A and B) E. L. Mansvelt, I. M. M. Roos, and M. J. Hattingh (1500×), (C) D. Cooke, provided by E. Hellman, Texas A&M University, (D) E. Hellman, and (E) E. Alves, Federal University of Lavras, Brazil.]

Molecular (protein & nucleic acid) changes in diseased plants

The most common effect of pathogenesis are changes in cell membrane permeability i.e. loss of **electrolytes** from the cell. Transcription of cellular DNA into messenger RNA and translation of messenger RNA to produce proteins are two of the most basic and precisely controlled processes in the biology of any normal cell. Increases in protein synthesis in infected tissues have been observed primarily in hosts resistant to the pathogen and reach their highest levels in the early stages of infection.

Effect on Transcription

Several pathogens, particularly viruses and fungal obligate parasites, such as rusts and powdery mildews, affect the transcription process in infected cells. In some cases, pathogens affect ranscription by changing the composition, structure, or function of the chromatin associated with the cell DNA. In some diseases, especially those caused by viruses, the pathogen, through its own enzyme or by modifying the host enzyme (RNA polymerase) that makes RNA, utilizes the host cell nucleotides and machinery to make its own (rather than host) RNA. In several diseases, the activity of ribonucleases (enzymes that break down RNA) is increased, perhaps by formation in infected plants of new kinds of ribonucleases not known to be produced in healthy plants. Finally, in several diseases, infected plants, particularly resistant ones, seem to contain higher levels of RNA than healthy plants, especially in the early stages of infection. It is generally believed that greater RNA levels and, therefore, increased transcription in cells indicate an increased synthesis of substances involved in the defense mechanisms of plant cells.

Effect on Translation

Infected plant tissues often have increased activity in several enzymes, particularly those associated with the generation of energy (respiration) or with the production or oxidation of various phenolic compounds, some of which may be involved in (defense) reactions to infection. Although a certain amount of some of these enzymes (proteins) may be present in the cell at the time of infection, several are produced *de novo*, necessitating increased levels of transcription and translation activity.

Increases in protein synthesis in infected tissues have been observed primarily in hosts resistant to the pathogen and reach their highest levels in the early stages of infection, i.e., in the first few minutes and up to 2–20 hours after inoculation. If resistant tissues are treated before or during infection with inhibitors of protein synthesis, their resistance to the pathogen is reduced. These observations suggest that much of the increased protein synthesis in plants attacked by pathogens reflects the increased production of enzymes and other proteins involved in the defense reactions of plants.

8. Predisposition, survival of pathogen in nature and its spread, disease epidemics

Predisposition

Yarwood (1976) defined predisposition as 'The tendency of treatments and conditions acting before inoculation or before introduction of the incitant, to affect susceptibility to biotic and abiotic pathogens.'

Sources of survival of pathogens

1) Infected host as reservoir of inoculum (or) survival in vital association with living plants.
2) Survival as saprophytes outside the host.
3) Survival by means of specialized resting structures in or on the host or outside the host.
4) Survival in association with insects, nematodes and fungi

1. Survival in vital association with living plants

Survival of the plant pathogens in vital association with living plants is grouped into

a. Seed

The pathogen of loose smut of wheat, *Ustilago nuda tritici*, enters the stigma and style and infects the young seed, in which it survives as mycelium. The seed-infecting pathogens that cause loose smut of wheat and loose smut of barley are strikingly different from other smut fungi that attack cereal crops. Most of the others survive from season to season either in nonpathogenic association with seed or as spores in the soil. *Colletotrichum lindemuthianum*, the causative organism of bean anthracnose, can also infect the seed; unless the seed is killed, the fungus in newly sprouted bean seedlings initiates new infections. The bacteria that cause bean blights and bacterial blight of cotton survive the winter

in infected seed. In Mexico, the fungus of late blight of potatoes (*Phytophthora infestans*) produces oospores but in colder regions of the world, the fungus overwinters as mycelium in diseased tubers.

b. Collateral hosts

Collateral hosts are those, which are susceptible to the plant pathogens of crop plants and provide adequate facilities for their growth and reproduction of these pathogens. Weeds, which survive and live during non-cropping season provide for the continuous growth and multiplication of the pathogen. For example, the fungal pathogen for blast disease of rice, *Pyricularia oryzae* can infect the grass weeds like *Bracguarua mutica, Dinebra retroflexa, Leersia hexandra, Panicum repens*, etc., and survive during off-season of rice-crop.

c. Alternate hosts

The role of alternate hosts is not important as of collateral hosts. However, when a pathogen has very wide host-range and is tolerant to wide range of weather conditions the alternate hosts become very, important source of survival of the pathogen. These alternate hosts are very important for the completion of the life cycle of heteroecious rust pathogens. e.g. in temperate regions the alternate host *Berberis vulgaris* of *Puccinia graminis tritici* (black/stem. rust pathogen on wheat), the barberry bush, grows side by side with the cultivated host, wheat.

d. Self sown crops

Self-sown plants, voluntary crops and early sown crops are reservoirs of many plant pathogens e.g., groundnut rust pathogen, *Puccinia arachidis* and ring mosaic of groundnut caused by tomato spotted wilt virus. Self-sown rice plants harbour the pathogen as well as vector. e,g., rice tungro virus and its vector, *Nephotettix virescens*.

Survival of plant pathogens:

Most plant pathogens can survive only by repeatedly reinfecting host plants. This process of repeated infection is known as an infection chain. If the infection chain is broken the pathogen dies out. The Swiss plant pathologist Ernst Gd.umann (1950) in his book Principl.es oJ Plant In Jection divided infection chains into two types depending on how the inoculum of the pathogen survives between leaving the infected host (the distributor host) and arriving on the host to be infected (the receiver host). In continuous infection chains the pathogen survives by continually infecting susceptible plants in an uninterrupted fashion.

Discontinuous infection chains occur when the disease cycle is interrupted by an epiphytic phase, a saprophytic phase or a resting stage.

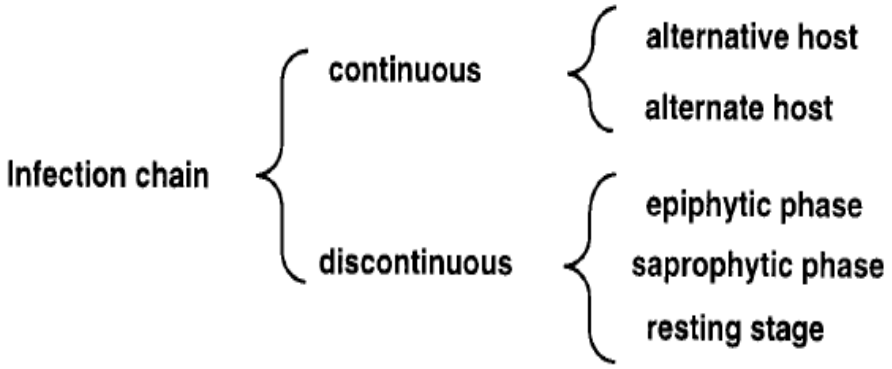


Figure 12.1 Five types of infection chains available to plant pathogens.

Continuous infection chains involving the same or alternative hosts

Continuous infection chains involving the same or alternative hosts occur when the parasite survives by continually infecting plants of the same host species or plant species other than the main crop species. Such plants are called alternative hosts and are often related to the crop species. If the alternative host, which is often a weed species, does not show symptoms of disease, it is called a disease carrier. The parasite does not form resting structures nor does it survive saprophytically or epiphytically on plant surfaces. Survival therefore is dependent upon the presence of susceptible host plants. Such infection chains are common in all major groups of plant parasites.

In tropical regions where year-round cropping of plant species such as rice and root crops (e.g. cassava, sweet potato, taro and yams) is a common practice, the continuous presence of host plants enables parasites to survive by continuously infecting new hosts. In temperate regions, it is not uncommon for self-sown, volunteer crop plants to grow along roadsides, fencelines and irrigation channels and as weeds in paddocks during periods when the main crop is not growing. These self-sown plants enable parasites to survive during intercrop periods and serve as sources of inoculum when the main crop is grown. The wheat growing areas of northern NSW and southern Queensland, where crops are sown in late autumn/early winter and harvested in late spring/early summer. are often referred to as rust liable areas. This is because the summer storms which are prevalent in the region allow seed that has fallen to the ground during the previous season's harvest to germinate. Susceptible self-sown plants become infected and this enables the fungus to oversummer. In the southern regions of Australia the climate is more Mediterranean-like with winter rains and summer drought. Under these conditions self-sown wheat plants do not occur in summer (except in irrigation areas), the fungus cannot survive and epidemics are rare. However, grasses such as barley grass (Hordeum Leporinum) and common wheat grass (Agropgron scabrum) can serve as alternative hosts to various special forms of Puccinia graminis. A similar infection chain occurs with other pathogens of wheat.

Many plant pathogens survive intercrop periods by infecting alternative hosts. For example, the black rot bacterium of brassicas (Xanthomonas campestrus pv. campestris) can survive on related weed species such as wild radish. Similarly, root-knot and lesion nematodes have a wide host range and infected alternative
hosts play a major role in their survival.

Continuous infection chains involving alternate hosts

Some plant pathogens cannot be directly transmitted from an infected plant to another plant of the same species. They must alternate between two completely unrelated biological species. Even with the modern technologies available today, scientists cannot transmit most plant viruses and phytoplasmas directly from
one plant to another. They can only be transmitted via a vector, usually but not always, an insect. In many cases viruses enter into a biological relationship with their vector and in some instances multiply in the vector for more details on virus-vector relationships). The vector therefore acts as an alternate or intermediate host and the infection chain alternates between the two unrelated species. Thus, the infection chain consists of plant-insect vector-plant. If the alternate host {the vector) is absent the infection chain is broken. Some heteroecious rust fungi also require an alternate host in the infection chain. They cannot complete their disease cycle in the absence of the alternate host. An example is the European pear or cluster rust fungus (Ggmno sporangium) which is present in Europe, Asia Minor and North Africa. It has been introduced into North America but is currently absent from the Southern Hemisphere. The fungus forms clusters of aecia opposite spermagonia on the underside of pear leaves (Purus communis) for more detail on the plant rusts). The aeciospores cannot infect pear. They can only infect the alternate host, the g,.rnnosperm Juniper (Juniperus spp.). Teliospores formed on the alternate host cannot re-infect juniper plants, they can only infect pear. The fungus does not form urediniospores and therefore cannot reproduce asexually on Junipents.

Discontinuous infection chains involving an epiphytic phase

Several fungal and bacterial pathogens survive on the surfaces of their hosts and other plants in a non-parasitic relationship as epiphytes. For example, the peach leaf curl fungus (*Taphrina dejormans*) causes leaves to develop yellow to reddish areas which progressively thicken and pucker, causing the leaf to curl and drop prematurely. Naked asci containing ascospores are produced on the upper surface of the curled leaves. Ascospores germinate to form budding, yeast-like conidia that can only infect very young leaves which are unavailable at the time when the budding colonies are formed.

The fungus survives during the summer months (when only mature leaves are present on trees) and through the winter months (when no leaves are present on the deciduous peach trees) as an epiphyte on the bark and on the scales of the following year's leaf buds. In the following spring, when the leaf buds swell and young leaves begin to emerge, the budding cells infect the young leaves and form a mycelium in young, undifferentiated host tissue. The fungus produces asci in the early summer and the life cycle is completed. Satisfactory control of peach leaf curl can be achieved by a single application of a protectant fungicide (e.g. copper based sprays)just before bud-burst in the spring.

Bacterial canker of stone fruit caused by Pseudomonas sgrtngae pv. Syringae causes sunken cankers, which frequently exude gum, on the woody tissue of affected trees. Infection can lead to premature death of whole trees or infected branches. The bacterium overwinters in cankers and in spring it multiplies and spreads by rain splash to leaves where large epiphytic populations develop. These epiphytic populations provide the main source of inoculum for wound and leaf scar infections in the following autumn. The same bacterium can also cause bacterial blight of peas. Again, the

bacterium is present as part of the normal leaf-surface microflora of healthy peas. Infection occurs following wounding of plants.

Discontinuous infection chains involving a saprophytic phase

Many plant pathogenic bacteria and fungi survive during intercrop periods as saprophytes on diseased plant debris or on organic material present in or on soil. For example, the apple scab fungus (Venturia tnaequalls) infects apple leaves and fruits during spring and summer. The fungus overwinters as pseudothecial initials in infected leaves and fruit that fall to the ground during autumn. Following the union of mycelium of compatible mating types in autumn, black pseudothecia develop in the fallen leaves. The fungus then overwinters in the fallen leaves. In the following spring, the pseudothecia produce ascospores which serve as the primary inoculum which starts the disease cycle in the new season. The primary infections resulting from ascospore infection produce conidia which serve as the secondary inoculum that contributes towards the development of epidemics. Disease levels can be reduced by preventing the fungus from overwintering in fallen leaves. This can be achieved by spraying with a systemic fungicide before leaves fall during autumn so that pseudothecial development is prevented. The application of nitrogen (e.g. urea) to the leaf litter accelerates leaf decomposition under mild winter conditions reducing the survival rate in leaves.

Many root-infecting pathogens of annual crops have a saprophytic phase in their life cycle. When infected plants die, either as a result of disease or natural senescence, the pathogen survives saprophytically on the infected debris. For example the take-all fungus of wheat (GaeumannomAces gramtnis var. tritici) persists as a saprophyte in infected host debris during the intercrop period. Some pathogens that survive in diseased plant debris compete poorly as saprophytes with the normal microbial inhabitants of soil. Consequently, their numbers decline during the intercrop period. Such pathogens have been called root inhabitants or soil lnvaders.

Other plant pathogenic bacteria {e.g. *Agrobacterium rhizogenes* which causes crown gall, *Ralstonta sotanacean-um* which causes bacterial wilt and Streptomgces scabies which causes common scab of potato) and fungi (e.g. damping-off fungi and several species of *Fusarium, Pythium and Rhizoctonia*) can compete very effectively as saprophytes with the normal soil microflora. These parasites are often referred to as soil inhabitants because they are part of the normal soil microflora.

Discontinuous infection chains involving a resting stage

Plant pathogenic bacteria, phytoplasmas and viruses do not form resting structures and can survive only by means of continuous infection chains or in the case of bacteria as saprophytes or epiphytes. Plant pathogenic bacteria do not form endospores. Some viruses retain their infectiousness in infected plant debris for long periods of time. For example, leaves infected with tobacco mosaic virus retain their infectiousness when kept dry under laboratory conditions for over 50 years. However, most viruses lose their infectiousness soon after their host plants die.

Fungi and nematodes on the other hand often form resting structures that enable them to survive, often for long periods, during the absence of suitable host plants or when the environment is unfavourable. The downy mildews produce oospores, the rusts and smuts form teliospores and many fungi produce chlamydospores. In some instances, resting spores can survive for periods of up to twenty years or more. The resting spores of Plasmodiophora brassicae (the cause of club root of brassicas) and Spongospora subterranea (the cause of powdery scab of potato) can survive in soil for many years. Moreover, the spores of both species are stimulated to germinate only by exudates secreted from the roots of certain plant species reducing the probability of spores germinating in a 'no win' situation when potential hosts are absent. Many fungi produce sclerotia (e.g. Sclerotium roLfsii, ScLerotinta scLerotiorum, VerticilLium dahliae) which can survive in soil or in infected plant debris for periods ranging from months to many years, depending on the species. Other resting structures formed by fungi include sexual fruiting structures such as cleistothecia, perithecia and pseudothecia.

Nematodes also have adaptations that enable survival for long periods in soil in the absence of host plants. Eggs of the cyst nematodes (*Globodera* and *Heterodera* spp.) survive in egg cysts while those of root-knot nematodes (*Meloidogyne* spp.) survive in gelatinous egg masses. Both structures reduce the rate of egg desiccation. Some species such as *Angutna funesta* and *Ditglenchus dipsaci* can survive for several years in an anhydrobiotic state (metabolically inactive with loss of water from body fluids). Some cyst nematodes (e.g. the potato cyst nematode, *Globodera rostochiensrs*) will not hatch in the absence of host root exudates.

Some fungal pathogens infect their host, enter into a dormant state and resume activity at a later stage of host development. For example, the loose smut fungus of wheat (*Ustilago tritici*) infects developing wheat embryos during flowering. Within a week of flowering, the embryo becomes resistant to infection.

The fungus survives as dormant mycelium within the embryo of the seed. When infected seed germinates, the pathogen is activated. As plants approach maturity, the inflorescence is replaced by masses of smut teliospores. Some of the ripe-rots or postharvest rots of fruits are caused by fungi that infect the young developing fruit and then enter into a quiescent or latent state. They later resume activity when fruits commence to ripen. The black spot fungus of citrus, *Guignardia cttricarpa*, is an example of such a fungus. Ascospores produced in pseudothecia on leaves that fell to the ground 6 weeks to 6 months previously infect very immature citrus fruits (between fruit set and 4 months development). From the appressorium, an infection peg penetrates the cuticle and forms a small mass of subcuticular (between the cuticle and epidermis) mycelium. The fungus then remains in a quiescent state for about six months until the fruits are fully grown and mature. It resumes growth and grows into the rind tissue to produce black spot symptoms. Thus, the disease cycle involves a saprophytic state in fallen leaves and a resting state in immature fruits.

A. Survival as saprophytes

The ability to live saprophytically enables many plant pathogens to survive in the absence of growing susceptible plants. Saprophytic survival usually occurs in the soil. Waksman (1971) distinguished between soil inhabitants soil invaders; the former comprise the basic fungal flora of the soil, whereas the latter are short-lived exotics. As applied to the root infecting fungi soil inhabitants are unspecialized parasites with a wide host range that are able to survive-indefinitely in the soil as saprophytes; soil invaders (root inhabiting fungi) are more specialized parasites that survive in soils inclose association with their hosts. Most plant pathogenic fungi and bacteria are soil invaders, but some pathogens, notably *Rhizoctonia solani* and *Pythium debaryanum* that cause seedling blights and root rots, live saprophytically in the soils.

B. Survival by means of specialized resting structures

Enduring structures of plant pathogens may be as simple as conidia or as complex as perithecia. Apparently, ascospores or conidia derived from them, serve to carry the pathogen causing peach- leaf curl (*Taphrina deformans*) over the winter. Conidia of *Alternaria solani*, the pathogen of early blight of potato and tomato, survive for eighteen months in dried diseased leaves. Specialized thick-walled chlamydospores of *Fusarium* and other Imperfect fungi, spores of many smut fungi and the amphiospores, uredospores and teliospores of certain rust fungi also are important enduring structures. The resting spores of *Plasmodiophora brassicae* may survive for ten years in soils infested upon the disintegration of clubbed roots.

C. Survival in association with Insect, nematodes and fungi

Many insects are carriers of inocula during the growing season and several important plant pathogens survive between growing seasons within insects. Some bacterial plant pathogens may survive within the insect body and over winter therein. The com flea beetle, *Chaetocnema pulicaria* Melsh carries inside its body, the com wilt pathogen, *Xanthomonas stewartii* and thus helps in its overwintering. The cucumber beetles, *Diabrotica vitata* Fabr. and *D. duodecimpunctata* Oliv., which chew the plant parts affected by *Erwiniatra cheiphila* carry the pathogen inside their body, where it over winters.

Plant viruses like wheat mosaic, wheat spindle streak virus, lettuce big vein, tobacco necrosis, tobacco rattle and tobacco ring spot viruses survive with nematodes or fungi found in the soil between crop seasons. Tobacco ring spot virus is associated with the nematode, *Xiphinema americanum*. The fungus, *Polymyxa graminis* (Barley yellow mosaic, oat yellow mosaic, wheat soil-borne mosaic, wheat spindle-streak mosaic) and *Spongospora subterranea* (potato mop top) carry the viruses internally and transmit.them through their resting spore.

Transmission of Plant Diseases:

Rational and most effective control of parasitic plant diseases is possible only if –

1. the disease is correctly diagnosed,
2. the nature of transmission of the disease is known and
3. life cycle stages of the involved parasite i.e its mode of reproduction active structures produced under the favourable condition for repaid and wide dispersal and the structures produced to overcome adverse condition are known.

The detail study of life cycles of different parasites being beyond the scope of this article it would be quite sufficient to understand and remember. one of the general modes of reproduction of different plant parasites viz.fungi, bacteria, viruses (not true parasites) and nematodes and the structures which enable these parasites to survive under most

unfavourable condition like the off season of suitable host crop(s) and / or exceptional high temperature and dry weather conditions prevailing in the summer months in a tropical country like India.

All parasitic as well as viral diseases are transmissible, the parasites or issues being infectious to suitable host plants with ability to spread from host to host and from one area to another. The microscopic parasites or sub-microscopic infectious agents viz. Viruses causing plant diseases are technically termed as 'pathogens'.

Wherever a disease is established in a particular area or country, transmission of the pathogen from host to host or from one place to another is termed as 'dissemination' or 'dispersal' of the pathogen. Dissemination of plant diseases is recognized in relation to different phases of diseases as under:

1. **Primary infection:** Contact of a pathogen with a suitable host plant and initiation of the disease first time in the season of a crop is called 'primary infection'. Often a few or several plants in the crop are likely to get primarily infected.
2. **Secondary spread**: When a plant or few plants are primarily infected, rapid multiplication of the pathogen sets in under favorable climatic conditions, which helps 'secondary spread' of the disease.

In case of fungal diseases the primary infections are usually caused by the resting or dormant structures of the fungal pathogen while the secondary spread occurs through the asexually produced, countless and short-lived spores

Although in majority of plant diseases the above two phases occur sequentially, primary infections occurring only once in the season and secondary spread often repeating several times in the same season thus causing rapid. Transmission over wide areas, this type of cycle is not observed in many other diseases. In such cases there is no secondary spread in the same season i.e. the pathogen multiplies only once during the crop period and the spread of disease is observed only during the following crop season by way of increased primary infection. Mildews, leaf spots, blasts, blights etc are the common examples of former type while some smut disease of cereal or grain crops where black powder is formed in place of grains or inflorescence fall in the latter type. Control measures, therefore, in the former type need to be directed to avoid the primary infection and also to check the secondary spread.

Modes of primary infections:

According to the general mode of primary infection plant diseases are recognized as:-

1. Soil borne
2. Seed borne, including diseases carried with planting material.
3. Wind borne
4. Insect borne etc.

This knowledge is helpful in adopting suitable control measures.

Continuous and discontinuous transmission: -

Transmission of disease is termed as 'continuous' when it occurs naturally by way of growth, multiplication and spread of the pathogen in an area or country where the disease is established. At times, however, in an area or country where a particular disease has never occurred, it may get introduced through the agency of man carrying diseased material to a new locality or to a distant country for the purpose of introduction of new plants, crops, varieties etc. such transmission, of course, is unnatural and regarded as 'discontinuous' transmission. Altogether different control measures viz. Quarantine Regulations are enforced to check such discontinuous transmission.

Direct and indirect transmission:-

For classifying the methods of disease transmission in relation to the methods of suitable control measures, the following two groups can be conveniently recognized.

1. **Direct transmission:** - Disease transmission where the pathogen is carried externally or internally on the seed or planting material like cuttings, sets, tubers, bulbs etc.
2. **Indirect transmission:-** The pathogen spreading itself by way of its persistent growth or certain structures of the pathogen carried independently by natural agencies like wind, water, animals, insects, mites, nematodes, birds etc. are the different methods of indirect transmissions.

Direct transmission: -

1. **Internal transmission through seed or planting material:-** False smut disease as well as Helminthosporin Blight disease of wheat are the common examples of fungal diseases carried internally through apparently healthy seed. Ring rot and Brown rot of potato caused by bacteria are carried internally through the tubers. The well known whip smut and red rot of sugarcane are fungal diseases carried internally in the planting sets. Mosaic and leaf roll of potato which are viral diseases are also carried inside the infected tubers.

2. **External transmission through seed or planting material:-** In this mode of transmission the pathogen is carried externally over the surface of seed or vegetatively propagated plant parts like sets, tubers, bulbs etc. or may even be carried as a physical mixture of fungal structures with the seed. The common grain smut of jowar is an example of the former type while the fungal structures called 'sclerotia' having the size of a grain or slightly bigger in case of the Ergot disease of bajra are often likely to be transmitted in the form of physical mixture with the seed.

Indirect transmission: -

1. **Autonomous transmission:-** It takes place by continuous and persistent growth of the threads or 'hyphae' of the causal fungi in soil, characteristic of several wood rotting fungi attacking forest trees and some fruit plants. Some root rotting fungi infecting certain seasonal crops also are transmitted by this method. The autonomous dispersal of such soil fungi may range from few cm. To several (8 to 10) meters in a single season. Some plant parasitic nematodes also exhibit active but limited mobility in the soil.
2. **Wind dispersal: -** Fungal spores produced externally on host surfaces are most easily carried by wind currents and this is the most dangerous mode of transmission of plant pathogenic fungi like those causing powdery and downy mildews, leaf spots, blasts, blights and rust diseases. The black stem rust disease of wheat in India perpetuates on wild grasses in the Nilgiri hills in the south India from where the rust spores are carried to south, central & then to north India by wind currents every year. Spores may be carried from low to very high altitudes of 12,000 to 14,000 feet and from short distances to very long distances of several hundred kilometers.

   Extensive and severe epidemics of plant diseases are mostly the results of wind transmission of the pathogens. Wind dissemination involves four stages relating to the spores viz. Production of countless spores, their liberation in the wind currents, dispersal alongwith the wind and deposition on new susceptible host surfaces where they cause infection under favourable climatic conditions. Apart from spores, bits of fungal threads and nematode cysts are also amenable to wind transmission in certain cases.
3. **Water dissemination:** Disease transmission through the agency of water in different ways is comparatively less important as compared to the wind transmission. Splashing rain drops mostly transmit the foliar diseases from leaf to leaf, from shoot to shoot and even from plant to plant in case of closely spaced crops. Such transmission is usually accompanied by wind dispersal as well. Plant pathogens requiring high humidity conditions like the fungi causing downy mildew diseases or bacteria causing canker of citrus are well adapted to this kind of short distance water dispersal.

   Certain soil inhabiting pathogenic fungi and bacteria causing root and collar rots, wilts, foot, rots, etc are likely to be transmitted to much longer distances through the agencies like irrigation water, streams and rivers, etc. It is also an important agency in transmission of seeds of higher flowering parasites like dodder and striga.
4. **Animals:** Farm animals serve as disease transmitting agents in some cases. They are likely to carry the pathogen externally on their body surface, particularly on legs and hoofs, etc. or internally through their intestinal tract. Commonly, the soil inhabiting fungi causing rots and wilts are carried externally while certain smut fungi causing diseases to grain crops are transmitted through the intestinal tract.
5. **Birds:** Although birds play a very minor role in disease transmission, in cases of dispersal of seeds of higher flowering parasite. Loranthus sp. Parasitising certain trees like mango, etc. their role is of great significance. They transmit loranthus both externally and internally.
6. **Implements and Tools:** Farm implements used for cultivation of soil are often likely to transmit plant pathogens from one place to another. The pathogens in this case are usually carried in the form of bits of plant disease debris lying in the soil. Similarly tools used for carrying out operations like cutting, pruning, budding, grafting, thinning,etc. also help in the transmission of certain diseases from plant to plant. Several viral diseases are disseminated through the budding and grafting operations.
7. **Insects:** Most of the viral diseases of plants are transmitted through the agency of different insects. Both types of insects viz. sucking and chewing or/biting are capable of transmitting viral diseases. The transmission may be simply `mechanical' or it may be `biological'. In the latter case the specific insect and the specific viral pathogen have some kind of association or relationship between the two. Insects in such cases are called the `vectors' for the particular viral pathogen. In case of mechanical transmission the pathogen is simply carried externally or internally by the insect.

   Viruses carried `biologically' by the insect vectors are of two types:

1. Non-persistent-viral pathogen requiring no latent or incubation period in the insect body.
2. Persistent: viral pathogens requiring certain incubation period inside the vector body before they are inoculated or transmitted to healthy host.The insects responsible for transmission of viral diseases belong to the species of aphids, jassids (leaf hoppers), white flies, mealy bugs, etc. Certain bacterial and several fungal pathogens are also known to be carried by insects.

1. **Mites:** Mites in contrast to insects are wingless anthropods resembling ticks and having four pairs of legs and no antennae. It is suspected that some viral diseases of chillies, tomato, brinjal, etc. have vector relationship with mites.
2. **Nematodes:** Nematodes have been observed to transmit viral, bacterial and fungal plant diseases. Nematodes feeding externally on host plant roots cause injuries to roots which become the avenues for entrance of fungal and bacterial pathogens infecting plant roots. The Fan-leaf virus of grapevine is a well known example of transmission through a species of nematodes.
3. **Biological transmission**: Dodder which is higher flowering parasite is known to transmit certain viral diseases which remain `persistent' in the dodder plant. The flowering parasite after acquiring the virus from infected plant does not show any symptom itself but remains capable of transmitting the virus to healthy hosts.
4. **Human dispersal**: Man is often responsible for transmission of plant diseases in two ways viz.

1. Workers handling seedlings, other planting material or fruits are likely to get personally in contact with plant pathogens like fungi or bacteria. While handling the diseased material and unknowingly and indirectly transmit the pathogens to healthy seedlings or plant parts through his contaminated hands. This is a kind of `continuous' mode of transmission.
2. The other or`discontinous' mode of transmission for which only man is responsible is the most efficient and equally dangerous phenomenon of transmission of plant diseases between distant geographical areas often separated by physical barriers like oceans, mountains or deserts, etc. Such long distances transmission of a disease to an area or country hitherto free from the disease is usually accomplished by the transport of infected seed, nursery stock or timber, etc. Thus it is a kind of direct transmission through propagating material.

In the history of plant pathology this mode of transmission has often resulted in to some of the worst plant disease epidemics in new areas, the local host plant stock being not adapted to the pathogen and consequently remaining highly susceptible. Fungal diseases viz. Late blight of potato and downy mildew of grapes, Bunchy top of banana, which is a viral disease and Bacterial blight of paddy are the examples of some severe and important diseases introducing in India from other countries.

Practically all the countries of the world have suffered from such introduction of new plant diseases through the agency of man. Concerned international authorities, therefore, are engaged in promoting studies in this respect and designing regulations to reduce the danger.

Disease Epidemics:

When a pathogen spreads to and affects many individuals within a population over a relatively large area and within a relatively short time, the phenomenon is called an epidemic. An epidemic has been defined as any increase of disease in a population.

A similar definition of an epidemic is the dynamics of change in plant disease in time and space. The study of epidemics and of the factors that influence them is called epidemiology. Epidemiology is concerned simultaneously with populations of pathogens and host plants as they occur in an evolving environment, i.e., the classic disease triangle. As a result, epidemiology is also concerned with population genetics of host resistance and with the evolutionary potential of pathogen populations to produce pathogen races that may be more virulent to host varieties or more resistant to pesticides. Epidemiology, however, must also take into account other biotic and abiotic factors, such as an environment strongly influenced by human activity, particularly as it relates to disease management.

Plant disease epidemics, sometimes called epiphytotics, occur annually on most crops in many parts of the world. Most epidemics are more or less localized and cause minor to moderate losses. Some epidemics are kept in check naturally, e.g., by changes in weather conditions. Others are kept in check by chemical sprays and other control measures. Occasionally, however, some epidemics appear suddenly, go out of control, and become extremely widespread or severe on a particular plant species. Some plant disease epidemics, e.g., wheat rusts, southern corn leaf blight (Fig. 8-1), and grape downy mildew, have caused tremendous losses of produce over rather large areas. Others, e.g., chestnut blight (Fig. 1-8), Dutch elm disease, and coffee rust, have threatened to eliminate certain plant species from entire

continents. Still others have caused untold suffering to humans. *The Irish potato famine of 1845–1846 was caused by the Phytophthora late blight epidemic of potato, and the Bengal famine of 1943 was caused by the Cochliobolus (Helminthosporium) brown spot epidemic of rice.* Epiphytotic, rate of increase of disease is mathematically analogous to compound interest in money eg rusts of cereals, powdery mildews

The elements of an epidemic:

Plant disease epidemics develop as a result of the timely combination of the same elements that result in plant disease: susceptible host plants, a virulent pathogen, and favorable environmental conditions over a relatively long period of time. Humans may unwittingly help initiate and develop epidemics through some of their activities, e.g., by topping or pruning plants in wet weather.

More frequently, humans may stop the initiation and development of epidemics by using appropriate control measures under situations in which epidemics would almost certainly occur without human intervention. Thus, the chance of an epidemic increases when the susceptibility of the host and virulence of the pathogen are greater, as the environmental conditions approach the optimum level for pathogen growth, reproduction, and spread, and as the duration of all favorable combinations is prolonged or repeated.

The interaction of the four components can be visualized as a tetrahedron, or pyramid, in which each plane represents one of the components. This figure is referred to as the disease tetrahedron or disease pyramid (Fig. 8-2). The effect of time on disease development becomes apparent when one considers the importance of the time of year (i.e., the climatic conditions and stage of growth when host and pathogen may coexist), the duration and frequency of favorable temperature and rains, the time of appearance of the vector, the duration of the infection cycle of a particular disease, and so on.

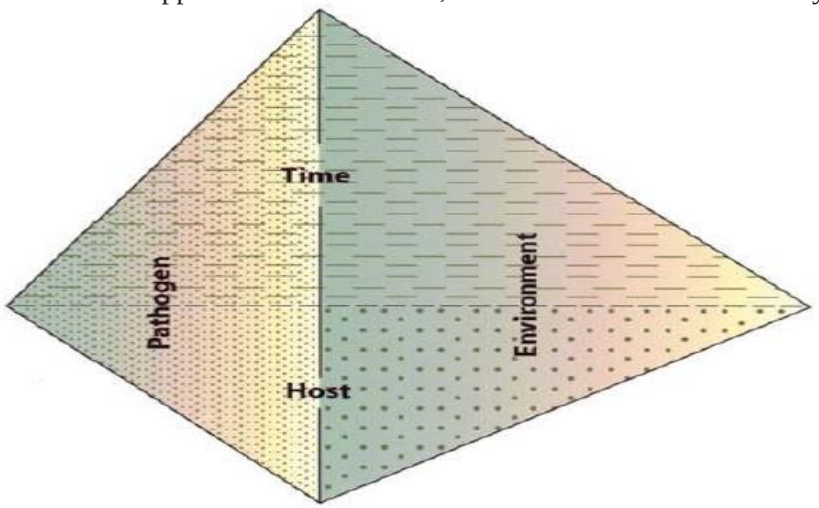


FIGURE 8-2 The disease tetrahedron.

Host factors that affect the development of epidemics:

Several internal and external factors of particular host plants play an important role in the development of epidemics involving those hosts.

i) Levels of Genetic Resistance or Susceptibility of the Host

Obviously, host plants carrying race-specific (vertical) resistance do not allow a pathogen to become established in them, and thus no epidemic can develop. Host plants carrying partial (horizontal) resistance will probably become infected, but the rate at which the disease and the epidemic will develop depends on the level of resistance and the environmental conditions.

Susceptible host plants lacking genes for resistance against the pathogen provide the ideal substrate for establishment and development of new infections. Therefore, in the presence of a virulent pathogen and a favorable environment, susceptible hosts favor the development of disease epidemics.

ii) Degree of Genetic Uniformity of Host Plants

When genetically uniform host plants, particularly with regard to the genes associated with disease resistance, are grown over large areas, a greater likelihood exists that a new pathogen race will appear that can attack their genome and result in an epidemic. This phenomenon has been observed repeatedly, for example, in the *Cochliobolus* (*Helminthosporium*) blight on Victoria oats and in southern corn leaf blight on corn carrying Texas male-sterile

cytoplasm. For similar reasons of genetic uniformity, the highest rates of epidemic development generally occur in vegetatively propagated crops, intermediate rates in self-pollinated
crops, and the lowest rates in cross-pollinated crops. This explains why most epidemics develop rather slowly in natural populations, where plants of varying genetic makeup are intermingled.

iii) Type of Crop

In diseases of annual crops, such as corn, vegetables, rice, and cotton, and in foliar, blossom, or fruit diseases of trees and vines, epidemics generally develop much more rapidly (usually in a few weeks) than they do in diseases of branches and stems of perennial woody crops such as fruit and forest trees. Some epidemics of fruit and forest trees, e.g., tristeza in citrus, pear decline, Dutch elm disease, and chestnut blight, take years to develop.

iv) Age of Host Plants

Plants change in their reaction (susceptibility or resistance) to disease with age. The change of resistance with age is known as ontogenic resistance. In some plant–pathogen combinations, e.g., *Pythium* damping off and root rots, downy mildews, peach leaf curl, systemic smuts, rusts, bacterial blights, and viral infections, the hosts (or their parts) are susceptible only during the growth period and become resistant during the adult period (adult resistance). With several diseases, such as rusts and viral infections, plant parts are actually quite resistant to infection while still very young, become more susceptible later in their growth, and then become resistant again before they are fully expanded.

Pathogen factors that affect development of epidemics:

i) Levels of Virulence

Virulent pathogens capable of infecting the host rapidly ensure a faster production of larger amounts of inoculum, and, thereby, disease, than pathogens of lesser virulence.

ii) Quantity of Inoculum near Hosts

The greater the number of pathogen propagules (bacteria, fungal spores and sclerotia, nematode eggs, virus infected plants, etc.) within or near fields of host plants, the more inoculum reaches the hosts and at an earlier time, there by increasing the chances of an epidemic greatly.

iii) Type of Reproduction of the Pathogen

All pathogens produce many offspring. Some of them, such as most fungi, bacteria, and viruses, produce a great many offspring, while a few fungi, all nematodes, and all parasitic plants produce relatively small numbers of offspring. Some plant pathogenic fungi, bacteria, and viruses have short reproduction cycles and therefore are polycyclic, i.e., they can produce many generations in a single growing season. Polycyclic pathogens include fungi that cause rusts, mildews, and leaf spots and are responsible for most of the sudden, catastrophic plant disease epidemics in the world. Some soil fungi, such as *Fusarium* and *Verticillium*, and most nematodes usually have one to a few (up to four) reproductive cycles per growing season. For these latter pathogens, the smaller number of offspring and, especially, the conditions of their dispersal limit their potential to cause sudden and widespread epidemics in a single season.

iv) Ecology of the Pathogen

Some pathogens, such as most fungi and all parasitic higher plants, produce their inoculum (spores and seeds, respectively) on the surface of the aerial parts of the host. From there, spores and seeds can be dispersed with ease over a range of distances and can cause wide spread epidemics. Other pathogens, such as vascular fungi and bacteria, mollicutes, viruses, and protozoa, reproduce inside the plant. In this case, spread of the pathogen is rare or impossible without the help of vectors. Therefore, such pathogens can cause epidemics only when vectors are plentiful and active.

v) Mode of Spread of the Pathogen

The spores of many plant pathogenic fungi, such as those causing rusts, mildews, and leaf spots, are released into the air and can be dispersed by air breezes or strong winds over distances varying from a few centimeters up to several kilometers. These kinds of fungi are responsible for the most frequent and most widespread epidemics.

In terms of their ability to cause sudden and widespread epidemics, the next most important group of pathogens includes those whose inoculum is carried by airborne vectors. Many of the viruses are transmitted by aphids, whiteflies, and some other insects. Mollicutes and fastidious bacteria are transmitted by leafhoppers, plant hoppers, or psyllids. Some fungi (such as the cause of Dutch elm disease), bacteria (such as the cause of bacterial wilt of cucurbits), and even nematodes (such as the cause of pine wilt disease) are disseminated primarily by beetles. Pathogens that are transmitted by windblown rain (primarily fungi causing diseases such as anthracnoses and apple scab, and most bacteria) are almost annually responsible for severe but somewhat localized epidemics within a field, a country, or a valley. Pathogens carried with the seed or other vegetative propagative organs (such as tubers or bulbs)

are often placed in the midst of susceptible plants, but their ability to cause epidemics depends on the effectiveness of their subsequent transmission to new plants.

Environmental factors that affect development of epidemics:

The majority of plant diseases occur wherever the host is grown but, usually, do not develop into severe and widespread epidemics. The concurrent presence in the same areas of susceptible plants and virulent pathogens does not always guarantee numerous infections, much less the development of an epidemic. This fact dramatizes the controlling influence of the environment on the development of epidemics. The environment may affect the availability, growth stage, succulence, and genetic susceptibility of the host plants. It may also affect the survival, vigor, rate of multiplication, sporulation, and ease, direction, and distance of dispersal of the pathogen, as well as the rate of spore germination and penetration.

i) Moisture

Abundant, prolonged, or repeated high moisture, whether in the form of rain, dew, or high humidity, is the dominant factor in the development of most epidemics of diseases caused by oomycetesand fungi (blights, downy mildews, leaf spots, rusts, and anthracnoses), bacteria (leaf spots, blights, soft rots), and nematodes. Moisture not only promotes new succulent and susceptible growth in the host, but, more importantly, it increases sporulation of fungi and multiplication of bacteria. Moisture facilitates spore release by many fungi and the oozing of bacteria to the host surface, and it enables spores to germinate and zoospores, bacteria, and nematodes to move. The presence of high levels of moisture allows all these events to take place constantly and repeatedly and leads to epidemics.

ii) Temperature

Epidemics are sometimes favored by temperatures higher or lower than the optimum for the plant because they reduce the plant's level of partial resistance. At certain levels, temperatures may even reduce or eliminate the race-specific resistance of host plants. Plants growing at such temperatures become "stressed" and predisposed to disease, provided the pathogen remains vigorous.

Low temperature reduces the amount of inoculum of oomycete fungi, bacteria, and nematodes that survives cold winters. High temperature reduces the inoculum of viruses and mollicutes that survives hot summer temperatures. In addition, low temperatures reduce the number of vectors that survive the winter. Low temperatures occurring during the growing season can reduce the activity of vectors.

The most common effect of temperature on epidemics, however, is its effect on the pathogen during the different stages of pathogenesis, i.e., spore germination or egg hatching, host penetration, pathogen growth or reproduction, invasion of the host, and sporulation. When temperature stays within a favorable range for each of these stages, a polycyclic pathogen can complete its infection cycle within a very short time (usually in a few days). As a result, polycyclic pathogens can produce many infection cycles within a growing season. Because the amount of inoculum is multiplied manyfold (perhaps 100 times or more) with each infection cycle and because some of the new inoculum is likely to spread to new plants, more infection cycles result in more plants becoming infected by more and more pathogens, thus leading to the development of a severe epidemic.

In reality, moisture and temperature must be favourable and act together in the initiation and development of the vast majority of plant diseases and plant disease epidemics.

Effect of human cultural practices and control measures:

Many activities of humans have a direct or indirect effect on plant disease epidemics, some of them favouring and some reducing the frequency and the rate of epidemics.

i) Site Selection and Preparation

Low-lying and poorly drained and aerated fields, especially if near other infected fields, tend to favor the appearance and development of epidemics.

ii) Selection of Propagative Material

The use of seed, nursery stock, and other propagative material that carries various pathogens increases the amount of initial inoculum within the crop and favors the development of epidemics greatly. The use of pathogen-free or treated propagative material can reduce the chance of epidemics greatly.

iii) Cultural Practices

Continuous monoculture, large acreages planted to the same variety of crop, high levels of nitrogen fertilization, no-till culture, dense plantings, overhead irrigation, injury by herbicide application, and poor sanitation all increase the possibility and severity of epidemics.

iv) Disease Control Measures

Chemical sprays, cultural practices (such as sanitation and crop rotation), biological controls (such as using resistant varieties), and other control measures reduce or eliminate the possibility of an epidemic. Sometimes, however, certain controls, e.g., the use of a certain chemical or planting of a certain variety, may lead to selection of virulent strains of the pathogen that either are resistant to the chemical or can overcome the resistance of the variety and thus lead to epidemics.

Patterns of epidemics:

Interactions of the structural elements of epidemics, as influenced over time by factors of the environment and by human interference, are expressed in patterns and rates. The pattern of an epidemic in terms of the numbers of lesions, the amount of diseased tissue, or the numbers of diseased plants is given by a curve, called the **disease–progress curve**, that shows the progress of the epidemic over time. The point of origin and the shape of a disease–progress curve reveal information about the time of appearance and amount of inoculum, changes in host susceptibility during the growing period, recurrent weather events, and the effectiveness of cultural and control measures.

Disease–progress curves, because they are affected by weather, variety, and so on, vary somewhat with location and time, but they are generally characteristic for some groups of diseases. For example, a saturation-type curve is characteristic for monocyclic diseases, a sigmoid curve is characteristic for polycyclic diseases, and a bimodal curve is characteristic for diseases affecting different organs (blossoms, fruit) of the plant. Knowledge of disease–progress curves also allows disease forecasting and selection of the best control strategy for the particular disease and time.

Comparison of epidemics:

For better comparison of epidemics of the same disease at different times, different locations, or under different management practices or to compare different diseases, the patterns obtained for disease–progress curves and disease–gradient curves are frequently transformed mathematically into straight lines. The slopes of these lines can then be used to calculate epidemic rates.

In monocyclic diseases, the amount of inoculum does not increase significantly during the season. In such diseases, therefore, the rate of disease increase is affected only by the inherent ability of the pathogen to induce disease and by the ability of the environmental factors and cultural practices to influence host resistance and the virulence of the pathogen.

In contrast, the initial inoculum for diseases caused by polycyclic pathogens, although extremely important,
has relatively less importance than the number of infection cycles in the final disease outcome. Pathogens that have many infection cycles also have numerous opportunities to interact with the host. Therefore, the same factors mentioned earlier, namely the inherent ability of the pathogen to induce disease, environmental factors, host resistance, and cultural practices, have an opportunity to influence the dispersal, penetration, multiplication, size of lesion, rate of lesion formation, and rate and amount of sporulation, but they can do that not once but several times during the same growth season. The continuous or, sometimes, intermit-tent increase of the amount of inoculum and disease may result in highly variable infection rates for individual short-term intervals during the growth season, and quite variable epidemic rates for the entire season.

In addition to the epidemics caused by monocyclic and polycyclic pathogens, there are also polyetic epidemics. Pathogens causing polyetic epidemics are present for one year or more in the infected plant before they produce effective inoculum

Development of epidemics:

For a disease to become significant in a field, particularly if it is to spread over a large area and develop into a severe epidemic, specific combinations of environmental factors must occur either constantly or repeatedly, and at frequent intervals, over a large area. Even in a single, small field that contains the pathogen, plants almost never become severely diseased from just one set of favorable environmental conditions. It takes repeated infection cycles and considerable time before a pathogen produces enough individuals to cause an economically severe epidemic in the field. Once large populations of the pathogen are available, however, they can attack, spread to nearby fields, and cause a severe epidemic in a very short time, often in just a few days. A plant disease epidemic can occur in a garden, a greenhouse, or a small field, but “epidemic” generally implies the development and rapid spread of a pathogen on a particular kind of crop plant cultivated over a large area, such as a large field, a valley, a section of a country, the entire country, or even part of a continent. Therefore, the first component of a plant disease epidemic is a large area planted to a genetically uniform crop plant, with the plants and the fields being close together. The second component of an epidemic is the presence or appearance of a virulent pathogen.

Such cohabitations of host plants and pathogens occur, of course, daily in countless locations. Most of these, however, cause local diseases of varying severity, destroy crop plants to a limited extent, and do not develop into epidemics. Epidemics develop only when the combinations and progression of the right sets of conditions occur. These include appropriate temperature, moisture, and wind or insect vector coinciding with the susceptible stage or stages of the plant and with the production, spread, inoculation, penetration, infection, and reproduction of the pathogen.

9. Principles of plant disease control: exclusion, eradication, protection and therapy

Plant diseases have caused severe losses to humans in several ways. Starvation and uprooting of families resulted from the Irish famine caused by potato late blight (caused by *Phytophthora infestans*). A valued resource was lost with the virtual elimination of the American chestnut by chestnut blight (caused by *Cryphonectria parasitica*). And direct economic loss such as the estimated one billion dollars lost in one year to American corn growers from southern corn leaf blight (caused by *Cochliobolus maydis*, anamorph *Bipolaris maydis*). Many plant diseases cause less dramatic losses annually throughout the world but collectively constitute sizable losses to farmers and can reduce the aesthetic values of landscape plants and home gardens.

The goal of plant disease management is to reduce the economic and aesthetic damage caused by plant diseases. Traditionally, this has been called plant disease control, but current social and environmental values deem "control" as being absolute and the term too rigid. More multifaceted approaches to disease management, and integrated disease management, have resulted from this shift in attitude, however. Single, often severe, measures, such as pesticide applications, soil fumigation or burning are no longer in common use. Further, disease management procedures are frequently determined by disease forecasting or disease modeling rather than on either a calendar or prescription basis. Disease management might be viewed as proactive whereas disease control is reactive, although it is often difficult to distinguish between the two concepts, especially in the application of specific measures.

One early proposal by H. H. Whetzel included four general disease control principles, **exclusion**, **eradication**, **protection** and **immunization** (the latter principle is more appropriately called **resistance** since plants do not have an immune system in the same sense as animals). These principles have been expanded or altered to some extent by others. They are still valid and are detailed here but students should investigate other systems such as those proposed by Gäumann, Sharvelle, or the National Academy of Science and use the one(s) that they believe are applicable.

Exclusion:

This principle is defined as any measure that prevents the introduction of a disease-causing agent (pathogen) into a region, farm, or planting. The basic strategy assumes that most pathogens can travel only short distances without the aid of some other agent such as humans or other vector, and that natural barriers like oceans, deserts, and mountains create obstacles to their natural spread. In many cases pathogens are moved with their host plants or even on non host material such as soil, packing material or shipping containers. Unfortunately, exclusion measures usually only delay the entry of a pathogen, although exclusion may provide time to plan how to manage the pathogen when it ultimately arrives. Karnal bunt (caused by *Tilletia indica*) of wheat is an example of a pathogen originally from India that was anticipated. Measures were established to prevent its introduction, but it finally found its way into the United States. Soybean rust (caused by *Phakopsora pachyrhizi*) has been found recently in the south eastern U.S. and precautions have been undertaken to prevent further spread. Due to its destructiveness, South American leaf blight (SALB) (caused by *Microcyclus ulei*) is a feared disease in the major rubber producing region of Indonesia, and contingency plans have been proposed to chemically defoliate rubber trees by aerial application of herbicides if the pathogen is detected. It is hoped that this would prevent establishment of the pathogen in the region.

In the United States, the Animal and Plant Health Inspection Service (APHIS), a division of the U.S. Department of Agriculture, is responsible for promulgating and enforcing plant quarantine measures. There are also state agencies that deal with local quarantines. Internationally, eight regional plant protection organizations (PPOs) were established in 1951 by the International Plant Protection Convention sponsored by the Food and Agricultural Organization of the United Nations. This was revised in 1997 and now includes nine regional PPOs. The European and Mediterranean Plant Protection Organization (EPPO) is the oldest of the regional PPOs. The regional PPOs have no regulatory

authority such as APHIS or other governmental agency, but function to develop strategies against the introduction and spread of pests and to coordinate the use of phytosanitary regulations to ensure agreement among the different member countries.

An important and practical strategy for excluding pathogens is to produce pathogen-free seed or planting stock through certification programs for seeds and vegetatively propagated plant materials such as potatoes, grapes, tree fruits, etc. These programs utilize technologies that include isolation of production areas, field inspections, and removal of suspect plants to produce and maintain pathogen-free stocks. Planting stock that is freed of pathogens can be increased by tissue culture and micropropagation techniques as well as be maintained in protective enclosures such as green houses to exclude pathogens and their vectors. Exclusion may be accomplished by something as simple as cleaning farming equipment (Figure 2) to remove contaminated debris and soil that can harbor pathogens such as *Verticillium*, nematodes or other soil borne organisms and prevent their introduction into non-infested fields.

Eradication:

This principle aims at eliminating a pathogen after it is introduced into an area but before it has become well established or widely spread. It can be applied to individual plants, seed lots, fields or regions but generally is not effective over large geographic areas. Two large attempts at pathogen eradication in the United States were the golden nematode (*Globodera rostochiensis*) program on Long Island, New York and the citrus canker (caused by *Xanthomonas axonopodis* pv. *citri* and pv. *aurantifolii*) program in Florida. However, neither of these attempts was a lasting success.

Eradication of the golden nematode involved removing infested soil, fumigating soil in infested fields and eventually abandoning infested potato fields for housing developments and other uses. Citrus canker eradication involved widespread removal and burning of diseased trees and, in some cases, destruction of entire citrus groves and nurseries. The disease appeared to be contained and the pathogen eradicated, but the disease has reappeared and new attempts at eradication are ongoing.

Eradication can also be on a more modest scale such as the removal of apple or pear branches infected by the fire blight bacterium (*Erwinia amylovora*) or pruning to remove blister rust cankers (caused by *Cronartium ribicola*) on white pine branches. Or, it can be the sorting and removal of diseased flower bulbs, corms or rhizomes. Hot water seed-treatment of cereal seeds to kill smut mycelium in the seed and heat treatment to eliminate viruses from fruit tree budwood for grafting are other examples of pathogen eradication.

Two programs that are actually forms of protection and not pathogen eradication are barberry eradication for reducing stem rust (caused by *Puccinia graminis*) of wheat and *Ribes* eradication for preventing white pine blister rust. The strategy is that removing these alternate hosts breaks the disease cycles and prevents infection of the economically more valuable host. These two examples are mentioned here because they are frequently cited as eradication measures. However, stem rust can readily spread from wheat to wheat in many regions by the uredinial stage although elimination of the aecial host, barberry, may deter or diminish the development of pathogenic races of the rust. The white pine blister rust fungus is perennial in the pine host and eradication of the alternate host only protects non infected trees but does not necessarily eliminate the pathogen from the area.

Eradication may also be accomplished by destroying weeds that are reservoirs of various pathogens or their insect vectors. Elimination of potato cull piles is an effective method of eradicating overwintering inoculum of the late blight pathogen.

Soil fumigation has been a widely used eradication strategy. This technology involves introducing gas-forming chemicals such as carbon disulfide, methyl bromide, or chloropicrin into soil to kill target pathogens. However, undesirable side effects such as killing beneficial organisms, contamination of groundwater, and toxicity of these chemicals have resulted in less reliance on this approach for disease management. Volatile fumigants like methyl bromide are injected into soil and sealed with a plastic film. Some water-soluble fumigants like metam-sodium can be injected into the soil and the soil simply compacted to form a seal.

Crop rotation is a frequently used strategy to reduce the quantity of a pathogen, usually soil-borne organisms, in a cropping area. Take-all of wheat (caused by *Gaeumannomyces graminis*) and soybean cyst nematode (*Heterodera glycines*) are two examples of soilborne diseases that are easily managed by short rotations of 1 and 2 years, respectively, out of susceptible crops, which may include susceptible weed hosts such as grasses in the case of take-all. Burning is an effective means of eradicating pathogens and is often required by law to dispose of diseased elm trees

affected by Dutch elm disease (DED) citrus trees infected by citrus canker or of bean fields infected by halo blight bacteria (*Pseudomonas syringae* pv. *phaseolicola*). Propane flaming can effectively destroy *Verticillium* microsclerotia in mint stems, and flaming potato stems prior to harvest may prevent tuber infection by the late blight pathogen. However, burning agricultural fields is controversial because the smoke creates human health and safety and environmental concerns.

Protection:

This principle depends on establishing a barrier between the pathogen and the host plant or the susceptible part of the host plant. It is usually thought of as a chemical barrier, e.g., a fungicide, bactericide or nematicide, but it can also be a physical, spatial, or temporal barrier. The specific strategies employed assume that pathogens are present and that infection will occur without the intervention of protective measures. For example, bananas are covered with plastic sleeves as soon as the fruit are set to protect the fruit from various pests including fruit decay fungi.

Protection often involves some cultural practice that modifies the environment, such as tillage, drainage, irrigation, or altering soil pH. It may also involve changing date or depth of seeding, plant spacing, pruning and thinning, or other practices that allow plants to escape infection or reduce severity of disease. Raising planting beds to assure good soil water drainage is an example of cultural management of plant diseases such as root and stem rots.

Fungicides have been used for more than a hundred years and new fungicides continue to be developed. **Bordeaux mixture**, a basic copper sulfate fungicide, was the first widely used fungicide and is still used today in various forms. The earliest fungicides were simple elements like sulfur or metallic compounds of copper or mercury, and these are generally classed as inorganic fungicides. In the early to mid-1900s organic fungicides such as thiram, captan, and the bis dithiocarbamates were developed. These are broad-spectrum, contact or protectant fungicides that control a wide range of fungal diseases. Starting in the 1960s the "systemic" fungicides were developed. Most of these are not truly systemic in plants but have some limited mobility, usually translaminar, and often give some post-infection benefits. Some of the "systemic" fungicides move upward in the plant's vascular system, but currently only one (fosetyl-Al) has ambimobile distribution (both upward and downward) that would constitute a truly systemic fungicide. In addition to the SBI and DMI fungicides mentioned earlier, a recent group of systemic fungicides are the strobilurins. Some fungicides have narrow ranges of activity and are used primarily for control of specific groups of diseases such as downy mildews, rusts, smuts or powdery mildews while others are active against a wider range of diseases.

One liability of these recent narrow-range fungicides is that they often have single-site modes of action, (that is, their site-specific activity is controlled by one or a few genes), and thus are especially prone to development of fungicide resistance in the pathogen. Several management strategies have been developed to combat fungicide resistance. These include using mixtures of single-site and multi-site fungicides, alternating applications of fungicides with different modes of action, applying fungicides only when needed instead of on either a calendar or prescription basis, and applying the recommended dosage and not attempting to cut costs by reducing the recommended amount of fungicide applied.

Fungicides can be applied by any of several methods: ground sprayers, airplanes or through irrigation systems, but to be effective applications must be done properly. First, the fungicide must be legally registered for use on the plant involved and against the target disease. Several different chemicals may be registered for the same crop or disease. If the different fungicides are similar in effectiveness, cost, ease of application, and safety, then timing of application becomes the most critical factor. If applied too early much of the chemical will be wasted before it can be effective; if applied too late, it will be largely ineffective. The benefits of properly applied fungicides can often be striking. Distribution of the spray droplets is important; the finer the spray the more complete the coverage on the plant surface. However, very small droplets form a mist that is easily displaced by wind.

Many cultural practices can be modified to manage the occurrence, intensity or severity of plant diseases. These include selection of suitable growing sites for the crop, adequate tillage to bury pathogen-infested plant residues, rotation to nonsusceptible crops, selecting pathogen-free planting stocks, orientation of plantings to improve exposure to sun and air currents, pruning and thinning to eliminate sources of infection and improve aeration in and around susceptible plants, water management on both plants and in soil, adequate nutrition, proper cultivation to improve root growth and avoid plant injury, and sanitation procedures to eliminate sources of inoculum.

Biological control involves the use of one living organism to control another, and this management technology has received much attention in recent times. However, the number of biological agents registered for use is relatively small, success has been limited, and application has been largely restricted to intensively managed, high value crops such as greenhouse plants. Two examples of effective biological control are the use of the fungus *Peniophora gigantea* to inoculate tree stumps to prevent infection of adjacent trees by the wood decay fungus *Heterobasidion annosum*, and the application of the nonpathogenic (i.e., non-tumor-producing) bacterium*Agrobacterium radiobacter* to fruit trees before planting to prevent infection by the crown gall bacterium (*Agrobacterium tumefaciens*)

Avoidance:

It involves avoiding disease by planting at time when, or in areas where inoculums is absent or ineffective due to environmental conditions. The major aim is to enable the host to avoid contact with the pathogen or to ensure that the susceptible stage of the plant does not coincide with favourable conditions for the pathogen. The main practices under avoidance are choice of geographical area, selection of the field, choice of sowing/ planting time, selection of seed and planting material, short duration / disease escaping varieties and modification of agronomic/cultural practices. The potato cultivation at high altitude is relatively free from viruses; as prevailing environmental conditions do not permit the buildup of vector populations. Similarly, early planting of potato or wheat, in indo Gangetic plains may escape late blight or stem rust damage respectively.

Resistance:

Use of disease-resistant plants is the ideal method to manage plant diseases, if plants of satisfactory quality and adapted to the growing region with adequate levels of durable resistance are available. The use of disease-resistant plants eliminates the need for additional efforts to reduce disease losses unless other diseases are additionally present. Resistant plants are usually derived by standard breeding procedures of selection and/or hybridization. A few disease-resistant lines have been obtained by inducing mutations with x-rays or chemicals. There is also interest in chemicals called “plant activators” that induce plant defense responses called systemic acquired resistance (SAR) and induced resistance. Recently, resistant plants have been developed through the use of genetic engineering (e.g., resistance to the *Papaya ringspot virus*).

Selection of resistant plants involves subjecting plants to high levels of disease pressure and using the surviving plants as sources of disease resistance. Plants that survive this pressure often have genetic resistance that can be utilized directly by propagation or as sources of resistance to develop resistant plants that also have the requisite qualities for that crop. Hybridization is a tactic where a plant having the desired agronomic or horticultural qualities, but is susceptible to a disease, is crossed with a plant that is resistant but which may or may not have the other desirable characteristics such as size, yield, flavor, aesthetics, etc.

Disease escape occurs when susceptible plants do not become diseased for some reason. This may be due to some anatomical or physical character, such as the occurrence of leaf hairs, thick cuticle, or modified stomata, or they may be environmental, in which conditions are not conducive to disease development. Although disease escape based on some anatomical feature is useful occasionally, escape more often complicates the process of developing disease resistant plants.

Development of disease-resistant plants has been relatively successful with annual and biennial plants, but less so with perennials, primarily because of the longer time required to develop and test the progeny. Woody perennials, such as ornamental, forest, and orchard trees have been especially difficult for plant breeders to develop useful disease resistance. For example, chestnut blight and Dutch elm disease have devastated two valued native trees. In both cases there have been extensive attempts to develop resistant trees, usually by creating hybrids with exotic chestnut or elm trees, and some resistant selections have resulted. Unfortunately, these generally lack the desirable qualities, such as nut flavor or tree forms characteristic of the native trees. Another introduced disease that has impacted native trees is white pine blister rust. There has been an intense effort for more than 50 years to select and improve rust-resistant pines from the surviving population. These trees are now being planted for reforestation, but it will be another 50 or so years, when these trees have matured to produce a timber crop, before the success of this program is known.

Development of resistance has been most successful against the more specialized pathogens such as rust fungi, smut fungi, powdery mildew fungi, and viruses, but less so against general pathogens such as many blight, canker, root rot and leaf spotting pathogens.

A major problem with genetically resistant plants is that host-differentiated pathogenic races can be selected, so that many breeding programs become continuous processes to develop disease resistant plant lines. Disease resistance conferred by a single major gene is sometimes called specific or qualitative resistance and is race-specific. This type of resistance is often unstable, and emergence of a pathogenic race that can attack that genotype can completely overcome this type of resistance. Quantitative resistance or general resistance derives from many different genes for resistance with additive effects to provide more stable (or durable) resistance to pathogens.

There are several strategies to minimize this race development and resistance failure. These include methods of gene deployment, where different genetic plant types are interspersed on a regional basis to avoid a genetic monoculture, or planting mixtures of cultivars having different genetic compositions to ensure that some component of the crop will be resistant to the disease.

A recent and controversial technique in developing disease resistant plants is the insertion of genes from other organisms into plants to impart some characteristic. For example, genes from the bacterium *Bacillus thuringiensis* have been inserted into plants to protect against insect attacks. Plants with these inserted genes are called genetically-modified organisms (GMOs), and have caused concern that unanticipated, and perhaps detrimental, characteristics, such as unforeseen allergens, may also be transferred to the new plants. However, unforeseen and undesirable qualities also can be transmitted by conventional plant breeding techniques. The potato cultivar Lenape was developed in part because of its resistance to *Potato virus A* and resistance to late blight tuber infection. After it was released it was discovered that the tubers contained very high levels of solanine, a toxic alkaloid. The wheat cultivar Paha had resistance to stripe rust (caused by *Puccinia striiformis*) but also was very susceptible to flag smut (caused by *Urocystis agropyri*). Both of these plant cultivars, developed by conventional breeding methods, were quickly taken out of production. There is much interest in the genetic engineering of disease-resistant plants and some success has been obtained with several virus diseases, the best known of which is papaya ring spot. This approach to plant disease management will likely expand, especially for widely grown crops such as wheat, corn, soybeans, rice, and the like, as social, legal, and economic obstacles are overcome.

Immunization:

Development by natural or artificial means of an immune or highly resistant plant population in the area infected with the pathogen to be combated

Therapy

It is the treatment of infected host plant, which is attempted in case of economically important horticulture plants. As a principle of plant disease control, it provides an opportunity to cure or rejuvenate the diseased host plant by use of physical or chemical agents.

Under the concept of disease management these principles have been classified into following five categories:

1. Management of physical environment (cultural control)
2. Management of associated micro biota (biological antagonism)
3. Management of host genes (host resistance)
4. Management with chemicals (Chemical control)
5. Management with therapy (Physical, chemical etc)

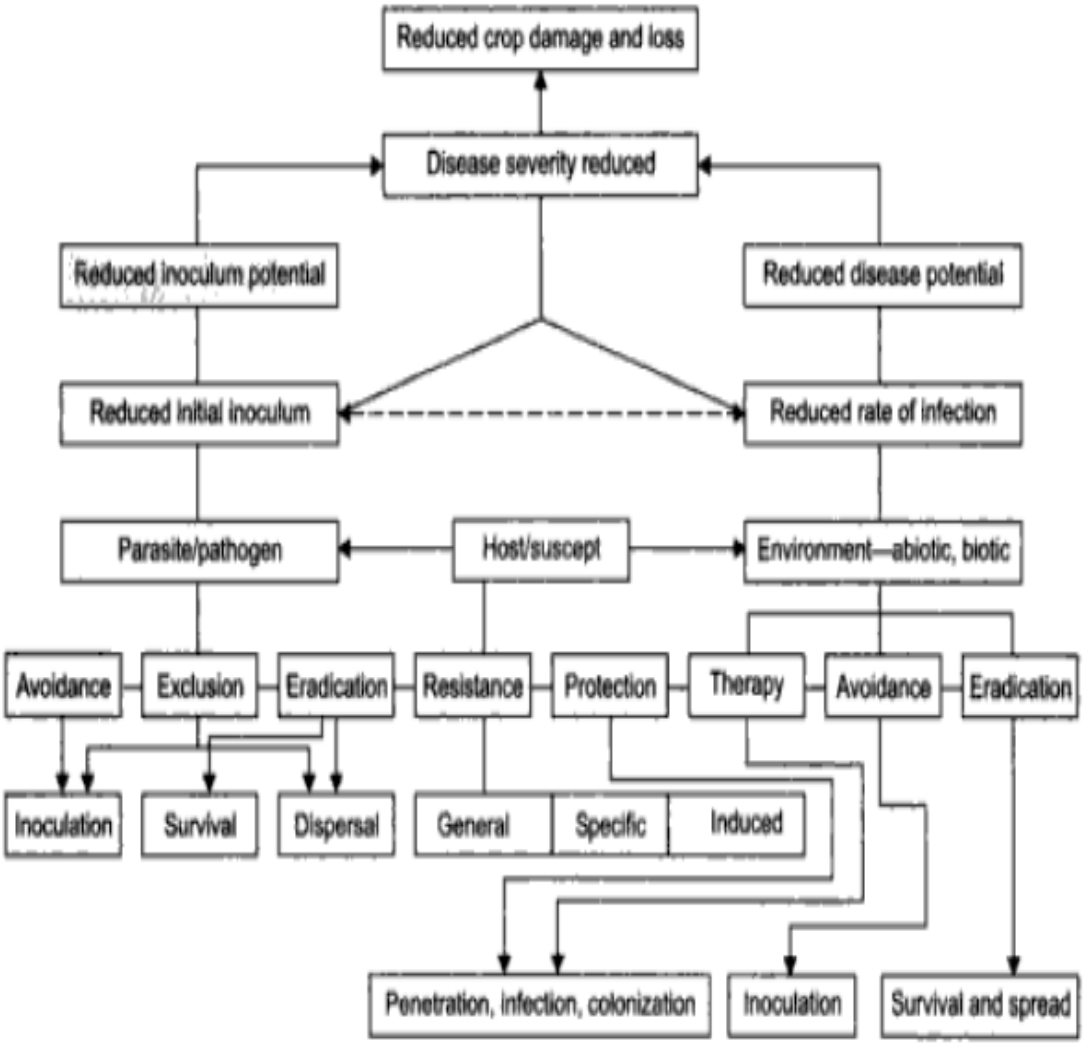
The six principles that characterize the modern concept of plant disease management should be viewed from three stand points

(a) Reduction in the initial inoculums or the rate of disease development.

(b) Management of the pathogen population, the cure or induce defense of the suscept or modify the environment as it influences disease and

(c) Interruption of dispersal, survival or the course of disease development.

These interactions are originally proposed by Baker (1968) and Roberts and Boothroyd (1972) and subsequently modified for the readers are illustrated as below:



10. Strategies of plant disease management with special emphasis on cultural and biological management; plant quarantine; integrated pest management

Depending on the nature of the agents employed, the control methods are: **Regulatory control** measures aim at excluding a pathogen from a host or from a certain geographic area. Most **cultural control** methods aim at helping plants avoid contact with a pathogen, creating environmental conditions unfavorable to the pathogen or avoiding favorable ones, and eradicating or reducing the amount of a pathogen in a plant, a field, or an area. Most **biological** and some **cultural control** methods aim at improving the resistance of the host or favoring microorganisms antagonistic to the pathogen. A new type of biological control involves the transfer of genetic material (DNA) into plants and the generation of transgenic plants that exhibit resistance to a certain disease(s). **Physical** and **chemical** methods aim at protecting the plants from pathogen inoculum that has arrived, or is likely to arrive, or curing an infection that is already in progress. Some chemicals operate by activating the defenses of the plant (systemic acquired resistance) against pathogens.

Methods of control vary considerably from one disease to another, depending on the kind of pathogen, the host, the interaction of the two, and many other variables.

Cultural management:

a) **Selection of Geographical area** which on the basis of the favourable temperature and humidity requirement for a particular crop but unflavored to the fungi and bacteria.

b) Selection of field:
Many soil borne diseases are controlled by proper selection of the field. It is quite possible that a particular field soil contains a pathogen species. In that case the particular crop is not sown in that field for several years. The causal organism of Red rot of sugarcane *Colletotrichum falcatum* survives in the soil for several months. Water drainage is also taken care of while selecting the field.

c) Choice of the time of sowing:
The susceptible stage of plant growth and the favourable environment for pathogen should not match at the same time.

d) Disease escaping varieties:
Certain varieties of crop due to their growth characteristics are able to escape from disease. This disease escaping characteristics of the crop is not genetic rather it is due to growth habits and time of maturation. Early maturing variety of pea is capable of escaping powdery mildew and rust.

e) Selection of seed:
To avoid seed borne diseases, healthy and disease free seeds are essential.

f) Crop rotation:
Crop rotation is essential for controlling soil borne diseases and pathogens.

g) Roguing:
Removal and Destruction of Diseased Plant Organs, eradication of alternate and collateral hosts and sanitation of Fields.

h) Modification of cultural Practices:
Cultural practices such as – distance between the plants, time and frequency of irrigation, transplantation time and method, mixed cropping, amount and property of fertilizer and compost etc. can be changed to reduce losses caused by the disease.

i) Eradication of Insect Vectors:
Insects serve as vectors for many diseases. Eradication of such insect vectors is essential for the control of pathogens.

Biological management:

Use of antagonists or biocontrol agents, eg. *Trichoderma harzianum, Pseudomonas fluorescens*
Mode of action: parasitism, predation, competition, lysis, antibiosis
Control of soil-borne diseases- using suppressive soil containing antagonistic microorganisms, Post-harvest disease- using yeast, *Candida oleophila*
Control of nematodes using predaceous fungi, *Dactylella* spp.
Control of insect pest using entomogenous fungi, *Beauveria* spp. Control of aphids using trap plants

Physical Methods:

a) The hot water treatment method of Jensen was developed in 1887 which was used to control loose smut disease of wheat, barley and Oats. Until the development of systemic fungicide hot water treatment was the only method to control loose smut. Hot water treatment is also effective in the control of nematodes.
b) Solar energy treatment to control loose smut was first developed by Lutlzra. In this method seeds are first rinsed or soaked in water for 4-5 hrs. before drying them in scorching sun.
c) Hot air treatment for the control of virus in propagating stocks was first developed by Kunkal in Peach yellow.

Chemical Methods:

a) Seed treatment with fungicide before transplanting.

i. Soil treating chemicals:
It is used for controlling such soil borne diseases which attack on seeds or seedlings. The examples of such chemicals are – Formaldehyde, Captan, Thiram, Zineb, Organo-mercurials, PCNB, Ethylene dibromide, vapam etc.

ii. For Externally seed borne diseases, chemicals such as formalin, copper carbonate, captan, organo-mercurials (Agrosan GN and Ceresan) are used for seed treatment.

iii. For Internally seed borne diseases (i.e. loose smut), hot water treatment and solar treatment are used.

iv. Systemic Organic Compounds are effective chemicals for controlling both externally and internally seed borne diseases eg. Oxanthin derivatives (Plantvax and Vitavax), Benlate, Bavistin, Demosan.
v. For controlling air borne diseases, foliar application of chemicals is more effective.
vi. The common copper fungicides are: Perenox, Perelan, Blitox, Cuprokyt, Cuprosanand Fytolan. Its use is comparatively better than that of Bordeaux mixture.

b) Seed dressing with organomercurials and systemic fungicides.

Plant quarantine:

Insects, mites, nematodes, fungi, bacteria, viruses, MLOs and other organisms are known to attack various crops of economic importance. These pests and pathogens not only reduce the quantity but also spoil the quality of the produce to a considerable extent. About crop losses due to the pests and diseases, legendary wheat pathologist, E.C. Stakman (1969) said, 'Weeds, insect-pests and plant diseases reduce the potential agricultural production in the U.S. by 23 percent. The potential food that has been aborted, spoiled or damaged would be enough to feed at least 75 million human beings. In Europe, it could feed Norway, Sweden, Finland, Denmark, Netherlands, Belgium, Czechoslovakia, Switzerland and Portugal or the four Scandinavian countries and the Great Britain. In Africa, it could feed Congo, Tanzania, Kenya, Ethiopia and Sudan'. Indeed, the magnitude of losses caused by various pests and diseases to our crops is frightening. The situation in most of the developing world may still be worse. All out efforts are, therefore, required to atleast reduce these losses through proper management of crops and through pests and disease management.

Various methods of pest/disease control are: exclusion, eradication, protection, therapy, resistance, and biological control. Exclusion or 'keeping out' is fundamental to the concept of plant quarantine while eradication methods are employed to eliminate a newly established pest/pathogen. **Plant quarantine** may, therefore, be defined as 'Rules and regulations promulgated by governments to regulate the introduction of plants, planting materials, plant products, soil, living organisms, etc. with a view to prevent inadvertent introduction of exotic pests, weeds and pathogens harmful to the agriculture or the environment of a country/region, and if introduced, to prevent their establishment and further spread'. Plant quarantine is thus designed as a safeguard against harmful pests/pathogens exotic to a country or a region.

Complementary role of plant quarantine:

Plant genetic stocks are a global resource meant for the welfare of humanity. Plant introductions could be in the form of new crops or new varieties for crop diversification, or germplasm in the form of primitive landraces or wild/weedy relatives of crop plants. Germplasm from centres of origin and crop diversity may possess valuable genes for resistance against pests/pathogens, high yield, early maturity, cold, drought or salinity tolerance and quality traits like increased oil, protein contents, etc. Plant quarantine services are charged with the responsibility of preventing entry of hazardous pests, pathogens and weeds, but to deny entry to the valuable genetic resources would be against national interest. These activities are meant to help agricultural development and they are complementary to each other. Too much conservatism on the part of plant quarantine officials and too liberal an attitude on the part of plant introduction officials/breeders would be harmful. Plant quarantine officials must strive to provide adequate safeguards to allow smooth flow of germplasm resources in a healthy state. They should also try to ensure that the germplasm, when received in quarantine station, is processed promptly and that the delays in release, if any, are purely due to biological considerations alone. At the same time, circumvention of plant quarantine must be avoided at all cost even if it means delay in release or rejection of certain materials based on biological consideration. The two should work in unison as members of a single team. Together they should decide the type, quantity and source of the material, and also the required quarantine safeguards. The plant quarantine officials should conduct research on developing sensitive and reliable methods of detection and salvaging treatments, or find alternatives to permit introduction of even high risk genera, if introduction of such materials is in the national interest.

Quarantine regulations:

Plant quarantine regulations are promulgated by the national and the state governments to prevent the introduction and spread of harmful pests and pathogens. Plant quarantine will be justified only when the pest has no natural means of spread and when they are based on biological considerations only, i.e., pest/pathogen introduction risks and the available safeguards.

In general, risks are more with the introduction of vegetative propagules than with true seed. In case of true seed, risks are more with deep-seated infections than with the surface borne contamination of pests/pathogens. Again, risks are far

greater with pathogens like viruses, downy mildews, smuts and many bacteria carried inside the seed without any external symptoms. When vegetative propagules are introduced, rooted plants, and other underground plant parts like rhizomes, suckers, runners, etc. carry higher risks than bud wood, scions and un rooted cuttings. In any case, bulk introductions are always risky as thorough examination and treatment in such cases is very difficult and planting area is far too large to prevent the establishment and spread of the introduced pest/disease.

Based on these factors, plant quarantine regulates the introductions as follows:

1. *Complete embargo/prohibition:* When the pest risk is very high, the safeguards available in the country are not adequate and, therefore, import is prohibited.

2. *Post-entry quarantine:* The risk is very high but adequate safeguards in the form of post-entry isolation growing facilities are available.

3. *Restricted:* Pest risk is not high and import permit is required stipulating conditions for entry, inspection and treatment.

4. *Unrestricted:* Import permit is not required, and material may enter without restriction.

While formulating quarantine regulations, local conditions like crop spectrum and environmental conditions are also to be considered. Since quarantine regulations are designed to break the life cycle of the pest/pathogen involved, the presence of alternate or collateral hosts in the country of import and their introduction should also be taken into account.

Plant quarantine system in India:

Plant quarantine activities in India are carried out under the Destructive Insects and Pests Act (DIP Act) of 1914, and the rules and regulations framed from time to time there under by the Govt. of India (Anonymous, 1975). Seed was not covered under the DIP Act until 1984, when the Govt. of India brought forward a comprehensive 'Plants, Fruits and Seeds (Regulation of Import into India) Order, 1984' which came into force in June 1985 (Anonymous, 1985).

With a view to provide the farmers the best planting materials available in the world for maximising productivity per unit area and to encourage the private seed industry in India not only to meet the internal requirements but also to develop export potential for high quality planting materials, the Government of India announced a 'New Policy on Seed Development' in September 1988. The new policy covers the import of seeds/planting materials of wheat, paddy, coarse cereals, oilseeds, pulses, vegetables, flowers, ornamentals and fruit crops; procedures for their import and the related plant quarantine procedures/requirements.

This policy also states that absolutely no compromise shall be made with the requirements of plant quarantine procedures to prevent entry into the country of exotic pests, diseases and weeds detrimental to Indian agriculture. Therefore, to meet the plant quarantine requirements in respect to the provisions of the new seed policy, the Govt. of India brought forward, through Gazette Notification, the updated Plants, Fruits and Seeds (Regulation of Import into India) Order, 1989 (Anonymous, 1989).

The main features of the existing plant quarantine regulations in India are as follows:

1. No consignment of seeds/planting materials shall be imported into India without a valid 'Import Permit', which is to be issued by a competent authority, to be notified by the Central Government from time to time in the Official Gazette.

2. No consignment of seeds/planting materials shall be imported into India unless accompained by a 'Phytosanitary Certificate', issued by the official Plant Quarantine Service of the source country.

3. All consignments of plants and seeds for sowing/propagation/planting purposes shall be imported into India through land customs station, seaport, airport at Amritsar, Bombay, Calcutta, Delhi and Madras, and such other entry points as may be specifically notified by the Central Government from time to time, where these shall be inspected and, if necessary, fumigated, disinfested/disinfected by authorised plant quarantine officials, before quarantine clearance.

4. Seeds/planting materials requiring isolation growing under detention, shall be grown in post-entry quarantine facility approved and certified by the Designated Inspection Authority (DIA) to conform to the conditions laid down by the Plant Protection Adviser to the Govt. of India.

5. Hay, straw or any other materials of plant origin shall not be used as packing material.

6. Import of soil, earth, sand, compost, and plant debris accompanying seeds/planting materials shall not be permitted. However, soil can be imported for research purposes under a special permit issued by the Plant Protection Adviser to the Govt. of India.

The DIP Act empowers the Central Government to make rules for regulating the import of seeds/planting materials into India and also the movement of the materials from one State to another within the country. The State Governments are also empowered to enact rules/regulations to regulate the movement of materials from one

region/area to another within a State.

The Directorate of Plant Protection, Quarantine & Storage, headed by the Plant Protection Adviser to the Govt. of India, is primarily responsible for enforcing the quarantine rules and regulations framed under the DIP Act in the country. For this purpose, plant quarantine and fumigation stations have been established at various international airports, seaports and land custom stations where the incoming consignments are inspected, fumigated or otherwise disinfested/disinfected before release to indentors. Consignments of plants/seeds for sowing/planting/propagation purposes, however, can only be imported through Amritsar, Bombay, Calcutta, Delhi and Madras quarantine stations, where facilities in respect to well equipped laboratories, quarantine green-houses and trained scientific and technical manpower are being strengthened to adequately meet the quarantine needs. Quarantine and fumigation stations under the Directorate of Plant Protection, Quarantine & Storage handle bulk imports for commerce and for planting.

Integrated Pest Management:

IPM is a sustainable approach to managing pest and diseases by combining the use of all practical methods of control including biological, cultural, physical and chemical methods, in a way that minimizes economic, health and environmental risks. This type of management programme to control plant diseases is called Integrated Disease Management i.e., IDM or Integrated Pest Management i.e., IPM.

Procedure of Integrated Plant Disease Management:

The procedure of management is different in respect of the principal component:

A. Management of Host:

1) It includes:Procedure to improve plant vigour.
2) Induction of disease resistance, and
3) Breeding for disease resistance.

B. Management of Pathogen:

It includes:

1) Eradication or reduction of inoculum.
2) Application of chemicals on plant surface, to discourage the pathogen.
3) Prevention through legislation (i.e., keeping the pathogen away from the host) by quarantine, etc.

C. Management of Environment:

It includes:

1) Crop management.
2) Soil management, and
3) Water management.

According to G. N. Agrios (1997), the main goals of an integrated plant disease control program are:

1. To eliminate or reduce the amount of initial inoculum.
2. To reduce the effectiveness of initial inoculum.
3. To increase the resistance of the host.
4. To delay the onset of disease, and
5. To slow down the secondary cycle.

Programmes of Integrated Plant Disease Management:

The programme of Integrated Plant Disease Control can be taken against a particular common disease, such as late blight disease of potato or against all diseases affecting a particular crop such, as Potato, Apple, Citrus etc.

Integrated Management in an Annual Crop:

The following programmes must be undertaken for annual plants like potato against different diseases like late blight (fungus), brown rot and ring rot (bacteria), leaf roll of potato (virus – potato virus-1), etc.:

(a) Stock tuber should be free from any disease i.e., a healthy tuber is needed.

(b) Field should be cleaned from previous years plant debris and tubers, if any, where patho•gen may

perpetuate for the next season.

(c) Crop rotation must be done with legume or any other crop plant, except Solanaceous members.

(d) Plant should be protected from disease through foliar spray at regular intervals.

(e) Make the soil loose by ploughing and expose it to sunlight for few days in summer months and/or fumigate the soil with chemicals.

A. Integrated Management in a Perennial Crop:

For perennial crop like orchard crop (peach, apple, pear etc.); following programme should be undertaken:

(a) Nursery-stock should be fumigated (to eradicate the nematode, if present).

(b) The stock should be fumigated (to eradicate the nematode, if present).

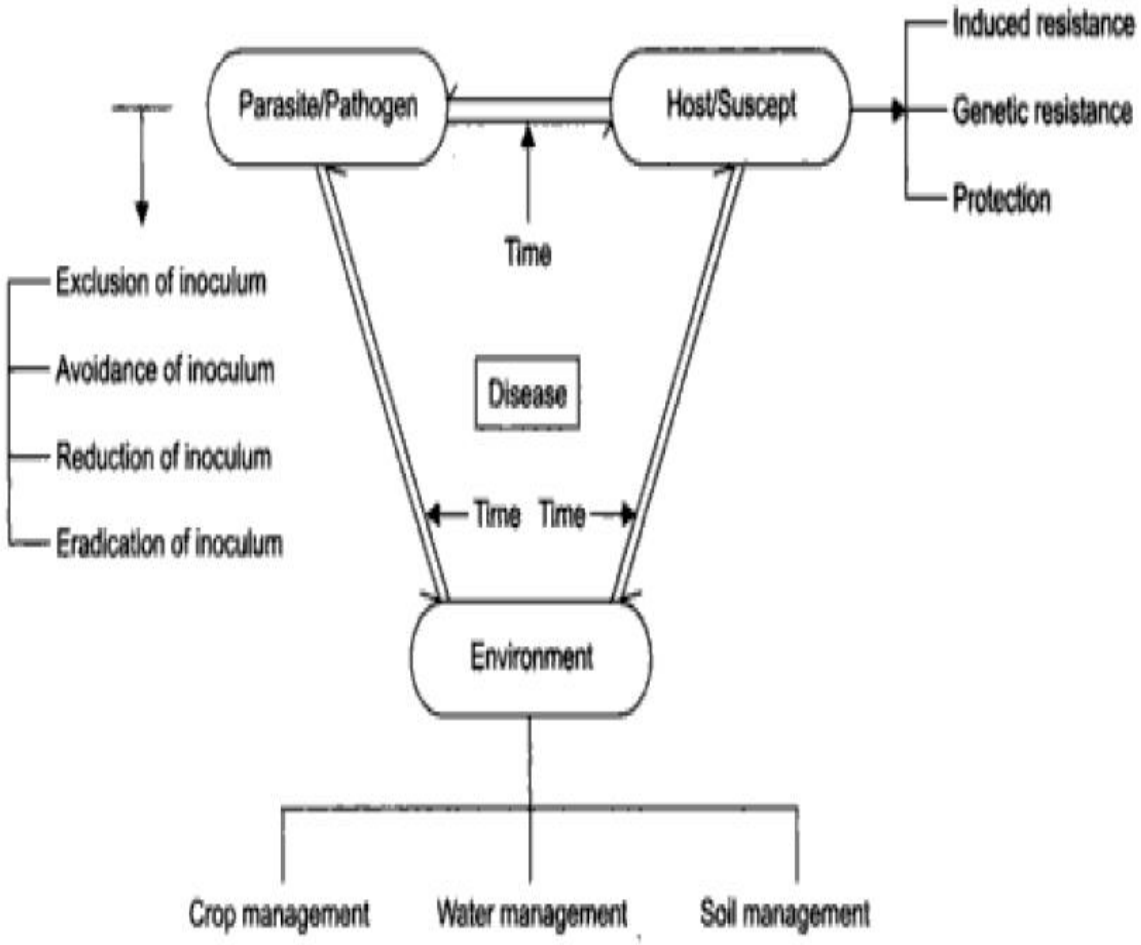
(c) The growing field should be fumigated before planting to eradicate any pathogen like *Armillaria*, *Phytophthora*, if present.

(d) Proper drainage in the field should be maintained.

(e) Stock should not grow in old field near the old plants that may carry different pathogens like canker, etc.

(f) The field should be properly irrigated, and supplemented by fertilisers.

(g) Plants should be sprayed regularly to protect them from different diseases and insect pests.



11. Fungicides: types and uses

Fungicides are biocidal chemical compounds or biological organisms used to kill parasitic fungi or their spores. A fungistatic inhibits their growth. Fungi can cause serious damage in agriculture, resulting in critical losses of yield, quality, and profit. Fungicides are used both in agriculture and to fight fungal infections in animals. Chemicals used to control oomycetes, which are not fungi, are also referred to as fungicides, as oomycetes use the same mechanisms as fungi to infect plants.[

Based on chemical nature: **inorganic** & **organic**

Based on time of application: **protectant** (use before infection) & **therapeutant** (use after infection)

Based on uptake by and mobility within plant tissue: **Systemic** & **non-systemic fungicides**

Bordeaux mixture

It is a mixture of copper sulphate: quick lime (or hydrated lime): water :: 8:8:100. Copper is the only ingredient in the Bordeaux mixture that is toxic to pathogens and, sometimes, to plants, whereas the role of lime is primarily that of a "safener." It controls many fungal (includingoomycete) and bacterial leaf spots, blights, anthracnoses, downy mildews, and cankers. Copper ion (Cu^{2+}) is toxic to all cells because it reacts with sulfhydryl (-SH) groups of certain amino acids and causes denaturation of proteins and enzymes.

Organic protectant fungicides

Thiram used as seed treatment, soil application to control damping off, seedling blights **Pentachloro nitrobenzene** used as a dip or in the furrow at planting time and selectively toxic to *Rhizoctonia, Sclerotium & Plasmodiophora*

Captan used as Leaf spots, blights, and fruit rots on fruit crops, vegetables, ornamentals. The reactive group of captan is –S-C(Cl)3 which interferes with the metabolic processes of sulfur containing enzymes and coenzymes

Systemic fungicides

Metalaxyl affect nucleic acid (rRNA) synthesis by inhibiting the activity of RNA polymerase I; block uridine incorporation into the RNA chain against Oomycetes, for control of damping off, downy mildew, late blight of potato

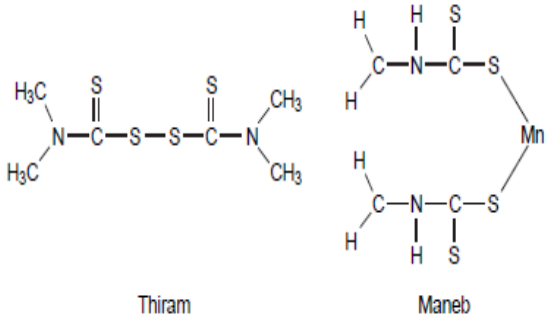
Carbendazim inhibits mitosis and cell division in target fungi. It interferes microtubule assembly by binding with β-tubulin. As a result non-disjunction or chromosome loss may occur causing death of the fungal pathogens

Plantvax & Vitavax are the first systemic fungicides to be discovered in 1966 for complete control of internally seed-borne infection of loose smut (*Ustilago nuda*) in barley. They cause dysfunction of succinate dehydrogenase (SDH) in the tricarboxylic cycle and mitochondrial electron transport chain, inhibiting the activity of Complex II and respiration in fungal cells **Fosetyl-Al** prevents the spore germination and penetration of pathogen into the plant through blocking its mycelial growth and spore production and improve the natural defense mechanism of the plant and induce phytoalexin production against Oomycetes

Edifenphos inhibit lipid biosynthesis in fungal cell membrane and inhibit chitin biosynthesis

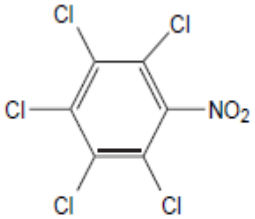
Morpholines & triazoles inhibit sterol biosynthesis

Fungicides are sold commercially as a mixture of active ingredient (that which kills the fungus) and other substances, (i.e., carriers, diluents, solvents, wetting agents, emulsifiers, etc.). The formulation indicates the portion of the product that is active ingredient and the physical form of the product.

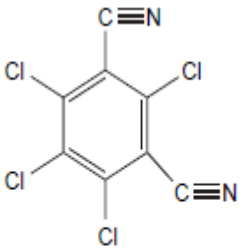


Thiram

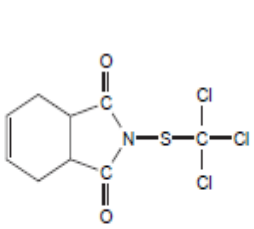
Maneb



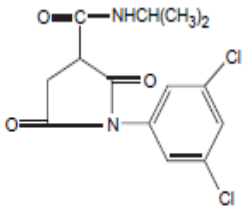
Pentachloro-nitrobenzene



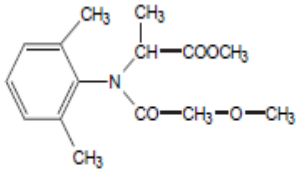
Chlorothalonil



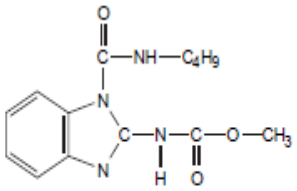
Captan



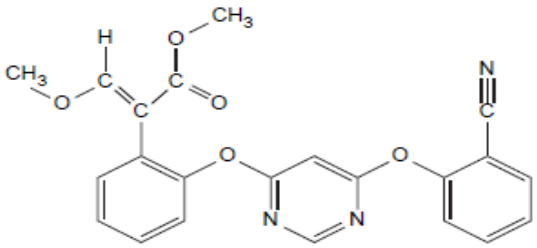
Iprodione



Metalaxyl



Benomyl



Azoxystrobin

Role of Fungicides in Disease Management:

Plant diseases are best managed by integrating a number of control practices that may include: crop rotation, selection of disease-tolerant or disease-resistant crop cultivars (cultivars genetically less susceptible than other cultivars), time of planting, level of fertilization, micro-climate modification, sanitation, and application of fungicides. Fungicides are often a vital part of disease management as (a) they control many diseases satisfactorily, (b) cultural practices often do not provide adequate disease control, (c) resistant cultivars are not available or not accepted in the marketplace for many diseases, and (d) certain high value crops have an extremely low tolerance for disease symptoms.

In contrast with most human medicines, most fungicides need to be applied before disease occurs or at the first appearance of symptoms to be effective. Unlike with many diseases of humans and animals, applying fungicides cannot heal symptoms already present, even if the pathogen is killed. This is because plants grow and develop differently than animals. Fungicides typically only protect new uninfected growth from disease. Few fungicides are effective against pathogens after they have infected a plant. Those that do have “curative” properties, which means they are active against pathogens that have already infected the plant, have limited ability to do so, often only being active on a pathogen within a few days of infection.

Many fungicides have targeted activity that imparts high efficacy against specific pathogens, which means low potential for toxicity to humans and other organisms, but also results in a high risk of pathogens developing resistance to the fungicide. A resistant pathogen is less sensitive to the action of the fungicide, which results in the fungicide being less effective or even ineffective. Fungicides that are designed to target specific enzymes or proteins made by fungi do not damage plant tissue, thus they can penetrate and move inside leaves enabling curative properties and increasing the amount of plant tissue protected to more than just where fungicide was deposit when applied. Since the mode of action of these fungicides is so specific, small genetic changes in fungi can overcome the effectiveness of these fungicides and pathogen populations can become resistant to future applications. Disease management strategies that rely heavily upon curative application of fungicides often lead to more resistance problems due to (a) the large size of the pathogen population when the application is made from which resistant individuals are being selected and (b) the difficultly in eradicating a pathogen entirely from inside the plant. Fungicide resistance is covered in more detail in a separate section.

Growers often use disease forecasting systems or action thresholds, when these are available, to ensure fungicides are applied when needed and to avoid the expense and possible environmental impact of unnecessary applications. Forecasting systems have been developed for a number of diseases based on an understanding of the environmental

conditions favorable for their development. Typically these are based on temperature and relative humidity or leaf wetness in the area where the crop is grown. Threshold-based fungicide programs involve routinely scouting the crop for symptoms, then applying fungicides when the amount of symptoms reaches a critical level beyond which the disease cannot be controlled adequately. An example of a critical level is one disease spot per five leaves examined. Knowledge of the disease cycle of the pathogen is important when developing and using forecasting systems and thresholds. Important aspects of the disease cycle include whether the disease is monocyclic (one generation per year) or polycyclic (multiple generations) and latent period (time between infection and production of new inoculum).

Economics often influence the choice of fungicide and application timing. Expensive fungicides and numerous applications are used on valuable plantings that might incur substantial economic loss in the absence of treatment, such as fruit trees and golf courses. Recognizing that with some diseases crop yield is not impacted when severity is low, an economic threshold is used to determine when fungicide treatment is needed. The crop tolerance level, or damage threshold, can vary depending upon the stage of the crop development when attacked, crop management practices, location and climatic conditions.

Application Methods

Fungicides are applied as dust, granules, gas, and, most commonly, liquid. They are applied to:

(a) Seed, bulbs, roots of transplants, and other propagative organs. These treatments are usually done by the seed company. Some treatments need to be done by the grower on-site at the time of planting. The goal is to kill pathogens that are on the planting material or to protect the young plant from pathogens in the soil.
(b) Soil either in-furrow at planting, after planting as a soil drench (including through drip irrigation), or as a directed spray around the base of the plant.
(c) Foliage and other aboveground parts of plants by means of a sprayer.
(d) Inside of trees via trunk injection.(f) Harvested produce, as a dip or spray in the packinghouse.
Fungicides are used as a formulated product consisting of an active ingredient plus inert ingredients that improve the performance of the product. Fungicides are typically mixed with water then applied by spraying. Application equipment ranges from small hand-held and back-pack sprayers to large spray units carried by tractors or aircraft (Figures 16-22). A few fungicides are applied as dusts. Fungicides can also be applied in greenhouses as smoke, mist, fog or aerosol. Coverage of all parts of the plant susceptible to the disease is critical because very few fungicides can move adequately throughout a plant. Advancements are continually being made to nozzles and sprayers to improve coverage (Figures 17 and 19).

For many diseases, effective control necessitates multiple applications of fungicides, sometimes as frequently as every 5 days. Repeated applications are needed to protect new growth and to replace fungicide lost from the plant by chemical decomposition, UV-light degradation, and erosion by wind and water.

Fungicide Resistance:

Fungicide resistance is a stable, heritable trait that results in a reduction in sensitivity to a fungicide by an individual fungus. This ability is obtained through evolutionary processes. Fungicides with single-site mode of action are at relatively high risk for resistance development compared to those with multi-side mode of action. Most fungicides being developed today have a single-site mode of action because this is associated with lower potential for negative impact on the environment, including non-target organisms.

When fungicide resistance results from modification of a single major gene, pathogen subpopulations are either sensitive or highly resistant to the pesticide. Resistance in this case is seen as complete loss of disease control that cannot be regained by using higher rates or more frequent fungicide applications. This type of resistance is commonly referred to as "**qualitative resistance**".

When fungicide resistance results from modification of several interacting genes, pathogen isolates exhibit a range in sensitivity to the fungicide depending on the number of gene changes. Variation in sensitivity within the population is continuous. Resistance in this case is seen as an erosion of disease control that can be regained by using higher rates or more frequent applications. Long-term selection for resistance in the pathogen by repeated applications may eventually result in the highest labeled rates and/or shortest application intervals not being able to adequately control the disease.

This type of fungicide resistance is commonly referred to as “quantitative resistance”. Comments about resistance risk of fungicides are included.
Fungal isolates that are resistant to one fungicide are often also resistant to other closely-related fungicides, even when they have not been exposed to these other fungicides, because these fungicides all have similar mode of action. This is called cross resistance. Fungicides with the same Group Code are likely to exhibit cross resistance. Occasionally negative cross resistance occurs between unrelated fungicides because the genetic change that confers resistance to one fungicide makes the resistant isolate more sensitive to another fungicide.

Managing fungicide resistance is critically important to extend the period of time that an at-risk fungicide is effective. The primary goal of resistance management is to delay its development rather than to manage resistant fungal strains after they have been selected. Therefore, resistance management programs need to be implemented when at-risk fungicides first become available for commercial use. The objective of resistance management is to minimize use of the at-risk fungicide without sacrificing disease control. This is accomplished by using the at-risk fungicide with other fungicides and with non-chemical control measures, such as disease resistant cultivars, in an integrated disease management program.

It is critical to use an effective disease management program to delay the build-up of resistant strains. At-risk fungicides should be used at the manufacturer’s recommended rate (full rate) and application interval. Using full rates is expected to minimize selection of strains with intermediate fungicide sensitivity when resistance involves several genes (quantitative resistance). At-risk fungicides should be used in alternation with other at-risk fungicides with different modes of action or different chemical groups, and they should be combined or alternated with fungicides that have a low resistance risk.

When one crop could serve as a source of inoculum for a subsequent crop, the alternation scheme among at-risk fungicides should be continued between successive crops such that the first at-risk fungicide applied to a crop belongs to a different cross-resistance group than the last at-risk fungicide applied to the previous crop. Some at-risk fungicides are formulated as premix products with other fungicides to manage resistance. At-risk fungicides should be used only when needed most. The most critical time to use them for resistance management is early in an epidemic when the pathogen population is small. Multi-site contact fungicides should be used alone late in the growing season, where they have been shown to provide sufficient disease control to protect yield. Another important component of resistance management is assessing disease control and reporting any loss of efficacy potentially due to resistance.

To promote resistance management, companies registering fungicides are voluntarily putting on the labels guidelines developed recently by EPA through a joint effort with the Canadian Pest Management Regulatory Agency (PMRA) under the North American Free Trade Agreement (NAFTA).

12. Study of some plant diseases with reference to symptoms, etiology and control measures

Fungal diseases:

Wart disease of potato

Wart disease of potato also known as Black wart of potato, was first described in 1895 from Hungary. In India, the disease was first reported from Darjeeling by Ganguly and Paul (1953) and continues to be restricted to that area.

Symptoms of Wart Disease:

The symptoms of the disease appear only on underground parts except roots of the plant i.e. tubers, buds of stems, and stolons. These are characterised by warty, tuberous and dirty Cauliflower like outgrowths on infected parts.

The warty outgrowths vary in size from small

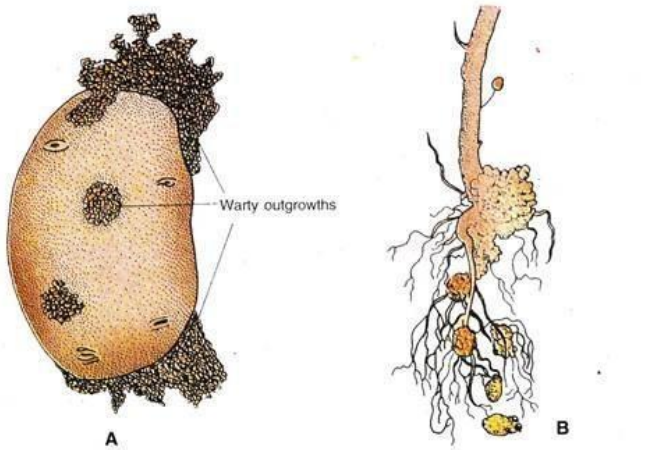


Fig. 22.1. Symptoms of wart disease of potato on a potato tuber (A) and underground parts of a plant (B).

protuberances to large intricately branched systems. Early in the growing season they are green or greenish-white in colour if exposed to light but are cream coloured or black on underground parts.
On the tubers, mostly the warts are larger than the tuber itself covering the whole tuber. In advanced stages, the warts become dark black in colour and may sometimes be attacked by saprophytic fungi. The wart usually consists of distorted proliferated branched structures grown together into a mass of hypertrophied tissue,

Causal Organism:

The causal organism is *Synchytrium endobioticum* (Schilb.) Peres.- a holocarpic endoparasite.

Disease Cycle:

The primary infection of the young potato crops available in the field takes place through the germination of resting spores present in the soil into Zoospores.

These zoospores after swimming for a while land on the underground shoot parts, withdraw their flagella and enter the host cells where they settle down at the bottom of the host cells.

Each of the neighbouring cells start dividing repeatedly followed by swelling, ultimately resulting in the formation of wart. There warts are the main Symptoms and contain the material for secondary infection. The sporangia contained in the warts produce zoospores upon disintegration of the hosts.

These zoospores are responsible for secondary infection. If environmental conditions are favourable, the zoospores are formed repeatedly and secondary infections may take place as well causing serious damage to the potato crop.

When the weather conditions become unfavourable and the crop season approaches towards the end, the zoospores function as planogametes, fuse and produce resting sporangia. While the crop is harvested, the resting sporangia remain buried in the soil and perennate waiting for the favourable conditions to return back.

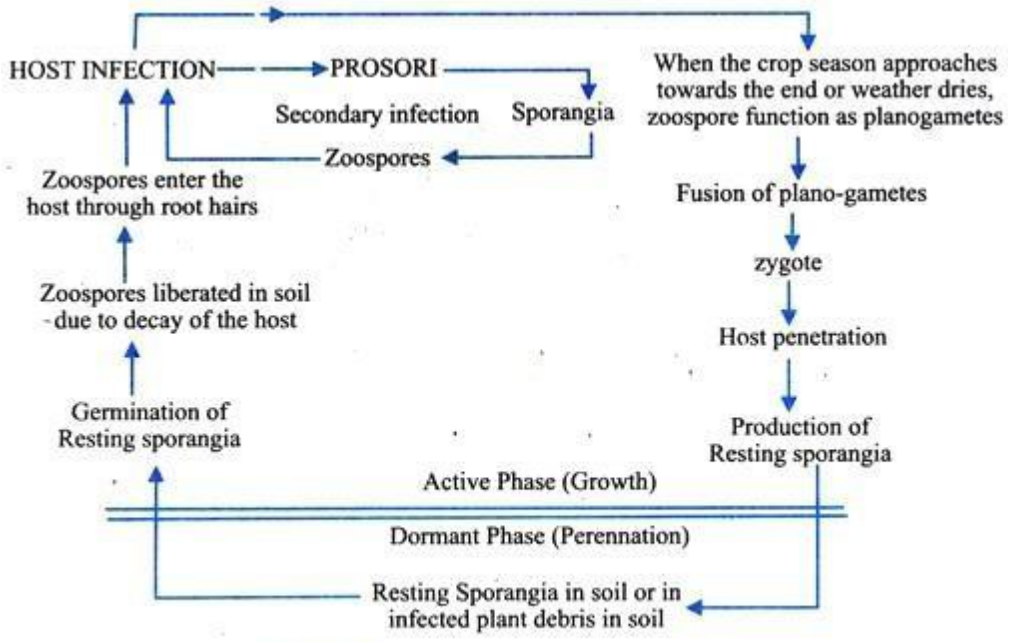


Fig. 22.2. Disease cycle of wart disease of Potato.

Control Measures of Wart Disease:

It is very difficult to control the disease once it has been introduced in the field. However, some of the control measures practised are listed below:

(i) Entry of diseased material into healthy areas should be prevented.

(ii) The diseased potato tubers should be discarded.

(iii) Soil treatment may control the disease to a large extent. These include steam sterilisation and application of mercuric chloride—copper sulphate and 5 percent formaline. But these are very costly.

(iv) Cultivation of disease resistant varieties continuously for 8-10 years is the only effective control measure.

Early blight of Potato

Early blight is a common foliage disease of potato and tomato. According to Mathur, Singh and Nagarkote (1971), there have been outbreaks of early blight of potato during the past-5 or 6 years in the plains of Uttar Pradesh. The pathogen causes injury to the leaves as a leaf spot disease and instigates permature defoliation. Finally it incites tuber rot of potato and fruit rot of tomato.

Symptoms of Early Blight Disease:

The disease appears on the leaflets, 3-4 weeks after the crop is sown as small, isolated, scattered pale brown to dark spots, oval or angular in shape mostly up to 3 or 4 mm in diameter. Each spot is usually delimited by a narrow chlorotic marginal zone which fades into the normal green The chlorotic zone increases with the increase in size of the spot. As a rule the oldest (lowest) leaves are affected first and the disease progresses upwards.

The necrotic tissue of the spot often shows a series of concentric ridges which produce a target- board effect, a symptom characteristic of this disease. The number of spots on the leaflets may be a few but if the conditions are favourable the spots increase in number and size involving the entire leaf surface.

Under humid conditions, the diseased areas coalesce and big rotting patches appear on the leaf surface. In severe cases of infection the leaves dry up, shrivel and drop off. Falling of leaves starts with the older (lower) ones until a few remain at the top.

Sometimes there is complete defoliation. Stems and petioles may also develop brown to dark lesions which may finally lead to either worthless plants or collapse of the entire over-ground portion of the plant.

Folsom and Bonds (1925) reported infection and rotting of potato tubers. The surface lesions on the potato tubers are a little darker than the healthy skin and slightly sunken.

Causal Organism:

The pathogen causing early blight of potato and tomato is a form-species *Altemana solani* (Ell. and Martin) Jones and Grout of form class Deuteromycetes.

The mycelium consists of light brown, slender, septate sparsely branched hyphae which become dark-coloured with age.

The conidiophores which are relatively short (50- 90 µ long and 9 µ broad) and dark-coloured arise from the older diseased tissue of the host and emerge through the stomata. There are 5-10 transverse septa and a few longitudinal ones. Each conidium develops from a bud formed on the terminal cell of the conidiophore.

Disease Cycle:

The mycelium and conidia of the pathogen remain viable for a considerable time, the former for about a year or more in the infected dry leaves and the latter for 17 months at room temperature.

The source of primary inoculum is the infected plant debris such as the dried leaves, stems, potato tubers and contaminated tomato seeds. Primary infection may be brought about by conidia

(A) or mycelium from the infected debris in the soil.

According to Walker (1969), the conidia germinate (B) at the optimum temperature of 28° to 30°C within 35 to 45 minutes. The germ tubes gain entry into the lower leaves of the host plant through stomata. Walker (1969) reported that the fungus penetrates the host leaf and stem directly through the epidermis (C). The incubation period varies from 48 to 72 hours. The primary infection usually appears on the foliage as leaf spots within 2 or 3 days under favourable conditions of temperature and moisture (D).

Secondary spread immediately follows through conidia produced on the primary spots within 5-7 days after infection. Conidia start forming when the spots are about 3 mm in diameter. Rand (1917) reported that heavy dew with rains now and then promote abundant sporulation.

The mature conidia are readily detached and dispersed through the agency of air currents, water and insects. Flees, beetles and Colorado bettles are reported to assist infection by carrying conidia on their bodies and facilitate penetration of germ tubes by inflicting wounds on the host surface.

Control Measures of Early Blight Disease:

As in the case of other soil borne diseases, crop rotation and field sanitation are the two rational measures which provide effective check against primary infection from spores in the infected plant debris. Infected dry leaves and dead haulms should be raked together and burned immediately after harvest.

Application of regular spray schedule is another effective measure. When the plants are 15- 20 cm high, weekly sprays (with Bordeaux mixture or other suitable fungicides) throughout the period of plant growth effectively control the early blight. Mathur, Singh and Nagarkote (1971) found that spraying potato crop with Dithane M-45 was most effective against early blight disease.

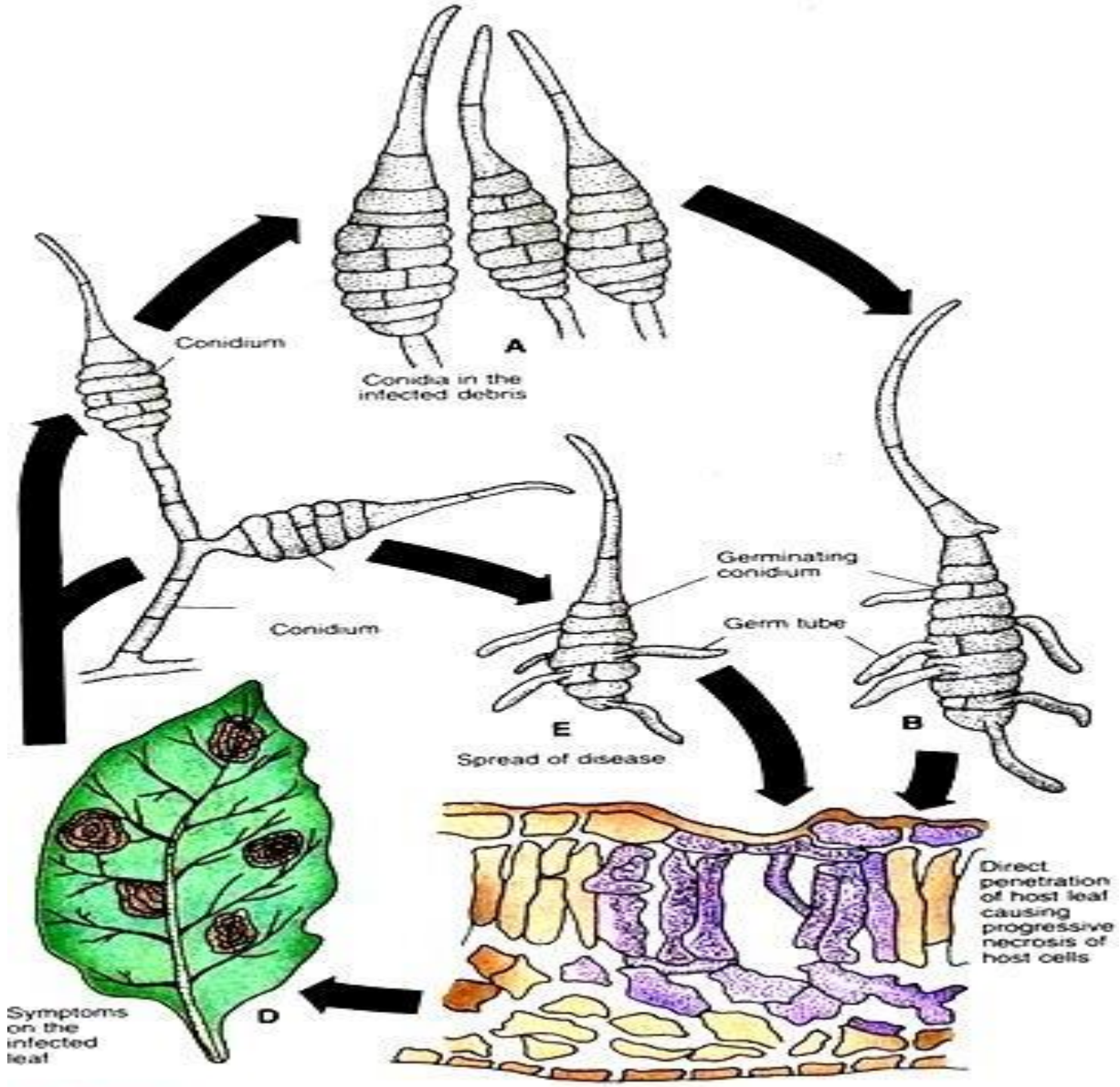


Fig. 22.23 (A–B). Early blight of potato—Pictorial disease cycle of *Alternaria solani*

Downy Mildew of Grape:

The best information available indicates that the downy mildew of grape is endemic in North America, where it was for the first time reported by Schweinitz in 1837.

The disease is confined largely to species and varieties of grapes (*Vitis*) although it has been reported also on the five-leaved ivy (*Parthertocissus quinquefolia* Planch.), and on English ivy (*P. tricuspidata* Planch.).

Symptoms of Downy Mildew of Grape:

The disease attacks all green parts of the plant leaf blades, petioles, tendrils, green shoots, and fruits at different stages of development. Early symptoms of the disease on the leaves consist of round light-green spots of an oily appearance on the upper surface, which enlarge even 1 /2 cm or more in diameter. In moist weather it persists; in extremely dry weather it may disappear. Later the spots become yellow, or variegated with tints of yellow and yellowish-brown forming patches of irregular shape.

On the stems, the lesions are brown and sunken, and along with the death of the affected parts, portions of the wine become brittle and break off easily.

Flowers may be completely blighted by early attacks of the disease. Fruits may be attacked when young or when approaching maturity. The young fruits show brownish spots and later become covered with downy mildew, their growth is checked. They may also assume a reddish- brown colour and failing to ripen, develop a soft root.

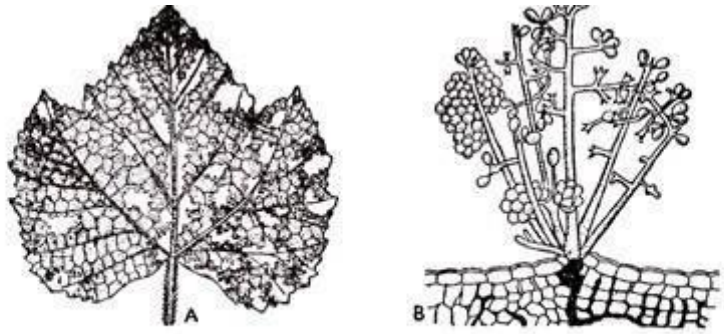


Fig. 361. Downy mildew of grape A. A diseased leaf. B. Section of a diseased leaf with sporangiophores emerging from a stoma.

Causal Organism of Downy Mildew of Grape:

Plasmopara viticola (Berk, and Curt.) Berl. The characteristic aseptate, intercellular mycelium produces knob-like haustoria to absorb food from the host cells. Fasciculate- sporangiophores arise from the hyphae in the intercellular spaces just beneath the lower epidermis and emerge through the stomata.

The oospores are produced from the mycelium embedded within the leaf tissue. The oospore consists of a thick endospore wall surrounded by a thinner rough exposure wall.

Disease Cycle of Downy Mildew of Grape:

In general, the sporangia are not adopted for long survival but serve to spread the disease in localities where the leaves may remain on the vines all the year round. In such cases infection by sporangia may be continuous from season to season. On the other hand, the oospores are capable of survival in the soil for at least a year.

Oospores which remain in the fallen leaves, vine debris, or shrivelled fruits on the ground are probably responsible for the primary infections which break out in the spring. Moreover the fungus may, in some localities, survive in the form of mycelium perennating in the winter buds.

The oospores germinate at a temperature range of 13°C to 33 °C. Primary infections are believed to occur when the zoospores formed during oospore germination, are conveyed by splashing raindrops from the soils on to the lowermost leaves of the vines.

It may so happen that the sporangia produced during oospore germination may themselves be carried on the

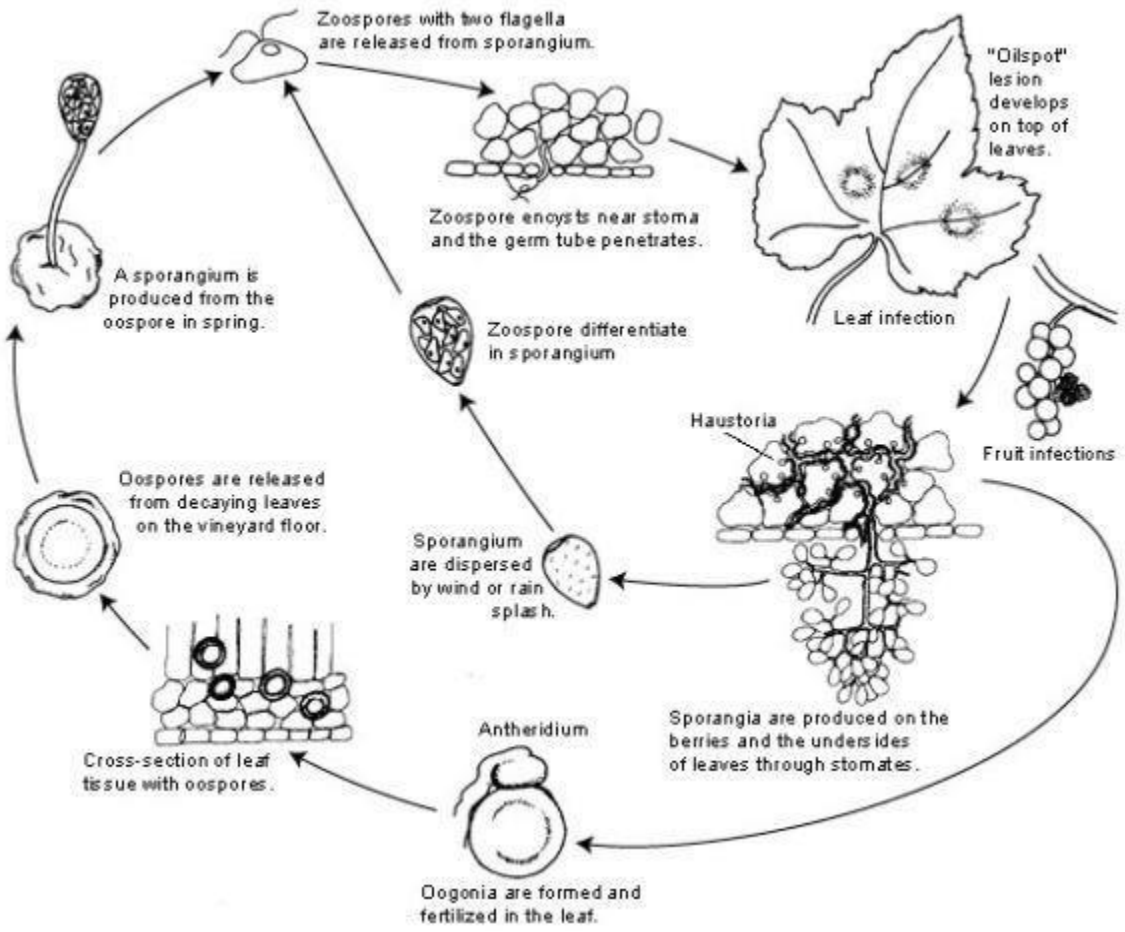
leaves near the ground and then zoospores are produced there.

Host petetration of the pathogen takes place through the stomata. The mycelium invades the intercellular spaces, ramifies in the host tissue deriving its nutrients in a large measure through haustoria which penetrate the cell lumen. In almost all cases, infection of the leaves takes place through the lower surface.

The optimum temperature for the development of the disease is 18°C to 24°C. Prolonged warm wet weather is conducive to an epidemic of the disease.

They also serve as the source of secondary inocula of the disease. Through the secondary inocula, secondary infections are induced resulting in secondary cycles.

Disease cycle of Downy mildew of grape is very similar to that of Downy mildew of pea, except that the sporangia germinate by secondary zoospores.



Control of Downy Mildew of Grape:

The chief methods of control of the disease are as follows:

(i) Sanitation:

Destruction of old leaves is recommended to eradicate the source of inoculum since the causal organism of the disease usually hibernates as oospores in the fallen leaves.

(ii) Spraying of Bordeaux Mixture:

Bordeaux mixture, 5-5-50 strength is usually recommended as a protective spray to the susceptible parts; but even up

to 8-8-50 is often recommended for epiphytotic conditions. Sporangia or zoospores absorb copper until a toxic limit is reached, the more epiphytotic the disease, the stronger must be the spray to be effective.

(iii) Spraying of Other Fungicides:

The spread of the disease can be effec•tively controlled by spraying the vines with 0.3 per cent. Blitox-50, Dithane Z-78, Ferbam or Captan first when the shoots are 6 to 8 inches high, again when they are about to flower, and finally when the fruits are just about to change their colour.

(iv) Improved Cultivation Practices:

Certain improved methods of cultivation are helpful to control the disease incidence. Vineyards should be sufficiently open to allow the foliage to dry quickly after rains. This method not only ensures free access of air but facilitates spraying as well. The risk of infection from resting oospores is reduced.

(v) Use of Resistant Varieties:

The use of resistant varieties is of course a very useful practice. But even with resistant vines, spraying with Bordeaux mixture is advisable.

Powdery mildew of rose:

Causal Agent: The fungus, *Sphaerotheca pannosa* var. rosae

Hosts: Rose

Symptoms: Powdery mildew produces a powdery, whitish coating on the leaves and other plant parts. In case of severe attack, plants may become stunted, leaves may curl, become dried, and drop. Unopened buds, young stems, and thorns may be entirely overgrown with the powdery coating. Only in very rare cases does death of the plant result from infection. Generally, the most favorable conditions for powdery mildew infection are as follows: daytime temperature near 80F (27C) and a relative humidity of 97-100%. These conditions prevail during most of the early part of the growing season, thus control must be made on a regular basis.

Control: During seasons of low rainfall in the spring and very early summer when the daytime temperatures are below 80F, control may not be needed until later. Thoroughly applied fungicides can effectively control powdery mildew. To avoid having to spray for powdery mildew control, resistant cultivars can be grown. Please contact your local county extension office for current information.

White Rust of Crucifers:

White rust or white blisters disease is one of the common diseases of crucifer crops. It is worldwide in distribution occurring in all the areas wherever crop is cultivated. Both wild and cultivated varieties are attacked.

Symptoms of White Rust Disease:

The disease affects all the aerial parts of the plant, the roots are not attacked. Symptoms may appear as a result of two types of infection: Local and Systemic. In case of local infection, isolated spots or pustules appear on leaves or stems or inflorescence. The pustules are of variable size, measuring 1 -2 mm in diameter and are raised shiny white areas.These may arise in close proximity and coalesce to form large irregular patches.

In systemic infections, young stems and inflorescence are infected. The fungus becomes systemic in these

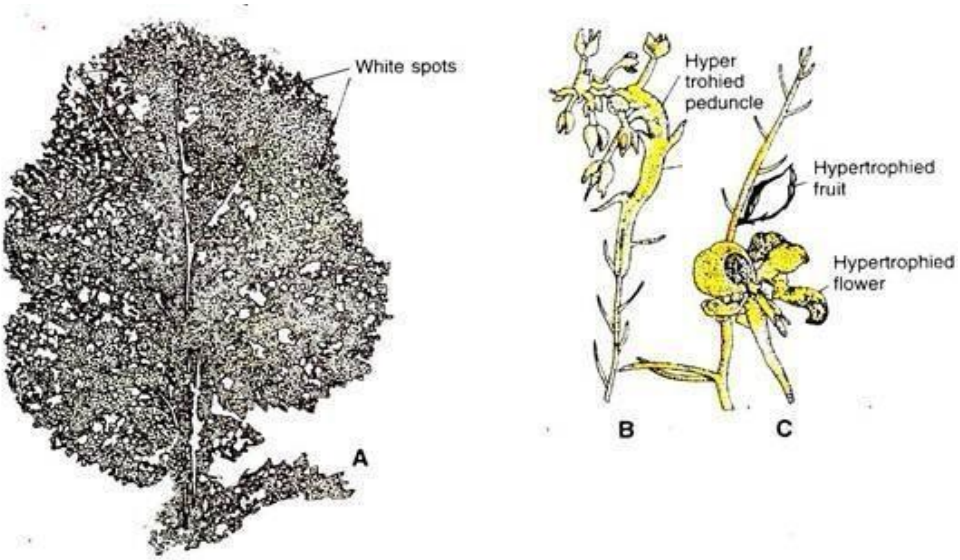


Fig. 22.3. Symptoms of white rust disease on leaves (A) and inflorescence (B and C).

parts and the affected tissues are stimulated to various types of deformities.

The peduncle and pedicel may become enormously thickened upto 12-15 times, the normal diameter. Floral parts become fleshy, swollen, green or violet in colour, the stamens falling off early.

Causal Organism:

The causal organism *Albugo Candida* (Lev.) Kunze or Cystopus candidus Lev. is an obligate parasite.

Disease Cycle:

The primary infection occurs due to oospores perennating in the soil or due to mycelium perennating on perennial hosts. These serve as primary inoculum when the environmental conditions are favourable.

Oospores germinate in presence of water to form a vesicle in which a large number of zoospores are formed. These zoospores swim in a film of water and land on the suitable host, germinate by germ tubes, enter the host and establish infection. The mycelium in the host is intercellular with globose haustoria. Conidiophores, which are clavate, and formed at the tip of hyphae, begin to produce conidiosporangia in basipetal succession.

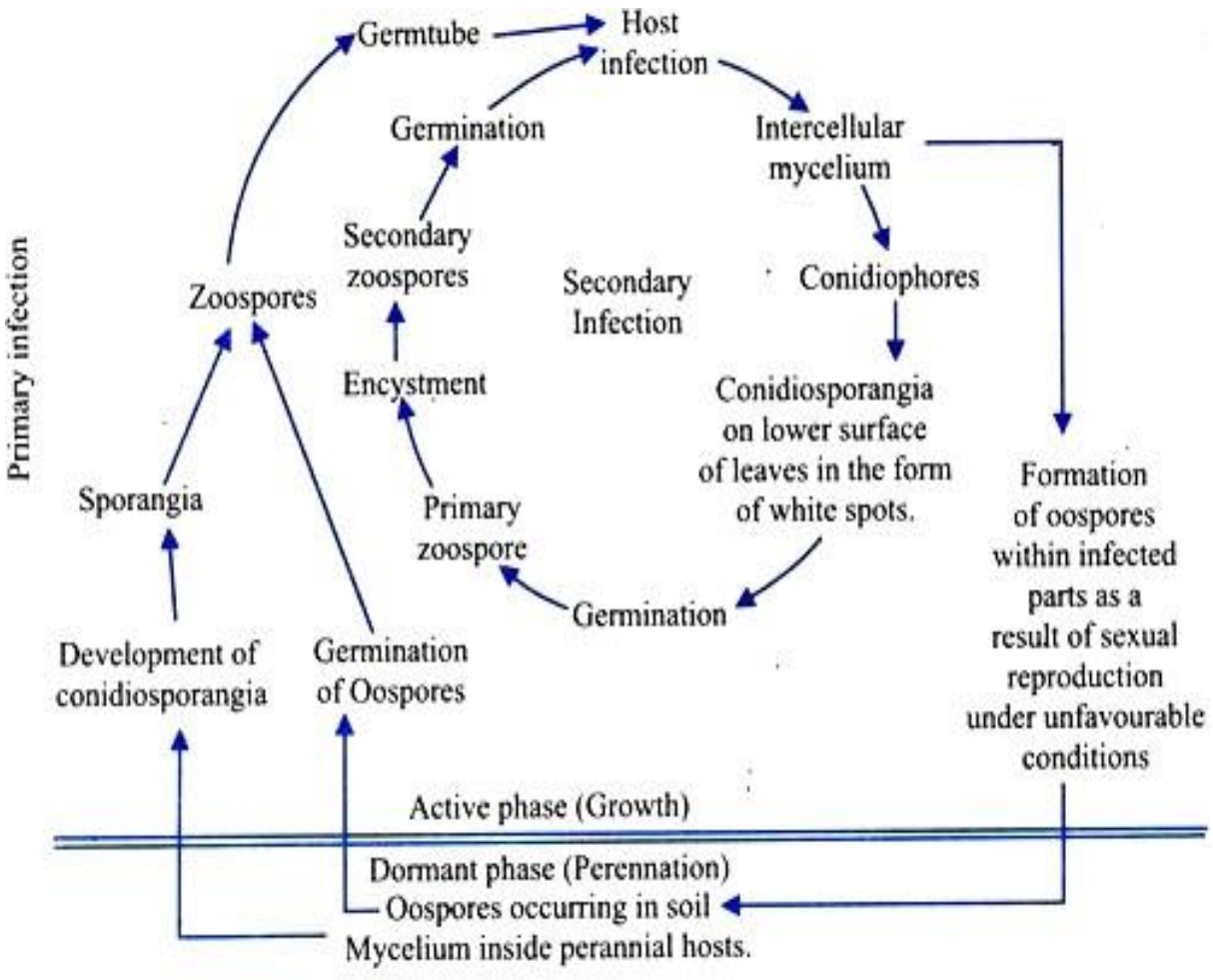


Fig. 22.4. White rust of crucifers–Disease cycle.

The conidiosporangia produced during early phase of the growing season cause secondary infection in the host. These are blown away by wind or any other agency, land on the host surface and germinate to form zoospores.

The zoospores germinate by formation of germ tubes which enter the host and cause secondary infection. If the

conditions are favourable, this is repeated.

Control Measures of White Rust Disease:
The disease may be controlled by the following methods:

(i) Clean cultivation and destruction of weed should be practised.

(ii) Crop rotation will avoid the soil borne primary inoculum.

(iii) Spraying with 0.8 percent Bordeaux mixture or Dithane M-45 (0.2%) may be undertaken to check the spread of the disease.

(iv) Disease resistant varieties be preferred.

Leaf Curl of Peaches Symptoms of the Disease:
Symptoms first appear in early spring when the leaves come out of the bud. Some of the leaves appear twisted, puckered, thickened, curled downwards and often greatly distorted. Affected leaves are reddish or purplish in the beginning, later turning to reddish yellow or yellowish grey and finally to yellow to brown.

Affected diseased leaves fall off prematurely and in serious infections, tree become naked due to premature defoliation of leaves in the early spring itself. Blossoms, young fruits and current year's twigs also get affected and diseased and fall off prematurely.

Causal Organism:
This disease is caused by *Taphrina deformans* (Berk) Tulasne. The fungus is characterised by intercellular sub-cuticular mycelium, absence of fruit body and the asci produced naked on the host surface.

Disease Cycle:
Primary infection takes place through ascospores or blastospores or conidia which are present in the soil of the orchards. Due to rains, these ascospores or blastospores or conidia are washed, splashed or blown onto young tissues where they germinate and cause infection.

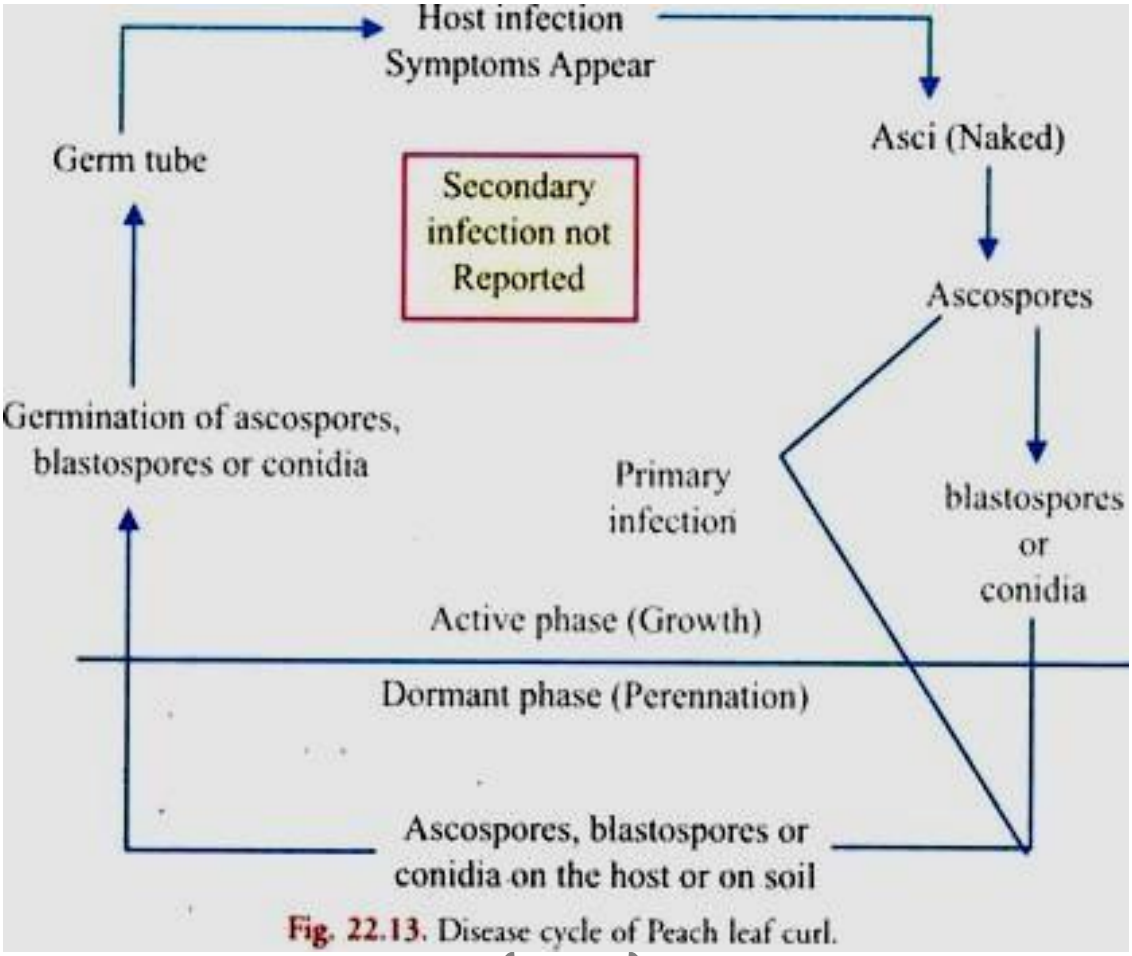


Fig. 22.13. Disease cycle of Peach leaf curl.

At the time of penetration the, germ tube becomes binucleate due to mitotic nuclear division. The binucleate nucleus upon entering the host tissue forms the binucleate mycelium. After the establishment of the infection inside the host, disease develops and symptoms become apparent.

Soon, the asci and ascospores are formed on host surface. Ascospores upon release usually germinate to produce blastospores or conidia.

Secondary infection has not been reported as the fungus can infect only the younger tissues. Older tissues generally become resistant to infection.

Control Measures of the Disease:

(i) Sanitation of the trees and orchards is important in controlling the disease.

(ii) The tree must not have diseased leaves. Therefore, as soon as the disease leaf is noticed, it should be plucked and burnt.

(iii) Fallen infected leaves should be collected and burnt.

(iv) Spraying with lime sulphur or copper fungicides helps in controlling the disease. Among the fungicides, Bordeaux mixture (4: 4: 50), Perenox or Fytolan (0.3%) Blitox-50 (0.25%) are commonly used. One spraying in autumn and another just before opening of the buds considerably reduce the incidence of the disease.

(v) Spraying with Cuman five times a year brings about total control of the disease.

Rust of Linseed:

Rust of linseed is a common disease of flax growing areas of the world. In India flax is a major oilseed crop cultivated in almost all the states. The disease generally appears in February or later but Butler (1918) has observed the disease in central India in early November.

Symptoms of the Rust of Linseed:

The leaves are the first to show the symptoms and gradually all the aerial parts of the plant get infected. Large, orange coloured pustules generally appear on the leaves. Small pustules are initially surrounded by chlorotic areas. Little necrosis of the leaves is at first observed but it grows, becomes more general and the leaves prematurely die.

The pustules on the leaves are uredopustules containing uredospores. Uredopustules may also appear on stems. While the uredopustules on leaves are round and small, those on stems are elongated and irregular.

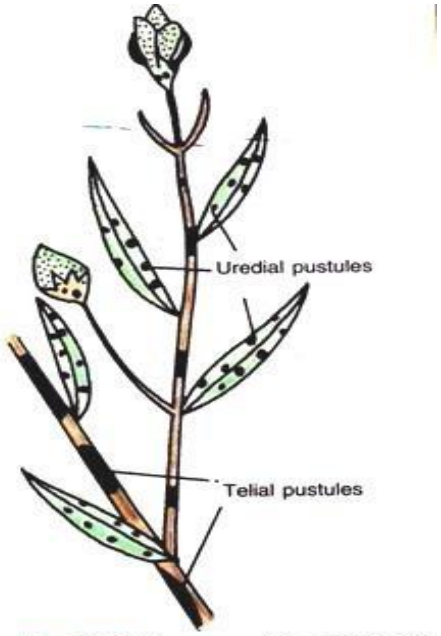


Fig. 22.18. Symptoms of Rust of Linseed.

Causal Organism:

The causal organism is *Melampsora lini* (Pers.) Lev.-an autoecious rust.

Disease Cycle:

In temperate countries, primary infection takes place through basidiospores which are produced as a result of germination of teleutospores perennating in the soil. But in tropical countries, the teleutospores, produced at the end of growing season i.e. April-May, lose their viability due to excessive heat of summers. Uredospores too are killed due to excessive temperatures. It is presumed that the uredospores produced on linseed at hills come down to plains to cause infection. Thus the primary inoculum, windblown, fall on the host, germinate and cause infection.

Because the transportation of uredospores from hills takes some time, the outbreak of the disease in plains takes place only after 2-3 months of the sowing of the crop.Uredospores produced as a result of primary infection cause secondary infection. Teleutospores produced at the end of the growing season appear to have no role to play in the continuance of the life cycle of the rust.

Control Measures of the Rust of Linseed:

(i) Use of disease resistant vars. like NP (RR) 9, 10, 56, 95, 218, 279B 279K3, 368, 381, 389, 415 and 501 is the only effective method to control the disease.

(ii) Seed treatment to kill the teleutospores in hills has also been suggested.

(iii) Avoidance of excessive nitrogenous manures is recommended.

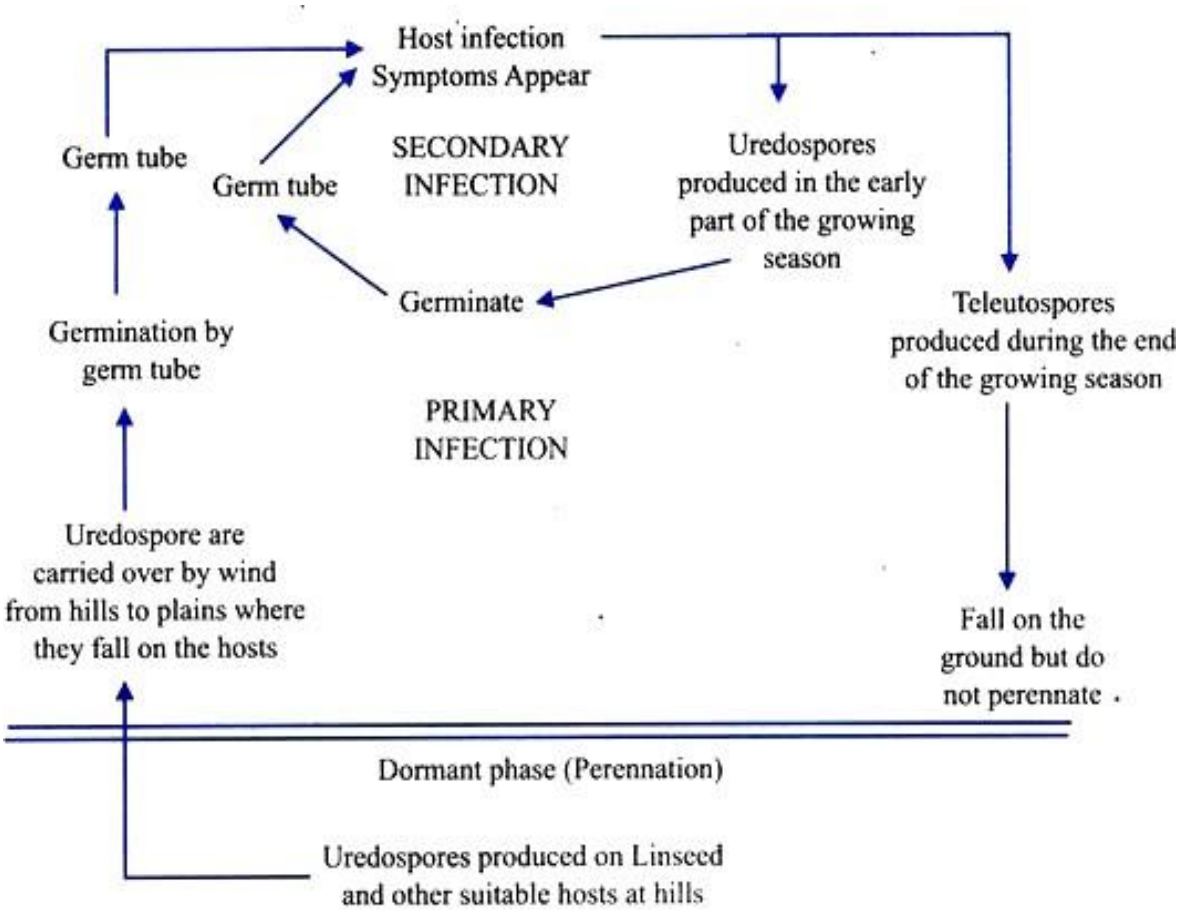


Fig. 22.19. Disease cycle of Rust of Linseed.

Sugarcane smut:

Sugarcane smut is a fungal disease of sugarcane caused by the fungus ***Sporisorium scitamineum***. The disease is known as culmicolous, which describes the outgrowth of fungus of the stalk on the cane. It attacks several sugarcane species and has been reported to occur on a few other grass species as well, but not to a critical amount. The most recognizable characteristic of this disease is a black or gray growth that is referred to as a "smut whip"

Symptoms:

Two to four months after the fungus has infected the plant, black whip-like structures, instead of a spindle leaf, emerge from the meristem, or growing point, of the plant. The developing whip is a mixture of plant tissue and fungal tissue. The whip reaches maturity between the sixth and the seventh month. When spores that are contained inside the whip are released, the core of the whip remains behind and is a straw-like color.

Plants infected with the fungus usually appear to have thin stalks and are often stunted. They end up tillering much more than normal and this result in leaves that are more slender and much weaker. They sometimes appear more grass-like than non-infected plants. Less common symptoms of the disease are stem or leaf galls and proliferating buds.

Disease cycle:
Sugarcane smut is disseminated via teliospores that are produced in the smut whip. These teliospores located either in the soil or on the plant, germinate in the presence of water. After germination they produce promycelium and undergo meiosis to create four haploid sporidia. Sugarcane smut is bipolar and therefore produces two different mating types of sporida. For infection to occur, two sporida from different mating types must come together and form a dikaryon. This dikaryon then produces hyphae that penetrate the bud scales of the sugarcane plant and infect the meristematic tissue. The fungus grows within the meristematic tissue and induces formation of flowering structures which it colonises to produce its teliopores. The flowering structures, usually typical grass arrows, are transformed into a whip like sorus that grows out between the leaf sheaths. At first it is covered by a thin silvery peridium (this is the host tissue) which easily peels back when desiccated to expose the sooty black-brown teliospores. These teliospores are then dispersed via wind and the cycle continues.

Control:

The management of sugarcane smut is done through the use of resistant cultivars, fungicide and using disease free planting stock. Control is mainly accomplished through the use of resistant cultivars in areas where the disease is present. Fungicides also are used in the control of this disease, but typically resistant cultivars are preferred due to the cost of fungicides. In areas where this disease is not yet found it is important to use disease-free planting stock so as not to introduce the pathogen. Important regulations are sometimes implemented by governments to help prevent the spread of the disease. Quarantines are also implemented in areas that are infected.

Red Rot of Sugarcane:
It is a serious and destructive disease of sugarcane. It occurs in tropical and subtropical regions of the world where sugarcane is cultivated extensively. In India, it occurs in most of the sugarcane growing states particularly Bihar, Uttar Pradesh, Madhya Pradesh, Haryana and Punjab.

Symptoms of Red Rot Disease:
The symptoms of the disease become apparent after the rainy season on the stems (B) and leaves (C). The earliest symptoms are the yellowing and drooping of the upper leaves (near the tip).

In the later stages the stems shrivel, the rind loses its bright colour, becomes dull in appearance and longitudinally wrinkled. If the diseased stems are split open lengthwise the white pith will be found reddened with patches of white running transversely across.

At the advanced stage of disease blood red lesions with dark margins develop on the mid ribs of the host leaves (B). With the formation of asexual fruit bodies (acervuli), these lesions become covered with powdery masses of conidia which serve to spread the disease.

Causal Organism:
The causal agent of the red rot disease of sugarcane is the form-species *Colletotrichum falcatum* Went, of form-class Fungi Imperfecti. The perfect or sexual stage of the fungus has also been found by Arx and Muller. It is described in the Ascomycetcs as *Glomerella tucumanensis*.

Disease Cycle:
The incitant lives from one growing season to the next on the debris of the diseased plants. The conidia are short-lived and thus play no role in the perennation of the pathogen, the thick-walled chlamydospores and perithecia are considered as probable means of survival.

Chona (1950) and later Chona and Nariani (1952) reported that the fungus is capable of growing and producing acervuli in the soil. It thus survives in the active stage for 3 or 4 months. The survival of the mycelium for this limited period is sufficient to provide easy catching of the succeeding crop because sugarcane practically has no dead season.

The conidia serve as a secondary means of infection and spread of the disease. They get detached and are dispersed through the agency of wind, water and insects. On reaching the surface of the healthy sugarcane plants, they germinate immediately in the moisture retained in the enclosing sheaths.

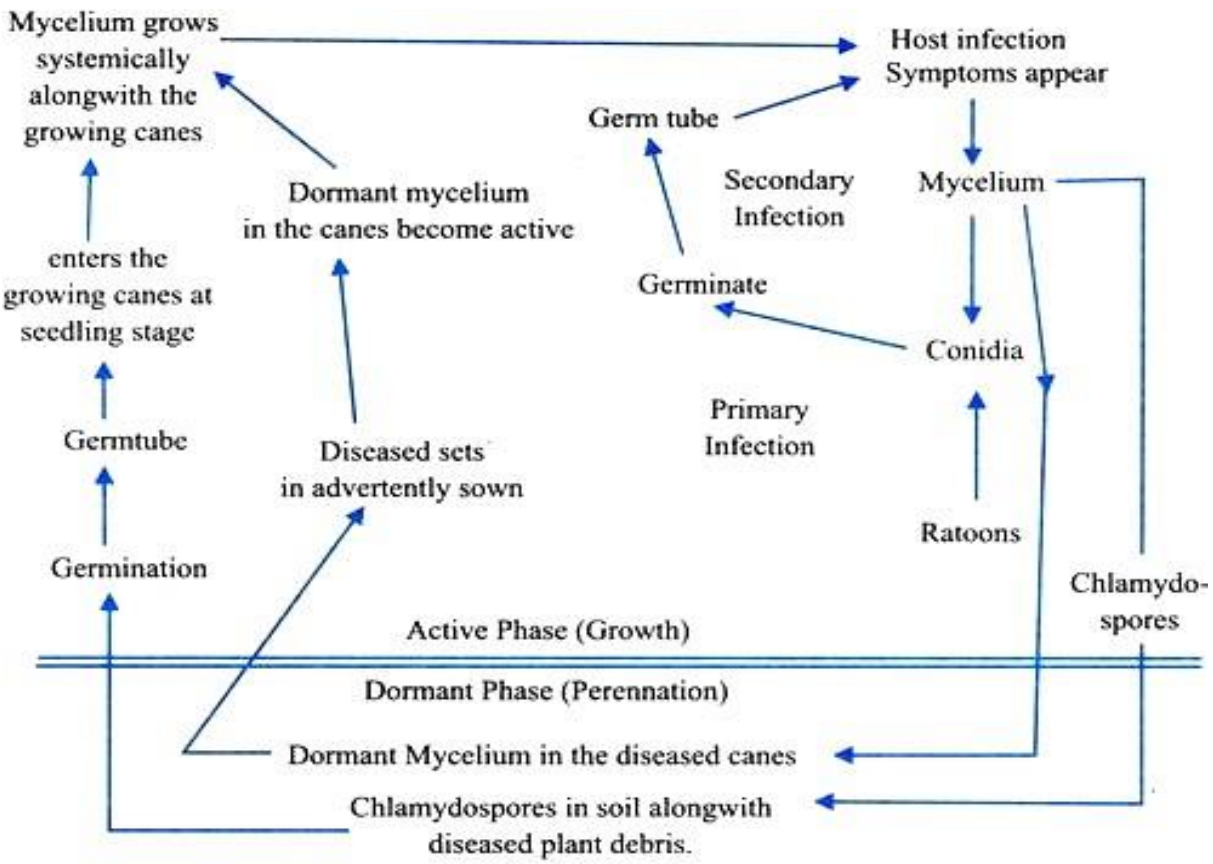


Fig. 22.27. Disease cycle of Red Rot of Sugarcane.

Control Measures of Red Rot Disease:

Field sanitation is an important measure to prevent the build source of primary inoculum. It consists in the collection and burning of sugarcane trash in the field. The other equally important preventive measure is the use of sound and healthy seed sets.

Long rotation of crops minimizes soil borne infection. The use of resistant varieties is the most effective method of controlling the disease. The following CO and BO varieties have been recommended to be resistant to red rot disease, Co. 846, Co. 951, Co. 1148, Co. 561, B.O.3, B.O. 7 and B.O. 32.

Tikka Diseases of Groundnut:

One of the best known leaf spot diseases is that of *Arachis hypogea* L. (groundnut). Popularly it is called the tikka disease. The tikka disease is a serious disease occurring in areas where the groundnut crop is grown in India.

Causal Organism:

Tikka disease of groundnuts is caused by two species of *Cercospora: Cercospora personata* (Berk. & Curt.) Elle and Eve., now known as *Cercosporidium personatum* (Berk. & Curt.) Deighton and *Cercospora arachidicola* Hori. The two form-species differ from each other with respect to the size, shape and colour of necrotic lesions they produce, conidia formation and the nature of the mycelium.

Symptoms of Tikka Disease:

B. personatum

1. Leaf spots are small, more circular and about 1-6 mm in diameter (A).
2. The necrotic lesions appear on both the leaf surfaces and change from dark brown to dark in colour.
3. Young spots lack bright yellow halo which, however, develops around the older ones.
4. The mycelium consists of hyphae which are entirely internal.

5. The septate hyphae ramify in the intercellular spaces and obtain nutrition by sending branched haustoria into the mesophyll (both spongy and palisade) cells.

6. The unbranched conidiophores arise in tufts from a dense, globular, and brown to black stroma (23-30 µ in dia.) and emerge by rupturing the host epidermis (B).

7. They are geniculate, olivaceous brown, 24-54 p long and 5-8 µ broad and are either aseptate or septate with 1-2 septa.

8. The conidiophores are confined to the lower surface of the host leaf and are seen arranged in concentric circles in the tuft.

C. arachidicola:

1. The leaf spots are comparatively larger in size, irregularly circular in outline and 4-10 mm in diameter.

2. The leaf spots are often confluent and necrotic lesions occur on both the surfaces. Those on the upper surface are reddish brown to black and on the lower surface are light brown.

3. There is a yellow halo around each spot but halos on the lower surface are less distinct.

Disease Cycle:

Primary infection takes place by soil borne conidia, which in the soil and on seeds in the shells. The spread of the disease during the season is by means of wind disseminated conidia.

Penetration is direct through the epidermis and also by way of stomata. Chiefly it occurs through the upper epidermis. However, there is possibility of infection through both the leaf surfaces.

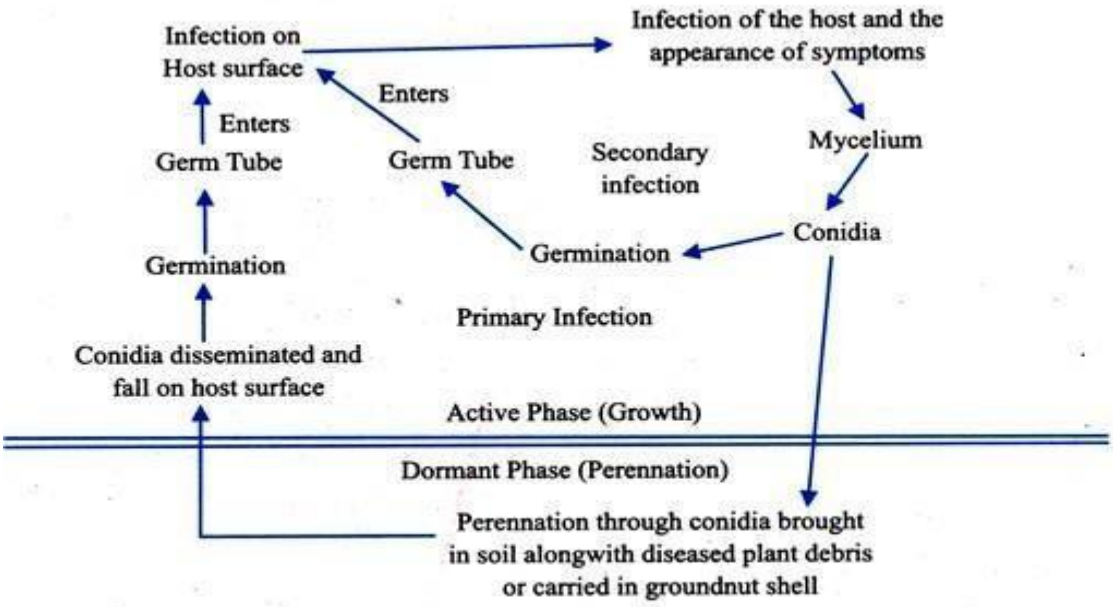


Fig. 22.25. Disease cycle of Tikka disease of Groundnuts.

Control Measures of Tikka Disease:

Rotation of crops, seed treatment and disposal of infected host debris by burning or burying in deep pits eliminate chances of primary infection from the soil borne inoculum. The seeds within the shells are disinfected with sulphuric acid.

Without shells, they are soaked for half an hour in 0.5% copper sulphate solution. Agrosan GN dressing of naked seeds is equally effective.

To check secondary spread of disease in the field, spraying with suitable fungicides is the only remedy. Among the fungicides the use of Bordeaux mixture has given good results.

Panama disease of banana

Panama disease is a plant disease of the roots of banana plants. It is a type of *fusarium* wilt, caused by the fungal pathogen ***Fusarium oxysporum*** f. sp. cubense (Foc). The pathogen is resistant to fungicide and cannot be controlled chemically. During the 1950s, Panama disease wiped out most commercial Gros Michel banana production.

Symptoms

Two external symptoms help characterize Panama disease of banana:

- Yellow leaf syndrome, the yellowing of the border of the leaves which eventually leads to bending of the petiole.
- Green leaf syndrome, which occurs in certain cultivars, marked by the persistence of the green color of the leaves followed by the bending of the petiole as in yellow leaf syndrome. Internally, the disease is characterized by vascular discoloration. This begins in the roots and rhizomes with a yellowing that proceeds to a red or brown color in the pseudostem.

Fusarium wilt has no symptoms on the growing buds or suckers, no exudates visible within the plant, and no symptoms in the fruit. Bacterial wilt can be characterized by distorted or necrotic buds, bacterial ooze within the plant, and fruit rot and necrosis.

Once a banana plant is infected, it continues to grow and any new leaves will be pale in color. Recovery is rare, but if it does occur, any new emerging suckers will already be infected and can propagate disease if planted.

Disease cycle:

Modern banana plants are reproduced asexually, by replanting the plant's basal shoot that grows after the original plant has been cut down.

The disease is dispersed by spores or infected material that travel in surface water. One of the biggest issues in spreading the disease is the means by which new banana plants are planted. Suckers are taken from one plant and clonally propagated to grow new trees. About 30 to 40% of suckers from a diseased plant are infected and not all show symptoms, so the chance of growing a new, already infected plant is fairly high. Finally, the disease is known to infect certain weeds without showing symptoms, meaning it can survive in the absence of banana plants and remain undetected in a place where bananas are planted later.

The disease survives in chlamydospores which are produced as the plant dies and can survive in the soil for upwards of 30 years. When the environment is ideal and there are host roots available, these chlamydospores will germinate and hyphae will penetrate the roots, initiating infection. There is in increase in the number of symptomatic plants when inflorescences emerge and the highest disease incidence occurs right before harvest. Once infected, microconidia are produced and present within the vessels of the plant’s vascular system. Macroconidia are another asexual spore that tends to be found on the surface of plants killed by Panama disease. Infection is systemic, moving through the vascular system and causing yellowing and buckling that starts in older leaves and progresses to younger leaves until the entire plant dies.

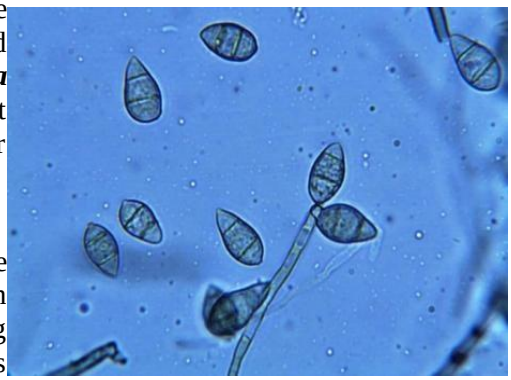
Controls:

Currently, fungicides and other chemical and biological control agents have proven fairly unsuccessful, or only successful in vitro or in greenhouses, in the face of Panama disease of bananas. The most commonly used practices include mostly sanitation and quarantine practices to prevent the spread of Panama disease out of infected fields. However, the most effective tool against Panama disease is the development of banana trees resistant to Fusarium oxysporum f. sp. Cubense. Unfortunately, the clonal reproduction of banana has led to a consequential lack of other varieties. Efforts are being made to produce resistant varieties, but with bananas being triploids which do not produce seeds, this is not an easy task. Creating clones from tissue cultures, rather than suckers, has proven somewhat successful in breeding resistant varieties, however, these tend to have decreased success in stress-tolerance, yield, or other beneficial traits necessary for commercial varieties. Nevertheless, these efforts are leading to the best control measure for Panama disease of banana.

Recently, an R gene (RGA2) was transformed into Cavendish bananas which confers disease resistance to *Fusarium* wilt tropical race 4. This is the first case of successful resistance in the field and is a promising step towards preventing the loss of the Cavendish cultivars that are a huge portion of banana export production and subsistence of many communities.

Rice Blast:

Rice (*Oryza sativa* L.) is the major staple food for nearly one half of the world's population. The rice crop suffer from a number of diseases among them rice blast caused by one of the most devastating agricultural pathogens in the world, a fungus called ***Magnaporthe grisea*** (Hebert) Barr [anomorph: ***Pyricularia grisea*** (Cooke) Sacc.], is one of the most important, causing significant losses in yield. Rice blast was probably first recorded as rice fever disease in China in 1637.

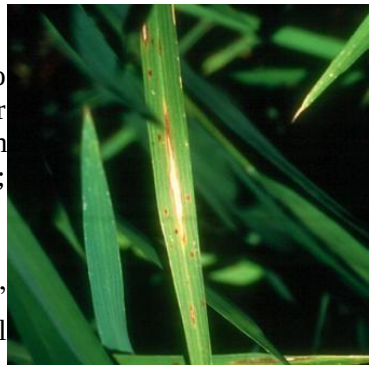


Symptoms of Rice Blast:

Rice blast is caused by *Magnaporthe grisea* (Hebert) Barr. All the above ground parts of the plant can be attacked by the fungus at any growth stages. However, Seedling stage, rapid tillering stage after transplanting and flower emergence stage were identified as the most susceptible ones to blast.

The disease can be described based on the part of the plant infected as follow:

i. Leaf Blast:



On the leaves the lesion/ spots first appear as minute brown specks, and then grow to become spindle-shaped, pointed at both ends. The center of the spots is usually gray or whitish with brown or reddish- brown margin. Fully developed lesions reach 1-1.5 cm long, 0.3-0.5 broad. Under favorable conditions, lesions enlarge and coalesce; eventually kill the leaves.

ii. Collar Rot:

Infection at the junction of the leaf blade and sheath in the typical brown "collar rot" symptom. A severe collar rot can cause the leaf to die completely. When collar rot kill the flag or penultimate leaf it may have a significant impact on yield.

iii. Neck Blast:

This occur when the pathogen infect the neck of the panicle to cause a typical "neck rot" or rotten neck blast symptom. The infected neck is griddled by a grayish brown lesion and the panicle falls down if the infection is severe. If the neck blast occurs before the milk stage, the entire panicle may die prematurely, leaving it white and completely unfilled.

iv. Panicle Blast:

The pathogen also causes brown lesions on the branches on the panicles and on the spikelets pedicles, resulting in "panicle blast". Infection of the neck, panicle branches, and spikelets pedicles may occur together or may occur separately.

v. Node Blast:

The fungus may also attack the stem at nodes, node blast in which the stem bend and break at the node causing spikelets sterility.

Sheath blight of paddy caused by *Rhizoctonia solani* AG1, banded sclerotial disease, rhizoctonia decline.

Disease cycle:

The pathogen infects as a spore that produces lesions or spots on parts of the rice plant such as the leaf, leaf collar, panicle, culm and culm nodes. Using a structure called an appressorium, the pathogen penetrates the plant. *M. grisea* then sporulates from the diseased rice tissue to be dispersed as conidiospores. After overwintering in

sources such as rice straw and stubble, the cycle repeats.

A single cycle can be completed in about a week under favorable conditions where one lesion can generate up to thousands of spores in a single night. With the ability to continue to produce the spores for over 20 days, rice blast lesions can be devastating to susceptible rice crops.

Controls:
The fungus has been able to establish resistance to both chemical treatments and genetic resistance in some types of rice developed by plant breeders. It is thought that the fungus can achieve this by genetic change through mutation. In order to most effectively control infection by *M. grisea*, an integrated management program should be implemented to avoid overuse of a single control method and fight against genetic resistance. For example, eliminating crop residue could reduce the occurrence of overwintering and discourage inoculation in subsequent seasons. Another strategy would be to plant resistant rice varieties that are not as susceptible to infection by *M. grisea*. Knowledge of the pathogenicity of *M. grisea* and its need for free moisture suggest other control strategies such as regulated irrigation and a combination of chemical treatments with different modes of action. Managing the amount of water supplied to the crops limits spore mobility thus dampening the opportunity for infection. Chemical controls such as Carpropamid have been shown to prevent penetration of the appressoria into rice epidermal cells, leaving the grain unaffected.

Rice Sheath Blight
Causal organism: *Rhizoctonia solani* AG1-1A Kühn (Teleomorph: Thanatephorus cucumeris (A. B. Frank) Donk.)

Symptoms
Early symptoms usually develop on the leaf sheaths at or just above the water line as circular, oval or ellipsoid, water-soaked spots which are greenish-gray in color. As the disease progresses, they enlarge and tend to coalesce forming larger lesions with grayish white centers surrounded by tan to dark brown irregular borders or outlines. Infection can spread to leaf blades and cause irregular lesions with dark green, brown, or yellow-orange margins. The lesions can develop

extensively and coalesce on partial or whole leaf blades, which may produce a rattlesnake skin pattern. These damaged tissues interrupt the normal flow of water and nutrients to the plant tissues above (leaves and panicles).

Controls:
Field sanitation: Levels of inoculum can be reduced by destroying weed hosts and other collateral hosts that could harbor sclerotia. However, this approach is not very effective or not feasible for management of sheath blight.

Management practices to avoid dense canopy: High seeding rate and overuse of nitrogen fertilizer usually increase stand and induce excessive vegetative growth and canopy density, creating a moist microclimate favorable for disease development. Therefore, avoiding high seeding rates and excessive application of fertilizers, especially nitrogen, can reduce the damage caused by sheath blight.

Crop rotation: Continuous rice or rotation with alternate hosts of the fungus such as soybeans increases inoculum in field soils. Fallow periods, along with efforts at reducing the inoculum by destroying collateral and weed hosts that could harbor sclerotia are viable management practices.

Chemical Control
Fungicides can be one of the most effective tools for control of sheath blight. In the United States, several fungicides, including azoxystrobin, provide excellent control of this disease. A single fungicide application is currently recommended to reduce production costs and maximize production returns. The timing of the application is critical for effective control of sheath blight. The disease should be scouted and monitored periodically during the development of the rice crop through heading. The application should be made during the growth stage between panicle differentiation plus five days and heading when the disease level reaches the economic threshold level.

Bacterial diseases:

1. Leaf blight of paddy caused by *Xanthomonas oryzae* pv *oryzae*

Xanthomonas oryzae: Pseudomonaceae

Order: Pseudomonales

A distinct yellowish discoloration and drying of the leaf margins are the typical symptoms of the rice bacterial blight. The leaf appears green with broad pale margins from a distance.

A bacterial disease on rice was first reported 75 years ago from Japan and Philippines. It was described in 1922 as *Pseudomonas oryzae.* In 1949, it was renamed as *Xanthomonas oryzae.* It remained a minor disease till 1950. In India, the bacterial blight was first reported in 1959 from Pune. The bacterium was isolated in 1961 from Tanjore. The disease was however taken seriously only after 1962 when a major outbreak occurred in Bihar and parts of north India.

Morphological Characters: The earliest symptom of the disease is the appearance of dull greenish water soaked or yellow spots, 5-10mm in length on the leaf towards the tip or along the margins. This leads to drying and discoloration of the tips or margins of rice leaves. As the disease progresses, several of these lesions coalesce to form straw-brown large lesions or blighted portions. The inner margin of the blighted patch in contact with the adjoining green part of the leat is ragged or wavy. Occasionally, the lesions may extend tip downward along the midrib itself. Small droplets of bacterial ooze may be found in the affected portions. These are pale amber in colour. When dried, these droplets form minute crusts and impart a rough touch to the leaves when passed between the fingers. In severe cases all the leaves are affected. If such leaves are cut and put in clear water, a turbid ooze of the bacterium streaming out of the leaves can be seen. The bacterium is rod shaped with a single polar flagellum.

Diversity: Several strains with different levels of virulence are known from India. The reaction of rice varieties to the different strains under varied ecological conditions is poorly understood.

Distribution: Widely distributed in Asia. It is known from all over the country affecting both indigenous and exotic varieties of rice.

Habitat Preference: Plants in well watered shaded areas and in nitrogen rich soils are the most affected by the bacteria.

Life Cycle: Primary infection is through seeds, the bacteria remaining in the husk or endosperm. It may also remain in plant debris infecting the nursery. The bacterium also infects certain grasses, which in turn serve as source for further infection. After infection, the bacterium becomes systemic and vascular not showing out till weeks after transplanting becoming most severe at the time of flowering. A combination of rainy weather, dull windy days and temperature of 22-26°C are most conducive for the bacterium.

Human Significance: The disease affects most varieties of rice all over the country causing severe crop losses (up to 60%).

Survey Method: Standing rice crops with severe infection show the typical blast symptoms even at a distance. Under less severe conditions small quadrats, say Imxlm, can be randomly placed in selected rice fields and the number of infected tillers of rice can be counted directly.

Management:

Management of bacterial leaf blight is most commonly done by planting disease resistant rice plants. PSB Rc82 is the standard variety of rice used in Southeast Asia, and the use of this cultivar enables the harvest of an estimated 0.8 million metric tons of rice per cropping season that would have otherwise been lost to bacterial leaf blight. Macassane, a new variety released in 2011, has been shown to have improved resistance to bacterial leaf blight and is being used currently in Mozambique.

Traditional treatments, such as the applications of copper compounds or antibiotics, are largely ineffective in the control of bacterial leaf blight. Increasingly, rice is being genetically engineered for resistance to the disease, as treatment proves difficult. More than 30 genes have been identified as being associated with resistance to bacterial leaf blight, and have been given names Xa1 to Xa33.

Biological control methods are relatively recent developments which are not currently in common use. They may be used in the future to reduce damage done by bacterial leaf blight, with experimental data showing up to a 64% reduction in damage.

2. Moko disease of banana caused by *Ralstonia solanacearum* race 2,

Causal organism: *Ralstonia solanacearum* (Yubucchi et al.)

It was first recorded in Guyana in 1840. In India, the disease was first reported from West Bengal. Susceptible

varieties are the Robusta and Poovan from Tamilnadu.

Symptoms:

Yellowing of younger leaves is the primary symptom. Yellowing progresses downward gradually leading to drooping and drying of leaves Fruit bunch size gets reduced with immature and irregular ripening of fruits. Infected fruits show cracking with bacterial ooze Vascular browning of the fruit along with light coloured vascular discolouration is common.

Etiology:

Pathogen is gram negative, lophotrichus bacteria. (More than one polar flagellum), multiplication occurs by bacterial fission.

Mode of Survival and spread:

Primary source of inoculum: The bacterium is soil/water borne and also spreads through infected suckers/rhizomes. It survives in susceptible host like banana and heliconia.

Secondary source of inoculum: Bacterial cells spread through irrigation water and also through suckers used for planting.

FIGURE 12-26 Bacterial wilt (Moko disease) of banana caused by *Ralstonia solanacearum*. Banana plants showing different stages of bacteria wilt, including wilted foliage only (A), infection of stalk and early infection of banana fruit (B), and thorough invasion and destruction of banana fruit (C). (D) Invasion and discoloration of several vascular bundles in the banana pseudostem. (E) Early (right) and later invasion and destruction of the contents of infected bananas (left). (F) Colonies of *R. solanacearum* growing on a specialized nutrient medium. [Photographs courtesy of H. D. Thurston, Cornell University.]

Epidemiology:
Soil temp 28-320c, relative humidity 87-92%, PH slightly acidic to neutral, clay loam and sandy loam soil and susceptible variety.

Life Cycle:
The bacteria survive through infected rhizomes and also in soil for 6 months to 2 years. The spread is through use of infected rhizomes, cutting machetes at the time of planting, and through insects which carry bacteria from oozing suckers and male flowers and bracts to healthy inflorescence and other parts of the plant. Entry into the host is mainly through injuries such as those caused during various cultural operations and attack of insects and nematodes. The bacteria multiply rapidly in the xylem. Auxin balance of the plant is disturbed. IAA is synthesized by the bacterium and by the host and accumulates due to inhibition of the auxin degrading system. Loss of virulence in the bacterium is generally accompanied

Management:

a) Use disease free planting material
b) Use resistant varieties like Robusta and Grand naine.
c) Infected plants should be collected and burnt.
d) Give proper drainage and avoid movement of water from infected to healthy plants
e) Drip irrigation method reduces the spread of bacteria.
f) Chemicals: Apply Copper Oxychloride @ 3gm per lit and Sreptocycline @ 0.5 gm per lit as a soil drench
g) Use bio- agent like Pseudomonas fluorescens.

ii. Viral diseases:

Tungro is the most serious virus disease of rice in south and southeast Asia from Pakistan to the Philippines. Tungro (yellow-orange) is the result of concurrent infection by two viruses: the single-stranded RNA virus *rice tungro spherical virus* (RTSV) and the double-stranded DNA virus *rice tungro bacilliform virus* (RTBV) (Figs. 14-44A and 14-44C). Both viruses are transmitted by several leafhoppers (Fig. 14-44B), particularly *Nephotettix virescens*, in the semipersistent manner. The RTSV RNA consists of about 12.4 kilobases, which encodes a 393-kilodalton polyprotein that is cleaved into several smaller proteins. The protein coat is made of two types of protein molecules. Tungro-infected rice plants are stunted and show mottling and yellow-orange discoloration of the leaves (Fig. 14-44A). Typical tungro symptoms can be caused by RTBV, but they are intensified by the presence of RTSV. RTSV often occurs alone but causes only very mild symptoms. The disease caused by RTSV alone was earlier known as rice waika disease and the virus as *rice waika virus*. Also, although both viruses are transmitted by leafhoppers in the semipersistent manner, only RTSV can be transmitted alone by leafhoppers, whereas RTBV transmission by leafhoppers is possible only when RTSV is also present in the donor plant.

iii. Disease caused by nematode:

Root knot of tomato a nematode (*Meloidogyne* spp.):
Root-knot nematodes occur throughout the world, especially in areas with warm or hot climates and short or mild winters, and in greenhouses everywhere. They attack more than 2,000 species of plants, including almost all cultivated plants, and reduce world crop production by about 5%. Losses in individual fields, however, may be much higher.
Root-knot nematodes damage plants by devitalizing root tips and causing the formation of swellings of the roots. These effects not only deprive plants of nutrients, but also disfigure and reduce the market value of many root crops. When susceptible plants are infected at the seedling stage, losses are heavy and may result in complete destruction of the crop. Infections of older plants may have only slight effects on yield or may reduce yields considerably.

Symptoms
Aboveground symptoms are reduced growth and fewer, small, pale green, or yellowish leaves that tend to wilt in warm weather. Blossoms and fruits are few and of poor quality. Affected plants usually linger through the growing season and are seldom killed prematurely.
Characteristic symptoms of the disease appear on the underground parts of the plants. Infected roots develop the typical root-knot galls that are two to several times as large in diameter as the healthy root. Several infections along the root give the root a rough, clubbed appearance. Roots infected by certain
species of the nematode also develop a bushy root

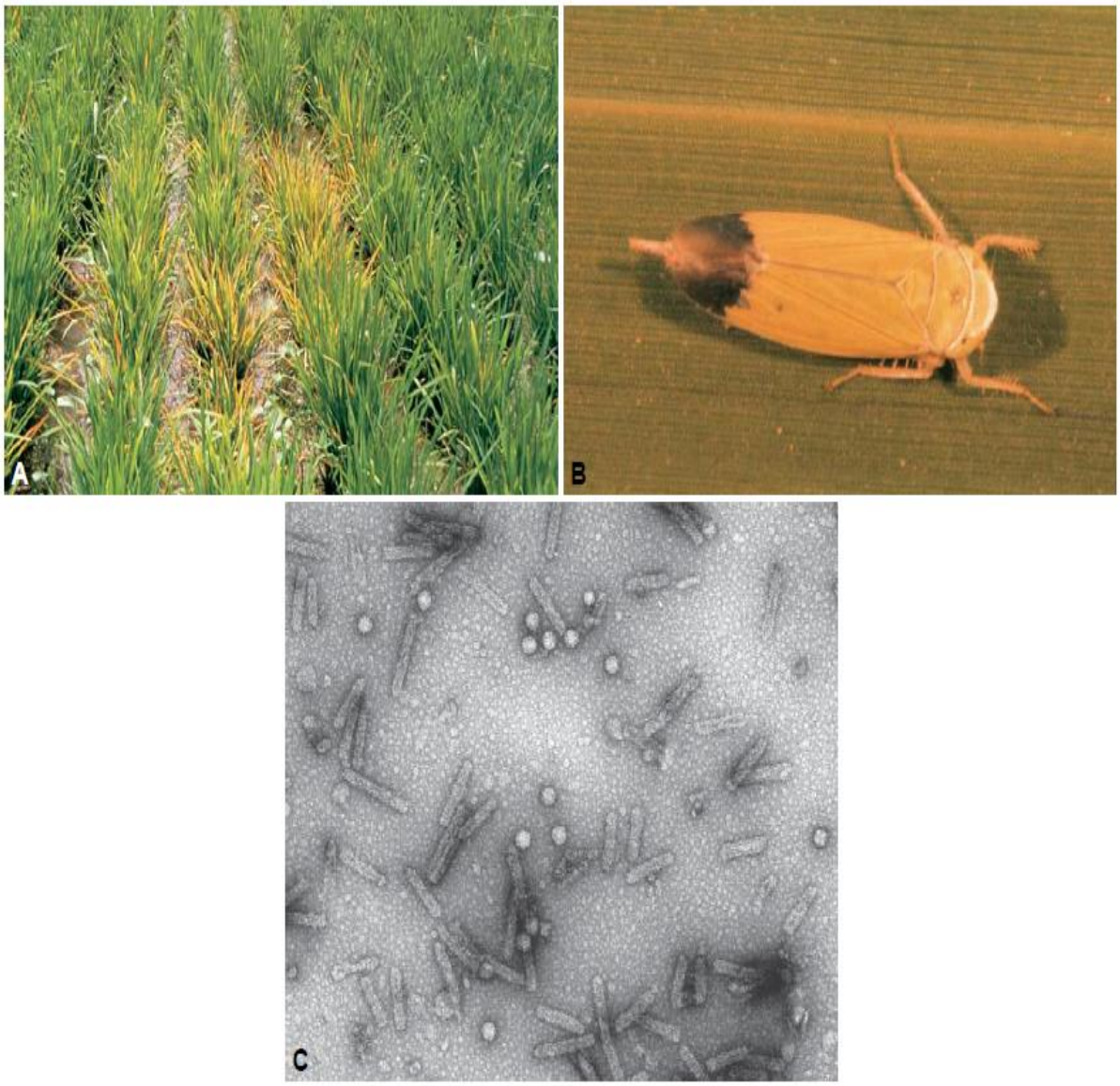


FIGURE 14-44 (A) Rice tungro-infected rice plants in the field showing stunting and yellow-orange coloration. (B) Female of the leafhopper vector of the tungro viruses. (C) Purified particles of the spherical (waikavirus) and bacilliform (badnavirus) viruses that together cause the rice tungro disease. (Photographs courtesy of H. Hibino.)

The Pathogen: **Meloidogyne *spp.***

The male and female root-knot nematodes are easily distinguishable morphologically. The males are wormlike and about 1.2 to 1.5 millimeters long by 30 to 36 micrometers in diameter. The females are pear shaped and about 0.40 to 1.30 millimeters long by 0.27 to 0.75 millimeters wide.

Each female lays approximately 500 eggs in a gelatinous substance. The first- and second-stage juveniles are wormlike and develop inside each egg.

The second-stage juvenile emerges from the egg into the soil. This is the only infective stage of the nematode. If it reaches a susceptible host, the juvenile enters the root, becomes sedentary, and grows thick like a sausage. The nematode feeds on the cells around its head by inserting its stylet and secreting saliva into the cells. The saliva stimulates cell enlargement and also liquefies part of the contents of the cells, which are then withdrawn by the nematode through its stylet.

A life cycle is completed in 25 days at 27°C, but it takes longer at lower or higher temperatures. When the eggs hatch, the infective second-stage juveniles migrate to adjacent parts of the root and cause new infections in the same root or

infect other roots of the same plants or roots of other plants. Most root-knot nematodes are found in the root zone from 5 to 25 centimeters below the surface.

Root-knot nematodes are spread primarily by water or by soil clinging to farm equipment or on infected propagating stock transported into uninfested areas.

Development of Disease

Second-stage juveniles enter roots behind the root tip and keep moving until they reach positions behind the growing point. There, they settle with their head in the developing vascular cylinder. In older roots the head is usually in the pericycle. Cells near the path of the juveniles begin to enlarge. Two or 3 days

after the juvenile has become established, some of the cells around its head begin to enlarge. Their nuclei divide, but no cell walls are laid down. The existing walls between some of the cells break down and disappear, giving rise to giant cells. Enlargement and coalescing of cells continues for 2 to 3 weeks, and the giant cells invade the surrounding tissues irregularly.

Each gall usually contains three to six giant cells, which are due to substances contained in the saliva secreted by the nematode in the giant cells during feeding.

The giant cells attract nutrients from surrounding cells and serve as feeder cells for the nematode. The giant cells crush xylem elements already present but degenerate when nematodes cease to feed or die. In the early stages of gall development the cortical cells enlarge in size and, later, they also divide rapidly. Swelling of the root results from excessive enlargement and division of all types of cells surrounding the giant cells and from enlargement of the nematode.

As the females enlarge and produce their egg sacs, they push outward, split the cortex, and may become exposed on the surface of the root or remain completely covered, depending on the position of the nematode in relation to the root surface.

In addition to the disturbance caused to plants by the nematode galls themselves, damage to infected plants is frequently increased by certain parasitic fungi, which can easily attack the weakened root tissues and the hypertrophied, undifferentiated cells of the galls.

Moreover, some fungi, e.g., *Fusarium*, *Rhizoctonia*, and the oomycete *Pythium*, grow and reproduce much faster in the galls than in other areas of the root, thus inducing an earlier breakdown of the root tissues.

Control

Root knot can be controlled effectively in the greenhouse with steam sterilization of the soil or soil fumigation with nematicides. In the field the best control of root knot is obtained by fumigating the soil with approved chemical nematicides. Each treatment usually gives satisfactory control of root knot for one season.

In several crops, varieties resistant to root-knot nematodes are also available. Transgenic plants producing inhibitors to certain nematode proteinases have shown promising resistance to the nematode and their use may prove practical in the future. Several cultural practices, such as crop rotation, fallow soil, soil solarization, and certain soil amendments, are also helpful in reducing root-knot losses. Biological control of root knot has been obtained experimentally by treating nematode infested soil with endospores of the bacterium *Pasteuria penetrans*, which is an obligate parasite of some plant parasitic nematodes, or with preparations of the fungus *Trichoderma harzianum*; by treating transplants or infested soils with spores of the fungus *Dactylella oviparasitica*, which parasitizes the eggs of *Meloidogyne* nematodes; and in some experiments by treating transplants or infested soils with spores of the vesicular arbuscular mycorrhizal fungi *Gigaspora* and *Glomus*.

Fairly good experimental control of root knot has also been obtained by mixing essential oils from plant spices into nematode-infested soil before planting and through an increase in plants of their local and systemic-induced resistance to root knot nematodes by mixing in the soil or spraying the plants with amino-butyric acid and other amino acids.

iv. Disease caused by mycoplasma-like organism:

Symptoms of Little Leaf Disease:

The main symptom of the disease is the production of very short leaves by affected plant. The petioles are so much reduced in size that leaves appear sticking to the stem. Such leaves are narrow, soft, smooth and yellowish in colour.

Newly formed leaves are further reduced in size. The internodes are shortened and at the same time large number of axillary buds are stimulated to grow into short branches with small leaves. This gives whole plant a bushy appearance. Usually such plant unable to form flowers. Fruiting is very rare.

Causal Organism:

Mycoplasma like organism (MLO).

Disease Cycle:

The disease is transmitted through by the vector Cestius phycitis. Artificially the disease has been transmitted successfully to tomato, potato and tobacco. Probably during the season of Brinjal crop, the causal agent survives on weed hosts and from there it is transmitted to main crop by its insect vector.

Control Measures of Little Leaf Disease:

Since no effective control measure is found it is better to eradicate the weed host and remove the diseased Brinjal plants. Tetra-cycline has been reported to control the disease.

Field sanitation Eradication of volunteer crops/plants previous season and Solanaceous weeds that can carry the disease. Plant barrier crop (Maize) In northern province, plant brinjal in late June or early July in order to avoid high populations of the vectors Plant tolerant variety (Padagoda) Keep plant healthy and vigorous Use good pest management practices to conserve the natural enemies such as lace wing, damsel bug, minute pirate bug Avoid cultivating alternative host plants (e.g. Chilli) to prevent build-up of the vectors Plant in correct spacing (90 X 75 cm) Observe for small leaves and short internodes at top Witches broom or rosetting Visit daily and observe the mobility of green hoppers Observe under surface of the leaves for vectors

Early destruction of infected plants. Uproot and burn infected plants. Control vector

Suggested reading

1. Agrios, George N. (1972). Plant Pathology (5th ed.). Academic Press.
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4. Martinelli, F., Scalenghe, R., Davino, S., Panno, S., Scuderi, G., Ruisi, P., Villa, P., Stroppiana, D., Boschetti, M., Goulart, L.R., Davis, C.E., Dandekar, A.M. (2014). "Advanced methods of plant disease detection. A review". Agronomy for Sustainable Development. **35**: 1–25. doi:10.1007/s13593-014-0246-1.
5. Creamer, Rebecca; H. Hubble; A. Lewis (May 2005). "Curtovirus Infection of Chile Pepper in New Mexico". Plant Diseases. **89** (5): 480–486. doi:10.1094/PD-89-0480.
6. https://www.apsnet.org/edcenter/intropp/lessons/fungi/ascomycetes/.../RiceSheath.asp x

Assignments

1. Define inoculum potential
2. What are the major host specific toxin?
3. Explain the role of cell wall degrading enzymes in host pathogen interaction. Discuss different physiological and chemical methods of crop protection Give a short note on structural defense mechanism.
4. What is Pr proteins?
5. Mention two systematic Fungicides and its role
6. Resistant of fungicides
7. Describe symptoms, causal organism, disease cycle and control of red rot of sugarcane disease. What is IPM?
8. Describe about the plant quarantine methods
9. Describe about the disease triangle
10. Different types of diseases cycle caused by deferent pathogens
11. Different stages about disease epidemic
12. Discuss about the different types of fungal toxins
13. Details recognition mechanism and signal transduction during plant-pathogen interaction

All the materials are self written and collected from eBooks, journals and websites.

POST GRADUATE DEGREE PROGRAMME (CBCS)
IN
BOTANY

SEMESTER - III

Course: BOHCT 3.2

(Plant Molecular Biology & Biotechnology)

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING
UNIVERSITY OF KALYANI
KALYANI – 741 235,
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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 UGC Regulations, 2017 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.) Sankar Kumar Ghosh, 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.

Prof Manas Mohan Adhikary
Director
Directorate of Open and Distance Learning
University of Kalyani

SYLLABUS
COURSE – BOHCT 3.2
Plant Molecular Biology & Biotechnology
(Full Marks – 75)

Course	Group	Details Contents Structure		Study hour
BOHCT3.2	Plant Molecular Biology	**Unit 1. Protein structure, function and protein analysis**	1. Protein structure and function, amino acid sequencing, protein analysis (electrophoresis, s-value crystallography, mass spectrophotometry, x-ray crystallography)	1
		Unit 2. Protein sorting	2. Protein sorting: protein targeting in organelles	1
		Unit 3. Nucleic acid and Recombinant DNA Technology	3. Nucleic acid: Structure, chemical and physical properties, nuclear, chloroplast and mitochondrial Genome 4. Recombinant DNA Technology: Restriction enzymes, cloning vectors, construction of recombinant DNA	1
		Unit 4. Biology of RNA types	5. Biology of RNA types: Ribosomal RNA, transfer RNA and messenger RNA; post m-RNA modifications, RNA splicing, Regulatory RNAs and related phenomenon: si-RNA, mi-RNA, Ribozyme, antisense RNA, RNAi, Riboswitch	1
		Unit 5. Blotting techniques	6. Blotting techniques: Southern, Northern and Western Blot; DNA fingerprinting, DNA foot printing, basic idea of proteomics and genomics, c-DNA and genomic library	1
		Unit 6. Bioinformatics	7. Bioinformatics: Definition, importance, constituents, application in genomics	1
	Biotechnology	**Unit 1. Plant tissue culture and gentic transformation**	1. Plant tissue culture: Cellular totipotency; organogenesis, somatic embryogenesis, Role of SERK and LEC genes during SE; haploidy and DH populations in crop improvement. 2. Genetic transformation: *Agrobacterium* mediated (co-culture, in planta, agroinfection); Direct method (PEG, electroporation, particle gun method); Reporter genes-screenable and selectable markers	1
		Unit 2. Biotechnological applications for crop management	3. Biotechnological applications for crop management: Approaches to improve shelf life of fruits and vegetables; herbicide resistance; insect and pest management.	1
		Unit 3. Secondary metabolite production in plant cultures and Micropropagation	4. Secondary metabolite production in plant cultures: Types of secondary metabolites; culture systems used for secondary metabolite production; improving secondary metabolite production in culture; hairy root culture. 5. Micropropagation: Production of virus free plants, virus free assessment methods, genetic assessment by RAPD and ISSR markers, certification for quality plants.	1
		Unit 4 Fermentation technology and Immobilization of microbial enzymes and whole cells	6. Fermentation technology: application of fermentation; batch, fed batch and their continuous cultures of microbes; Bioreactors: Principles and their design; microbial strain improvement. 7. Immobilization of microbial enzymes and whole cells and their applications in industries.	1

Course	Group	Details Contents Structure		Study hour
BOHCT3.2	Biotechnology	**Unit 5. Microbes as food and in food processing, Biofertilizers and biopesticides**	8. Microbes as food and in food processing, single cell protein. 9. Biofertilizers and biopesticides in agriculture.	1
		Unit 6. Environmental biotechnology and Regulatory issues in biotechnology	10. Environmental biotechnology: Treatment of waste & waste water; bioremediation 11. Regulatory issues in biotechnology: Intellectual property rights (IPR): Patents, plant variety protections & geographical indications, WTO & TRIPPS, Bio safety and Food safety of GMO - a brief outline.	1

Content

COURSE – BOHCT 3.2

(Plant Molecular Biology & Biotechnology)

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

Group – Group A (Plant Molecular Biology)

Content Structure

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1. Introduction
2. Course Objectives
3. Protein structure and function, amino acid sequencing, protein analysis (electrophoresis, s-value crystallography, mass spectrophotometry, x-ray crystallography)
4. Protein sorting: protein targeting in organelles
5. Nucleic acid: Structure, chemical and physical properties, nuclear, chloroplast and mitochondrial Genome
6. Recombinant DNA Technology: Restriction enzymes, cloning vectors, construction of recombinant DNA
7. Biology of RNA types: Ribosomal RNA, transfer RNA and messenger RNA; post m-RNA modifications, RNA splicing, Regulatory RNAs and related phenomenon: si-RNA, mi-RNA, Ribozyme, antisense RNA, RNAi, RiboswitchPopulation samples, sampling methods
8. Blotting techniques: Southern, Northern and Western Blot; DNA fingerprinting, DNA foot printing, basic idea of proteomics and genomics, c-DNA and genomic library
9. Bioinformatics: Definition, importance, constituents, application in genomics
10. Let’s sum up
11. Suggested Reading
12. Assignment

1. Introduction

Molecular Biology is the branch of biology that studies the structure and function of macro molecules that encode and regulate the flow of genetic information used by living organisms. This course will focus on the structure and content of the three genomes found in plant cells, gene structure, expression, and regulation. Other topics addressed in this class are transposable elements, and plant transformation procedures. A brief introduction to bioinformatics is also included.

2. Course Objectives

You should gather knowledge after studying the course:

This course aims to:

1. Provide students with a solid understanding of the relationship between structure and function of protein.
2. Knowledge about the basic methods and approaches used in molecular biology, blooting techniques, DNA fingerprinting, DNA foot printing
3. Familiarize students with the utilization of bioinformatics resources

3. Protein structure and function, amino acid sequencing, protein analysis (electrophoresis, s-value crystallography, mass spectrophotometry, x-ray crystallography)

Amino acid:
They have a carboxyl group and an amino group bonded to the same carbon atom (the a carbon). They differ from each other in their side chains, or **R groups**, which vary in structure, size, and electric charge, and which influence the solubility of the amino acids in water. The common amino acids of proteins have been assigned three letter abbreviations and one-letter Symbol, which are used as shorthand to indicate the composition and sequence of amino acids polyrnerized in proteins.

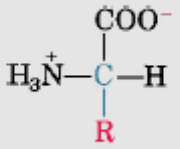
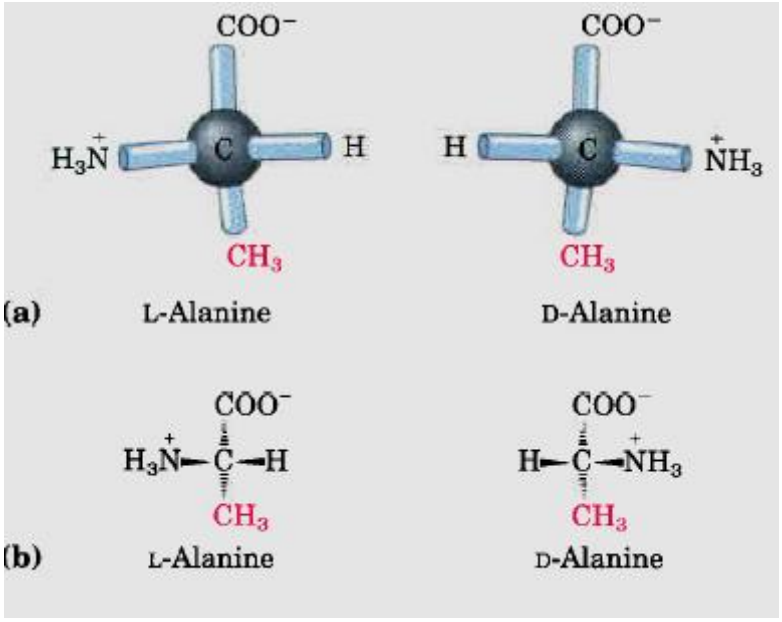


FIGURE 3–2 General structure of an amino acid. This structure is common to all but one of the α-amino acids. (Proline, a cyclic amino acid, is the exception.) The R group, or side chain (red), attached to the α carbon (blue) is different in each amino acid.

For all the common amino acids except glycine, the carbon is bonded to four different groups: a carboxyl group, an amino group, an R group, and a hydrogen atom (In glycine, the R group is another hydrogen atom). The a-carbon atom is thus a **chiral center**. Because of the tetrahedral arrangement of the bonding orbitals around the a-carbon atom, the four different groups can occupy two unique spatial arrangements, and thus amino acids have two possible stereoisomers. Since they are nonsuperposable mirror images of each other, the two forms represent a class of stereoisomers called **enantiomers.** All molecules with a chiral center are also **optically active**-that is, they rotate plane polarized light.



Stereoisomerism in α-amino acids

The amino acid residuises in proteins are L-Stereoisomers:
Nearly all biological compounds with a chiral center occur naturally in only one stereoisomeric form, either D or L. The amino acid residues in protein molecules are exclusively L-stereoisomers. D-Amino acid residues have been found in only a few, generally small peptides, including some peptides of bacterial cell walls and certain peptide antibiotics.
Amino acids can be classified by R Group

The topic can be simplified by groupng the amino acids into flve main classes based on the properties of their R groups, in particular, their polarity, or tendency to interact with water at biological pH (near pH 7.0). The polarity of the R groups varies widely, from nonpolar and hydrophobic (water-insoluble) to highly polar and hydrophilic (water-soluble).

Nonpolar, Aliphatic R Groups: The R groups in this class of amino acids are nonpolar and hydrophobic The side chains of **alanine, valine, leucine**, and **isoleucine** tend to cluster together within proteins, stabilizing protein structure by means of hydrophobic interactions. **Glycine** has the simplest structure. Although it is most easily grouped with the nonpolar amino acids, its very small side chain makes no real contribution to hydrophobic interactions. Methionine, one of the two sulfur-containing amino acids,has a nonpolar thioether group in its side chain. Proline has an aliphatic side chain with a distinctive cyclic structure. The secondary amino (imino) group of proline residues is held in a rigid conformation that reduces the structural flexibility of polypeptide regions containing proline.

Aromatic R Groups: **Phenylalanine, tyrosine**, and **tryptophan**, with their aromatic side chains, are relatively nonpolar (hydrophobic). All can participate in hydrophobic interactions. The hydroxyl group of tyrosine can form hydrogen bonds, and it is an important functional group in some enzymes. Tyrosine and tryptophan are signiflcantly more polar than phenylalanine because of the tyrosine hydroxyl group and the nitrogen of the tryptophan indole ring .

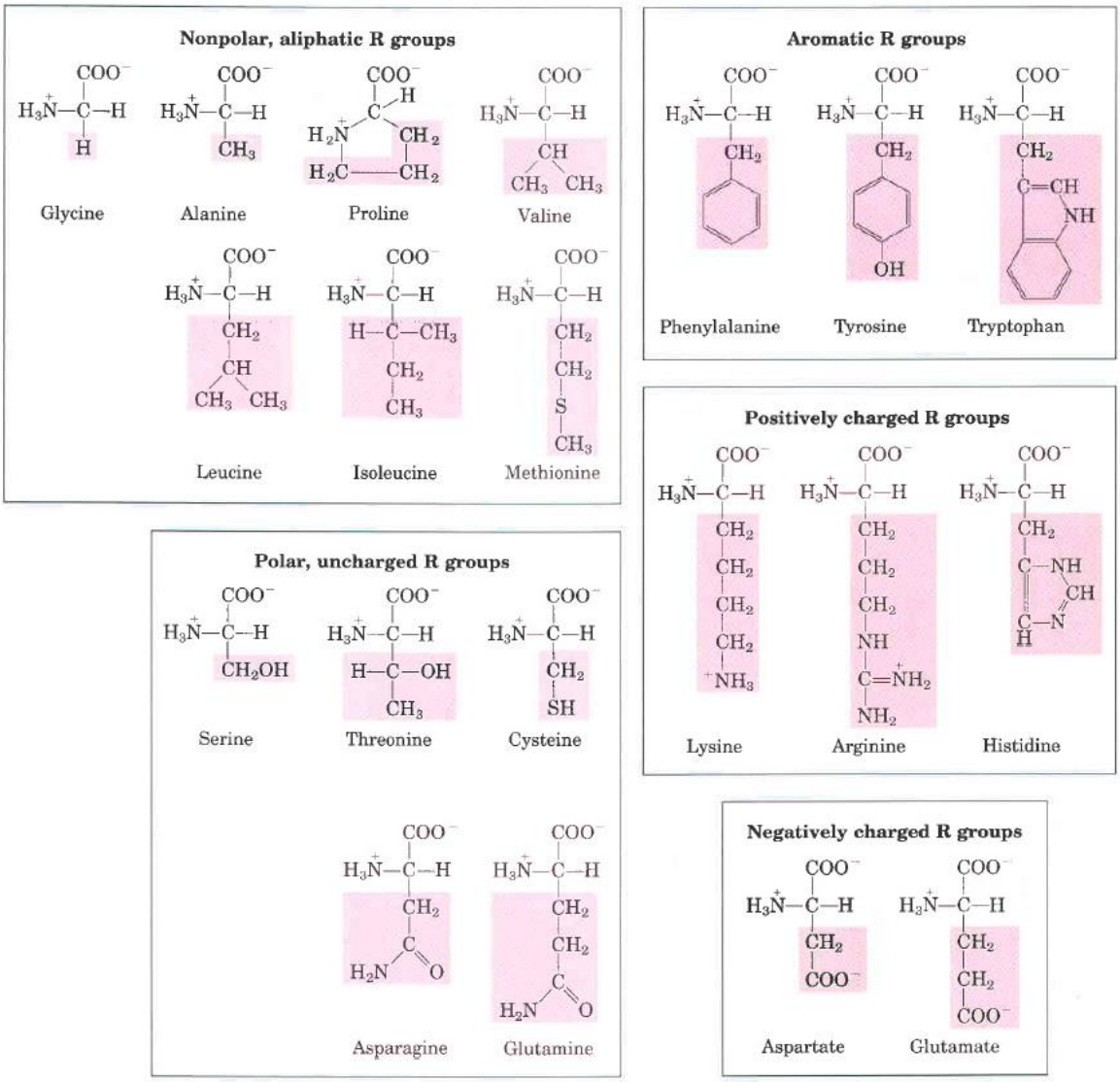


FIGURE 3–5 The 20 common amino acids of proteins. The structural formulas show the state of ionization that would predominate at pH 7.0. The unshaded portions are those common to all the amino acids; the portions shaded in pink are the R groups. Although the R group of histidine is shown uncharged, its pK_a (see Table 3–1) is such that a small but significant fraction of these groups are positively charged at pH 7.0. The protonated form of histidine is shown above the graph in Fig. 3–12b.

Polar, Uncharged R Groups: The R groups of these amino acids are more soluble in water, or more hydrophilic, than those of the nonpolar amino acids, because they contain functional groups that form hydrogen bonds with water. This class of amino acids includes s**erine, threonine, cysteine, asparagine**, and **glutamine**. The polarity of serine and threonine is contributed by their hydroxyl groups; that of cysteine by its sulfhydryl group, which is a weak acid and can make weak hydrogen bonds with oxygen or nitrogen; and that of asparagine and glutamine by their amide groups.

Positively Charged (Basic) R Groups: The most hydrophilic R groups are those that are either positively or negatively charged. The amino acids in which the R groups have signiflcant positive charge at pH 7.0 are lysine, which has a second primary amino group at the a position on its aliphatic chain; **arginine**, which has a positively charged guanidinium group; and **histidine**, which has an aromatic imidazole group.

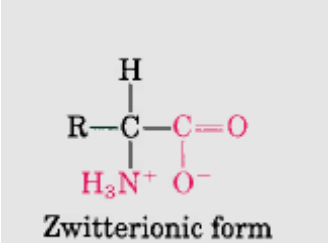
Negatively Charged (Acidic) R Groups: The two amino acids having R groups with a net negative charge at pH 7.0 are **aspartate** and **glutamate**, each of which has a second carboxyl group.

Uncommon amino acids:

Among these uncommon amino acids are **4-hydroxyproline**, a derivative of proline, and **5-hydroxylysine**, derived from lysine. The former is found in plant cell wall proteins, and both are found in collagen, a fibrous protein of connective tissues. **G-N-Methyllysine** is a constituent of myosin, a contractile protein of muscle. Another important uncolnmon amino acid is **1-carboxyglutamate**, found in the bloodclotting protein prothrombin and in certain other proteins that bind Ca^{2+} as part of their biological function.

Amino acids can act as acids and bases:

The amino and carboxyl groups of amino acids, along with the ionizable R groups of some amino acids, function as weak acids and bases. When an amino acid lacking an ionizable R group is dissolved in water at neutral pH, it exists in solution as the dipolar ion, or **zwitterion** (German for "hybrid ion"), which can act as either an acid or a base. Substances having this dual (acid-base) nature are **amphoteric** and are often called **ampholytes**.



Zwitterionic form

Protein:

Protein structure is the three-dimensional arrangement of atoms in an amino acid-chain molecule. Proteins are polymers – specifically polypeptides – formed from sequences of amino acids, the monomers of the polymer. A single amino acid monomer may also be called a residue indicating a repeating unit of a polymer. Proteins form by amino acids undergoing condensation reactions, in which the amino acids lose one water molecule per reaction in order to attach to one another with a peptide bond.

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 (—NH2) of one amino acid con-denses 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 —NH2 group is called as N-terminal end.

Although there may be hundreds of amino acids in a single polypeptide chain but funda-mentally 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).

In 1839, Dutch chemist **G.J. Mulder** was first to describe about proteins. The term protein is derived from a Greek word proteios, meaning **first place**.

Peptide Bond:
The α- carboxyl group of one amino acid (with side chain R1) forms a covalent peptide bond with α-amino group ofmanother amino acid (with side chain R2) by removal of a molecule of water.The result is Dipeptide. The dipeptide can then form a second peptide bond with a third amino acid (with side chain R3) to give **tripeptide**. Repetition of this process generates a polypeptide or protein of specific aminoacid sequence. Has 40% double bond character, caused by resonance. Polypeptide backbone is the repeating sequence of the N-CC- N-C-C… in the peptide bond. The side chain or R group is not part of the backbone or the peptide bond.

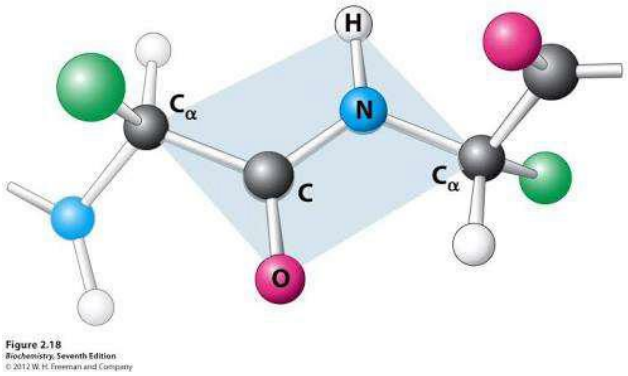


Figure 2.18
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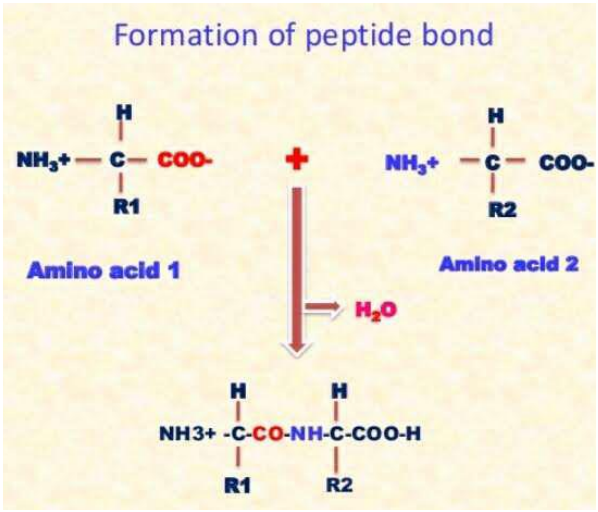
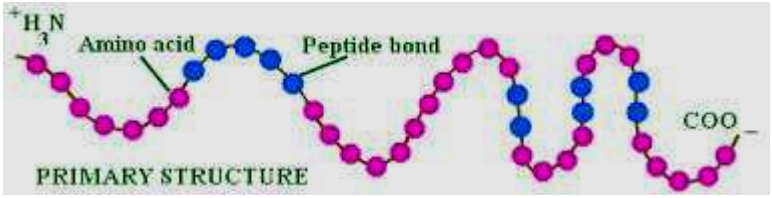


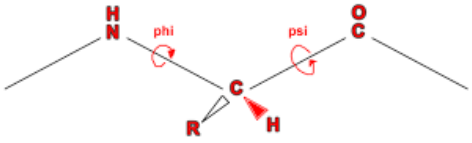
Primary Structure:
Proteins are made up of polypeptide chains, which are amino acids joined together with peptide bonds. The unique sequence of amino acids that make up a protein or polypeptide chain is called the Primary Structure. Each component amino acid in a polypeptide is called a **"residue" or "moiety**". By convention the primary structure of protein starts from the amino terminal (N) end and ends in the carboxyl terminal (C) end.



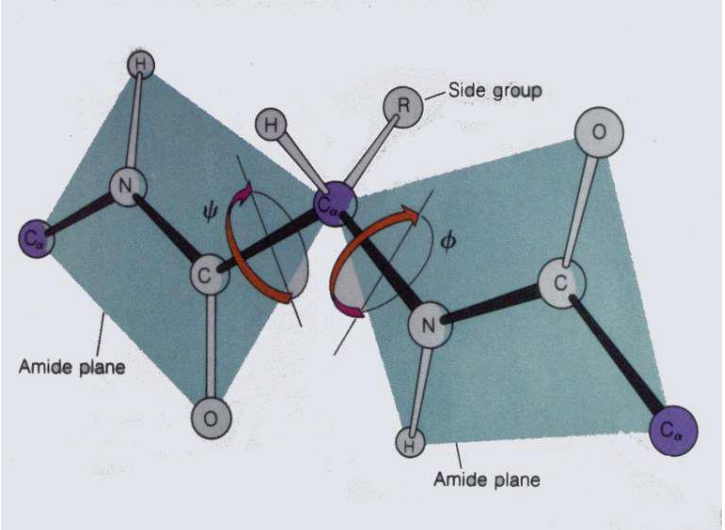
Secondary Structure:
It is a local, regularly occurring structure in proteins and is mainly formed through hydrogen bonds between backbone atoms. Pauling & Corey studied the secondary structures and proposed 2 conformations

- α helix
- β sheets.

Polypeptide chain conformation:
The backbone or main chain of a protein refers to the atoms that participate in peptide bonds, ignoring the side chains of the aminoacid residues. The only reasonable free movements are rotations around the Cα – N bond (measured as Φ) and the Cα –C bond (measured as Ψ). These angles are both defined as 180° when the polypeptide chain is in full conformation. The conformation of the backbone can therefore be described by the **torsion angles** (also called dihedral angles or rotational angles).



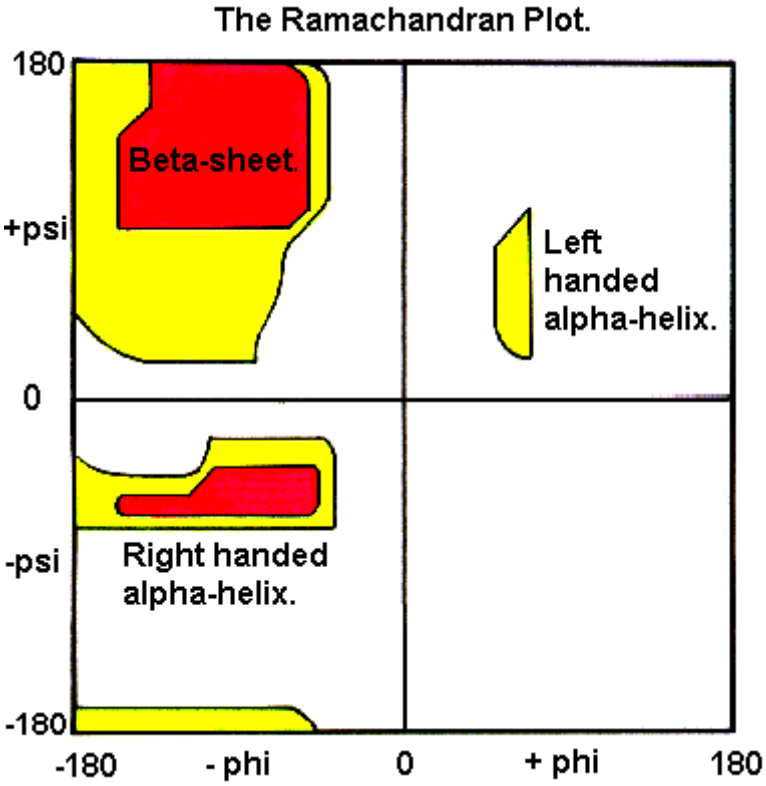
It is the angle between two intersecting planes. It helps to maintain the protein structure



Ramachandran Plot:

A **Ramachandran plot** (also known as a **Ramachandran diagram** or a [φ,ψ] plot), originally developed in 1963 by G. N. Ramachandran. A Ramachandran plot is a way to visualize backbone dihedral angles ψ against φ of amino acid residues in protein structure. A Ramachandran plot can be used to show which values, or conformations, of the ψ and φ angles are possible for an aminoacid residue in a protein and to show the empirical distribution of datapoints observed in a single structure.

The darkest areas correspond to the "core" regions representing the most favorable combinations of phi-psi values.



White regions : Sterically disallowed for all amino acids except glycine.

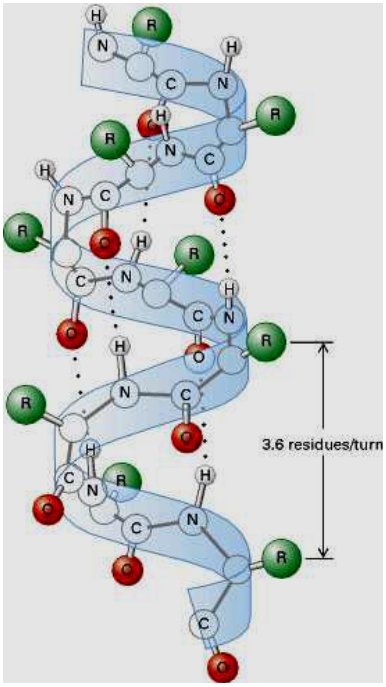
Red regions : allowed regions namely the α-helical and β-sheet conformations.

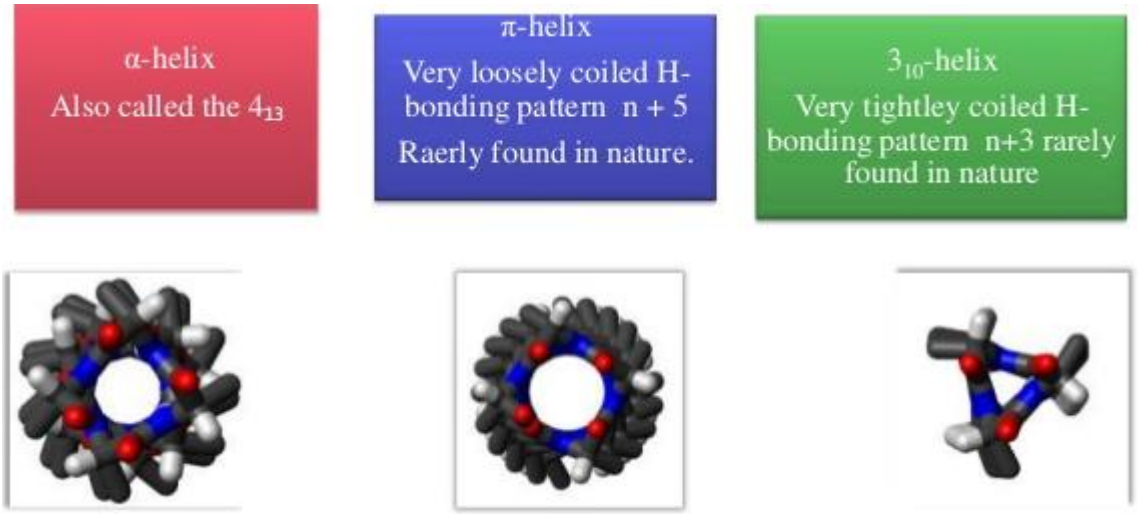
Yellow areas : outer limit

Alpha 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.

- Right handed spiral structure.
- Tightly packed, coiled polypeptide
- backbone core.
- Side chain extends outwards.
- Stabilized by H bonding that are arranged such that the peptide Carbonyl oxygen (nth residue) and amide hydrogen(n+4 th residue).
- Amino acids per turn – 3.6
- Pitch is 5.4 A°
- Alpha helical segments, are found in many globular proteins like myoglobin,troponin C.
- Length ~12 residues and ~3 helical turns.
- phi = -60 degrees, psi = -45 degrees , falls within the fully allowed regions of the Ramachandran diagram.





Types of α-helix

Beta Pleated Sheet:

Pauling and Corey predicted a second type of repetitive structure, the β conformation. This is a more extended conformation of polypeptide chains, and its structure has been confirmed by x-ray analysis. In the β conformation, the backbone of the polypeptide chain is extended into a zigzag rather than helical structure. The zigzag polypeptide chains can be arranged side by side to form a structure resembling a series of pleats. In this arrangement is called **β sheet**, hydrogen bonds form between adjacent segments of polypeptide chain. The individual segments that form a β sheet are usually nearby on the polypeptide chain, but can also be quite distant from each other in the linear sequence of the polypeptide; they may even be in different polypeptide chains. The basic unit of a beta sheet is a beta strand with approximate backbone dihedral angles phi = -120 and psi = +120. They are stabilized by H bond b/w N-H and carbonyl groups of adjacent chains. The adjacent pollpeptide chains in a β sheet can be either parallel or antiparallel.

Antiparallel beta sheet – neighboring hydrogen bonded polypeptide chains run in opposite direction.

Parallel beta sheet - hydrogen bonded chains extend in the same direction.

The idealized structures correspond to ϕ= -119º, ψ= +113º (parallel) and ϕ= -139º, ψ= +135º (antiparallel), these values vary some what in real proteins, resulting in structural variation, as seen above for α helices.

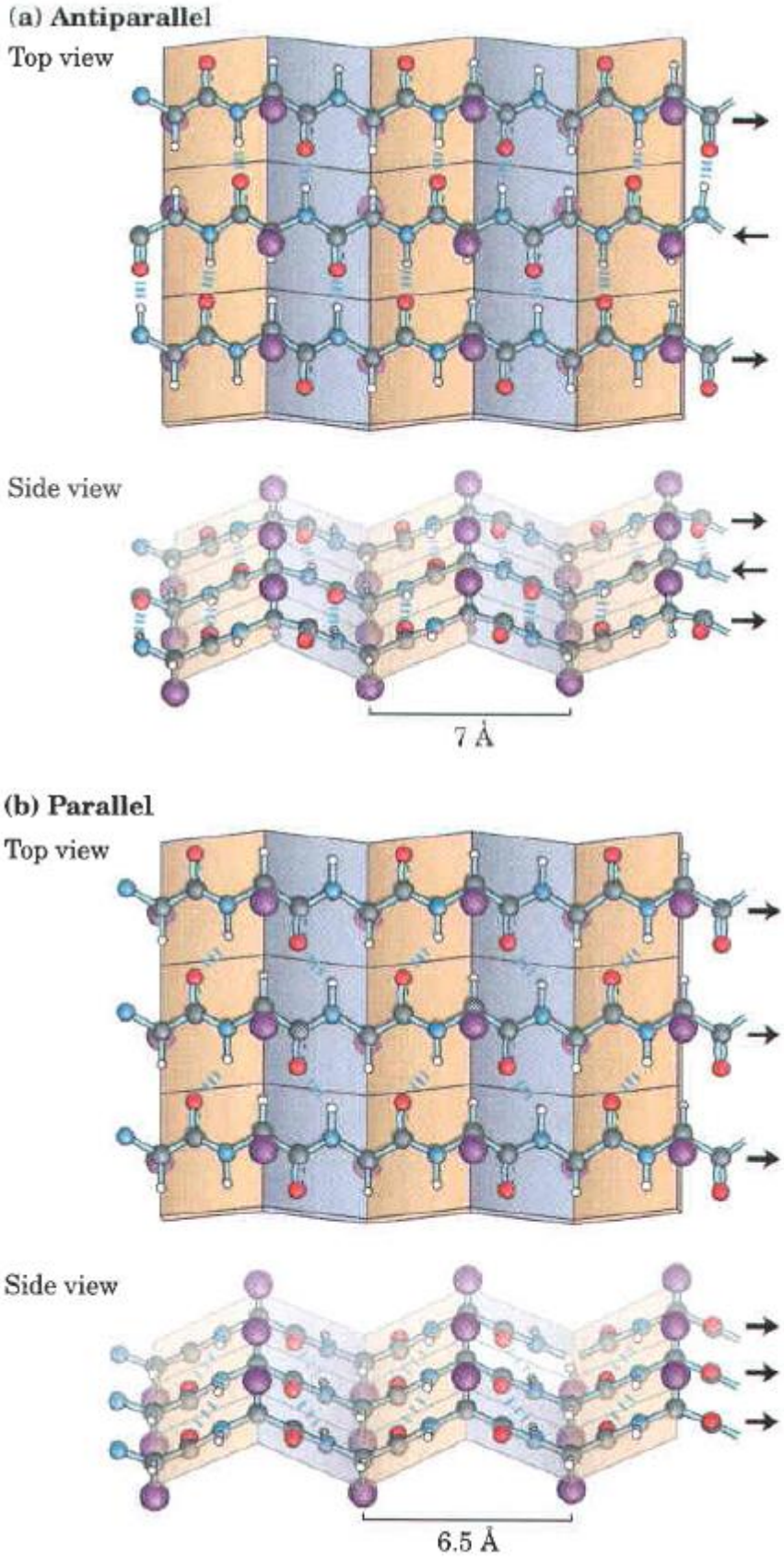
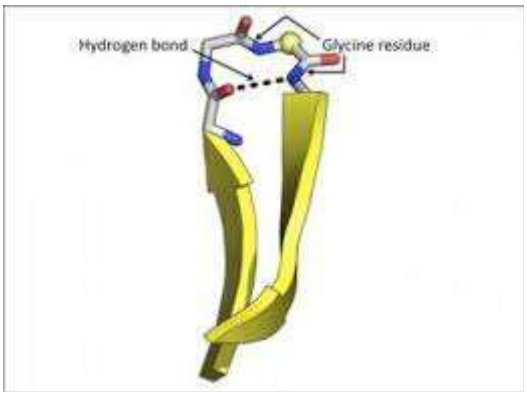


FIGURE 4–6 The β conformation of polypeptide chains. These top and

Beta turns:



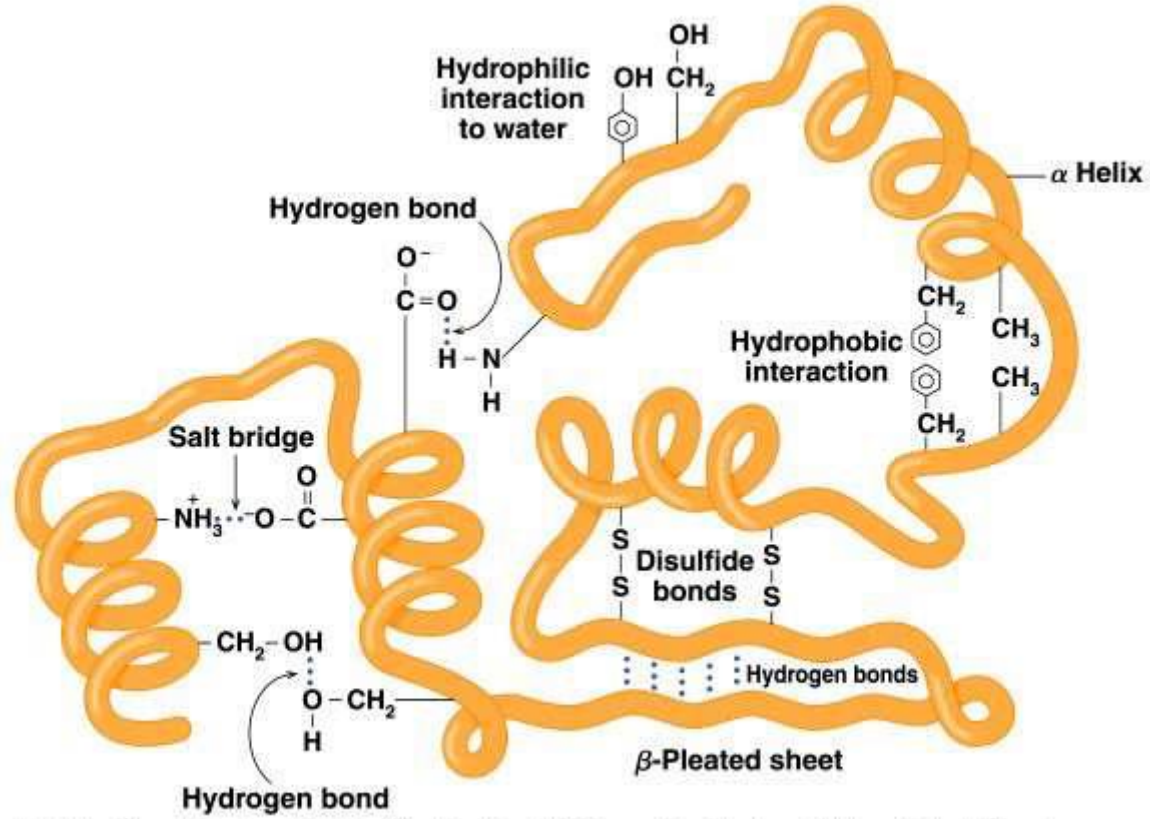
Also known as beta bends or tight turns. In a beta turn, a tight loop is formed when the carbonyl oxygen of one residue forms a hydrogen bond with the amide proton of an amino acid three residues down the chain. This hydrogen bond stabilizes the beta bend structure. Proline and Glycine are frequently found in beta turns, proline because its cyclic structure is ideally suited for the beta turn, and glycine because, with the smallest side chain of all the amino acids, it is the most sterically flexible. A beta turn is a means by which the protein can reverse the direction of its peptide chain. Beta turns often promote the formation of antiparallel beta sheets.

Tertiary Structure:

The final 3D structure of a protein is its Tertiary Structure, which pertains to the shaping of the secondary structure. This may involve coiling or pleating, often with straight chains of amino acids in between.

Tertiary structure is held together by four different bonds and interactions:

- **Disulphide Bonds** - Where two Cysteine amino acids are found together, a strong double bond (S=S) is formed between the Sulphur atoms within the Cysteine monomers.
- **Ionic Bonds** - If two oppositely charged 'R' groups (+ve and -ve) are found close to each other, and ionic bond forms between them.
- **Hydrogen Bonds** - Your typical everyday Hydrogen bonds.
- **Hydrophobic and Hydrophilic Interactions** - Some amino acids may be hydrophobic while others are hydrophilic. In a water based environment, a globular protein will orientate itself such that it's hydrophobic parts are towards its centre and its hydrophilic parts are towards its edges.



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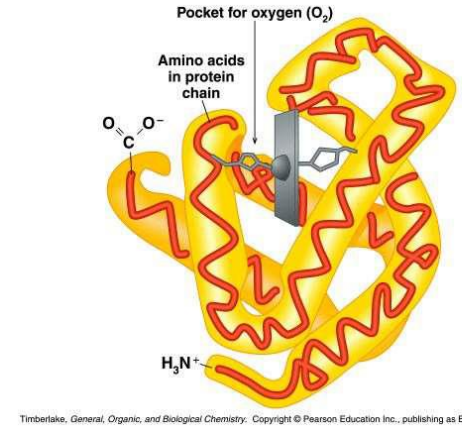
Tertiary structure can be broken by the action of heat. Increasing the kinetic energy of protein with a tertiary structure makes it vibrate more, and so the bonds that maintain its shape (which are mainly weak, non-covalent bonds) will be more likely to break. When a protein loses its shape in this way it is said to be **Denatured**. Even when cool the protein will not (or is highly unlikely to) form its original complex shape.

Proteins with a 3D structure fall into two main types:

Globular - These tend to form ball-like structures where hydrophobic parts are towards the centre and hydrophilic are towards the edges, which makes them water soluble. They usually have metabolic roles, for example: enzymes in all organisms, plasma proteins and antibodies in mammals. For example, myoglobin is a globular protein that stores oxygen in the muscles. Myoglobin is a single peptide chain that is mostly α-helix the O2 binding pocket is formed by a heme group and specific amino acid side-chains that are brought into position by the tertiary structure



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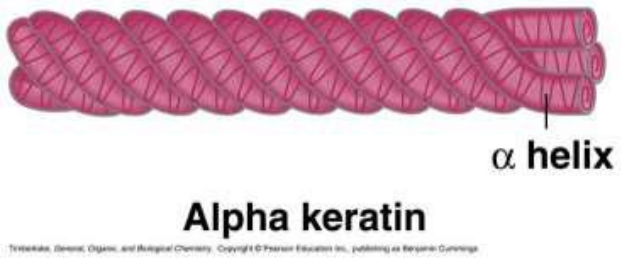
Fibrous - They proteins form long fibres and mostly consist of repeated sequences of amino acids which are insoluble in water. They usually have structural roles, such as: Collagen in bone and cartilage, Keratin in fingernails and hair.

For example,

α-keratins are fibrous proteins that make hair, fur, nails and skin. Hair is made of twined fibrils, which are braids of three α-helices (similar to the triple helixmstructure of collagen). The α-helices are held together by disulfide bonds.



β-keratins are fibrous proteins found in feathers and scales that are made up mostly of β-pleated sheets.

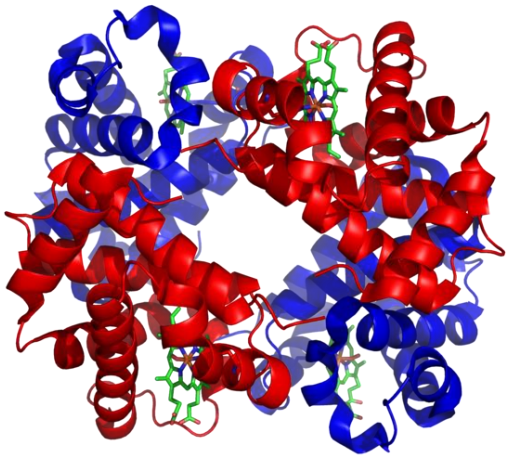
Quaternary structure:

The quaternary protein structure involves the clustering of several individual peptide or protein chains into a final specific shape. A variety of bonding interactions including hydrogen bonding, salt bridges, and disulfide bonds hold the various chains into a particular geometry. Two kinds of quaternary structures: both are multisubunit proteins.

Homodimer: association between identical polypeptide chains.

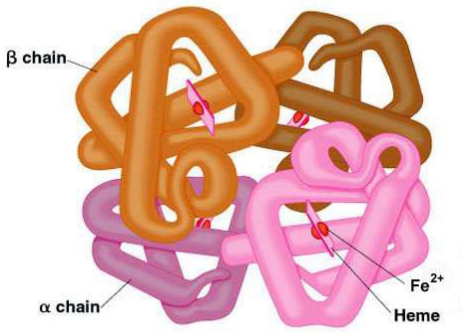
Heterodimer: interactions between subunits of very different structures.

The interactions within multi subunits are the same as that found in tertiary and secondary structures



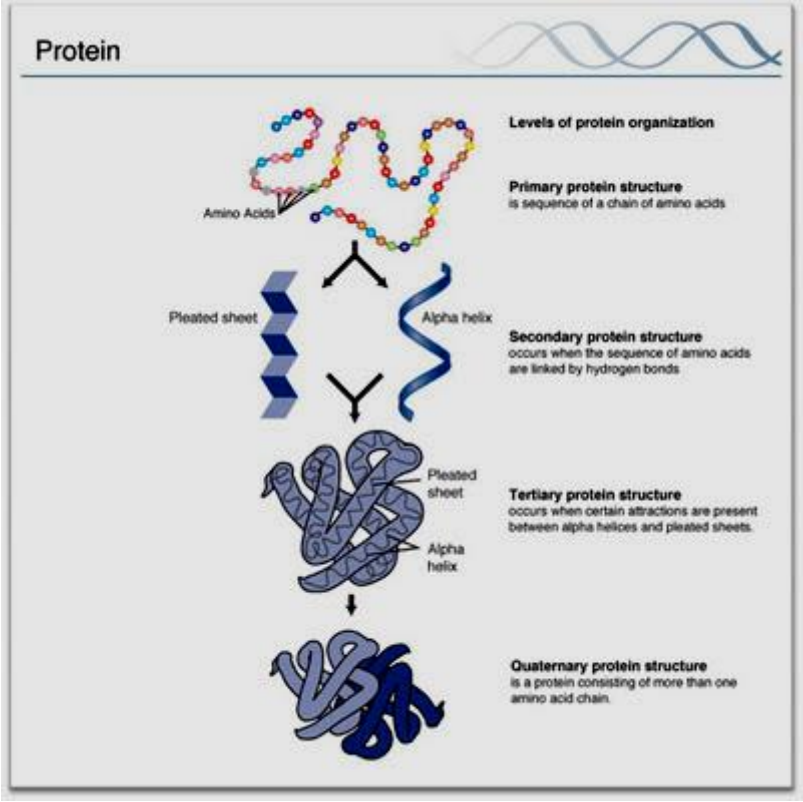
Haemoglobin:

Haemoglobin is a water soluble globular protein which is composed of two α polypeptide chains, two β polypeptide chains and an inorganic prosthetic heme group. Its function is to carry oxygen around in the blood, and it is facilitated in doing so by the presence of the heme group which contains a Fe^{2+} ion, onto which the oxygen molecules can bind.



Collagen:

Collagen is a fibrous protein consisting of three polypeptide chains wound around each other. Each of the three chains is a coil itself. Hydrogen bonds form between these coils, which are around 1000 amino acids in length, which gives the structure strength. This is important given collagen's role, as structural protein. This strength is increased by the fact that collagen molecules form further chains with other collagen molecules and form Covalent Cross Links with each other, which are staggered along the molecules to further increase stability. Collagen molecules wrapped around each other form Collagen Fibrils which themselves form Collagen Fibres.



Several key properties enable proteins to participate in such a wide **range of functions**. These are:

1. Proteins are linear polymers built of monomer units called amino acids. The construction of a vast array of macromolecules from a limited number of monomer building blocks is a recurring theme in biochemistry. Does protein function depend on the linear sequence of amino acids? The function of a protein is directly dependent on its threedimensional structure. Remarkably, proteins spontaneously fold up into three- dimensional structures that are determined by the sequence of amino acids in the protein polymer. Thus, proteins are the embodiment of the transition from the one- dimensional world of sequences to the three-dimensional world of molecules capable of diverse activities.
2. Proteins contain a wide range of functional groups. These functional groups include alcohols, thiols, thioethers, carboxylic acids, carboxamides, and a variety of basic groups. When combined in various sequences, this array of functional groups accounts for the broad spectrum of protein function.

For instance, the chemical reactivity associated with these groups is essential to the function of enzymes, the proteins that catalyze specific chemical reactions in biological systems.

3. Proteins can interact with one another and with other biological macromolecules to form complex assemblies. The proteins within these assemblies can act synergistically to generate capabilities not afforded by the individual component proteins. These assemblies include macromolecular machines that carry out the accurate replication of DNA, the transmission of signals within cells, and many other essential processes.

Amino acid sequencing:

Amino acid sequence is the order in which amino acid residues, connected by peptide bonds, lie in the chain in peptides and proteins. The sequence is generally reported from the N-terminal end containing free amino group to the C-terminal end containing free carboxyl group. Shorter peptides are sequenced with automated procedures but larger proteins must be sequenced in smaller segments.

Importance of amino acid sequencing:

- Knowledge of a protein's amino acid sequence is prerequisite for determining its three-dimensional structure and is essential for understanding its molecular mechanism of action.
- Many inherited diseases are caused by mutations that result in an amino acid change in a protein. Amino acid sequence analysis can assist in the development of diagnostic tests and effective therapies.
- Sequence comparisons among analogous proteins from different species yield insights into protein function and reveal evolutionary relationships among the proteins and the organisms that produce them.

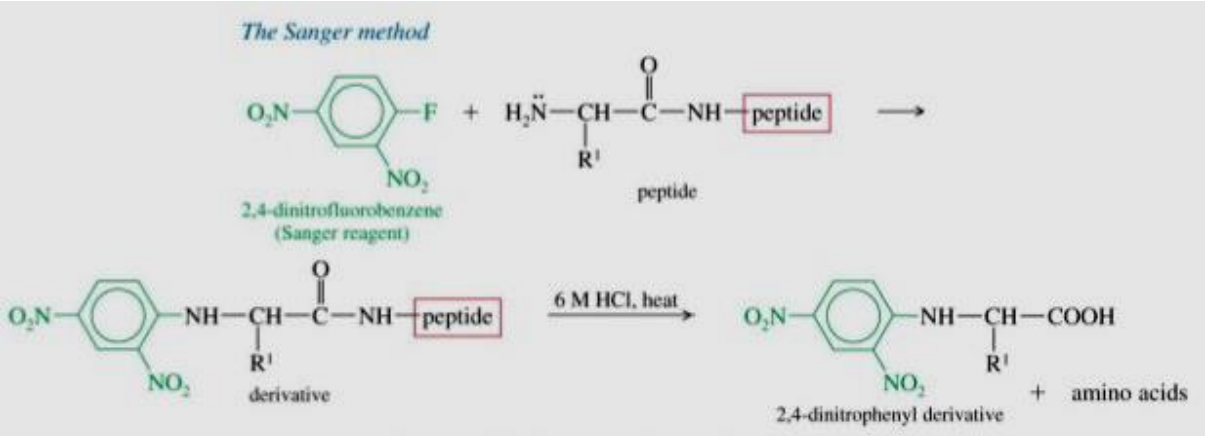
Frederick Sanger determined the first known protein sequence, that of hormone insulin in 1953. Since then, many additional proteins have been sequenced, and the sequences of many more proteins have been inferred from their DNA sequences. Sanger was awarded the Nobel Prize in 1958.

N-terminal analysis:

Each polypeptide chain (if it is not chemically blocked) has an Nterminal residue. Identifying this "end group" can establish the number of chemically distinct polypeptides in a protein. For example, insulin has equal amounts of the N-terminal residues Gly and Phe, which indicates that it has equal numbers of two nonidentical polypeptide chains.

Methods to determine n-terminal Amino acid

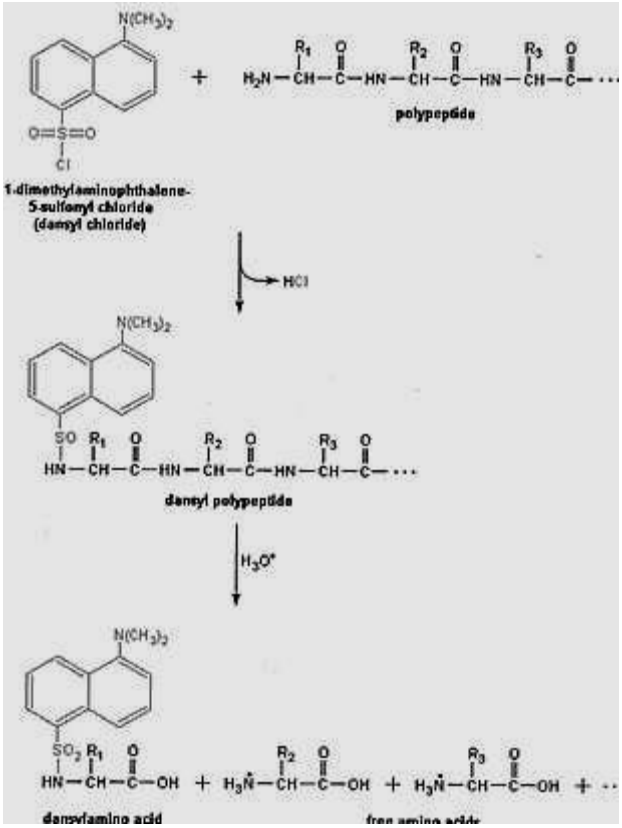
Sanger's method: Sanger developed the reagent 1-fluoro-2,4-dinitrobenzene (FDNB). After treatment of protein with FDNB , the amino terminal residue is labeled with FDNB and the polypeptide is hydrolyzed to its constituent amino acid . The labeled amino acid is identified.



N-terminal Analysis with Dansyl Chloride:

- Reagent: 1-dimethyl aminophthalene-5-sulfonyl chloride (dansyl chloride)
- Dansyl polypeptide chain is prepared
- Acidic hydrolysis liberates all amino acid and the N terminal dansyl amino acid
- Amino acids are separated
- Fluorescence of the dansyl amino acid is detected

- Type of aa is obtained from comparison with standard dansylated amino acid



Disadvantage:

The hydrolysis stage destroys the polypeptide , these procedures cannot be used to sequence a polypeptide beyond its amino-terminal residue.

Advantage:

It can help to determine the number of chemically distinct polypeptides in a protein , provided each has a different amino terminal residue.

Edman degradation:

The Edman degradation procedure labels and removes only the amino terminal residue from a peptide, leaving all other peptide bonds intact. The peptide is reacted with phenylisothiocyanate under mildly alkaline conditions, which converts the amino terminal amino acid to a **phenylthiocarbamoyl (PTC) adduct**. The peptide bond next to the PTC adduct is then cleaved in a step carried out in anhydrous trifluoroacetic acid, with removal of the amino- terminal amino acid as an anilinothiazolinone derivative. The derivatized amino acid is extracted with organic solvents, converted to the more stable phenylthiohydantoin derivative by treatment with aqueous acid and then identified.

- After removal and identification of the amino terminal residue, the new amino-terminal residue so exposed can be labeled, removed, and identified through the same series of reactions .This procedure is repeated until the entire sequence is determined.
- The Edman degradation is carried out in a machine, called a **sequenator** that mixes reagents in the proper proportions, separates the products, identifies them, and records the results.
- These methods are extremely sensitive. Often, the complete amino acid sequence can be determined starting with only a few micrograms of protein.

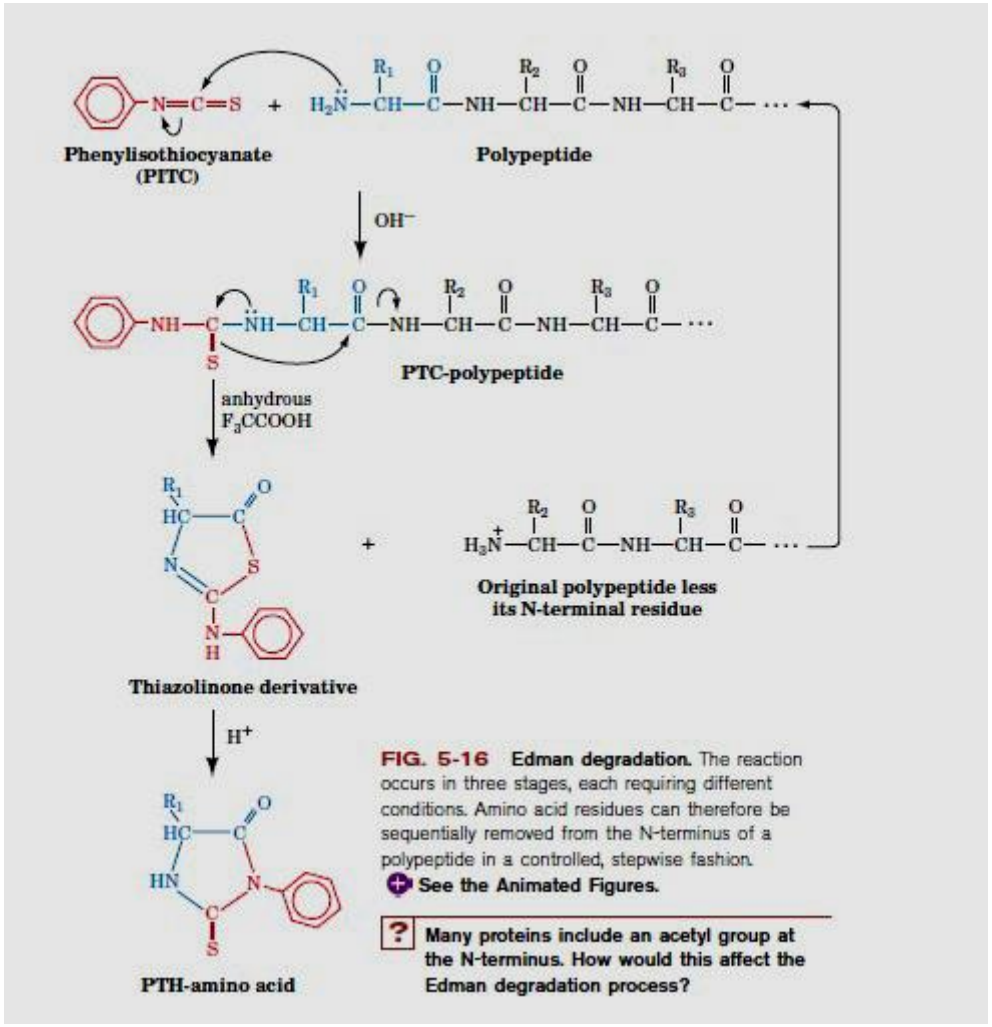
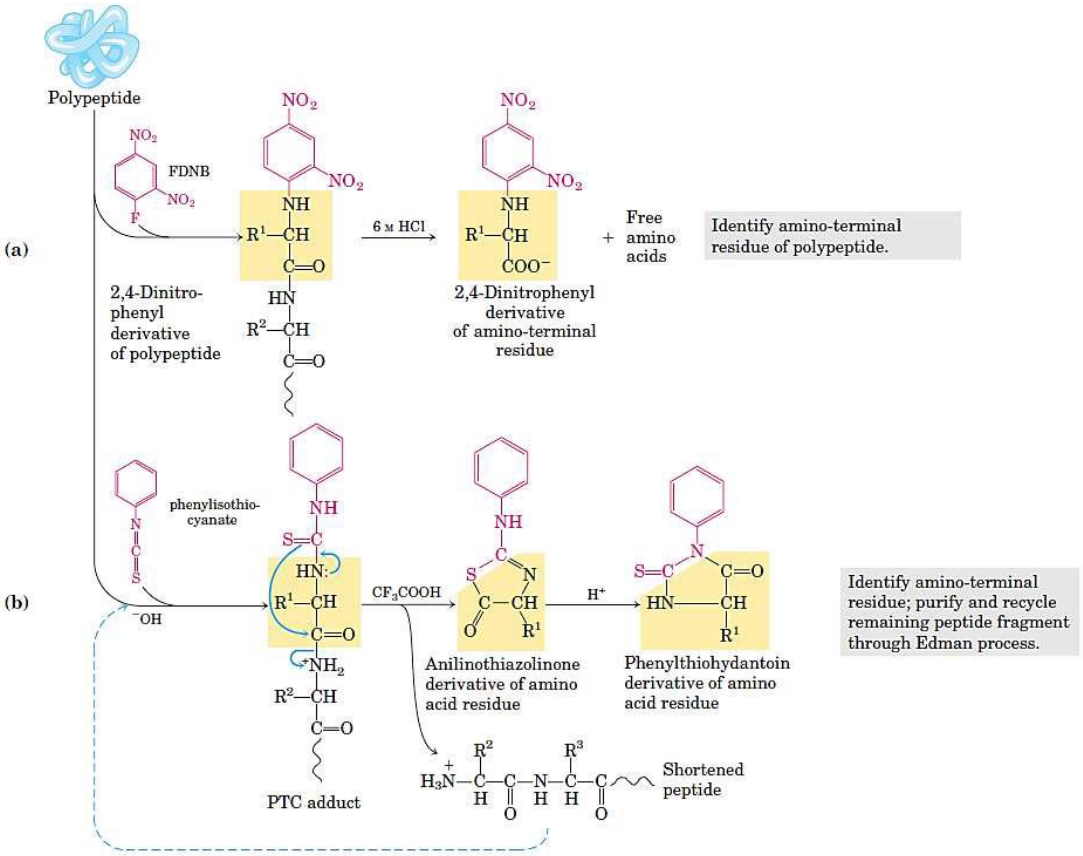


FIG. 5-16 **Edman degradation.** The reaction occurs in three stages, each requiring different conditions. Amino acid residues can therefore be sequentially removed from the N-terminus of a polypeptide in a controlled, stepwise fashion.
See the Animated Figures.

Many proteins include an acetyl group at the N-terminus. How would this affect the Edman degradation process?



C- Terminal Analysis:

There are two methods used for C-terminal determination. They are Hydrazine method and carboxy peptidase method.

Carboxypeptidases are enzymes that cleave amino acid residues from the C-termini of polypeptides in a successive fashion. Four carboxypeptidases are in general use: A, B, C, and Y.

Carboxypeptidase A (from bovine pancreas) works well inhydrolyzing the Cterminal peptide bond of all residues except proline, arginine, and lysine. The analogous enzyme from hog pancreas.

carboxypeptidase B, is effective only when Arg or Lys are the C-terminal residues. Thus, a mixture of carboxypeptidases A and B liberates any C-terminal amino acid except proline.

Carboxypeptidase C from citrus leaves and carboxypeptidase Y from yeast act on any C-terminal residue.

Sequencing of large proteins:

Breaking Disulfide Bonds: Disulfide bonds interfere with the sequencing procedure. If a cystine residue has one of its peptide bonds cleaved by the Edman procedure, it may remain attached to another polypeptide strand via its disulfide bond. Disulfide bonds also interfere with the enzymatic or chemical cleavage of the polypeptide.

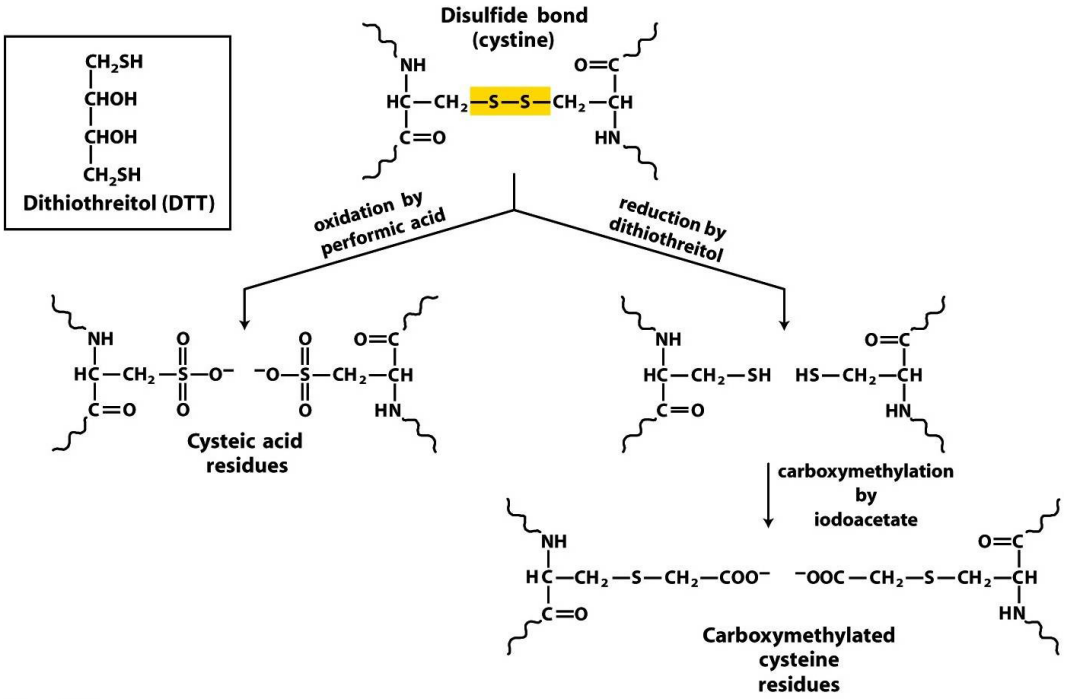


Figure 3-26
Lehninger Principles of Biochemistry, Fifth Edition

Cleaving polypeptide chains:

Enzymes called proteases catalyze the hydrolytic cleavage of peptide bonds.

REAGENT	CLEAVAGE POINTS
trypsin	Lys, Arg (C)
chymotrypsin	Phe, Trp, Tyr(C)
Asp-N-protease	Asp , Glu (C)
Pepsin	Leu , Phe, Trp, Tyr (N)
Elastase	Ala , Gly , Ser (C)
Cyanogen bromide	Met (C)
Endoproteinase Lys C	Lys (C)

Protein analysis by Electrophoresis:

What is Gel Electrophoresis?

Gel electrophoresis is a method for separation and analysis of macromolecules (DNA, RNA and proteins) and their fragments, based on their size and charge.

- Electrophoresis is a separation technique that is based on the movement of charged particles in an electric field.
- The term electrophoresis was coined from a Greek word **"Phoresis**" which means "**Being Carried Away**".
- Hence literal meaning of the word electrophoresis means "to carry with electricity."
- This electro kinetic phenomenon was observed for the first time in 1807 by **Reuss**.
- Electrophoresis through agarose or polyacrylamide gels is a standard method used to separate, identify and purify biopolymers, since both these gels are porous in nature.
- Polyacrylamide gels are chemically cross-linked gels formed by the polymerization of acrylamide with a cross-linking agent, usually N,N'-methylenebisacrylamide.
- The reaction is a free radical polymerization, usually carried out with ammonium persulfate as the initiator and **N,N,N',N'-tetramethylethylendiamine (TEMED)** as the catalyst.
- Polyacrylamide gel electrophoresis (PAGE) is a technique widely used in biochemistry, forensic chemistry, genetics, molecular biology and biotechnology to separate biological macromolecules, usually proteins or nucleic acids, according to their electrophoretic mobility.
- The most commonly used form of polyacrylamide gel electrophoresis is the Sodium dodecyl suplhate Polyacrylamide gel electrophoresis (SDS- PAGE) used mostly for the separation of proteins.

SDS-Page:

Sodium Dodecyl Sulfate (SDS) polyacrylamide gel electrophoresis is mostly used to separate proteins accordingly by size. This is one of the most powerful techniques to separate proteins on the basis of their molecular weight.

Principle:

This technique uses anionic detergent Sodium Dodecyl Sulfate (SDS) which dissociates proteins into their individual polypeptide subunits and gives a uniform negative charge along each denatured polypeptide. SDS also performs another important task.

It forces polypeptides to extend their conformations to achieve similar charge: mass ratio. SDS treatment therefore eliminates the effects of differences in shape so that chain length, which reflects their molecular mass, is the sole determinant of migration rate of proteins in the process of electrophoresis.

When these denatured polypeptides are loaded at the cathode end of an electrophoretic tank having polyacrylamide gel (as the supporting media) and subjected to an electric field, then we get clear bands of proteins arranged in an decreasing order of their molecular mass from the cathode to anode.

The rate of movement is influenced by the gel's pore size and the strength of electric field. In SDS-PAGE the vertical gel apparatus is mostly used. Although it is used to separate proteins on a routine basis, SDS-PAGE can also be used to separate DNA and RNA molecules.

Instrumentation:

Physical Apparatus:

This includes the physical body of the experimental set-up. It is of following two types:

a. Electrophoretic Apparatus:

Vertical horizontal tank with electrodes, gel cassettes, Teflon spacers, clips, pipette or sy- ringe, comb, acrylic cover.

b. Power Supply:

A power supply of 100-200 volts is needed. This is ideal for running and transferring protein resolving gels

c. Staining Box:

These are trays in which the gels are stained and made up of clear plastics. These are resistant to most organic dyes, silver and other stains.

Chemical Components:

This includes the following:

(a) Supporting Media:

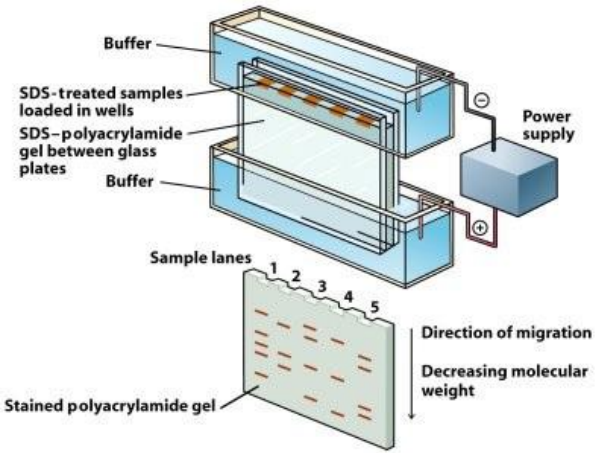
SDS-PAGE acrylamide is used as the supporting medium. It is a white crystalline powder, when acrylamide dissolves in water, it undergoes polymerization reaction to form a net-like structure called polyacrylamide. Polyacrylamide is a polymer ($CF_2CHCONH_2$-) formed from acrylamide subunits that can also be readily cross-linked.

This type of electrophoresis has a discontinuous system of gel, i.e., we have two different systems of gels present in the electrophoretic tank physically placed one over another.

These are as follows:

Resolving Gel:

This is also called separating or running gel. The separating gel constitutes about 2/3rd of the length of gel plate and is prepared by 5-10% of acrylamide. The pores in this gel (which is formed after the polyacrylamide is cross- linked) are numerous and smaller in diameter which impacts sieving property to this gel.



Stacking gel:

Stacking gel is poured on the top of resolving gel and a gel comb is inserted which forms the well. It is the upper layer of gel and constitutes 1/3rd of the gel plate. The percentage of acrylamide is chosen depending on the size of protein that one wishes to identify or probe in the sample.

The smaller the known weight, the higher the percentage that should be used. Generally, the percentage of acrylamide in stacking gel is 2-5%. It is highly porous and devoid of molecular sieving action.

(b) Buffer:

Two types of buffers are used in SDS-PAGE. The lower reservoir (which has the running gel) has amine buffers. It is adjusted by using HCl. The upper reservoir (which has stacking gel) also has amine buffers but its pH is slightly above that of running gel buffer and is adjusted with glycine instead of HCl.

(c) Dissociating Agent:

SDS is the most common dissociating agent used to denature native proteins to individual polypeptides. When a protein mixture is heated to 100°C in presence of SDS, the detergent wraps around the polypeptide backbone.

It binds to polypeptides in a constant weight ratio of 1.4 g/g of polypeptide. In this process, the intrinsic charges of polypeptides becomes negligible when compared to the negative charges contributed by SDS. Thus, polypeptides after treatment become rod-like structure possessing uniform charge density, that is the same net negative charge per unit length.

(d) Stains:

The stains are used to see the bands of separated proteins after the process of electrophoresis. **Coomassie Brilliant Blue R-250 (CBB)** is the most popular protein stain. It is an anionic dye, which binds with proteins non-specifically.

Proteins in the gel are fixed by acetic acid and simultaneously stained. The excess dye in- corporated in the gel can be removed by de-staining with the same solution but without the dye. The proteins are detected as blue bands on a clear background.

Procedure:

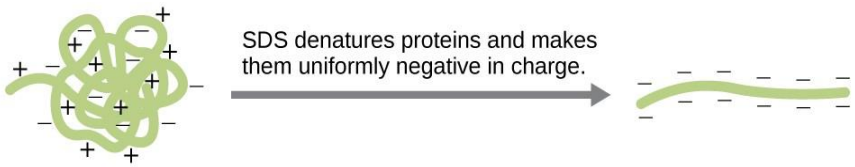
The solution of proteins to be analysed is first mixed with SDS, an anionic detergent, an an- ionic detergent which denatures secondary structure. Besides addition of SDS, proteins may optionally be boiled in the presence of a reducing agent, such as Di-Thio-Threitol (DTT) or 2-mercaptoethanol, which further denatures the proteins by reducing disulfide linkages, thus overcoming some forms of tertiary protein folding, and breaking up quaternary protein structure (Oligomeric subunits).

This is known as reducing SDS-PAGE, and is most commonly used. Non-reducing SDS- PAGE (no boiling and no reducing agent) may be used when native structure is important in further analysis (e.g., enzyme activity, shown by the use of zymograms). The denatured proteins are subsequently loaded into the wells of stacking gel flooded with stacking buffer.

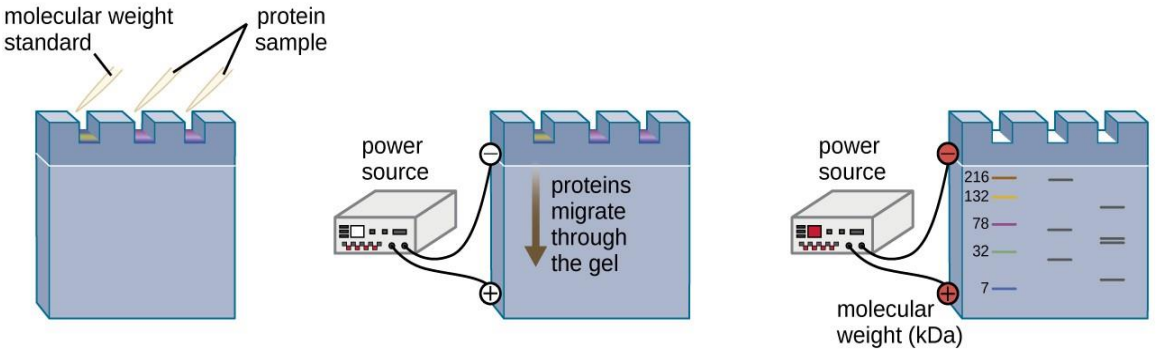
This end is connected with the cathode of power supply. Then an electric current is applied across the gel, causing negatively charged proteins to migrate across the gel towards anode. After crossing the stacking gel, denatured proteins enter the running gel which has its own buffer system (running buffer).

Depending on their size, each protein will move differently through the gel matrix: short proteins will more easily fit through the pores in the gel, while larger ones will have more difficulty.

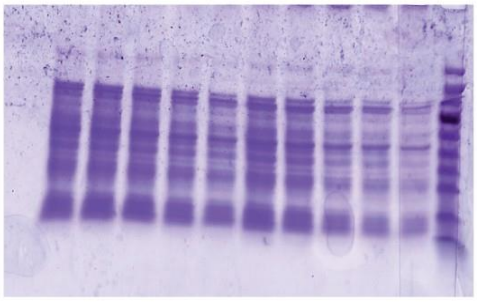
After the separation is over the gel is gently taken out and transferred to the staining box and treated with the staining dye, e.g., CBB R-250. Excess of stains are removed by de-staining using acetic acid solution. The bands appear to be blue stained which are then analysed according to the need of the experiment.



(a)



(b)



(c)

Application:
SDS-PAGE has many applications. It is mostly used for following purposes:

1. Establishing protein size
2. Protein identification
3. Determining sample purity
4. Identifying disulfide bonds
5. Quantifying proteins
6. Blotting applications

Advantages of SDS-PAGE:
SDS-PAGE has following advantages:

1. Mobility of the molecules is high and separation is rapid.
2. All the proteins are negatively charged; therefore, all migrate towards anode.
3. The proteins treated with SDS fixed dyes are better than the native proteins.
4. SDS solubilizes all proteins, including very hydrophobic and even denatured proteins.

Other Types of PAGE
Blue Native PAGE (BN-PAGE)
BN-PAGE is used to separate and characterize large protein complexes in their native and active forms. Originally described by Schagger and von Jagow (1987), this technique relies on the solubilization of protein complexes with mild, neutral detergents and the binding of negatively charged Coomassie (Brilliant) Blue G-250, stain to their surfaces. This imparts a high charge-to-mass ratio that allows the protein complexes to migrate to the anode as they do in SDS-PAGE. Coomassie Blue does not, however, denature and dissociate protein complexes the way SDS does.
Zymogram PAGE
Zymogram PAGE is used to detect and characterize collagenases and other proteases within the gel. Gels are cast with gelatin or casein, which acts as a substrate for the enzymes that are separated in the gel under nonreducing conditions. The proteins are run with denaturing SDS in order to separate by molecular weight. After renaturing the enzymes and then allowing them to break down the substrate, zymogram gels are stained with Coomassie (Brilliant) Blue R-250 stain, which stains the substrate while leaving clear areas around active proteases.
Isoelectric Focusing (IEF)
IEF combines the use of an electric field with a pH gradient to separate proteins according to their isoelectric point (pI). When a protein moves through a pH gradient, its net charge changes in response to the pH it encounters. Under the influence of an electric field, a protein in a pH gradient migrates to a position until its net charge is zero (see 2-D Electrophoresis for more details).
2-D Electrophoresis
The sequential application of different electrophoresis techniques produces a multi-dimensional separation. The most common 2-D technique (O'Farrell 1975) subjects protein samples first to denaturing IEF on a tube gel or IPG gel strip (for separation by pI), and then to SDS-PAGE for further separation by molecular weight. High-resolution 2-D methods enable separation of thousands of polypeptides in a single slab gel. The resulting spots can be visualized by gel staining, or they can be transferred to a membrane support for total protein staining or analysis with specific antibody detection.

Mass spectrophotometry:
Mass spectrometry (MS) is an analytical technique that measures the **mass-to-charge ratio of ions**. The results are typically presented as a mass spectrum, a plot of intensity as a function of the mass-to-charge ratio. Mass spectrometry is used in many different fields and is applied to pure samples as well as complex mixtures.
A mass spectrum is a plot of the ion signal as a function of the mass-to-charge ratio. These spectra are used to determine the elemental or isotopic signature of a sample, the masses of particles and of molecules, and to elucidate the chemical identity or structure of molecules and other chemical compounds.
In a typical MS procedure, a sample, which may be solid, liquid, or gaseous, is ionized, for example by bombarding it with electrons. This may cause some of the sample's molecules to break into

charged fragments or simply become charged without fragmenting. These ions are then separated according to their mass-to-charge ratio, for example by accelerating them and subjecting them to an electric or magnetic field: ions of the same mass-to-charge ratio will undergo the same amount of deflection. The ions are detected by a mechanism capable of detecting charged particles, such as an electron multiplier. Results are displayed as spectra of the signal intensity of detected ions as a function of the mass-to-charge ratio. The atoms or molecules in the sample can be identified by correlating known masses (e.g. an entire molecule) to the identified masses or through a characteristic fragmentation pattern.

Basic Principle

A mass spectrometer generates multiple ions from the sample under investigation; it then separates them according to their specific mass-to-charge ratio (m/z), and then records the relative abundance of each ion type.

The first step in the mass spectrometric analysis of compounds is the production of gas phase ions of the compound, basically by electron ionization. This molecular ion undergoes fragmentation. Each primary product ion derived from the molecular ion, in turn, undergoes fragmentation, and so on. The ions are separated in the mass spectrometer according to their mass-to-charge ratio, and are detected in proportion to their abundance. A mass spectrum of the molecule is thus produced. It displays the result in the form of a plot of ion abundance versus mass-to-charge ratio. Ions provide information concerning the nature and the structure of their precursor molecule. In the spectrum of a pure compound, the molecular ion, if present, appears at the highest value of m/z (followed by ions containing heavier isotopes) and gives the molecular mass of the compound.

Components

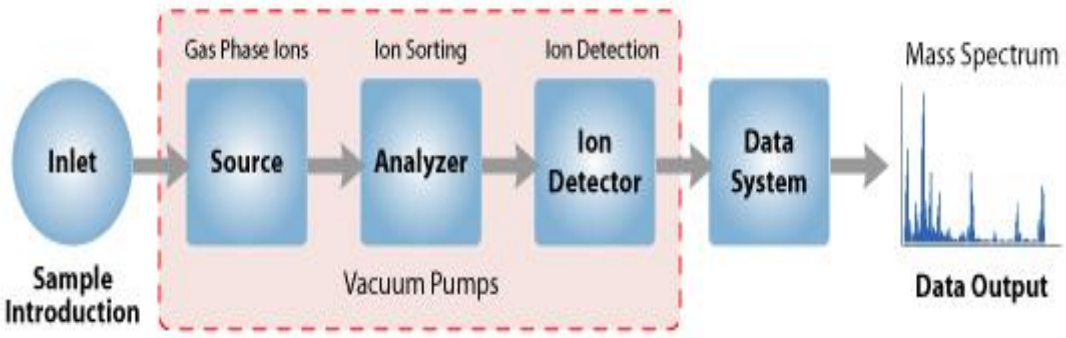
The instrument consists of three major components:

Ion Source: For producing gaseous ions from the substance being studied.

Analyzer: For resolving the ions into their characteristics mass components according to their mass-to-charge ratio.

Detector System: For detecting the ions and recording the relative abundance of each of the resolved ionic species.

In addition, a sample introduction system is necessary to admit the samples to be studied to the ion source while maintaining the high vacuum requirements (~10^{-6} to 10^{-8} mm of mercury) of the technique; and a computer is required to control the instrument, acquire and manipulate data, and compare spectra to reference libraries.



Components of a Mass Spectrometer

With all the above components, a mass spectrometer should always perform the following processes:

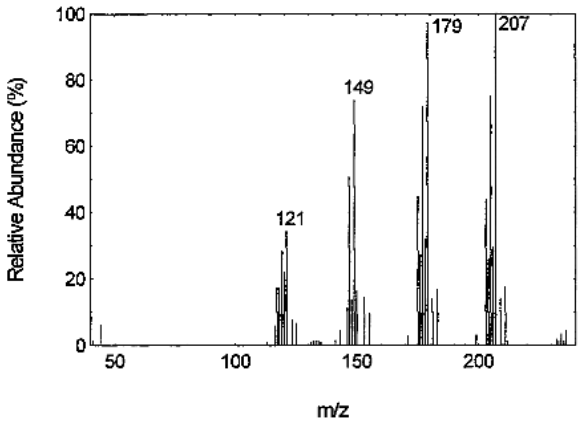
- Produce ions from the sample in the ionization source.
- Separate these ions according to their mass-to-charge ratio in the mass analyzer.
- Eventually, fragment the selected ions and analyze the fragments in a second analyzer.
- Detect the ions emerging from the last analyzer and measure their abundance with the detector that converts the ions into electrical signals.
- Process the signals from the detector that are transmitted to the computer and control the instrument using feedback.

Calculations and Deductions:

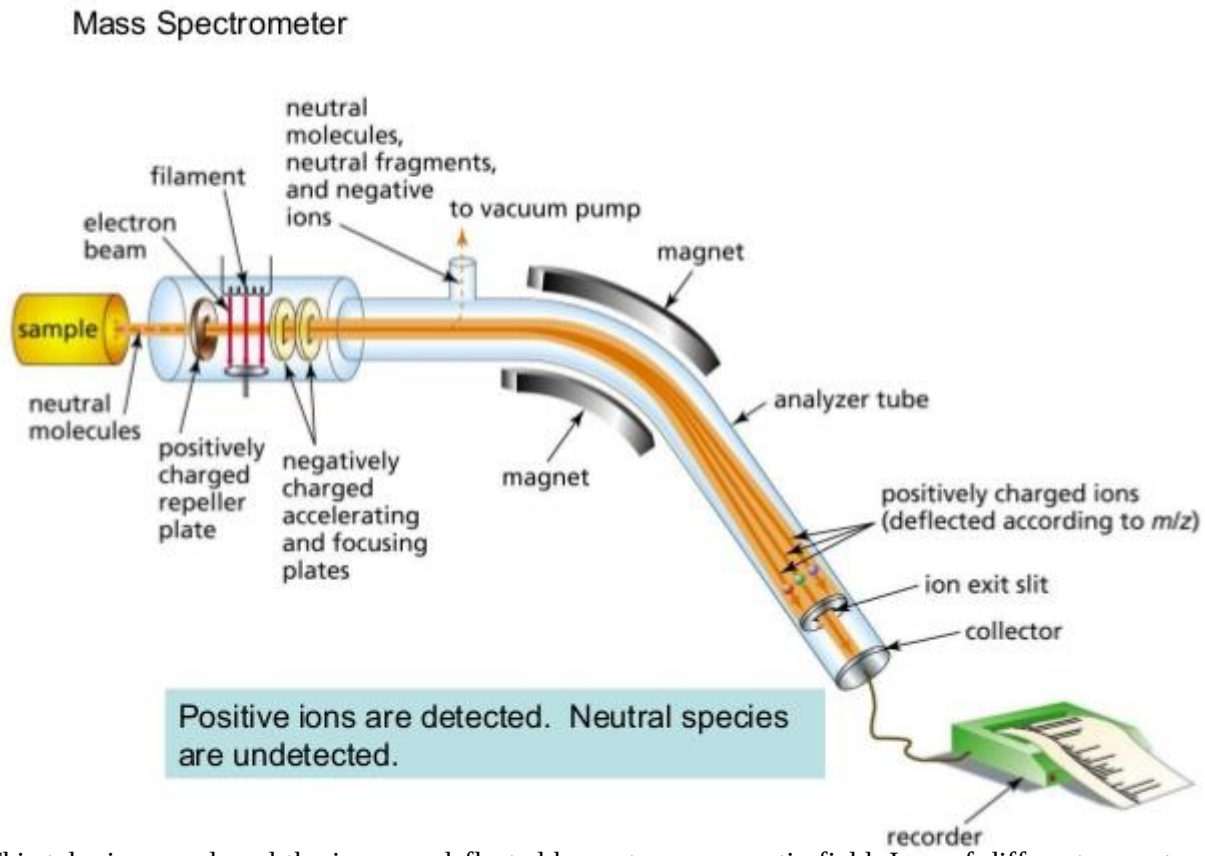
Given a sample of a single element, the relative atomic mass can be calculated by looking at the peaks and performing a simple mean calculation.

The relative molecular mass can be deduced by looking for the Molecular Ion peak, since this will be the peak caused by the whole molecule.

The structure of a molecule can be deduced by looking at the smaller peaks and inferring the structure of those, given the likely combinations of atoms present that could produce that mass. This is because these peaks will be cause by fragments of the whole molecule. The stronger the bond between atoms, the less likely it is to break, and so the lower the abundance of the fragments that would be formed by the breaking of that bond.



In reality, peaks will not be perfectly clear because of the varying mass of individual atoms. However, smaller peaks like this can help to determine the structure of the larger ones.

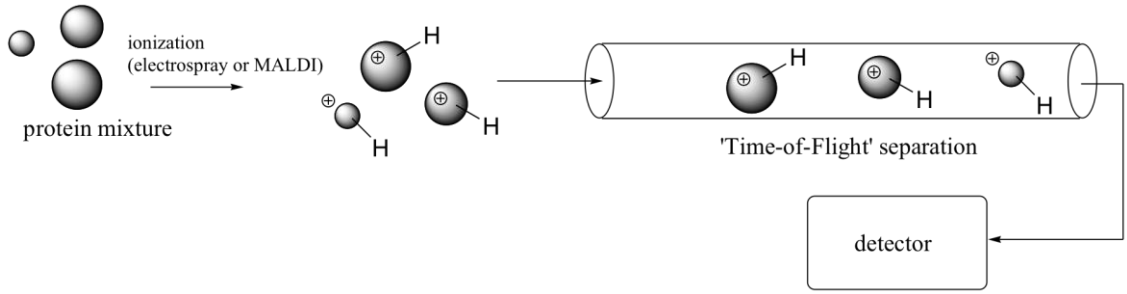


This tube is curved, and the ions are deflected by a strong magnetic field. Ions of different mass to charge (m/z) ratios are deflected to a different extent, resulting in a 'sorting' of ions by mass (virtually all ions have charges of z = +1, so sorting by the mass to charge ratio is the same thing as sorting by mass). A detector at the end of the curved flight tube records and quantifies the sorted ions.

Mass spectrometry of proteins - applications in proteomics

Electron ionization mass spectrometry is generally not very useful for analyzing biomolecules: their high polarity makes it difficult to get them into the vapor phase, the first step in EIMS. Mass spectrometry of biomolecules has undergone a revolution over the past few decades, with many new ionization and separation techniques being developed. Generally, the strategy for biomolecule analysis involves soft ionization, in which much less energy (compared to techniques such as EIMS)

is imparted to the molecule being analyzed during the ionization process. Usually, soft ionization involves adding protons rather than removing electrons: the cations formed in this way are significantly less energetic than the radical cations formed by removal of an electron. The result of soft ionization is that little or no fragmentation occurs, so the mass being measured is that of an intact molecule. Typically, large biomolecules are digested into smaller pieces using chemical or enzymatic methods, then their masses determined by 'soft' MS.
New developments in soft ionization MS technology have made it easier to detect and identify proteins that are present in very small quantities in biological samples. In electrospray ionization (ESI), the protein sample, in solution, is sprayed into a tube and the molecules are induced by an electric field to pick up extra protons from the solvent. Another common 'soft ionization' method is 'matrix-assisted laser desorption ionization' (MALDI). Here, the protein sample is adsorbed onto a solid matrix, and protonation is achieved with a laser.



Typically, both electrospray ionization and MALDI are used in conjunction with a time-of-flight (TOF) mass analyzer component.
The proteins are accelerated by an electrode through a column, and separation is achieved because lighter ions travel at greater velocity than heavier ions with the same overall charge. In this way, the many proteins in a complex biological sample (such as blood plasma, urine, etc.) can be separated and their individual masses determined very accurately. Modern protein MS is extremely sensitive – recently, scientists were even able to detect the presence of Tyrannosaurus rex protein in a fossilized skeleton! (Science 2007, 316, 277).
Soft ionization mass spectrometry has become in recent years an increasingly important tool in the field of proteomics. Traditionally, protein biochemists tend to study the structure and function of individual proteins. Proteomics researchers, in contrast, want to learn more about how large numbers of proteins in a living system interact with each other, and how they respond to changes in the state of the organism. One important subfield of proteomics is the search for protein 'biomarkers' for human disease: in other words, proteins which are present in greater quantities in the tissues of a sick person than in a healthy person. Detection in a healthy person of a known biomarker for a disease such as diabetes or cancer could provide doctors with an early warning that the patient may be especially susceptible to the disease, so that preventive measures could be taken to prevent or delay onset.
In a 2005 study, MALDI-TOF mass spectrometry was used to compare fluid samples from lung transplant recipients who had suffered from tissue rejection to samples from recipients who had not suffered rejection. Three peptides (short proteins) were found to be present at elevated levels specifically in the tissue rejection samples. It is hoped that these peptides might serve as biomarkers to identify patients who are at increased risk of rejecting their transplanted lungs. (Proteomics 2005, 5, 1705).

X-ray crystallography:
X-ray crystallography (XRC) is a technique used to determine the atomic and molecular structure of a crystal, in which the crystalline structure causes a beam of incident X-rays to diffract into many specific directions. By measuring the angles and intensities of these diffracted beams, a crystallographer can produce a three-dimensional picture of the density of electrons within the crystal. From this electron density, the mean positions of the atoms in the

crystal can be determined, as well as their chemical bonds, their crystallographic disorder, and various other information.

History:

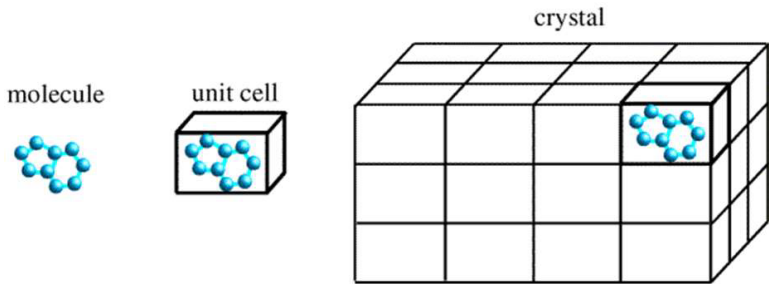
The English physicist **Sir William Henry Bragg** pioneered the determination of crystal structure by X-ray diffraction methods. Using X-ray crystal data, Dr. James Watson and Dr. Francis Crick were able to determine the helix structure of DNA in 1953.

Why X rays?

An electromagnetic wave of high energy and very short wavelength, which is able to pass through many materials opaque to light.(wavelength 1 angstrom). The wavelength of X-ray photons is on the order of the distance between atomic nuclei in solids (bonds are roughly 1.5-2.5 Å). You can think of it like the waves fit nice between the atoms and "fill" the crystal.

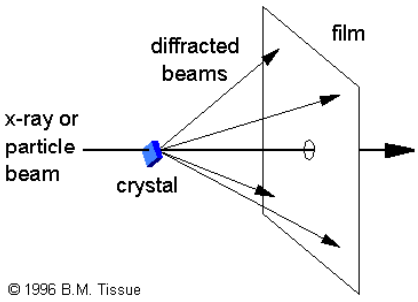
Why Crystal?

- Researchers crystallize an atom or molecule, because the precise position of each atom in a molecule can only be determined if the molecule is crystallized.
- If the molecule or atom is not in a crystallized form, the X-rays will diffract unpredictably and the data retrieved will be too difficult if not impossible to understand.



X-ray diffraction:

- Diffraction is the slight bending of light as it passes around the edge of an object.
- X-ray crystallography uses the uniformity of light diffraction of crystals to determine the structure of molecule or atom
- Then X-ray beam is used to hit the crystallized molecule.
- The electron surrounding the molecule diffract as the X-rays hit them.
- This forms a pattern. This type of pattern is known as X-ray diffraction pattern.

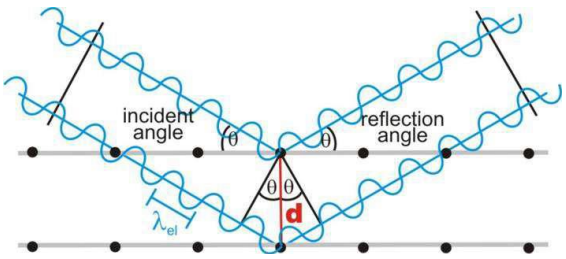


Bragg's Law

There is a definite relationship between the angle at which a beam of X rays must fall on the parallel planes of atoms in a crystal in order that there be strong reflection, the wavelength of the X rays, and the distance between the crystal planes

$$2d \sin\Theta = n\lambda$$

Here d is the spacing between diffracting planes, Θ is the incident angle, n is any integer, and λ is the wavelength of the beam

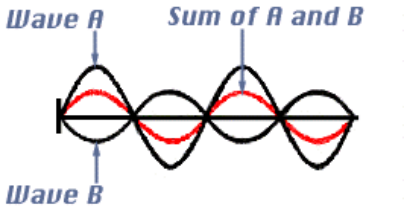


Interference:

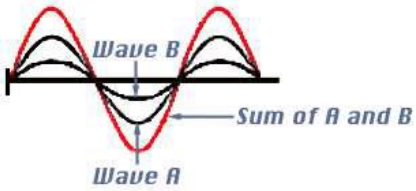
When an incident x-ray beam hits a scatterer, scattered x-rays are emitted in all directions. Most of the scattering waves fronts are out of phase interfere destructively. Some sets of wave fronts are in phase and interfere constructively.

A crystal is composed of many repeating unit cells in 3-dimensions, and therefore, acts like a 3-dimensional diffraction grating. The constructive interference from a diffracting crystal is observed as a pattern of points on the detector. The relative positions of these points are related mathematically to the crystal's unit cell dimensions.

Destructive Interference

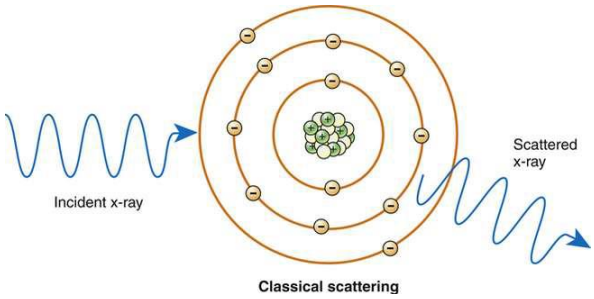


Constructive Interference



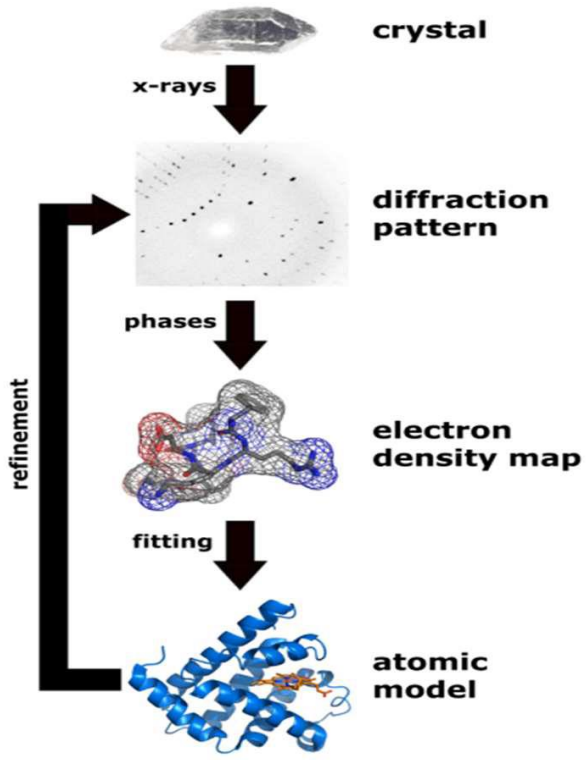
Interactions between X-rays and atoms:

X-rays are scattered almost exclusively by the electrons in the atoms, not by the nuclei. The incident electromagnetic wave exerts a force on the electrons. This causes the electrons to oscillate with the same frequency as the incident radiation. The oscillating electrons act as radiation scatters and emit radiation at the same frequency as the incident radiation.



Procedure:

1. The first-and often most difficult-step is to obtain an adequate crystal of the material under study.
2. The crystal should be sufficiently large (typically larger than 0.1 mm in all dimensions), pure in composition and regular in structure, with no significant internal imperfections such as cracks.
3. Researchers crystallize an atom or molecule, because the precise position of each atom in a molecule can only be determined if the molecule is crystallized.
4. The crystal is placed in an intense beam of X-rays, usually of a single wavelength (monochromatic Xrays), producing the regular pattern of reflections.
5. As the crystal is gradually rotated, previous reflections disappear and new ones appear; the intensity of every spot is recorded at every orientation of the crystal.
6. Multiple data sets may have to be collected, with each set covering slightly more than half a full rotation of the crystal and typically containing tens of thousands of reflections.
7. In the third step, these data are combined computationally with complementary



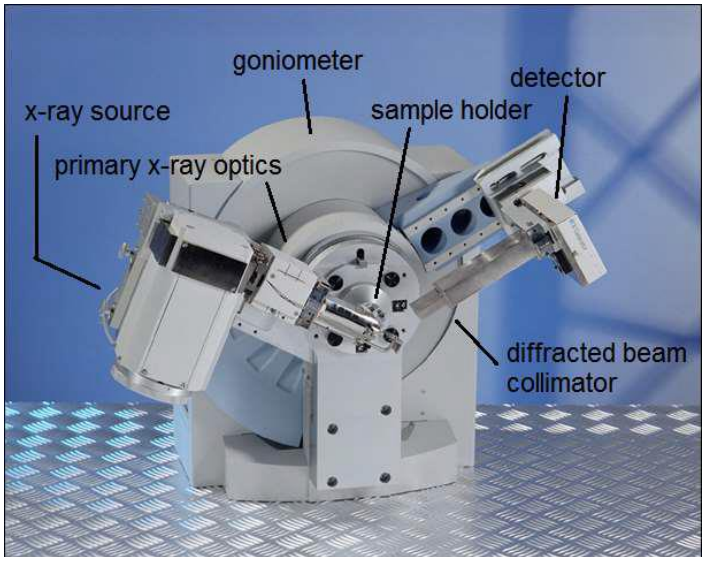
chemical information to produce and refine a model of the arrangement of atoms within the crystal. The final, refined model of the atomic arrangement-now called a crystal structure is usually stored in a public database.

8. After the diffraction pattern is obtained, the data is then processed by a computer and the structure of the atom or molecule is deduced and visualized.

Instrumentation and Limitations:

Generally a typical x-ray diffraction contain below parts:

- X-ray source
- Detector
- Crystal on the end of mounting needle
- Liquid nitrogen steam to keep crystal cold
- Movable mount to rotate crystal
- Collimator



- **X-ray Tube:** the source of X Rays
- **Incident-beam optics:** condition the X-ray beam before it hits the sample
- **The goniometer**: the platform that holds and moves the sample, optics, detector, and/or tube
- **The sample & sample holder**
- **Receiving-side optics:** condition the X-ray beam after it has encountered the sample
- **Detector:** count the number of X Rays scattered by the sample.

Limitations:

- Small-molecule crystallography typically involves crystals with fewer than 100 atoms in their asymmetric unit; such crystal structures are usually so well resolved that the atoms can be discerned as isolated "blobs." Of electron density.
- By contrast, macromolecular crystallography often involves tens of thousands of atoms in the unit cell. Such crystal structures are generally less well-resolved (more "smeared out"); the atoms and chemical bonds appear as tubes of electron density, rather than as isolated atoms.
- In general, small molecules are also easier to crystallize than macromolecules; however, X-ray crystallography has proven possible even for viruses with hundreds of thousands of atoms.

Application of X ray Crystallography

XRD is a nondestructive technique

- To identify crystalline phases and orientation
- To determine structural properties.
- To measure thickness of thin films and multi-layers
- To determine atomic arrangement
- X-ray diffraction is most widely used for the identification of unknown crystalline materials (e.g. minerals, inorganic compounds). Determination of unknown solids is critical to studies in geology, environmental science, material science, engineering and biology.

- ❖ Identification of fine-grained minerals such as clays and mixed layer clays that are difficult to determine optically.
- ❖ Determination of unit cell dimensions measurement of sample purity

Applications of X ray Crystallography in proteomics:

Protein structure determination:

Protein structure determination refers to finding the exact orientations and arrangements of different amino acids present in the protein. X ray crystallography helps us to determine the structure of proteins which further helps us to even determine its function.

Protein interaction studies

Protein interaction refers to the way in which two or more proteins interact with each other. Studies include the orientation, site of action and the major amino acids (of protein) taking part in a particular reaction ray crystallography helps to determine there Interactions.

Conformational studies

It is necessary to determine the arrangements as it determines the structure and function of Protein X ray crystallography is an efficient technique to determine it. Conformational studies refer to spatial arrangements of atoms in a molecule that can come about through free rotation of atoms about a chemical bond.

Enzyme catalysis determination:

Enzymes are protein. Determination of structure (specially active site's) and type of amino acids present in active sites determines catalytic activities, Interaction level of enzymes. X ray crystallography helps us to determine and predict the catalytic efficiency of enzymes.

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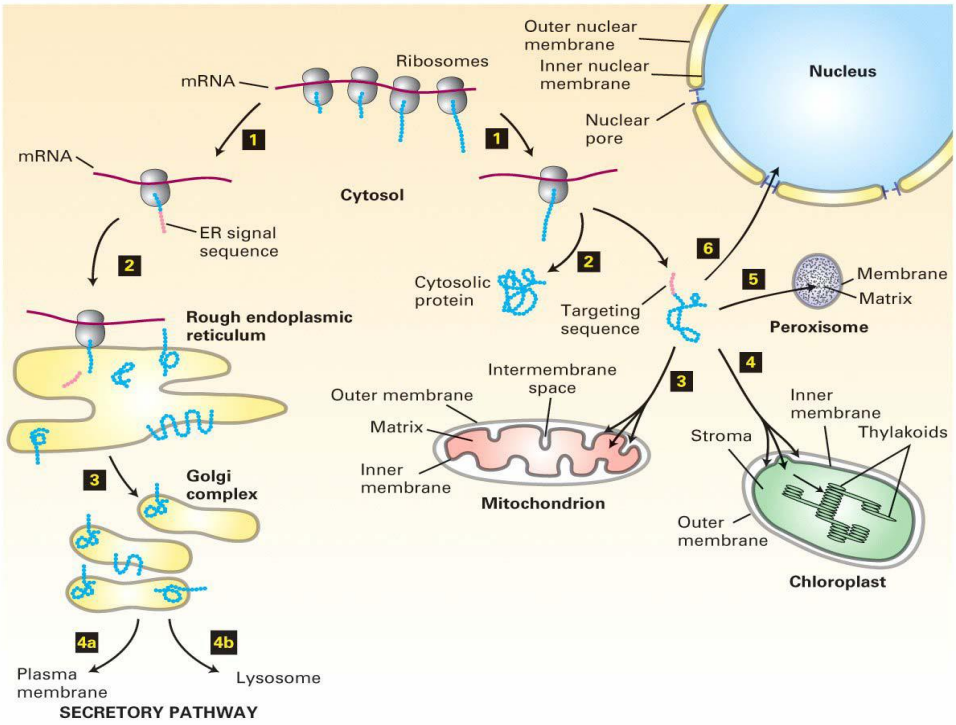
4. Protein sorting: protein targeting in organelles.

Introduction:

Both in prokaryotes and eukaryotes, newly synthesized proteins must be delivered to a specific sub cellular location or exported from the cell for correct activity. Protein targeting is necessary for proteins that are destined to work outside the cytoplasm. This delivery process is carried out based on information contained in the protein itself. Correct sorting is crucial for the cell; errors can lead to diseases.

The endomembrane system and secretory pathway:

Proteins destined for any part of the endomembrane system (or the outside of the cell) are brought to the ER during translation and fed in as they're made.



Types of Sorting Signals in Proteins

1. **Signal Sequence**
   - continuous sequence of 15-60 amino acids (aa's)
   - sometimes removed from finished protein
   - sometimes a part of finished protein
2. **Signal Patch**
   - specific 3D arrangement of atoms on protein surface; aa's distant
   - persist in finished protein

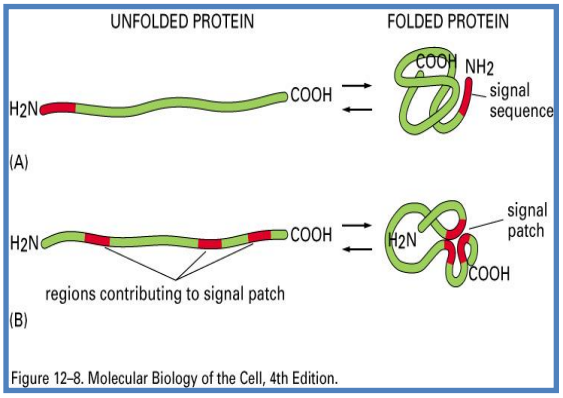


Figure 12–8. Molecular Biology of the Cell, 4th Edition.

Signal peptides:

The signal peptide that sends a protein into the endoplasmic reticulum during translation is a series of hydrophobic ("water-fearing") amino acids, usually found near the beginning (N- terminus) of the protein. When this sequence sticks out of the ribosome, it's recognized by a protein complex called the signal-recognition particle (SRP), which takes the ribosome, to the ER. There, the ribosome feeds its amino acid chain into the ER lumen (interior) as it's made.

1. Signal recognition particle (SRP) binds to the signal peptide as it emerges from the ribosome.
2. SRP brings the ribosome to the ER by binding to a receptor on the ER surface. The receptor is associated with other proteins that make a pore.
3. The ribosome resumes translating, feeding the polypeptide through the pore and into the ER lumen (interior).
4. An enzyme associated with the pore snips off the signal peptide.
5. Translation continues, and the growing amino acid chain slides into the ER lumen.
6. The completed polypeptide is released into the ER lumen, where it floats freely.
7. In some cases, the signal peptide is snipped off during translation and the finished protein is released into the interior of the ER (as shown above). In other cases, the signal peptide or another stretch of hydrophobic amino acids gets embedded in the ER membrane. This creates a transmembrane (membrane-crossing) segment that anchors the protein to the membrane.

TABLE 12–3 Some Typical Signal Sequences

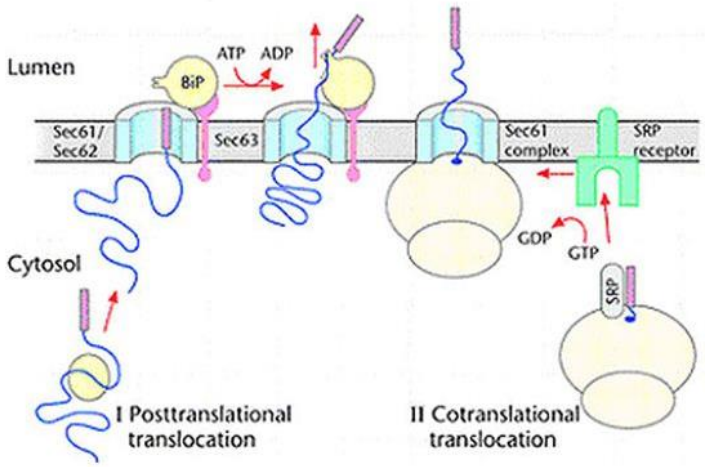
FUNCTION OF SIGNAL SEQUENCE	EXAMPLE OF SIGNAL SEQUENCE
Import into nucleus	-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-
Export from nucleus	-Leu-Ala-Leu-Lys-Leu-Ala-Gly-Leu-Asp-Ile-
Import into mitochondria	$^{+}H_3N$-Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-Cys-Ser-Ser-Arg-Tyr-Leu-Leu-
Import into plastid	$^{+}H_3N$-Met-Val-Ala-Met-Ala-Met-Ala-Ser-Leu-Gln-Ser-Ser-Met-Ser-Ser-Leu-Ser-Leu-Ser-Ser-Asn-Ser-Phe-Leu-Gly-Gln-Pro-Leu-Ser-Pro-Ile-Thr-Leu-Ser-Pro-Phe-Leu-Gln-Gly-
Import into peroxisomes	-Ser-Lys-Leu-COO^{-}
Import into ER	$^{+}H_3N$-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Leu-Val-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln-
Return to ER	-Lys-Asp-Glu-Leu-COO^{-}

Some characteristic features of the different classes of signal sequences are highlighted in color. Where they are known to be important for the function of the signal sequence, positively charged amino acids are shown in *red* and negatively charged amino acids are shown in green. Similarly, important hydrophobic amino acids are shown in *yellow* and hydroxylated amino acids are shown in *blue*. $^{+}H_3N$ indicates the N-terminus of a protein; COO^{-} indicates the C-terminus.

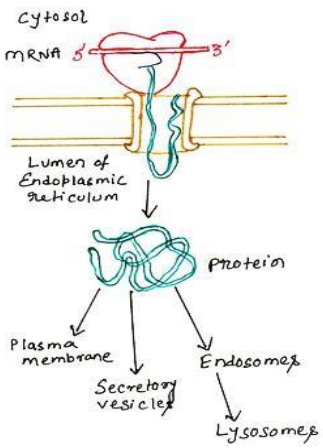
Protein translocation

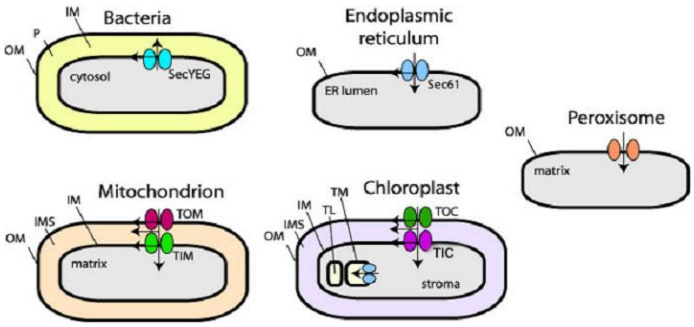
In 1970, **Günter Blobel** conducted experiments on the translocation of proteins across membranes. He was awarded the Nobel Prize (1999) for his findings. He discovered that many proteins have a

signal sequence, that is, a short amino acid sequence at one end that functions like a postal code for the target organelle.
Sorting or translocation of protein can occur in two ways:
1. Co-translational: Synthesised protein is transferred to an SRP receptor on the endoplasmic reticulum (ER), a membrane-enclosed organelle. There, the nascent protein is inserted into the translocation complex
2. Post-translational: Even though most proteins are co translationally translocated, some are translated in the cytosol and later transported to their destination. This occurs for proteins that go to a mitochondrion, a chloroplast, or a peroxisome.



Transport through the endomembrane system:
Eukaryotic cells posses distinct membrane bound organelles which are absent in prokaryotic cells. The membrane bound organelles have different functions and these organelles provide discrete compartments in which specific cellular activities take place. The complex internal organization of eukaryotic cells generates hardship for transport of proteins to their destinations.
Many proteins destined for the endoplasmic reticulum, the Golgi apparatus, lysosomes, the plasma membrane and secretion from the cell are synthesized on ribosomes that are bound to the membrane of endoplasmic reticulum.





IM-inner membrane, IMS- inner membrane space, P- Periplasm, OM- outer membrane, TL- thylakoid lumen, TM- thylakoid membrane and Sec YEG, Sec61, TOM, TIM, TOC are the protein subunits of the translocation systems.

Protein targeting to the endoplasmic reticulum:
The signal sequences cover about twenty amino acids, including a stretch of hydrophobic residues, usually at the amino terminus of the polypeptide chain. As soon as the signal sequences of the

growing polypeptide chain emerge from the ribosome, they are recognised and bound by a signal recognition particle (SRP) consisting of six polypeptides and a small cytoplasmic RNA (srp RNA). Then the complex containing the growing polypeptide chain, ribosome, and SRP is specifically targeted to the endoplasmic reticulum membrane by an interaction with a membrane-bounrd receptor, the SRP receptor or docking protein. In the next step, the SRP is released from both the ribosome and the signal sequence, where GTP (guanosine triphosphate) plays a key role. The ribosome then binds to a protein translocation complex in the membrane of the endoplasmic reticulum, and the signal sequence is inserted into a membrane channel or translocon. The translocons are complexes of three transmembrane proteins, known as Sec61 proteins. Transfer of the ribosome from the SRP to the translocon allows translation to resume, and the growing polypeptide chain is transferred directly into the translocon channel and across the membrane of the endoplasmic reticulum as translation proceeds. As translocation proceeds, the signal sequence is cleaved by the signal peptidase and the polypeptide is released into the lumen of the endoplasmic reticulum. Finally, GTP hydrolysis leads to the dissociation of the SRP from its receptor, and a new targeting cycle can begin. The actual transfer of the polypeptide through the membrane does not require the SRP or its receptor and commences only after their disengagement. Two basic functions are done by the SRP, where first it targets the polypeptide chain to the Endoplasmic reticulum membrane by interacting both with the signal sequence and with the translocation apparatus and secondly it keeps the bound signal sequence segregated from the rest of the polypeptide chain and thereby prevents aberrant, premature folding.

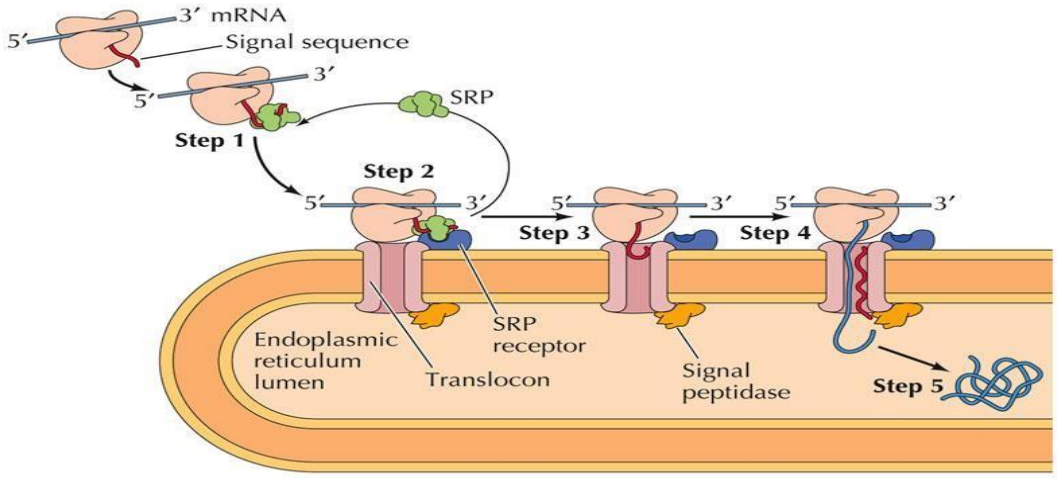


Figure: The co-translational pathway of transport of secretory proteins to the endoplasmic reticulum

Some proteins in mammals and many proteins in yeast are transported through post- translational pathway. These proteins are synthesized on free cytosolic ribosomes and these proteins do not require a signal recognition particle (SRP) for their transport. Their signal sequences are recognised by distinct receptor proteins associated with the translocon in the endoplasmic reticulum membrane. The polypeptide chains are remained in an unfolded conformation by the cytosolic Hsp70 chaperones.

ER and Protein Trafficking

Signal Sequence is Removed from Soluble Proteins

Two signaling functions:

1) Directs protein to ER membrane
2) Serves as “start transfer signal” to open pore

Signal peptidase removes terminal ER signal sequence upon release of protein into the lumen

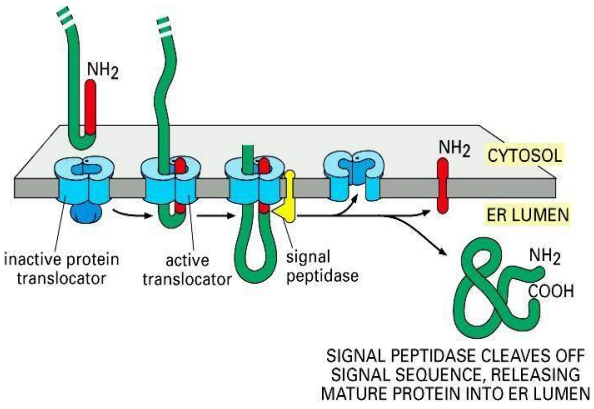


Figure 12–46. Molecular Biology of the Cell, 4th Edition.

Single Pass Transmembrane Proteins

1. N-terminal signal sequence initiates trans-location and additional hydrophobic "stop sequence anchors protein in membrane
2. Signal sequence is internal and remains in lipid bilayer after release from translocator
3. Internal signal sequence in opposite orientation
4. Orientation of start-transfer sequence governed by distribution of nearby charged aa

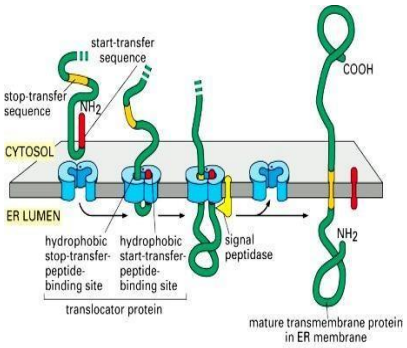


Figure 12–47. Molecular Biology of the Cell, 4th Edition.

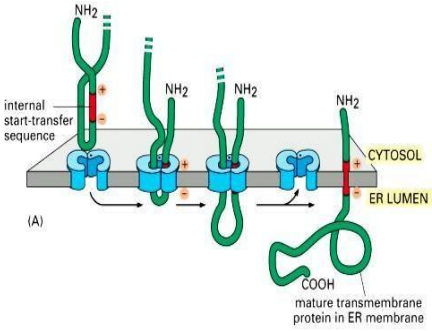


Figure 12–48 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

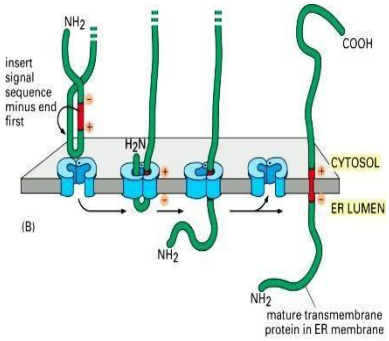


Figure 12–48 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

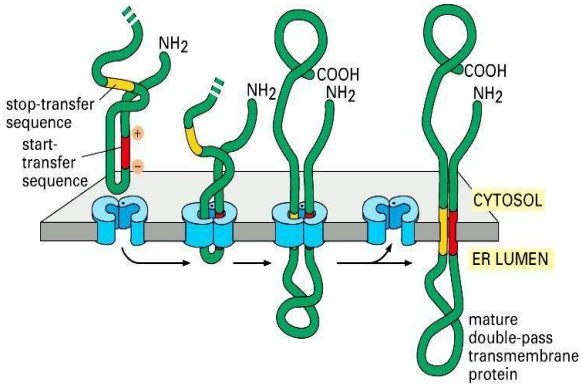


Figure 12–49. Molecular Biology of the Cell, 4th Edition.

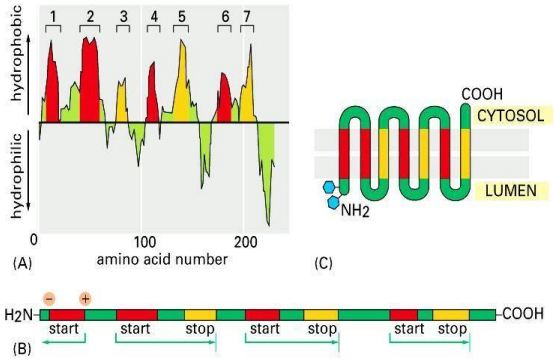


Figure 12–50. Molecular Biology of the Cell, 4th Edition.

Multipass Transmembrane Proteins

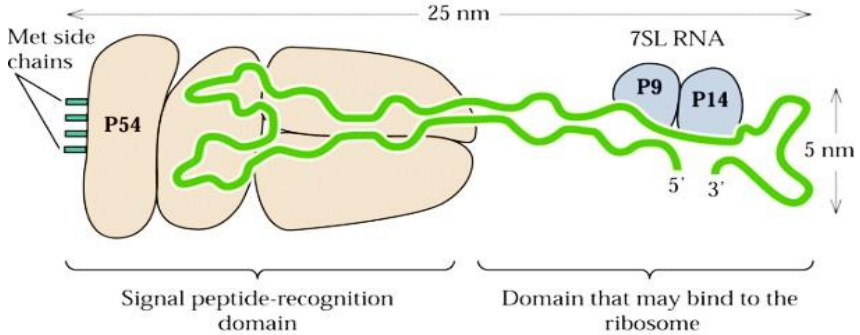
- Combinations of start- and stop-transfer signals determine topology
- Whether hydrophobic signal sequence is a start- or stop-transfer sequence depends upon its location in polypeptide chain
- All copies of same polypeptide have same orientation

The signal and receptor machine:

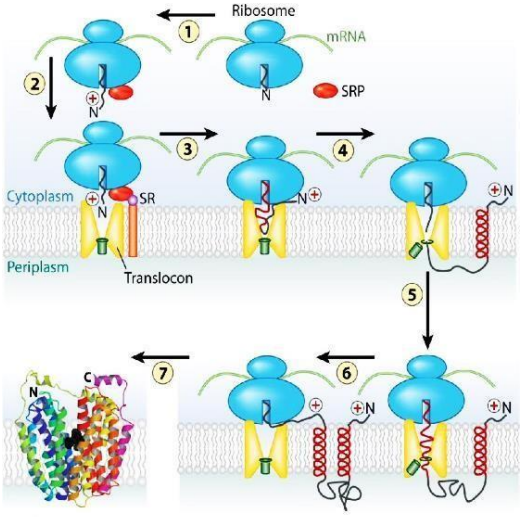
a) The signal peptide is often a hydrophobic stretch of 20-40 AA at the N-terminus of the protein. Cleaved after translocation into ER.

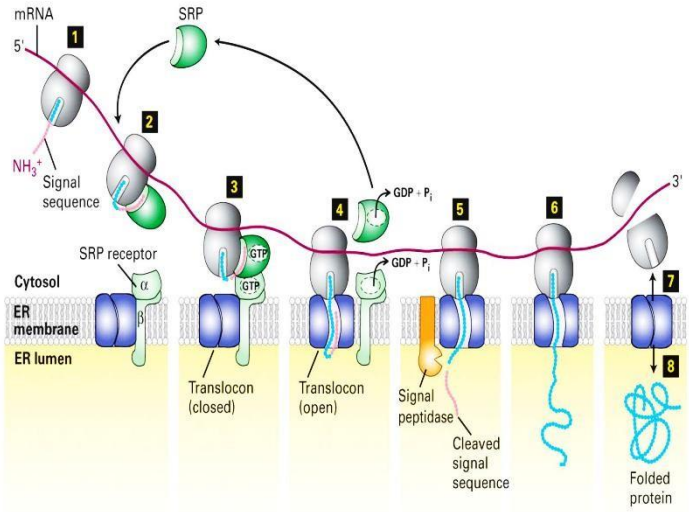
b) The SRP is a protein-RNA complex (1 RNA of 300 base long and 5 proteins). SRP54 is the major component that recognizes and binds the signal peptide. It is a GTPase.

SRP is normally located in the cytosol but can associate to the membrane by interacting with its receptor in the ER membrane.

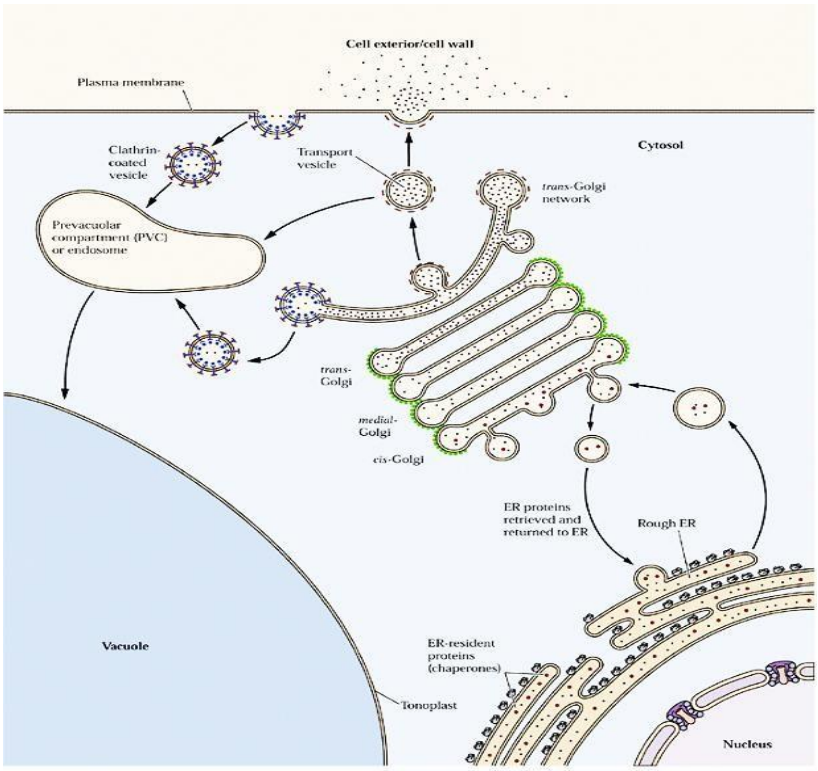


The SRP receptor: is an integral membrane protein
Complex of two subunits that are both GTPases.
The complex interacts with SRP—as docking site. One of the SRP receptor proteins also interacts with signal peptide.
The translocon: the protein channel - As the SRP and its receptor interacts, a third protein complex comes into play.
This translocon also serves as "receptor" for ribosome that in turn serves as a gating machine to block the channel from leakage of ER content to the cytosol.



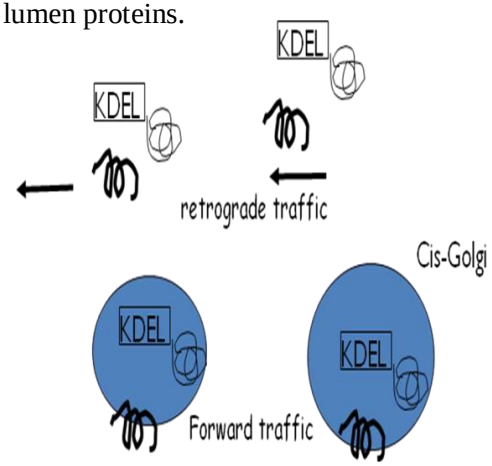


From ER to Golgi and beyond vesicular transport:
Cargo proteins are transported in a sealed container that is made of membrane vesicle.



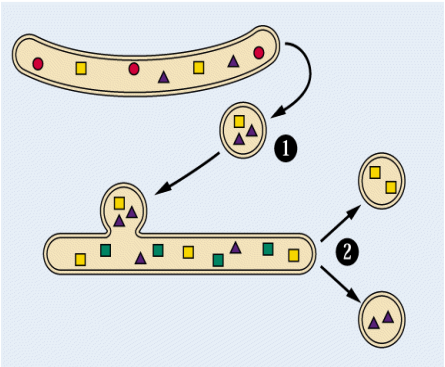
From ER to cis-Golgi:
a) The Golgi complex: three separated compartments: (Close to ER) cis/medial/trans (farther from ER)
b) Which proteins move forward from ER—cis Golgi? Forward traffic

c) How does ER residents find ER destination? The retrograde traffic and ER retention signal KDEL at the C-terminus of the ER lumen proteins.



From cis to medial and trans Golgi network (TGN):

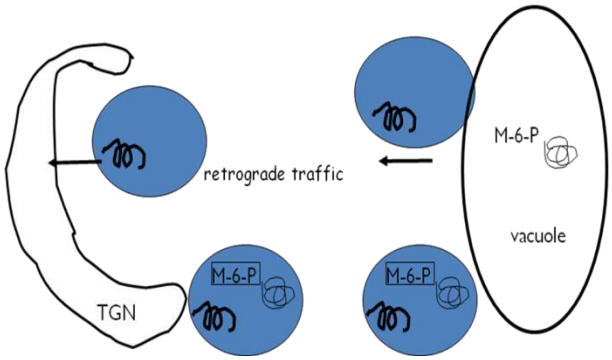
- Resident proteins stayed behind and other proteins move forward to the TGN.
- An important modification in Golgi is O-linked glycosylation following N-linked sugar addition in ER.
- Different compartments of Golgi (cis/medial/trans) have different enzymes that do different things to the protein passengers.



From TGN to vacuole or Plasma membrane/cell wall

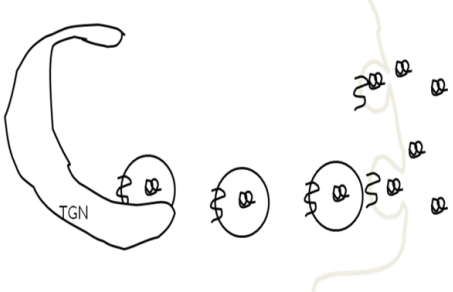
1) ***From TGN to lysosome/vacuole: a receptor-mediated pathway***

In animals and yeast: The receptors recognize a specific sugar label called mannose-6- phosphate added to the protein in cis-Golgi. The enzyme recognizes the specific protein substrates and phosphorylates the mannose-OH group. Such M-6-P serves as ligand for the receptors in the vesicular membrane.

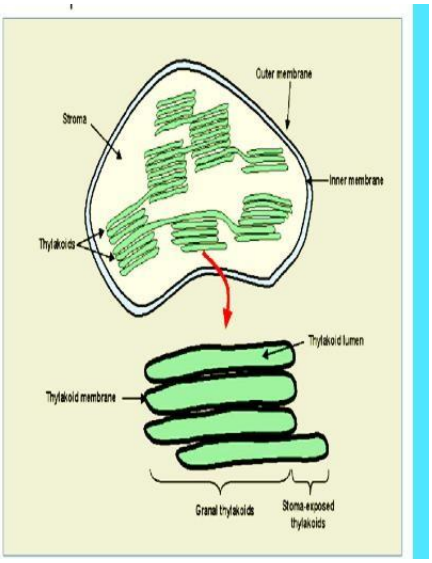


2) ***From TGN to PM/CW: a default pathway***

All passengers without the M-6-P tag will be deposited to the vesicles that will fuse with plasma membrane and soluble proteins secreted out of the cell.



Sorting of proteins to chloroplast



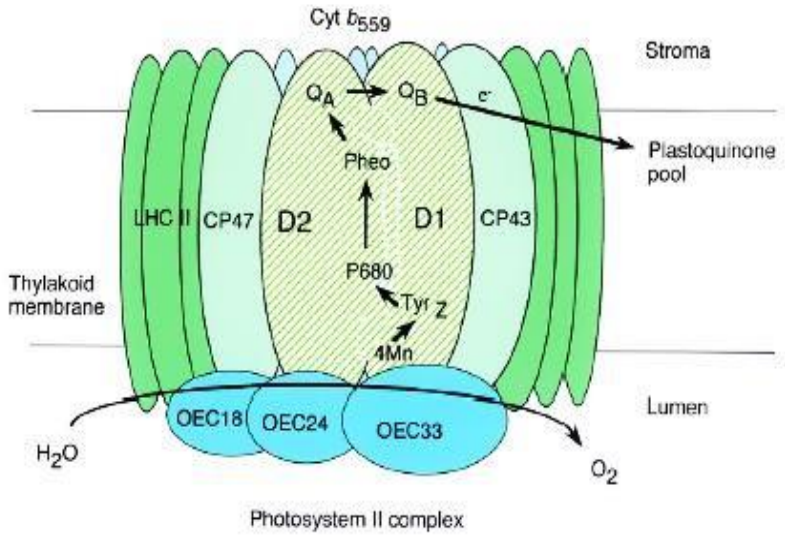
- The preprotein for **chloroplast** may contain a stromal import sequence or a stromal and thylakoid targeting sequence.
- The majority of preproteins are translocated through the Toc and Tic complexes located within the chloroplast envelope.
- In the stroma the stromal import sequence is cleaved off and folding as well as intra- chloroplast sorting to thylakoids continues.
- Proteins targeted to the envelope of chloroplasts usually lack cleavable sorting sequence.

3 pathways for protein targeting into and across thylakoid membranes (to lumen)

1. secA-dependent
2. pH gradient-dependent (or Tat pathway)
3. SRP-dependent

secA-dependent pathway for protein targeting:

- Involves a soluble, secA (bacterial gene) homologue
- requires ATP
- pH gradient stimulates
- Examples of proteins transported this way:
  - Plastocyanin
  - OE33 : 33 kDa protein of the oxygen evolving component of PSII (OEC)



Photosystem II complex

OEC (or OE) proteins of PSII mediate water splitting: Found in thylakoid lumen

pH gradient-dependent (or Tat pathway):

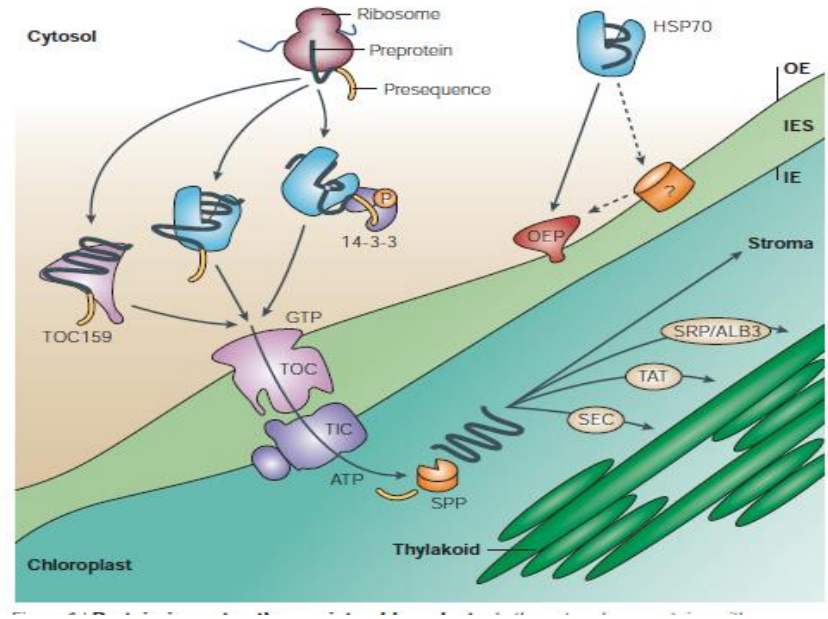
- Requires the pH gradient across thylakoid membrane (generated by photosynthesis)
- Examples of proteins transported by this pathway:
  - OE24 and OE17 subunits of the OEC
- Transit peptides of these proteins have twin-arginine (Tat) motif that is essential for transport across thylakoids
  - also occurs in bacteria
  -

SRP dependent pathway for protein targeting:

- Involves a signal recognition particle (SRP)-like protein (cSRP54)

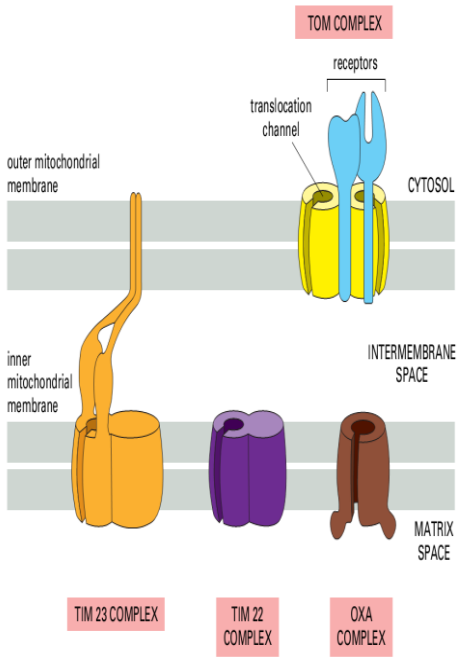
- SRP occurs in prokaryotes and eukaryotes
- Green plant chloroplast SRP does not have an RNA subunit

❖ requires GTP
❖ pH gradient stimulates
❖ Examples of proteins transported by this pathway-
- LHCPs: light-harvesting chlorophyll proteins (cab genes)



Sorting of proteins in mitochondria:

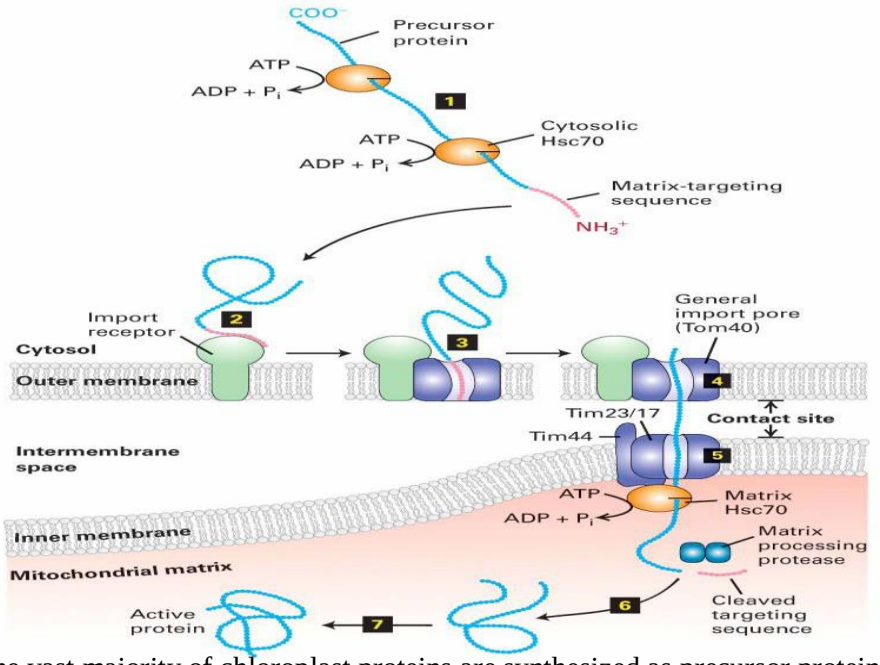
❖ Most mitochondrial proteins are encoded by nuclear DNA.
❖ Only very few are encoded by mitochondrial DNA and synthesized on mitochondrial ribosomes.
❖ Usually located at N-terminus of precursor polypeptide
❖ Usually removed in mitochondrial matrix
❖ Protein translocation across mitochondrial membranes is mediated by multi-subunit protein complexes that function as **protein translocators**.
❖ ***TOM ,TIM 23,TIM22 ,OXA***
❖ TOM transports-**mitochondrial precursor proteins** , nucleus-encoded mitochondrial proteins.
❖ TIM23-proteins **into the matrix space**.
❖ TIM22-**mediates the insertion of a subclass of inner membrane proteins**, including the carrier protein that transports ADP, ATP, and phosphate.
❖ OXA-mediates the **insertion of inner membrane proteins**.



Process:

1. Mitochondrial proteins are synthesized in cytosol as precursors

2. Bind to cytosolic **chaperones (Hsp 70)** to keep them unfolded until they ready to be translocated
3. Energy from ATP
4. Some outer membrane proteins insert themselves in the membrane while in transit
5. Intermembrane space proteins remain there and fold
6. Protein destined to matrix passes through Tom 40 and then Tim (inner membrane translocon).



7. The vast majority of chloroplast proteins are synthesized as precursor proteins (preproteins) in the cytosol and are imported post-translationally into the organelle.
8. Most proteins that are destined for the **thylakoid membrane**,
9. Preproteins that contain a cleavable transit peptide are recognized in a GTP-regulated manner12 by receptorsof the outer-envelope translocon, which is called **theTOC complex**.
10. The preproteins cross the outer envelope through an aqueous pore and are then transferred to the translocon in the inner envelope,which is called **the TIC complex**
11. The TOC and TIC translocons function together during the translocation process Completion of import requires energy, which probably comes from the ATP-dependent functioning of molecular chaperones in the stroma.
12. The stromal processing **peptidase** then cleaves the transit sequence to produce the mature form of the protein, which can fold into its native form.

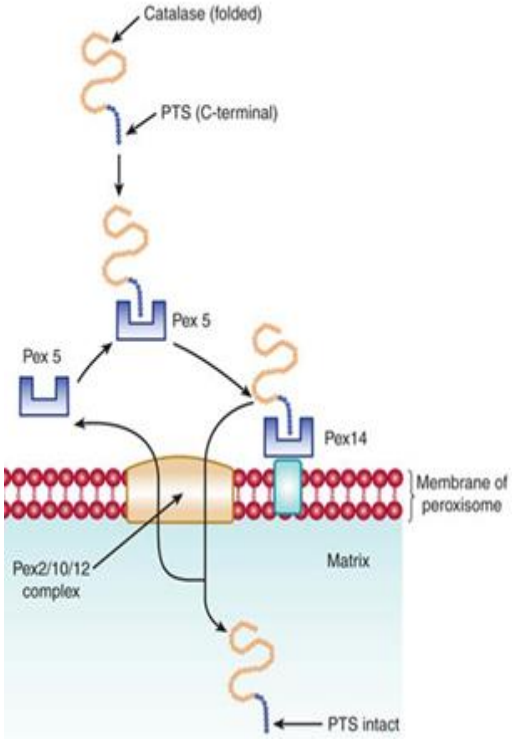
Sorting of proteins to both chloroplasts and mitochondria:

- Mitochondria and chloroplasts are **double-membrane-enclosed organelles**.
- They specialize in the synthesis of ATP, using energy derived from **electron transport** and **oxidative phosphorylation in mitochondria** and **from photosynthesis in chloroplasts**.
- Both organelles contain their **own DNA, ribosomes**, and other components required for protein synthesis.
- Their **growth depends** mainly on the import of **proteins from the cytosol**.

Sorting of proteins to peroxisomes

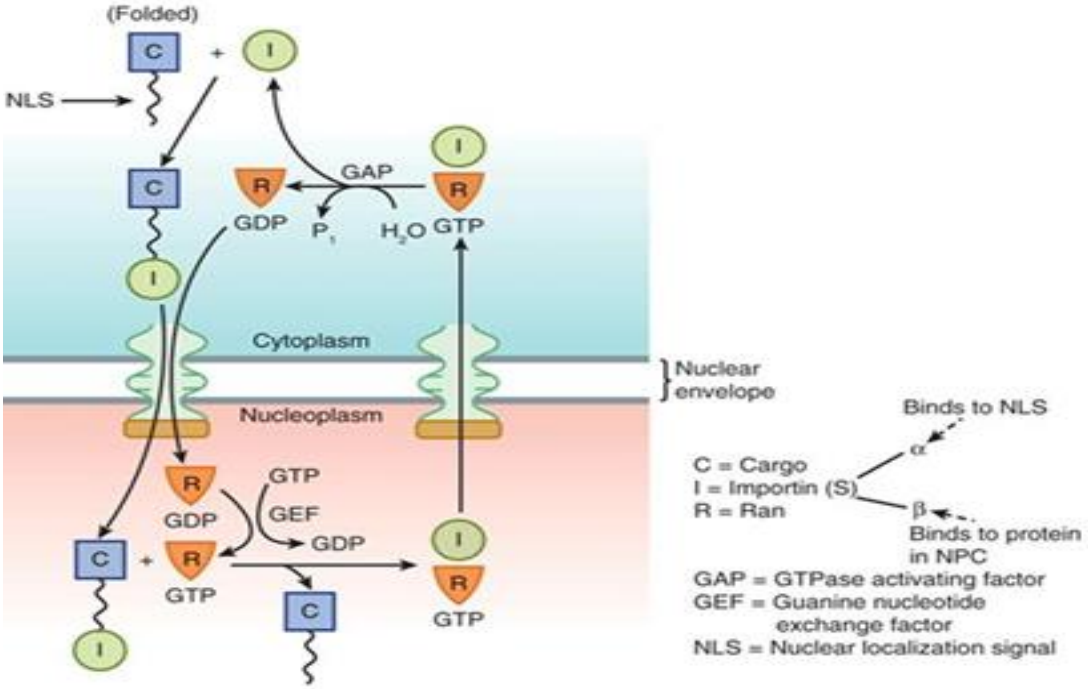
- Single membrane organelle
- Matrix contains oxidative enzymes
  - Lipid oxidation without ATP production
- Proteins encoded by nuclear DNA (all have to be imported)
- Peroxins - peroxisome transport receptors
- Bind to proteins with PTS1 and dock to the translocation channel

- The complex is transported through the membrane
- Protein is released and recycled
- **Peroxisome targeting signal 1 (PTS1):** a C-terminal tripeptide with a consensus sequence. The most common PTS1 is serine-lysine-leucine (SKL). Most peroxisomal matrix proteins possess a PTS1 type signal.
- **Peroxisome targeting signal 2 (PTS2):** a nonapeptide located near the N-terminus with a consensus sequence



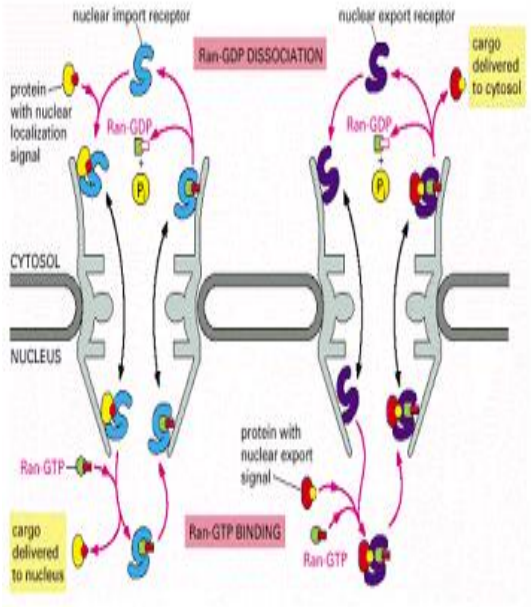
Transport into the nucleus

- All proteins found in the nucleus are synthesized in the cytoplasm
- Examples:
  - Histones
  - Ribosomal proteins
  - DNA and RNA polymerases
  - Transcription factors
- Transport requires nuclear localization sequences (NLS)
- Transport occurs through the nuclear pores
  - Nuclear import receptor (Importin α and β)
  - Energy from GTP
  - GTPase Ran
- Fully folded proteins are transported
- Importin α and β bind to the protein to be transported
  - Nuclear localization signal binds to importin α
- The complex is translocated through the nuclear membrane
- Activated Ran (GTP) causes the complex to dissociate
- Ran transports importin β back to cytosol
- Importin α becomes a part of export receptor



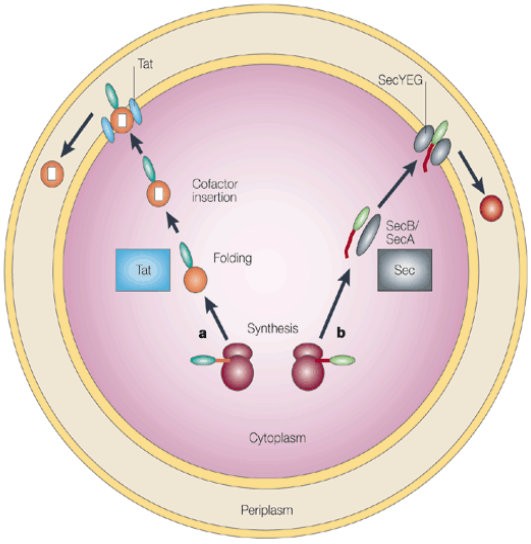
Import and export protein to nucleus:

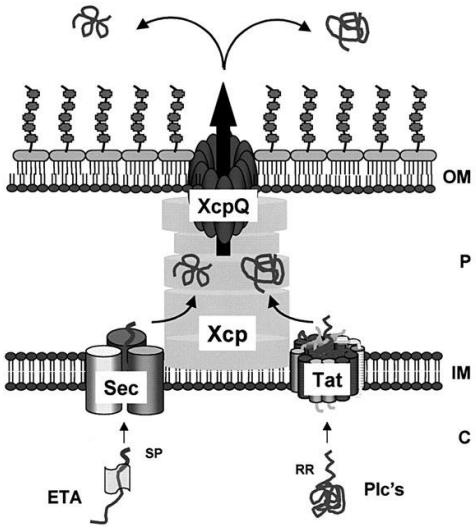
- Protein encodes a receptor protein that is specialized for the transport of a group of nuclear proteins sharing structurally similar nuclear localization signals.
- Nuclear import receptors do not always bind to nuclear proteins directly. Additional adaptor proteins are sometimes used that bridge between the import receptors and the nuclear localization signals on the proteins to be transported.
- **Export -ribosomal subunits and RNA molecules.**
- **For import and export requires energy**



Protein targeting in *E. coli*:

1. Tat: for periplasmic redox proteins & thylakoid lumen
2. Sec pathway
3. Periplasmic proteins with the correct signals (exposed after cleaving signal peptide) are exported by XcpQ system





5. Nucleic acid: Structure, chemical and physical properties, nuclear, chloroplast and mitochondrial Genome

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.

Francis H.C. Crick

James D. Watson

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.

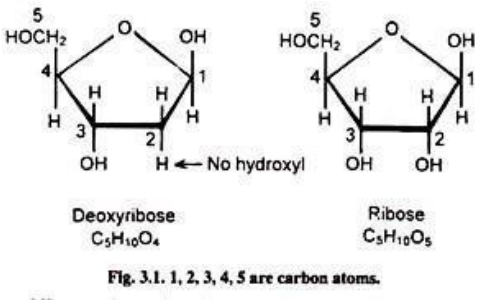


Fig. 3.1. 1, 2, 3, 4, 5 are carbon atoms.

Nitrogen Bases:

Nitrogen bases in DNA are of two kinds, Purine and Pyrimidine.

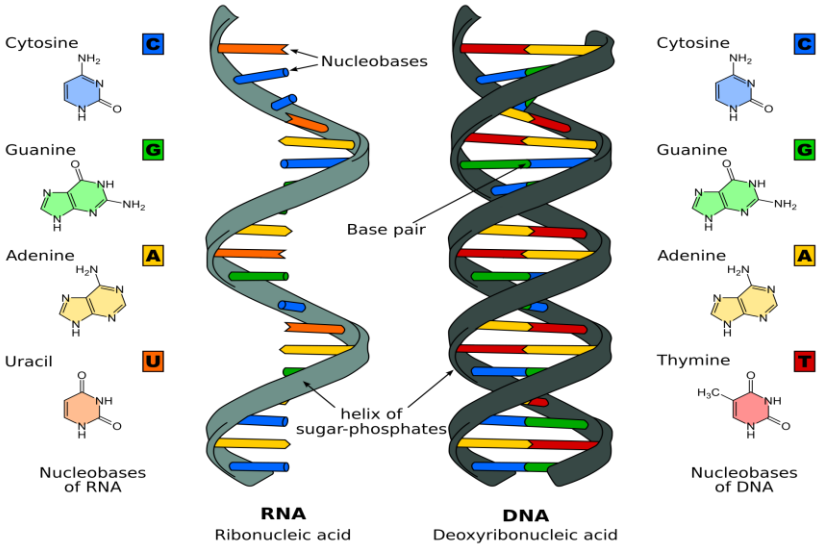
Purine:

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.

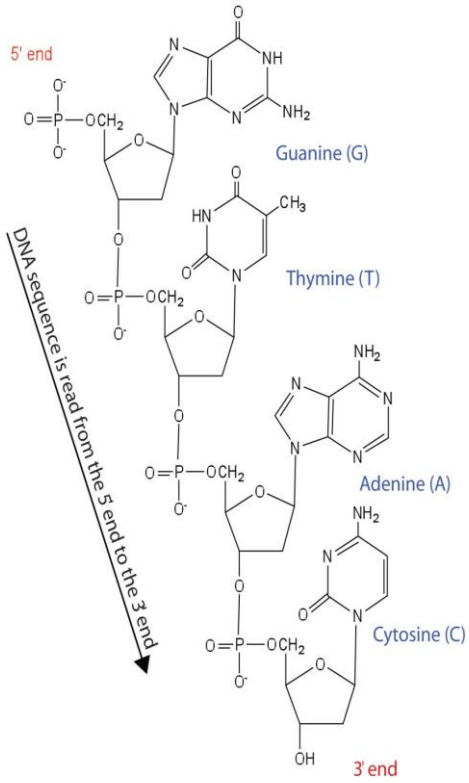


In this way DNA consists of four bases A, G, C, T and RNA has A, G, C, U.

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.



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.

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.

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, dGTP, dCTP and dTTP.

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 (4N).

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 T2 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.

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.

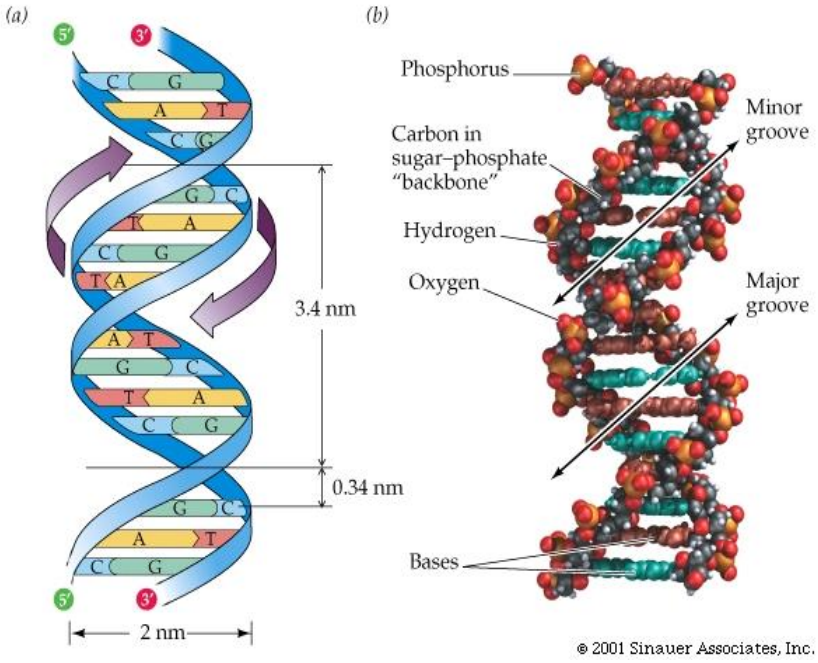
Following are the other important properties revealed by X-ray diffraction data:

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. The Z- form DNA makes one complete turn at 45A as compared to 34 A in B-DNA.

Characteristics	A form of DNA	B form of DNA	Z form of DNA
Helical sense	Right handed	Right handed	Left handed
Diameter	26Å	20Å	18Å
Rise per turn of helix	28Å	36Å	44Å
Base pairs per helical turn	11 base pairs	10 base pairs	12 base pairs
Helix rise per base pair	2.6Å	3.6Å	3.7Å
Base tilt normal to the helix axis	20^0	6^0	7^0
Glycosyl bond conformation	Anti	Anti	Anti for pyrimidine and syn for purines

Table: Differences between various forms of DNA (A-DNA, B-DNA and Z-DNA

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.
Circular DNA:
Most bacterial chromosomes and many viruses have DNA which is in the form of a closed circle. The two ends of the double helix get covalently sealed to form a closed circle. E. coli has a circular chromosome of about 5 million base pairs.
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.
Structure of RNA:
The primary structure of RNA is the same as that of DNA. It is also a polynucleotide chain with 5′-3′ sugar phosphate links. But the sugar is ribose and generally it exists as a single-stranded molecule. For that reason, it does not have the one-to-one ratio between the complementary bases. The amount of purines is not equal to that of pyrimidines.
One of the four major bases in RNA is uracil (U) instead of thymine. Biophysical properties of RNA show much less secondary structure. However, when a sequence of bases is followed by a complementary sequence in the same chain, the polynucleotide may fold back on itself to generate an antiparallel duplex structure, known as a hairpin.
It has a stem, the base-paired double helical region, and a loop with unpaired bases at one end. In these regions G can also pair with U, but it is not as strong as G-C pair.
Seconadary structure found in some RNAs
Cells contain three types of RNA-messenger RNA (mRNA), ribosomal RNA(rRNA) and transfer RNA (tRNA). Messenger RNA serves as the template for protein synthesis. It is a very heterogeneous class of molecules and very unstable. It constitutes 2 – 5 per cent of the total RNA of the normal cell. It was first detected by Hershey (1956). The name and concept of messenger RNA was first given by F. Jacob and J. Monod (1961).
When molten DNA is slowly cooled with some specific m-RNA, DNA- RNA hybrid molecules are formed, suggesting that m-RNA is formed from the template strand of the DNA duplex.

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.

Chemical and Physical Properties of Nucleic Acids:

- Effect of Acid
- Effect of alkali
- Chemical denaturation
- Viscosity
- Buoyant density
- Thermal Stability
- Optical absorption
- Solubility

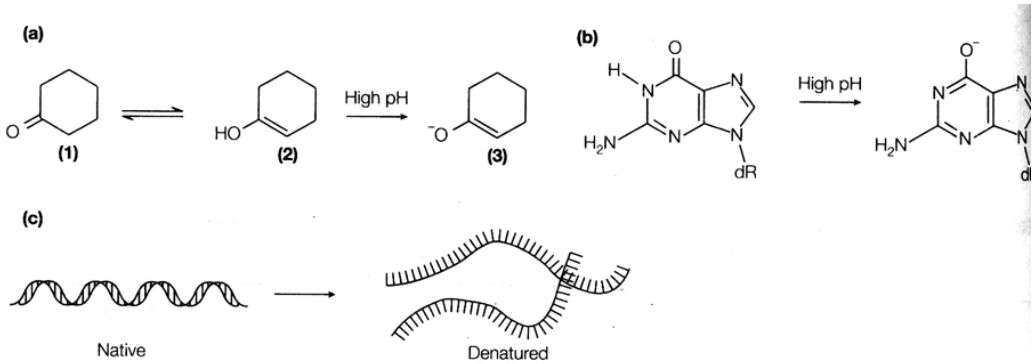
Effect of Acid:

Strong acid + high temperature: completely hydrolyzed to bases, riboses/deoxyrobose, and phosphate.

pH 3-4 : apurinic nucleic acids [glycosylic bonds attaching purine bases(A and G) to the ribose ring are broken], can be generated by formic acid.

Effect of Alkali:

- High pH (> 7-8) has small effects on DNA structure
- High pH changes the tautomeric state of the bases



Base pairing is not stable anymore because of the change of tautomeric states of the bases, resulting in DNA denaturation.

Chemical Denaturation:

Urea (H_2NCONH_2): Used as denaturing agent in PAGE

Formamide ($HCONH_2$): denaturing agent in Northern blot

Disrupting the hydrogen bonding of the bulk water solution

↓

Hydrophobic effect (due to aromatic bases) is reduced

↓

Denaturation of strands in double helical structure

Viscosity:

Very often, for preparative of analytical purposes, one uses the sedimentation equilibrium in a CsCl gradient established during the centrifugation; this gives a density gradient ranging generally from 1.65 to about 1.75 g/ml.

The DNA is concentrated in a band at the place where the density of CsCl solution is equal to its own and this density is generally determined by comparison with a DNA of known density, centrifuged in the same gradient. The density of a DNA at the sedimentation equilibrium increases with greater percentages of G-C pairs of the DNA (because G-C pairs are more dense then A-T pairs).

The DNA solutions have a very high viscosity due to the considerable length and relative rigidity of the double helix. The denaturation of DNA molecules can be followed up with the help of viscosity measurements.

Buoyant Density:

When DNA is centrifuged at high speeds in a concentrated solution of caesium chloride- (CsCI), the CsCl will form a density gradient (ascending) and the DNA will remain stationary or buoyant at a

point in the tube where its density is equal to the density of CsCI at that point. Different DNA will have different densities, which again depend upon the GsC content of that DNA. Higher the G = C content, higher is the buoyant density of that DNA and vice versa.
Measurement of these two characters, viz., melting temperature and buoyant density will enable us to calculate the proportions of G ≡ C and A = T pairs in that DNA, which indirectly helps in deducting the gene sequence.

Buoyant density (DNA)

1.7 g cm^{-3}, a similar density to 8M CsCl

Use in Purifications of DNA: equilibrium density gradient centrifugation

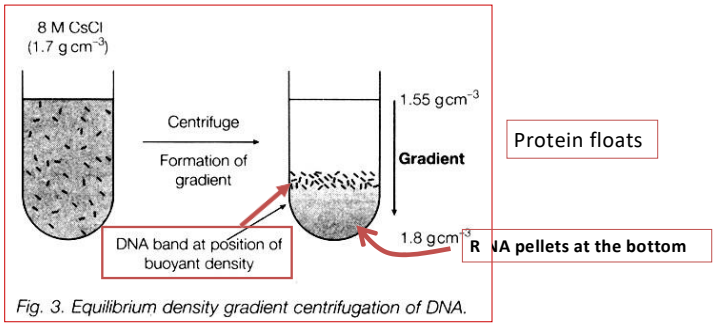
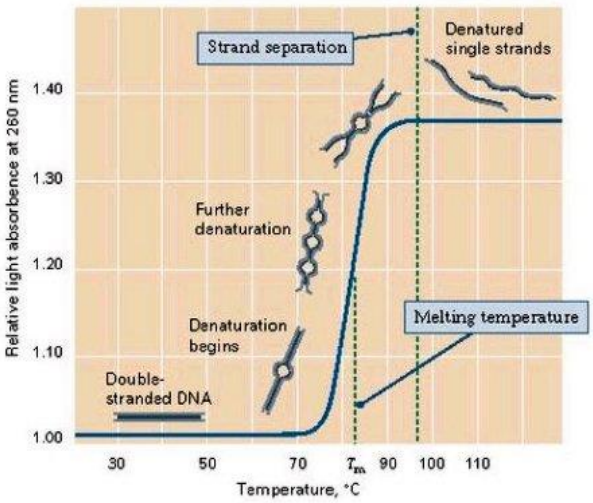


Fig. 3. Equilibrium density gradient centrifugation of DNA.



Heat denaturation and Tm:
When DNA were heated to certain temperature, the absorption value at 260nm would increased sharply, which indicates that the double strand helix DNA was separated into single strand.

Tm (melting temperature of DNA):
The temperature where UV absorption increase to an half of maximum value in DNA denaturation.

Spectroscopic Properties of Nucleic Acids (Optical Absorption):

UV absorption:

- Nucleic acids absorb UV light due to the aromatic bases
- The wavelength of maximum absorption by both DNA and RNA is 260 nm (lmax = 260 nm)
- Applications: detection, quantitation, assessment of purity (A_{260}/A_{280})

Hypochromicity: caused by the fixing of the bases in a hydrophobic environment by stacking, this makes these bases less accessible to UV absorption. Ds-DNA, ss DNA/RNA, nucleotide.

What is a Genome and Gene?
A **genome** is an organism's complete set of DNA. Each genome contains all of the information needed to build and maintain that organism. A **gene** is a locus (or region) of DNA that encodes a functional RNA or protein product, and is the molecular unit of heredity.

Genome organization:

- Prokaryotes
  - Most genome is coding
  - Small amount of non-coding is regulatory sequences
- Eukaryotes
  - Most genome is non-coding (98%)
  - Regulatory sequences
  - Introns

- Repetitive DNA

Prokaryotic genome:

- Example: *E. coli*
  - ✓ 89% coding
  - ✓ 4,285 genes
  - ✓ 122 structural RNA genes
  - ✓ Prophage remains
  - ✓ Insertion sequence (IS) elements
  - ✓ Horizontal transfers

Prokaryotic genome organization

- Haploid circular genomes (0.5-10 Mbp, 500-10000 genes)
- Operons: polycistronic transcription units
- Environment-specific genes on plasmids and other types of mobile genetic elements
- Usually asexual reproduction, great variety of recombination mechanisms
- Transcription and translation take place in the same compartment

Eukaryotic genome:

- Most genome is non-coding (98%)
- Regulatory sequences
- Introns
- Repetitive DNA
- Example: *C. elegans*
  - ✓ 10 chromosomes
  - ✓ 19,099 genes
  - ✓ Coding region – 27%
  - ✓ Average of 5 introns/gene
  - ✓ Both long and short duplications

Eukaryotic genome organization

1. Multiple genomes: nuclear, plastid: mitochondria, chloroplasts
2. Plastid genomes resemble prokaryotic genomes
3. Multiple linear chromosomes, total size 5- 10,000 MB, 5000 to 50000 genes
4. Monocistronic transcription units
5. Discontinuous coding regions (introns and exons)
6. Large amounts of non-coding DNA
7. Transcription and translation take place in different compartments
8. Variety of RNAs: Coding (mRNA, rRNA, tRNA), Non-coding (snRNA, snoRNA, microRNAs, etc).
9. Often diploid genomes and obligatory sexual reproduction
10. Standard mechanism of recombination: meiosis

Nuclear genome:

Repetitive DNA sequences

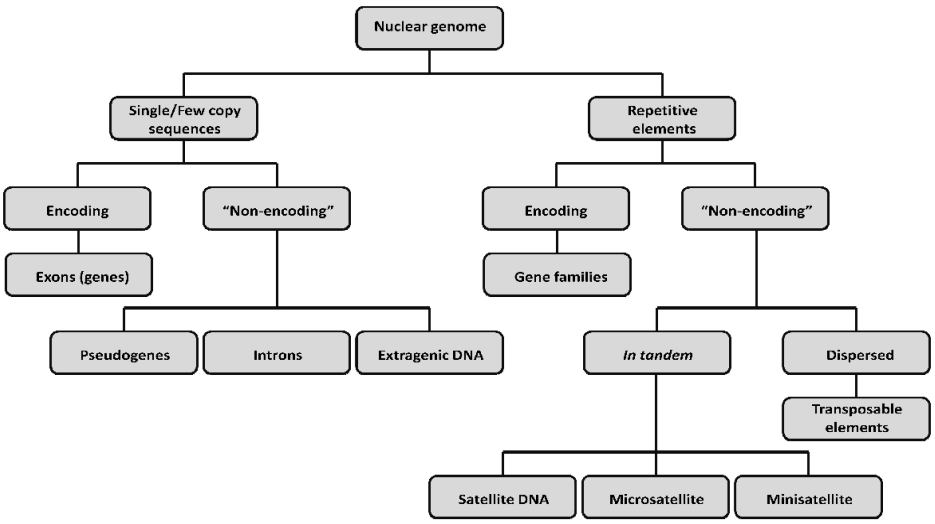
The repetitive DNA sequences are present in several to a million copies per genome. Except few genes, majority of repetitive DNA sequences are non-coding sequences. These sequences make eukaryotic genome voluminous. The number of copies of a DNA sequence present in one genome is called repetition frequency (f). Around 45% of the human genome and almost 40% of the mouse genome are composed of repeated DNA sequences. It has been suggested that the event of unequal crossing- over could be the main mechanism for the evolution of repetitive DNAs. Repetitive DNA sequences are grouped into the following two classes: (i) highly repetitive DNA and (ii) moderately repetitive DNA.

(i) Highly repetitive DNA:

It is also called simple sequence DNA. These short sequences, often less than 10 bp in length, are present in hundreds of thousands to millions copies that are repeated in tandem and clustered in certain regions of the chromosome, especially at centromeres and telomeres.

(ii) Moderately repetitive DNA:

Moderately repetitive DNA consists of sequences from 150 to 300 bp in length (although they may be longer) that are repeated many thousands of times.
Much of the moderately repetitive DNA has no known function in the cell while some of these moderately repetitive sequences perform important functions for the cell; for example, the genes for ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs) make up a part of the moderately repetitive DNA.



Moderately repetitive DNA is of two types of repeats.
Tandem repeat sequences appear one after another and tend to be clustered at particular locations on the chromosomes.
Interspersed repeat sequences are scattered throughout the genome. An example of an interspersed repeat is the Alu sequence, a 200 bp sequence that is present more than a million times and comprises 11% of the human genome.
Short repeats, such as the Alu sequences, are called SINEs (short interspersed elements).
Longer interspersed repeats consisting of several thousand base pairs are called LINEs (long interspersed elements). One class of LINE, called LINE1, comprises about 17% of the human genome.
Satellite DNAs-
Satellite DNAs are short sequences ranging from 5 to 200 bp and it consists of very large arrays of tandemly repeating, non-coding DNA. Satellite DNA is the main component of functional centromeres, and forms the main structural constituent of heterochromatin. These highly repetitive sequences are GC-rich DNA due to which they show lower buoyant density than that of bulk of the DNA of the genome and hence appear as a distinct 'satellite' band from the main band of DNA during density gradient centrifugation (hence the name satellite DNA). The term satellite DNA is essentially synonymous with simple sequence DNA. There is complex satellite DNA with longer repeat units. For example, alfa and beta satellite DNA found in humans have a repeat unit of 171 bp and ~ 68 bp respectively. Satellite DNAs are species specific and represent less than 10% of the genome.
Depending upon the number of base pairs involved in repeat regions, satellite DNA is of two types, microsatellite sequences and minisatellite sequences.
Microsatellite DNA:
These include the shortest sequences one to five base pairs long, present in clusters of about 50 to 100 base pairs in length. They are dispersed evenly throughout the DNA. The human genome contains about 30,000 different microsatellite loci.
Minisatellite DNA:
These usually occur in clusters with about 3000 repeats, their size ranging from 12 to 100 bp in length. Minisatellite sequences occupy shorter stretches of the genome than the satellite sequences.

Minisatellites are often unstable and the number of copies of minisatellites can increase or decrease from one generation to the next.

Unique DNA Sequences

Unique sequences are non-repeated DNA sequences, sometimes called single-copy sequences, present as single or few copies per haploid genome. These sequences can be coding sequences, gene and gene families or noncoding sequences (introns, regulatory elements, pacer DNA etc.). In prokaryotes, with the exception of few sequences such as ribosomal RNA genes, transfer RNA genes, almost the entire genome consists of unique sequences and the majority of the sequences are coding gene sequences (solitary genes). In eukaryotes, most of the protein coding genes are unique sequences and called solitary genes. A group of genes that encodes similar proteins (not identical) form a gene family. Globins, actins, myosins, collagens, tubulins, integrins, protein kinase and vertebrate immunoglobins are some important examples of such gene families. The multigene families are formed due to gene duplication and mutation in the duplicated genes. The portion of unique DNA in cells of various species may vary from 70% (man) to only 8% (rye). In human, these unique DNAs contribute ~50% of the total genome and only ~1.5 % DNAs constitutes coding regions within genes that code for proteins. This 26 is because 95% of the gene is made up of the non-protein coding DNAs called introns. Recently discovered miRNA genes, which code small structural RNAs, are also classified under as a unique sequence of DNA. These structural RNAs regulate the expression of other genes.

C-value paradox-

C-value: 'C-value' of an organism is the total amount of DNA present within its haploid genome. It is usually represented in base pairs (bp). The term C-value was given by Hawson Swift in 1950. In C-value, the 'C' stands for the 'constant or characteristic' of DNA content and is measured in picograms. It ranges from 6.6×105 for an alga to 1011 for some plants and amphibians.

C-value Paradox

The size of the genome (C-value) depends on the organism. It is essentially constant within species, but varies widely among species. There is not a strong correlation between organism complexity and genome size. This is known as the C-value paradox.

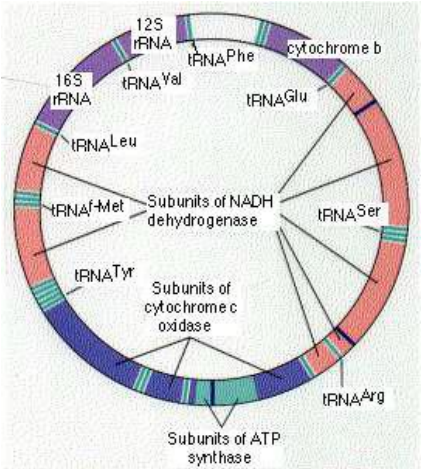
In general, bacterial genomes are smaller than eukaryotic genomes. Within bacteria, genomes range from 580 kb to 13 Mb, thus there is 20–30-fold size variation within prokaryotes.

A few eukaryotic genomes fall in the size range of bacteria (e.g. yeast), but most are much larger. The size range of eukaryotic genomes is 8.8 Mb to ≈700 Gb. This is 80,000-fold size variation.

The correlation between genome size and organism complexity exists well across the lower eukaryotes. While, a group of higher eukaryotes show the contradictory relationship 21 between C-value and its complexity, for example, genome size of Xenopus, (an amphibian) is closer to the genome size of human (most complex organism) and tulips have 40 times larger genome than humans. Similarly even the number of chromosomes in an organism cannot explain the complexity of different organisms. Genome size or C value or DNA content per haploid genome cannot explain the complexity of the organisms which have evolved during evolution over the years due to multiple factors.

The Mitochondrial Genome

- The genome of human mitochondria contains 16,569 base pairs of DNA organized in a closed circle.
- These encode:
- 2 ribosomal RNA (rRNA) molecules
- 22 transfer RNA (tRNA) molecules
- 13 polypeptides



The 13 polypeptides participate in building several protein complexes embedded in the inner mitochondrial membrane.

7 subunits that make up the mitochondrial NADH dehydrogenase

3 subunits of cytochrome c oxidase

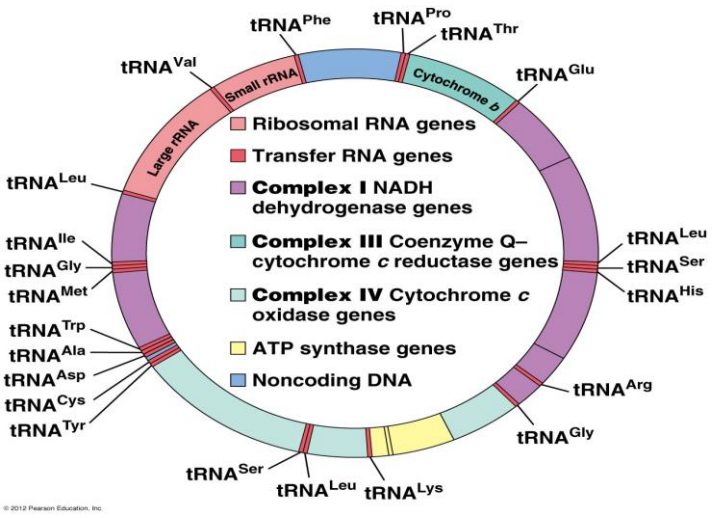
2 subunits of ATP synthase cytochrome b

All these gene products are used within the mitochondrion, but the mitochondrion also needs proteins encoded by nuclear genes. These proteins (e.g., cytochrome c and the DNA polymerases used within the mitochondrion) are synthesized in the cytosol and then imported into the mitochondrion.

- The mitochondrial genome is a circle, 16.6 kb of DNA. A typical bacterial genome is 2-4 Mbp.
- The two strands are notably different in base composition, leading to one strand being "heavy" (the H strand) and the other light (the L strand).
- Both strands encode genes, although more are on the Hstrand.
- A short region (1121 bp), the D loop (D = "displacement"), is a DNA triple helix: there are 2 overlapping copies of the H strand there.
- The D loop is also the site where most of replication and transcription is controlled.
- Genes are tightly packed, with almost no non-coding DNA outside of the D loop. In one case, two genes overlap: they share 43 bp, using different reading frames. Human mitochondrial genes contain no introns, although introns are found in the mitochondria of other groups (plants, forinstance).
- Genes: Total of 37. 22 tRNAs, 2 rRNAs, 13 polypeptides.
- tRNA: only 60 of the 64 codons code for amino acids. 8 tRNAs cover all 4 3rd base positions with the same amino acid, and the remaining 14 tRNAs each cover two 3rd base positions (purines or pyrimidines). Thus, all 60 codons are covered.
- rRNA: 16S and 23S which are standard sizes for bacterial rRNAs. Bacterial ribosomes don't use 5S or 5.8S rRNAs.
- polypeptides: all are components of the electron transport chain. Other components are encoded in the nucleus and transported to the mitochondria after translation.

Chloroplast genome:

- Chloroplast DNA (cpDNA) is also known as plastid DNA (ptDNA).
- Circular double stranded DNA molecule
- Ct genomes are relatively larger
  - ✓ 140kb in higher plants.
  - ✓ 200kb in lower eukaryotes.
- Multiple copies of genome per organelle.
- Vary in size ,
- But are large enough to code 50-100 proteins as well as rRNAs & tRNAs
- cpDNA regions includes Large Single-Copy (LSC) & Small Single-Copy (SSC) regions, and Inverted Repeats (IRA & IRB).



- Variation in length mainly due to presence of inverted repeat (IR)
- Conifers and a group of legumes lack Inverted Repeats.

The ctDNA is double stranded circular, and devoid of histones and other proteins. In many cases, the GC content of cpDNA differs from that of nuclear DNA and mitochondrial DNA. Complete cpDNA sequences have been determined in tobacco (155, 844 bp) and rice (135, 42 bp).

Multiple copies of cpDNA are present in the nucleoid region of each chloroplast. In the green alga Chlamydomonas, one chloroplast contains 500 to 1500 cpDNA molecules. Chloroplasts divide by growing and then dividing into two daughter chloroplasts.

The proportion of introns in chloroplast DNA could be high, 38% in Euglena. Among the expressed genes in chloroplast genome, 70 to 90% of the genes encode proteins including those involved in photosynthesis, four genes code for rRNAs (one each for 16S, 23S, 4.5S and 5S), and about 30 genes encode tRNAs.

Chloroplast genome also contains genes for some of the proteins required for transcription and translation of the encoded genes, and most importantly, genes for photosynthesis. Most of the proteins

in chloroplasts are encoded by the nuclear genes. The mRNA transcripts of the chloroplast genes are translated according to the standard genetic code.
However, the primary structures of several RNA transcripts are found to go through editing consisting of C to U transitions, that cause mRNA sequence to deviate from the sequence in the corresponding gene. Editing makes it difficult to convert chloroplast nucleotide sequences into amino acid sequences of the corresponding protein.
Most of the cpDNAs studied share a common feature, that is, a 10 to 24 kb segment present in two identical copies as an inverted repeat. The cpDNA also contains two copies of each of the rRNA genes which are located in these two identical repeat sequences in an inverted orientation.
Other genes that are found in the repeated sequence are therefore, also duplicated in the chloroplast genome. The location of these repeats defines a short single copy (SSC) region and a long single copy (LSC) region in chloroplast genome.
Chloroplast protein synthesis uses organelle-specific 70S ribosomes consisting of 50S and 30S subunits. The 50S subunit contains one copy each of 23S, 5S and 4.5S rRNAs, while the 30S subunit contains one copy of a 16S rRNA.
Among the ribosomal proteins, some are encoded by the nuclear DNA, some by the chloroplast genome. About 100 open reading frames (ORFs), putative protein coding genes, have been identified by computer analysis. Protein synthesis is similar to that in prokaryotes

6. Recombinant DNA Technology: Restriction enzymes, cloning vectors, construction of recombinant DNA

Recombinant DNA (rDNA) is a form of artificial DNA that is created by combining two or more sequences that would not normally occur together through the process of gene splicing.
Recombinant DNA technology is a technology which allows DNA to be produced via artificial means. The procedure has been used to change DNA in living organisms and may have even more practical uses in the future.
Stanley N. Cohen, who received the Nobel Prize in Medicine in 1986 for his work on discoveries of growth factors. **Stanley N. Cohen** and **Herbert Boyer** constructed the first recombinant DNA using bacterial DNA and plasmids.
Restriction enzymes:
These are also known as molecular scissors, used for cutting of DNA. The cutting of DNA at specific locations became possible with the discovery of molecular scissors, i.e., restriction enzymes. In the year 1963, the two enzymes responsible for restricting the growth of bacteriophage in E. coli were isolated. One of these added methyl groups to DNA, while the other cuts the DNA. Later was termed as restriction endonucleases.
The first restriction endonuclease was isolated by Smith Wilcox and Kelley in 1968 was Hind II. It was found that it always cuts DNA molecules at a particular point by recognising a specific sequence of six base pairs known as recognition sequence for Hind II. Today, more than 900 restriction enzymes have been isolated from over 230 bacterial strains each of which recognise different recognition sequences.
Restriction endonuclease enzymes occur naturally in bacteria as a chemical weapon against the invading viruses. They cut both strands of DNA when certain foreign nucleotides are introduced in the cell. Endonucleases break strands of DNA at internal positions in random manner.
The first observations on the existence of restriction enzymes was made by Arber and Dussoix in 1962, and proposed model to explain the restriction phenomenon. Their views on restriction enzymes affirmed that certain bacterial strains contained endonucleases able to cleave unprotected DNA. In addition, several other strains contained a modification system responsible for protecting their own DNA.
Some of the observations were made by W. Arber and his associates while studying the efficiency of plating of the bacteriophage lambda on different strains of Escherichia coli. They even demonstrated

that restriction endonucleases were able to cleave DNA from other strains while exempting that of the original strains.

They are also associated with modifying enzymes, which methylate the DNA. Methylated DNA escape cleavage by endonucleases, and prevents the cell from degrading its own DNA. Thus, invading foreign DNA in bacteria that has not been correctly methylated will be degraded.

In 1970, Smith, Wilcox and Kelly have characterized and purified restriction enzymes and elucidated their recognition and cleavage site of a more useful restriction enzyme, Hind II.

Naming of Restriction Enzymes:

The convention for naming these enzymes proceeds in a way that the first letter of the name comes from the genes and the second two letters come from the species of prokaryotic cell, from which they were isolated, e.g., Eco RI comes from E. coli RY13. The letter 'R' is derived from the name of strain. Roman numbers following the names, indicate the order in which the enzymes were isolated from that strain of bacteria.

Naming exercise of RE enzymes is based on following rules:

1. Each RE enzyme is named by a three-letter code.
2. The first letter of this code is derived from the first epithet (first letter of name) of the genus name. It is printed in italics.
3. The second and third letters are from the first two letters of its species name. They are also printed in italics.
4. This is followed by the strain number. If a particular strain has more than one restriction enzyme, these will be identified by Roman numerals as I, II, III, etc.

For example, the enzyme Eco RI was isolated from the bacterium *Escherichia* (E) *coli* (co) strain RY13 (R) and it was the first endonuclease (I). R also indicates antibiotic resistant plasmid of the bacterium. Likewise, Hind II from *Haemophilus influenzae* strain Rd and Bgl I from *Bacillus globigii*. A few restriction endonuclease enzymes and their sources are given below:

Table 55.3. A few restriction endonuclease enzymes, their sources and recognition sites.

Name of the restriction endonuclease enzyme	Source (Microorganisms)	Recognition sequence and cleavage site
1. *Aat* II	*Acetobacter aceti*	GACGT↓C
2. *Bcl* I	*Bacillus Caldoyticus*	T↓GATCA
3. *Cvn* I	*Chromatium vinosum*	CC↓TNAGG
4. *Eco* RI	*Escherichia coli* RY13	G↓AATTC
5. *Eco* RII	*Escherichia coli R*245	↓CCTGG
6. *Hind* II	*Haemophilus influenzae* Rd	GTP_{y} ↓ PuAC*
7. *Hind* III	*Haemophilus influenzae* Rd	A↓AGCTT
8. *Kpn* I	*Klebsiella pneumoniae* OK	GGTAC↓C
9. *Nop* I	*Nocardia opaca*	G↓TC GAC
10. *Nsp* B II	*Nostoc*	C(A/C)G↓C(T/G)G

Restriction enzymes belong to a larger class of enzymes called nucleases, which are of two types:

(a) Exonucleases remove nucleotides from the ends of the DNA either (5′ or 3′) in one strand of duplex.

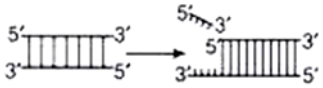
(b) Endonucleases make cuts at specific positions within DNA. Each restriction endonucleases function by 'inspecting' the length of a DNA sequence.

Once it finds its specific recognition sequence, it will bind to the DNA and cut each of the two strands of the double helix at specific points in their sugar phosphate backbones.

Differences between Exonucleases and Endonucleases

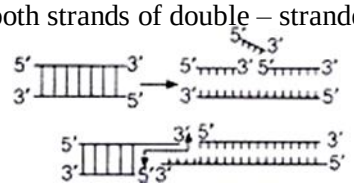
Exonucleases:

1. These nucleases cleave base pairs of DNA at their terminal ends
2. They act on single – strand of DNA or gaps in double –stranded DNA. They do not cut RNA



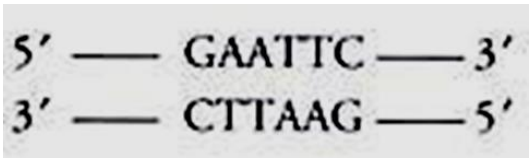
Endonucleases:

1. They cleave DNA at any Pont except the terminal ends
2. They cleave one strand or both strands of double – stranded DNA. They may cut RNA

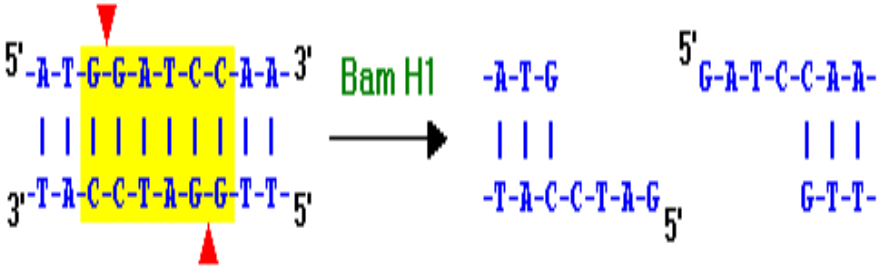


Each restriction endonuclease recognizes a specific palindromic nucleotide sequences in the DNA. Recognition sites of most restriction enzymes have a twofold rotational symmetry. Restriction enzymes have corresponding symmetry to facilitate recognition and usually cleave the DNA on the axis of symmetry.

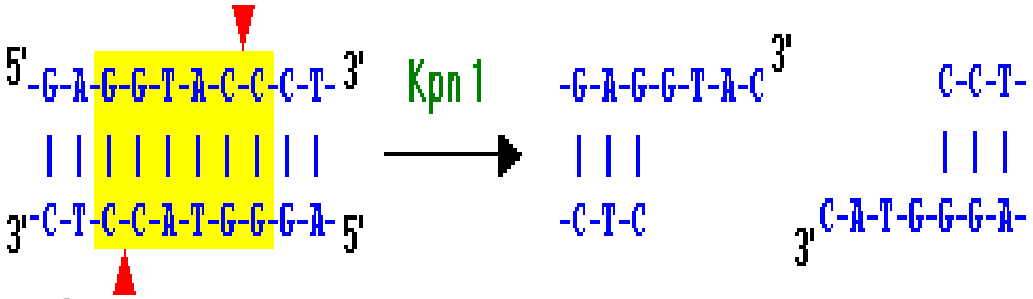
Palindrome in the DNA is a groups of letters that forms the same words when read both forward and backward. For example, the following sequences read the same on the two strands in 5′ → 3′ direction as well as 3′ → 5′ direction.



5' overhangs: The enzyme cuts asymmetrically within the recognition site such that a short single-stranded segment extends from the 5' ends. Bam HI cuts in this manner.

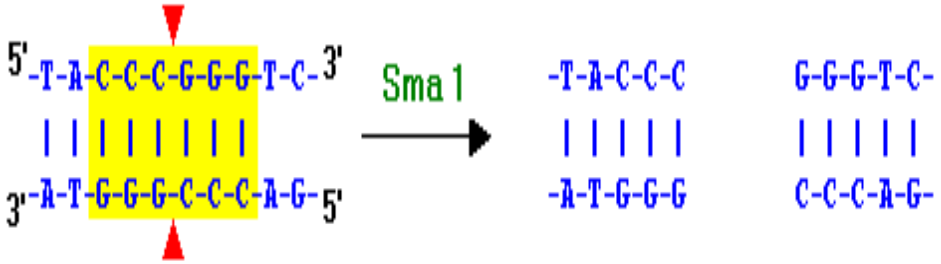


3' overhangs: Again, we see asymmetrical cutting within the recognition site, but the result is a single-stranded overhang from the two 3' ends. KpnI cuts in this manner.



Blunt cut ends:

Enzymes that cut at precisely opposite sites in the two strands of DNA generate blunt ends without overhangs. SmaI is an example of an enzyme that generates blunt ends.



Isoschizomers and Neochischizomers:

- ✓ Restriction enzymes that have the same recognition sequence as well as the same cleavage site are **Isoschizomers.** Eg.Sphl and Bbul
- ✓ SphI (CGTAC/G) and BbuI (CGTAC/G)
- ✓ Restriction enzymes that have the same recognition sequence but cleave the DNA at a different site within that sequence are **Neochizomers.** Eg:SmaI and XmaI
- ✓ Isocaudomers are pairs of restriction enzymes that have slightly different recognition sequences but upon cleavage generate identical termini. For example the enzymes **Mbo I and BamH I** are isocaudomers:

Types:

Naturally occurring restriction endonucleases are categorized into four groups (Types I, II III, and IV) based on their composition and enzyme cofactor requirements, the nature of their target sequence, and the position of their DNA cleavage site relative to the target sequence. All types of enzymes recognize specific short DNA sequences and carry out the endonucleolytic cleavage of DNA to give specific fragments with terminal 5'-phosphates. They differ in their recognition sequence, subunit composition, cleavage position, and cofactor requirements, as summarised below:

Type I enzymes (EC 3.1.21.3)

- ❖ Capable of both restriction and modification activities
- ❖ The cofactors S-Adenosyl methionine (AdoMet), ATP, and Mg2+, are required for their full activity
- ❖ Contain,
    - two R (restriction) subunits,
    - two M (methylation) subunits and
    - one S (specificity) subunit
- ❖ Cleave DNA at random length from recognition site

Type II enzymes (EC 3.1.21.4)

- ❖ Mostly used for gene analysis and cloning
- ❖ More than 3500 REs
- ❖ Recognize 4-8 bp sequences
- ❖ Need Mg 2+ as cofactor
- ❖ Cut in close proximity of the recognition site
- ❖ Single function (restriction digestion) enzymes independent of methylase.
- ❖ Homodimers
- ❖ ATP hydrolysis is not required
- ❖ Examples: EcoRI, EcoRII, BamHI, HindIII

Type III enzymes (EC 3.1.21.5)

- ❖ Large enzymes
- ❖ Combination restriction-and-modification
- ❖ Cleave outside of their recognition sequences
- ❖ Require two recognition sequences in opposite orientations within the same DNA moleculecle.
- ❖ Require ATP (but do not hydrolyse it); S-adenosyl-L-methionine stimulates the reaction but is not required

Type IV enzymes

- ❖ Cleave only normal and modified DNA (methylated, hydroxymethylated and glucosyl-hydroxymethylated bases).
- ❖ Recognition sequences have not been well defined
- ❖ Cleavage takes place ~30 bp away from one of the sites

Artificial Restriction Enzymes

- ❖ Generated by fusing a natural or engineered DNA binding domain to a nuclease domain
- ❖ Can target large DNA sites (up to 36 bp)

- ❖ Can be engineered to bind to desired DNA sequences

Mechanism of Action of Restriction Enzymes:

A restriction enzyme (or restriction endonuclease) is an enzyme that cuts double-stranded DNA. The enzyme makes two incisions, one through each of the sugar- phosphate backbones (i.e., each strand) of the double helix without damaging the nitrogenous bases.

The chemical bonds that the enzymes cleave can be reformed by other enzymes known as ligases, so that restriction fragments carved from different chromosomes or genes can be spliced together, provided their ends are complementary.

Restriction enzymes cut the strand of DNA a little away from the center of the palindrome sites, but between the same two bases on the opposite strands. This leaves single-stranded portion at the ends. There are overhanging stretches called sticky ends on each strand as given in above figure.

Restriction endonucleases are also used in genetic engineering to form recombinant molecules of DNA, which are composed of DNA from different sources or genomes. The resultant DNA fragments have the same sticky ends, which are complementary to each other, therefore can be joined together (end-to-end) using DNA ligases, when cut by the same restriction enzyme.

Many of the procedures of molecular biology and genetic engineering rely on restriction enzymes. The term restriction comes from the fact that these enzymes were discovered in *E. coli* strains that appeared to be restricting the infection by certain bacteriophages.

Cloning vectors:

Criteria of an Ideal Vector:

Vectors are those DNA molecules that can carry a foreign DNA fragment when inserted into it. A vector must possess certain minimum qualifications to be an efficient agent for the transfer, maintenance and amplification of the passenger DNA.

1. The vector should be small and easy to isolate.
2. They must have one or more origins of replication so that they will stably maintain themselves within host cell.
3. Vector should have one or more unique restriction sites into which the recombinant DNA can be inserted.
4. They should have a selectable marker (antibiotic resistance gene) which allows recognition of transformants.
5. Vector DNA can be introduced into a cell.
6. The vector should not be toxic to host cell.

History of Cloning Vectors:

Herbert Boyer, Keiichi Itakura, and Arthur Riggs were three scientists working in the Boyer's lab, University of California, where they recognized a general cloning vector. This cloning vector had restriction sites for cloning foreign DNA and also, the expression of antibiotic resistance genes for the screening of recombinant/ transformed cells. The first vector used for cloning purposes was pBR322, a plasmid. It was small in size, nearly 4kB, and had two selectable markers.

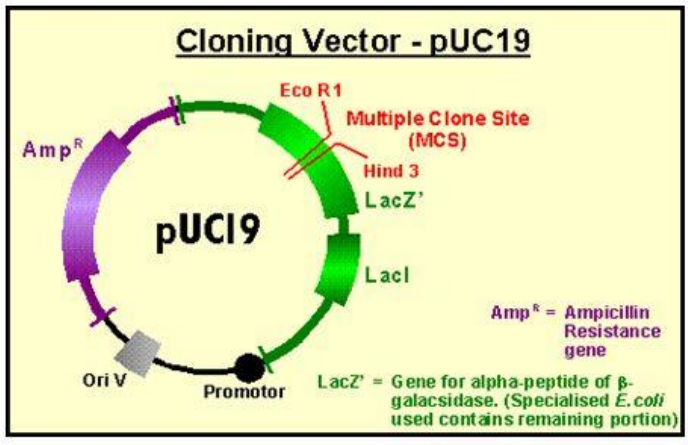
Features of Cloning Vectors

1. Origin of Replication (ori)

- A specific set/ sequence of nucleotides where replication initiates.
- For autonomous replication inside the host cell.
- Foreign DNA attached to ori also begins to replicate.

2. Cloning Site

- Point of entry or analysis for genetic engineering.
- Vector DNA at this site is digested and foreign DNA is



Cloning vector

inserted with the aid of restriction enzymes.

- Recent works have discovered plasmids with multiple cloning sites (MCS) which harbour up to 20 restriction sites.

3. Selectable Marker

- Gene that confers resistance to particular antibiotics or selective agent which, under normal conditions, is fatal for the host organism.
- Confers the host cell the property to survive and propagate in culture medium containing the particular antibiotics.

4. Marker or Reporter Gene

- Permits the screening of successful clones or recombinant cells.
- Utilised extensively in blue-white selection.

5. Inability to Transfer via Conjugation

Vectors must not enable recombinant DNA to escape to the natural population of bacterial cells.

Types of Cloning Vectors:

A. Plasmids

- Plasmids were the first vectors to be used in gene cloning.
- They are naturally occurring and autonomously replicating extra-chromosomal double-stranded circular DNA molecules. However, not all plasmids are circular in origin.
- Plasmids
- They are present in bacteria, archaea, and eukaryotes.
- The size of plasmids ranges from 1.0 kb to 250 kb.
- DNA insert of up to 10 kb can be cloned in the plasmids.
- The plasmids have high copy number which is useful for production of greater yield of recombinant plasmid for subsequent experiments.
- The low copy number plasmids are exploited under certain conditions like the cloned gene produces the protein which is toxic to the cells.
- Plasmids only encode those proteins which are essential for their own replication. These protein-encoding genes are located near the ori.

Examples: pBR322, pUC18, F plasmid, Col plasmid.

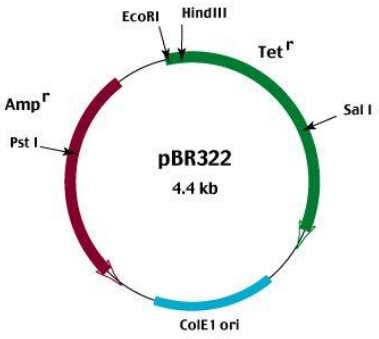
Nomenclature of plasmid cloning vector: pBR322 cloning vector has the following elements:

p= plasmid

B= Bolivar (name of the scientist)

R= Rodriguez (name of the scientist)

322= number of plasmid discovered in the same lab



pBR322 plasmid

Advantages of using Plasmids as vectors:

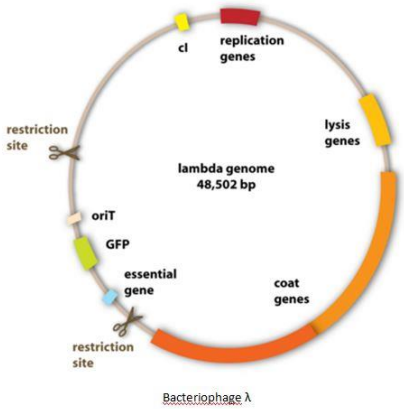
- Easy to manipulate and isolate because of small size.
- More stable because of circular configuration.
- Replicate independent of the host.
- High copy number.
- Detection easy because of antibiotic-resistant genes.

Disadvantages of using Plasmids as vectors:

- Large fragments cannot be cloned.
- Size range is only 0 to 10kb.
- Standard methods of transformation are inefficient.

B. Bacteriophage:

- Bacteriophages or phages are viruses which infect bacterial cells.
- The most common bacteriophages utilized in gene cloning are Phage λ and M13 Phage.
- A maximum of 53 kb DNA can be packaged into the phage.



Bacteriophage λ

- If the vector DNA is too small, it cannot be packaged properly into the phage.

Examples: Phage Lambda, M13 Phage, etc.

Phage Lambda λ:

- It has head, tail, and tail fibers.
- Its genome consists of 48.5 kb of DNA and 12 bp ss DNA which comprise of sticky ends at both the terminals. Since these ends are complementary, they are cohesive and also referred to as cos sites.
- Infection by λ phage requires adsorption of tail fibers on the cell surface, contraction of the tail, and injection of the DNA inside the cell.

M13 Phage

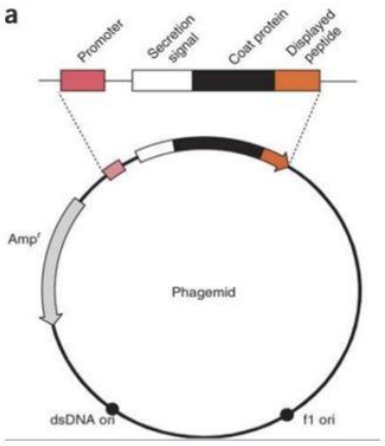
- These vectors are used for obtaining single-stranded copies of the cloned DNA.
- They are utilized in DNA sequencing and in vitro mutagenesis.
- M13 phages are derived from filamentous bacteriophage M13. The genome of M13 is 6.4 kb.
- DNA inserts of large sizes can be cloned.
- From the double-stranded inserts, pure single-stranded DNA copies are obtained.

Advantages of using Phage Vectors

- They are way more efficient than plasmids for cloning large inserts.
- Screening of phage plaques is much easier than identification of recombinant bacterial colonies.

C. Phagemids or Phasmid

- They are prepared artificially.
- Phasmid contains the F1 origin of replication from F1 phage.
- They are generally used as a cloning vector in combination with M13 phage.
- It replicates as a plasmid and gets packaged in the form of single-stranded DNA in viral particles.



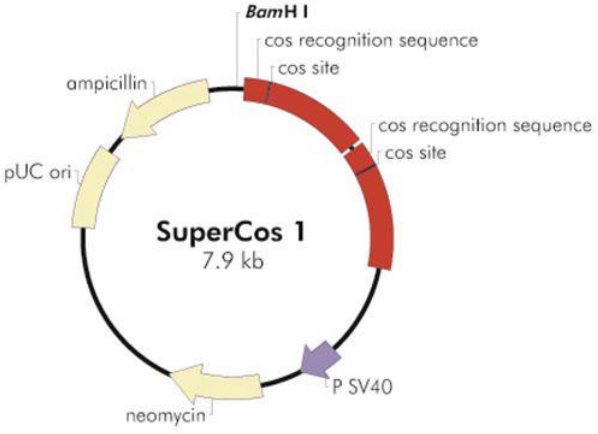
Phagemid

Advantages of using Phagemids:

- They contain multiple cloning sites.
- An inducible lac gene promoter is present.
- Blue-white colony selection is observed.

D. Cosmids

- Cosmids are plasmids.
- They are capable of incorporating the bacteriophage λ DNA segment. This DNA segment contains cohesive terminal sites (cos sites).
- Cos sites are necessary for efficient packaging of DNA into λ phage particles.
- Large DNA fragments of size varying from 25 to 45 kb can be cloned.
- They are also packaged into λ This permits the foreign DNA fragment or genes to be introduced into the host organism by the mechanism of transduction.



Cosmid vector

Advantages of using cosmids as vectors:

- They have high transformation efficiency and are capable of producing a large number of clones from a small quantity of DNA.
- Also, they can carry up to 45 kb of insert compared to 25 kb carried by plasmids and λ.

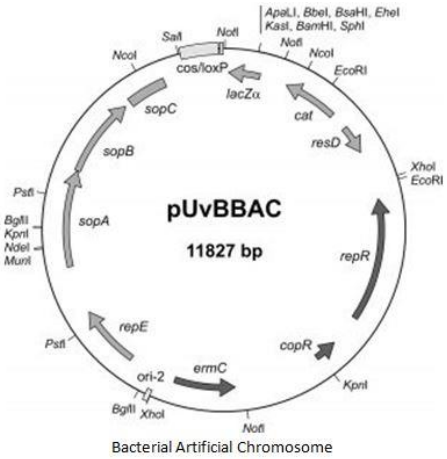
Disadvantages of using cosmids as vectors:

- Cosmids cannot accept more than 50 kb of the insert.

- Cosmid VectorCosmid Vector

E. Bacterial Artificial Chromosomes (BACs)

- Bacterial artificial chromosomes are similar to E. coli plasmid vectors.
- They contain ori and genes which encode ori binding proteins. These proteins are critical for BAC replication.
- It is derived from naturally occurring F' plasmid.
- The DNA insert size varies between 150 to 350 kb.



Bacterial Artificial Chromosome

Advantages of BACs:

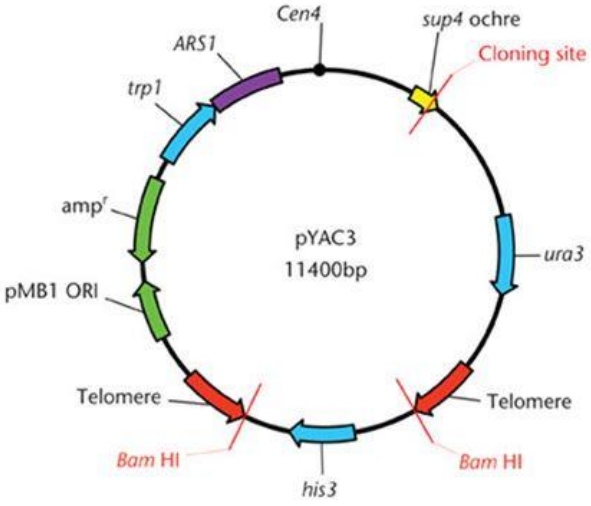
- They are capable of accommodating large sequences without any risk of rearrangement.
- BACs are frequently used for studies of genetic or infectious disorders.
- High yield of DNA clones is obtained.

Disadvantages of BACs:

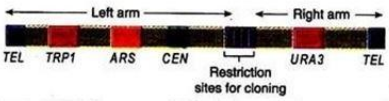
- They are present in low copy number.
- The eukaryotic DNA inserts with repetitive sequences are structurally unstable in BACs often resulting in deletion or rearrangement.

F. Yeast Artificial Chromosomes (YACs)

- A large DNA insert of up to 200 kb can be cloned.
- They are used for cloning inside eukaryotic cells. These act as eukaryotic chromosomes inside the host eukaryotic cell.
- It possesses the yeast telomere at each end.
- A yeast centromere sequence (CEN) is present which allows proper segregation during meiosis.
- The ori is bacterial in origin.
- Both yeast and bacterial cells can be used as hosts.



Yeast Artificial Chromosome



Structure of yeast artificial chromosome (YAC) cloning vector. It contains a telomere (TEL) at each end, a yeast centromere sequence (CEN), a yeast selectable marker for each arm (*TRP1* and *URA3*), a sequence that allows autonomous replication in yeast (ARS) and restriction sites for cloning

Yeast Artificial Chromosome

Advantages of using YACs:

- A large amount of DNA can be cloned.
- Physical maps of large genomes like the human genome can be constructed.

Disadvantages of using YACs:

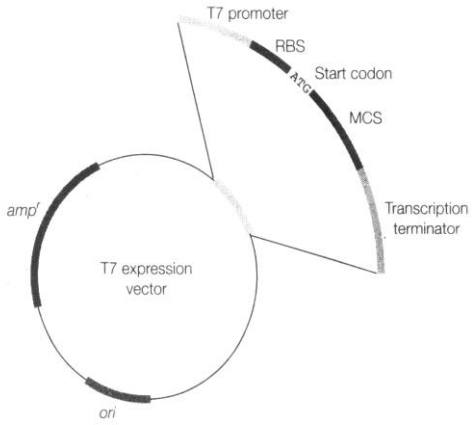
- Overall transformation efficiency is low.
- The yield of cloned DNA is also low.

Advantages of BACs over YACs

- Comparatively stable.
- Easy to transform.
- Simple purification required.
- User- friendly.
- Aid in the development of vaccines.

G. Expression Vectors

- Express the DNA insert producing specific protein.
- They have prokaryotic promoter.
- Ribosome binding site.
- Origin of replication.



- Antibiotic resistance gene.
- Expression vectors with strong promoters.
- Inducible Expression Vectors.
- Eukaryotic expression vectors.

H. Human Artificial Chromosome (HACs)

- Human artificial chromosomes are artificially synthesized.
- They are utilized for gene transfer or gene delivery into human cells.
- It can carry large amounts of DNA inserts.
- They are used extensively in expression studies and determining the function of the human chromosomes.

Advantages of using HACs:

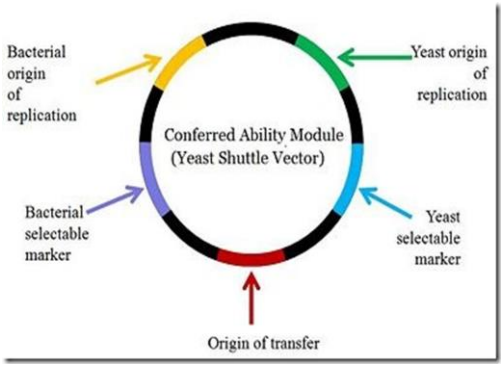
- No upper limit on DNA that can be cloned.
- it avoids the possibility of insertional mutagenesis.

Summary of vectors and what they can carry.

The size of DNA that vector can carry	Vector
0 – 10 kb	Standard plasmid
0 – 23 Kb	Lambda Bacteriophage
30 – 44 Kb	Cosmid
70 – 100 Kb	Bacteriophage P1
130 – 150 Kb	P1 Artificial chromosome PAC
Maximum 300 Kb	Bacterial Artificial Chromosome BAC
0.2 – 2 Mb	Yeast Artificial Chromosome YAC

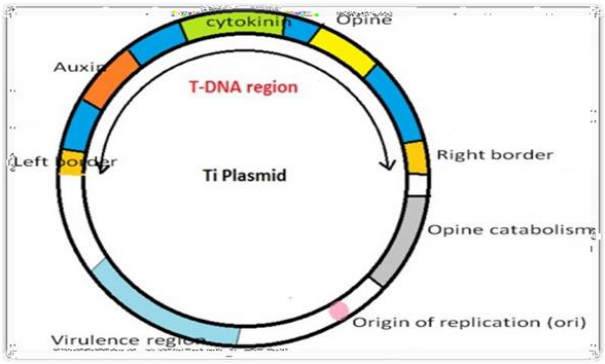
Shuttle vectors:

Vectors contain sequences required for replication and selection in both *E. coli* and the desired host cells, so that the construction and many other manipulations of the recombinant plasmids can be completed in *E. coli*. Most of the eukaryotic vectors are constructed as shuttle vectors.



Recombinant Ti plasmid:

1. Place the target gene in the T-DNA region of a Ti plasmid, then transform the recombinant Ti plasmid.
2. Recombinant T-DNA transformed into the A. tumefaciens cell carrying a modified Ti plasmid without T-DNA (T-DNA that are responsible for crown gall formation. The deleted T-DNA is called disarmed T-DNA shuttle vector).



Construction of recombinant DNA:

Tools for Constructing Recombinant DNA:

Recombinat DNA technology utilizes a number of biological tools to achieve its objectives, most important of them being the enzymes. Important biological tools for rec DNA technology are:

A. Enzymes:
    a) Restriction Endonucleases
    b) Exonucleases
    c) DNA ligases
    d) DNA polymerase
B. Cloning Vector
C. Host organism
D. DNA insert or foreign DNA
E. Linker and adaptor sequences

Enzymes:

A number of specific enzymes are utilized to achieve the objectives of rec DNA technology.

The enzymology of genetic engineering includes the following types of enzymes:

(a) Restriction Endonuclease:

These enzymes serve as important tools to cut DNA molecules at specific sites, which is the basic need for rec DNA technology.

These are the enzymes that produce internal cuts (cleavage) in the strands of DNA, only within or near some specific sites called recognition sites/recognition sequences/ restriction sites 01 target sites. Such recognition sequences are specific for each restriction enzyme. Restriction endonuclease enzymes are the first necessity for rec DNA technology.

The presence of restriction enzymes was first of all reported by W. Arber in the year 1962. He found that when the DNA of a phage was introduced into a host bacterium, it was fragmented into small pieces. This led him to postulate the presence of restriction enzymes. The first true restriction endonuclease was isolated in 1970s from the bacterium E. coli by Meselson and Yuan.

Another important breakthrough was the discovery of restriction enzyme Hind-II in 1970s by Kelly, Smith and Nathans. They isolated it from -the bacterium Haemophilus influenza. In the year 1978, the Nobel Prize for Physiology and Medicine was given to Smith, Arber and Nathans for the discovery of endonucleases.

(b) Exonucleases:

Exonuclease is an enzyme that removes nucleotides from the ends of a nucleic acid molecule. An exonuclease removes nucleotide from the 5′ or 3′ end of a DNA molecule. An exonuclease never produces internal cuts in DNA.

In rec DNA technology, various types of exonucleases are employed like Exonuclease Bal31, *E. coli* exonuclease III, Lambda exonuclease, etc.

Exonculease Bal31 are employed for making the DNA fragment with blunt ends shorter from both its ends.

(c) DNA ligase:

The function of these enzymes is to join two fragments of DNA by synthesizing the phosphodiester bond. They function to repair the single stranded nicks in DNA double helix and in rec DNA technology they are employed for sealing the nicks between adjacent nucleotides. This enzyme is also termed as molecular glue.

(d) DNA polymerases:

These are the enzymes which synthesize a new complementary DNA strand of an existing DNA or RNA template. A few important types of DNA polymerases are used routinely in genetic engineering. One such enzyme is DNA polymerase, which prepared from *E coli*. The Klenow fragment of DNA polymerase-I .s employed to make the protruding ends double-stranded by extension of the shorter strand.

Another type of DNA polymerase used in genetic engineering is Taq DNA polymerase which is used in PCR (Polymerase Chain Reaction).

Reverse transcriptase is also an important type of DNA polymerase enzyme for genetic engineering. It uses RNA as a template for synthesizing a new DNA strand called as cDNA a e complementary DNA). Its main use is in the formation of cDNA libraries. Apart from all these above mentioned enzymes, a few other enzymes also mark their importance in genetic engineering.

(e) Terminal deoxynucleotidyl transferase enzyme:

It adds single stranded sequences to 3′-terminus of the DNA molecule. One or more deoxynbonucleotides (dATP, dGTP, dl IP, dCTP) are added onto the 3′-end of the blunt- ended fragments.

(f) Alkaline Phosphatase Enzyme:

It functions to remove the phosphate group from the 5′-end of a DNA molecule.

(g) Polynucleotide Kinase Enzyme:

It has an effect reverse to that of Alkaline Phosphatase, i.e. it functions to add phosphate group to the 5′-terminus of a DNA molecule.

Cloning Vectors:

It is another important natural tool which geneticists use in rec DNA technology. The cloning vector is the DNA molecule capable of replication in a host organism, into which the target DNA is introduced producing the rec DNA molecule.

A cloning vector may also be termed as a cloning vehicle or earner DNA or simply as a vector or a vehicle a great variety of cloning vectors are present for use with E. coli is the host organism. Different types of DNA molecules may be used as cloning vehicles such as they may be plasmids, bacteriophages, cosmids, phasmids or artificial chromosomes.

Host Organism:

A good host organism is an essential tool tor genetic engineering. Most widely used host for rec DNA technology is the bacterium E. coli. because cloning and isolation of DNA inserts is very easy in this host. A good host organism is the one winch easy to transform and in which the replication of rec DNA is easier. There should not be any interfering element against the replication of rec DNA in the host cells

DNA Insert or Foreign DNA:

The desired DNA segment which is to be cloned is called as DNA insert or foreign DNA or target DNA. The selection of a suitable target DNA is the very first step of rec DNA technology. The target DNA (gene) may be of viral, plant, animal or bacterial origin.

Following points must be kept in mind while selecting the foreign DNA:

1. It can be easily extracted from source.
2. It can be easily introduced into the vector.
3. The genes should be beneficial for commercial or research point of view.

Linker and Adaptor Sequences:

Linkers and adaptors are the DNA molecules which help in the modifications of cut ends of DNA fragments. These can be joined to the cut ends and hence produce modifications as desired.

Both are short, chemically synthesized, double stranded DNA sequences. Linkers have (within them) one or more restriction endonuclease sites and adaptors have one or both sticky ends. Different types of linkers and adaptors are used for different purposes.

Steps in Recombinant DNA Technology:

The basic 7 steps involved in gene cloning are:

1. Isolation of DNA [gene of interest] fragments to be cloned.
2. Insertion of isolated DNA into a suitable vector to form recombinant DNA.
3. Introduction of recombinant DNA into a suitable organism known as host.

4. Selection of transformed host cells and identification of the clone containing the gene of interest.
5. Multiplication/Expression of the introduced Gene in the host.
6. Isolation of multiple gene copies/Protein expressed by the gene.
7. Purification of the isolated gene copy/protein

Isolation of the DNA fragment or gene:

- The target DNA or gene to be cloned must be first isolated. A gene of interest is a fragment of gene whose prod-uct (a protein, enzyme or a hormone) interests us. For example, gene encoding for the hormone insulin.
- The desired gene may be isolated by using restriction endonuclease (RE) enzyme, which cut DNA at specific recognition nucleotide se-quences known as restriction sites towards the inner region (hence endonuclease) producing blunt or sticky ends.
- Sometimes, reverse transcriptase enzyme may also be used which synthesizes complementary DNA strand of the desired gene using its mRNA.

Selection of suitable cloning vector

- The vector is a carrier molecule which can carry the gene of interest (GI) into a host, replicate there along with the GI making its multiple copies.
- The cloning vectors are limited to the size of insert that they can carry. Depending on the size and the application of the insert the suitable vector is selected.
- The different types of vectors available for cloning are plasmids, bacteriophages, bacterial artificial chromosomes (BACs), yeast artificial chromosomes (YACs) and mammalian artificial chromosomes (MACs).
- However, the most commonly used cloning vectors include plasmids and bacteriophages (phage λ) beside all the other available vectors.

Formation of Recombinant DNA

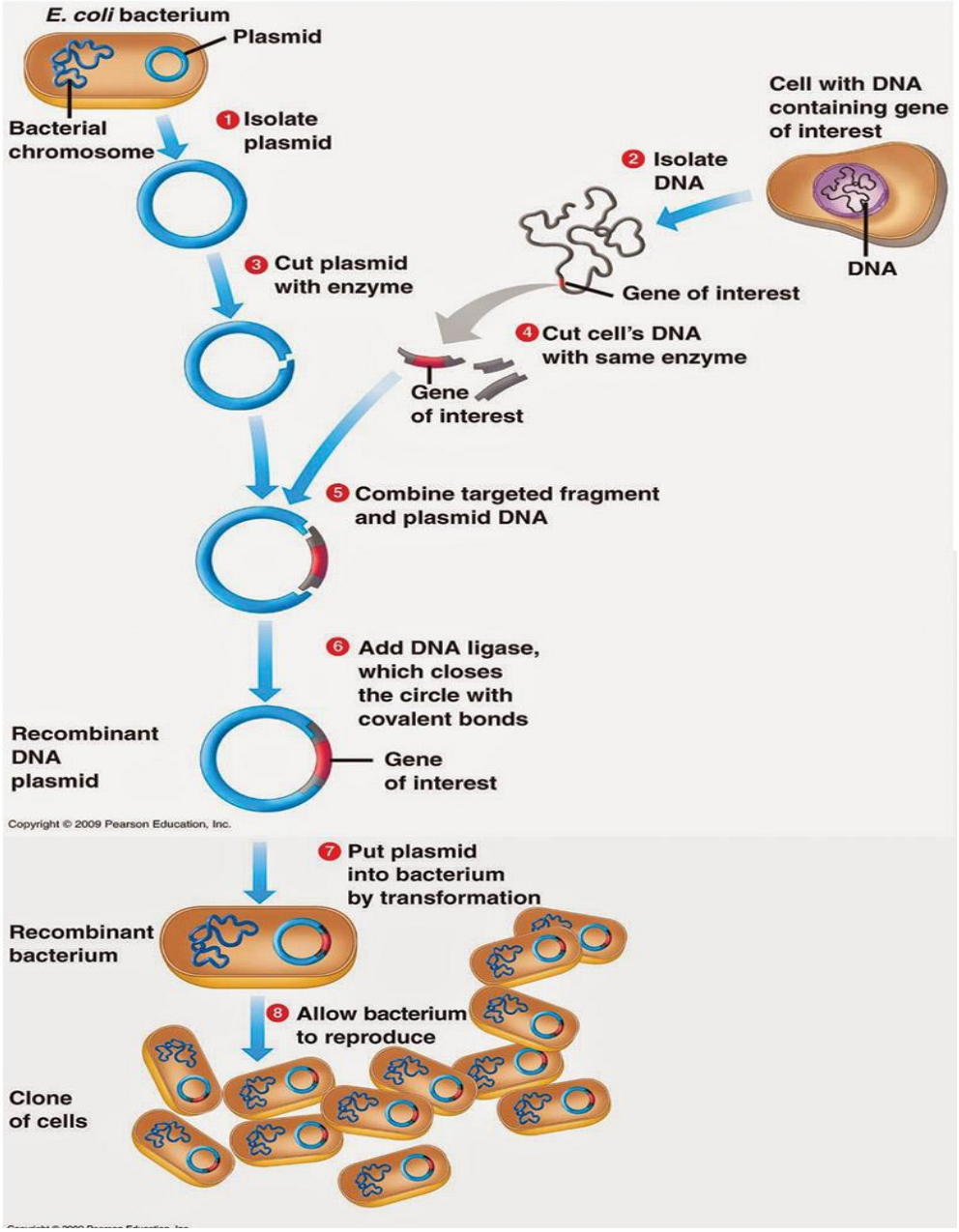
- The plasmid vector is cut open by the same RE enzyme used for isolation of donor DNA fragment.
- The mixture of donor DNA fragment and plasmid vector are mixed together.
- In the presence of DNA ligase, base pairing of donor DNA fragment and plasmid vector occurs.
- The result-ing DNA molecule is a hybrid of two DNA molecules – the GI and the vector. In the ter-minology of genetics this intermixing of dif-ferent DNA strands is called recombination.
- Hence, this new hybrid DNA molecule is also called a recombinant DNA molecule and the technology is referred to as the recom-binant DNA technology.

Transformation of recombinant vector into suitable host

- The recombinant vector is transformed into suitable host cell mostly, a bacterial cell.
- This is done either for one or both of the following reasons:
- To replicate the recombinant DNA mol-ecule in order to get the multiple copies of the GI.
- To allow the expression of the GI such that it produces its needed protein product.
- Some bacteria are naturally transformable; they take up the recombinant vector automatically.
- For example: *Bacillus*, *Haemophillus*, *Helicobacter pylori*, which are naturally competent.
- Some other bacteria, on the other hand require the incorporation by artificial methods such as Ca^{++} ion treatment, electroporation, etc.

Isolation of Recombinant Cells

- The transformation process generates a mixed population of transformed and non-trans-formed host cells.
- The selection process involves filtering the transformed host cells only.
- For isolation of recombinant cell from non-recombinant cell, marker gene of plasmid vector is employed.
- For examples, PBR322 plasmid vector contains different marker gene (Ampicillin resistant gene and Tetracycline resistant gene. When pst1 RE is used it knock out Ampicillin resistant gene from the plasmid, so that the recombinant cell become sensitive to Ampicillin.



Multiplication of Selected Host Cells

- Once transformed host cells are separated by the screening process; becomes necessary to provide them optimum parameters to grow and multiply.
- In this step the transformed host cells are introduced into fresh culture media .
- At this stage the host cells divide and re-divide along with the replication of the recom-binant DNA carried by them.
- If the aim is obtaining numerous copies of GI, then simply replication of the host cell is allowed. But for obtaining the product of interest, favourable conditions must be provided such that the GI in the vector expresses the product of interest.

Isolation and Purification of the Product

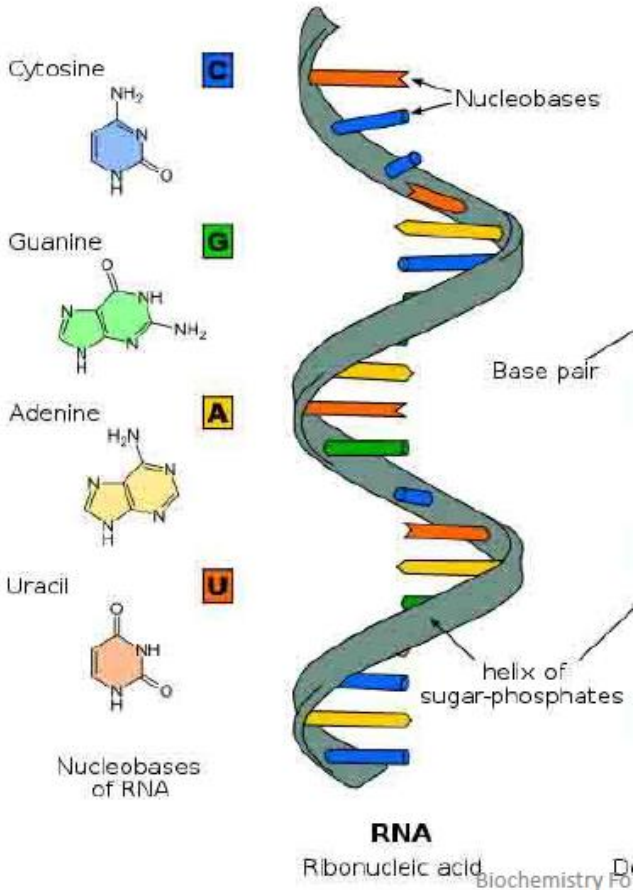
- The next step involves isolation of the multiplied GI attached with the vector or of the protein encoded by it.
- This is followed by purification of the isolated gene copy/protein.

7. Biology of RNA types: Ribosomal RNA, transfer RNA and messenger RNA; post m-RNA modifications, RNA splicing, Regulatory RNAs and related phenomenon: si-RNA, mi-RNA, Ribozyme, antisense RNA, RNAi, Riboswitch

Ribonucleic acid (RNA) is a polymeric molecule essential in various biological roles in coding, decoding, regulation and expression of genes. RNA and DNA are nucleic acids, and, along with lipids, proteins and carbohydrates, constitute the four major macromolecules essential for all known forms of life.

Structure of RNA

- Ribonucleic acid, or RNA is one of the major biological macromolecules that are essential for all known forms of life (along with DNA and proteins)
- Each nucleotide in RNA contains a ribose sugar, with carbons numbered 1' through 5'. A base is attached to the 1' position, in general, adenine (A), cytosine (C), guanine(G), and uracil (U).
- Back bone is sugar and phosphate group
- Nitrogenous bases linked to sugar moiety project fromthe backbone
- Nitrogenous bases are linked to pentose sugar through
- N-glycosidic linkage to form a nucleoside
- Phosphate group is linked with 3'OH of nucleoside through phosphoester linkage
- 2 nucleotides are linked through 3'-5'-phosphodiester linkage to form a dinucleotide
- More and more such groups will be linked to form a polynucleotide chain
- Such a polymer has a free phosphate moiety at 5' end of ribose sugar and it is called as 5'-end of polynucleotide chain
- At other end, ribose has free 3'-OH group which is called as the 3'-end of polynucleotide chain
- In RNA, every nucleotide has an additional-OH present at 2'-position of ribose



Types of RNA:

In all prokaryotic and eukaryotic organisms, three main classes of RNA molecules exist-

1) Messenger RNA(m RNA)
2) Transfer RNA (t RNA)
3) Ribosomal RNA (r RNA)

The other are –

- small nuclear RNA (SnRNA),
- micro RNA(mi RNA) and
- small interfering RNA(Si RNA) and
- heterogeneous nuclear RNA (hnRNA).

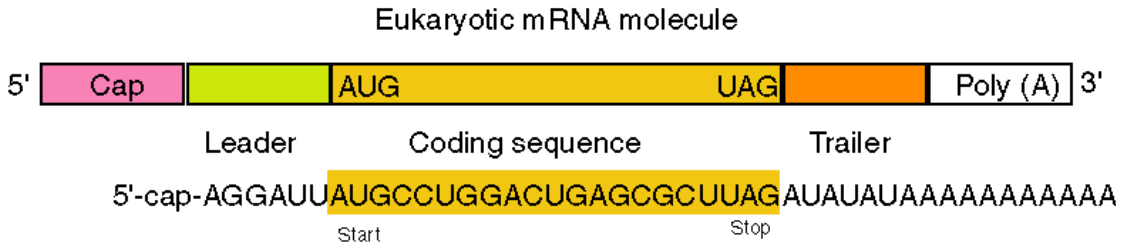
Messenger RNA (m-RNA):

- Comprises only 5% of the RNA in the cell

- Most heterogeneous in size and base sequence
- All members of the class function as messengers carrying the information in a gene to the protein synthesizing machinery

Structural Characteristics of m-RNA:

- The 5' terminal end is capped by 7- methyl guanosine triphosphate cap.
- The cap is involved in the recognition of mRNA by the translating machinery
- It stabilizes m RNA by protecting it from 5' exonuclease
- The 3'end of most m-RNAs have a polymer of Adenylate residues(20-250)
- The tail prevents the attack by 3' exonucleases
- Histones and interferons do not contain poly A tails
- On both 5' and 3' end there are non coding sequences which are not translated (NCS)
- The intervening region between non coding sequences present between 5' and 3' end is called coding region. This region encodes for the synthesis of a protein.
- The m- RNA molecules are formed with the help of DNA template during the process of transcription.
- The sequence of nucleotides in m RNA is complementary to the sequence of nucleotides on template DNA.
- The sequence carried on m -RNA is read in the form of codons.
- A codon is made up of 3 nucleotides
- The m-RNA is formed after processing of heterogeneous nuclear RNA



Transfer RNA (t- RNA)

- Transfer RNA is the smallest of three major species of RNA molecules
- They have 74-95 nucleotide residues
- They are synthesized by the nuclear processing of a precursor molecule
- They transfer the amino acids from cytoplasm to the protein synthesizing machinery, hence the name t RNA.
- They are easily soluble, hence called "Soluble RNA or s RNA
- They are also called Adapter molecules, since they act as adapters for the translation of the sequence of nucleotides of the m RNA in to specific amino acids
- There are at least 20 species of t RNA one corresponding to each of the 20 amino acids required for protein synthesis.

Structural characteristics of t- RNA

1) Primary structure- The nucleotide sequence of all the t RNA molecules allows extensive intrastand complimentarity that generates a secondary structure.

2) Secondary structure- Each single t- RNA shows extensive internal base pairing and acquires a clover leaf like structure. The structure is stabilized by hydrogen bonding between the bases and is a consistent feature. Secondary structure (Clover leaf structure) of all t-RNA contain 5 main arms or loops which are as follows

a) Acceptor arm
b) Anticodon arm
c) D HU arm
d) TΨ C arm
e) Extra arm

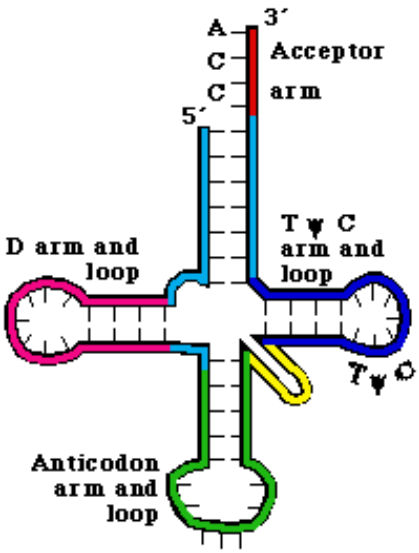
a) Acceptor arm

- The acceptor arm is at 3' end
- It has 7 base pairs

- The end sequence is unpaired Cytosine, Cytosine-Adenine at the 3' end
- The 3' OH group terminal of Adenine binds with carboxyl group of amino acids
- The t RNA bound with amino acid is called Amino acyl t RNA
- CCA attachment is done post transcriptionally

b) Anticodon arm

- Lies at the opposite end of acceptor arm
- 5 base pairs long
- Recognizes the triplet codon present in the m RNA
- Base sequence of anticodon arm is complementary to the base sequence of m RNA codon.
- Due to complimentarity it can bind specifically with m RNA by hydrogen bonds.



c) DHU arm

- It has 3-4 base pairs
- Serves as the recognition site for the enzyme (amino acyl t RNA synthetase) that adds the amino acid to the acceptor arm.

d) TΨC arm

- This arm is opposite to DHU arm
- Since it contains pseudo uridine that is why it is so named
- It is involved in the binding of t RNA to the ribosomes

e) Extra arm or Variable arm

- About 75 % of t RNA molecules possess a short extra arm
- If about 3-5 base pairs are present the t-RNA is said to be belonging to class 1. Majority t -RNA belong to class 1.
- The t –RNA belonging to class 2 have long extra arm, 13-21 base pairs in length.

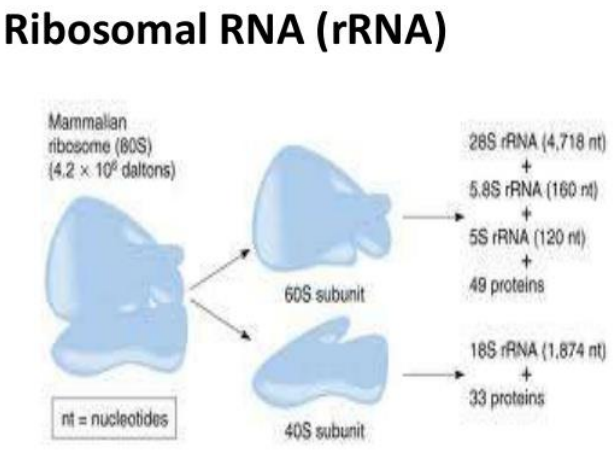
Tertiary structure of t- RNA

The L shaped tertiary structure is formed by further folding of the clover leaf due to hydrogen bonds between T and D arms. The base paired double helical stems get arranged in to two double helical columns, continuous and perpendicular to one another.

Ribosomal RNA (rRNA):

The mammalian ribosome contains two major nucleoprotein subunits—a larger one with a molecular weight of 2.8 x 106 (60S) and a smaller subunit with a molecular weight of 1.4 x 106 (40S).

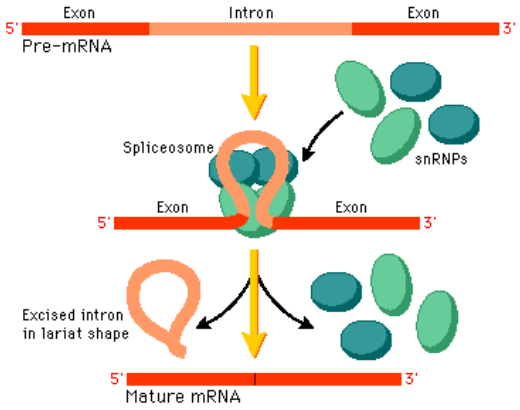
- The 60S subunit contains a 5S ribosomal RNA (rRNA), a 5.8S rRNA, and a 28S rRNA; there are also probably more than 50 specific polypeptides.
- The 40S subunit is smaller and contains a single 18S rRNA and approximately 30 distinct polypeptide chains.
- All of the ribosomal RNA molecules except the 5S rRNA are processed from a single 45S precursor RNA molecule in the nucleolus.
- 5S rRNA is independently transcribed.
- The functions of the ribosomal RNA molecules in the ribosomal particle are not fully understood, but they are necessary for ribosomal assembly and seem to play key roles in the binding of mRNA to ribosomes and its translation



- Recent studies suggest that an rRNA component performs the peptidyl transferase activity and thus is an enzyme (a ribozyme).

Small RNA

Most of these molecules are complexed with proteins to form ribonucleoproteins and are distributed in the nucleus, in the cytoplasm, or in both. They range in size from 20 to 300 nucleotides and are present in 100,000–1,000,000 copies per cell.



Small nuclear RNAs (snRNA): Many RNAs are involved in modifying other RNAs. Introns are spliced out of pre-mRNA by spliceosomes, which contain several small nuclear RNAs (snRNA), or the introns can be ribozymes that are spliced by them. Of the several snRNAs, U1, U2, U4, U5, and U6 are involved in intron removal and the processing of hnRNA into mRNA. The U7 snRNA is involved in production of the correct 3' ends of histone mRNA—which lacks a poly(A) tail.

Small nucleolar RNAs (snoRNA): RNA can also be altered by having its nucleotides modified to nucleotides other than A, C, G and U. In eukaryotes, modifications of RNA nucleotides are in general directed by small nucleolar RNAs (snoRNA; 60–300 nt), found in the nucleolus and cajal bodies. snoRNAs associate with enzymes and guide them to a spot on an RNA by base pairing to that RNA. These enzymes then perform the nucleotide modification. rRNAs and tRNAs are extensively modified, but snRNAs and mRNAs can also be the target of base modification. RNA can also be methylated.

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, the dicer enzymes and closely related effector complexes for post- transcriptional repression of protein synthesis. On the other hand, siRNAs and miRNAs differ in their molecular origins. In the cytoplasm the dicer enzymes split the dsRNA primer molecules. The finished siRNAs in animals are usually 21-22 nitrogen bases long, similar to miRNAs.

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*.

Micro-RNAs genes produce short (~22 nitrogen bases) ss segments that fold over on themselves forming a short section of dsRNA in hairpin like structure. Humans express over 460 genetically encoded miRNAs. These miRNAs make up more than 1 % of human genome and may regulate over 30% of all protein coding genes. Micro-RNAs can pair exactly with a mRNA and cause its cleavage and destruction, or it can pair partially with mRNA and produce translational inhibition (block the ribosome). It is presumed that they are involved in regulating development by controlling as transcriptional factor.

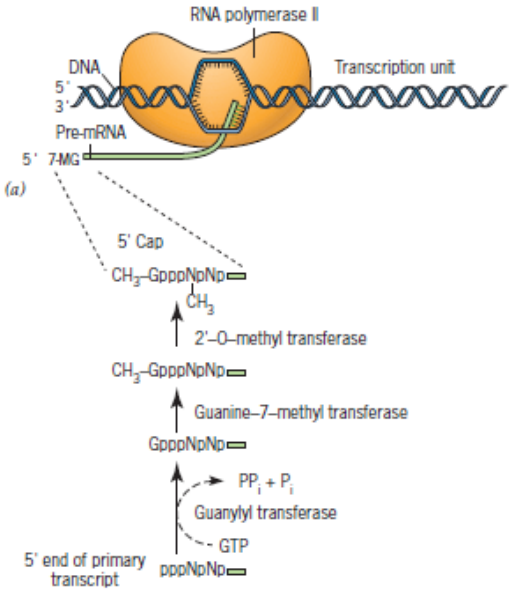
Ribozymes:

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.

Ribozymes are involved in the transformation of large precursor molecules of tRNA, rRNA and mRNA into smaller final products. In their active form, ribozymes are complexed with protein molecules, e.g., the enzyme ribonuclease-P (RNAse-P) is found in all living

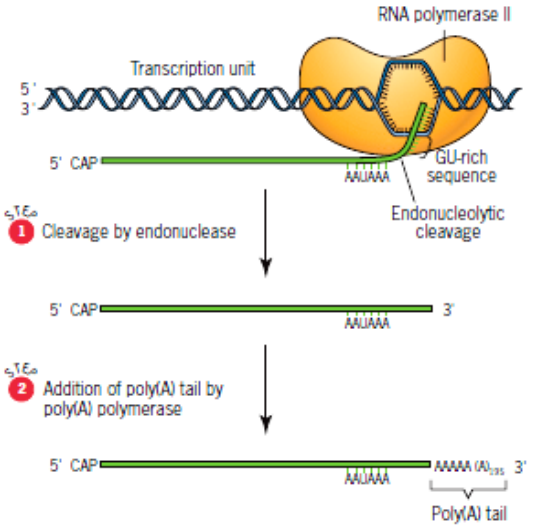
Pre-mRNA processing:

Capping: Early in the elongation process, the 5' ends of eukaryotic pre-mRNAs are modified by the addition of 7-methyl guanosine (7-MG) caps. These 7-MG caps are added when the growing RNA chains are only about 30 nucleotides long. The 7-MG cap contains an unusual 5-5' triphosphate linkage and two or more methyl groups. These 5' caps are added co-transcriptionally by the biosynthetic pathway. The 7-MG caps are recognized by protein factors involved in the initiation of translation and also help protect the growing RNA chains from degradation by nucleases.

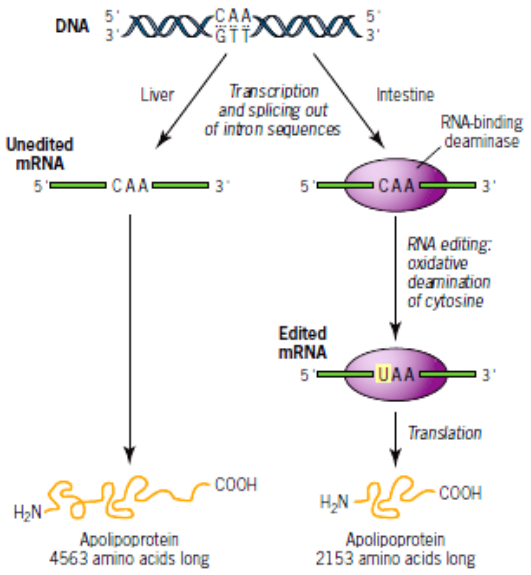


(b) **Pathway of biosynthesis of the 7-MG cap.**

Poly A tail: The cleavage event that produces the 3 ' end of a transcript usually occurs at a site 11 to 30 nucleotides downstream from a conserved polyadenylation signal, consensus AAUAAA, and upstream from a GU-rich sequence located near the end of the transcript. After cleavage, the enzyme poly (A) polymerase adds poly(A) tails, tracts of adenosine monophosphate residues about 200 nucleotides long, to the 3' ends of the transcripts. The addition of poly(A) tails to eukaryotic mRNAs is called polyadenylation. To examine the polyadenylation signal of the human HBB (globin) gene, check out Solve It: Formation of the 3'-Terminus of an RNA Polymerase II Transcript. The formation of poly (A) tails on transcripts requires a specificity component that recognizes and binds to the AAUAAA sequence, a stimulatory factor that binds to the GU-rich sequence, an endonuclease, and the poly(A) polymerase. These proteins form a multimeric complex that carries out both the cleavage and the polyadenylation in tightly coupled reactions. The poly (A) tails of eukaryotic mRNAs enhance their stability and play an important role in their transport from the nucleus to the cytoplasm.



RNA editing: Normally, the genetic information is not altered in the mRNA intermediary. However, the discovery of RNA editing has shown that exceptions do occur.



RNA editing processes alter the information content of gene transcripts in two ways: (1) by changing the structures of individual bases and (2) by inserting or deleting uridine monophosphate residues.

The first type of RNA editing, which results in the substitution of one base for another base, is rare. This type of editing was discovered in studies of the apolipoprotein-B (apo-B) genes and mRNAs in rabbits and humans. Apolipoproteins are blood proteins that transport certain types of fat molecules in the circulatory system. In the liver, the apo-B mRNA

encodes a large protein 4563 amino acids long. In the intestine, the apo-B mRNA directs the synthesis of a protein only 2153 amino acids long. Here, a C residue in the pre-mRNA is converted to a U, generating an internal UAA translation– termination codon, which results in the truncated apolipoprotein.

Splicing:

This process takes place in the nucleus and involves the removal of noncoding intron sequences from pre-mRNAs to produce mature mRNAs in which the coding sequences, corresponding to the exons, are continuous. The mature spliced mRNA, an accurate template for protein synthesis, is then exported to the cyto-plasm where it acts as a template for protein synthesis.

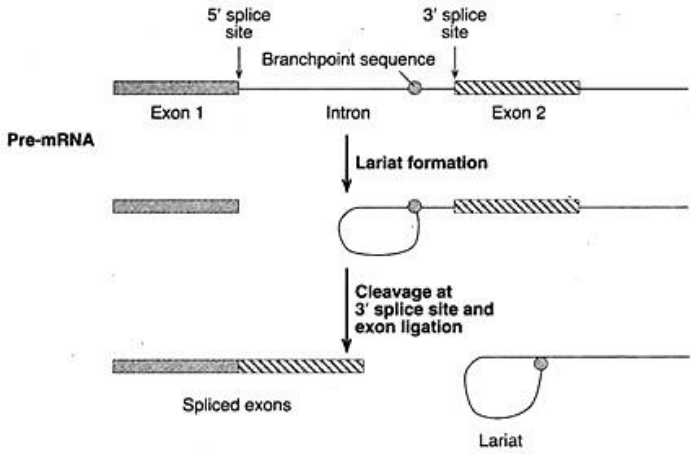


Fig. 16.8A: Splicing of pre-mRNA in eukaryotes

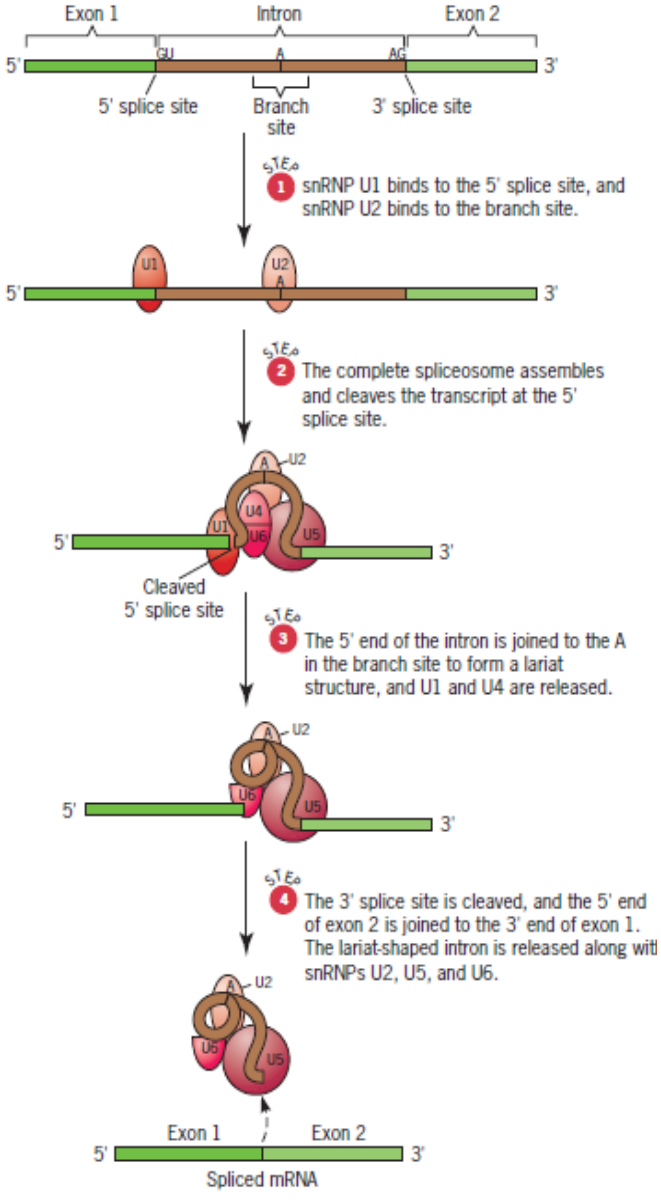
Splicing depends on the presence of signal sequences in the pre-mRNA. In almost all genes, the first two nucleotides at the 5′ end of an intron are GT and the last two at the 3′ end are AG. These are part of larger signal sequences present at the 5′ and 3′ ends of the introns. The complete 5′ signal sequence is 5′ AGGTAAGT 3′ and the 3′ sequence is 5′ YYYYYYNCAG 3′ (Y = pyrimidine, N = any nucleotide).

A branch point sequence is present in vertebrates, in the introns 10-40 bases upstream of the 3′ signal sequence. A more specific sequence 5′ UACUAAC 3′, occurs in introns of yeast. Splicing occurs in two steps (Fig. 16.8A). In the first step, the 2′ hydroxyl group of the adenine of the branch point sequence attacks the phosphodiester bond 5′ to the G of the GT (5′ splice site).

Splicing a pre-mRNA in Eukaryotes

The bond is bro-ken releasing the 5′ end of the intron and attach-ing it to the branch point sequence. The intron now forms a tailed loop structure called a lariat. In the second step, the 3′ end of the Intron is cleaved after G of the AG (3′ splice site), the intron is released and the two exon sequences are joined together.

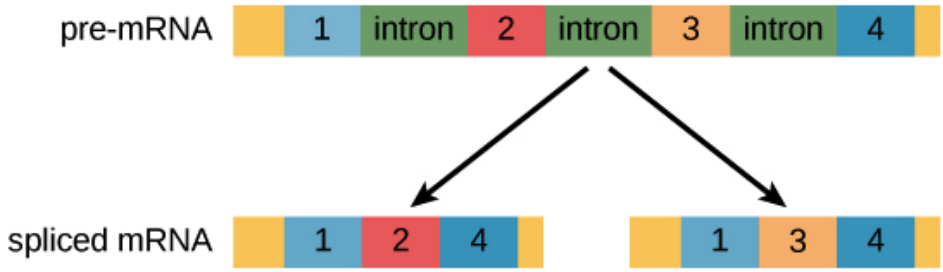
Splicing is catalyzed by a group of molecules called small nuclear ribonucleoproteins (snRNPs) – U1, U2, U4, U5 and U6. These are composed of small RNA molecules rich in uracil, called U RNAs or small nuclear RNAs (snRNAs) that exist complexed with proteins. The U1 snRNP binds to the 5′ splice site and the U2 snRNP binds to the branch point sequence.



The remaining snRNPs, U5 and U4/U6, then form a complex with U1 and U2 causing the intron to loop out and the exons to be brought together. The combination of the pre-mRNA and the snRNPs is called the spliceosome and this is responsible for folding the pre-mRNA into the correct conformation for splicing (Fig. 16.8B).
The spliceosome also catalyzes the cutting and joining reactions that excise the intron and ligate the exons. Once splicing is completed the spliceosome dissociates.

Alternative splicing

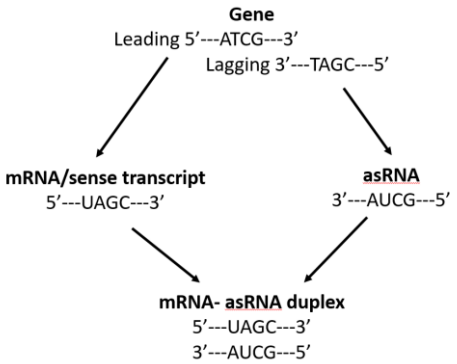
Most pre-mRNA molecules have sections that are removed from the molecule, called introns, and sections that are linked or together to make the final mRNA, called exons. This process is called splicing.
In the process of alternative splicing, different portions of an mRNA can be selected for use as exons. This allows either of two (or more) mRNA molecules to be made from one pre-mRNA.



Alternative splicing 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.

Antisense RNA

Antisense RNA (asRNA), also referred to as antisense transcript, natural antisense transcript (NAT) or antisense oligonucleotide, is a single stranded RNA that is complementary to a protein coding messenger RNA (mRNA) with which it hybridizes, and thereby blocks its translation into protein. as RNAs (which occur naturally) have been found in both prokaryotes and eukaryotes, antisense transcripts can be classified into short (<200 nucleotides) and long (>200 nucleotides) non-coding RNAs (ncRNAs). The primary function of asRNA is regulating gene expression. asRNAs may also be produced synthetically and have found wide spread use as research tools for gene knockdown. They may also have therapeutic applications.



RNA interference

RNA interference (RNAi)is a mechanism where the presence of certain fragments of dsRNA interferes with the expression of a particular gene which shares a homologous sequence with this dsRNA.
Before RNAi was well characterized, it was called by other names, including:

- Post Transcriptional Gene Silencing (PTGS)
- Co-supression
- Virus Induced Gene Silencing (VIGS) In Virus
- Quelling In Fungi

RNAi has become a powerful and more reliable technique to inhibit the expression of targeted genes in a precise manner and also determine gene loss-of-function phenotype, when no mutant alleles are unavailable.

- RNAi pathway is well conserved across eukaryotes.

- ❖ The use of RNAi to reduce expression in plants has been a common procedure for many years. Single-stranded antisense RNA was introduced into plant cells that hybridized to the cognate, single-stranded, sense messenger RNA. While scientists first believed that the resulting dsRNA helix could not be translated into a protein, it is now clear that the dsRNA triggered the RNAi response. The use of dsRNA became more widespread after the discovery of the RNAi machinery, first in petunia and later in round worms (Caenorhabditis elegans).
- Because lin-4 encoded no protein, she deduced that it must be these transcripts that are causing the silencing by RNA-RNA interactions.
- The second small RNA wasn't discovered until 2000.

What are small ncRNAs?

Two flavors of small non-coding RNA:

1. Micro RNA (miRNA)
2. Short interfering RNA (siRNA)

Properties of small non-coding RNA:

1. Involved in silencing other mRNA transcripts.
2. Called "small" because they are usually only about 21-24 nucleotides long.
3. Synthesized by first cutting up longer precursor sequences (like the 61 nt one that Lee discovered).
4. Silence an mRNA by base pairing with some sequence on the mRNA.

Salient features of RNA interference machinery

- ❖ The RNA interference machinery processed long dsRNA into small dsRNAs (19–24 nt) using an endonuclease (Dicer), separates the two strands, and then proceeds to destroy other single-stranded RNA molecules that are complementary to one of those sequences.
- ❖ The dsRNAs direct the creation of small interfering RNAs (siRNAs) which target RNA-degrading enzymes (RNAses) to destroy transcripts complementary to the siRNAs.
- ❖ The life cycle and replication of many RNA viruses involves a double-stranded RNA stage, so it is likely that part of the RNA interference machinery evolved as a defense against these viruses.
- ❖ The machinery is however also used by the cell itself to regulate gene activity: certain parts of the genome are transcribed into microRNA, short RNA molecules that fold back on themselves in a hairpin shape to create a double strand. When the RNA interference machinery detects these double strands, it will also destroy all mRNAs that match the microRNA, thus preventing their translation and lowering the activity of many other genes.

Small Regulatory RNAs

Small Regulatory RNAs in eukaryotes fall into two main groups-

- ❖ - miRNAs [microRNAs]
- ❖ - siRNAs [short interfering RNAs]
- ❖ Both of these RNAs are non-coding. i.e. they are untranslated and therefore do not specify a polypeptide product.

miRNAs :

- ❖ MicroRNAs are ssRNA regulatory molecules about 21-23 nucleotide (nt) long that derive from RNA transcripts.
- ❖ miRNAs are coded by genes in the genome of all multicellular eukaryotes, as well as some unicellular ones (budding yeast- Saccharomyces cerevisiae).
- ❖ In humans, several hundred miRNA genes are scattered throughout all the chromosomes except of the Y chromosome.
- ❖ ~30% of mammalian miRNA genes are located in intergenic regions i.e. between protein-coding genes of the genome.
- ❖ They are transcribed by RNA polymerase II resulting in capped, polyadenylated transcripts.
- ❖ Some miRNA genes are located in transposons.
- ❖ Rests are situated within other genes- many are in introns of protein-coding genes, while some are in introns and exons of non-protein coding genes.
- ❖ In all cases, the miRNA sequence is transcribed by an RNA polymerase as part of the transcript of the host gene.

- In few cases, an intron-located miRNA gene is transcribed independently by RNA polymerase II.

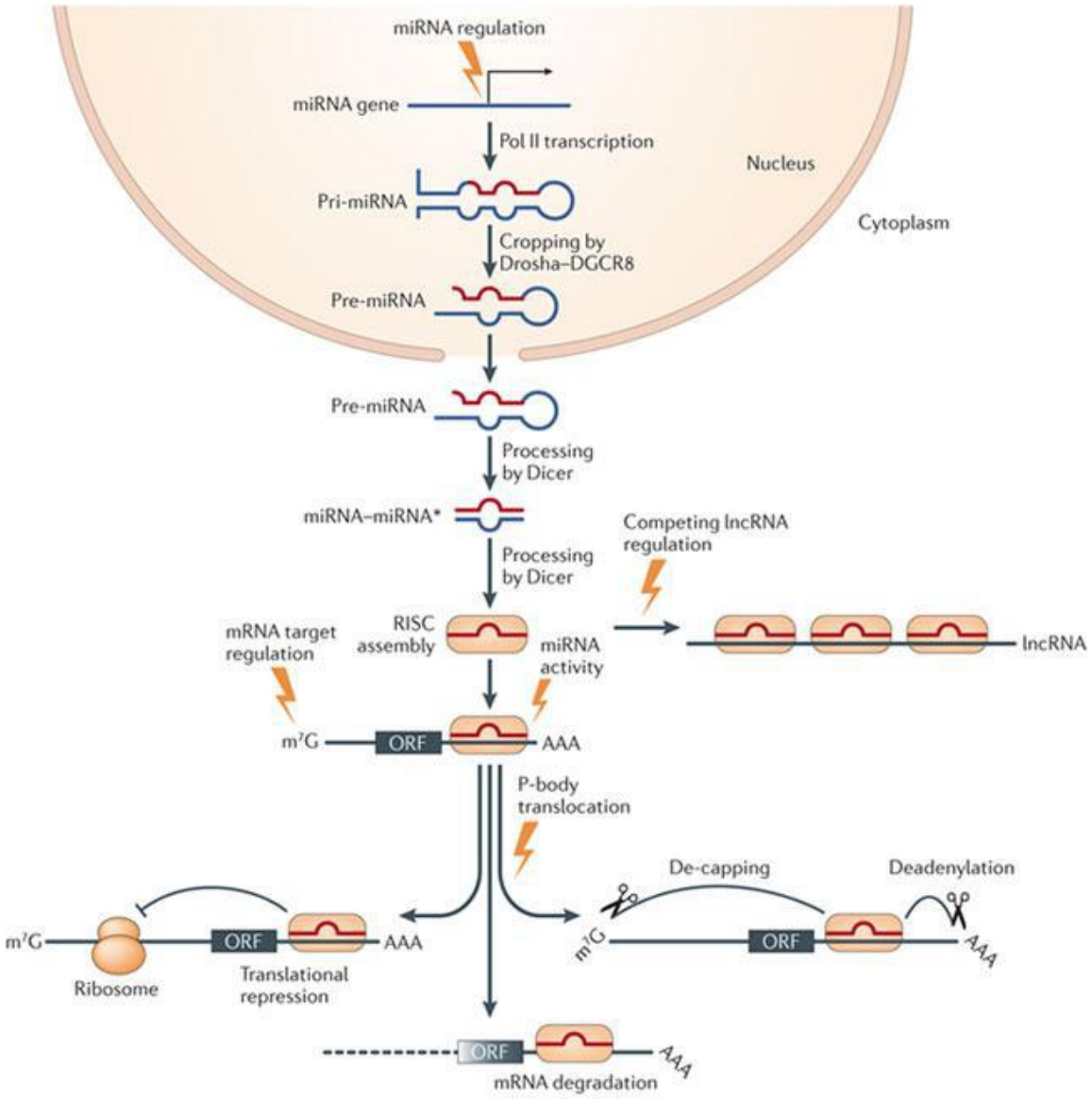
MicroRNAs (miRNAs) mediated gene silencing

- The transcript containing an miRNA is called the primary miRNA transcript or pri-miRNA.
- The pri-miRNA molecule contains a hairpin structure about 70 nt long, within which is the eventual miRNA.
- The hairpin is cut out of the pri-miRNA in the nucleus by the dsRNA-specific endonuclease Drosha complexed to an accessory protein (Pasha in Drosophila).
- Drosha makes staggered cuts resulting in a ~2 nt 3' single-stranded overhang.
- The excised hairpin- pre-miRNA is exported rapidly to the cytoplasm.
- In the cytoplasm, another dsRNA-specific endonuclease, Dicer, complexed to an accessory protein (Loq in Drosophila), makes staggered cuts in the pre-miRNA, releasing a short miRNA:miRNA* dsRNA consisting of some the former paired sides of the hairpin.
- The two RNA strands are imperfectly paired: "miRNA" is the mature miRNA strand that subsequently functions in the cell for RNA silencing, while miRNA* is its partial complement and does not function in RNA silencing.
- Because the miRNA directs RNA silencing, it is termed the guide strand, while the miRNA* is termed the passenger strand.
- Next the dsRNA, Dicer and accessory protein bind to Ago1, a member of the Argonaute family of protein and other proteins to form the pre-microRNA-induced silencing complex, or pre-miRISC.
- Ago1 is another RNA endonuclease; more generally called Slicer. It makes a single cut within the miRNA* passenger strand.
- A helicase that is part of the pre-miRISC then unwinds to two pieces from the miRNA guide strand, and they dissociate from the complex.
- The result is the mature miRISC, the ribonucleoprotein complex that can silence gene expression.

How Does an miRISC function in posttrancriptional gene silencing?

- The miRNA in the miRISC is a trans-acting RNA regulatory molecule, meaning that it targets mRNAs that are not the same as the RNA molecules from which the miRNA is derived. This is one distinguishing feature of miRNAs compared with siRNAs.
- An miRISC binds to a target mRNA through complementary base pairing involving the miRNA.
- Usually, the sequence to which the miRNA binds are short sequence in the 3' UTR of the mRNA
- An mRNA molecule may have one or more sequences in its 3' UTR to which the same miRNA can bind and/or it may have several sequences in its 3' UTR to which several different miRNAs can bind. The latter raises the possibility of regulating the expression of the same gene (through its mRNA) by various combinations of miRNA regulator molecules.
- Here, one miRISC is shown binding to a 3' UTR sequence for simplicity.
- Binding of most of the miRISC to their target mRNAs involves imperfect pairing between the miRNA and the 3' UTR region of the mRNA.
- Such pairing triggers translational repression – translation of that mRNA becomes inhibited.
- The translationally repressed mRNA with its associated miRISC(s) is then sequestered from the translation machinery by becoming or moving into a P body.
- P body is a cytoplasmically located aggregate of translationally repressed mRNAs complexed with proteins, and proteins for mRNA decapping and mRNA degradation.
- The mRNAs is P bodies may be degraded using the contained mRNA degradation machinery or stored in ribonucleoprotein complexes.
- Stored mRNAs can be returned to translation at a later time. Whether degraded or stored the effect of miRNA action is to reduce the expression of the gene encoding the targeted mRNA at the translational level.
- In plants, binding of most miRISCs to their target mRNAs involves perfect or near-perfect pairing between much of the miRNA and the 3' UTR region of the mRNA.

- Perfect pairing triggers mRNA degradation rather than translational repression.
- Here, the Ago1 Slicer protein cuts the target mRNA into two and the mRNA-miRISC complex forms, or it is moved to a P body where degradation of the mRNA is completed.



Riboswitch:

RNA domains at the front of messenger RNA, referred to as riboswitches, can directly interact with small molecules and can control gene expression. The vast majority of riboswitches have been found in bacteria and so far it is only in bacteria that experimental evidence for riboswitch operation exists.

- Three distinct tricks for switching gene expression have been revealed:
- the RNA element can cause premature termination of transcription of the mRNA,
- it can block ribosomes from translating the mRNA,
- or it can even cleave the mRNA and thereby promote its destruction. This involves an RNA unit directly binding a small-molecule metabolite, which switches the RNA into a conformation that activates its intrinsic self-cleavage activity. This "ribozyme riboswitch" represents a new type of biological activity for a catalytic RNA.
- Many antibiotics bind to ribosomal RNAs and selectively inhibit bacterial growth.

- ❖ Riboswitches might also be targeted by new classes of antibiotics.

Given the significant role that riboswitches play in bacterial genetic control and the fact that they have evolved to bind metabolites, drug compounds could be created that disrupt bacterial genetic control. Engineered riboswitches might function as designer genetic control elements.

TPP riboswitch: this riboswitch binds TPP (thiamin pyrophosphate inorder to regulate the transport and synthesis of thiamin as well asother metabolites with similar properties.

Lysine riboswitch : binds to lysine and regulates its biosynthesis, catabolism, and transport.

Glycine riboswitch : this riboswitch regulates glycine metabolism. This is the only riboswitch known currently to be able to perform cooperative binding.

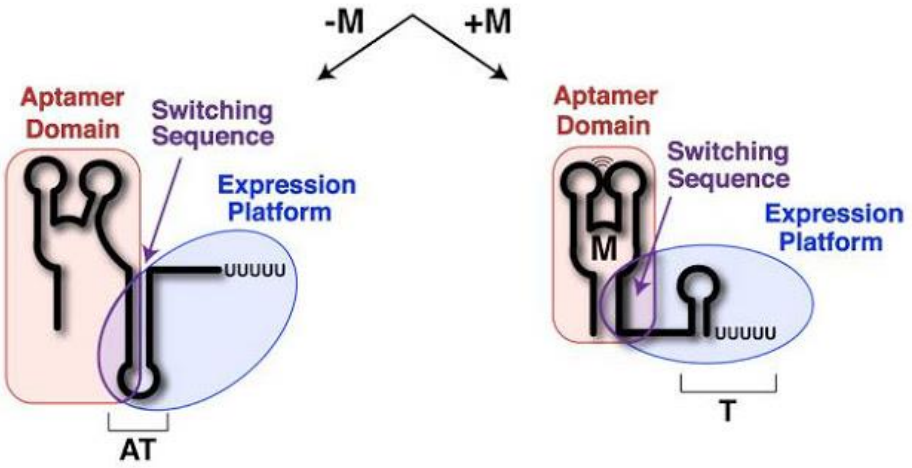
FMN riboswitch : this riboswitch binds FMN (flavin mononucleotide) in order to regulate the transport and synthesis of riboflavin.

Purine riboswitch : binds purines to regulate its transport and metabolism. Different forms of this riboswitch are able to bind either guanine or adenine depending on the pyrimidine in the riboswitch.

Cobalamin riboswitch : this riboswitch binds adenosylcobalamin, the coenzyme form of B12 vitamin, in order to moderate the synthesis and transport of cobalamin and other similar metabolites. as well as many others such as SAM riboswitch, PreQ1 riboswitch, SAH riboswitch, glmS riboswitch, and cyclic di-GMP riboswitch

Structure:

- ➢ Two domains
- ➢ Aptamer-(Ligand binding domain)
- ➢ Ligand recognation and binding.
- ➢ Highly conserved.
- ➢ Expression platform.
    - Less conserved
    - Adopts two mutually exclusive conformation.
    - Shine-dalgano sequence locates in this domain.



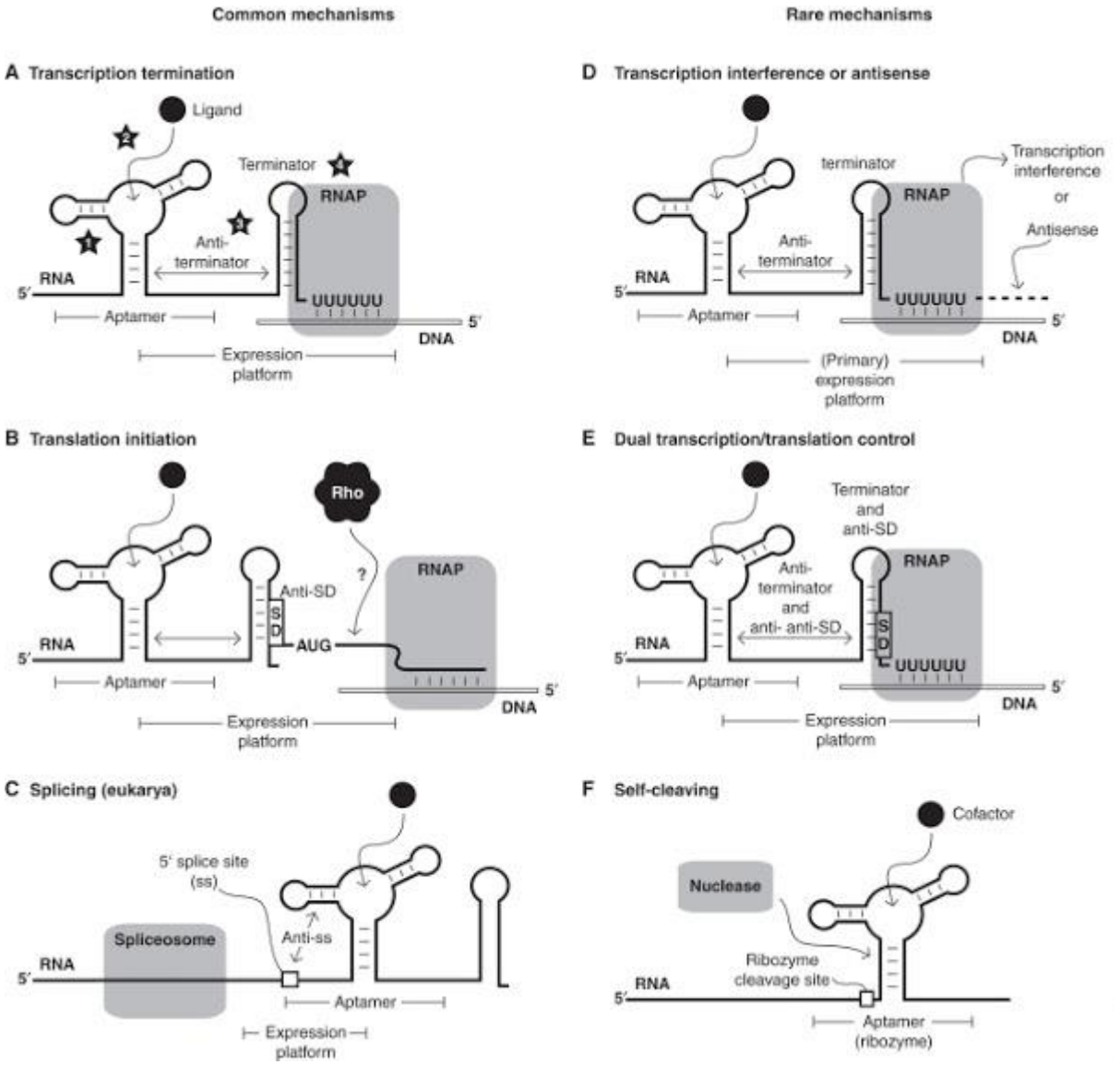
Mechanism:

Three mechanisms:

A.The terminator loop reduces the stability of either the mRNA:RNA polymerase interaction and/or of the DNA:RNA hybrid causing the RNA polymerase to dissociate, terminating transcription prematurely

B. When no metabolite is bound, the Shine-Dalgarno (SD) site is exposed- ribosome can bind and initiate translation. Binding of the metabolite to the 5' leader region of the mRNA induces the formation of an SD:anti-SD stem-loop structure that masks the ribosome binding site such that initial step of translation,, is not achieved .

C. that the conformational change induced by the binding of the ligand to the riboswitch brings adjacent nucleotides in line with each other in an orientation that favours cleavage-ribozyme action eg. Glms box.



Applications

- Synthetic analogs of riboswitch ligands could be engineered to shut off central metabolic pathways, arresting the growth of the bacteria-less toxic as RNA is targeted instead of protein.
- Can be used in the synthetic aptamers that led to their discovery: as molecular chemosensors for measuring chemical composition or biochemical secretions
- Using riboswitch fusions to trans-genes as a means to regulate gene inserts through small molecule inducersThis could have widespread applications in genetic research, and even in medicine and gene therapy.
- Use of riboswitches in taxonomic studies-Though regions of riboswitches are well-conserved, there are distinct variable regions that have been indicated as being dependant on taxonomy.

8. Blotting techniques: Southern, Northern and Western Blot; DNA fingerprinting, DNA foot printing, basic idea of proteomics and genomics, c-DNA and genomic library.

Biochemistry studies molecules such as DNA, RNA and proteins. Blotting techniques are what scientists use to separate these types of molecules. In cells, they exist as a mixture. Blotting allows

researchers to find one protein among many, like a needle in a haystack. Blotting is generally done by letting a mixture of DNA, RNA or protein flow through a slab of gel. This gel allows small molecules to move faster than bigger ones. The separated molecules are then pressed against a membrane, which helps move the molecules from the gel onto the membrane. The molecules stick to the membrane, but stay in the same location, apart from each other, as if they were still in the gel.

Southern blot

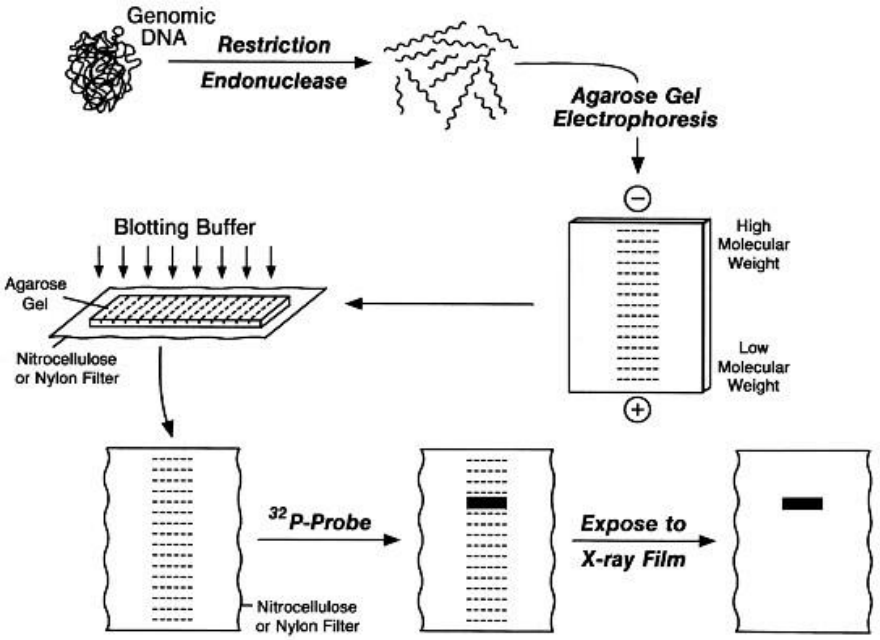
Southern blotting is the original blotting technique, which started the naming system. It was invented by Edwin Southern. The Southern blot is used to detect the amount of DNA in a mixture. Just as with protein and RNA, the DNA of a cell can be released when that cell is broken open. Southern blotting separates DNA from different cell types by size. The DNA from each sample is spread into neat, parallel lanes. Individual pieces of DNA can be detected using a radioactive or fluorescent probe, which is designed to bind only to that piece of DNA. The energy signal from a radioactive probe, or the flashes of light from a fluorescent signal, tell researchers how much of that piece of DNA is in each sample.

Principle

- Southern blotting is an example of RFLP (restriction fragment length polymorphism). It was developed by Edward M. Southern (1975). Southern blotting is a hybridization technique for identification of particular size of DNA from the mixture of other similar molecules. This technique is based on the principle of separation of DNA fragments by gel electrophoresis and identified by labelled probe hybridization.
- Basically, the DNA fragments are separated on the basis of size and charge during electrophoresis. Separated DNA fragments after transferring on nylon membrane, the desired DNA is detected using specific DNA probe that is complementary to the desired DNA.
- A hybridization probe is a short (100-500bp), single stranded DNA. The probes are labeled with a marker so that they can be detected after hybridization.

Procedure/ Steps

1. Restriction digest: by RE enzyme and amplification by PCR
2. Gel electrophoresis: SDS gel electrophoresis
3. Denaturation: Treating with HCl and NaOH
4. Blotting
5. Baking and Blocking with casein in BSA
6. Hybridization using labelled probes
7. Visualization by autoradiogram



Step I: Restriction digest:

- The DNA is fragmentized by using suitable restriction enzyme. RE cuts the DNA at specific site generating fragments
- The number of fragments of DNA obtained by restriction digest is amplified by PCR

Step II: Gel electrophoresis

- The desired DNA fragments is separated by gel electrophoresis

Step III: Denaturation

- The SDS gel after electrophoresis is then soaked in alkali (NaOH) or acid (HCl) to denature the double stranded DNA fragments.
- DNA strands get separated

Step IV: Blotting

- The separated strands of DNA is then transferred to positively charged membrane nylon membrane (Nitrocellulose paper) by the process of blotting.

Step V: Baking and blocking

- After the DNA of interest bound on the membrane, it is baked on autoclave to fix in the membrane.
- The membrane is then treated with casein or Bovine serum albumin (BSA) which saturates all the binding site of membrane

Step VI: Hybridization with labelled probes

- The DNA bound to membrane is then treated with labelled probe
- The labelled probe contains the complementary sequences to the gene of interest
- The probe bind with complementary DNA on the membrane since all other non-specific binding site on the membrane has been blocked by BSA or casein.

Step VII: Visualization by Autoradiogram

- The membrane bound DNA labelled with probe can be visualized under autoradiogram which give pattern of bands.

Application of Southern blotting:

1. Southern blotting technique is used to detect DNA in given sample.
2. DNA finger printing is an example of southern blotting.
3. Used for paternity testing, criminal identification, victim identification
4. To isolate and identify desire gene of interest.
5. Used in restriction fragment length polymorphism
6. To identify mutation or gene rearrangement in the sequence of DNA
7. Used in diagnosis of disease caused by genetic defects
8. Used to identify infectious agents

Northern blot

Northern blotting is used to detect RNA. Cells can be broken open to release their RNA. The RNA from different cell types can be run on separate lanes on a gel. The gel spreads the different RNA by size. These neat, parallel rows of RNA allow a researcher to compare which cell type has how much of which RNA. This method allows a researcher to determine if cells from a certain disease have more of this RNA or less of that RNA. Northern blotting may reveal how a disease is working at the level of RNA production.

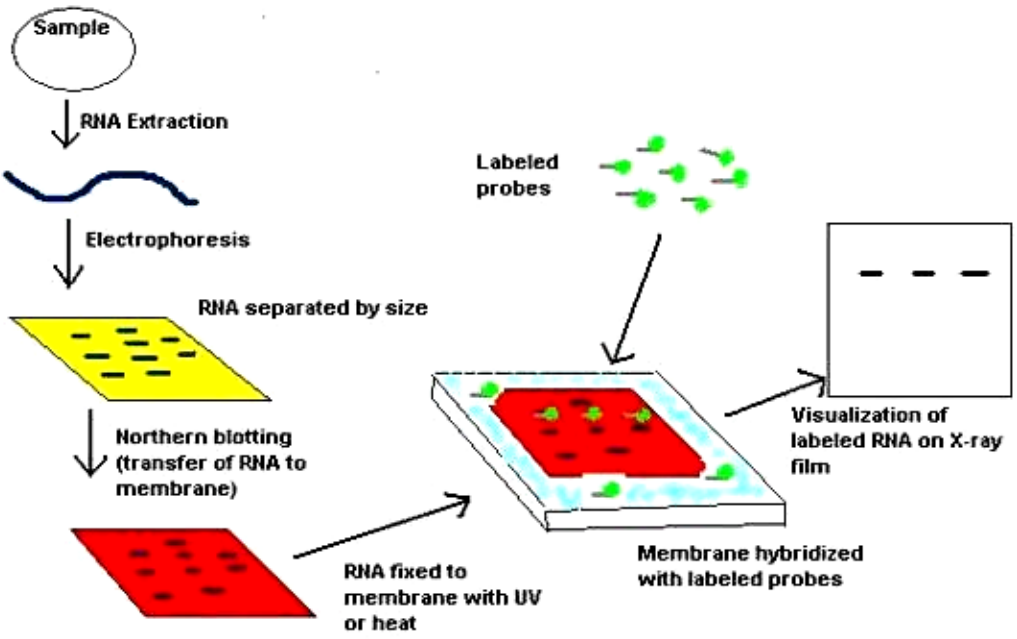
Principle

As all normal blotting technique, northern blotting starts with the electrophoresis to separate RNA samples by size. Electrophoresis separates the RNA molecules based on the charge of the nucleic acids. The charge in the nucleic acids is proportional to the size of the nucleic acid sequence. Thus the electrophoresis membrane separates the Nucleic acid sequence according to the size of the RNA sequence. In cases where our target sequence is an mRNA, the sample can be isolated through oligo cellulose chromatographic techniques, as mRNA are characterized by the poly(A)-tail. Since gel molecules are fragile in nature, the separated sequences are transferred to the nylon membranes. The selection of nylon membrane is contributed to the factor that nucleic acids are negatively charged in nature. Once the RNA molecules are transferred it is immobilized by covalent linkage. The probe is

then added, the probe can be complementary an ss DNA sequence. Formamide is generally used as a blotting buffer as it reduces the annealing temperature.

Procedure:

1. The tissue or culture sample collected is first homogenized. The samples may be representative of different types of culture for comparison or it can be for the study of different stages of growth inside the culture.
2. The RNA sequence is separated in the electrophoresis unit an agarose gel is used for the purpose of the nucleic acid separation.
3. Now the separated RNA sequence is transferred to the nylon membrane. This is done by two mechanisms capillary action and the ionic interaction.
4. The transfer operation is done by keeping the gel in the following order. First, the agarose gel is placed on the bottom of the stack, followed by the blotting membrane. On top of these paper towels a mild weight (glass plate) is placed. The entire setup is kept in a beaker containing transfer buffer.
5. RNA transferred to the nylon membrane is then fixed using UV radiation.
6. The fixed nylon membrane is then mixed with probes. The probes are specifically designed for the gene of interest, so that they will hybridize with RNA sequences on the blot corresponding to the sequence of interest.
7. The blot membrane is washed to remove unwanted probe
8. Labeled probe is detected by chemiluminescence or autoradiography. The result will be dark bands in x ray film.



Applications:

Northern blotting allows one to observe a particular gene's expression pattern between tissues, organs, developmental stages, environmental stress levels, pathogen infection, and over the course of treatment. The technique has been used to show overexpression of oncogenes and downregulation of tumor-suppressor genes in cancerous cells when compared to 'normal' tissue, as well as the gene expression in the rejection of transplanted organs. If an upregulated gene is observed by an abundance of mRNA on the northern blot the sample can then be sequenced to determine if the gene is known to researchers or if it is a novel finding.

The expression patterns obtained under given conditions can provide insight into the function of that gene. Since the RNA is first separated by size, if only one probe type is used variance in the level of

each band on the membrane can provide insight into the size of the product, suggesting alternative splice products of the same gene or repetitive sequence motifs.The variance in size of a gene product can also indicate deletions or errors in transcript processing. By altering the probe target used along the known sequence it is possible to determine which region of the RNA is missing.
BlotBase is an online database publishing northern blots. BlotBase has over 700 published northern blots of human and mouse samples, in over 650 genes across more than 25 different tissue types.Northern blots can be searched by a blot ID, paper reference, gene identifier, or by tissue. The results of a search provide the blot ID, species, tissue, gene, expression level, blot image (if available), and links to the publication that the work originated from. This new database provides sharing of information between members of the science community that was not previously seen in northern blotting as it was in sequence analysis, genome determination, protein structure, etc.

Advantages and disadvantages

Analysis of gene expression can be done by several different methods including RT-PCR, RNase protection assays, microarrays, RNA-Seq, serial analysis of gene expression (SAGE), as well as northern blotting. Microarrays are quite commonly used and are usually consistent with data obtained from northern blots; however, at times northern blotting is able to detect small changes in gene expression that microarrays cannot. The advantage that microarrays have over northern blots is that thousands of genes can be visualized at a time, while northern blotting is usually looking at one or a small number of genes.
A problem in northern blotting is often sample degradation by RNases (both endogenous to the sample and through environmental contamination), which can be avoided by proper sterilization of glassware and the use of RNase inhibitors such as DEPC (diethylpyrocarbonate). The chemicals used in most northern blots can be a risk to the researcher, since formaldehyde, radioactive material, ethidium bromide, DEPC, and UV light are all harmful under certain exposures. Compared to RT-PCR, northern blotting has a low sensitivity, but it also has a high specificity, which is important to reduce false positive results.
The advantages of using northern blotting include the detection of RNA size, the observation of alternate splice products, the use of probes with partial homology, the quality and quantity of RNA can be measured on the gel prior to blotting, and the membranes can be stored and reprobed for years after blotting.
For northern blotting for the detection of acetylcholinesterase mRNA the nonradioactive technique was compared to a radioactive technique and found as sensitive as the radioactive one, but requires no protection against radiation and is less time consuming.

Western blot:

Western blotting is a common technique for separating proteins by size, but in straight columns. These parallel columns allow researchers to compare the amount of a protein across different samples that are run right next to each other, like bowling lanes. For example, if you were testing the effect of different amounts of a drug on cell growth, you would treat four different groups of cells with a different amount of drug. Then you could break the cells open and run the proteins of each group in separate lanes on a gel. Spreading the proteins out in this way allows you to see what an increasing concentration of drugs does to a certain protein.

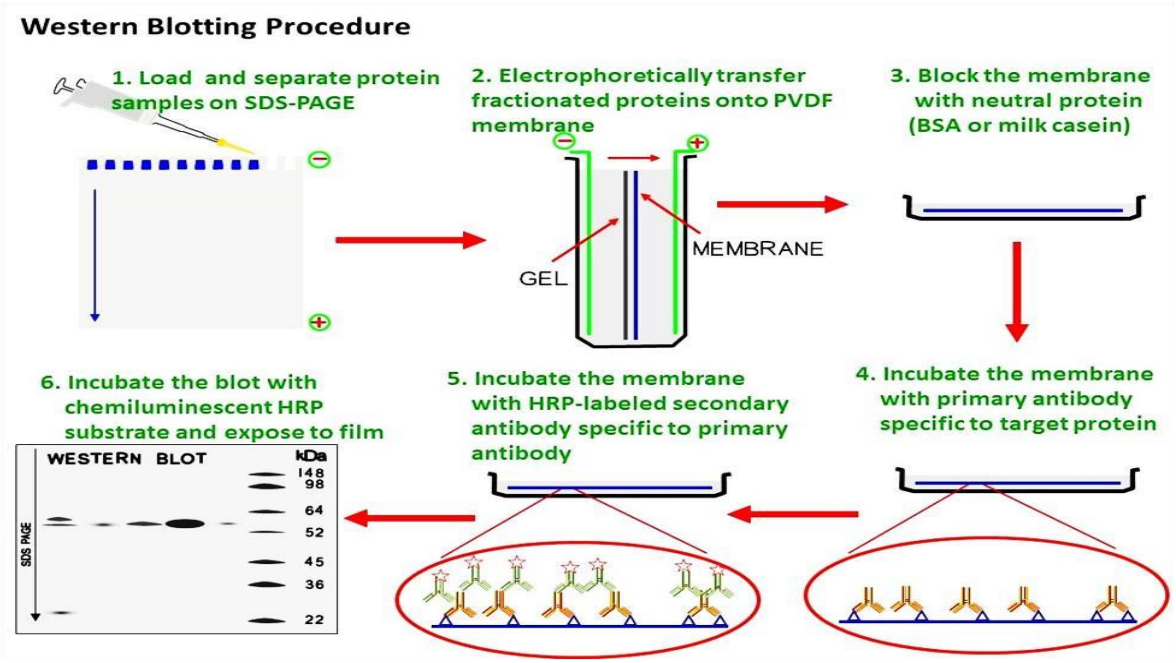
Principle

- Western blotting technique is used for identification of particular protein from the mixture of protein.
- In this method labelled antibody against particular protein is used identify the desired protein, so it is a specific test. Western blotting is also known as immunoblotting because it uses antibodies to detect the protein.

Procedure/Steps

1. Extraction of protein
2. Gel electrophoresis: SDS PAGE
3. Blotting: electrical or capillary blotting
4. Blocking: BSA
5. Treatment with primary antibody

6. Treatment with secondary antibody(enzyme labelled anti Ab)
7. Treatment with specific substrate; if enzyme is alkaline phosphatase, substrate is p-nitro phenyl phosphate which give color.



Step I: Extraction of Protein

- Cell lysate is most common sample for western blotting.
- Protein is extracted from cell by mechanical or chemical lysis of cell. This step is also known as tissue preparation.
- To prevent denaturing of protein protease inhibitor is used.
- The concentration of protein is determined by spectroscopy.
- When sufficient amount of protein sample is obtained, it is diluted in loading buffer containing glycerol which helps to sink the sample in well.
- Tracking dye (bromothymol blue) is also added in sample to monitor the movement of proteins.

Step II: Gel electrophoresis

- The sample is loaded in well of SDS-PAGE Sodium dodecyl sulfate- poly-acrylamide gel electrophoresis.
- The proteins are separated on the basis of electric charge, isoelectric point, molecular weight, or combination of these all.
- The small size protein moves faster than large size protein.
- Protein is negatively charged, so they move toward positive (anode) pole as electric current is applied.

Step III: Blotting

- The nitrocellulose membrane is placed on the gel. The separated protein from gel get transferred to nitrocellulose paper by capillary action. This type of blotting is time consuming and may take 1-2 days
- For fast and more efficient transfer of desired protein from the gel to nitrocellulose paper electro-blotting can be used.
- In electro-blotting nitrocellulose membrane is sandwich between gel and cassette of filter paper and then electric current is passed through the gel causing transfer of protein to the membrane.

Step IV: Blocking

- Blocking is very important step in western blotting.

- Antibodies are also protein so they are likely to bind the nitrocellulose paper. So before adding the primary antibody the membrane is non-specifically saturated or masked by using casein or Bovine serum albumin (BSA).

Step V: Treatment with Primary Antibody

- The primary antibody (1° Ab) is specific to desired protein so it form Ag-Ab complex

Step VI: Treatment with secondary antibody

- The secondary antibody is enzyme labelled. For eg. alkaline phosphatase or Horseradish peroxidase (HRP) is labelled with secondary antibody.
- Secondary antibody (2° Ab) is antibody against primary antibody (anti-antibody) so it can bind with Ag-Ab complex.

Step VII: Treatment with suitable substrate

- To visualize the enzyme action, the reaction mixture is incubated with specific substrate.
- The enzyme convert the substrate to give visible colored product, so band of color can be visualized in the membrane.
- Western blotting is also a quantitative test to determine the amount of protein in sample.

Application:

1. To determine the size and amount of protein in given sample.
2. Disease diagnosis: detects antibody against virus or bacteria in serum.
3. Western blotting technique is the confirmatory test for HIV. It detects anti HIV antibody in patient's serum.
4. Useful to detect defective proteins. For eg Prions disease.
5. Definitive test for Creutzfeldt-Jacob disease, Lyme disease, Hepatitis B and Herpes.

Other blots

The three main blotting techniques -- Western, Northern and Southern -- have been modified in different ways to detect slightly different molecules. The Western blot vs the Southern blot, for example. detects protein and DNA, respectively. Each modified technique is generally done the usual way, but uses a different method to detect the molecule that is being spread out into the parallel lanes. Southwestern blots detect molecules of protein stuck to DNA. Northwestern blots detect molecules of protein stuck to RNA. Farwestern blots detect molecules of protein stuck to other proteins.

DNA-fingerprinting

It has been a subject of human interest since primitive times when man used to hunt for his food with the help of animal's foot prints. Science of fingerprinting was first used by Sir William Herschel as a method of identification in 1858. In India the science of fingerprints was discovered by chance during a murder investigation in Jalpaiguri in 1897.

Alec Jeffreys (1984) invented the DNA fingerprinting technique at Leicester University, United Kingdom. Dr. V.K. Kashyap and Dr. Lalji Singh started the DNA fingerprinting technology in India at CCMB (Centre for Cell and Molecular Biology) Hyderabad.

What is DNA-fingerprinting?

DNA-fingerprinting (also called DNA typing or DNA profiling). It is a technique of determin-ing nucleotide sequences of certain areas of DNA which are unique to each individual. Each person has a unique DNA fingerprint.

Unlike a conventional fingerprint that occurs only on the fingertips and can be altered by surgery, a DNA fingerprint is the same for every cell, tissue and organ of a person. It cannot be changed by any known treatment. The ideal way to distinguish an individual — from other people would be his or her entire genomic DNA sequence.

Principle of DNA Fingerprinting:

By their differences, about 0.1% or 3 x 106 base pairs (out of 3 x 109 bp) provide individuality to each human being. Human genome pos-sesses numerous small noncoding but inheritable sequences of bases which are repeated many times. These sequences occur near telomere, centromeres, Y chromosome and hetero-chromatic area. The area with same sequence of bases repeated several times is called repetitive DNA.

They can be separated as satellite from the bulk DNA during density gradient centrifugation and hence called satellite DNA. In satellite DNA, repetition of bases is in tandem. Depending upon length,

base composition and numbers of tandemly re-petitive units, satellite DNAs have subcat-egories like microsatellites and mini-satellites. Satellite DNAs show poly-morphism. The term polymorphism is used when a variant at a locus is present with a frequency of more than 0.01 population.
Variations occur due to mutations. While mutations in genes produce alleles with dif-ferent expressions, mutations in noncoding repetitive DNA have no immediate impact.
These mutations in the noncoding sequences have piled up with time and form the basis of DNA polymorphism (variation at genetic level arises due to mutations). DNA polymorphism is the basis of genetic mapping of human genome as well as DNA finger printing.
Short nucleotide repeats in the DNA are very specific in each individual and vary in
number from person to person but are inherited. These are the 'Variable Number Tandem Repeats' (VNTRs). These are also called "minisatellites". Each individual inherits these repeats from his/her parents which are used as genetic markers in a personal identity test.
For example, a child might inherit a chromosome with six tandem repeats from the mother and the same tandem repeated four times in the homologous chromosome inherited from the father. One half of VNTR alleles of the child resemble that of the mother and other half with that of the father.

DNA Fingerprinting Steps

- **Extracting DNA from Cells**

To perform DNA fingerprinting, you must first have a DNA sample!
In order to procure this, a sample containing genetic material must be treated with different chemicals. Common sample types used today include blood and cheek swabs.
These samples must be treated with a series of chemicals to break open cell membranes, expose the DNA sample, and remove unwanted components – such as lipids and proteins – until relatively pure DNA emerges.

- **PCR Amplification (Optional)**

If the amount of DNA in a sample is small, scientists may wish to perform PCR – Polymerase Chain Reaction – amplification of the sample.
PCR is an ingenuous technology which essentially mimics the process of DNA replication carried out by cells. Nucleotides and DNA polymerase enzymes are added, along with "primer" pieces of DNA which will bind to the sample DNA and give the polymerases a starting point.
PCR "cycles" can be repeated until the sample DNA has been copied many times in the lab if necessary.

- **Treatment with Restriction Enzymes**

Once sufficient DNA has been isolated, and amplified if necessary, it must be cut with restriction enzymes to isolate the VNTRs.
Restriction enzymes are enzymes that attach to specific DNA sequences and create breaks in the DNA strands.
Bacterial cells use restriction enzymes for protection against DNA viruses which may invade the cell and hijack its machinery, but scientists have taken advantage of these special enzymes and used them to make DNA profiling and even genetic engineering possible.
In genetic engineering, DNA is cut up with restriction enzymes and then "sewn" back together by ligases to create new, recombinant DNA sequences.
In DNA profiling, however, only the cutting part is needed. Once the DNA has been cut to isolate the VNTRs, it's time to run the resulting DNA fragments on a gel to see how long they are.

- **Gel Electrophoresis**

Gel electrophoresis is a brilliant technology that separates molecules by size. The "gel" in question is a material that molecules can pass through, but only at a slow speed.
Just as air resistance slows a big truck more than it does a motorcycle, the resistance offered by the electrophoresis gel slows large molecules down more than small ones. The effect of the gel is so precise that scientists can tell exactly how big a molecule is by seeing how far it moves within a given gel in a set amount of time.
In this case, measuring the size of the DNA fragments from the sample that has been treated with restriction enzyme will tell scientists how many copies of each VTNR repeat the sample DNA contains.

It's called "electrophoresis" because, to make the molecules move through the gel, an electrical current is applied. Because the sugar-phosphate backbone of the DNA has a negative electrical charge, the electrical current tugs the DNA along with it through the gel

By looking at how many DNA fragments the restriction enzymes produced and the sizes of these fragments, the scientists can "fingerprint" the DNA donor.

- **Transfer onto Southern Blot**

Now that the DNA fragments have been separated by size, they must be transferred to a medium where scientists can "read" and record the results of the electrophoresis.

To do this, scientists treat the gel with a weak acid, which breaks up the DNA fragments into individual nucleic acids that will more easily rub off onto paper.

They then "blot" the DNA fragments onto nitrocellulose paper, which fixes them in place.

- **Treatment with Radioactive Probe**

Now that the DNA is fixed onto the blot paper, it is treated with a special probe chemical that sticks to the desired DNA fragments. This chemical is radioactive, which means that it will create a visible record when exposed to X-ray paper.

This method of blotting DNA fragments onto nitrocellulose paper and then treating it with a radioactive probe was discovered by a scientist name Ed Southern – hence the name "Southern blot."

Amusingly, the fact that the Southern blot is named after a scientist has nothing to do with directions didn't stop scientists from naming similar methods "northern" and "western" blots in honor of the Southern blot.

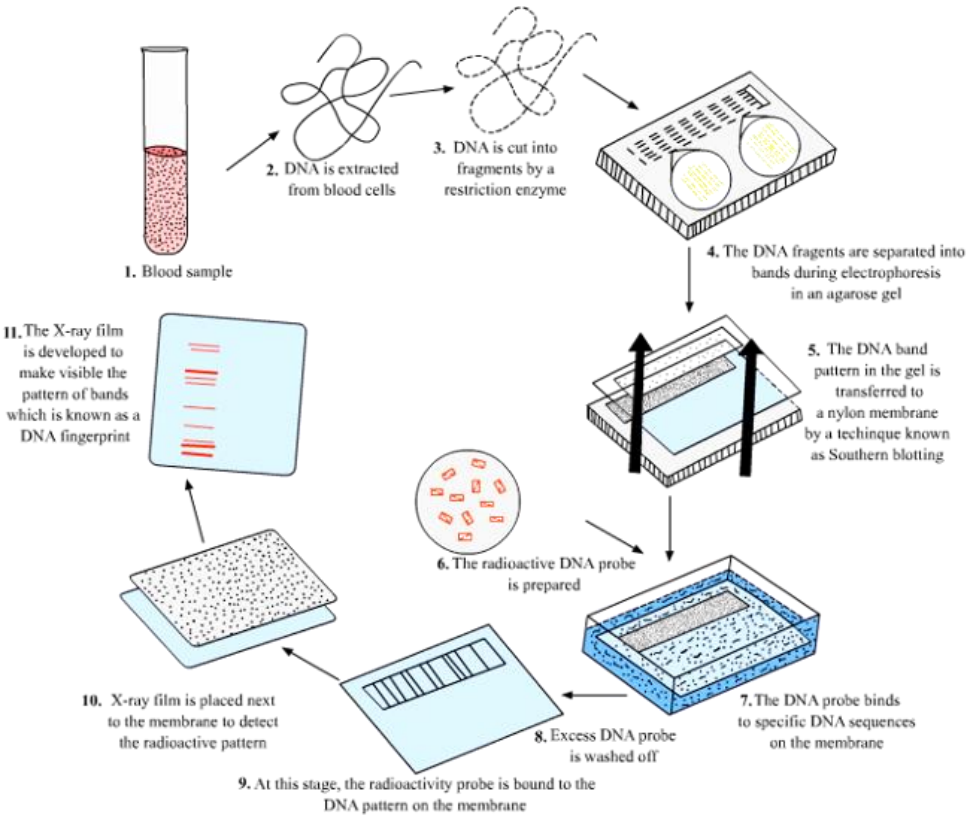
- **X-Ray Film Exposure**

The last step of the process is to turn the information from the DNA fragments into a visible record. This is done by exposing the blot paper, with its radioactive DNA bands, to X-ray film.

X-ray film is "developed" by radiation, just like camera film is developed by visible light, resulting in a visual record of the pattern produced by the person's DNA "fingerprint."

To ensure a clear imprint, scientists often leave the X-ray film exposed to the weakly radioactive Southern blot paper for a day or more.

Once the image has been developed and fixed to prevent further light exposure from changing the image, this "fingerprint" can be used to determine if two DNA samples are the same or similar!



Steps in DNA fingerprinting techinque

Applications of DNA Fingerprinting:

(i) Individuality:

Like skin finger printing (der- matoglyphics), DNA finger printing can help to distinguish one human being from another with exception of monozygotic twins,

(ii) Paternity/Maternity Disputes:

DNA finger print-ing can identify the real genetic mother, father and the offspring,

(iii) Human Lineage:

DNA from various probables is being studied to find out human lineage,

(iv) Hereditary Diseases:

The technique is being used to identify genes connected with hereditary diseases,

(v) Forensics:

DNA finger printing is very useful in the detection of crime and legal pursuits. DNA fingerprinting has proved that Dhanu, the human bomb, was the real murderer of Shri Rajiv Gandhi, the former Prime Minister of India,

(vi) Sociology:

It can identify racial groups, their origin, historical migration and invasions. Genography is the study of migratory history of human species.

DNA footprinting

DNA footprinting is a method of investigating the sequence specificity of DNA- binding proteins in vitro. This technique can be used to study protein-DNA interactions both outside and within cells.

The regulation of transcription has been studied extensively, and yet there is still much that is not known. Transcription factors and associated proteins that bind promoters, enhancers, or silencers to drive or repress transcription are fundamental to understanding the unique regulation of individual genes within the genome. Techniques like DNA footprinting help elucidate which proteins bind to these associated regions of DNA and unravel the complexities of transcriptional control.

In 1978, David Galas and Albert Schmitz developed the DNA footprinting technique to study the binding specificity of the lac repressor protein. It was originally a modification of the Maxam-Gilbert chemical sequencing technique.

Methods and Procedure

The simplest application of this technique is to assess whether a given protein binds to a region of interest within a DNA molecule. Polymerase chain reaction (PCR) amplifies and label region of interest that contains a potential protein-binding site, ideally amplicon is between 50 and 200 base pairs in length. Add protein of interest to a portion of the labeled template DNA; a portion should remain separate without protein, for later comparison. Add a cleavage agent to both portions of DNA template. The cleavage agent is a chemical or

enzyme that will cut at random locations in a sequence independent manner. The reaction should occur just long enough to cut each DNA molecule in only one location. A protein that specifically binds a region within the DNA template will protect the DNA it is bound to from the cleavage agent. Run both samples side by side on a polyacrylamide gel electrophoresis. The portion of DNA template without protein will be cut at random locations, and thus when it is run on a gel, will produce a ladder-like distribution. The DNA template with the protein will result in ladder distribution with a break in it, the "footprint", where the DNA has been protected from the cleavage agent. Note: Maxam-Gilbert chemical DNA sequencing can be run alongside the samples on the polyacrylamide gel to allow the prediction of the exact location of ligand binding site.

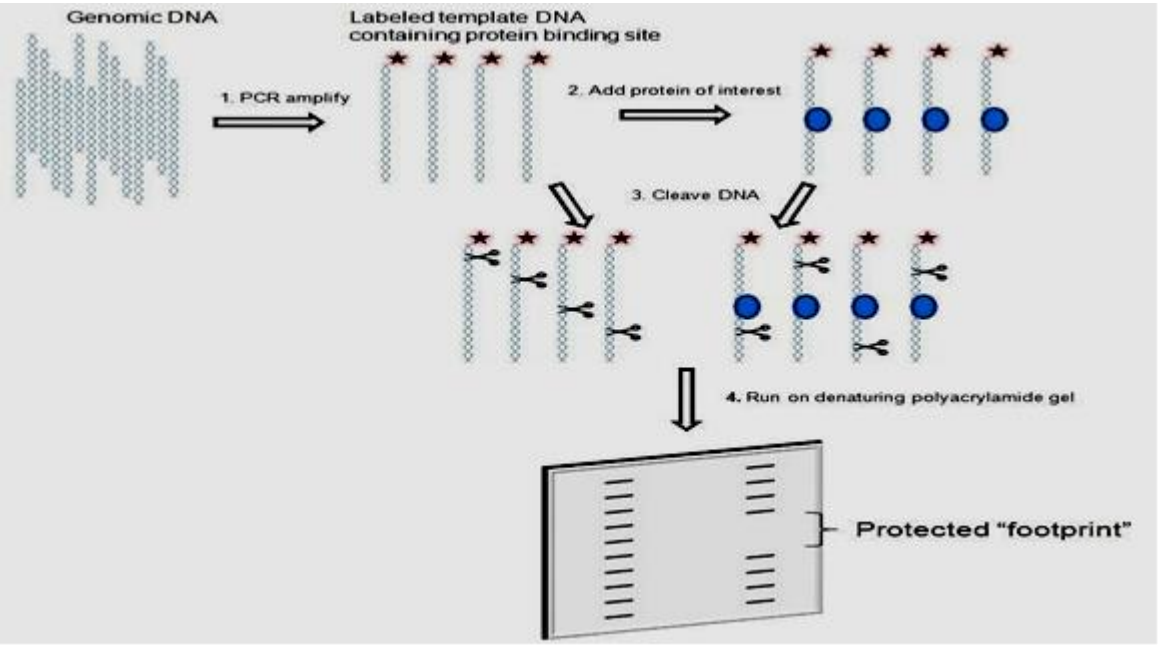
Labeling

The DNA template labeled at the 3' or 5' end, depending on the location of the binding site(s). Labels that can be used are: radioactivity and fluorescence. Radioactivity has been traditionally used to label DNA fragments for footprinting analysis, as the method was originally developed from the Maxam-Gilbert chemical sequencing technique. Radioactive labeling is very sensitive and is optimal for visualizing small amounts of DNA. Fluorescence is a desirable advancement due to the hazards of using radio-chemicals. However, it has been more difficult to optimize because it is not always sensitive enough to detect the low concentrations of the target DNA strands used in DNA footprinting

experiments. Electrophoretic sequencing gels or capillary electrophoresis have been successful in analyzing footprinting of fluorescent tagged fragments.

Cleavage agent

A variety of cleavage agents can be chosen. a desirable agent is one that is sequence neutral, easy to use, and is easy to control. Unfortunately no available agents meet all of these standards, so an appropriate agent can be chosen, depending on your DNA sequence and ligand of interest. The following cleavage agents are described in detail:DNase I is a large protein that functions as a double-strand endonuclease. It binds the minor groove of DNA and cleaves the phosphodiester backbone. It is a good cleavage agent for footprinting because its size makes it easily physically hindered. Thus is more likely to have its action blocked by a bound protein on a DNA sequence. In addition, the DNase I enzyme is easily controlled by adding EDTA to stop the reaction. There are however some limitations in using DNase I. The enzyme does not cut DNA randomly; its activity is affected by local DNA structure and sequence and therefore results in an uneven ladder. This can limit the precision of predicting a protein's binding site on the DNA molecule. Hydroxyl radicals are created from the Fenton reaction, which involves reducing Fe2+ with H2O2 to form free hydroxyl molecules. These hydroxyl molecules react with the DNA backbone, resulting in a break. Due to their small size, the resulting DNA footprint has high resolution. Unlike DNase I they have no sequence dependence and result in a much more evenly distributed ladder. The negative aspect of using hydroxyl radicals is that they are more time consuming to use, due to a slower reaction and digestion time. Ultraviolet irradiation can be used to excite nucleic acids and create photoreactions, which results in damaged bases in the DNA strand. Photoreactions can include: single strand breaks, interactions between or within DNA strands, reactions with solvents, or crosslinks with proteins. The workflow for this method has an additional step, once both your protected and unprotected DNA have been treated, there is subsequent primer extension of the cleaved products. The extension will terminate upon reaching a damaged base, and thus when the PCR products are run side-by-side on a gel; the protected sample will show an additional band where the DNA was crosslinked with a bound protein. Advantages of using UV are that it reacts very quickly and can therefore capture interactions that are only momentary. Additionally it can be applied to in vivo experiments, because UV can penetrate cell membranes. A disadvantage is that the gel can be difficult to interpret, as the bound protein does not protect the DNA, it merely alters the photoreactions in the vicinity



Application

1. In vivo footprinting

In vivo footprinting is a technique used to analyze the protein-DNA interactions that are occurring in a cell at a given time point. DNase I can be used as a cleavage agent if the cellular membrane has

been permeabilized. However the most common cleavage agent used is UV irradiation because it penetrates the cell membrane without disrupting cell state and can thus capture interactions that are sensitive to cellular changes. Once the DNA has been cleaved or damaged by UV, the cells can be lysed and DNA purified for analysis of a region of interest. Ligation-mediated PCR is an alternative method to footprint in vivo. Once a cleavage agent has been used on the genomic DNA, resulting in single strand breaks, and the DNA is isolated, a linker is added onto the break points. A region of interest is amplified between the linker and a gene-specific primer, and when run on a polyacrylamide gel, will have a footprint where a protein was bound. In vivo footprinting combined with immunoprecipitation can be used to assess protein specificity at many locations throughout the genome. The DNA bound to a protein of interest can be immunoprecipitated with an antibody to that protein, and then specific region binding can be assessed using the DNA footprinting technique.

2. Quantitative footprinting

The DNA footprinting technique can be modified to assess the binding strength of a protein to a region of DNA. Using varying concentrations of the protein for the footprinting experiment, the appearance of the footprint can be observed as the concentrations increase and the proteins binding affinity can then be estimated.

3. Detection by capillary electrophoresis

To adapt the footprinting technique to updated detection methods, the labelled DNA fragments are detected by a capillary electrophoresis device instead of being run on a polyacrylamide gel. If the DNA fragment to be analyzed is produced by polymerase chain reaction (PCR), it is straightforward to couple a fluorescent molecule such as carboxyfluorescein (FAM) to the primers. This way, the fragments produced by DNaseI digestion will contain FAM, and will be detectable by the capillary electrophoresis machine.

Genomics:

The term genomics was first used by Thomas Roderick in 1986. It refers to the study of structure and function of entire genome of a living organism. The complete genetic content of an organism is genome, and the DNA obtained is called genomic DNA. This genomic DNA of prokaryote contains the entire coding region and can be sequenced, whereas the DNA of eukaryotes includes both intron and exon sequences (coding sequence) as well as noncoding regulatory sequences such as promo-ter, and enhancer sequences.

The subject genomics is the complete analysis of the entire genome of a chosen organism which involves the study of physical structure of the orga-nism's genome or the genetic makeup of an organism to know the number of genes present and the type of genes, i.e., to study the function of different genes.

Whole Genome Sequence Data:

Complete nucleotide sequences of nuclear, mito-chondrial and chloroplast genomes have already been worked out in large number of prokaryotes and several eukaryotes. By the year 2005, among prokaryotes, approx. 1400 viral genomes, 250 bacterial genomes (230 eubacteria and 20 archaea), 500 mitochondr-ial genomes, 35 chloroplast genomes have been fully sequenced.

Among the eukaryotes namely the whole genome of *Saccharomyces cerevisiae* (yeast), *Coenorhabditis elegans* (nematode), fruitfly (*Drosophila melanogaster*), Human (*Homo sapiens*), Crucifer weed (*Arabidopsis thaliana*) and rice (*Oryza sativa*) have been sequenced already and data available for annotation studies.

The sequence data of eukaryotic nuclear genome is an important source of identi-fication, discovery and isolation of important genes. This data is very much helpful in variety of application relevant to animal, plant and microbial biotechnology.

Types of Genomics:

The discipline of genomics consists of two parts, viz. structural genomics and functional genomics. These are defined as under:

i. Structural Genomics:

It deals with the study of the structure of entire genome of a living organism. In other words, it deals with the study of the genetic structure of each chromosome of the genome. It determines the size of

the genome of a species in mega-bases [Mb] and also the genes present in the entire genome of a species.

ii. Functional Genomics:

The study of function of all genes present in the entire genome is known as functional genomics. It deals with transcriptome and proteome. The transcriptome refers to complete set of RNAs transcribed from a genome and proteome refers to complete set of proteins encoded by a genome.

iii. Comparative Genomics:

It deals with the study of multiple whole genomes for understanding the differences and similarities between all the genes of the multiple species. For example in the phylogenic tree analysis two specialized concepts are the rooted and unrooted trees.

Phylogenetic trees are designed to reveal evolutionary relationship among DNA or protein sequences. In unrooted trees, when distantly related sequence for comparison has not included when an unrooted tree is required.

Epigenomics

Epigenomics is the study of the complete set of epigenetic modifications on the genetic material of a cell, known as the epigenome. Epigenetic modifications are reversible modifications on a cell's DNA or histones that affect gene expression without altering the DNA sequence. Two of the most characterized epigenetic modifications are DNA methylation and histone modification. Epigenetic modifications play an important role in gene expression and regulation, and are involved in numerous cellular processes such as in differentiation/development and tumorigenesis. The study of epigenetics on a global level has been made possible only recently through the adaptation of genomic high-throughput assays.

Metagenomics

Environmental Shotgun Sequencing (ESS) is a key technique in metagenomics. (A) Sampling from habitat; (B) filtering particles, typically by size; (C) Lysis and DNA extraction; (D) cloning and library construction; (E) sequencing the clones; (F) sequence assembly into contigs and scaffolds.

Metagenomics is the study of metagenomes, genetic material recovered directly from environmental samples. The broad field may also be referred to as environmental genomics, ecogenomics or community genomics. While traditional microbiology and microbial genome sequencing rely upon cultivated clonal cultures, early environmental gene sequencing cloned specific genes (often the 16S rRNA gene) to produce a profile of diversity in a natural sample. Such work revealed that the vast majority of microbial biodiversity had been missed by cultivation-based methods. Recent studies use "shotgun" Sanger sequencing or massively parallel pyrosequencing to get largely unbiased samples of all genes from all the members of the sampled communities. Because of its power to reveal the previously hidden diversity of microscopic life, metagenomics offers a powerful lens for viewing the microbial world that has the potential to revolutionize understanding of the entire living world.

Role of Genomics in Crop Plants:

The discipline of genomics is of recent origin. The genome mapping was first completed in a free living bacteria *Haemophillus influenza* in 1995. Later on genome sequencing work was intensified both in prokaryotes and eukaryotes. In plants, genome sequencing was first completed in Arabidopsis thaliana (a weedy relative of mustard followed by rice (*Oriza sativa*).

Now genome sequencing work has been completed in more than 40 crop plants. The list of some field crops, fruit crops and other plant species in which genome sequencing work has

S.No.	Species	Botanical Name	Genome Size [Mb]	Gene No.
		A. FIELD CROPS		
1.	Arabidopsis	*Arabidopsis thaliana*	120	27416
2.	Rice	*Oryza sativa*	370	40577
3.	Corn	*Zea mays*	2500	>32000
4.	Sorghum	*Sorghum bicolor*	700	34496
5.	Cucumber	*Cucumis sativa*	243.5	26682
6.	Soybean	*Glycine max*	950	46430
7.	Caster bean	*Ricinus communis*	320	31237
8.	Pigeon pea	*Cajanus cajan*	833	48680
9.	Potato	*Solanum tuberosum*	844	39031
10.	Cannabis	*Cannabis sativa*	534	30,000

been completed is presented below.

In plants, the smallest genome size has been reported in *Arabidopsis thaliana* (120 Mb) and the largest in corn (2500). Thus, in plants the genome size investigated so far varies from 120 Mb to 2500 Mb. After gene sequencing, function is assigned to individual gene through various molecular techniques.

Genes to be mapped:

In genome research, both types of genes, viz., major genes (oligogenes) and minor genes (polygenes) can be easily mapped. The mapping of polygenic traits is possible by genome mapping techniques which is not possible by conventional gene mapping techniques such recombination mapping and deletion mapping.

The genome mapping is done for morphological, productivity, resistance, quality, agronomic and some special traits as discussed below.

i. Morphological Characters:

It includes highly heritable traits such as shape, size and color of leaf, flower, calyx, corolla, etc. It also includes surface of leaf and stem (hairiness and smoothness).

ii. Productivity traits:

Such characters differ from species to species.

iii. Resistance Traits:

Such characters include resistance to diseases, insects, drought, soil salinity, soil alkalinity, soil acidity, heat, frost, water logging, cold, etc.

iv. Quality Traits:

Such traits include nutritional quality, market quality and keeping quality.

v. Agronomic Traits:

Such traits include earliness, plant height, plant type, etc.

vi. Special Characters:

Such characters include genes controlling male sterility, self-incompatibility, photo and thermo insensitivity, toxic substances, apomixes, adaptation, etc.

Genome Mapping Laboratories:

The genome mapping work is carried out by collaborative efforts of various International and National Research Laboratories of public domain. The list of some organizations where genome sequencing and mapping work on different crop species is carried out is presented in Table.

TABLE 35.2. List of some Genome Sequencing Organization

S.No.	*Name of Crop*	*Scientific name*	*Name of Genome Project*	*In Collaboration with*
1.	Arabidopsis	*Arabiddopsis thaliana*	Arabidopsis Genome Initiative	USA, China and Japan.
2.	Brassica	*Brassica* Spp.	Brassica Genome Gate way	UK, USA, France, Germany, Australia, South Korea, Poland and China.
3.	Wheat	*Tricum aestivum*	International Wheat genome	UK Scientists from Uni. of Liverpool, Bristol and John Innes.
4.	Rice	*Oriza* Spp.	International Rice Genome Sequencing Project	Mexico, USA, China and Japan
5.	Cotton	*Gossypium* Spp.	International Cotton Genome Initiative Project	USA, France, Australia
6.	Soybean	*Glycine max*	Uni. Purdue, North Carolina Uni, and 18 other centres	United States of America
7.	Potato	*Solanum tuberosum*	Potato Genome Sequencing Consortium	USA, UK, Russia, China, India, Poland, Chile,New Zealand, Nether land, Peru, etc.
8.	Tomato	*Lycopersicum esculentum*	Tomato Genome Consortium	China, japan, Germany,Korea, Spain, UK, USA, Israel, Netherland & Others
9.	Pigeon pea	*Cajanus cajan*	International Initiative for Pigeonpea Genomics	India (ICRISAT), China, USA, Mexico, etc.
10.	Apple	*Malus domesticus*	Apple Genome sequencing Initiative	Washington State Uni. USA and NRI COMPETITIVE GRANT

Genome Mapping in India:

In India, the functional genome research projects are looked after by the Depart of Biotechnology [DBT] and ICAR. The DBT has initiated such work on several crops such as rice, wheat, maize, chick pea, banana, tomato, *Brassica*, etc. The ICAR has created genome mapping facilities for rice at NRCPB, IARI, New Delhi. In India, the genome mapping work is carried out at the following centres.
i. National Research Centre for Plant Biotechnology, IARI, New Delhi.
ii. International Centre for Genetic Engineering and Biotechnology, New Delhi.
iii. Jawahar Lal Nehru University, New Delhi.
iv. National Botanical Research Institute, Lucknow.

Role of Genomics in Crop Improvement:

Genomics has several practical applications in crop improvement. Genome mapping is useful in several ways. It is useful or provides information about genome size, gene number, gene mapping, gene sequencing, evolution of crop plants, gene cloning, identification of DNA markers, marker assisted selection, transgenic breeding, construction of linkage maps and QTL mapping.
All these aspects are briefly discussed as follows:

i. Genome Size:

Genome mapping is a very useful technique for determining the genome size in various plant species. In the plant species studied so far, the largest genome size has been reported in maize (2500 Mb) and the smallest in *Arabidopsis thaliana* (120 Mb).

ii. Gene Number:

Genome mapping provide information about gene number in a species. In crop plants studied so far, the maximum number of genes has been reported in rice (56,000).

iii. Gene Mapping:

Genome research is very much useful in mapping/tagging of genes on the different chromosomes of a genome. In other words, it helps in large scale discovery of new gene in a genome.

iv. Gene Sequencing:

Genome mapping helps in determining the order of genes on the chromosomes. The order of genes is determined on each chromosome of a genome.

v. Evolution:

Genome mapping provide information about the evolution of different species. It measures the association between different genomes and thus provides information about the relatedness or evolutionary biology of crop plants.

vi. Gene Cloning:

Genome research is very much useful in making multiple copies of a gene and transfer of the same from one genotype to another. Thus, it aids in specific gene transfer.

vii. Identification of DNA Markers:

The genome mapping techniques are useful in identification of DNA markers which can be used in molecular breeding i.e. marker assisted selection. The mapping populations developed from inter-specific crosses have high polymorphism for DNA markers than those mapping populations derived from intra-specific crosses.

viii. Marker Assisted Selection:

Marker assisted selection refers to indirect selection for a desired phenotype based on band pattern of linked DNA markers. The improvement of crop plants using such selection is cal molecular breeding. Various DNA markers used for such purpose include RFLP, AFLP, ISSR, etc.
The effect of DNA marker is correlated with morphological markers and then selection is made for particular trait. The selection based on DNA markers is more reliable because DNA markers are not influenced by environmental factors.

ix. Transgenic Breeding:

Genome mapping is useful in gene cloning. The gene of interest can be cloned and used in developing transgenic plants (genetically engineered plants). Transgenic breeding permits direct gene transfer bypassing sexual process.

x. Construction of Linkage maps:

Genome mapping helps in construction of linkage groups. The linkage groups can be constructed from the information of gene mapping and gene sequencing.

xi. QTL Mapping:

The genome mapping techniques is widely used for mapping of quantitative trait loci (QTL). The mapping of QTL or polygenic traits is not possible by conventional methods, viz., recombination mapping and deletion mapping techniques.

Limitations of Genomics:

These days, the genome mapping of crop plants is gaining increasing importance. It has several useful applications as discussed above. However, there are some limitations of genome mapping such high cost, high technical skill, laborious work, availability of limited genes and lake of proper markers. These are briefly presented below:

i. Expensive Technique:

The genome research requires well equipped sophisticated laboratory with costly chemicals and glassware. Thus lot of funds is required for carrying genomic research. Lack of adequate funds sometimes becomes limiting factor in the progress of such project.

ii. High Technical Skill:

The genome mapping work requires high technical skill. It requires training of scientists in the specialized field of genomics. It also requires International collaboration with other leading genome research laboratories which sometimes becomes limiting factor. The international collaboration is possible if the crop on which genome research work is to be carried out is of global significance.

iii. Laborious Work:

The genome mapping requires detection of various DNA markers (RFL, AFLP, RAPD, SSR, etc.) which is a laborious and time consuming work. Huge populations related to F2, RILs, NILs and doubled haploids need to be screened for such purpose. This limits the progress of the work.

iv. Limited Genes Available:

Firstly, limited number of genes and promoters are available for development of transgenic. Secondly, such genes are protected under Intellectual Property Rights and, therefore, cannot be used for developing transgenic plants.

v. Lack of Proper Markers:

Most of the useful agronomic traits are governed by polygenes and are complex in nature. Tightly linked DNA markers are yet to be identified for such characters.

10. Future Thrusts of Genomics:

Considerable research work on genome sequencing and mapping has been done on various crop plants so far. In future, for rapid progress of genome sequencing work several points need consideration. Important points include funding, training, material sharing, research priorities, important traits, selection of species, etc.

Proteomics:

The entire protein component of a given organism is called ‘proteome’, the term coined by Wasinger in 1995. A proteome is a quantitatively expressed protein of a genome that provides information on the gene products that are translated, amount of products and any post translational modifications.

Proteomics is an emerging area of research in the post-genomic era, which involves identifying the structures and functions of all proteins of a proteome. It is sometimes also treated as structural based functional genomics.

Types of Proteomics:

i. Structural Proteomics:

One of the main targets of proteomics investigation is to map the structure of protein complexes or the proteins present in a specific cellular organelle known as cell map or structural proteins. Structural proteomics attempt to identify all the proteins within a protein complex and characterization all protein-protein interactions. Isolation of specific protein complex by purification can simplify the proteomic analysis.

ii. Functional Proteomics:

It mainly includes isolation of protein complexes or the use of protein ligands to isolate specific types of proteins. It allows selected groups of proteins to be studied its chracteristics which can provide important information about protein signalling and disease mechanism etc.

Expression proteomics

Expression proteomics includes the analysis of protein expression at larger scale. It helps identify main proteins in a particular sample, and those proteins differentially expressed in related samples—such as diseased vs. healthy tissue. If a protein is found only in a diseased sample then it can be a useful drug target or diagnostic marker. Proteins with same or similar expression profiles may also be functionally related. There are technologies such as 2D- PAGE and mass spectrometry that are used in expression proteomics.

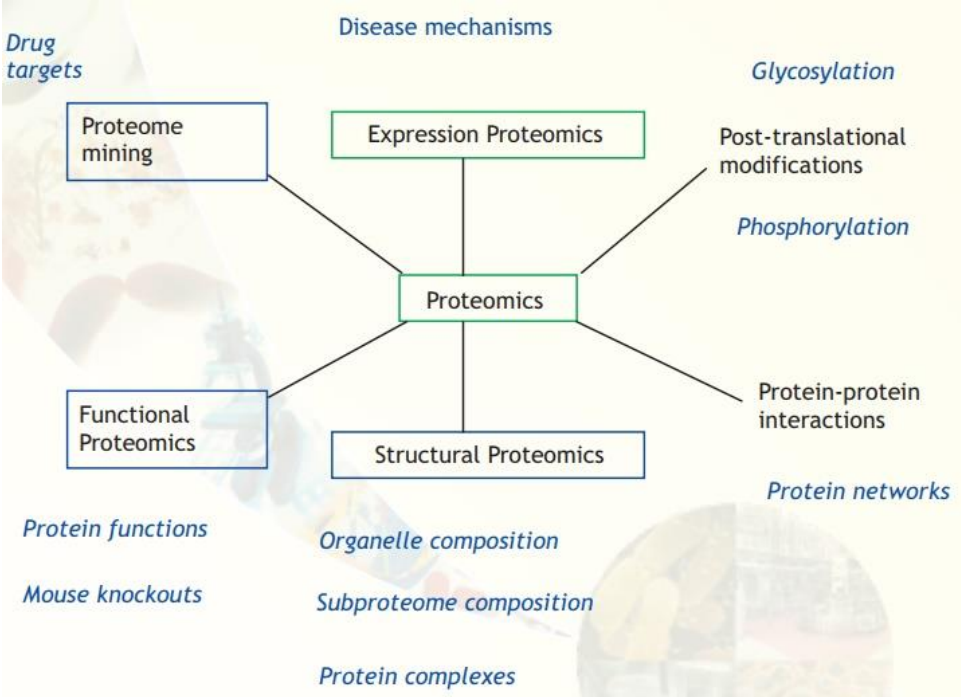


Fig. 5. Types of proteomics and the scientific knowledge that can be gained from them.

Significance of Proteomics:

i. Protein profiling:

Bioinformatics has been widely employed in protein-profiling, where question of protein structural information for the purpose of protein identification, characterization and database is carried out. The spectrum of protein expressed in a cell type provides the cell with its unique identity. It explores how the protein complement changes in a cell type during development in response to environmental stress.

ii. Protein arrays:

Protein microarrays facilitate the detection of protein protein interaction and protein expression profiling. Several protein microarray examples indicate that protein arrays hold great promise for the global analysis of protein-protein and protein-ligand interaction.

iii. Proteomics to a phosphorylation:

In post-translational modification of protein, mass spectrometer (MS) can be used to identify novel phosphorylation. Measure changes in phosphorylation state of protein takes place in response to an effective and determining phosphorylation sites in proteins.

Identification of phosphorylation sites can provide information about the mechanism of enzyme regulation and protein kinase and phosphotases involved. A proteomics approach for this process has an advantage that one can study all the phosphorylating proteins in a cell at the same time.

iv. Proteome mining:

Proteome mining is a functional proteomic approach used to extract information from the analysis of specific sub-proteomics. In principle, it is based on the assumption. In principle, it is based oil the assumption that all drug like molecule selectively compete with a natural cellular ligand for a binding site on a protein target.

Genomics	Proteomics
Genomics is the study of genome of an organism. Genome represents the entire genes of an organism or a cell type	Proteomics is the study of proteome of an organism. Proteome refers to the entire protein set coded by the genome of an organism or a cell type
Genomics include mapping, sequencing and analysis of genome	Proteomics include characterization of all proteins of an organism or study of structure and function of proteins
Genomics can be broadly classified into structural and functional genomics **a)Structural genomics:** is the study of the structure of all genes and its relative position on the chromosome **b)Functional genomics:** study of function of all genes or the role of these genes in regulating metabolic activities of the cell	Proteomics can be classified into structural functional and expression proteomics **a)Structural proteomics:** is the study of the structure of proteins and their location in the cell **b)Functional proteomics:** study of function of all proteins which primarily include protein-protein interaction and interaction of proteins with other biomolecules **c) Expression proteomics:** is the study of identification and quantification or expression level of proteins of the cell at different developmental stages or at different environmental conditions
Techniques in genomics include a) gene sequencing strategies like directed gene sequencing, whole genome short gun sequencing, b)Construction of ESTs (expressed sequence Tags), c) Identification of single nucleotide polymorphisms (SNPs), d) Analysis and interpretation of sequenced data using different databases and software.	Techniques in proteomics include a) protein extraction, electrophoretic separation, digestion of separated proteins into small fragments using trypsin, mass spectroscopy to find out amino acid sequences and finally protein identification using standard databases. b) Protein 3D structure prediction using software. c) Protein expression study using protein microarray.
Thrust areas in Genomics: Genome Sequencing projects of many organisms including Human Genome Project	Proteome database development like SWISS-2D PAGE and software development for computer aided drug design

c-DNA and genomic library:

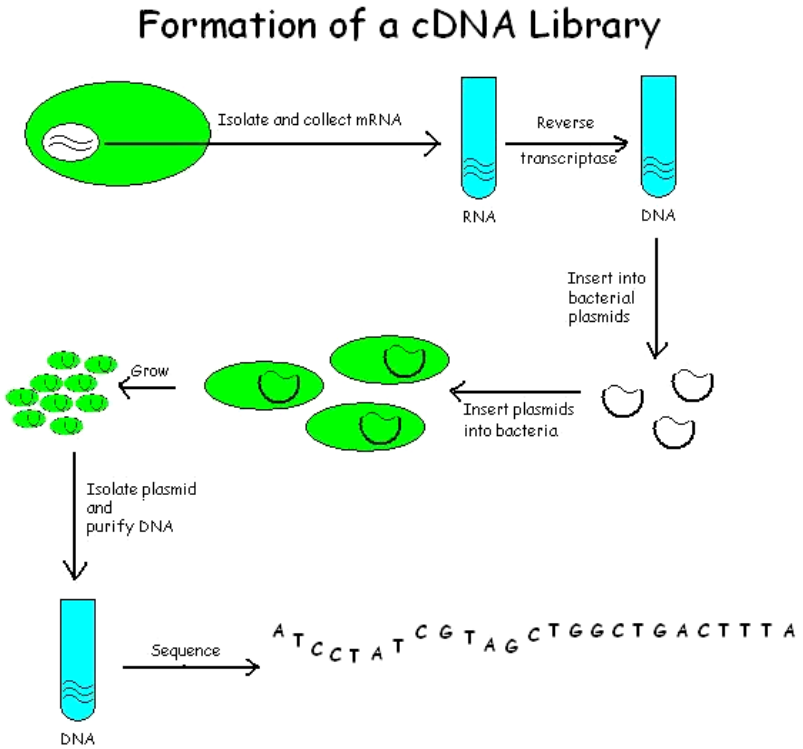
c-DNA

In genetics, complementary DNA (cDNA) is DNA synthesized from a single stranded RNA (e.g., messenger RNA (mRNA) or microRNA) template in a reaction catalyzed by the enzyme reverse transcriptase. cDNA is often used to clone eukaryotic genes in prokaryotes. When scientists want to express a specific protein in a cell that does not normally express that protein (i.e., heterologousexpression), they will transfer the cDNA that codes for the protein to the recipient cell. cDNA is also produced naturally by retroviruses(such as HIV-1, HIV- 2, simian immunodeficiency virus, etc.) and then integrated into the host's genome, where it creates a provirus.

The term cDNA is also used, typically in a bioinformatics context, to refer to an mRNA transcript's sequence, expressed as DNA bases (GCAT) rather than RNA bases (GCAU).
cDNA is derived from mRNA, so it contains only exons, with no introns.

Synthesis

Although there are several methods for doing so, cDNA is most often synthesized from mature (fully spliced) mRNA using the enzyme reverse transcriptase. This enzyme, which naturally occurs in retroviruses, operates on a single strand of mRNA, generating its complementary DNA based on the pairing of RNA base pairs (A, U, G and C) to their DNA complements (T, A, C and G, respectively).
To obtain eukaryotic cDNA whose introns have been removed:
1. A eukaryotic cell transcribes the DNA (from genes) into RNA (pre-mRNA).
2. The same cell processes the pre-mRNA strands by removing introns, and adding a poly-A tail and 5' Methyl-Guanine cap (this is known as post-transcriptional modification)
3. This mixture of mature mRNA strands is extracted from the cell. The poly-A tail of the post-transcriptional mRNA can be taken advantage of with oligo(dT) beads in an affinity chromatography assay.
4. A poly-T oligonucleotide primer is hybridized onto the poly-A tail of the mature mRNA template, or random hexamer primers can be added which contain every possible 6 base single strand of DNA and can therefore hybridize anywhere on the
RNA (Reverse transcriptase requires this double-stranded segment as a primer to start its operation.)
5. Reverse transcriptase is added, along with deoxynucleotide triphosphates (A, T, G, C). This synthesizes one complementary strand of DNA hybridized to the original mRNA strand.
6. To synthesize an additional DNA strand, traditionally one would digest the RNA of the hybrid strand, using an enzyme like RNase H, or through alkali digestion method.
7. After digestion of the RNA, a single stranded DNA (ssDNA) is left and because single stranded nucleic acids are hydrophobic, it tends to loop around itself. It is likely that the ssDNA forms a hairpin loop at the 3' end.
8. From the hairpin loop, a DNA polymerase can then use it as a primer to transcribe a complementary sequence for the ss cDNA.
9. Now, you should be left with a double stranded cDNA with identical sequence as the mRNA of interest.



Advantages of cDNA Library:

A cDNA library has two additional advantages. First, it is enriched with fragments from ac-tively transcribed genes. Second, introns do not interrupt the cloned sequences; introns would pose a problem when the goal is to pro-duce a eukaryotic protein in bacteria, because most bacteria have no means of removing the introns.

Disadvantages of cDNA Library:

The disadvantage of a cDNA library is that it contains only sequences that are present in mature mRNA. Introns and any other se-quences that are altered after transcription are not present; sequences, such as promoters and enhancers, that are not transcribed into RNA also are not present in a cDNA library.

It is also important to note that the cDNA library represents only those gene sequences ex-pressed in the tissue from which the RNA was isolated. Furthermore, the frequency of a par-ticular DNA sequence in a cDNA library depends on the abundance of the corresponding mRNA in the given tissue. In contrast, almost all genes are present at the same frequency in a genomic DNA library.

Applications of cDNA Library:

Following are the applications of cDNA librar-ies:

1. Discovery of novel genes.
2. Cloning of full-length cDNA molecules for in vitro study of gene function.
3. Study of the repertoire of mRNAs expressed in different cells or tissues.
4. Study of alternative splicing in different cells or tissues.

Genomic library:

A genomic library is a collection of the total genomic DNA from a single organism. The DNA is stored in a population of identical vectors, each containing a different insert of DNA. In order to construct a genomic library, the organism's DNA is extracted from cells and then digested with a restriction enzyme to cut the DNA into fragments of a specific size. The fragments are then inserted into the vector using DNA ligase. Next, the vector DNA can be taken up by a host organism - commonly a population of Escherichia coli or yeast - with each cell containing only one vector molecule. Using a host cell to carry the vector allows for easy amplification and retrieval of specific clones from the library for analysis.

There are several kinds of vectors available with various insert capacities. Generally, libraries made from organisms with larger genomes require vectors featuring larger inserts, thereby fewer vector molecules are needed to make the library. Researchers can choose a vector also considering the ideal insert size to find a desired number of clones necessary for full genome coverage.

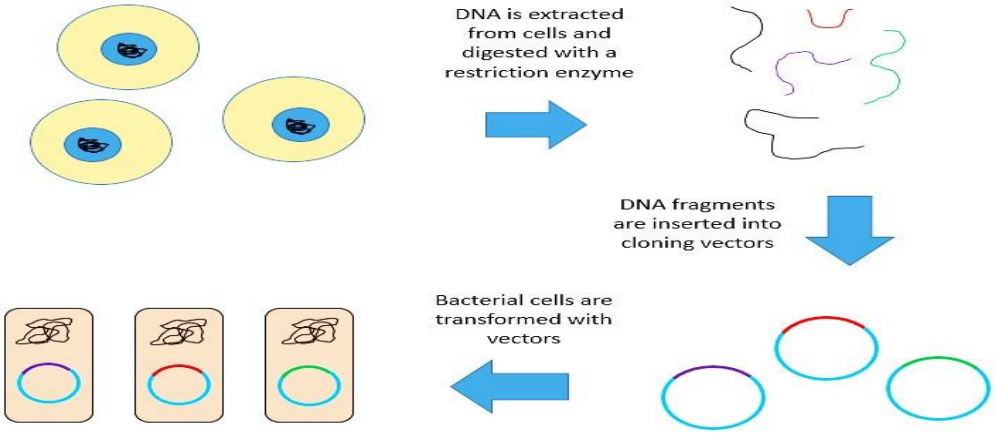
Genomic libraries are commonly used for sequencing applications. They have played an important role in the whole genome sequencing of several organisms, including the human genome and several model organisms.

The first DNA-based genome ever fully sequenced was achieved by two-time Nobel Prize winner, Frederick Sanger, in 1977. Sanger and his team of scientists created a library of the bacteriophage, phi X 174, for use in DNA sequencing. The importance of this success contributed to the ever-increasing demand for sequencing genomes to research gene therapy. Teams are now able to catalog polymorphisms in genomes and investigate those candidate genes contributing to maladies such as Parkinson's disease, Alzheimer's disease, multiple sclerosis, rheumatoid arthritis, and Type 1 diabetes. These are due to the advance of genome- wide association studies from the ability to create and sequence genomic libraries. Prior, linkage and candidate-gene studies were some of the only approaches.

Genomic library construction:

Construction of a genomic library involves creating many recombinant DNA molecules. An organism's genomic DNA is extracted and then digested with a restriction enzyme. For organisms with very small genomes (~10 kb), the digested fragments can be separated by gel electrophoresis. The separated fragments can then be excised and cloned into the vector separately. However, when a large genome is digested with a restriction enzyme, there are far too many fragments to excise individually. The entire set of fragments must be cloned together with the vector, and separation of clones can occur after. In either case, the fragments are ligated into a vector that has been digested

with the same restriction enzyme. The vector containing the inserted fragments of genomic DNA can then be introduced into a host organism.
Below are the steps for creating a genomic library from a large genome.
1. Extract and purify DNA.
2. Digest the DNA with a restriction enzyme. This creates fragments that are similar in size, each containing one or more genes.
3. Insert the fragments of DNA into vectors that were cut with the same restriction enzyme. Use the enzyme DNA ligase to seal the DNA fragments into the vector. This creates a large pool of recombinant molecules.
4. These recombinant molecules are taken up by a host bacterium by transformation, creating a DNA library.



Applications of Genomic Library:
Genomic library has following applications:
1. It helps in the determination of the com-plete genome sequence of a given organism.
2. It serves as a source of genomic sequence for generation of transgenic animals through genetic engineering.
3. It helps in the study of the function of regu-latory sequences in vitro.
4. It helps in the study of genetic mutations in cancer tissues.
5. Genomic library helps in identification of the novel pharmaceutical important genes.
6. It helps us in understanding the complex-ity of genomes.

9. Bioinformatics: Definition, importance, constituents, application in genomics

Bioinformatics:
Bioinformatics is currently defined as the study of information content and information flow in biological systems and processes. It serves as the bridge between observations (data) in diverse biologically-related disciplines and the deriva-tions of understanding (information) about how the systems or processes function and sub-sequently the application (knowledge).
Though Hwa Lim, Father of Bioinformatics, coined the word ‘bio/informatique’ in 1987, but Temple Smith used the term ‘Bioinformatics’ in 1991.
In Silico Biology, a new area of Biology, has been developed in recent years because of gene-ration of data in the field of genetics at an unprecedented exponential rate; the manage-ment and use of which requires the increasing use of computers and the relevant software.
Computational Biology, another term often used interchangeably with bioinformatics, although the former typically focuses on algo-rithm development and specific computational methods, while the latter focuses more on hypothesis testing and discovery in the biologi-cal domain.

Systems Biology, another area of research, emerged due to availability of enormous amount of molecular data and bioinformatics tools crea-ting unprecedented opportunities to assemble and integrate this data into networks of genes, proteins and bio-chemical pathways.

Bioinformatics involves collection, storage, retrieval and analysis of biological data that has a lot of applications in pharmaceutical, agricultural and food industries, and in molecular genetics research.

Biological data are generated from various genome sequencing projects, obtained by different techniques like ONA sequencing (genome and EST), 2D gel electrophoresis, mass spectroscopy (MS, MALDI, LC-MS), protein crystallization, microarrays (e.g., cDNA, oligos, pep-tide), molecular markers (e.g., RFLP, RAPD, AFLP, SNP).

Thus bioinformatics is an interface of bio-logical sciences, mathematics, physical sciences and computer sciences, i.e., the integrated field of biology and information technology.

Bioinformatics in Institutes, Web-sites, Databases, Tools:

i. Institutes:

Major public domain bioinformatics facilities are:

(a) NCBI – National Centre for Biotechno-logy Information, USA.

(b) EBI – European Bioinformatics Institute, UK.

(c) SIB – Swiss Institute of Bioinformatics, Switzerland.

(d) Genome NET (KEGG & DDBJ), Japan.

ii. Websites:

Some important websites commonly used for bioinformatics are depicted in Table.

iii. Databases:

Bioinformatics is involved in storing the sequence information in different nucleic acid and protein databases which can be assessed by people all over the world through network technology.

Protein databases:

The major protein databases are:

PDB, SWISS-PROT, PROSITE, ExPASy, PIR, PRINTS, BLOCKS, PRODOM, Pfam, Inter Pro.

Nucleic acid databases:

The major nucleic acid databases are: Gen Bank, DDBJ, Ref Seq, dbEST, NDB, CSD, EMBL.

iv. Tools for Genetic Studies:

In order to deal with molecular data, a wide range of software's are now available which facilitated analysis of data in user-friendly marther.

These tools are classified into four classes:

a. **Statistical-analysis tools**

Table 19.2: Two major tools for statistical analysis of data for genetic studies

Name	Features
SPSS	Produces results in understandable/presentable formats (in tabular and graphical form), and also allows sharing of results with others using a variety of reporting methods.
SAS	Used for a variety of statistical analyses, information storage and retrieval, data modification and programming, report writing, simple and advanced statistics, and file handling. The SAS/STAT software used for all kinds of statistical analyses; SAS/ETS software is used for time series analysis and SAS/IML is used for interactive matrix manipulation.

b. **Genome analysis tools**

Table 19.3: Tools for genome analysis (From P. K. Gupta)

Purpose	Tools and their features
(i) Tools for molecular-marker development	
SSR mining	TROLL (tandem repeat occurrence locator); TRF (tandem repeat finder); SSRFinder; MSL (microsatellite locator); SSRIT (SSR identification tool); SSRSEARCH; MISA (Microsatellite); Sputnik; SSR Primer, etc.
SNP mining	SNP pipeline (PHRED, PHRAP and DEMIGLACE); PolyPhred; PolyBayes; AutoSNP; SEAN; PARSESNP; SniPpER; SNPF (Single Nucleotide Polymorphism Finder); TRACE_DIFF, etc.
Primer designing	(i) Normal PCR - Primer3, Prima; (ii) site-directed mutagenesis - Primer Generator, PrimerX; (iii) RNAi - RNAi Design.
(ii) Tools for large-scale genome sequencing	
Base calling/quality assignment	PHRED: (i) calls bases (base calling means assigning bases to peaks of different colours) and (ii) assigns quality values to the bases.
Fragment assembly	PHRAP: (i) assembles shotgun DNA sequences, constructs contigs, and provides extensive information about assembly including quality values for contig sequences.
Vector trimming	Cross match; compares a set of sequences to a set of vector sequences and trims vector sequences to produce vector-masked versions of these sequences.
Viewing and editing contigs	Consed/Autofinish: allows viewing, editing, and finishing sequence assemblies created with PHRAP.
Sequence assembly program	CAP3: clips 5′ and 3′ low-quality regions of sequences; uses base quality values (Q-vlaues) in computation of overlaps between sequences; multiple sequence alignments, and generation of consensus sequences.
(iii) Tools for map construction, map alignment and QTL/LD analysis	
Map preparation	MAPMAKER, JoinMap, etc.
QTL analysis	(i) single locus analysis: QTL Cartographer, PLABQTL; (ii) two locus analysis: QTLMapper/QTLNetwork, PseudoMarker, etc.
LD analysis	LDA, TASSEL, STRUCTURE, GDA, (LD estimation); LDMAP, ALLASS (LD mapping); QTDT (association tests and TDT), etc.
MAP comparison	cMAP: for comparing the order of loci in different maps for the same species or in the maps of related genera (for comparative genomics).
Anchoring of BAC contigs to genetic maps	iMAP: allows unambiguous assignment of BAC contigs to the genetic map, i.e., assists in preparing genetically anchored BAC contig maps.

c. Sequence alignment tools

Table 19.4: List of sequence alignment tools and their specific features (From P. K. Gupta)

Name of tool	Features
(i) Pair-wise sequence alignment tools	
FASTA family	(i) [fastaa] and [ssearch], compare a protein sequence against a protein database or a DNA sequence against a DNA database. (ii) [fastx/fasty], translates a DNA sequence into amino acid sequence of a protein and compares such a protein against a protein database. (iii) [tfastx/tfasty], compares a protein sequence against a DNA sequence translated in three forward and three reverse frames.
PatMatch	Searches for short nucleotide or peptide sequences (3-30 nt or amino acid).
BLAST (earlier versions)	(i) BLASTp compares protein sequence against a protein sequence; (ii) BLASTn compares a nucleotide sequence against a nuclotide sequence; (iii) BLASTx compares a nucleotide sequence translated in all reading frames against a protein sequence; (iv) tBLASTn compares a protein sequence against a nucleotide sequence translated in all reading frames; (v) tBLASTx compares the six frame translations of a nucleotide sequence against the six frame translations of a nucleotide sequence; (vi) BLASTz compares long stretches of nucleotides (>2 kb).
BLAST (later versions)	(i) VecScreen (for match with sequence of plasmids, phage, cosmids, BACs, PACs and YACs). (ii) IgBLAST (for analysis of immunoglobulin sequences). (iii) MegaBLAST (for comparing a set of ESTs with a set of genes, and grouping them in clusters). (iv) SNP BLAST (for match query sequence with sequence carrying SNPs available in the SNP database, dbSNP). (v) PowerBlast (for alignment of query sequence with all sequences rather than one to one alignment).
ParAlign	Exploits parallelism involving division of a major task into small tasks that are performed in parallel to perform a very rapid computation.
ProteinEngine and Transeq	Used first for translating DNA sequences into proteins, which are then used for further similarity searches and for the study of 3-D structures encoded.

d. Genome annotation tools

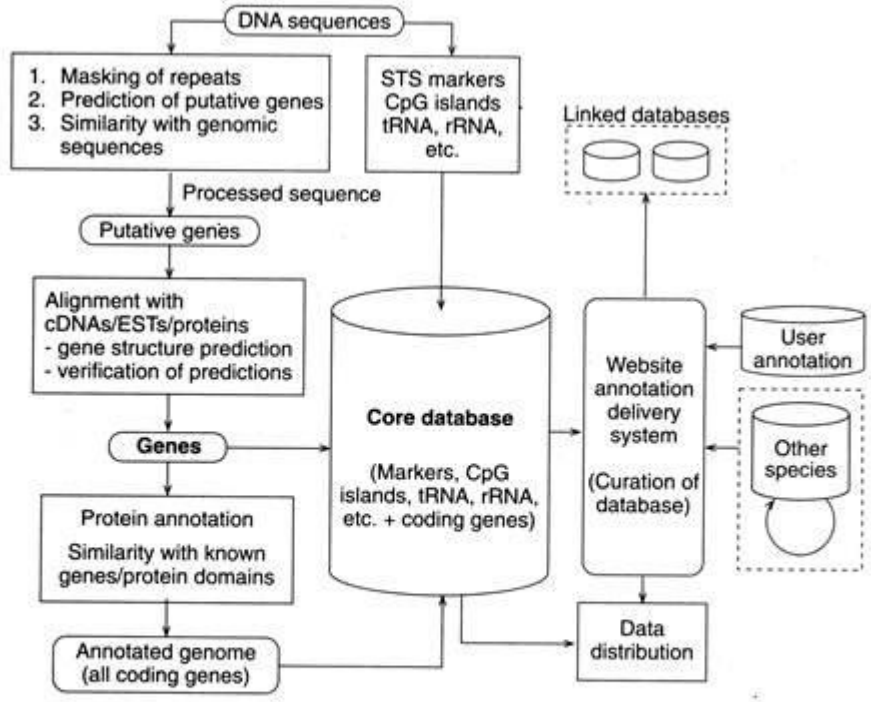


Fig. 19.3: Basic outline of an automatic genome annotation pipeline and delivery system. The diagram can be partitioned into (i) analysis pipeline stage (shown on the left), and (ii) delivery system (shown on the right); the data is distributed between the two stages as the central core database. The sequence data enters the pipeline and flows down through the gene prediction analysis before being stored in the core database. The website delivery system is a key feature as it serves to integrate the gene predictions and features from the core database alongside multiple external databases (From P. K. Gupta)

Importance:

i. Sequence mapping of biomolecules (DNA, RNA, proteins).
ii. Identification of nucleotide sequences of functional genes.
iii. Finding of sites that can be cut by restriction enzymes.
iv. Designing of primer sequence for polymerase chain reaction.
v. Prediction of functional gene products.
vi. To trace the evolutionary trees of genes.
vii. For the prediction of 3-dimensional structure of proteins.
viii. Molecular modelling of biomolecules.
ix. Designing of drugs for medical treatment.
x. Handling of vast biological data which otherwise is not possible.
xi. Development of models for the functioning various cells, tissues and organs.

The above mentioned importances however, may be treated as incomplete, since at present there is no field in biological sciences that does not involve bioinformatics.

In the context of genomics, annotation is the process of marking the genes and other biological features in a DNA sequence. This process needs to be automated because most
genomes are too large to annotate by hand, not to mention the desire to annotate as many genomes as possible, as the rate of sequencing has ceased to pose a bottleneck. Annotation is made possible by the fact that genes have recognisable start and stop regions, although the exact sequence found in these regions can vary between genes.

The first description of a comprehensive genome annotation system was published in 1995 by the team at The Institute for Genomic Research that performed the first complete sequencing and analysis of the genome of a free-living organism, the bacterium Haemophilus influenzae. Owen White designed and built a software system to identify the genes encoding all proteins, transfer RNAs, ribosomal RNAs (and other sites) and to make initial functional assignments. Most current genome

annotation systems work similarly, but the programs available for analysis of genomic DNA, such as the GeneMark program trained and used to find protein-coding genes in Haemophilus influenzae, are constantly changing and improving.

Following the goals that the Human Genome Project left to achieve after its closure in 2003, a new project developed by the National Human Genome Research Institute in the U.S appeared. The so-called ENCODE project is a collaborative data collection of the functional elements of the human genome that uses next-generation DNA-sequencing technologies and genomic tiling arrays, technologies able to automatically generate large amounts of data at a dramatically reduced per-base cost but with the same accuracy (base call error) and fidelity (assembly error).

Constituent

Bioinformatics comprises three components:

1. **Creation of databases:**

This involves the organizing, storage and management the biological data sets. The databases are accessible to researchers to know the existing information and submit new entries, e.g. protein sequence data bank for molecular structure. Databases will be of no use until analysed.

2. **Development of algorithms and statistics:**

This involves the development of tools and resources to determine the relationship among the members of large data sets e.g. comparison of protein sequence data with the already existing protein sequences.

3. **Analysis of data and interpretation:**

The appropriate use of components 1 and 2 (given above) to analyse the data and interpret the results in a biologically meaningful manner. This includes DNA, RNA and protein sequences, protein structure, gene expression profiles and biochemical pathways.

Application in genomics

Genomics is the study of complex sets of genes, their expression and the most vital role they play in biology. The most important application of bioinformatics in genomics is the Human Genome Project through which more than 30,000 genes have been identified and secured through the sequencing of chemical base pairs which make up the DNA. It has thus enabled us to obtain necessary knowledge as to how these genes inter-relate and what functions they perform. Cures for many diseases are being discovered through this inter-relation where bioinformatics, no doubt, plays a pivotal role.

In the context of genomics, annotation is the process of marking the genes and other biological features in a DNA sequence. This process needs to be automated because most genomes are too large to annotate by hand, not to mention the desire to annotate as many genomes as possible, as the rate of sequencing has ceased to pose a bottleneck. Annotation is made possible by the fact that genes have recognisable start and stop regions, although the exact sequence found in these regions can vary between genes.

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10. Let's sum up

- Amino acids have a carboxyl group and an amino group bonded to the same carbon atom (the a carbon). They differ from each other in their side chains, or R groups, which vary in structure, size, and electric charge, and which influence the solubility of the amino acids in water.
- Proteins are organic nitrogenous compounds in which a large number of amino acids are joined together by peptide linkages to form long polypeptide chains. Proteins are made up of polypeptide chains, which are amino acids joined together with peptide bonds.
- Amino acid sequence is the order in which amino acid residues, connected by peptide bonds, lie in the chain in peptides and proteins. The sequence is generally reported from the N-terminal end containing free amino group to the C-terminal end containing free carboxyl group.
- Gel electrophoresis is a method for separation and analysis of macromolecules (DNA, RNA and proteins) and their fragments, based on their size and charge. Mass spectrometry is an important method for the accurate mass determination and characterization of proteins, and a variety of methods and instrumentations have been developed for its many uses.
- X-ray crystallography is a technique used for determining the atomic and molecular structure of a crystal, in which the crystalline structure cause a beam of incident X-rays to diffract into many specific directions.
- A DNA nucleotide consists of a deoxyribose sugar, a phosphate group, and a nitrogenous base. RNA consists of a ribose sugar, a phosphate group, and a nitrogenous base.
- Recombinant DNA technology is a technology which allows DNA to be produced via artificial means. The procedure has been used to change DNA in living organisms and may have even more practical uses in the future.
- Naturally occurring restriction endonucleases are categorized into four groups (Types I, II III, and IV) based on their composition and enzyme cofactor requirements, the nature of their target sequence, and the position of their DNA cleavage site relative to the target sequence.
- In all prokaryotic and eukaryotic organisms, three main classes of RNA molecules exist-1) Messenger RNA(m RNA) 2) Transfer RNA (t RNA) 3) Ribosomal RNA (r RNA)
- Large-scale changes take place in precursor mRNA. These changes are called processing of mRNA. Both 5′-end 3′-end of mRNA are modified. Non-coding regions are removed by splicing. The changes lead to the formation of mature mRNA which takes part in protein synthesis.
- An exogenous dsRNA or endogenous pre-miRNA can be processed by dicer and incorporated into the RNA-induced silencing complex (RISC), which targets single-stranded messenger RNA mol-ecules and triggers translational repression; incorporation into the RNA-induced transcriptional silencing complex (RITS) induces genome maintenance activities such as histone methylation and chromatin reorganization.
- In molecular biology, a riboswitch is a regulatory segment of a messenger RNA molecule that binds a small molecule, resulting in a change in production of the proteins encoded by the mRNA.
- Blotting technique is an extremely powerful tool for analyzing gene structure and used to study gene expression, once cloned cDNA is isolated. There are three important types of blotting techniques are: a) Southern Blotting b) Northern Blotting c) Western Blotting.
- DNA fingerprinting is a laboratory technique used to establish a link between biological evidence and a suspect in a criminal investigation.

- Genomics provides an overview of the complete set of genetic instructions provided by the DNA, while transcriptomics looks into gene expression patterns. Proteomics studies dynamic protein products and their interactions, while metabolomics is also an intermediate step in understanding organism's entire metabolism.

- A genomic library is a collection of independently isolated vector linked DNA fragments derived from a single organism. Complementary DNA (cDNA) libraries can also be prepared by iso-lating mRNAs from tissues which are actively synthesizing proteins, like roots and leaves in plants, ovaries or reticulocytes in mammals, etc.

- Bioinformatics is the computer aided study of biology and genetics. In other words, it refers to computer based study of genetics and other biological information.

11. Suggested Readings

1. Bruce Alberts, Alexander Johnson, Julian Lewis, David Morgan, Martin Raff, Keith Roberts, Peter Walter. Molecular Biology of the Cell. 2015. 6th Ed: Garland Science.
2. Watson, J.D., Baker, T.A., Bell, S.P., Gann, A., Levine, M. & Losick, R. Molecular Biology of the Gene (5thed.) 2004. Pearson Education Inc.
3. Harvey Lodish , Arnold Berk, Chris A. Kaiser , Monty Krieger , Anthony Bretscher , Hidde Ploegh , Angelika Amon , Kelsey C. Martin. Molecular Cell Biology. 2013. 8th Ed. Freeman.
4. Gerald Karp. Cell Biology. 2013. 7th Ed. International Student Version. Wiley.
5. Snustad, D.P. & Simmons, M.J. Principles of Genetics, John Wiley & Sons.
6. Klug, W.S. & Cummings, M.R. Concepts of Genetics, 2003, Pearson Education.
7. B.D. Singh 1996. Fundamentals Of Genetics, Kalyani Publishers.
8. Lewin, B. Genes VIII, 2004, Pearson Educational International.
9. Cooper, G.M. The Cell, A molecular approach (2nd ed.), 2000, ASM Press.
10. Gupta, P.K. Genetics, 2007, Rastogi Publications.
11. Kar, D.K. and Halder, S. Cell Biology, Genetics and Molecular Biology 2008, New Central Book Agency.
12. http://www.biologydiscussion.com/
13. https://en.wikipedia.org/
14. https://www.onlinebiologynotes.com
15.

12. Assignment

1. What is meant by Ramachandran plot?
2. Briefly describe secondary structure of protein.
3. What is isoelectic point?
4. Define enantiomers.
5. Give an account on edman degradation methods of amino acid sequencing.
6. What are the criteria of a ideal vectors?
7. What is the full form of TEMED?
8. How does a miRISC function in posttrancriptional gene silencing?
9. Distinguish between stacking and resolving gel.
10. Name the stain used in SDS-PAGE.
11. What is IEF?
12. How does mass spectrometry helps in the study of protein structure?
13. Discuss RNA processing in eukaryotes to produce mRNA from pre mRNA.
14. Describe the procedure of X-ray crystallography.
15. What is Bragg's Law?
16. Briefly describe pathways for protein targeting into and across thylakoid membranes.

17. Write a short note on sorting of proteins in mitochondria.
18. Mention the characteristic features of Watson and Crick model of DNA.
19. Differentiate between B and Z form oDNA.
20. Write a short note on mitochondrial genome.
21. What are different types of restriction enzymes?
22. What is isoschizomers?
23. Enumerate the steps of recombinant DNA construction.
24. What is cosmid?
25. Differentiate between BAC abd YAC.
26. What is shuttle vector?
27. Write a short note on t-RNA.
28. What is riboswitch? Mention its mechanism of action.
29. What are the principles of Southern blotting? Explain the procedure of its.
30. How c-DNA library is constructed?
31. Enumerate the steps of DNA fingerprinting. Mention its application.
32. What is meant by expression proteomics?
33. Write a short note on genomics.
34. Give a basic outline of genome annotationpipeline and delivery system.
35. Name two bioinformatics institute of worlds.
36. Differentiate between genomics and proteomics.

COURSE – BOHCT 3.2

Plant Molecular Biology & Biotechnology

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

Group B (Biotechnology)

Content Structure

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1. Introduction
2. Course Objectives
3. Plant tissue culture: Cellular totipotency; organogenesis, somatic embryogenesis, Role of SERK and LEC genes during SE; haploidy and DH populations in crop improvement.
4. Genetic transformation: Agrobacterium mediated (co-culture, in planta, agroinfection); Direct method (PEG, electroporation, particle gun method); Reporter genes- screenable and selectable markers.
5. Biotechnological applications for crop management: Approaches to improve shelf life of fruits and vegetables; herbicide resistance; insect and pest management.
6. Secondary metabolite production in plant cultures: Types of secondary metabolites; culture systems used for secondary metabolite production; improving secondary metabolite production in culture; hairy root culture.
7. Micropropagation: Production of virus free plants, virus free assessment methods, genetic assessment by RAPD and ISSR markers.
8. Fermentation technology: application of fermentation; batch, fed batch and their continuous cultures of microbes; Bioreactors: Principles and their design; microbial strain improvement.
9. Immobilization of microbial enzymes and whole cells and their applications in industries.
10. Microbes as food and in food processing, single cell protein.
11. Biofertilizers and biopesticides in agriculture.
12. Environmental biotechnology: Treatment of waste & waste water; bioremediation
13. Regulatory issues in biotechnology: Intellectual property rights (IPR): Patents, plant variety protections & geographical indications, WTO & TRIPPS, Bio safety and Food safety of GMO - a brief outline.
14. Let’s sum up
15. Suggested Reading
16. Assignment

1. Introduction

Biotechnology is technology based on biology, especially when used in agriculture, food science, and medicine. The UN Convention on Biological Diversity has come up with one of many definitions of biotechnology: "Biotechnology means any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use."
Traditional pharmaceutical drugs are small chemicals molecules that treat the symptoms of a disease or illness - one molecule directed at a single target. Biopharmaceuticals are large biological molecules known as proteins and these target the underlying mechanisms and pathways of a malady; it is a relatively young industry. They can deal with targets in humans that are not accessible with traditional medicines. Small molecules are manufactured by chemistry but large molecules are created by living cells: for example, - bacteria cells, yeast cell. Biotechnology has applications in four major industrial areas, including health care (medical), crop production and agriculture, non-food (industrial) uses of crops and other products (e.g. biodegradable plastics, vegetable oil, biofuels), and environmental uses.
This course is very important for students.

2. Course Objectives

This course aims to:

- To acquire knowledge and abilities to apply the biotechnological tools necessary in the development of products and services involving biological organisms or components, from genetic engineering techniques to the use of bioreactors and development of bioprocesses, along with applications to healthcare.
- Knowledge about the single cell protein, uses of biofertilizers and biopesticides in agriculture.

3. Plant tissue culture: Cellular totipotency; organogenesis, somatic embryogenesis, Role of SERK and LEC genes during SE; haploidy and DH populations in crop improvement.

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. Plant tissue culture 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 clear particular plants of viral and other infections and to quickly multiply these plants as 'cleaned stock' for horticulture and agriculture.

Plant tissue culture relies on the fact that many plant cells have the ability to regenerate a whole plant (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.

What conditions do plant cells need to multiply in vitro?

Tissue culture has several critical requirements:

- Appropriate tissue (some tissues culture better than others)
- A suitable growth medium containing energy sources and inorganic salts to supply cell growth needs. This can be liquid or semisolid
- Aseptic (sterile) conditions, as microorganisms grow much more quickly than plant and animal tissue and can overrun a culture.
- Growth regulators - in plants, both auxins & cytokinins.
- Frequent subculturing to ensure adequate nutrition and to avoid the build-up of waste metabolites

Cellular Totipotency:

Unlike an animal cell, a plant cell, even one that highly maturated and differentiated, retains the ability to change a meristematic state and differentiate into a whole plant if it has retained an intact membrane system and a viable nucleus. 1902 Haberlandt raised the totipotentiality concept of plant totipotency in his Book “Kulturversuche mit isolierten Pflanzenzellen” (Theoretically all plant cells are able to give rise to a complete plant).

Totipotency is the genetic potential of a plant cell to produce the entire plant. In other words, totipotency is the cell characteristic in which the potential for forming all the cell types in the adult organism is retained.

. However, all living plant cells retain the potential to revert back to the meristematic state and form new plants on exposure to favorable conditions, irrespective of their specialization and ploidy level (haploid, diploid or triploid). It has been a routine horticultural practice to use leaf, stem, and root cuttings as source material to regenerate new individuals for vegetative propagation of some plant species. Plant tissue culture has considerably enlarged the scope of regeneration of plants from highly differentiated and structurally and functionally specialized cells of leaves, roots, stem, floral parts, and endosperm. In vitro regeneration of plants is also possible from isolated gametic cells. The potentiality of differentiated and specialized cells to form complete plants like the zygote is referred to as Cellular Totipotency. The term was probably coined by T.H. Morgan (1901). However, it was the

famous German plant physiologist, Göttlieb Haberlandt, who in his famous address to the German Academy in 1902 introduced the concept of cellular totipotency and suggested that the terminally differentiated plant cells, as long as they contain the entire complement of chromosomes, should be capable of regenerating whole plants. In tissue cultures, cellular totipotency may be expressed via organogenesis (shoot differentiation) or embryogenesis (adventive embryony).

Organogenesis:

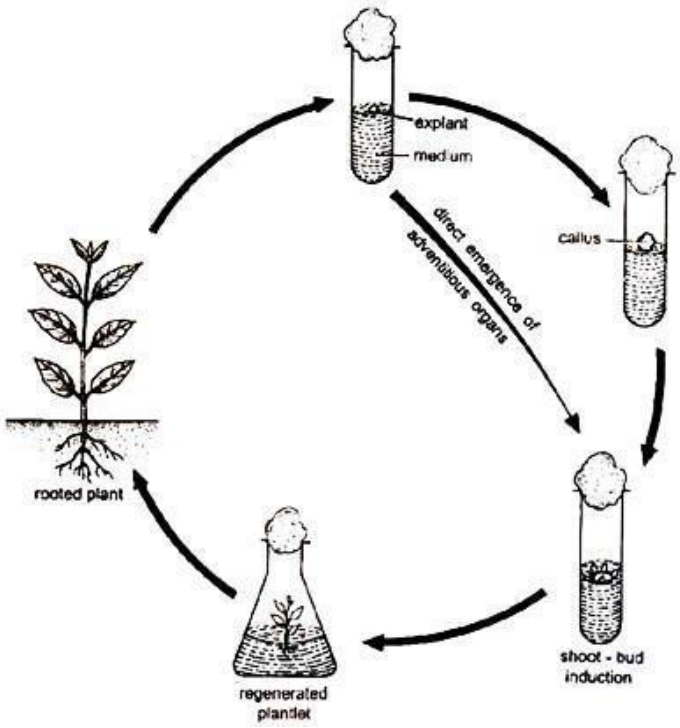
In plant tissue culture, organogenesis is a process of differentiation by which plant organs viz. roots, shoots, bud flowers, stem etc. are formed while adventitious refers to the development of organs (roots, buds, shoots, flowers etc.) or embryos (embryo like structures) from unusual points of origin of an organized explant where a preformed meristem is lacking. Adventitious shoots and roots are induced on tissues that normally do not produce these organs. Adventitious shoots are stem and leaf structures that arise naturally on plant tissues located in
sites other than at the normal leaf axil regions. Plant development through organogenesis is the formation of organs either de novo or adventitious in origin. Whole plant regeneration via organogenesis is a monopolar structure. Plant production through organogenesis can be achieved by two modes: (i) Organogenesis through callus formation with de novo origin and
emergence of adventitious organs directly from the explant.

Organogenesis through callus formation

Plant regeneration from cultured explants involves the initiation of basal callus and then shoot bud differentiation. Establishment of callus growth with subsequent organogenesis has been obtained from many species of plants and from numerous explants viz. cotyledons, hypocotyl, stem, leaf, shoot apex, root, young inflorescence, flower petals, petioles, embryos etc. cultured in vitro. For any given species or variety a particular explants may be necessary for successful plant regeneration. Explants from both mature and immature organs can be induced to form callus and then plant regeneration. However, explants with mitotically active cells are generally good for callus initiation. Immature tissues and organs are invariably more mopho-genetically plastic in vitro than mature tissues and organs. The size and shape of the explant is also crucial. It has been seen that a small percentage of cells in a given explant contribute to the formation of callus. Callus is produced on explants in vitro as a result of wounding and in response to hormones either endogenous or exogenously supplied in the medium.

Two modes of cell culture are generally used for organogenic path: (i) the cultivation of cell cultures on a solid medium and (ii) the cultivation of cell suspensions in liquid medium. Suspension culture should be subcultured at leat once a week while callus culture should be subcultured after3-4 weeks.

Plant growth regulators (PGRs) concentration in the culture medium is critical for morphogenesis. Auxin at a moderate to high concentration is the primary hormone used to produce callus. Often, 2,4-D , a very potentauxin, is used alone to imitate callus. In some species a high concentration of auxinand a low concentration of cytokinin in the medium promoteabundant cell proliferation with the formation of callus. Cytokinin if supplied are kinetic or benzyladenine. Callus tissue comprises a wide range of cell types and characteristically consists of irregularly differentiated, vacuolated cells interspersed with smaller more meristematic cells. The nature of any callus will depend on the explant tissue or tissues form which it has arisen and also on the composition of the medium



used to induce and maintain it.

Direct adventitious organ formation

The somatic tissues of higher plants are capable under certain conditions of regenerating adventitious buds. Adventitious buds are those which arise directly from a plant organ or a piece thereof without any intervening callus phase. Induction of adventitious shoots directly on roots and leaves, bulb scales and other organs of intact plants is a common method of propagation. The requirement of exogenous auxin and cytokinin in the process varies with the tissue system, apparently depending on the endogenous levels of the hormones in the tissue. Adventitious in vitro regeneration may give a much higher rate of shoot production than axillary shoots. Adventitious shoots proliferation is the most frequently used multiplication technique in micropropagation system.

What is Somatic Embryogenesis?

In plant tissue culture, the developmental pathway of numerous well-organised, small embryoids resembling the zygotic embryos from the embryo genic potential somatic plant cell of the callus tissue or cells of suspension culture is known as somatic embryogenesis.

The process was discovered for the first time in *Daucas carota* L. (carrot) by **Steward (1958), Reinert (1959)**.

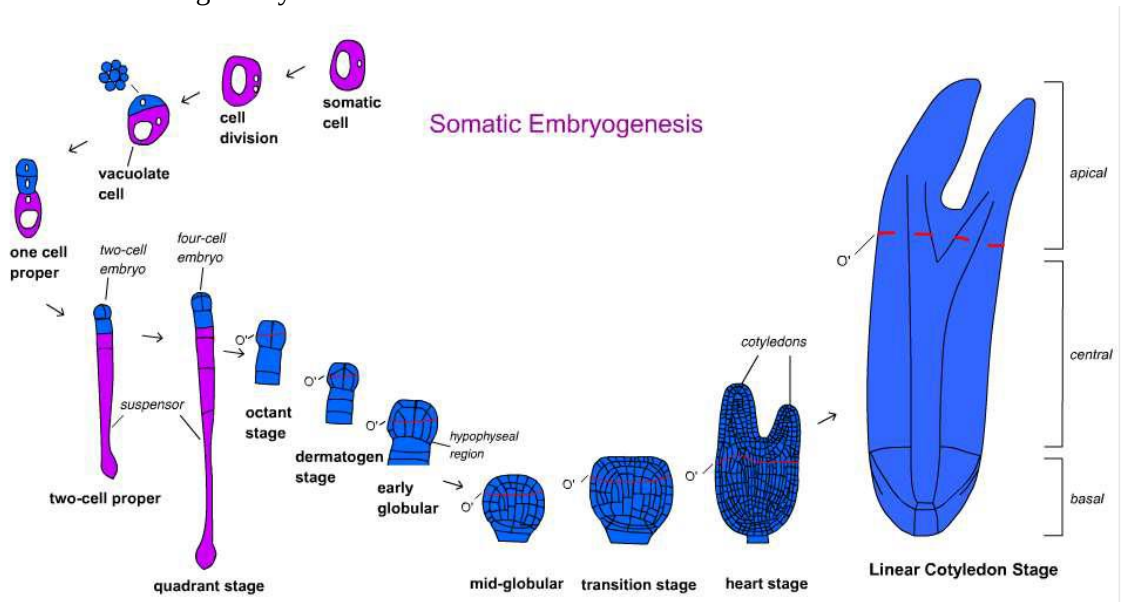
The most basic requirement for embryo development is the physical and chemical environment which is available only inside the '**Magic Bath'** of embryo sac. In vitro embryo can be developed if we provide the nutritional conditions same as in magic bath.

What is Embryo genic Potential?

The capability of the somatic plant cell of a culture to produce embryoids is known as embryo genic potential.

What is Embryoid?

Embryoid is a small, well-organised struc-ture comparable to the sexual embryo, which is produced in tissue culture of dividing embryo.



Stages of somatic embryogenesis

1. Induction

- An auxin, particularly 2, 4-D, is generally necessary to induce embryogenesis.
- Requirement of exogenous auxin for induction of SEs depends on nature of explants used with relative concentration of auxin.

2. Development

- After reinitiation of cell division and a period of cell proliferation in presence of auxin embryogenic cells are released into auxin free medium. These cells are in the clusters of cytoplasmic cells called Proembryonic mass of cells(PEMs).

3. Maturation

- The quality of SEs with regard to their germinability or conversion into plants is very poor. This is because the apparently normal looking SEs are actually incomplete in their development.
- Unlike seed embryos, SEs do not go through the final phase of embryogenesis, called embryo maturation which is characterised by accumulation of embryo specific reserve food materials and proteins which impart desiccation tolerance to the embryos; embryo size does not increase during this phase.
- ABA, which prevent precocious germination and promotes normal development of embryogenesis by triggering expression of genes which normally express during dryingdown stage of seeds (Dure et al,1981).

Sharp et. al. (1980) described mainly two routes for somatic embryogenesis:

1. Direct Embryogenesis:

The embryos initiate directly from the explant without callus formation and here some cells which are called as 'Pre-embryonic determined cells' (PEDC) initiates embryonic development, only those cells need to be released. Such cells are found mostly in embryonic tissues, certain tissues of young in vitro grown plants, hypocotyl, nucellus, embryo-sac, etc.

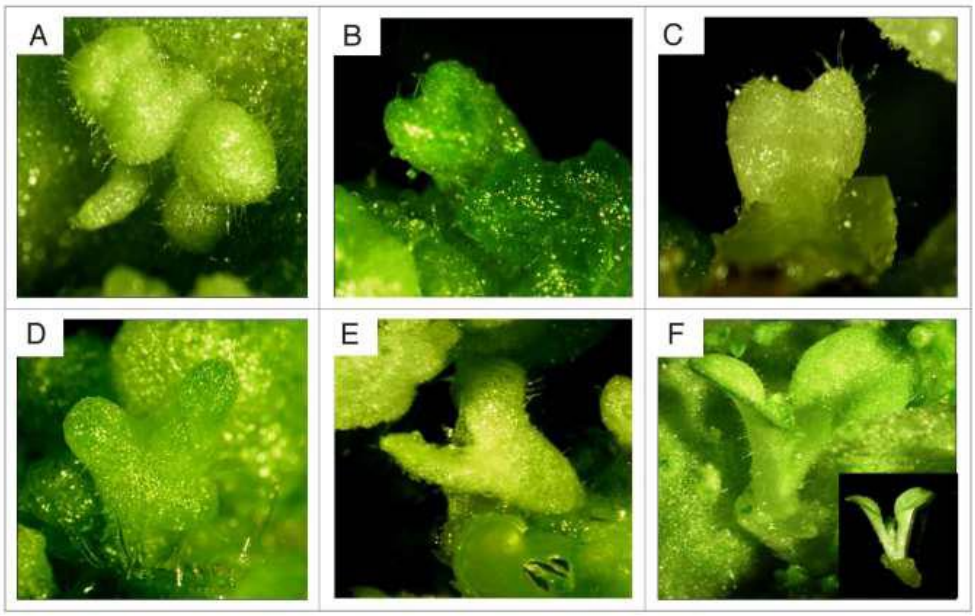


Figure 1. Various stages of direct somatic embryogenesis of *Nicotiana tabacum*. **(A)** Fused globular stage embryos; **(B)** early heart shape stage; **(C)** heart shaped stage; **(D)** early torpedo stage; **(E)** torpedo stage; **(F)** cotyledonary stage. Pictures were taken by Nikon LY-SM21500

2. Indirect Embryogenesis:

Here, the embryos are developed through cell proliferation i.e., callus formation. The cells from which embryos arise are called as 'Induced embryogenic determined cells' (IEDC). Here growth regulators with specific cul-tural conditions are required for initiation of callus and then redetermination of those cells into the embryo development.

Figure 1 - Primary callus induction, somatic embryogenesis and plant regeneration from immature embryos of maize cv. Gaurav (A) Inoculation of immature embryos in MS with 5 mg/L 2,4-D and 2 mg/l NAA + 1 mg/l BAP, (B) Callus proliferation in MS with 5 mg/L 2,4-D and 2 mg/l NAA + 1 mg/l BAP, (C-D) Globular shape observed during somatic embryogenesis (arrows) (E) Regenerating calli in MS medium with 5.0 mg/L 2,4-D. Culture showing mature green somatic embryos (arrow) (F) Root induction in MS medium with 5.0 mg/L 2,4-D (Joshi et al., 2010).

Somatic embryos	Zygotic embryos
1. SEs are formed by sporophytic cells. **2**. SEs store less amount of embryo specific reserves. **3**. A distinct suspensor is absent in SEs even if it is present it may not be functional as in seed embryos. **4**. Embryos have no vascular connections with the cultured explant. **5**. SEs generally lack a dormant phase and often show secondary embryogenesis and pluricotyledony. **6**. SEs show high rate of propagation .	**1**. Formed by fusion of gametic cells. **2**. Seed storage proteins, carbohydrates are the characterstic features. **3**. A well developed distinct suspensor is present. **4**. Embryos have vascular connections with the explant. **5**. They do not show secondary embryogenesis and pluricotyledony. **6**. Low rate of propagation than SEs.

Advantages:

- It is observable, as its various culture conditions can be controlled.
- Lack of material is not a limiting factor for experimentation.
- High propagation rate.
- Somaclonal variations.
- Germplasm conservation.
- Labour saving.
- Elimination of diseases and viruses

Disadvantages:

- Confined to few species.
- The somatic embryos show very poor germination because of their physiological and biochemical immaturity.
- Instability of cultured cells in long-term cultures is a major limitation in commercial exploitation and mass propagation of SEs.

Applications of Somatic Embryogenesis:

(i) Large Scale Propagation Compared to Zygotic Embryos:

Induction of somatic embryogenesis forms the ultimate goal in free cell suspension cultures relying on the totipotency of the cell and could reasonably be exploited for micro-propagation.

Each cell of the suspension cultures can be induced to produce somatic embryos which can be maintained in an arrested state of development by cold storage or using mitotic inhibitors until the time of sowing. Somatic embryo-genesis is highly desirable and holds out promise for rapid multiplication in a shorter time, with a shoot-root axis.

(ii) More Useful than Organogenesis:

The mass production of adventitious embryos in cell culture is still regarded by many as the ideal propagation system. The adventitious embryo is a bipolar structure that develops directly into a complete plantlet and there is no need for a separate rooting phase as with shoot culture.

(iii) Useful for Mutagenic Studies and Mutant Production:

The somatic embryos generally arise from single cells, so it may be advantageous for mutagenic studies. Also the plantlets arising from such somatic embryos are more homogeneous in nature, so the mutant gene expression can be studied well.

(iv) Useful for Genetic Manipulation Technique:

In plant biotechnological appli-cation, during foreign gene transfer if the transformed cell gives rise to plantlet via somatic embryogenesis then there is least possibility of chimera formation. So for transgenic plant production this method of multiplication system is very much useful.

(v) Useful for Pathogen-Free Plant Production:

Plants derived from this kind of somatic embryos may be free from viral or other pathogens. So it may be an alternative approach of disease free plant production.

(vi) A Good Source of Protoplast Culture:

Embryogenic cultures are specially valu-able in providing a source of regenerable protoplasts in the graminaceous and coniferous plants. Protoplasts from these cultures were induced to divide to form a cell mass from which the embryoids, even plantlets are regenerated on a suit-able nutrient medium.

(vii) Conservation of Genetic Resources:

Somatic embryos which originate from sin-gle cells and subsequently regenerate mostly genetically uniform plants are good materials for genetic resource conservation. Embryogenic cultures as well as somatic embryos remain viable upon storage at ambient temperature, cold storage or cryostorage.

Role of SERK and LEC genes during SE:

In plant biology, zygotic embryogenesis (ZE) is a model to study the expression of genes and translated proteins in the signal response during embryo development. However, SE has been found to be a useful tool as well, and can probe different questions surrounding these phenomena, and in a more controlled way. Due to the advantages of SE for the culturing of thousands of crop plants of economic importance, a field of study has been opened to understand the regulatory processes involved during the initiation and progress of different embryo developmental stages.

SERK

SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE (SERK) is a transmembrane receptor that has been implicated in triggering embryogenesis. It was isolated for the first time from Daucus carota suspension cells, where expression was up regulated in the globular stage of embryogenesis. SERK is activated by auto-phosphorylation, specifically in the residue threonine 468, glutathione S-transferee fusion and in vivo phosphorylation assays. SERK transduces the signal from cell membrane to obtain site, regulating the subsequent downstream proteins in the signal transduction pathway. To date, different experiments suggest the pivotal role of this protein in the initiation of embryogenic competence. For instance, the ortholog SERK of Arabidopsis enhances the ability to undergo the somatic embryo response. The histochemical and immunochemical techniques using β-glucoronidase detection have revealed the special localization of gene expression during indirect somatic embryogenesis. The expression was detected in four places: in embryos, in the outer layers of cotyledons and in the provascular and vascular strands of developing somatic embryos.

LEC

The LEAFY COTYLEDON (LEC) genes are clasiified into two classes. The first class is HAP3-related transcription factors, represented in Arabidopsis by LEC1 and L1L. The second class encodes B3 domain transcription factors, which are represented by LEC2, FUS3, and AB13 in Arabidopsis. Among the LEC genes LEC1 and LEC2 have been suggested to have complementary a partial redundancy to induce somatic embryos. They have similar but not identical functions, as lec1lec2

double mutants have synergistic phenotypes. Overexpressing phenotypes of LEC1 seedlings arrest embryo-like seedlings and fail to develop; cotyledon-like organs sometimes form in place of the first leaves. LEC1 has been associated with the maturation of embryos. In LEC2, embryo-like seedlings continued to proliferate, producing callus, cotyledon-like structures and leaf-like organs in addition to the somatic embryos. Transgenic plants expressing LEC2 ectopically develop somatic embryos, which have cotyledon-like and leaf-like structures.

Haploidy and DH populations in crop improvement:

What do you mean by doubled haploids?

Doubled haploids refer to the diploid lines which are obtained by doubling the chromosome number of a haploid line by colchicine treatment. Such condition is referred to as doubled haploids.

What are main features doubled haploids?

Main features of doubled haploids are briefly presented below:

i. Doubled haploids are represented as DH.

ii. Double haploids are developed from haploids by doubling the chromosome number of haploids by colchicine treatment.

iii. Double haploids are completely homozygous lines.

iv. Doubled haploids can be produced in both self pollinated and cross pollinated species.

v. Double haploid method allows to fix recombinant gametes directly as fertile homozygous lines.

vi. This is a rapid method of producing pure lines. The pure lines can be produced from a heterozygous parent in one generation. The development of pure lines or inbred lines by conventional methods takes 3-5 years.

vii. Doubled haploids can be released as cultivars after testing or can be used as parents of hybrids.

viii. Doubled haploid method reduces the time needed to develop and release new cultivars by 3-5 years in comparison with conventional breeding techniques.

ix. Doubled haploids are homozygous and homogeneous populations and hence their produce is highly uniform.

x. The doubled haploids obtained from recombinant lines generally have broad genetic base due to gene combination from two different sources.

xi. The doubled haploid method opens a unique way for the fixation hybrid vigour or heterosis in homozygous lines, which would avoid all problems associated with the production of hybrid seed. For example, from a cross between AAbb x aaBB genotypes we can get a haploid with AB genotype which can be fixed as doubled haploid (AABB).

xii. Doubled haploids segregate much simpler in the progeny of crosses being true breeding individuals.

Table 20.1 Differences between doubled Haploid and conventional methods of producing pure lines

Particulars	Doubled Haploid Method	Conventional Method
1. Time required for developing pure lines.	One year or one crop season	3-5 years.
2. Time required for developing cultivars	Two to three years	Seven-eight years
3. Fixation of heterosis	Possible	Not possible
4. Expenditure/cost involved	More than conventional method	Lesser than DH method.
5. Identification of recessive mutants	Very easy	Difficult

Application in Crop Improvement:

Doubled haploids (DH) are presently being used in breeding of a number of crop species. Doubled haploid cultivars have been developed in different countries such as Canada, USA, UK, Spain, China,

Hungry, and Italy etc. The maximum DH cultivars have been developed in Barley followed by Rapeseed, wheat, melon, pepper, Rice, Asparagus, Tobacco and Eggplant. Moreover, maximum doubled haploid cultivars have been released by anther culture.

Table 20.2 Number of doubled haploid cultivars released for commercial cultivation in different crops.

Name of Crop	Number of Cultivars Released	Developed
Barley (Hordeum vulgare)	115	Anther culture
Rapeseed (Brassica campestris)	47	Anther culture
Wheat (Triticum aestivum)	21	Anther culture
Melon (Cucurbita marchata)	9	Irradiated pollen
Pepper (Capsicum annum)	8	Anther culture
Rice (Oriza sativa)	8	Anther culture
Asparagus (Asparagus officinalis)	7	Irradiation
Tobacco (Nicotiana tabacum)	6	Irradiation
Eggplant (Solanum melongena)	5	Anther/Irradiation

What are uses of doubled haploids?
The use of doubled haploid method is gaining increasing importance these days looking to its various advantages.
Doubled haploids are used in:
i. Development of purelines
ii. Development of cultivars
iii. Development of hybrids as parents
iv. Construction of genetic maps
v. Gene tagging/locating genes
vi. Identification of molecular markers for trait selection.

What do you mean by haploid?
An individual having gametic chromosome number in the somatic cells is known as haploid and such condition is referred to as haploidy.
Main features of haploids are presented below:
i. Haploids occur spontaneously as well as can be induced artificially. The spontaneous haploids have been reported in 10 families covering 26 genera and 36 species. Main crops in which spontaneous haploids have been reported include tomato, cotton, coffee, beets, barley, flax, pearl millet, rapeseed, asparagus and wheat.
ii. Haploids are sterile due to lack of pairing partners of chromosomes,
iii. Haploids are represented by n, Haploids differ from monoploids. Monoploids have single copy of the genome and are represented by x. In a true diploid species both monoploid and haploid chromosome number is the same (n = x). Thus a monoploid is haploid but all haploids cannot be monoploids.
iv. Haploids are of different types; their detailed description is beyond the scope of this discussion.

How would you induce haploids?
Haploids can be induced in four principal ways, viz. by:
(i) Wide crossing,
(ii) Irradiation and – chemical treatment,
(iii) Selection of twins, and
(iv) By anther and pollen culture.
These are briefly discussed below:
(i) Wide Crossing:

It includes interspecific and intergeneric hybridization and is also known as distant hybridization. Interspecific crosses have resulted in development of haploids in wheat and barley. Use of maize as pollen parent for wheat has produced high frequency of haploids in wheat.

(ii) Irradiation and Chemical Treatment:

The pollination with irradiated pollen stimulates the unfertilized eggs to parthenogenic development. Such haploids have been developed in tabacco, wheat, snapdragon and oenothera. Application of a dye toluene blue (TB) to the pistils has induced haploids in Vinca rosea, tomato, maize and poplar.

(iii) Selection of Twins:

In some species haploids can be obtained from the screening of poly-embryonic seeds. Poly-embryonic seeds can produce three types of seedlings viz. haploid-haploid; diploid-diploid and haploid-diploid. This has been reported in capsicum and in some horticultural species.

(iv) Anther and Pollen Culture:

This method is widely used for production of haploids. Haploids develop directly from pollen grains in culture, either through direct formation of embryos from pollen grains or formation of callus and subsequent plant regeneration.

Application in Crop Improvement:

1. Development of homozygous lines:

It is now possible to develop homozygous lines within a span of few months or a year by employing anther/pollen culture. This is in contrast to the conventional plant breeding programme that might take several years (6-10 yrs). In this way, production of haploids is highly useful for research related to plant genetics and breeding.

2. Generation of exclusive male plants:

By the process of androgenesis to produce haploids, followed by chromosome doubling, it is possible to develop exclusive male plants. The male plants are particularly useful when their productivity and applications are much more than female plants.

3. Induction of mutations:

In general, majority of induced mutations are recessive and therefore are not expressed in diploid cells (due to the presence of dominant allele). Haploids provide a convenient system for the induction of mutations and selection of mutants with desired traits. In fact, the haploid cells can be cultured and handled in a fashion similar to microorganisms.

Mutants from several plant species that are resistant to antibiotics, toxins, herbicides etc. have been developed. When the haploid cells of tobacco plant (Nicotiana tabacum) were exposed to methionine sulfoximine (a mutagen), mutants which showed lower level of infection to Pseudomonas tabaci were produced.

4. Production of disease resistance plants:

Disease resistance genes can be introduced while producing haploids. The so developed haploids are screened for the desired resistance, and then diploidized.

Some examples of disease resistance plants are listed:

1. Hwansambye, a rice variety resistant to leaf blast, bacterial leaf blight and rice stripe tenui virus.
2. Barley accession Q21681 resistant to stem rust, leaf rust and powdery mildew.

More examples of disease resistance crops are given in Table 45.1.

TABLE 45.1 A selected list of improved varieties of crops developed by using anther culture

Crop	*Varieties*	*Improvements made*
Wheat (*Triticum aestivum*)	Lunghua 1, Zing Hua 1, Zing Hua 2 Huapei 1, Florin, Ambitus, Jingdan 2288	High yield, rust resistance, cold resistance, large spikes, more tillers.
Rice (*Oryza sativa*)	Tangfong 1, Xin Xiu, Zhog Hua 8 Zhong Hua 9, Hua yu 1, Hua Yu 2, Huapei Shanyou 63, Zhe keng 66, Ta Be 78, Nonhua 5, Hirohikari, Hirohonami.	High yield, good quality, disease resistance
Tobacco (*Nicotiana tabacum*)	Tanyu 1, Tanyu 2, Tanyu 3, F 211 Hai Hua 19, Hai Hua 30	Mild smoking, disease resistance
Brassica napus	Jai kisan	Low erucic acid

5. Production of insect resistance plants:
Some varieties of rice resistant to insects have been developed e.g. Hwacheongbyeo resistant to brown plant hopper. Other varieties of rice that are resistant to pests have also been produced.
6. Production of salt tolerance plants:
The plant species with salt tolerance are needed for their cultivation in some areas. Anther cultures have resulted in some varieties of rice and wheat with good salt tolerance e.g. wheat Hua Bain 124-4.
7. Cytogenetic research:
Haploids are useful in several areas of cytogenetic research. These include
i. Production of aneuploids
ii. Determination of the nature of ploidy
iii. Determination of basic chromosome number
iv. Evaluation of origin of chromosomes.
8. Induction of genetic variability:
Besides the development of haploid mutants, it is also possible to produce plants with various ploidy levels through androgenesis.
9. Doubled haploids in genome mapping:
Genome mapping, a recent development in molecular biology, can be more conveniently achieved by using doubled haploid plant species.
10. Evolutionary studies:
A comparison of di-haploids (doubled haploids) with diploid wild plant species will be useful to trace the evolutionary origin of various plants. The close evolutionary relationship between tomato and potato has been evaluated by this approach.

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4. Genetic transformation: *Agrobacterium* mediated (co-culture, in planta, agroinfection); Direct method (PEG, electroporation, particle gun method); Reporter genes- screenable and selectable markers

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In molecular biology, **genetic transformation** is a process by which the genetic material carried by an individual cell is altered by the incorporation of foreign (exogenous) DNA into its genome. Genetic transformation discovered by **British bacteriologist Frederick Griffith in 1928** has revolutionized molecular biology, but it was not until the recombinant DNA was produced from Escherichia coli with the use of biochemical scissor called restriction enzymes that genetic transformation of cells started.
In plant cells, gene transfer to plant cells achieved using two different methods:

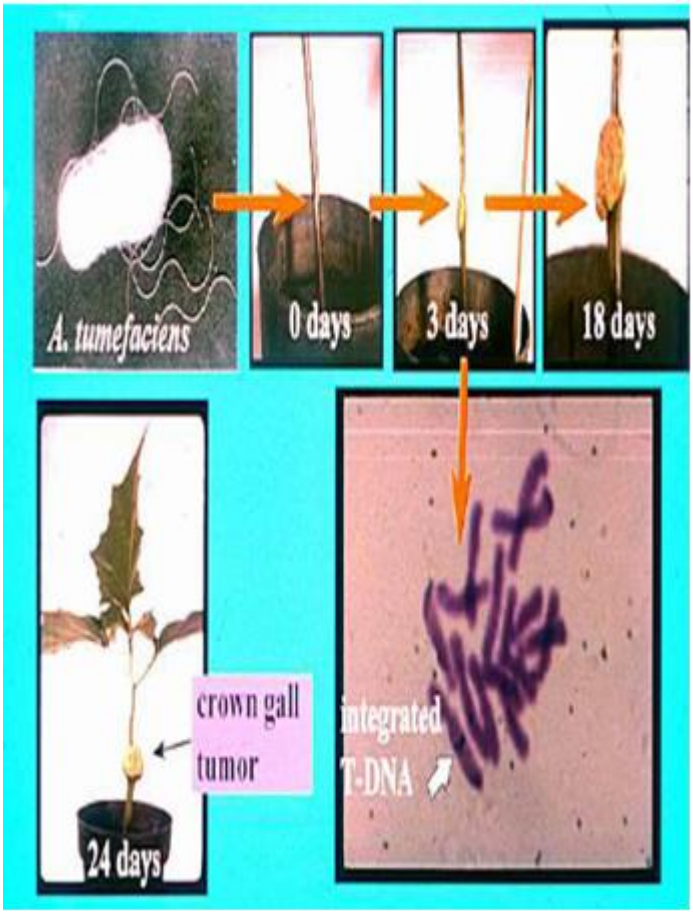
I. Indirect methods(Vector-mediated methods)
II. Direct methods (Vector less methods)

I. Indirect methods (Vector-mediated methods)

The vector mediated methods exploit the natural ability of certain bacteria (*Agrobacterium* species) and viruses to naturally transfer DNA to the genomes of infected plant cells.

***Agrobacterium* mediated gene transfer:**
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 *Agrobacterium* infects a plant, it transfers this T-DNA to a random site in the plant

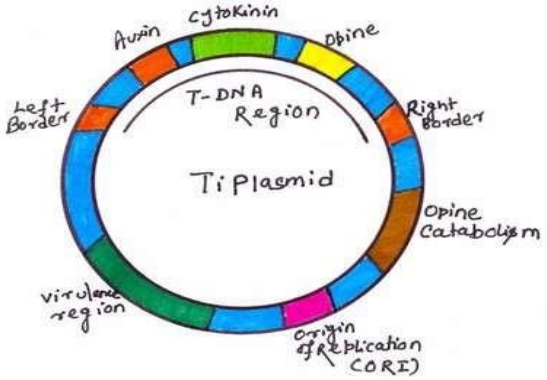
genome. When used in genetic engineering the bacterial T-DNA is removed from the bacterial plasmid and replaced with the desired foreign gene. The bacterium is a vector, enabling transportation of foreign genes into plants. This method works especially well for dicotyledonous plants like potatoes, tomatoes, and tobacco. *Agrobacteria* infection is less successful in crops like wheat and maize.



A.tumifaciens cause crown gall (tumor) and *A rhizogenes* hairy root disease in dicot plants by infecting through wounds on roots or stem at the soil surface. The bacterium contains Ti (Tumor inducing) and Ri (Root inducing) plasmids . Both these plasmids can transfer part of their DNA (T-DNA) into plant cell chromosome by which Plant cells become transformed by expression of T-DNA gene which induce disease.

Description of Agrobacterium as Natural Genetic Engineer

Agrobacterium is a soil plant pathogenic bacterium. This bacterial cell possesses a Ti plasmid in addition to bacterial chromosome. The bacteria can infect plants specifically dicots through wounds near soil surface. During infection a segment (T-DNA) of Ti plasmid gets transferred and become integrated in the plant cell genome. This T-DNA carries genes for expression of Disease.

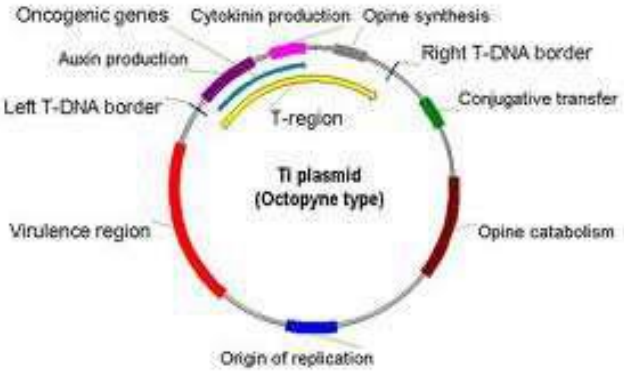


Components of Ti / Ri Plasmid:

- T- (Transferable) DNA region
- Vir (Virulence) region
- Host Specificity Region
- Ori (origin of Replication) region

The Ti plasmids are classified into different (about 14) types depending upon the specific opine being synthesized.(octapine/nopaline/Agropine).

The most important is T DNA region which carries genes encoding plant hormones and opines. The Vir region contains the virulence gene which helps in transfer of T DNA Crown gall disease results from transformation of the plant genome with this part of the plasmid in a process analogous to bacterial conjugation Third region of plasmid contains conjugation genes concerned with whole plasmid transfer between bacteria. Fourth region encodes genes concerned with opine utilization.

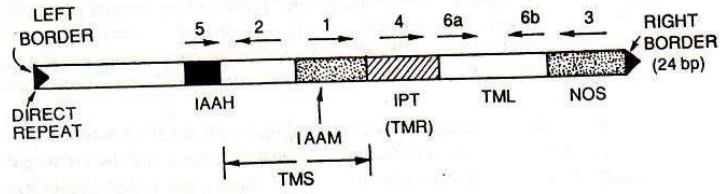


- It is ~200 kb megaplasmid
- T-DNA (15-40 kb) region contains genes for synthesis of Auxins,Cytokinins and Opines.
- Auxins and cytokinin genes are expressed in plant tissue inducing disease.

- Opines (unusual amino acids) produced by infected cells are used as nutrients by *Agrobacterium*
- T-DNA region is bordered on both sides by 25bp repeat which helps in its transfer to plant genome.
- Virulence Region contains about 8 operons having about 24- 25 genes
- These genes help in transfer of T-DNA
- Host specificity region has gene for conjugative transfer and opine catabolism
- Ti plasmid also has origin of replication

Organisation of T-DNA

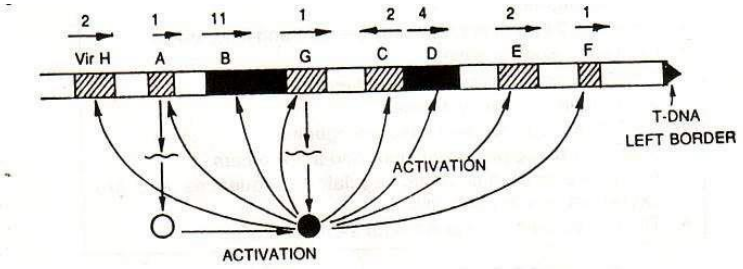
- T-DNA is ~ 23kb (15-40kb) segment bordered on both sides by 25bp direct repeat sequences.
- T-DNA contains genes for tumor induction (IAAM, IAAH & IPT) by forming auxins, cytokinins and Opines.
- All the genes in T-DNA region contain eukaryotic regulatory sequences, so are expressed only in plant cells



ORGANISATION OF T-DNA OF Ti PLASMID

Organisation of Vir region

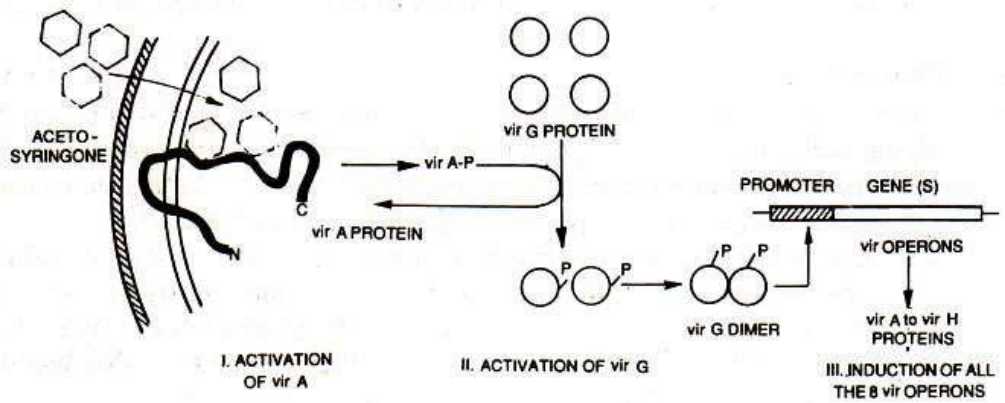
- Vir region contains 8 operons (VirA,B,C,D,E,F ,G &H) which together have 25 genes.
- Vir region mediates transfer of T-DNA into plant genome. It is itself not transferred.
- VirA and Vir G are constitutive operons encoding Vir A and VirG Proteins.
- Other Vir operons encode various proteins involved in T-DNA transfer.



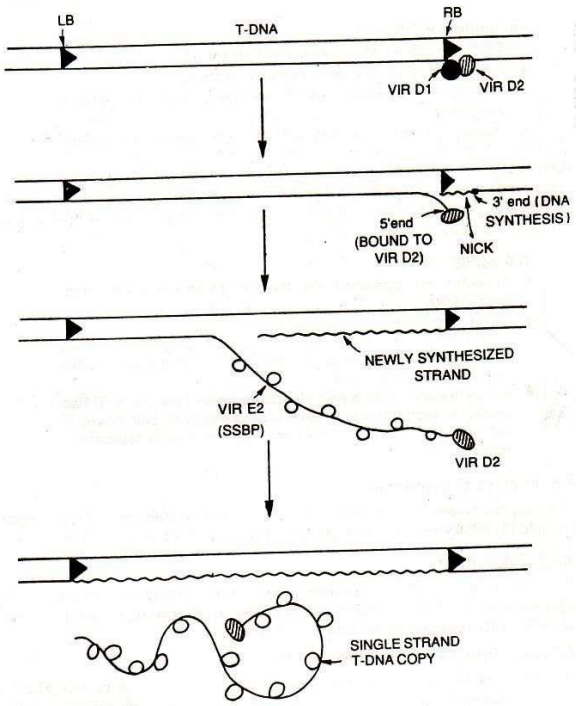
ORGANISATION OF Vir REGION OF Ti PLASMID

Mechanism of transfer of T-DNA

- Transfer of T-DNA is a step wise process.
- Vir region of Ti plasmid becomes activated by the phenolic signal molecules.
- Acetosyringone and α-hydroxyacetosyringone released by wounded tissue of dicot plants which constitute wound response as follows



- Acetosyringone and α-hydroxyacetosyringone bind with Vir A protein (located in the inner membrane) and activates it. It start functioning as autokinase to phosphorylate itself by ATP. Phosphorylated Vir A protein then phosphorylates Vir G protein which then dimerises.
- Phosphorylated Vir G protein has DNA binding function. It induces expression of rest of Vir operons
- Vir D1 protein has topoisomerase and endonuclease activity.
- It binds to right border sequence of T-DNA and facilitate the action of Vir D2 protein which is also endonuclease and nicks at the right border and remains bound to 5'end so generated.



Production of T-DNA copy (single stranded for transfer into plant cells)

LB- Left Border
RB- Right Border
SSBP-Single stranded binding proteins

- The 3'end produced at the site of nick serves as a primer for DNA synthesis in 5'----3' direction as a result of which one strand of
- T-DNA is displaced from the DNA duplex.
- The T-DNA strand is again nicked at the left border to generate a single strand copy of T-DNA.
- To this single strand copy Vir E 2 protein (single strand DNA binding proteins) bind for its protection against exonucleases
- Vir B operon consisting of 11 genes encode membrane bound Vir B proteins. These along with Vir D4 proteins participate in conjugal tube formation between bacterial and plant cells for transfer of
- T-DNA
- Vir D2 which remains bound to 5'end of T DNA has a signal sequence which drives it into the nucleus of plant cell.

Integration of t-dna into plant genome:

- T-DNA enters plant cell as a single stranded structure which is immediately converted into double stranded form.
- Vir E2 also has nuclear localization sequence and is responsible for transfer of T DNA into plant cell nucleus
- Double stranded T-DNA integrate at random sites in the host plant genome.
- For integration 23-79 base pair deletion takes place at the integration or target site
- After integration of T-DNA into plant genome ,the genes for auxins, cytokinins and opines express themselves which result in uncontrolled growth in the form of tumor

T-DNA transfer and integration

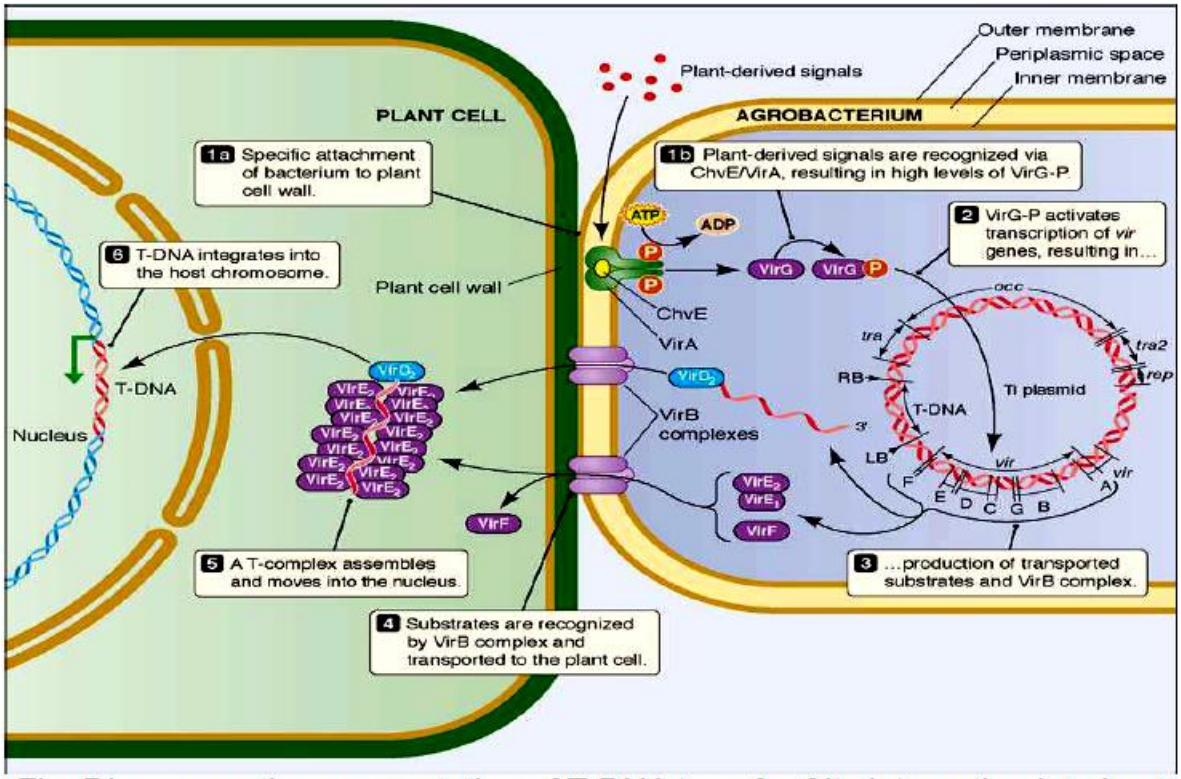


Fig: Diagrammatic representation of T-DNA transfer &its integration into host plant cell genome

Harnessing agrobacterium in transferring Foreign gene into plant

Part I of this presentation has made it clear that *Agrobacterium* has the potentiality to transfer prokaryotic DNA to eukaryotic Genome as a Natural Genetic Engineer

In part two of this presentation discussion will be made on strategies involved for harnessing *Agrobacterium* for introducing new (desired) gene into plant cells

Agrobacterium's ability to introduce its DNA into plant genome with efficiency makes Ti plasmid an attractive vector for gene transfer into plants After knowing about how T-DNA is transferred and what modifications are needed , Genetic engineers employed different strategies and before actual transfer of desired gene Genetically engineered Agrobacterium were produced with disarmed T-DNA region in which oncogenes and opine biosynthesis genes were replaced by desired (Foreign) gene and a selectable marker gene. Along with this some other sequences as unique restriction sites were added.

The natural Ti plasmids are unsuitable to be used directly as vectors for invitro manipulation due to following reasons

- Large size
- Tumor induction (Oncogenic) property
- Absence of unique restriction enzyme site

Now it is also well known that disarmed TDNA ,left and right borders along with genes of Vir region are essential elements for designing of transformation vectors

Now let us see the strategies involved for harnessing *Agrobacterium* for introduction of new/desired/foreign genes into plants

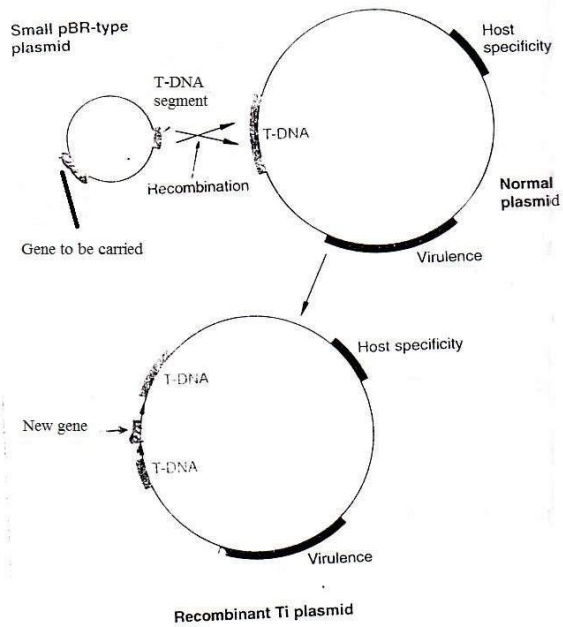
Only useful attributes of Mega Ti plasmid (~200kb) have been exploited in designing plant transformation vector.

For using *Agrobacterium* for in vitro genetic engineering purposes following manipulation are to be made

- ✓ Ti plasmids are disarmed .Genes from T DNA responsible for growth hormones (Oncogenes) and Opine formation are deleted so that no disease will be produced and as opines will not be synthesized further growth of *Agrobacterium* inside the host tissue stops
- ✓ The space between lB and RB is used to insert desired gene which is to be integrated and expressed in host tissue for transformation. The foreign gene is inserted by RDT
- ✓ A selectable marker gene providing resistance against antibiotic like kanamycin is also inserted in the T-DNA region
- ✓ The oncogenic problem can be solved by use of disarmed T-DNA from which these gene are removed

The large size problem of Ti plasmid can be tackled by any of following two ways using manipulated *Agrobacterium* vectors

1. The Co- integrated vector For this purpose a suitably modified E coli plasmid or vector is integrated into disarmed Ti plasmid (pTi), this gives rise to co-integrate vector. During disarming oncogenes of T-DNA are replaced by gene insert and other sequences of E coli plasmid. For this both plasmids are introduced into same Agrobacterium cell .Because of homology through recombination pBR plasmid is integrated into the T-DNA region. The gene to be cloned is therefore inserted into unique restriction site on the small pBR plasmid ,introduced into A tumifaciens cell carrying a Ti plasmid and natural recombination process left to integrate the new gene into T-DNA.



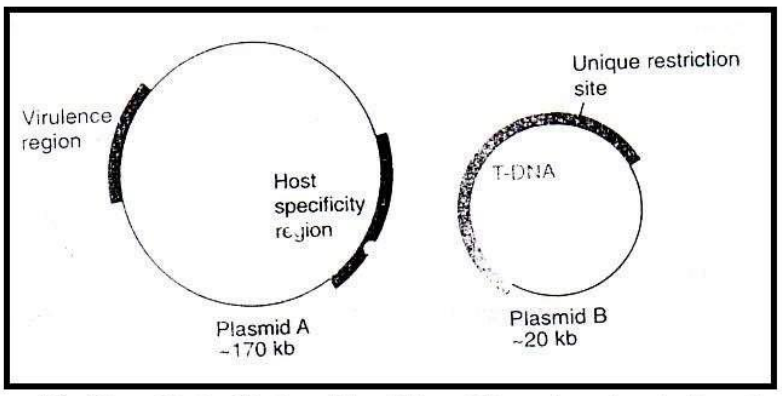
Recombinant Ti plasmid

The cointegration strategy-Uses a entirely new plasmid based on pBR322 or a similar E coli vector but containing a small portion of T-DNA. The homology between the new molecule and the Ti plasmid means thai if both are present in the same Agrobacterium tumifaciens cell,then recombination can integrate the pBR plasmid into the T-DNA region. The gene to be cloned is therefore inserted into a unique restriction site on the small pBR plasmid introduced into A.tumifaciens cell carrying a Ti plasmid, and the natural recombination process left to integrate the new gene into the T-DNA. Infection of the plant leads to insertion of the new gene, along with the rest of the T-DNA into plant chromosome.

ii) Binary (Two) Vector strategy (*Agrobacterium* containing two different recombinant plasmids)

First is *Agrobacterium E coli* shuttle vector containing disarmed. T DNA with 25 bp repeats flanking the gene insert to be introduced and a selective marker (often a Neo gene which provide cells resistence to antibiotic Kanamycin) This Mini plasmid (pBIN19) is designed to replicate in both E coli and Agrobacterium and is capable of conjugal transfer between two bacterial species also has a copy of lacZ gene containing multiple cloning sites

Second is helper Ti plasmid (p AL4404) from which T DNA segment has been removed. It has a functional Vir region. The Vir genes of the helper Ti induce the transfer of T DNA present on first plasmid in the same bacterial cell. As a result a gene insert within T-



The Binary Vector Strategy- Plasmid A and B complement each other when present in the same Agrobacterium tumifaciens cell. The T-DNA carried by

region is also transferred into plant cell. Many binary vectors have been developed which differ in size and source of 25bpnrepeat sequence, plant selection marker and cloning sites. In both cases (co-integrate or binary vector) ,the manipulated T-DNA is transferred to the plant genome efficiently

Transformation technique using *Agrobacterium*

- Some prerequisities for integration of foreign gene and production of transgenic plants are as follows
- The plant explants must produce acetosyringone to induce Vir genes for virulence
- Induced Agrobacterium should have access to cells that are competent for transformation or to take up DNA
- Cells must be meristematic, often transformed tissue or explants do not regenerate and are not totipotent, therefore transformation and regeneration competent cells should be taken as explants.
- The transformed explants cells should allow expression of transgene
- Disease causing genes should not be transferred and expressed, which is helped by disarmed T DNA

***Agrobacterium* gene transfer can be achieved in the following two ways**

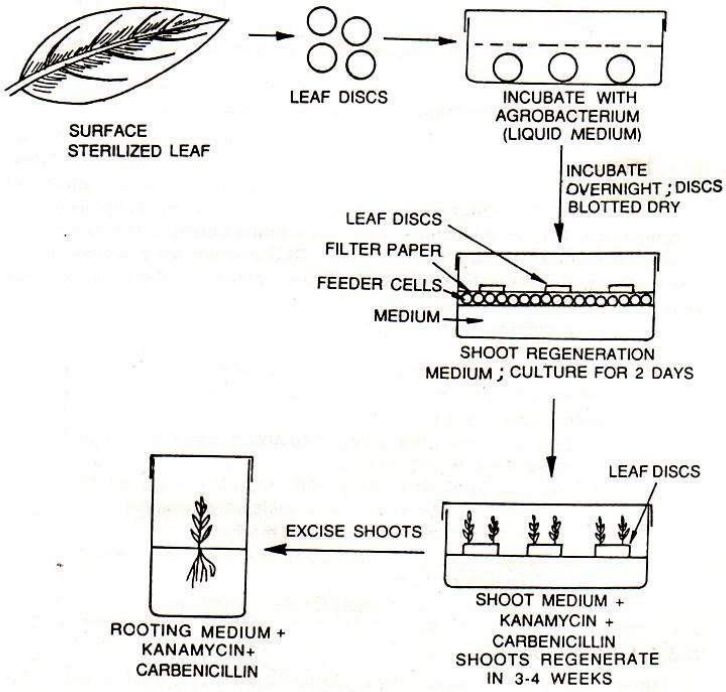
- Co culture with tissue explants
- In planta transfusion

Co- culture with tissue explants

For transformation explants like protoplast, callus , tissue slices, leaf disc, stem or floral tissue etc. can be co-cultured with genetically engineered. *Agrobacterium* with recombinant vectors for about 2 days. During co-culture acetosyringone(phenolic signal molecule) released from wounds (cut leaf disc) induce the Vir genes which bring about the transfer of recombinant T-DNA into many of the plant cells.

The explants can then be transferred to shoot inducing (regeneration medium) containing kanamycin and carbenicillin. Kanamycin allows only transformed plant cells to divide and regenerate shoots in about 3-4 weeks, while carbenicillin kills Agrobacterium cells.

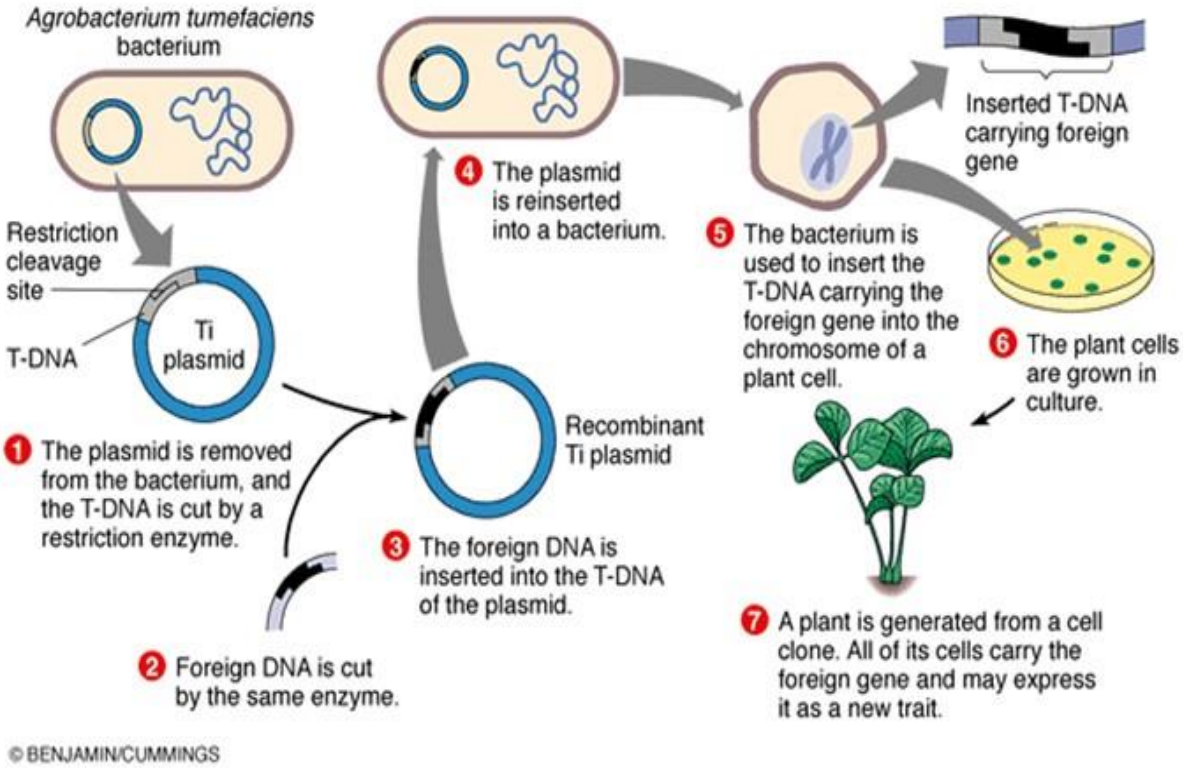
The shoots are separated and transferred to root inducing medium and finally after few weeks are transferred to soil.



By this co-culture method only dicot plants can be transformed. *Agrobacterium* mediated transformation of monocot plant (cereals) cells can be achieved by adding acetosyringone in the medium during co-culture

In planta transformation

Transformation can also be achieved by imbibition of seeds in fresh culturs of *Agrobacterium*. T-DNA becomes integrated into plant genome. It appears that *Agrobacterium* cells enter the seedling during germination, are retained within the plants, when flowers develop, the zygote or cells become transformed.



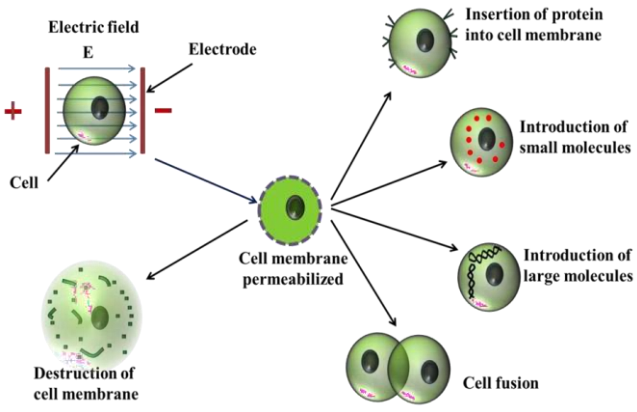
II. Direct Methods of Gene transfer (Vector less methods):

Vectorless methods use chemical or physical means to introduce DNA into plant cell. These methods are species and genotype independent in terms of DNA delivery.

Physical Methods

1. Electroporation:

Electroporation involves the creation of pores in the cell membrane using electrical pulses of high field strength. If DNA is present in the buffer solution at a sufficient concentration, it will be taken up through these pores. Plant cell electroporation generally utilizes the protoplast because thick plant cell walls restrict macromolecule movement. Electrical pulses are applied to a suspension of protoplasts with DNA placed between electrodes in an electroporation cuvette. Short high voltage electrical pulses induces the formation of transient micropores in cell membranes allowing DNA to enter the cell and then the nucleus.



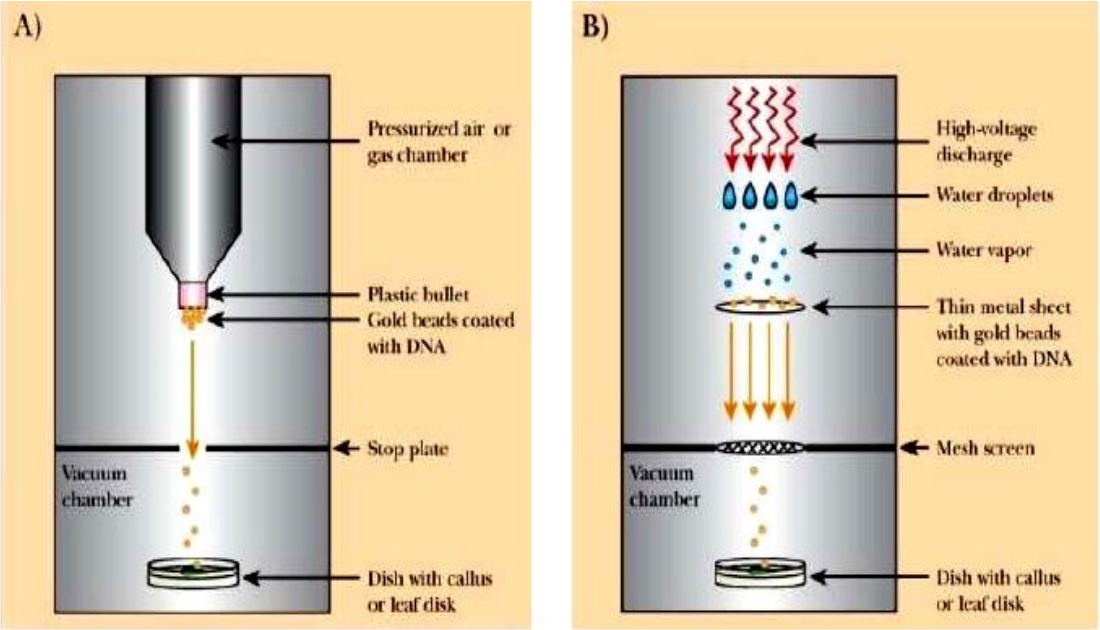
Advantage: Simple, fast, low cost.

Drawbacks: Low efficiency, requires laborious protocols, and transforms mainly protoplasts.

2. Particle bombardment:

- ❖ It is also known as microprojectile bombardment, biolistics, gene gun, etc.
- ❖ Foreign DNA coated with high velocity gold or tungsten particles to deliver DNA into cells.

- ❖ This method is widely being used because of its ability to transfer foreign DNA into the mammalian cells and microorganisms.
- ❖ It was developed by Prof. Sanford and coworkers of Cornell University (USA) in 1987.
- ❖ As the term denotes, it shoots foreign DNA into plant cells or tissue at a very high speed.
- ❖ This technique is most suitable for those plants which hardly regenerate and do not show sufficient response to gene transfer through *Agrobacterium* for example, rice, wheat corn, sorghum, chickpea and pigeon-pea.



Advantages

- It is clean and safe.
- The ability to engineer organized and potentiality regenerable tissue permits introduction of foreign genes into elite germplasm.
- Transient gene expression has been demonstrated in numerous tissues representing many different species.
- By this process transformation of recalcitrant species such as rice, maize, wheat etc can be done.
- Transformation frequency is very high.
- Gene transfer occurs without protoplast isolation.
- Suitable transformation of monocot plant.

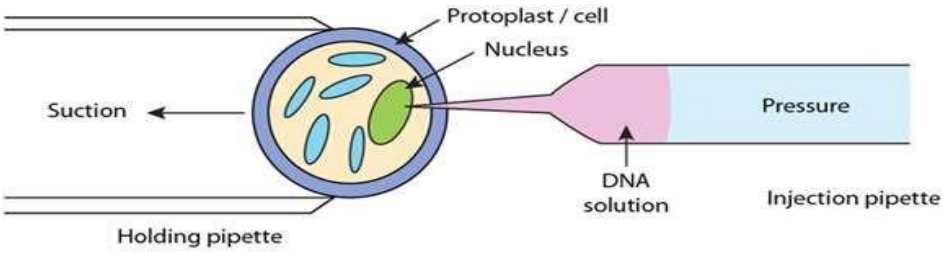
Disadvantages

- Limited by depth of penetration.
- Lack of control over the velocity of bombardment, which often lead to substantial damage to the target cells.
- The emerge of chimeral plants.
- Less ability to regenerate.
- Very expensive method.

3. Microinjection

- ❖ 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 into the 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 performed by trained and skilled personnel.

- e.g. **Tobacco, *Brassica napus*** has been successfully transformed by this approach.



Advantages

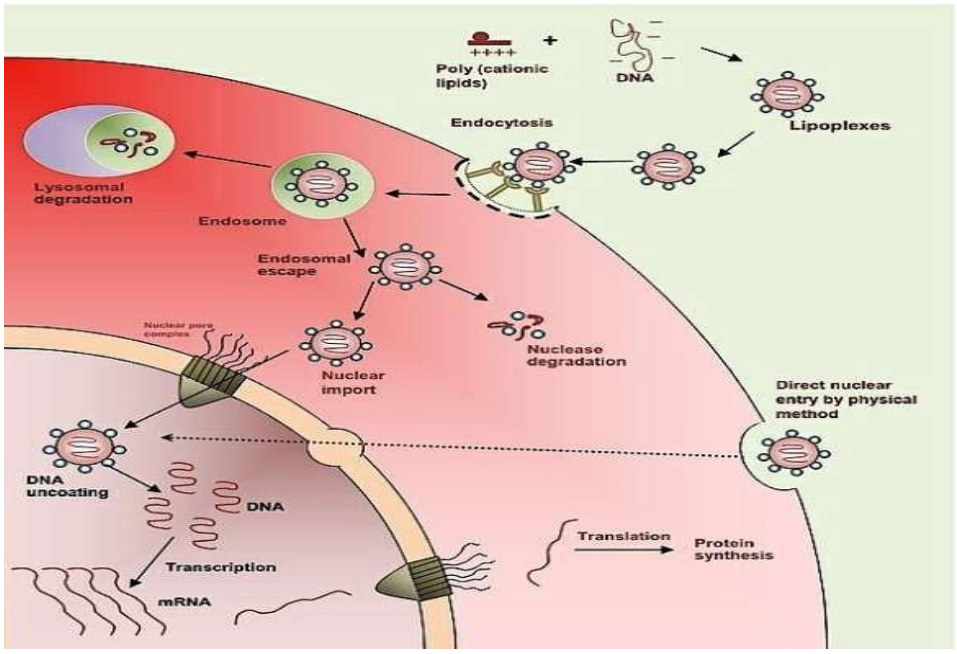
- The amount of DNA delivered per cell is not limited by the technique and can be optimized. This improves the chance for integrative transformation.
- The delivery is precise, again increasing the chance of integrative transformation.
- The small structures can be injected containing only a few cells and with high regeneration potential.
- Since it is a direct physical approach, it is host-range independent.

Disadvantages

- Injection can cause damage that affects embryonic survival and can result in quite high mortalities.
- Only one cell is targeted per injection.
- The handling requires specialized skill and instrumentation.
- Has low transformation rate.

4. Liposome mediated transformation

Liposome mediated transformation involves adhesion of liposomes to the protoplast surface, its fusion at the site of attachment and release of plasmids inside the cell.



Chemical gene mediated transfer:

1. Polyethylene glycol- mediated transformation

- Polyethylene glycol (PEG), in the presence of divalent cations, destabilizes the plasma membrane of protoplasts and renders it permeable to naked DNA.
- A large number of protoplasts can be simultaneously transformed.
- This technique can be successfully used for a wide variety of plant species.
- It has certain limitations:

i) The DNA is susceptible for degradation and rearrangement.
ii) Random integration of foreign DNA into genome may result in undesirable traits.
iii) Regeneration of plants from transformed protoplasts is a difficult task.

2. DEAE-Dextran mediated transfer

- The desirable DNA can be complexed with a high molecular weight polymer diethyl amino ethyl(DEAE)dextran and transferred.
- The major limitation of this approach is that it does not yield stable transformants.

Selectable marker genes-

The selection is based on the survival of transformed cells when grown on a medium containing a toxic substance (antibiotic, herbicide, antimetabolite). This is due to the fact that the selectable marker gene confers resistance to toxicity in the transformed cells, while the non-transformed cells will get killed.

Some of them are given below:

Selective agent	**Marker gene/ Reporter gene**	**Source of gene**
Kanamycin (antibiotic)	NPT (neomycin phosphotransferase)	*E. coli*
Streptomycin(antibiotic)	SPT(streptomycin phosphotransferase)	streptomyces
Hygromycin(antibiotic)	HPT(hygromycin phosphotransferase)	*E. coli*
Phosphinothricin(herbicide)	BAR(phosphinothricin acetyltransferase)	streptomyces
B-glucuronidase	gus	*E.coli*
Glyphosate(herbicide)	EPSP synthase	Plant/micro-organism
Bleomycin	Ble	*E. coli*

Reporter genes- An assay for the reporter gene is carried out by estimating the quantity of the protein it produces or the final products formed. Reporter genes are often used as an indication of whether a certain gene has been taken up by or expressed in the cell or organism population.

Some of the important ones are given below:

Opine synthase (ocs), β-Glucuronidase (gus), Bacterial luciferase (luxA),
Firefly luciferase (luc).

Screenable marker gene:

The screenable markers presently used are mostly derived from bacterial genes coding for an enzymes that is readily detected by the use of chromogenic or other substances. A screenable marker gene is functional only if an enzyme with comparable activity is not present in non- transformed/non-transfected cells. Several reporter genes are used and the most commonly used examples of reporter genes are:

- **Green fluorescent protein** makes cells glow green under UV light. A specialized microscope is required to see individual cells. Yellow and red versions are also available, so scientists can look at multiple genes at once. It is commonly used to measure gene expression.[2]
- **GUS assay (using β-glucuronidase)** is an excellent method for detecting a single cell by staining it blue without using any complicated equipment. The drawback is that the cells are killed in the process. It is particularly common in plant science.
- **Blue white screen** is used in both bacteria and eukaryotic cells. The bacterial lacZ gene encodes a beta-galactosidase enzyme. When media containing certain galactosides (e.g. X-gal) is added, cells expressing the gene convert the X-gal to a blue product and can be seen with the naked eye.
- **Chloramphenical acetyl transferase (CAT)** encoding gene of E.coli: The CAT gene encodes the enzyme chloramphenicol acetyltransferase, which transfer acetyl groups from acetyl CoA onto the antibiotic chloramphenicol.

• **Luciferase** encoding gene of firefly, Photinus pyralis, catalyses the oxidation of luciferin with the emission of yellow-green light which can be detected easily even at low levels.

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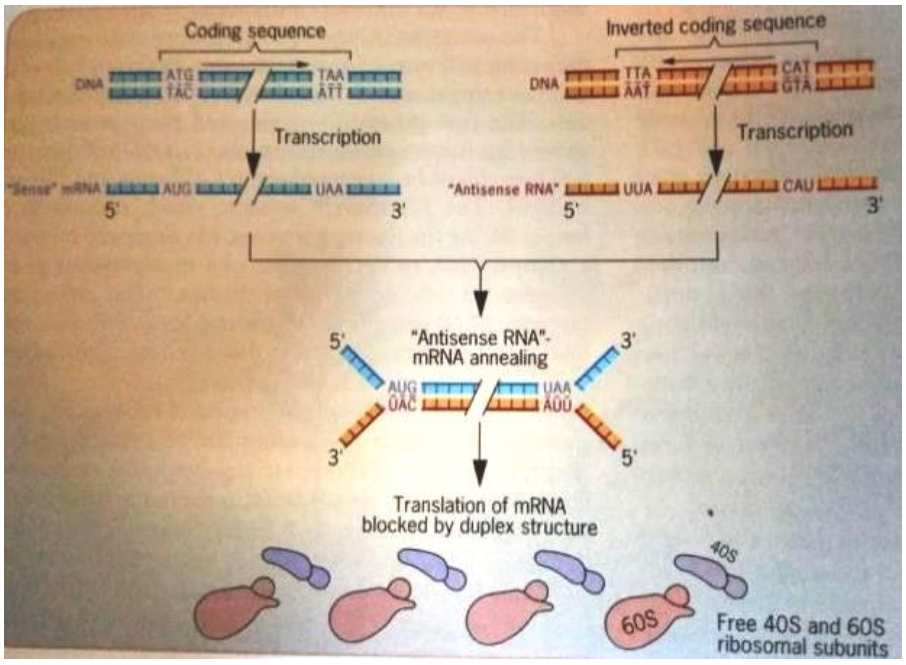
5. Biotechnological applications for crop management: Approaches to improve shelf life of fruits and vegetables; herbicide resistance; insect and pest management.

Approaches to improve shelf life of fruits and vegetables:

The importance of fruits and vegetables in the diet of mankind cannot be over emphasized. Typically, biotechnology technique such as genetic modification is used in fruits and vegetables to enable plants tolerate the biotic and abiotic stresses, and plant resistances to problematic pests and disease, which may provide higher nutritional contents, and extend the shelf life of the produce. Recently, recombinant DNA technology has been used by scientists to delay ripening in fruits and vegetables in order for farmers to have the flexibility in marketing their produce and ensure consumers good quality produce from their farms. Genes related to ethylene biosynthesis and cell wall degradation are the primary targets for the extension of the shelf life of the fruits and vegetables. The levels of endogenous gene expression can be reduced by the following 4 approaches: (i) Antisense RNA approach (ii) ribozyme (iii) gene disruption (iv) Over transcription leading to co-suppression.

Antisense RNA approach has proven useful to improve shelf life of fruits and vegetables. It has also been a commercial success. The antisense RNA method involves the synthesis of the RNA molecules that are complementary to the mRNA molecules produced by transcription of a given gene. The antisense is produced by inverting the orientation of the protein encoding region of a gene in relation to its promoter. A s result, the reference sense strand of the gene becomes oriented 3'-5' direction with reference to its promoter and is transcribed. The RNA produced by this gene has the same sequence as the antisense strand of the normal gene and is therefore known as antisense RNA.

A major problem in agriculture is perishing ability of its products. Various methods have been initiated and developed to increase the shelf life of crops especially for fruits and vegetables. One such successful technique is delaying the ripening of fruits and vegetables by manipulating the genes through genetic engineering.



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 synthesizes 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).

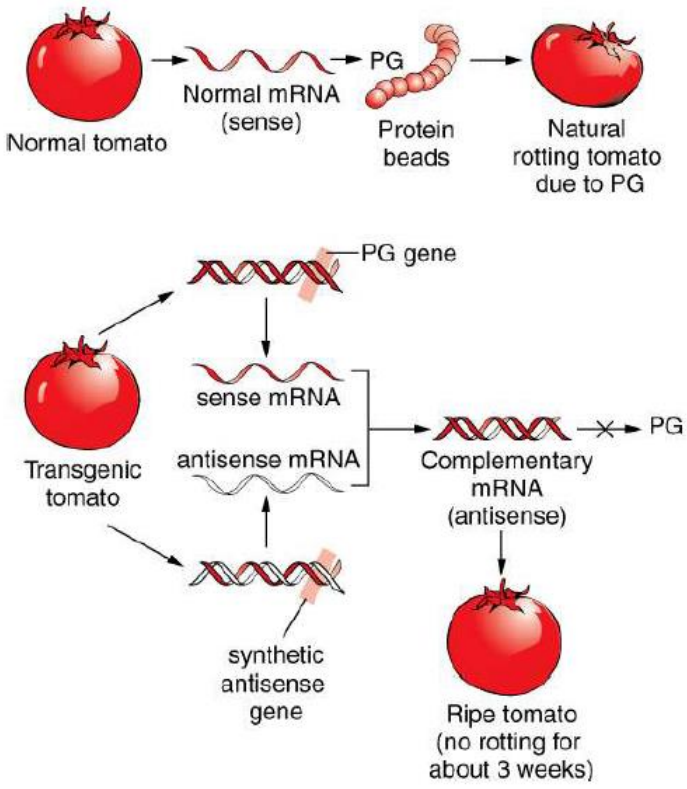
2) Transfer of PG gene to a vector bacteria and production of complementary DNA (cDNA) molecules.

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 is actively 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 PC antisense 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!

Herbicide Resistance:

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 worst 78 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.

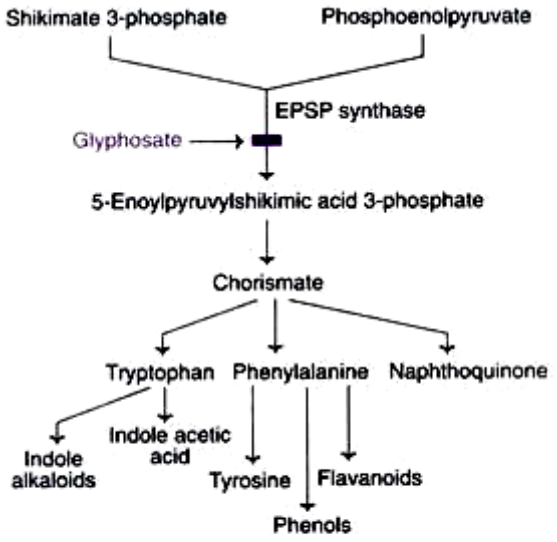


Fig. 50.5 : Shikimate pathway indicating the action of the herbicide glyphosate (EPSP synthase-5-Enolylpyruvylshikimate 3-phosphate synthase)

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.

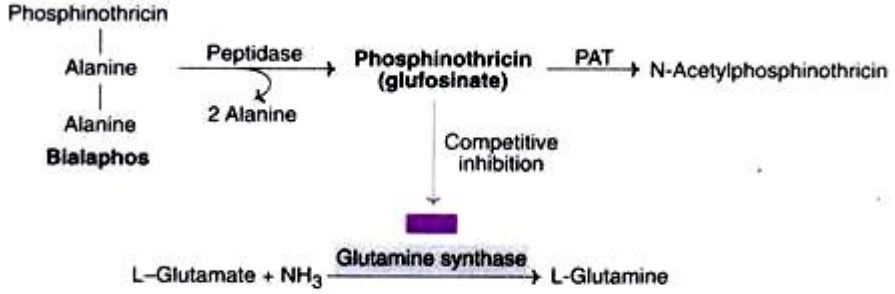


Fig. 50.7 : The formation, mode of action and detoxification of phosphinothricin (PAT-Phosphinothricin acetyl transferase).

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 of phosphinothricin 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 acetyl transferase (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 to phosphinothricin.

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 crops is 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 y-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 and now Bt.

Bt-based genetic transformation of plants:

It has been possible to genetically modify (GM) plants by inserting Bt genes and provide pest resistance 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:

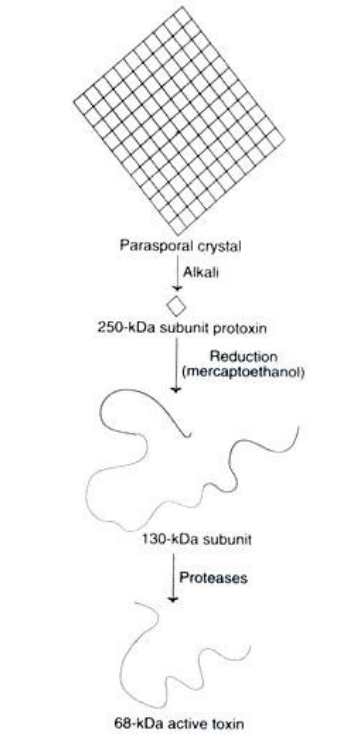
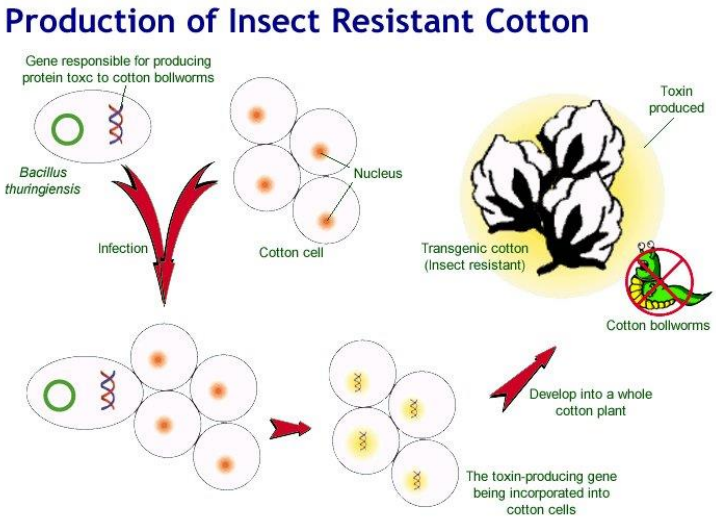


Fig. 50.2 : A diagrammatic representation of the formation of active toxin from the parasporal crystal of Bacillus thuringiensis.



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 yet sold commercially)

Golden Rice —The Provitamin A Enriched Rice:

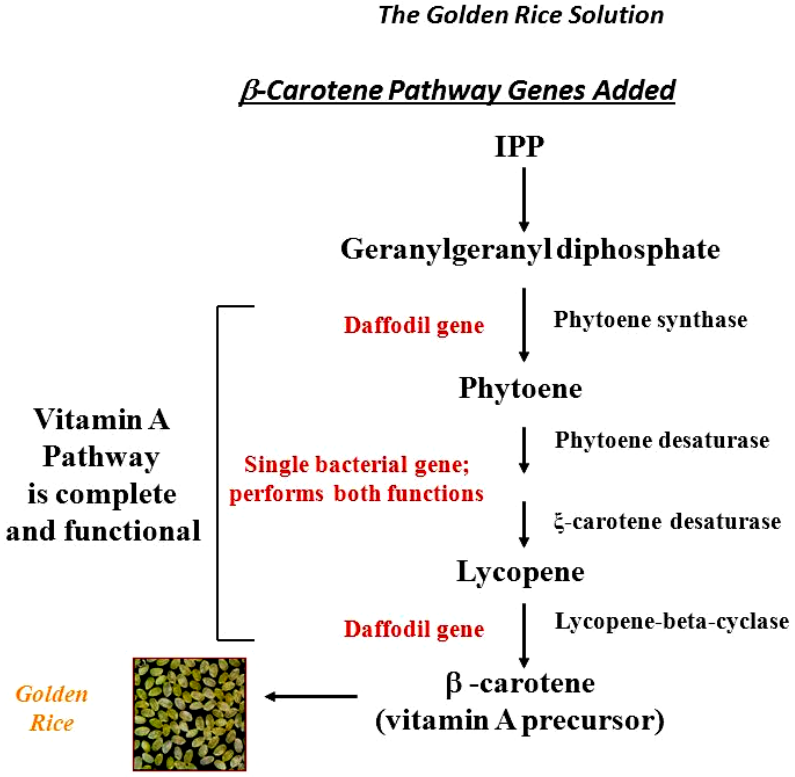
About one-third of the world's population is dependent on rice as staple food. The milled rice that is usually consumed is almost deficit in P-carotene, the pro-vitamin A. As such, vitamin A deficiency (causing night blindness) is major nutritional disorder world over, particularly in people subsisting 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.



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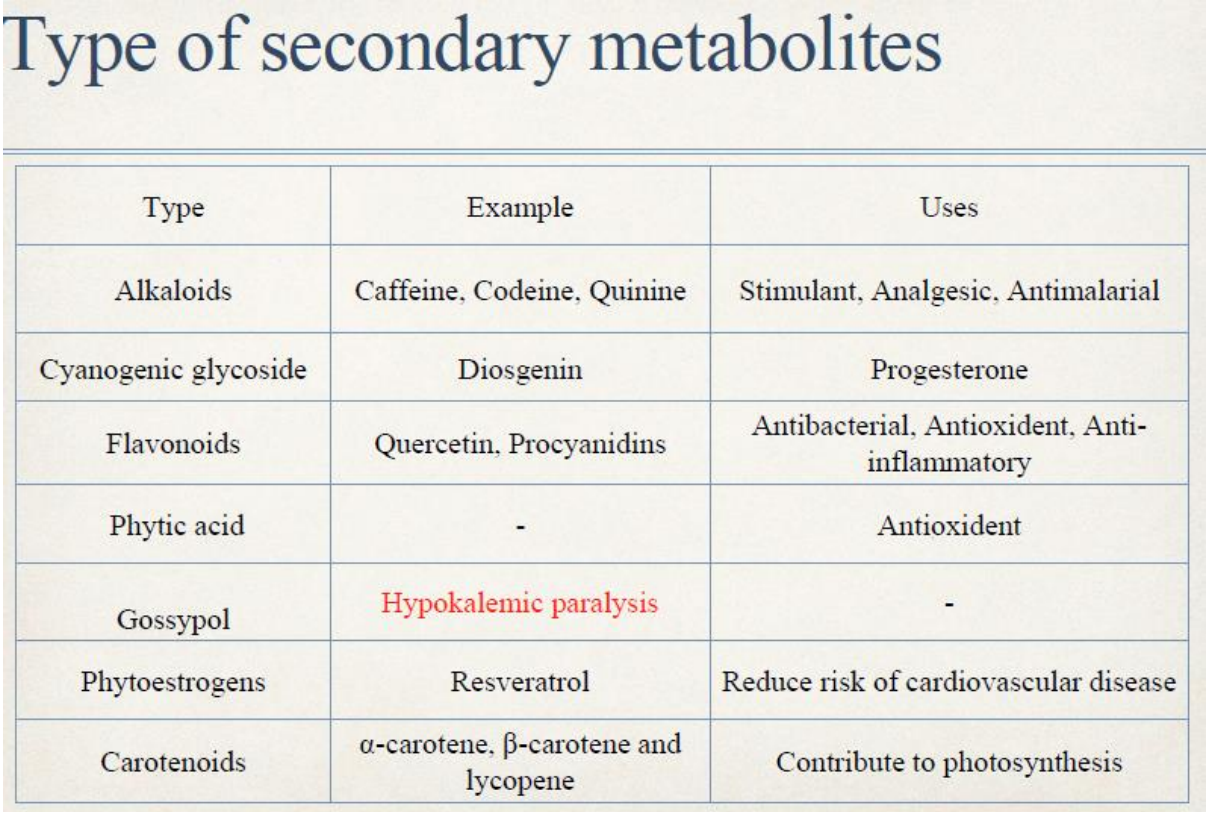
6. Secondary metabolite production in plant cultures: Types of secondary metabolites; culture systems used for secondary metabolite production; improving secondary metabolite production in culture; hairy root culture.

What are secondary metabolites?
Secondary metabolites are generally defined as small organic molecules produced by organisms that are not essential for their growth, development and reproduction. They may include pharmaceuticals, flavours, fragrance, food additives, feedstock etc. Possibly over 250,000 secondary metabolites in Plants. Classified based on common biosynthetic pathways where a chemical is derived. Four major classes: Alkaloids, glycosides, phenolics, terpenoids.
Why plants produce secondary metabolites?

- Plant hormones, which are secondary metabolites, are often used to regulate the metabolic activity within cells and oversee the overall development of the plant
- It protects plant against herbivores and microbial pathogens.
- It serves as attractants for pollination and seed dispersing animals.

Types of secondary metabolites:



Type	Example	Uses
Alkaloids	Caffeine, Codeine, Quinine	Stimulant, Analgesic, Antimalarial
Cyanogenic glycoside	Diosgenin	Progesterone
Flavonoids	Quercetin, Procyanidins	Antibacterial, Antioxident, Anti-inflammatory
Phytic acid	-	Antioxident
Gossypol	Hypokalemic paralysis	-
Phytoestrogens	Resveratrol	Reduce risk of cardiovascular disease
Carotenoids	α-carotene, β-carotene and lycopene	Contribute to photosynthesis

Alkaloids:

- These are commonly applied to basic nitrogenous compounds of plant origin that are physiologically active.
- Organic nitrogenous compounds with a limited distribution in native nature.
- Indole alkaloids is the largest group in this family, derived from tryptophan
- Widely used as medicine
- They are bitter in taste.
- Derived from amino acids.The amino acids that are most often serve as alkaloidal precursors are: **phenylalanine, tyrosine, tryptophan**, **histidine, anthranilic acid, lysine and ornithine**.
- Alkaloids form double salts with compounds of mercury, gold, platinum and other heavy metals. These salts are obtained as precipitate which are microcrystals.
- Insoluble or sparingly soluble in water, but the salts formed on reaction with acids are usually freely soluble.
- Most are crystalline solids although a few are amorphous.
- Free alkaloids are usually soluble in polar solvents like ether, chloroform
- Some alkaloids are liquid because of lacking of oxygen in their molecules. (e.g coniine, nicotine, spartenine)

Sources and Occurrence of Alkaloids:

- Alkaloids can occur in plant kingdoms; among the angiosperms,
- Leguminosae,
- Papaveraceae,
- Ranunculaceae,
- Rubiaceae,
- Solanaceae,
- Berberidaceae are outstanding alkaloid-yielding plants.

Classification:

Alkaloids are often divided into the following major groups:

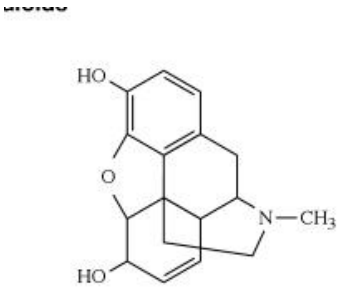
1. **"True alkaloids"** contain nitrogen in the heterocycle and originate from amino acids. Their characteristic examples are **atropine, nicotine, and morphine**. This group also includes some alkaloids that besides the nitrogen heterocycle contain terpene (e.g., evonine) or peptide fragments (e.g. ergotamine). The piperidine alkaloids coniine and coniceine may be regarded as true alkaloids although they do not originate from amino acids.
2. **"Protoalkaloids"**, which contain nitrogen (but not the nitrogen heterocycle) and also originate from amino acids. Examples include **mescaline, adrenaline and ephedrine**.
3. **Polyamine alkaloids –** derivatives of **putrescine, spermidine, and spermine**.
4. **Peptide and cyclopeptide alkaloids**.
5. **Pseudoalkaloids –** alkaloid-like compounds that do not originate from amino acids. This group includes terpene-like and steroid-like alkaloids, as well as purine-like alkaloids such as **caffeine, theobromine, theacrine and theophylline**.

Uses of Alkaloids in Plants:

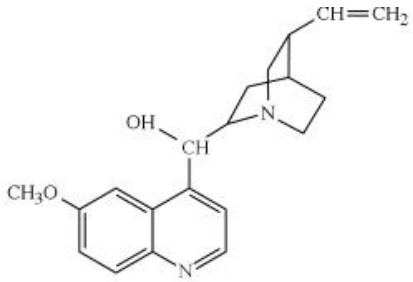
- Poisonous agents which protect plants against insects and herbivores
- End products of detoxification reactions representing a metabolic locking-up of compounds otherwise harmful to the plants
- For regulatory growth factors
- Reserve substance capable of supplying nitrogen or other elements necessary to the plant's economy

Pharmacologic action of Alkaloids

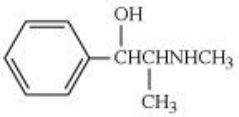
- ✓ Analgesic (morphine, codeine)
- ✓ Narcotics (strychnine, brucine which are central stimulant)
- ✓ Anti malarial (quinine)
- ✓ Anti pyretic
- ✓ Anti cancer (vincristine)
- ✓ Mydriatics (atropine)
- ✓ Anti inflammatory
- ✓ Miotics (physostigmine, pilocarpine)
- ✓ Ephedrine (rises in blood pressure, bronchodilator)
- ✓ Reserpine (produce fall in excessive hypertension



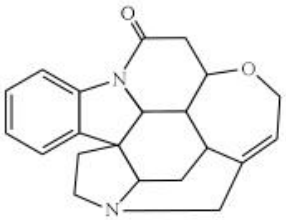
morphine



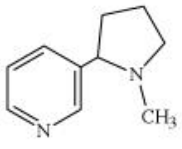
quinine



ephedrine



strychnine

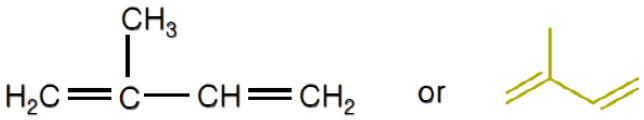


nicotine

Terpenes:

The terpenoids, sometimes called isoprenoids, are a large and diverse class of naturally occurring organic chemicals derived from terpenes. Most are multicyclic structures with oxygen-containing functional groups. About 60% of known natural products are terpenoids. Although sometimes used

interchangeably with "terpenes", terpenoids contain additional functional groups, usually O-containing. Terpenes are hydrocarbons.



Isoprene
(2-methyl-1,3-butadiene)

Plant terpenoids are used for their aromatic qualities and play a role in traditional herbal remedies. Terpenoids contribute to the scent of eucalyptus, the flavors of cinnamon, cloves, and ginger, the yellow color in sunflowers, and the red color in tomatoes. Well-known terpenoids include citral, menthol, camphor, salvinorin A in the plant Salvia divinorum, the cannabinoids found in cannabis, ginkgolide and bilobalide found in *Ginkgo biloba*, and the curcuminoids found in turmeric and mustard seed.

The steroids and sterols in animals are biologically produced from terpenoid precursors. Sometimes terpenoids are added to proteins, e.g., to enhance their attachment to the cell membrane; this is known as isoprenylation.

Types:

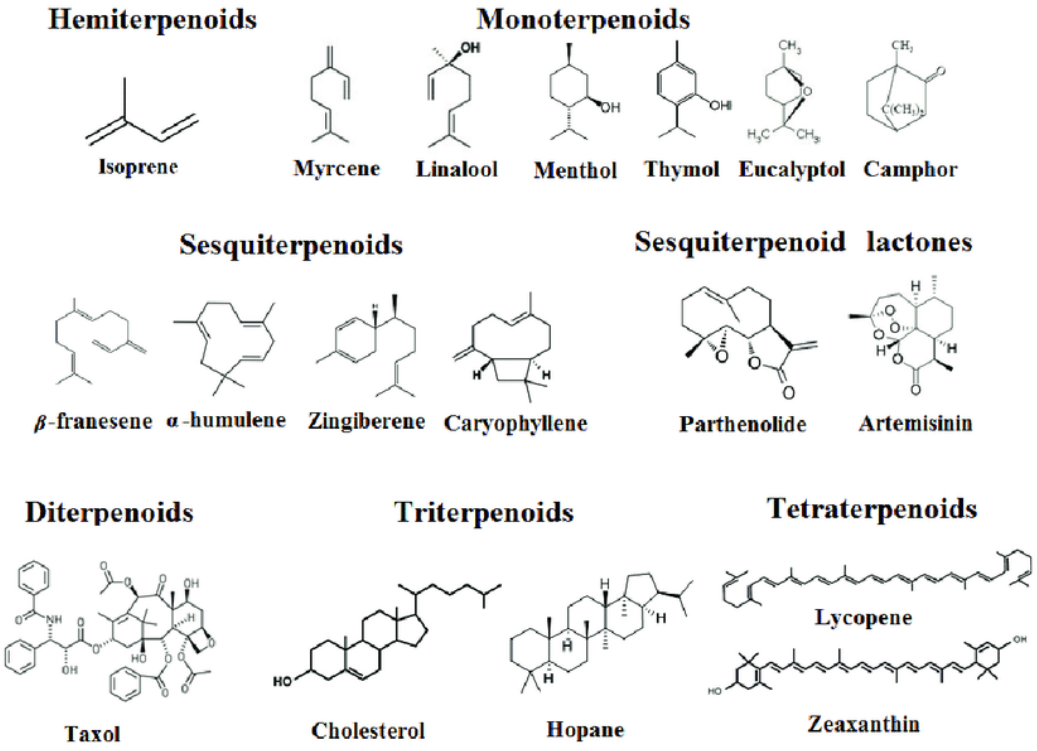
Terpenoids are modified terpenes, wherein methyl groups have been moved or removed, or oxygen atoms added. (Some authors use the term "terpene" more broadly, to include the terpenoids.) Just like terpenes, the terpenoids can be classified according to the number of isoprene units that comprise the parent terpene:

Hemiterpenes consist of a single isoprene unit. Isoprene itself is considered the only hemiterpene, but oxygen-containing derivatives such as prenol and iso valeric acid are hemiterpenoids.

Monoterpenes consist of two isoprene units and have the molecular formula $C_{10}H_{16}$. Examples of monoterpenes are: geraniol,limonene and terpineol.

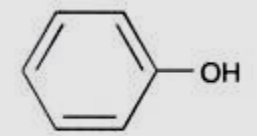
Sesquiterpenes consist of three isoprene units and have the molecular formula $C_{15}H_{24}$. Examples of sesquiterpenes are: humulene,farnesenes, farnesol.

Diterpenes are composed of four isoprene units and have the molecular formu la $C_{20}H_{32}$. They derive from geranylgeranyl pyrophosphate. Examples of diterpenes are cafestol, kahweol, cembrene and taxadiene (precursor of taxol).



Triterpenes consist of six isoprene units and have the molecular form ula $C_{30}H_{48}$. The linear triterpene squalene, the major constituent of shark liver oil, is derived from the reductive coupling of two molecules of farnesyl pyrophosphate. Squalene is then processed biosynthetically to generate either lanosterol or cycloartenol , the structural precursors to all the steroids.
Tetraterpenes contain eight isoprene units and have the molecular formula $C_{40}H_{64}$. Biologically important tetraterpenes include the acyclic lycopene, the monocyclic gamma-carotene, and the bicyclic alpha- and beta-carotenes.
Polyterpenes consist of long chains of many isoprene units,eg, Natural rubber.

Phenolics:
Thousands of organic compounds which contain one or more phenolic residue i.e., a functional hydroxyl group on a benzene ring are produced by plants and are called as phenolic compounds or plant phenolics. They include such well known substances as coumarins, lignins, flavonoids and tannins etc.



Phenol

Phenolic compounds are extremely diverse in chemical structure. Some of them are soluble in organic solvents. Some occur as carboxylic acids and glycosides and are soluble in water while others may be large complex polymers that are insoluble.

Biological Functions of Plant Phenolics:
Large number of phenolic compounds occur in plants as secondary metabolites which per-form the following functions:
(i) Some of them act as chemical deterrents against herbivores and pathogens.
(ii) Plant phenolics such as lignins provide mechanical strength to the plants and have significant proective functions in them.
(iii) Some phenolics play important role in plants in attracting pollinators and fruits & seeds dispersers.
(iv) Some plant phenolics play important role in allelopathy (Greek, allelon = of one an-other; pathos = diseases). Allelopathy is the influence of chemicals released by one plant species on another plant or animal with resulting benefits to the species which contains them.

Classification of Plant Phenolics:
Plant phenolics may be classified into major categories on the basis of the no. of C-atoms and basic arrangement of carbon skeletons in their structure as shown in Table 24.2.

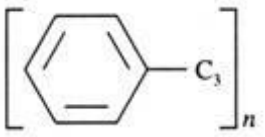
Table 24.2. Major Categories of Plant Phenolics.

No. of C-atoms	Basic carbon skeleton	Major category
6	C_6	Phenols
7	$C_6 - C_1$	Phenolic acids
8	$C_6 - C_2$	Phenylacetic acids, Hydroxy cinnamic acids, Phenyl propenes
9	$C_6 - C_3$	Coumarins, Isocoumarins, chromones
10	$C_6 - C_4$	Naphthoquinones
13	$C_6 - C_1 - C_6$	Xanthones, Stilbenes
14.	$C_6 - C_2 - C_6$	Anthraquinones
15.	$C_6 - C_3 - C_6$	Flavonoids
18.	$[C_6 - C_3]_2$	Lignans, Neolignans
30.	$[C_6 - C_3 - C_6]_2$	Biflavonoids
n	$[C_6 - C_3]_n$ $[C_6]_n$ $[C_6 - C_3 - C_6]_n$	Lignins Melanins Condensed tannins (Flavolans)

The phenolics containing 6-10 C-atoms and basic carbon skeletons C6, C6 – C1, C6 – C2, C6 – C3 and C6 – C4 as mentioned in the above table are sometimes grouped together as simple phenolic compounds or simple phenolics. Elaborations of basic carbon skeleton produce large array of phenolic compounds.

Lignin:

Lignin is highly complex and branched polymer of simple phenolic compounds with C6 – C3 basic carbon skeleton.



The structure of lignin is obscure. However, three phenyl propanoid alcohols viz., coniferyl alcohol, sinapyl alcohol and p-coumaryl alcohol are believed to be building blocks of lignin. These three building units of lignin are not joined in a simple repeating manner but have a highly branched and complex arrangement to form lignin. The proportion, of these three compounds in lignin varies among species, plant organs and even in different regions of the same cell wall.

Flavonoids:

Flavonoids are 15-C phenolic compounds widely distributed in plants and consist of C6 – C3 – C6 basic carbon skeleton.

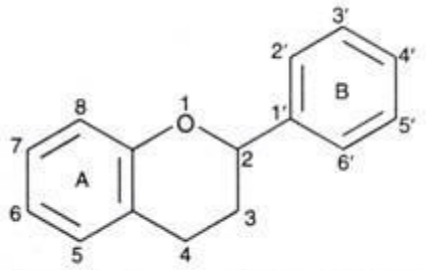
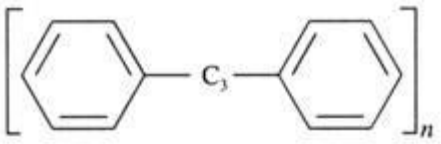


Fig. 24.18. Basic carbon skeleton of flavonoids

Based on degree of oxidation at various positions of the central ring, the flavonoids are classified into many subgroups. However, three of these subgroups are of particular interest in plant physiology viz., anthocyanins, flavonols & flavones and isoflavones (isoflavonoids).

Tannins:

Tannins are plant phenolic polymers which are widely distributed in higher plants. Bark, leaves, wood and unripe fruits of some plants are good sources of tannins. The compounds having a mol., wt. between 500 – 3000 and containing sufficient phenolic hydroxylic groups (1- 2% M.W.) to form effective cross links with proteins are considered as good tannin agents. An extract from oak bark (*Quercus* spp.) is a common tanning agent.



Culture systems used for secondary metabolite production:

In order to achieve industrial production of the desired metabolite, large scale cultivation of plant cells is required. Plant cells (20-150 µm in diameter) are generally 10-100 times larger than bacterial or fungal cell. When cultured, plant cells exhibit changes in volumes and thus variable shapes and sizes. Further, cultured cells have low growth rate and genetic instability. All these aspects have to be considered for mass cultivation of cells.

The following four different culture systems are widely used:

1. Free-cell suspension culture
2. Immobilized cell culture
3. Two-phase system culture
4. Hairy root culture.

1. Free-cell Suspension Culture:

Mass cultivation of plant cells is most frequently carried out by cell suspension cultures. Care should be taken to achieve good growth rate of cells and efficient formation of the desired secondary metabolite. Many specially designed bioreactors are in use for free-cell suspension cultures.

Some of these are listed below:
i. Batch bioreactors
ii. Continuous bioreactors
iii. Multistage bioreactors
iv. Airlift bioreactors
v. Stirred tank bioreactors.

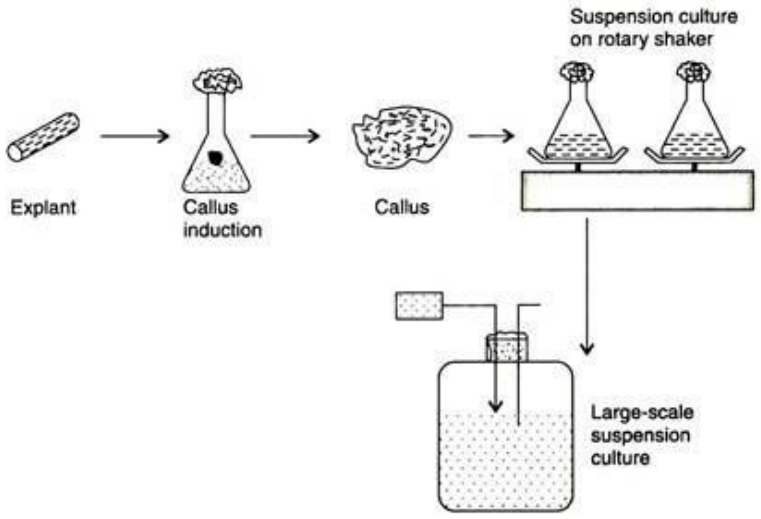


Fig. 12.2 Production of secondary metabolites by suspension culture

Two important aspects have to be considered for good success of suspension cultures.
1. Adequate and continuous oxygen supply.
2. Minimal generation of hydrodynamic stresses due to aeration agitation.

2. Immobilized Cell Cultures:

Plant cells can be made immobile or immovable and used in culture systems. The cells are physically immobilized by entrapment. Besides individual cells, it is also possible to immobilize aggregate cells or even calluses. Homogenous suspensions of cells are most suitable for immobilization.

Surface immobilized plant cell (SIPC) technique efficiently retains the cells and allows them to grow at a higher rate. Further, through immobilization, there is better cell-to-cell contact, and the cells are protected from high liquid shear stresses. All this helps in the maximal production the secondary metabolite.

The common methods adopted for entrapment of cells are briefly described:

1. Entrapment of cells in gels:

The cells or the protoplasts can be entrapped in several gels e.g., alginate, agar, agarose, carrageenin. The gels may be used either individually or in combination. The techniques employed for the immobilization of plant cells are comparable to those used for immobilization of microorganisms or other cells.

2. Entrapment of cells in nets or foams:

Polyurethane foams or nets with various pore sizes are used. The actively growing plant cells in suspension can be immobilized on these foams. The cells divide within the compartments of foam and form aggregates.

3. Entrapment of cells in hollow-fibre membranes:

Tubular hollow fibres composed of cellulose acetate silicone polycarbonate and organized into parallel bundles are used for immobilization of cells. It is possible to entrap cells within and between the fibres. Membrane entrapment is mechanically stable. However, it is more expensive than gel or foam immobilization.

Bioreactors for Use of Immobilized Cells:

Fluidized bed or fixed bed bioreactors are employed to use immobilized cells for large scale cultivation. In the fluidized-bed reactors, the immobilized cells are agitated by a flow of air or by

pumping the medium. In contrast, in the fixed-bed bioreactor, the immobilized cells are held stationary (not agitated) and perfused at a slow rate with an aerated culture medium.

Table: A selected list of the immobilized cells from selected plants and their utility to produce important bio-chemicals

Plant culture species	*Immobilization method*	*Substrate*	*Product*
Catharanthus roseus	Entrapment in agarose	Cathenamine	Ajmalicine
Digitalis lanata	Entrapment in alginate	Digitoxin	Digoxin
Capsicum frutescens	Entrapment in polyurethane foam	Sucrose	Capsaicin
Catheranthus roseus	Entrapment in alginate, agarose, carrageenin	Sucrose	Ajmalicine
Petunia hybrida	Entrapment in hollow fibres	Sucrose	Phenolics
Morinda citrifolia	Entrapment in alginate	Sucrose	Anthraquinone
Solanum aviculare	Attachment polyphenylene beads	Sucrose	Steroid glycosides
Glycine max	Entrapment in hollow fibre	Sucrose	Phenolics

3. Two-phase System Culture:

Plant cells can be cultivated in an aqueous two phase system for the production of secondary metabolites. In this technique, the cells are kept apart from the product by separation in the bioreactor. This is advantageous since the product can be removed continuously. Certain polymers (e.g., dextran and polyethylene glycol for the separation of phenolic compounds) are used for the separation of phases.

4. Hairy Root Culture:

Hairy root cultures are used for the production of root-associated metabolites. In general, these cultures have high growth rate and genetic stability. For the production of hairy root cultures, the explant material (plant tissue) is inoculated with the cells of the pathogenic bacterium, Agrobacterium rhizogenes. This organism contains root-inducing (Ri) plasmid that causes genetic transformation of plant tissues, which finally results in hairy root cultures. Hairy roots produced by plant tissues have metabolite features similar to that of normal roots. Hairy root cultures are most recent organ culture systems and are successfully used for the commercial production of secondary metabolites.

Improving secondary metabolite production in culture

Biochemical production by cultured cells can be increased chiefly by the following approaches:

1. Proper selection of cell lines

The heterogeneity within the cell population can be screened by selecting cell lines capable of accumulating higher level of metabolites.

2. Manipulation of medium

The constituents of culture medium, like nutrients, phytohormones and also the culture conditions, like temperature, light etc. influence the production of secondary metabolites. For e.g., if sucrose concentration is increased from 3% to 5%, production of rosamarinic acid is increased by five times. In case of shikonin production, IAA enhances the yield whereas 2,4- D and NAA are inhibitory.

3. Addition of Elicitors

Elicitors are the compounds which induce the production and accumulation of secondary metabolites in plant cells. Elicitors produced within the plant cells include cell wall derived polysaccharides, like pectin, pectic acid, cellulose etc. Product accumulation also occurs under stress conditions caused by physical or chemical agents like UV, low or high temperature, antibiotics, salts of heavy metals, high salt concentrations which are grouped under abiotic elicitors. Addition of these elicitors to the medium in low concentration enhances the production of secondary metabolites.

4. Addition of precursors

Precursors are the compounds, whether exogenous or endogenous, that can be converted by living system into useful compounds or secondary metabolites. It has been possible to enhance the biosynthesis of specific secondary metabolites by feeding precursors to cell cultures. For example, amino acids have been added to suspension culture media for production of tropane alkaloids, indole alkaloids. The amount of precursors is usually lower in callus and cell cultures than in differentiated

tissues. Phenylalanine acts as a precursor of rosmarinic acid; addition of phenylalanine to *Salvia officinalis* suspension cultures stimulated the production of rosmarinic acid and decreased the production time as well. Phenylalanine also acts as precursor of the N-benzoylphenylisoserine side chain of taxol; supplementation of Taxus cuspidata cultures with phenylalanine resulted in increased yields of taxol. The timing of precursor addition is critical for an optimum effect. The effects of feedback inhibition must surely be considered when adding products of a metabolic pathway to cultured cells.

5. Permeabilisation

Secondary metabolites produced in cells are often blocked in the vacuole. By manipulating the permeability of cell membrane, they can be secreted out to the media. Permeabilisation can be achieved by electric pulse, UV, pressure, sonication, heat, etc. Even charcoal can be added to medium to absorb secondary metabolites.

6. Immobilization

Cell cultures encapsulated in agarose and calcium alginate gels or entrapped in membranes are called immobilised plant cell cultures. Immobilization of plant cells allows better cell to cell contact and the cells are also protected from high shear stresses. These immobilized systems can effectively increase the productivity of secondary metabolites in a number of species. Elicitors can also be added to these systems to stimulate secondary metabolism.

In some cases, the secondary metabolites are synthesized in certain specialized cells, which may also serve as storage sites. These specialized cells are absent in callus and suspension cultures so that they are unable to produce the biochemicals in questions. In such cases, organized structures possessing the specialized cells are grown in vitro for isolation of the biochemicals. For example, the monoterpenes in *Mentha* spp. are synthesized and stored in epidermal oil glands of leaves. Mentha cell cultures lack these glands and as a result, are unable to accumulate high levels of monoterpenes. In contrast, shoot cultures of *Mentha* grown on GR free media can accumulate monoterpenes, but the yield is often lower than that from field grown plants.

Hairy root culture

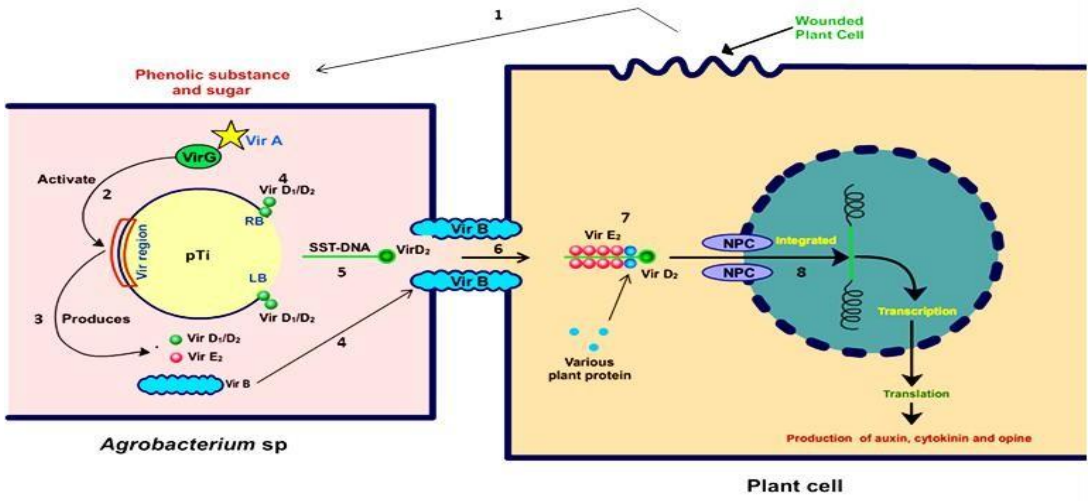
Plant remains major source of pharmaceuticals and fine chemicals and cell cultures have been viewed as promising alternatives to whole plant extraction for obtaining valuable chemicals. The major constraint with the cell culture is that they are genetically unstable and tend to produce low yield of secondary metabolites. A new method for enhancing secondary metabolite production is by transformation of cells or tissues using the natural vector system. Agrobacterium rhizogenes, the causative agent of hairy root disease, is a soil dwelling gram negative bacterium capable of entering a plant through a wound and causing a proliferation of secondary roots. The biosynthetic capacity of the hairy root cultures is equivalent or sometimes more to the corresponding plant roots. Therefore, hairy root cultures have been developed as an alternate source for the production of root biomass and to obtain root derived compounds.

Establishment of hairy root cultures

For the production of hairy root cultures, the explant material is inoculated with a suspension of *A. rhizogenes*. The bacterial suspension is generated by growing bacteria in Yeast Mannitol Broth (YMB) medium for 2 days at 25°C under shaking conditions. Thereafter, pelleting by centrifugation (5 x 10 rpm; 20 min) and resuspending the bacteria in YMB medium to form a thick suspension (approx. 1010 viable bacteria/ml). Transformation may be inducesd in aseptic seedlings or surface sterilized detached leaves, leaf-discs, petioles, stem segments, from greenhouse grown plants by scratching the leaf midrib or the stem of a

7. Use of organ cultures

plantlet with the needle of a hypodermic syringe containing a small (about 5-10 µl) droplet of thick bacterial suspension of *A. rhizogenes*. Roots may arise directly from that site of transformation or they may differentiate from a callus that may develop at that site. The roots are excised and used to initiate root cultures; usually a culture flask is inoculated with 3-4 roots of 2-3 cm in length. Hairy root cultures are easily developed in most dicot plants.



Wounded plant cell releases phenolic substances and sugar (1); which are sensed by Vir A, Vir A activates Vir G, Vir G induced for expression of Vir gene of Ri-plasmid (2); Vir gene produces all the Vir -protein (3); Vir D1 and Vir D2 are involved in ssT-DNA production from Ri-plasmid and its export (4) and (5); the ssT-DNA (associated with Vir D1 and Vir D2) with Vir E2 are exported through transfer apparatus Vir B (6); in plant cell, T-DNA coated with Vir E2 (7); various plant proteins influence the transfer of T-DNA + Vir D1 + Vir D2 + Vir E2 complex and integration of T-DNA to plant nuclear DNA(8). (LB= left border; RB= Right border; pRi = Ri plasmid, NPC = nuclear pore complex)

Properties of hairy roots

Hairy roots have following properties

- High degree of lateral branching
- Profusion of root hairs
- Absence of geotropism
- They have high growth rates in culture, due to their extensive branching, resulting in the presence of many meristems.
- They do not require conditioning of the medium.
- Hairy roots are genetically stable consequently they exhibit biochemical stability that leads to stable and high-level production of secondary metabolites.

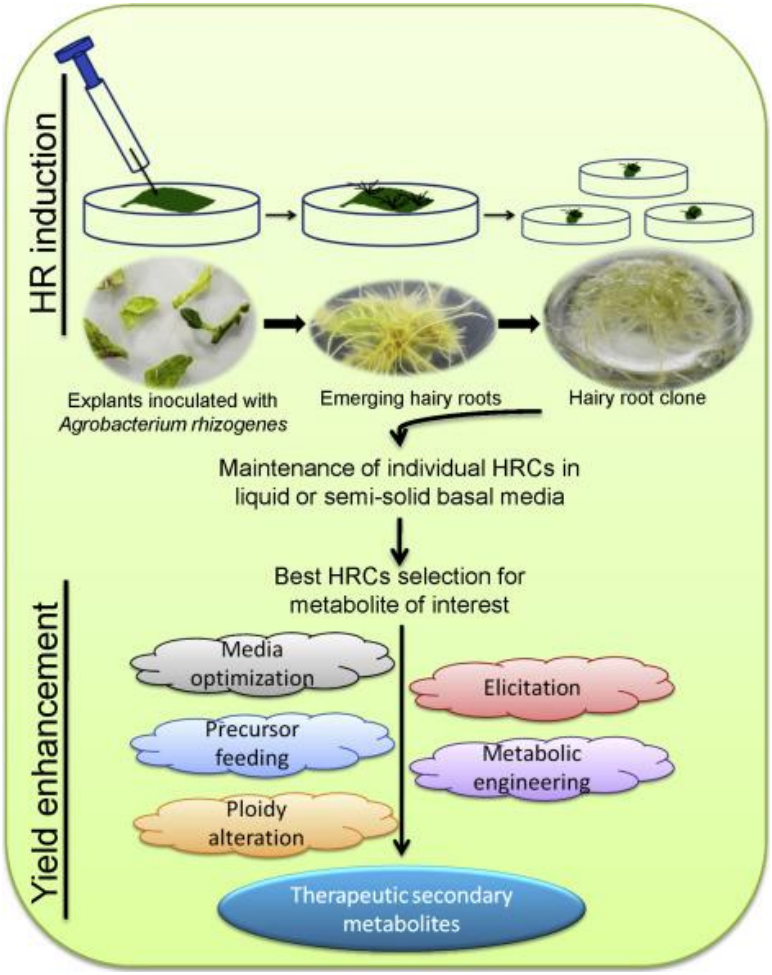
Application of hairy root cultures

1. **Production of secondary metabolites**

The hairy root system is stable and highly productive under hormone-free culture conditions. The fast growth, low doubling time, easy maintenance, and ability to synthesize a range of chemical compounds of hairy root cultures gives additional advantages as continuous sources for the production of plant secondary metabolites. Usually root cultures require an exogenous phytohormone supply and grow very gradually, resulting in the poor or insignificant synthesis of secondary metabolites. Hairy roots are also a valuable source of photochemical that is useful as pharmaceuticals, cosmetics, and food additives. These roots synthesize more than a single metabolite; prove economical for commercial production purposes. Many medicinal plants have been transformed successfully by *A. rhizogenes* and the hairy roots induced show a relatively high productivity of secondary metabolites, which are important pharmaceutical products. Sevon has summarized the most important alkaloids produced by hairy roots, including *Atropa belladonna* L., *Catharanthus trichophyllus* L., and *Datura candida* L.

Metabolic engineering offers new perspectives for improving the production of secondary metabolites by the over expression of single genes. This approach may lead to an increase of some enzymes involved in metabolism and, consequently, results in the accumulation of the target products. This method utilizes the foreign genes that encode enzyme activities not normally present in a plant. This may cause the modification of plant metabolic pathways. Two direct repeats of a bacterial lysine decarboxylase gene, expressed in the hairy roots of *Nicotiana tabacum*, have markedly increased the production of cadaverine and anabasine. The production of anthraquinone and alizarin in hairy roots of *Rubia peregrina* L. was enhanced by the introduction of isochorismate synthase. *Catharanthus*

roseus hairy roots harboring hamster 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) cDNA without the membrane-binding domain were found to produce more ajmalicine and catharanthine or serpentine and campesterol than the control.



2. **Production of compounds not found in untransformed roots**

Transformation may affect the metabolic pathway and produce new compounds that cannot be produced normally in untransformed roots. For example, the transformed hairy roots of *Scutellaria baicalensis* Georgi accumulated glucoside conjugates of flavonoids instead of the glucose conjugates accumulated in untransformed roots.

3. **Changing composition of metabolites**

The expression of an *Antirrhinum* dihydroflavonol reductase gene which resulted in changes in condensed tannin structure and its accumulation in root cultures of *L. corniculatus*. The analysis of selected root culture lines indicated the alteration of monomer levels during growth and development without changes in composition.

Table: Pharmaceutical products produced using hairy root cultures

Plant species	**Product**
Bidens spp.	Polyacetylenes
Cinchona ledgeriana	Quinoline alkaloids
Datura spp.	Tropane
Cassia spp.	Anthraquinones
Echinacea purpurea	Alkaloids

7. Micropropagation: Production of virus free plants, virus free assessment methods, genetic assessment by RAPD and ISSR markers.

Micropropagation:

Micropropagation is the practice of rapidly multiplying stock plant material to produce many progeny plants, using modern plant tissue culture methods.

Micropropagation is used to multiply 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 a stock plant which does not produce seeds, or does not respond well to vegetative reproduction.

Cornell University botanist Frederick Campion Steward discovered and pioneered micropropagation and plant tissue culture in the late 1950s and early 1960s.

Stages:

Stage 0: Mother plant selection, maintenance and preparation for culture initiation

Stage I: Initiation and establishing an aseptic culture

Stage II: Multiplication of suitable propagules

Stage III: Preparation for growth in the natural environment

Stage IV: Transfer to the natural environment

Applications of Micro propagation:

Micro propagation has become a suitable alternative to conventional methods of vegetative propagation of plants. There are several advantages of micro propagation.

High Rate of Plant Propagation:

Through micro propagation, a large number of plants can be grown from a piece of plant tissue within a short period. Another advantage is that micro propagation can be carried out throughout the year, irrespective of the seasonal variations. Further, for many plants that are highly resistant to conventional propagation, micro propagation is the suitable alternative. The small sized propagules obtained in micro propagation can be easily stored for many years (germplasm storage), and transported across international boundaries.

Production of Disease-free Plants:

It is possible to produce disease-free plants through micro propagation. Meristem tip cultures are generally employed to develop pathogen-free plants .In fact, micro propagation is successfully used for the production of virus-free plants of sweet potato (*Ipomea batatus*), cassava (*Manihot esculenta*) and yam (*Discorea rotundata*).

Production of Seeds in Some Crops:

Micro propagation, through axillary bud proliferation method, is suitable for seed production in some plants. This is required in certain plants where the limitation for' seed production is high degree of genetic conservation e.g. cauliflower, onion.

Cost-effective Process:

Micro propagation requires minimum growing space. Thus, millions of plant species can be maintained inside culture vials in a small room in a nursery. The production cost is relatively low particularly in developing countries (like India) where the manpower and labour charges are low.

Automated Micropropagation:

It has now become possible to automate micro propagation at various stages. In fact, bio- reactors have been set up for large scale multi-plication of shoots and bulbs. Some workers employ robots (in place of labourers) for micro- propagation, and this further reduces production cost of plants.

Disadvantages of Micro propagation:

Contamination of Cultures:

During the course of micro propagation, several slow-growing microorganisms (e.g. *Eswinia* sp, *Bacillus* sp) contaminate and grow in cultures. The microbial infection can be controlled by addition of antibiotics or fungicides. However, this will adversely influence propagation of plants.

Brewing of Medium:

Micro propagation of certain plants (e.g. woody perennials) is often associated with accumulation of growth inhibitory substances in the medium. Chemically, these substances are phenolic compounds, which can turn the medium into dark colour. Phenolic compounds are toxic and can inhibit the growth

of tissues. Brewing of the medium can be prevented by the addition of ascorbic acid or citric acid or polyvinyl pyrrolidone to the medium.

Genetic Variability:

When micro propagation is carried out through shoot tip cultures, genetic variability is very low. However, use of adventitious shoots is often associated with pronounced genetic variability.

Vitrification:

During the course of repeated in vitro shoot multiplication, the cultures exhibit water soaked or almost translucent leaves. Such shoots cannot grow and even may die. This phenomenon is referred to as vitrification. Vitrification may be prevented by increasing the agar concentration (from 0.6 to 1%) in the medium. However, increased agar concentration reduces the growth rate of tissues.

Cost Factor:

For some micro propagation techniques, expensive equipment, sophisticated facilities and trained manpower are needed. This limits its use.

Production of virus free plants:

Viruses are very small (submicroscopic) infectious particles (virions) composed of a protein coat and a nucleic acid core. They carry genetic information encoded in their nucleic acid, which typically specifies two or more proteins. Translation of the genome (to produce proteins) or transcription and replication (to produce more nucleic acid) takes place within the host cell and uses some of the host's biochemical "machinery".

Viruses cause many important plant diseases and are responsible for huge losses in crop production and quality in all parts of the world. Infected plants may show a range of symptoms ranging from leaf yellowing (either of the whole leaf or in a pattern of stripes or blotches), leaf distortion (e.g. curling) and/or other growth distortions (e.g. stunting of the whole plant, abnormalities in flower or fruit formation). Viral diseases occur in virtually all seed propagated crops.

There is no commercially available treatment to cure virus-infected plants.

To produce disease free plants a healthy nucleus stock is needed. However, when the whole population is infected then the only way to obtain a pathogen free plant is through tissue culture.

Virus replication is unable to keep pace with cell proliferation.

Apical meristems in the infected plants are generally either free or carry a very low concentration of the viruses. However, the titer of the viruses increases in the older tissues corresponding to the increase in the distance from the meristem tips.

Reasons attributed to the escape of the meristems by virus invasions are:

- Viruses **move rapidly in a plant body** through the vascular system which in meristems is absent.
- A high **metabolic activity** in the in the actively dividing meristematic cells does not allow virus replication
- A **high endogenous auxin** level in shoot apices may inhibit virus multiplication.
- A meristem-tip culture has also enabled plants to be freed from other pathogens including viroids, mycoplasmas, bacteria and fungi.

Methods of Virus Elimination:

A general term ‘virus free’ is used by commercial horticulturists for plants free of any type of virus. Virus free material can be re-infected if proper precautionary measures are not adopted.

1. Heat treatment
2. Meristem-tip culture
3. Chemical treatment
4. Other in vitro methods

1. Heat treatment

Before the advent of the meristem cultures the in vivo eradication of viruses from plants can be achieved by **heat treatment (thermotherapy)** of whole plants.

At temperature higher than optimum for many viruses in plant tissues are partially or completely inactivated with little or no injury to the host tissues. Heat treatment is given through hot water or hot air.

The hot water treatment effectively eliminates viruses in dormant buds, where as hot air treatment is recommended for elimination of viruses from actively growing shoots. The survival rate of the host tissue is better in hot air treatment.

Hot Air Treatment

1. Temperature: **35-40°C [few minutes to several months]**
2. Adequate supply of **humidity and light**
3. Plants must have **ample carbohydrate** reserves to withstand the heat treatment
4. The **temperature** of the air should be **gradually raised** during the first few days until the desired temperature is raised.
5. **Small cuttings are taken** from the shoot tips **immediately** after heat treatment and grafted onto healthy rootstocks.
6. Generally the **percentage** of plants that survive after heat treatment is **small.**

2. Meristem-tip culture

For purposes of virus elimination the chance are better if culture are initiated with shoot tips of smaller size comprising mostly meristematic cell.

Apical meristem: The portion of the shoot lying distal to the youngest leaf primordium measuring up to about 100 micro m in diameter and 250 micro m in length.

Shoot apex: The apical meristem together with one to three young leaf primordia measuring 100-500 micro m.

In most published work explants of larger size (100-1000 micro m long) have been cultured to raise virus free plants.

Under a stereoscopic microscope (8-40 magnification) with a suitable light source explants are placed on a Petri plate lined with a sterile moist filter paper to avoid desiccation.

Meristeem tips taken from terminal buds observed to give better result than those from axillary buds

The percentage of virus free plants can also depend on season, especially with crops which display periodic growth.

Although the apical meristems are often virus free, there may be exceptions. Some viruses are known to actually invade the meristimatic region of the growing tips in certain plants. i.e. TMV.

In such cases it has also been possible to obtain virus free plants by combining meristem tip culture with thermotherapy. The duration of heat treatment has to be decided carefully since excessive exposure can damage the plant tissues. To avoid deterioration of meristem tip cultures by continuous exposure to high temperature, the treatment with diurnal or daily cycle of high and low temperature can be tried. i.e. CMV in tissue cultures of *Nicotina rustica* and *Stellaria indica* can be inactivated by following the diurnal cycles of 40oC (16hrs) and then 22oC (8 hrs) per day.

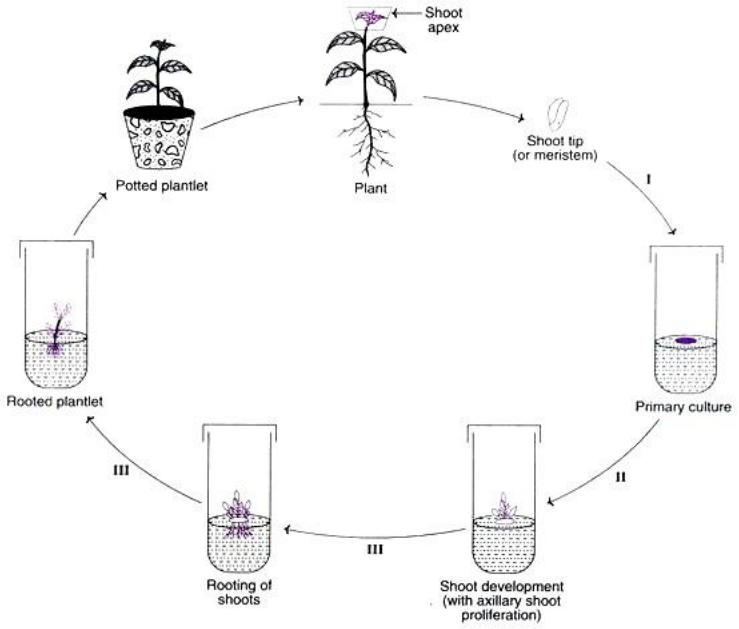


Fig. 47.2 : A diagrammatic representation of shoot tip (or meristem) culture in micropropagation
(Note : I, II and III represent stages in micropropagation)

Meristem-tip cultures are influenced by the following factors:

i. Physiological condition of the explant — actively growing buds are more effective.

ii. Thermotherapy prior to meristem-tip culture — for certain plants (possessing viruses in the meristematic regions), heat treatment is first given and then the meristem-tips are isolated and cultured.

iii. Culture medium —MS medium with low concentrations of auxins and cytokinins is ideal.

A selected list of the plants from which viruses have been eliminated by meristem cultures is given in Table.

TABLE 47.1 A selected list of the plants with virus elimitation by meristem cultures

Plant species	*Virus eliminated*
Solanum tuberosum (potato)	Leaf roll, potato viruses — A, X, Y, S
Nicotiana tabacum (tobacco)	Tobacco mosaic virus
Saccharum officinarum (sugar cane)	Mosaic virus
Allium sativum (garlic)	Mosaic virus
Anenas sativus (pineapple)	Mosaic virus
Brassica oleracea (cauliflower)	Cauliflower/mosaic virus turnip mosaic virus
Ipomoea batata (sweet potato)	Fealthery mottle virus
Ribes grassularia	Vein banding virus
Humulus lupulus	Hop latent virus
Armoracia rusticena	Turnip mosaic virus
Musa sp (Banana)	Cucumber mosaic virus
Hycinthus sp	Hycinth mosaic virus
Dahlia sp	Dahlia mosaic virus
Chrysanthemum sp	Virus B
Petunia sp	Tobacco mosaic virus
Iris sp	Iris mosaic virus
Cymbidium sp	Cymbidium mosaic virus
Fragaria sp	Pallidosis virus, yellow virus complex
Freesia sp	Freesia mosaic virus

3. Chemical treatment

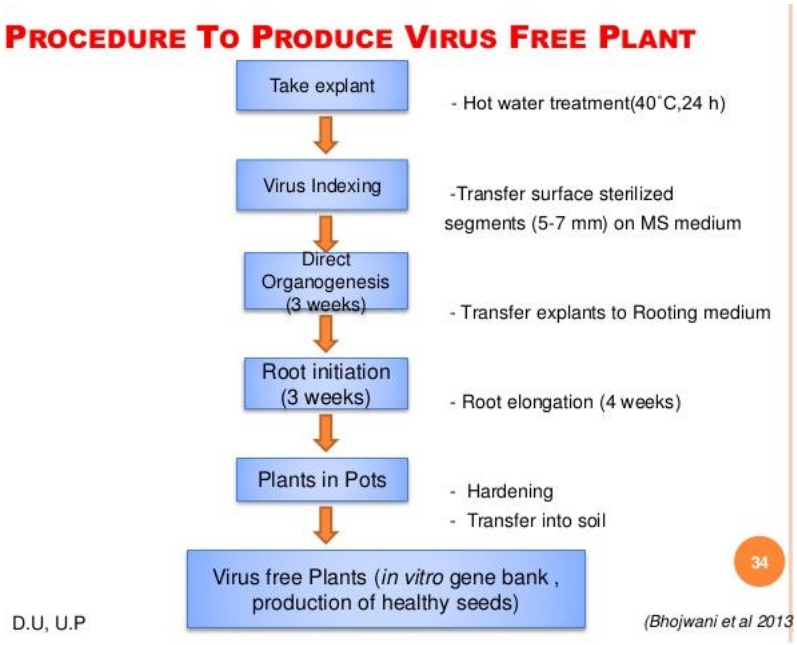
In the absence of effective therapeutic chemicals capable of eradicating virus from infected plants, there are reports of some attempts to suppress viruses in plant tissue and protoplast by the addition of some chemicals in the media. In some instances virus multiplication was suppressed by **the addition of cytokinins and other growth substances** while in others it was actually stimulated. Antimetabolites **malachite green, thiouraciol and acetylsalicylic acid** had little or a limited effect on virus elimination in meristem-tip regenerated plants, but the incorporation of nucleoside analog **rivavirin** (PVX,CMV, alfalfa mosic virus), **vidarbin, cyclohexamide** and **actinomycin-D** inhbit virus replication in plant protoplasts**.**

4. Cryotherapy

Cryotherapy is the prolonged exposure to a low temperature followed by shoot tip culture. It is a good method of virus elimination. Shoot tip cultures from *Chrysanthemum* plants treated with 5°C for 4 months yielded 67% plants free from *Chrysanthemum* stunt virus (CSV) and 22% plants free from *Chrysanthemum* chlorotic motile virus (CCMV). Cold treatment is preferable to heat treatment as it is less injurious to the plants and often more effective in virus elimination.

5. Electrotherapy

Electrotherapy assays were carried out either on infected in vivo or in vitro plants. Pulses of 15 V were applied for 5 min to 2- 3 cm long explants containing apical meristem. The meristems were then excised and placed on an MS culture medium. The efficiency of electrotherapy in producing virus-free regenerants from BSV-infected banana plants (cv. W. Bungulan) is 40-80 %.



Virus free assessment methods

Tissue culture offers great promise for the production of quality planting material on account of disease free plants produced through meristem culture. But all the plants obtained through meristem culture with or without the therapeutic treatments are not virus free. Testing plants for the presence or absence of viruses is known as virus indexing. Every meristem tip or callus derived plant must be tested before using it as a mother plant to produce virus free stock. The three methods which are followed for virus indexing are sap transmission test, serology and EM examination.

Sap transmission test

It is done by taking the saps (filtered leaf extract) from test plants and which may be used to inoculate highly sensitive and healthy indicator plants. An Indicator plant for a virus is that plant species or variety which is highly susceptible to the virus and readily develops the symptoms. The inoculated indicator plants are maintained in a green house or aphid- proof cages separated from each other and from other plants. Sap transmission test is the most sensitive test among the three methods and can be easily performed on a commercial scale.

Serological test

It is a highly sensitive and precise technique for virus indexing employing antibodies specific to the concerned viruses. This test is performed by adding a drop of centrifuged sap from a test plant to a drop of antiserum taken from the blood of a rabbit. If the virus is present, the precipitation will take place due to the presence of specific antibodies in the blood. The ELISA (enzyme linked immunosorbent assay) is one of the serological methods used to identify viruses based on antibody reaction. ELISA is the most convenient, rapid and efficient test especially when a large number of samples are to be handled.

EM (Electron microscopy) examinations

These are particularly useful for identifying latent viruses (viruses those exhibit no visible symptoms). This method is not usually implemented as specialized equipment and trained personnel are required to carry out EM studies. Immunosorbent Electrom Microscopy (ISEM) described combines both serology and EM studies for detection of viruse.

Genetic assessment by RAPD and ISSR markers:

Advances in molecular biology techniques have provided the basis for uncovering virtually unlimited numbers of DNA markers. The utility of DNA-based markers is generall determined by the technology that is used to reveal DNA-based polymorphism. Currently, the restriction fragment length polymorphism (RFLP) assay has been the choice for many species to measure genetic diversity and construct a genetic linkage map. However, an RFLP assay which detects DNA polymorphism through

restriction enzyme digestion, coupled with DNA hybridisation, is, in general, time consuming and laborious. Over the last decade, polymerase chain reaction (PCR) technology has become a widespread research technique and has led to the development of several novel genetic assays based on selective amplification of DNA. This popularity of PCR is primarily due to its apparent simplicity and high probability of success.

Unfortunately, because of the need for DNA sequence information, PCR assays are limited in their application. The discovery that PCR with random primers can be used to amplify a set of randomly distributed loci in any genome facilitated the development of genetic markers for a variety of purposes.

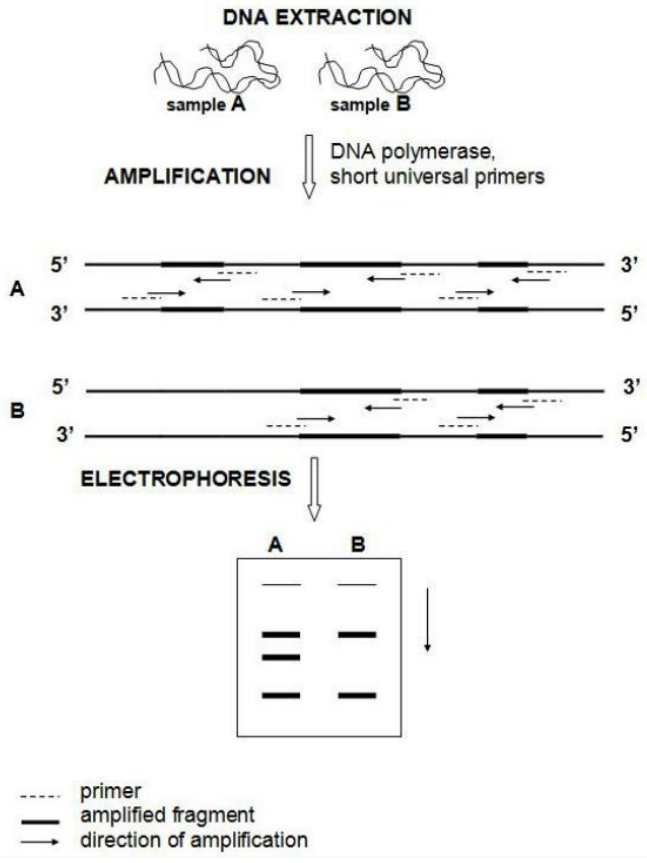
Randomly amplified polymorphic DNA markers (RAPD):

RAPD markers detect nucleotide sequence polymorphisms between individuals by employing a single, short (decamer), random oligonucleotide primers. DNA polymorphisms are detected due to the changes of nucleotide at or between oligonucleotide primer binding sites in the genome. RAPD are dominant markers that can detect several loci in a single assay ranging from 0.5 to 5 kb. DNA fragments are fractionated by agarose gel electrophoresis and visualized with ethidium bromide (EtBr) that scores differences in the amplification pattern by their presence (+) and absence (-) at particular loci. Simplicity in methodology, no prior sequence information for primer designing, cost efficiency as well as no requirement radioactive probes has made RAPD technique a promising tool for identification of markers linked to agronomically important traits. However, inherent problems of reproducibility, sensitivity to experimental conditions, presence of artefactual bands (false positives) and inability to distinguish heterozygous from homozygous individuals makes them less preferential tool for genome wide studies.

RAPD primer	Sequence (5′-3′)	No. of amplification products
2	AGACGCGTAG	7
3	TGGACCCACA	3
4	GTGGCTTCTC	5
5	GCGCAGTATC	8
6	CCACCGTACT	6
7	TCCGGCTGTT	6
8	TGTCCCGTTG	7
11	CACACGAGAC	7
12	CGTACACCAG	5

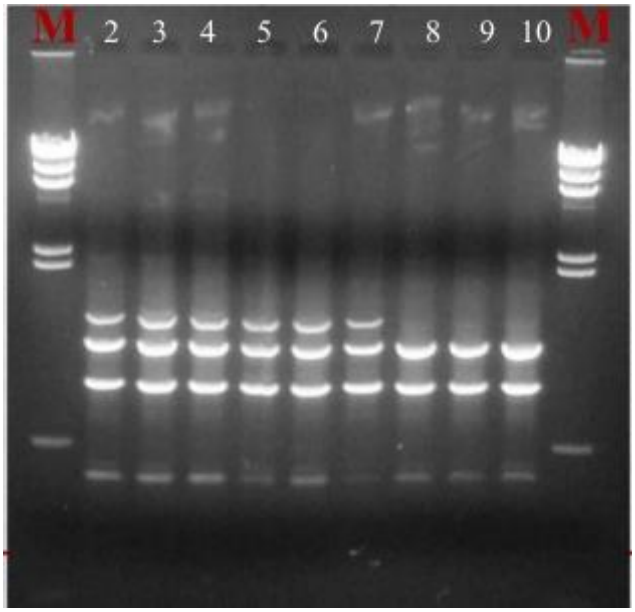
RAPD involves following steps:-

1. The DNA of a selected species is isolated
2. An excess of selected decaoligonucleotide added.
3. This mixture is kept in PCR equipment and is subjected to repeated cycles of DNA denaturation-renaturation-DNAreplication.
4. During this process, the decaoligonucleotidenwill pair with the homologous sequence present at different locations in the DNA.
5. DNA replication extends the decaoligonucleotide and copy the sequence continuous with the sequence with which the selected oligonucleotide has paired.
6. The repeated cycles of denaturation - renaturation-DNA replication will amplify this sequence of DNA.
7. Amplification will takes place only of those regions of the genome that



has the sequence complementary to the decaoligonucleotide at their both ends.

8. After several cycles of amplification the DNA is subjected to gel electrophoresis.
9. The amplified DNA will form a distinct band. it is detected by ethidium bromide staining and visible fluorescence's under U.V. light



Separated RAPD fragments

Advantages:

RAPD primers are readily available being universal. They provide moderately high genotyping throughput. This technique is simple PCR assay (no blotting and no radioactivity). It does not require special equipment. Only PCR is needed. The start-up cost is low.

RAPD marker assays can be performed using very small DNA samples (5 to 25 ng per sample). RAPD primers are universal and can be commercially purchased. RAPD markers can be easily shared between laboratories. Locus-specific, co-dominant PCR-based markers can be developed from RAPD markers. It provides more polymorphism than RFLPs.

Disadvantages:

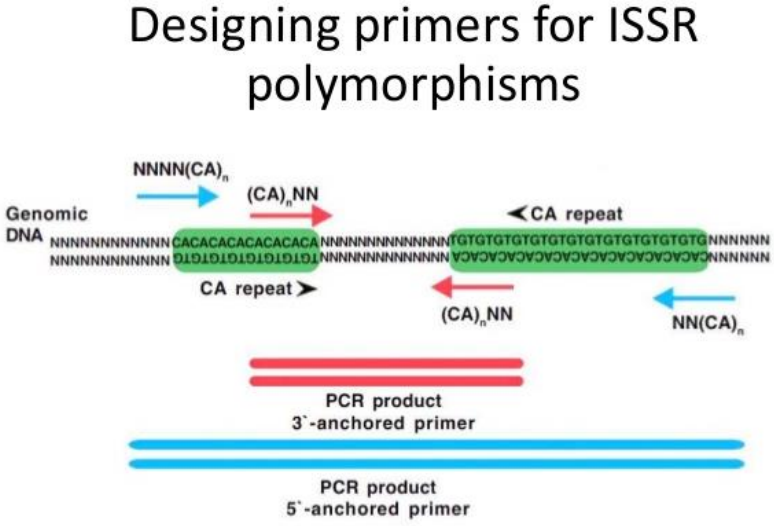
The detection of polymorphism is limited. The maximum polymorphic information content for any bi-allelic marker is 0.5. This technique only detects dominant markers. The reproducibility of RAPD assays across laboratories is often low. The homology of fragments across genotypes cannot be ascertained without mapping. It is not applicable in marker assisted breeding programme.

Uses:

This technique can be used in various ways such as for varietal identification, DNA fingerprinting, gene tagging and construction of linkage maps. It can also be used to study phylogenetic relationship among species and sub-species and assessment of variability in breeding populations.

Inter simple sequence repeat (ISSR):

ISSR involves the amplification of DNA fragments flanked by inversely oriented SSR-microsatellites. The DNA polymorphism depends on the abundance and hypervariability of microsatellites in the genome. This technique uses microsatellites consisting of di-, tri-, tetra- or penta-nucleotides as primers complementary to microsatellite regions in the genome and amplify inter simple sequence repeats of different sizes. Depending on the usage ISSR primers can be either unanchored or anchored at 3' or 5' end with 1–4 degenerate bases extended into the flanking sequences. Primer extension with 1–4 degenerate nucleotide at 3' or 5' end avoids internal priming and smear formation. The size of the amplified products varies between 200 and 2000 bp and can be separated using both agarose and polyacrylamide gel electrophoresis. The technique is simple, quick and need no sequence information for primer synthesis. ISSRs use longer semi-arbitrary



SSR primers (15–30 mers) as compared to RAPD primers (10 mers), which allow the subsequent use of high annealing temperature leading to higher stringency and greater band reproducibility. However, dominant inheritance and homoplasy are the main limitations of ISSRs.

During the last twenty years, tissue culture and molecular biology have experienced a dynamic synergism. While tissue culture has provided model systems for molecular biology studies, molecular biology techniques have been applied to address limitations of tissue culture systems. Molecular marker systems have investigated genetic stability or somaclonal variation of plants after long term preservation or cryopreservation under in vitro conditions, and also of plants coming from long term culture conditions or following a large number of subcultures. Quality control of tissue cultured plants, molecular breeding in in vitro conditions and determining the genes involved in tissue culture responsiveness are other areas of usage of molecular markers.

RAPD and ISSR markers have been extensively applied to serve the purposes in the following areas:

Determination of genetic stability or detection of somaclonal variation

Somaclonal variation is the variation observed among plants regenerated from in vitro culture. These variations are heritable, i.e., transmitted through meiosis, and are usually irreversible. Many factors such as plant growth regulator balance (auxin and cytokinin concentration), culture duration (subculture number), macro and micro elements used in in vitro culture, and physiological stress induce somaclonal variation under in vitro conditionsThe source of explants and their pattern of regeneration are known to play major roles in determining the extent of genetic or somatic variation. Plants regenerated from adventitious buds around axillary buds, or from other well developed meristematic tissue, show the lowest tendency for genetic variation, whereas more changes are detected in plants derived from callus compared with those coming from embryogenic tissue.

Genetic stability of plants after long term in vitro conservation and cryopreservation

Germplasm preservation is essential to maintain biodiversity and avoid genetic erosion. In vitro culture may provide an alternative to standard methods for the conservation of many woody plants. Genetic conservation is based on the assumption that the material is conserved under conditions ensuring genetic stability. However, many factors associated with in vitro culture conservation procedures may result in somatic variation. It is essential to observe genetic stability during in vitro conservations. Currently, RAPD and ISSR markers are being increasingly used for monitoring genetic stability of germplasm during their long-term conservation.

Genetic stability of plants after long subculture

In some plants the variable number of proliferation cycles increases the occurrence of the somaclonal variation, mainly, due to chromosomal abnormalities.

Molecular markers for quality control of in vitro plants

The micropropagation industry is expanding and somaclonal variation and bacterial and viral contamination is becoming a problem for commercial propagators of a genotype. It is necessary that the plant material produced through tissue culture be certified before being distributed to growers. Quality control of most micropropagated plants is being ensured using molecular markers (RAPD and ISSR) and DNA fingerprinting techniques. Polymorphism obtained in the progenies of tissue culture plants in comparison to the control mother explant source could be correlated with the apparent morphological changes. Although this is very time consuming, once done this will give an ideal marker system. These markers will further enhance the ability to understand the effect of different variables on the production of somaclonal variation in plants regenerated from tissue culture.

Developing molecular markers linked to a trait in question

The identification of gene or genomic regions that influence agriculturally related traits is very important in DNA marker-trait association study. This provides the basis for marker assisted selection (MAS) in plant breeding. Random molecular markers (RAPD and ISSR) are traditionally applied to establish a genetic linkage with a phenotypic trait. Having a tight linkage with the phenotypic trait RAPD and ISSR markers are the method of choice in many cases.

8. Fermentation technology: application of fermentation; batch, fed batch and their continuous cultures of microbes; Bioreactors: Principles and their design; microbial strain improvement.

Fermentation:

Fermentation is a metabolic process that produces chemical changes in organic substrates through the action of enzymes. In biochemistry, it is narrowly defined as the extraction of energy from carbohydrates in the absence of oxygen. In the context of food production, it may more broadly refer to any process in which the activity of microorganisms brings about a desirable change to a foodstuff or beverage. The science of fermentation is known as **zymology**.

In microorganisms, fermentation is the primary means of producing ATP by the degradation of organic nutrients anaerobically. Humans have used fermentation to produce foodstuffs and beverages since the Neolithic age. For example, fermentation is used for preservation in a process that produces lactic acid found in such sour foods as pickled cucumbers, kimchi, and yogurt, as well as for producing alcoholic beverages such as wine and beer**.** Fermentation also occurs within the gastrointestinal tracts of all animals, including humans.

Louis Pasteur in the 19th century used the term fermentation in a narrow sense to describe the changes brought about by yeasts and other microorganisms growing in the absence of air (anaerobically); he also recognized that ethyl alcohol and carbon dioxide are not the only products of fermentation.

Types of Fermentation:

Two types of fermentation are common:

a. Alcoholic Fermentation:

In this type, the pyruvic acid is first decarboxylated to acetaldehyde in the presence of enzyme carboxylase. Thiamine pyrophosphate (TPP) is required as co-factor.

$$2\,CH_3COCOOH \xrightarrow[TPP]{carboxylase} 2\,CH_3CHO + 2\,CO_2$$

Pyruvic acid → Acetaldehyde

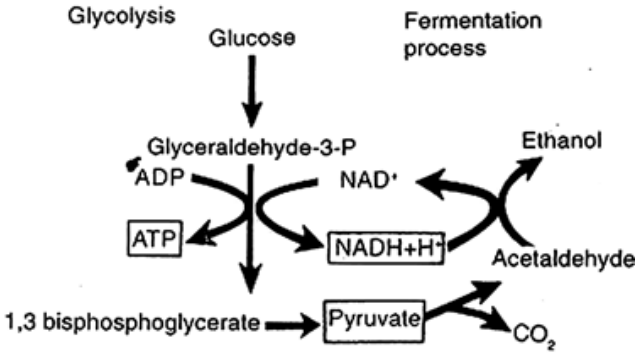


Fig. 4.7. Pathway of fermentation (anaerobic respiration) in yeast.

Acetaldehyde is then reduced to ethanol (ethyl alcohol) by the enzyme alcohol dehydro-genase. Coenzyme $NADH_2$ (produced in glycolysis) is oxidised.

$$2\,CH_3CHO + NADH_2 \xrightarrow[dehydrogenase]{alcoholic} CH_3CH_2OH + NAD$$

Acetaldehyde (Reduced) → Ethanol (Oxidised)

The overall equation for anaerobic respiration involving alcoholic fermentation is as follows:

$$C_6H_{12}O_6 \longrightarrow 2\,CH_3CH_2OH + 2\,CO_2$$

b. Lactic acid Fermentation:

This type of fermentation occurs in lactic acid bacteria, certain fungi and muscles. Here, pyruvic acid is converted into lactic acid by the enzyme lactic dehydrogenase. Coenzyme NADH2, produced in glycolysis, is oxidised.

$$CH_3CO.COOH + NADH_2 \xrightarrow[dehydrogenase]{lactic} CH_3CHOH.COOH + NAD$$

pyruvic acid → lactic acid

The overall equation for anaerobic respiration (lactic acid fermentation) is as follows:

$$\underset{\text{glucose}}{C_6H_{12}O_6} \longrightarrow \underset{\text{lactic acid}}{2\ CH_3CHOH.COOH}$$

There is net gain of only 2 ATP molecules (in glycolysis stage) during anaerobic respiration (or fermentation) and most of energy contained in glucose molecule is released as heat.

The range of fermentation process

There are five major groups of commercially important fermentations:

(i) Those that produce microbial cells (or biomass) as the product.
(ii) Those that produce microbial enzymes.
(iii) Those that produce microbial metabolites.
(iv) Those that produce recombinant products.
(v) Those that modify a compound which is added to the fermentation the transformation process.

The component parts of a fermentation process

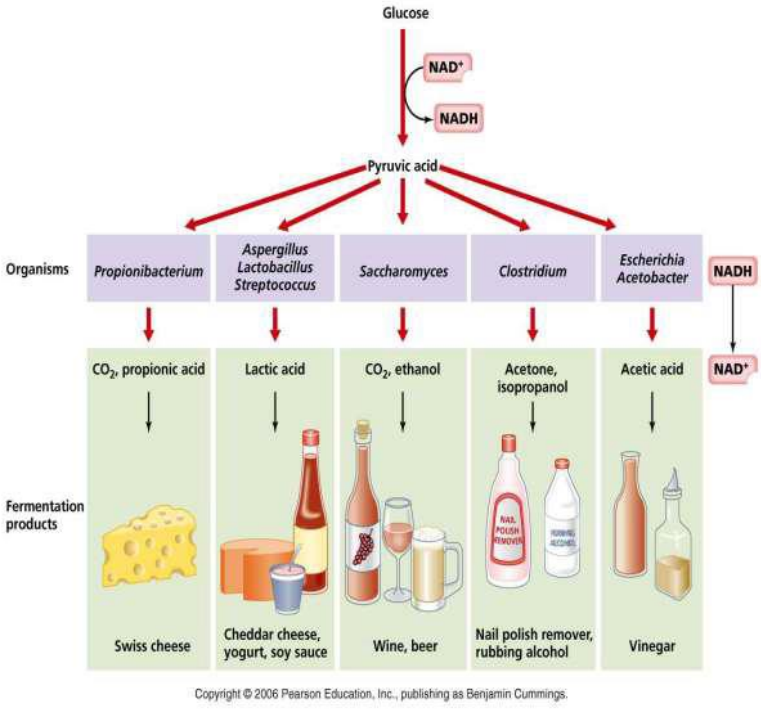
(i) The formulation of media to be used in culturing the process organism during the development of the inoculum and in the production fermenter.
(ii) The sterilization of the medium, fermenters and ancillary equipment.
(iii) The production of an active, pure culture in sufficient quantity to inoculate the production vessel.
(iv) The growth of the organism in the production fermenter under optimum conditions for product formation.
(v) The extraction of the product and its purification.
(vi) The disposal of effluents produced by the process.

Application of fermentation:

Many of the fermentive processes are of great importance in the household and industry. An example of the household is souring of milk or formation of curd. In the latter category are the alcoholic and acid fermentations.

The ripening of cheese, the retting of flax, sannhemp, ramie, and jute, the curing of tobacco and tea leaves, and the tanning of leather are some very important examples of commercial processes dependent upon fermentations brought about by different species of bacteria.

Alcoholic fermentation is used in brewing industry, where various types of beers, whisky and wines are produced, whereas CO2 of alcoholic fermentation is used in baking industry for making bread and biscuits. Vinegar is produced by fermentation activity of acetic acid bacteria. Cleaning of raw hides is done by fermentive activity of bacteria.



Products of Fermentation

Fermentation products include:

Food products: from milk (yogurt, kefir, fresh and ripened cheeses), fruits (wine, vinegar), vegetables (pickles, sauerkraut, soy sauce), meat (fermented sausages, salami)

Industrial chemicals: (solvents: acetone, butanol, ethanol, enzymes, amino acids)

Specialty chemicals (vitamins, pharmaceuticals)

Antibiotics:

Antibiotic	Produced by	Activity	Chemical nature
Amphotericin B	*Sterptomyces nodosus*	antifungal	polyene
bacitracin	*Bacillus subtilis*	Gram +	peptide
Celphosporin C	*Cephalosporium acermonium*	Gram+	peptide
cycloheximide	*S.griseus*	antifugal	peptide
fungimycin	*S.coelicolor*	antifugal	polyene
gentamycin	*Micromonospora purpurea*	Gram+	aminoglyoside
gramicidin	*Bacillus brevis*	Gram+	peptide
sterptomycin	*S.grseus*	Gram+, mycobacteria	aminoglcoside
trichomycin	*S.hachijoensis*	antifugal	polyene

Enzymes:

Enzymes	Source	Applications	Use in the form
Alpha-amylase	*Bacillus licheniformis*	Hydrolysis of strach to dextrans	Extracelluler,soluble
glucoamylase	*Aspergillus niger*	Dextran hydrolisis to glucose	Extracellular,soluble
Xylose isomerase	*Bacillus coagulans*	Pure glucose to equilibrium mixture of glucose + fructose	Immobilized whole cell
Alkaline protease	*B.licheniformis*	detergent	Extracellular,soluble
Neutral protease	*B.subtilis*	Protien digestion in brewing sub	extracellular.,soluble
lipase	*A.niger*	Detergent, lipid	Extracellular,soluble

i) Amino acid production-

a) Lysine
b) Glutamic acid

ii) Organic acids

a) Latic acid
b) Citric acid
c) Acidic acid

There are three different process of fermentation viz.:
(1) Batch fermentation
(2) Feb-batch fermentation and
(3) Continuous culture

Batch fermentation:

This term is attributed to that type of fermentation wherein there is change in culture medium, number of microorganisms and the amount of the product produced (i.e. the metabolite or target protein). In batch fermentation six phases of the microbial growth are seen.

(a) Lag phase:

Immediately after inoculation, there is no increase in the numbers of the microbial cells for some time and this period is called lag phase. This is in order that the organisms adjust to the new environment they are inoculated into.

(b) Acceleration phase:

The period when the cells just start increasing in numbers is known as acceleration phase.

(c) Log phase:

This is the time period when the cell numbers steadily increase.

(d) Deceleration phase:

The duration when the steady growth declines.

(e) Stationary phase:

The period where there is no change in the microbial cell number is the stationary phase. This phase is attained due to depletion of carbon source or accumulation of the end products.

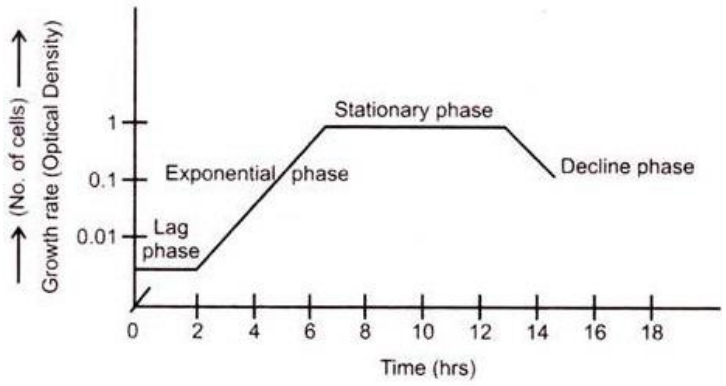


Fig. 3.1: Growth pattern of bacteria in batch culture

(f) Death phase:

The period in which the cell numbers decrease steadily is the death phase. This is due to death of the cells because of cessation of metabolic activity and depletion of energy re- sources. Depending upon the product required the different phases of the cell growth are maintained. For microbial mass the log phase is preferred. For production of secondary metabolites i.e. antibiotics, the stationary phase is preferred.

2. Fed-batch culture:

- Fed-batch culture is also called as semi-closed system of cultivation.
- In this technique, at first nutrient media is prepared and it is inoculated with culture organism and then incubated for particulate time.
- During the course of incubation a particular nutrient is added at intervals without removing the used up media.so the volume of culture increases continuously.
- Fed batch culture technique is applied in many types of fermentation process.
- In fermentation some nutrient is very essential for the process but when these nutrients are provided in higher concentration in the culture they inhibit the growth of bacteria ultimately ceasing the fermentation. Therefore such nutrients are kept in lower concentration initially and it is added slowly and continuously during the course of fermentation.

Advantage:

Fed batch culture gives greater product yields than batch culture technique.

Disadvantage:

Chance of contamination of culture is higher in fed-batch than batch culture technique.

3. Continuous culture technique

- Continuous culture technique is also called as open system of cultivation.
- In this technique fresh sterile medium is added continuously in the vessel and used up media with bacterial culture is removed continuously at the same rate. So the volume and bacterial density remain same in the cultivation vessel.
- In this technique, bacteria grow continuously in their log phase. This type of growth is known as steady state growth.
- The cell density in continuous culture remains constant and it is achieved by maintaining constant dilution and flow rate.

Types of approach to continuous culture

i. Chemostat:

- It is the most common type of approach which controls the population density and growth of culture.
- Two elements are used in chemostat, the dilution rate and concentration of limiting nutrient.
- In continuous culture, end products do not accumulates and nutrients are not completely depleted, therefore bacteria never reach in stationary phase because fresh nutrients are supplied continuously and end products are removed continuously.
- In chemostat, the liquid media contain some nutrient in growth limiting concentration and the concentration of limiting nutrient determines the rate of bacterial growth.
- During steady state chemostat, concentration of limiting nutrient remains constant because the rate of addition of nutrient equals the rate at which it is used by organism plus flow through outlet.

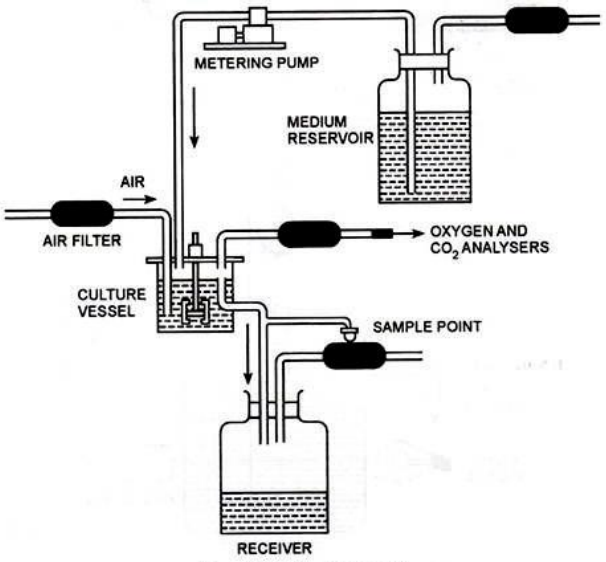


Fig. 18.25. The Chemostat

- To check whether there is constant cell density or not, concentration of that essential nutrient in the vessel is checked.
- If the concentration of that nutrient is altered then it indicates bacterial density is changing. Therefore in this case flow rate is adjusted to maintain constant cell density.

ii. Turbidostat:

- In turbidostat, a photoelectric device is used to monitor the cell density in the cultivation vessel.
- The optical sensing device measure the turbidity (absorbance) of the culture in the vessel.
- If concentration is altered, it is noticed by the photoelectric device and the flow rate is adjusted to
- Maintain constant cell density in the culture.

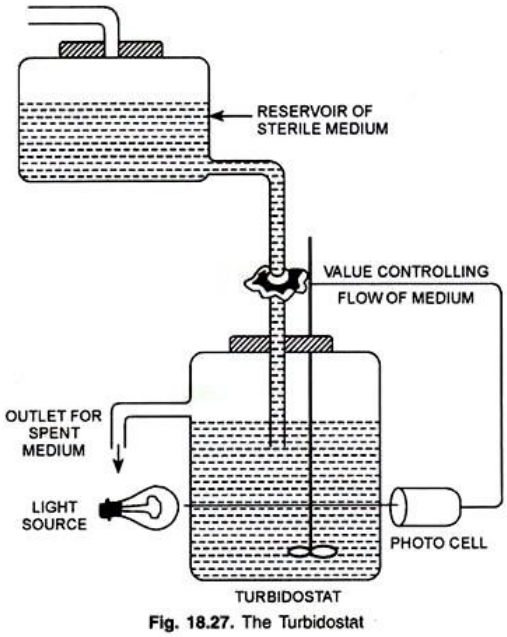


Fig. 18.27. The Turbidostat

Advantages of continuous fermentation:

1. The size of the bioreactor and other equipment used in continuous fermentation are relatively smaller compared to batch fermentation for the production of the same quantity of product.
2. The yield of the product is more consistent since the physiological state of the cells is uniform.
3. The 'down time' between two successive fermentations for cleaning and preparing the bioreactor for reuse is avoided in continuous fermentation.
4. Continuous fermentation can be run in a cost-effective manner.

Disadvantages of continuous fermentation:

Despite many advantages of continuous fermentation (described above), it is not very widely used in industries. Some of the drawbacks are listed.

1. Continuous fermentation may run continuously for a period of 500 to 1,000 hours. Maintenance of sterile conditions for such a long period is difficult.
2. The recombinant cells with plasmid constructs cannot function continuously and therefore the product yield decreases.
3. It is not easy to maintain the same quality of the culture medium for all the additions. Nutrient variations will alter the growth and physiology of the cells, and consequently the product yield.

Bioreactors:

A bioreactor (fermentor) is a closed vessel with adequate arrangement for aeration, agitation, temperature and pH control, and drain or overflow vent to remove the waste biomass of cultured microorganisms along-with their products.

A fermentor is used for commercial production in fermentation industries and is a device in which a substrate of low value is utilized by living cells or enzymes to generate a product of higher value. Fermentors are extensively used for food processing, fermentation, waste treatment, etc

Design of bioreactor:

All bioreactors deal with heterogeneous systems dealing with two or more phases, e.g., liquid, gas, solid. Therefore, optimal conditions for fermentation necessitate efficient transfer of mass, heat and momentum from one phase to the other. Chemical engineering principles are employed for design and operation of bioreactors.

A bioreactor should provide for the following:

(i) Agitation (for mixing of cells and medium),
(ii) Aeration (aerobic bioreactor); for O2 supply,
(iii) Regulation of factors like temperature, pH, pressure, aeration, nutrient feeding, liquid level etc.,
(iv) Sterilization and maintenance of sterility, and
(v) Withdrawal of cells/medium (for continuous bioreactor).

Modern fermentors are usually integrated with computers for efficient process monitoring, data acquisition, etc.

Generally, 20-25% of fermentor volume is left unfilled with medium as "head space" to allow for splashing, foaming and aeration. The fermentor design varies greatly depending on the type and the fermentation for which it is used. Bioreactors are so designed that they provide the best possible growth and biosynthesis for industrially important cultures and allow ease of manipulation for all operations.

Size of Fermentors:

The size of fermentors ranges from 1-2 litre laboratory fementors to 5,00,000 litre or, occasionally, even more, fermentors of upto 1.2 million litres have been used. The size of the fermentor used depends on the process and how it is operated. A summary of fermentor or size of fermentor (litres) Industrial product sizes for some common microbial fermentations is given in Table.

Construction of Fermentors:

Industrial fermentors can be divided into two major classes, anaerobic and aerobic. Anaerobic fermentors require little special equipment except for removal of heat generated during the fermentation process, whereas aerobic fermentors require much more elaborate equipment to ensure that mixing and adequate aeration are achieved.

Since most industrial fermentation process are aerobic, the construction of a typical aerobic fermentor is the following:

1.

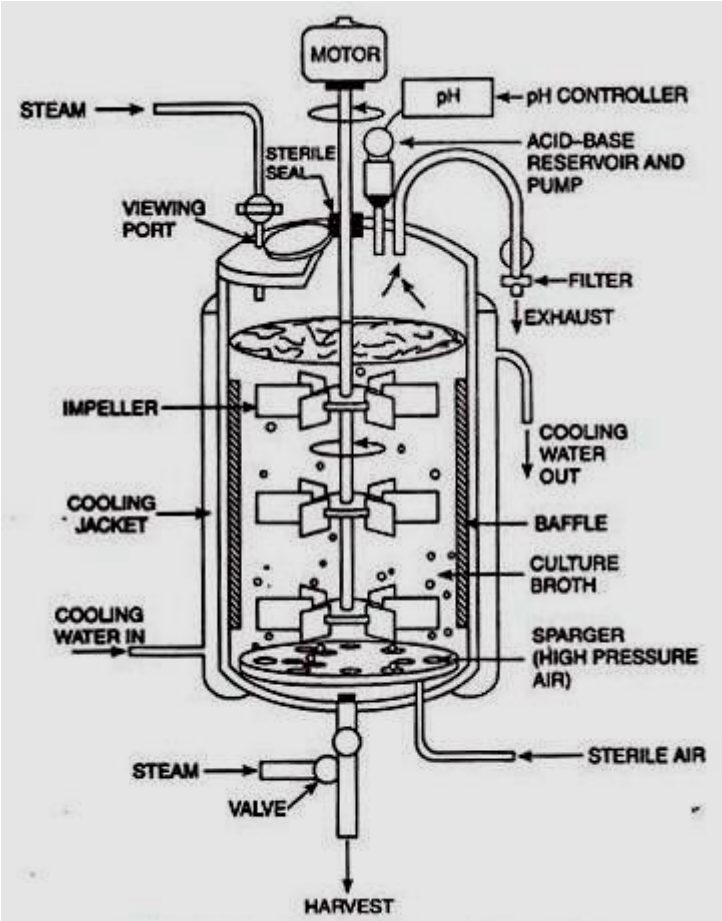


FIG. 39.1. An industrial aerobic fermentor (internal view)

Cooling Jacket:

Large-scale industrial fermentors are almost always constructed of stainless steel. A fermentor is a large cylinder closed at the top and the bottom and various pipes and valves are fitted into it. The fermentor is fitted externally with a cooling jacket through which steam (for sterilization) or cooling water (for cooling) is run.

Cooling jacket is necessary because sterilization of the nutrient medium and removal of the heat generated are obligatory for successful completion of the fermentation in the fermentor. For very large fermentors, insufficient heat transfer takes place through the jacket and therefore, internal coils are provided through which either steam or cooling water is run.

2. Aeration System:

Aeration system is one of the most critical part of a fermentor. In a fermentor with a high microbial population density, there is a tremendous oxygen demand by the culture, but oxygen being poorly soluble in water hardly transfers rapidly throughout the growth medium.

It is necessary, therefore, that elaborate precautions are taken using a good aeration system to ensure proper aeration an oxygen availability throughout the culture. However, two separate aeration devices are used to ensure proper aeration in fermentor. These devices are sparger and impeller.

The sparger is typically just a series of holes in a metal ring or a nozzle through which filter- sterilized air (or oxygen-enriched air) passes into the fermentor under high pressure. The air enters the fermentor as a series of tiny bubbles from which the oxygen passes by diffusion into the liquid culture medium.

The impeller (also called agitator) is an agitating device necessary for stirring of the fermenter.
The stirring accomplishes two things:
(i) It mixes the gas bubbles through the liquid culture medium and
(ii) It mixes the microbial cells through the liquid culture medium. In this way, the stirring ensures uniform access of microbial cells to the nutrients.
The size and position of the impeller in the fermentor depends upon the size of the fermentor. In tall fermentors, more than one impeller is needed if adequate aeration and agitation is to be obtained. Ideally, the impeller should be 1/3 of the fermentors diameter fitted above the base of the fermentor. The number of impeller may vary from size to size to the fermentor.

3. Baffles:

The baffles are normally incorporated into fermentors of all sizes to prevent a vortex and to improve aeration efficiency. They are metal strips roughly one-tenth of the fermentors diameter and attached radially to the walls.

4. Controlling Devices for Environmental Factors:

In any microbial fermentation, it is necessary not only to measure growth and product formation but also to control the process by altering environmental parameters as the process proceeds. For this purpose, various devices are used in a fermentor. Environmental factors that are frequently controlled includes temperature, oxygen concentration, pH, cells mass, levels of key nutrients, and product concentration.

Use of Computer in Fermentor:

Computer technology has produced a remarkable impact in fermentation work in recent years and the computers are used to model fermentation processes in industrial fermentors. Integration of computers into fermentation systems is based on the computers capacity for process monitoring, data acquisition, data storage, and error-detection.
Some typical, on-line data analysis functions include the acquisition measurements, verification of data, filtering, unit conversion, calculations of indirect measurements, differential integration calculations of estimated variables, data reduction, tabulation of results, graphical presentation of results, process stimulation and storage of data.

Types of Fermentor:

The fermentor (bioreactor) types used extensively in industries are the stirred tank fermentor, airlift fermentor, and bubble column fermentor.

(i) Stirred Tank Fermentor:

Stirred tank fermentors consists of a cylindrical vessel with a motor driven central saft that supports one or more impellers.

(ii) Airlift Fermentor:

In airlift fermentor the liquid culture volume of the vessel is divided into two interconnected zones by means of a baffle or draft tube. Only one of the two zones is sparged with air or other gas and this sparged zone is known as the riser.
The other zone that receives no gas is called down-comer. The bulk density of the gas-liquid dispersion in the gas-sparged riser tends to be lower than the bulk density in the down-comer, consequently the dispersion flows up in the riser zone and down-flow occurs in the down- comer.
Airlift fermentors are highly energy-efficient and are often used in large-scale manufacture of biopharmaceutical proteins obtained from fragile animal cells. Heat and mass transfer capabilities of airlift reactors are at least as good as those of other systems, and airlift reactors are more effective in suspending solids than are bubble column fermentors.

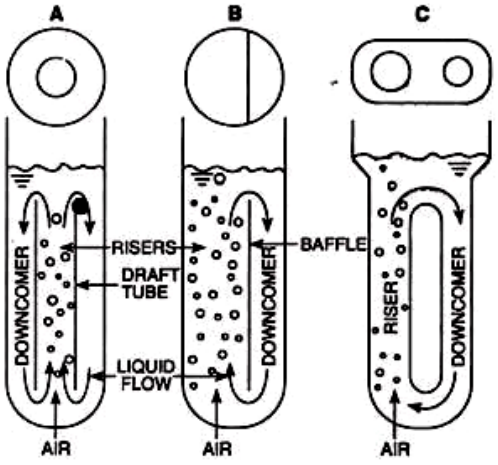


FIG. 39.2. Airlift fermentor. (A) Draft-tube internal loop configuration, (B) a split cylinder device, and (C) an external loop device.

All performance characteristics of airlift -fermentor are related ultimately to the gas injection rate and the resulting rate of liquid circulation. Usually, the rate of liquid circulation increases with the square root of the height of the airlift device.

Because the liquid circulation is driven by the gas hold-up difference between the riser and the down-comer, circulation is enhanced if there is little or no gas in the down-comer. All the gas in the down-comer comes from being entrained in with the liquid as it flows into the down-comer from the riser near the top of the reactor.

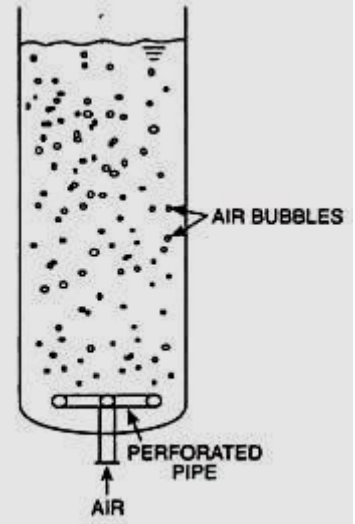


FIG. 39.3. A bubble column fermentor.

(iii) Bubble Column Fermentor:

A bubble column fermentor is usually cylindrical with an aspect (height-to- diameter) ratio of 4-6. Gas is sparged at the base of the column through perforated pipes, perforated plates, or sintered glass or metal micro-porous spargers.

O_2transfer, mixing and other performance factors are influenced mainly by the gas flow rate and the rheological properties of the fluid. Internal devices such as horizontal perforated plates, vertical baffles and corrugated sheet packing's may be placed in the vessel to improve mass transfer and modify the basic design.

The column diameter does not affect its behaviour so long as the diameter exceeds 0.1 m. One exception is the axial mixing performance. For a given gas flow rate, the mixing improves with increasing vessel diameter. Mass and heat transfer and the prevailing shear rate increase as gas flow rate is increased.

Microbial strain improvement:

Strain- A Strain is a group of species with one/ more characteristics that distinguish it from other sub groups of the same species of the strain. Each strain is identified by a name, number or letter. Example:- *E.coli* Strain K12.

Strain Improvement- The Science and Technology of manipulating and improving microbial strains in order to enhance their metabolic capacities is known as Strain Improvement

Ideal Characteristics of Strain

- Rapid growth
- Genetic stability
- Non-toxicity to humans
- Ability to use cheaper substrates
- Elimination of the production of compounds that may interfere with downstream processing
- To improve the use of carbon and nitrogen sources.
- Reduction of cultivation cost
- Shorter fermentation time.

Purpose of Strain Improvement

- Increase the productivities
- Regulating the activity of the enzymes
- Introducing new genetic properties into the organism by Recombinant DNA technology / Genetic engineering.

1. Methods of Strain Improvement:

A mutant requiring oleic acid for neomycin formation by Streptomyces fradiae showed a decrease in the intracellular level of neomycin precursors in the mutant. On the other hand supersensitive mutants of β-lactam antibiotics are another example.

Recent approaches towards strain improvement are given below:

(i) Role of Plasmid:

Plasmid genes are involved in antibiotic production in Streptomyces spp. Although, plasmids are involved in genetic characteristics on curing experiments. Involvement of plasmids in biosynthesis of aureothricin and kasugamycin in Str. kasuaensis was demonstrated more than decades ago by Okanishi (1970). The genetic study using Str. venezuelae ISP 5230 a chloramphenicol (CM) producer contains most of the structural genes for the CM biosynthetic steps treated between met and ilu on the

chromosome and the plasmid played role in increasing CM production. A linear plasmid like DNA (pSLA2) of 11.2 × 106 dalton molecular weight from Streptomyces sp. produced antibiotics.

(ii) Protoplast Fusion:

Protoplast fusion is one of the useful techniques for obtaining hybrids or recombinants of different microorganism strains. Various studies have been carried out by using protoplast fusion in *Streptomyces*, *Saccharomyces*, and fungi.

Protoplast formation in Sterptomyces was first reported by Okanishi and his team in the year 1966. Further, they have worked on formation stabilization and regeneration of protoplast of *Str. griseus* and *Str. venezuelae*. Fusion of yeast protoplasts has been reported with *Sacchromyces cerevisiae*. Technique for protoplast fusion in *Brevibacterium flavum*, has been used for strain improvement.

(iii) Mutation:

Screening after major subjection of a parent strain to physical or chemical mutagen greatly increased the probability of finding improved strain.

(a) Major mutations:

It involves the selection of mutants with a pronounced change in a biochemical character of practical interest. Such variants are commonly used in genetic studies and are generally low mutants'.

They are isolated routinely from population surviving after prolonged exposure to a mutagen, for example, selection of non-pigmented *Penicillium chrysogenum* strains with high penicillin production. The initial strain of *Sterptomyces griseus* (a streptomycin producing organism) synthesized the small amount of streptomycin but its variant was isolated which produced greater amount of streptomycin.

For further improvement it is also necessary to study the biosynthetic pathways which contribute to the identification of precursors as in case of a modified tetracycline synthesized by a mutant strain of *Str. aureofacies*.

The molecule got changed at the C-5 position and was almost devoid of antibiotic activity. Another mutant strain S-604 synthesized 6-dimethyl tetracycline, a new antibiotic, not elaborated by the parent strains, proved to have several advantages. Today it is one of leading commercial forms of tetracycline.

(b) Minor mutations:

It plays a dominant role in strains improvement. By definition such mutation affects only the amount of product synthesized. Such variants are usually phenotypically similar to the parent, with rapid and abundant mycelial and conidial development.

A 10 to 15% increase in conidial population exposed to moderate doses of a mutagen, obtained after repeated isolation of minor (positive) variants and using each succeeding strain for further mutation and selection Such increases have also been obtained by repeated selection without the introduction of mutagen In this case, the population to be tested must be large and assay for the desired product also must be accurate and specific.

This technique fetched importance in improving P. chrysogenum. For example, Wisconsin series were the famous Q-176 culture with significantly improved antibiotic titres, and strains BL3-D10, which does not produce the characteristic and trouble some chrysogenin pigment. All further mutant selections over the next decade were derived from Q-176.

2. Mutation Concept for Strain Development:

Strains selected as obvious variants after exposure to mutagen are usually inferior in their capacity for accumulation of antibiotic. Improvements are extremely few and their selection and evaluation is extremely important.

Mutagen dose is important. Mutants sought for major mutation rates are best isolated from populations surviving prolonged doses of mutagen, whereas variants for increased productivity are generally isolated from population surviving intermediates dose level.

Strains with enhanced altered morphology, etc. may be inherently better producers but may require considerable fermentation development. Step wise selection implies small increment in productivity, and the probability of getting hyper producing strains decreases.

Variant strains may require special propogation and preservation procedures and actual production gains depend also on stability and reliability of performance.

Though, strains may prove better in their productivity at laboratory scale, there is no guarantee that enhanced productivity will occur in production fermenters. The long term pilot plant studies are often necessary before any enhanced strains potential can be realized in actual production.

3. Isolation of Mutant Classes and Their Use in Microbial Processes:

(i) Localized Mutagenesis and Computation:

Localized mutagenesis affecting the small selected regions of the chromosomes, offers a promising new approach. Mutation programmes can be directed to maximize mutations in any marked area on the chromosome, specially the areas known to affect the formation of end products. Isolation of strains in unknown loci linked to the revertant site can be done by a heterokaryon method or by the use of temperature sensitive mutants.

(ii) Sexual and Parasexual Processes:

In fungi, the vegetative mycelium is haploid and can be propogated almost indefinitely by serial transfer of hyphal fragments and can also be propogated by asexual spores/conidia.

Two strains of opposite mating types (A or B) are required to initiate the sexual cycle and allow to mate by mixing the conidia of mating type A with mycelia on appropriate media. After a period of nuclear division and migration, fusion between A and B nuclei takes place. Each fused nucleus (diploid) undergoes meiosis to form four haploids, which divide mitotically into eight nuclei contained in ascus.

Few of the industrially important fungi form heterokaryon in which rare diploid nuclei result from the fusion of two haploid nuclei. This process is called Parasexuality. Although, recombination in fewer fragments in the parasexual cycle compared to the meiotic process it can occur by mitotic crossing over or by other mechanism.

The importance of mitotic crossing over or recombination is that it makes possible genetic analysis and controlled breeding in organisms with no sexual cycle. Stram improvement through parasexual cycle has been reported in *P. chrysogenum* and in one study; a homozygous diploid representing parent was an efficient producer of penicillin V.

Recombinant proteins:

These are the proteins produced by the transferred gene / transgene; they themselves are of commercial value. Ex: Insulin, Interferons etc. are produced in Bacteria

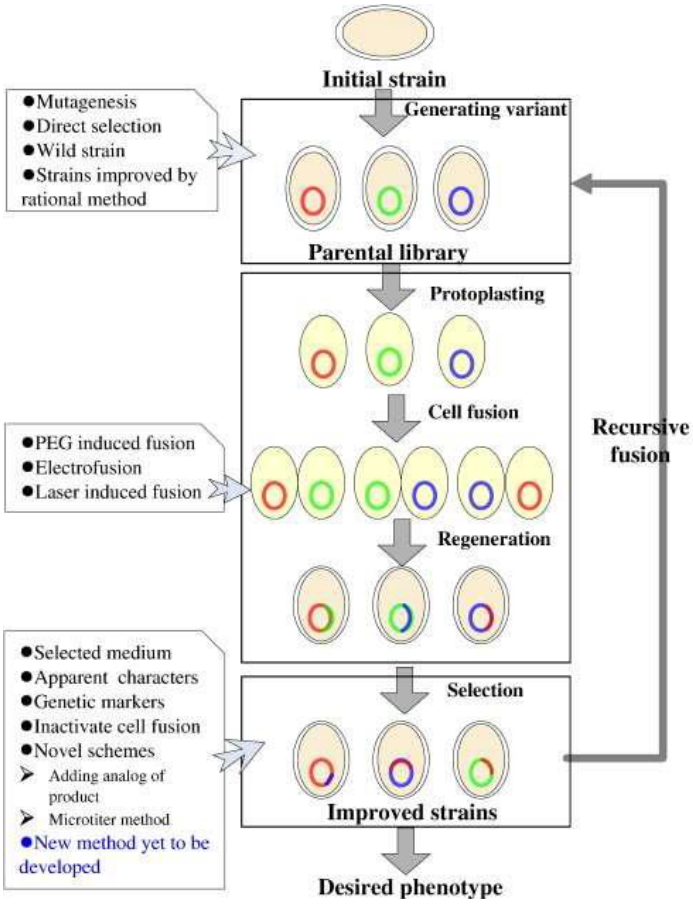
Metabolic Engineering:

When metabolic activities of an organism are modified by introducing into it transgenes, which affect enzymatic, transport and /or regulatory function of its cells its known as Metabolic Engineering. Ex: Over production of the amino acid Isoleucine in *Corynebacterium glutamicum* & Ethanol by *E.coli*.

Product Modification include the new enzymes which modifies the product of existing biosynthetic pathway e.g. Conversion of Cephalosporin C into 7-amino cephalosporanic acid by D-amino acid oxidase (in *A. chrysogenum*).

Completely new metabolite formation include in which all the genes of a new pathway are transferred e.g. *E.coli*, transfer of 2 genes for polyhydroxybutyrate synthesis from Alcaligenes eutrophus.

Enhance growth include enhanced substrate



utilization. e.g. *E.coli*, glutamate dehydrogenase into *M.methylotrophus* carbon conversion increased from 4% to 7%

Genome Shuffling

It is a novel technique for strain improvement that allows for recombination between multiple parents at each generation and several rounds of recursive genome fusion were carried out resulting in the final improved strain involving genetic trait from m ultiple initial strains.

9. Immobilization of microbial enzymes and whole cells and their applications in industries.

Immobilization of enzymes:

Immobilization of enzymes (or cells) refers to the technique of confining/anchoring the enzymes (or cells) in or on an inert support for their stability and functional reuse. By employing this technique, enzymes are made more efficient and cost-effective for their industrial use. Some workers regard immobilization as a goose with a golden egg in enzyme technology. Immobilized enzymes retain their structural conformation necessary for catalysis.

There are several advantages of immobilized enzymes:

a. Stable and more efficient in function.
b. Can be reused again and again.
c. Products are enzyme-free.
d. Ideal for multi-enzyme reaction systems.
e. Control of enzyme function is easy.
f. Suitable for industrial and medical use.
g. Minimize effluent disposal problems.

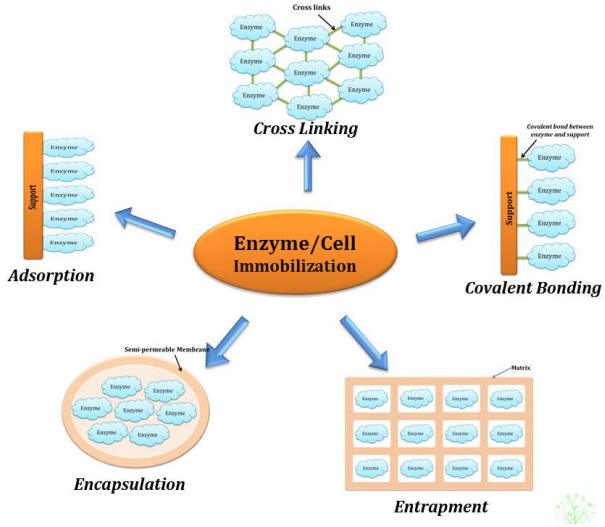
There are however, **certain disadvantages** also associated with immobilization.

a. The possibility of loss of biological activity of an enzyme during immobilization or while it is in use.
b. Immobilization is an expensive affair often requiring sophisticated equipment.

Immobilized enzymes are generally preferred over immobilized cells due to specificity to yield the products in pure form. However, there are several advantages of using immobilized multi-enzyme systems such as organelles and whole cells over immobilized enzymes. The immobilized cells possess the natural environment with cofactor availability (and also its regeneration capability) and are particularly suitable for multiple enzymatic reactions.

Methods of Immobilization:

The commonly employed techniques for immobilization of enzymes are—adsorption, entrapment, covalent binding and cross-linking.



Adsorption:

Adsorption involves the physical binding of enzymes (or cells) on the surface of an inert support. The support materials may be inorganic (e.g. alumina, silica gel, calcium phosphate gel, glass) or organic (starch, carboxymethyl cellulose, DEAE-cellulose, DEAE-sephadex).

Adsorption of enzyme molecules (on the inert support) involves weak forces such as van der Waals forces and hydrogen bonds. Therefore, the adsorbed enzymes can be easily removed by minor changes in pH, ionic strength or temperature. This is a disadvantage for industrial use of enzymes.

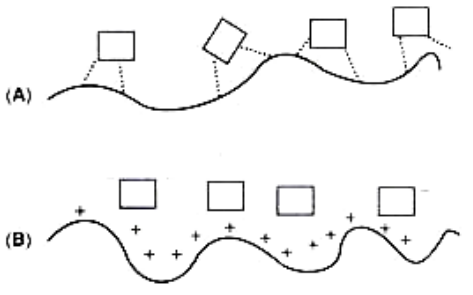


*Fig. 21.3 : Immobilization of enzymes by adsorption (A) By van der Waals forces (B) By hydrogen bonding (**Note** : Cloured blocks represent enzymes)*

Entrapment:

Enzymes can be immobilized by physical entrapment inside a polymer or a gel matrix. The size of the matrix pores is such that the enzyme is retained while the substrate and product molecules pass through. In this technique, commonly referred to as lattice entrapment, the enzyme (or cell) is not subjected to strong binding forces and structural distortions.

Some deactivation may however, occur during immobilization process due to changes in pH or temperature or addition of solvents. The matrices used for entrapping of enzymes include polyacrylamide gel, collagen, gelatin, starch, cellulose, silicone and rubber. Enzymes can be entrapped by several ways.

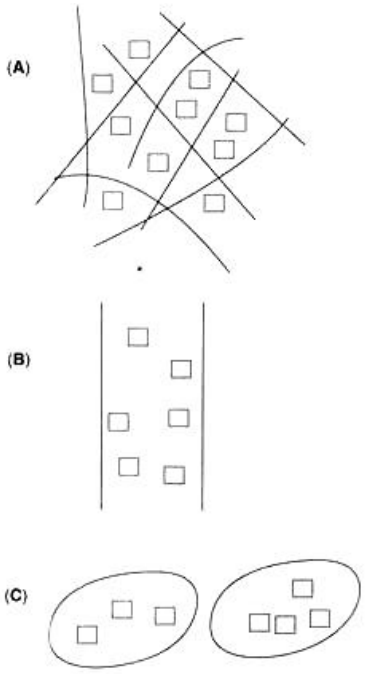


*Fig. 21.4 : Immobilization of enzymes by entrapment (A) Inclusion in gels (B) Inclusion in fibres (C) Inclusion in microcapsules (**Note** : Coloured blocks represent enzymes)*

1. Enzyme inclusion in gels:

This is an entrapment of enzymes inside the gels.

2. Enzyme inclusion in fibres:

The enzymes are trapped in a fibre format of the matrix.

3. Enzyme inclusion in microcapsules:

In this case, the enzymes are trapped inside a microcapsule matrix. The hydrophobic and hydrophilic forms of the matrix polymerise to form a microcapsule containing enzyme molecules inside. The major limitation for entrapment of enzymes is their leakage from the matrix. Most workers prefer to use the technique of entrapment for immobilization of whole cells. Entrapped cells are in use for industrial production of amino acids (L-isoleucine, L-aspartic acid), L-malic acid and hydroquinone.

Microencapsulation:

Microencapsulation is a type of entrapment. It refers to the process of spherical particle formation wherein a liquid or suspension is enclosed in a semipermeable membrane. The membrane may be polymeric, lipoidal, lipoprotein-based or non-ionic in nature. There are three distinct ways of microencapsulation.

1. Building of special membrane reactors.
2. Formation of emulsions.
3. Stabilization of emulsions to form microcapsules.

Microencapsulation is recently being used for immobilization of enzymes and mammalian cells. For instance, pancreatic cells grown in cultures can be immobilized by microencapsulation. Hybridoma cells have also been immobilized successfully by this technique.

Covalent Binding:

Immobilization of the enzymes can be achieved by creation of covalent bonds between the chemical groups of enzymes and the chemical groups of the support. This technique is widely used. However, covalent binding is often associated with loss of some enzyme activity. The inert support usually

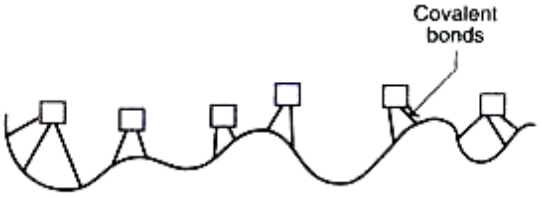


*Fig. 21.5 : A general representation of immobilization of enzymes by covalent binding (**Note** : coloured blocks represent enzymes).*

requires pretreatment (to form pre-activated support) before it binds to enzyme. The following are the common methods of covalent binding.

1. Cyanogen bromide activation:

The inert support materials (cellulose, sepharose, sephadex) containing glycol groups are activated by CNBr, which then bind to enzymes and immobilize them.

2. Diazotation:

Some of the support materials (amino benzyl cellulose, amino derivatives of polystyrene, aminosilanized porous glass) are subjected to diazotation on treatment with $NaNO_2$ and HCI. They, in turn, bind covalently to tyrosyl or histidyl groups of enzymes.

3. Peptide bond formation:

Enzyme immobilization can also be achieved by the formation of peptide bonds between the amino (or carboxyl) groups of the support and the carboxyl (or amino) groups of enzymes. The support material is first chemically treated to form active functional groups.

4. Activation by bi- or poly-functional reagents:

Some of the reagents such as glutaraldehyde can be used to create bonds between amino groups of enzymes and amino groups of support (e.g. aminoethylcellulose, albumin, amino alkylated porous glass).

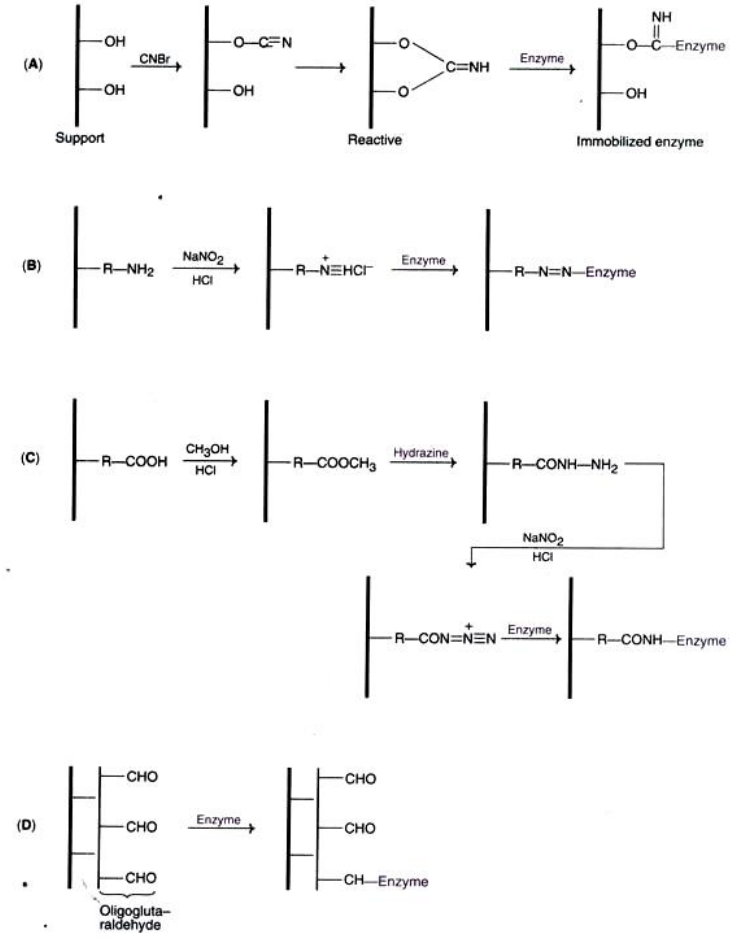


Fig. 21.6 : Immobilization of enzymes by covalent binding (A) Cyanogen bromide activation, (B) Diazotation, (C) Peptide bond formation, (D) Activation by bifunctional agent.

Cross-Linking:

The absence of a solid support is a characteristic feature of immobilization of enzymes by cross-linking. The enzyme molecules are immobilized by creating cross-links between them, through the involvement of poly-functional reagents. These reagents in fact react with the enzyme molecules and create bridges which form the backbone to hold enzyme molecules. There are several reagents in use for cross-linking. These include glutaraldehyde, diazobenzidine, hexamethylene diisocyanate and toluene di- isothiocyanate.

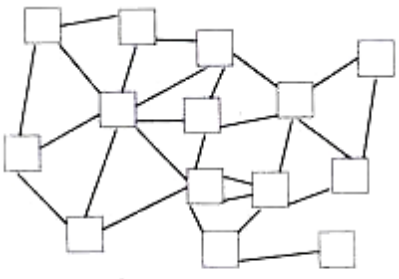


Fig. 21.7 : Immobilization of enzyme molecules by cross linking.

Glutaraldehyde is the most extensively used cross-linking reagent. It reacts with lysyl residues of the enzymes and forms a Schiff's base. The cross links formed between the enzyme and glutaraldehyde

are irreversible and can withstand extreme pH and temperature. Glutaraldehyde cross- linking has been successfully used to immobilize several industrial enzymes e.g. glucose isomerase, penicillin amidase. The technique of cross-linking is quite simple and cost-effective. But the disadvantage is that it involves the risk of denaturation of the enzyme by the poly-functional reagent.

Choice of Immobilization Technique:

The selection of a particular method for immobilization of enzymes is based on a trial and error approach to choose the ideal one. Among the factors that decide a technique, the enzyme catalytic activity, stability, regenerability and cost factor are important.

Immobilization of L-amino acid acylase:

L-Amino acid acylase was the first enzyme to be immobilized by a group of Japanese workers (Chibata and Tosa, 1969). More than 40 different immobilization methods were attempted by this group. Only three of them were found be useful. They were covalentbinding to iodoacetyl cellulose, ionic binding to DEAE-Sephadex and entrapment within polyacrylamide.

Stabilization of Soluble Enzymes:

Some of the enzymes cannot be immobilized and they have to be used in soluble form e.g. enzymes used in liquid detergents, some diagnostic reagents and food additives. Such enzymes can be stabilized by using certain additives or by chemical modifications. The stabilized enzymes have longer half-lives, although they cannot be recycled. Some important methods of enzyme stabilization are briefly described.

Solvent Stabilization:

Certain solvents at low concentrations stabilize the enzymes, while at high concentrations the enzymes get denatured e.g. acetone (5%) and ethanol (5%) can stabilize benzyl alcohol dehydrogenase.

Substrate Stabilization:

The active site of an enzyme can be stabilized by adding substrates e.g. starch stabilizes a- amylase; glucose stabilizes glucose isomerase.

Stabilization by Polymers:

Enzymes can be stabilized, particularly against increased temperature, by addition of polymers such as gelatin, albumin and polyethylene glycol.

Stabilization by Salts:

Stability of metalloenzymes can be achieved by adding salts such as Ca, Fe, Mn, Cu and Zn
e.g. proteases can be stabilized by adding calcium.

Stabilization by Chemical Modifications:

Enzymes can be stabilized by suitable chemical modifications without loss of biological activity. There are several types of chemical modifications.

a. Addition of poly-amino side chains e.g. polytyrosine, polyglycine.
b. Acylation of enzymes by adding groups such as acetyl, propionyl and succinyl

Stabilization by Rebuilding:

Theoretically, the stability of the enzymes is due to hydrophobic interactions in the core of the enzyme. It is therefore, proposed that enzymes can be stabilized by enhancing hydrophobic interactions. For this purpose, the enzyme is first unfold and then rebuilt in one of the following ways.

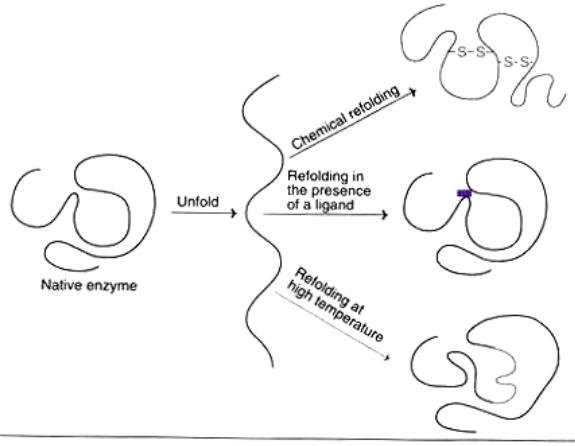


Fig. 21.8 : Stabilization of an enzyme by refolding.

1. The enzyme can be chemically treated (e.g. urea and a disulfide) and then refolded.
2. The refolding can be done in the presence of low molecular weight ligands.
3. For certain enzymes, refolding at higher temperatures (around 50°C) stabilize them.

Stabilization by Site-Directed Mutagenesis:

Site-directed mutagenesis has been successfully used to produce more stable and functionally more efficient enzymes e.g. subtilisin E.

Immobilization of Cells:

Immobilized individual enzymes can be successfully used for single-step reactions. They are, however, not suitable for multi-enzyme reactions and for the reactions requiring cofactors. The whole cells or cellular organelles can be immobilized to serve as multi-enzyme systems,

In addition, immobilized cells rather than enzymes are sometimes preferred even for single reactions, due to cost factor in isolating enzymes. For the enzymes which depend on the special arrangement of the membrane, cell immobilization is preferred.

Immobilized cells have been traditionally used for the treatment of sewage. The techniques employed for immobilization of cells are almost the same as that used for immobilization of enzymes with appropriate modifications. Entrapment and surface attachment techniques are commonly used. Gels, and to some extent membranes, are also employed.

Immobilized Viable Cells:

The viability of the cells can be preserved by mild immobilization. Such immobilized cells are particularly useful for fermentations. Sometimes mammalian cell cultures are made to function as immobilized viable cells.

Immobilized Non-viable Cells:

In many instances, immobilized non-viable cells are preferred over the enzymes or even the viable cells. This is mainly because of the costly isolation and purification processes. The best example is the immobilization of cells containing glucose isomerase for the industrial production of high fructose syrup. Other important examples of microbial biocatalysts and their applications are given in Table.

Table 21.5 Selected examples of immobilized cells (to bring out one or two enzyme reactions) in industrial applications

Immobilized microorganism (microbial biocatalyst)	*Application(s)*
Escherichia coli	For the synthesis of L-aspartic acid from fumaric acid and NH_3
Escherichia coli	For the production of L-tryptophan from indole and serine
Pseudomonas sp	Production of L-serine from glycine and methanol
Saccharomyces cerevisiae	Hydrolysis of sucrose
Saccharomyces sp	Large scale production of alcohol
Zymomonas mobilis	Synthesis of sorbitol and gluconic acid from glucose and fructose
Anthrobacter simplex	Synthesis of prednisolone from hydrocortisone
Pseudomonas chlororaphis	Production of acrylamide from acrylonitrile
Humicola sp	For the conversion of rifamycin B to rifamycin S
Bacteria and yeasts (several sp)	In biosensors

Limitations of Immobilizing Eukaryotic Cells:

Prokaryotic cells (particularly bacterial) are mainly used for immobilization. It is also possible to immobilize eukaryotic plant and animal cells. Due to the presence of cellular organelles, the metabolism of eukaryotic cells is slow. Thus, for the industrial production of biochemical, prokaryotic cells are preferred. However, for the production of complex proteins (e.g. immunoglobulin's) and for the proteins that undergo post- translational modifications, eukaryotic cells may be used.

Effect of Immobilization on Enzyme Properties:

Enzyme immobilization is frequently associated with alterations in enzyme properties, particularly the kinetic properties of enzymes.

Some of them are listed below:

1. There is a substantial decrease in the enzyme specificity. This may be due to conformational changes that occur when the enzyme gets immobilized.
2. The kinetic constants Km and Vmax of an immobilized enzyme differ from that of the native enzyme. This is because the conformational change of the enzyme will affect the affinity between enzyme and substrate.

Immobilized Enzyme Reactors:

The immobilized enzymes cells are utilized in the industrial processes in the form of enzyme reactors. They are broadly of two types — batch reactors and continuous reactors.

Batch Reactors:

In batch reactors, the immobilized enzymes and substrates are placed, and the reaction is allowed to take place under constant stirring. As the reaction is completed, the product is separated from the enzyme (usually by denaturation).

Soluble enzymes are commonly used in batch reactors. It is rather difficult to separate the soluble enzymes from the products; hence there is a limitation of their reuse. However, special techniques have been developed for recovery of soluble enzymes, although this may result in loss of enzyme activity.

Stirred tank reactors:

The simplest form of batch reactor is the stirred tank reactor. It is composed of a reactor fitted with a stirrer that allows good mixing, and appropriate temperature and pH control. However, there may occur loss of some enzyme activity. A modification of stirred tank reactor is basket reactor. In this system, the enzyme is retained over the impeller blades. Both stirred tank reactor and basket reactor have a well-mixed flow pattern.

Plug flow type reactors:

These reactors are alternatives to flow pattern type of reactors. The flow rate of fluids controlled by a plug system. The plug flow type reactors may be in the form of packed bed or fluidized bed. These reactors are particularly useful when there occurs inadequate product formation in flow type reactors. Further, plug flow reactors are also useful for obtaining kinetic data on the reaction systems.

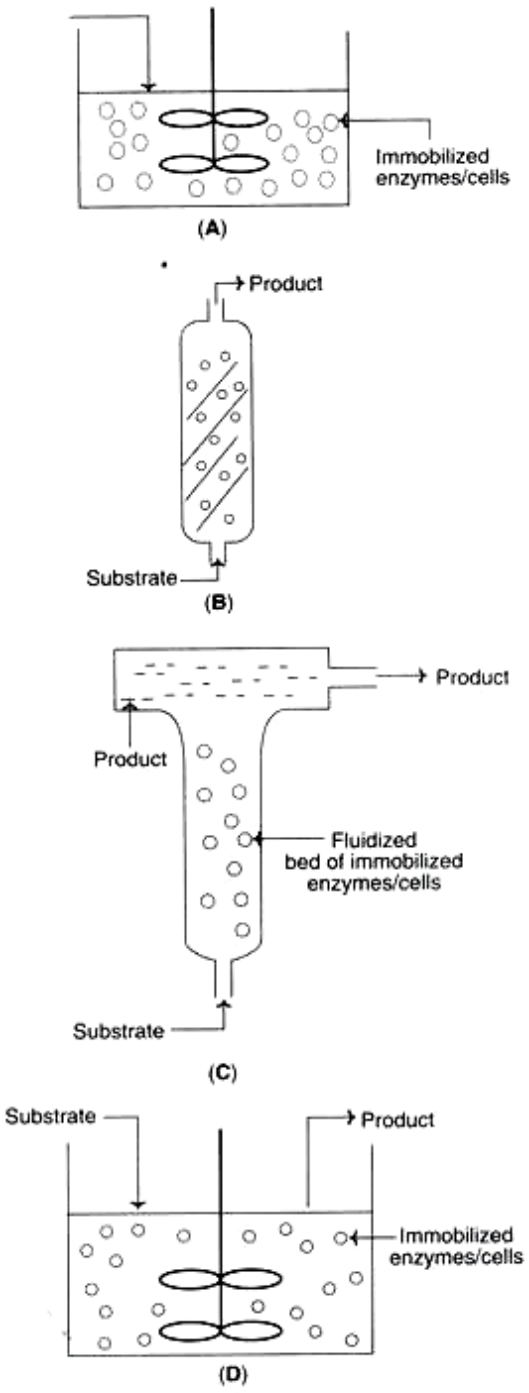


Fig. 21.9 : Immobilized enzyme (cell) reactors (A) Batch stirred tank reactor, (B) Packed bed reactc (C) Fluidized bed reactor, (D) Continuous stirred tank reactor.

Continuous Reactors:

In continuous enzyme reactors, the substrate is added continuously while the product is removed simultaneously. Immobilized enzymes can also be used for continuous operation. Continuous reactors have certain advantages over batch reactors. These include control over the product formation, convenient operation of the system and easy automation of the entire process. There are mainly two types of continuous reactors-continuous stirred tank reactor (CSTR) and plug reactor (PR). CSTR is ideal for good product formation.

Membrane Reactors:

Several membranes with a variety of chemical compositions can be used. The commonly used membrane materials include polysulfone, polyamide and cellulose acetate. The biocatalysts (enzymes or cells) are normally retained on the membranes of the reactor. The substrate is introduced into reactor while the product passes out. Good mixing in the reactor can be achieved by using stirrer. In a continuous membrane reactor, the biocatalysts are held over membrane layers on to which substrate molecules are passed.

In a recycle model membrane reactor, the contents (i.e. the solution containing enzymes, cofactors, and substrates along with freshly released product are recycled by using a pump. The product passes out which can be recovered.

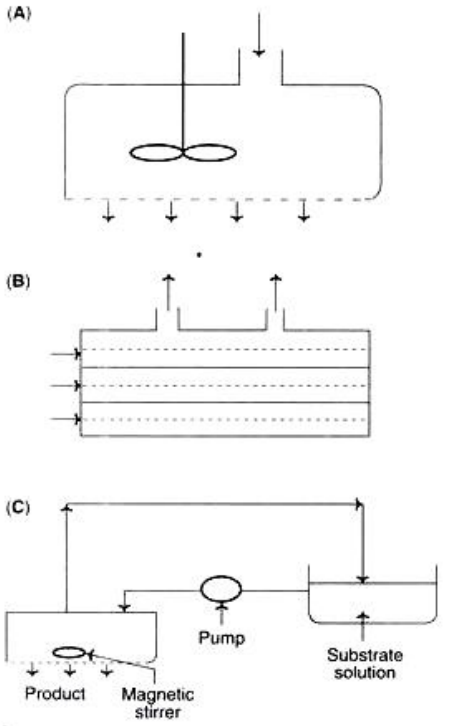


Fig. 21.10 : Membrane reactors (A) Batch membrane reactor, (B) Continuous membrane reactor, (C) Recycle membrane reactor (Coloured lines indicate membranes).

Applications of Immobilized Enzymes and Cells:

Immobilized enzymes and cells are very widely used for industrial, analytical and therapeutic purpose, besides their involvement in food production and exploring the knowledge of biochemistry, microbiology and other allied specialties. A brief account of the industrial applications of immobilized cells is given in Table.

Manufacture of Commercial Products:

A selected list of important immobilized enzymes and their industrial applications is given in Table. Some details on the manufacture of L-amino acids and high fructose syrup are given hereunder.

Table 21.6 A selected list of important immobilized enzymes and their industrial applications

Immobilized enzyme	*Application(s)*
Aminoacylase	Production of L-amino acids from D, L-acyl amino acids
Glucose isomerase	Production of high fructose syrup from glucose (or starch)
Amylase	Production of glucose from starch
Invertase	Splitting of sucrose to glucose and fructose
β-Galactosidase	Splitting of lactose to glucose and galactose
Penicillin acylase	Commercial production of semi-synthetic penicillins
Aspartase	Production of aspartic acid from fumaric acid
Fumarase	Synthesis of malic acid from fumaric acid
Histidine ammonia lyase	Production of urocanic acid from histidine
Ribonuclease	Synthesis of nucleotides from RNA
Nitrilase	Production of acrylamide from acrylonitrile

Production of L-Amino Acids:

L-Amino acids (and not D-amino acids) are very important for use in food and feed supplements and medical purposes. The chemical methods employed for their production result in a racemic mixture of D- and L-amino acids. They can be acylated to form D, L-acyl amino acids. The immobilized enzyme aminoacylase (frequently immobilized on DEAE sephadex) can selectively hydrolyse D, L-acyl amino acids to produce L-amino acids.

$$\text{D, L-Acyl amino acids} \xrightarrow{\text{Aminoacylase}} \text{L-Amino acids} + \text{D, L-Acyl amino acids}$$

The free L-amino acids can separated from the un-hydrolysed D-acyl amino acids. The latter can be recemized to D, L-acyl amino acids and recycled through the enzyme reactor containing immobilized aminoacylase. Huge quantities of L-methionine, L-phenylalanine L- tryptophan and L-valine are produced worldwide by this approach.

Production of High Fructose Syrup:

Fructose is the sweetest among the monosaccharide's, and has twice the sweetening strength of sucrose. Glucose is about 75% as sweet as sucrose. Therefore, glucose (the most abundant monosaccharide) cannot be a good substitute for sucrose for sweetening. Thus, there is a great demand for fructose which is very sweet, but has the same calorific value as that of glucose or sucrose.

High fructose syrup (HFS) contains approximately equivalent amounts of glucose and fructose. HFS is almost similar to sucrose from nutritional point of view. HFS is a good substitute for sugar in the preparation of soft drinks, processed foods and baking

High fructose syrup can be produced from glucose by employing an immobilized enzyme glucose isomerase. The starch containing raw materials (wheat, potato, corn) are subjected to hydrolysis to produce glucose. Glucose isomerase then isomerizes glucose to fructose. The product formed is HFS containing about 50% fructose. (Note: Some authors use the term high fructose corn syrup i.e. HFCS in place of HFS).

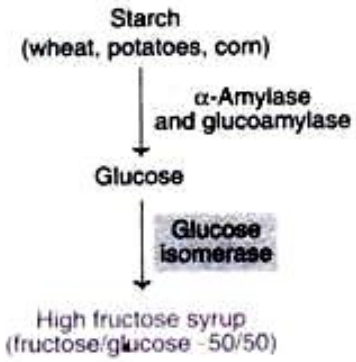


Fig. 21.11 : Production of high fructose syrup from starch (glucose isomerase is the immobilized enzyme).

Glucose isomerase:

This is an intracellular enzyme produced by a number of microorganisms. The species of Arthrobacter, Bacillus and Streptomyces are the preferred sources. Being an intracellular enzyme, the isolation of glucose isomerase without loss of biological activity requires special and costly techniques. Many a times, whole cells or partly broken cells are immobilized and used.

Immobilized Enzymes and Cells- Analytical Applications:

In Biochemical Analysis:

Immobilized enzymes (or cells) can be used for the development of precise and specific analytical techniques for the estimation of several biochemical compounds. The principle of analytical assay primarily involves the action of the immobilized enzyme on the substrate.

A decrease in the substrate concentration or an increase in the product level or an alteration in the cofactor concentration can be used for the assay. A selected list of examples of immobilized enzymes used in the assay of

TABLE 21.7 Selected examples of immobilized enzymes used in analytical biochemistry

Immobilized enzyme	*Substance assayed*
Glucose oxidase	Glucose
Urease	Urea
Cholesterol oxidase	Cholesterol
Lactate dehydrogenase	Lactate
Alcohol oxidase	Alcohol
Hexokinase	ATP
Galactose oxidase	Galactose
Penicillinase	Penicillin
Ascorbic acid oxidase	Ascorbic acid
L-Amino acid oxidase	L-Amino acids
Cephalosporinase	Cephalosporin
Monoamine oxidase	Monoamine

some substances is given in Table. Two types of detector systems are commonly employed.
Thermistors are heat measuring devices which can record the heat generated in an enzyme catalysed reaction. Electrode devices are used for measuring potential differences in the reaction system. In the Figuure, an enzyme thermistor and an enzyme electrode, along with a specific urease electrode are depicted.

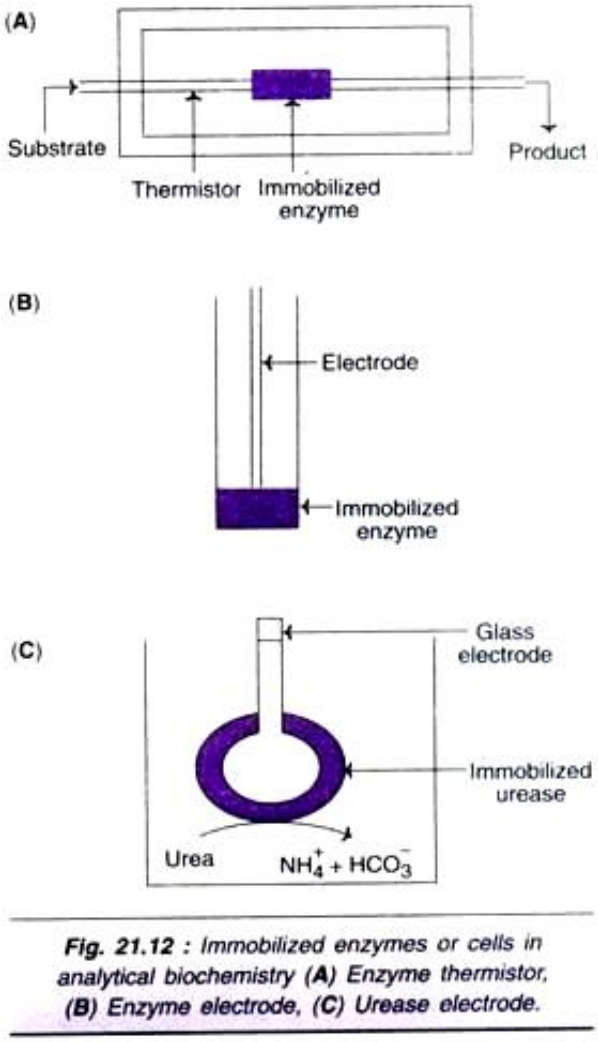


Fig. 21.12 : Immobilized enzymes or cells in analytical biochemistry (A) Enzyme thermistor, (B) Enzyme electrode, (C) Urease electrode.

In Affinity Chromatography and Purification:
Immobilized enzymes can be used in affinity chromatography. Based on the property of affinity, it is possible to purify several compounds e.g. antigens, antibodies, cofactors.

10. Microbes as food and in food processing, single cell protein

There are many useful application of microbes in the food industry. They influence the quality, availability and quantity of food. Microorganisms are used to change one substance to another which is used as food, such as milk to yoghurt and cheese, sugar to wine and bread.
Fermented Dairy Products:
Fermented milk is produced by inoculating pasteurised milk with specific culture of microorganisms. The different fermented dairy products include yoghurt and cheese.
Bacteria is used in Yoghurt Making:
Yoghurt is a dairy product which is produced by the bacterial fermentation of milk. Most commonly, cow's milk is used, though it can be made from any kind of milk. It can be prepared from a variety of milk including whole, skimmed, dried, evaporated or semi- skimmed milk.

The steps involved in yoghurt making are illustrated in Fig.

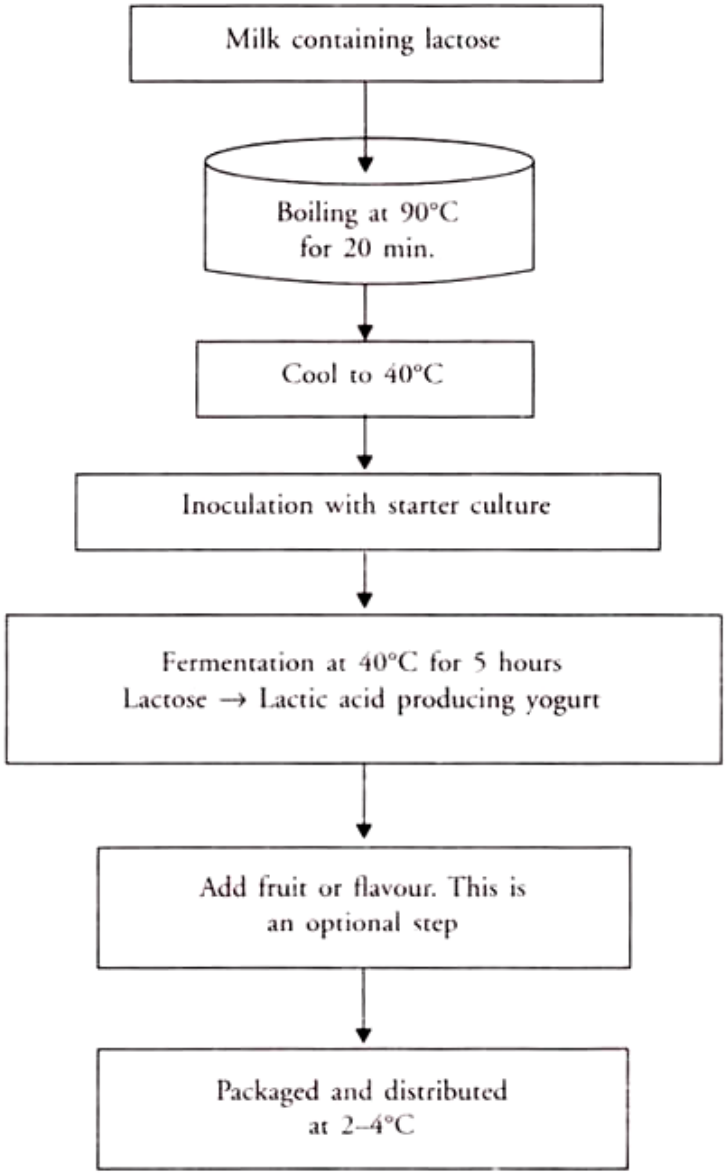


Fig. 1 Manufacture of yogurt.

The milk sugar, i.e. lactose is fermented into lactic acid by the friendly bacteria, Strepto- coccus salivarius, S. thermophilus and Lactobacillus bulgaricus. These bacteria are collectively known as lactic acid bacteria or LAB. The bacteria feed on the lactose and release lactic acid as a waste product. The acid cause the curdling of the milk protein, casein into a solid mass called curd. The gel like texture and taste of yoghurt is due to the fermentation of lactose to lactic acid. The increased acidity (pH = 4-5) also prevents the proliferation of other potentially pathogenic bacteria.

Both unpasteurised and pasteurized milk may be used for yoghurt making. The use of unpasteurised milk maintains the healthy balance of bacteria and enzymes of milk in its unprocessed state under very carefully controlled temperature and environmental conditions. To ensure complete fermentation two or more different bacteria may be used together.

Yoghurt is often sold sweetened and flavoured, or with fruit added at the bottom. The flavour varies in different countries.

a. Lassi is yoghurt-based beverage in India and is consumed either salty or sweet. Salty lassi is usually flavoured with ground- roasted cumin and black pepper powder, while the sweet variety is served with lemon, mango or other fruit juice.

b. A lassi-like, salty drink called ayran is popular in Turkey and Bulgaria and is prepared by mixing yoghurt with water and salt.

In India, Bulgaria and Turkey yogurt is prepared at home using a small amount of plain active culture yogurt as the starter culture. The milk is boiled to kill undesirable microbes. It is cooled to about 40°C. A tablespoon of starter culture is added and mixed thoroughly. It is left undisturbed for about 6 hours.

Bacteria and Fungi are used in Cheese Making:

Cheese is prepared by inoculating milk with a starter culture containing specific micro- organisms. Cheese is a solid food made from the milk of various animals, most commonly cows. Milk from goat, sheep, reindeer and water buffalo may also be used. There are several types of cheese.

Fermentation of milk leads to lactic acid production, which sours the milk. This leads to coagulation of milk protein, casein. The solid part of the milk produced by coagulation is known as curd and the liquid is known as whey.

The curds can be separated and pressed into desired shape and whey is used as food source for yeasts, which in turn can be processed as cattle feed and is rich in protein and vitamins. The cheese can be matured or ripened by the addition of bacteria or fungi or both. The bacteria added reduce the pH, alters texture and develops a flavour.

Coagulation can be controlled using rennet tablets, which contains the enzyme rennin. Rennin is an enzyme present in the stomach of Calves but now is also available in genetically engineered bacteria. Coagulation can also be done using acids such as vinegar or lemon juice.

Depending on the nature of the organism added, cheese is of the following types:

a. Cheddar cheese is prepared by the addition of bacteria to enhance its flavour and texture.
b. The use of mould fungi produces Roquefort cheese and blue cheese
c. A combination of both bacteria and fungi produces camembert cheese.
d. Swiss cheese is prepared by the addition of Propionibacterium sharmanii. The big holes in the cheese is because of the production of large amounts of CO2.

The natural colour of cheese ranges from off-white to yellow. Herbs and spices may also be added to the cheese. Other factors that contribute to a different flavours and styles of cheese are different levels of milk fat, variations in length of aging, different processing treatments and different breeds of cows, sheep or other mammals.

Cheese production steps:

Step 1. Coagulum Formation:

Milk coagulation occurs due to two distinct activities.

(i) Inoculation with bacterial cultures, e.g., *Streptococcus lactis* or *S. cremoris* for incubation at 31°C, or S. thermophilic combined- with Lactobacillus lactis. L. bulguricus or L. helveticus (for incubation at 50°C), results in lactose degradation to produce lactic acid, which lowers the pH to about 4.6.

(ii) Incubation with rennet cleaves K-casein into para-K-casein and caseino macropeptide. This cleavage occurs at a specific peptide bond between phenylalanine at position 105 and methionine at position 106 (-phe 105-met 106-), and leads to coagulation of α- and β-caseins and the K-casein hydrolysis products.

Traditionally, rennet obtained from the fourth stomach of unweaned calves has been used. But at present, rennet from microbial sources is used extensively, and is responsible for about 70% of US and 30% of the worldwide cheese productions. But the rennet obtained from Mucor miehei is relatively more thermostable and hence remains active during ripening, which often produces bitter off-flavours.

Therefore, it is treated with oxidising agents like H2O2, peracids, etc., which converts the methionine residues to their sulphoxides. This reduces the temperature tolerance of the enzyme by 10°C and makes M. miehei rennet more comparable to calf rennet. Attempts to clone calf chymosin gene in E. coli and Saccharomyces cerevisiae have been successful, but active renin is secreted only by the yeast cells.

Step 2. Separation of Curd:
The coagulum is heated to 37°C and cooled. This eliminates the remaining rennet activity and separates, to some extent the watery fluid called whey. The curd is separated from whey, salted, and mixed with proteases and/or lipases; alternatively, bricks of cheese may be inoculated with specific strains if fungi, e.g., Penicillium roquefortii, P. camembertii, etc. The bricks are pressed to remove excess moisture to enable proper ripening.

Step 3. Ripening:
Ripening procedures will vary with the type of cheese to be produced. The cheese bricks are inoculated with specific strains of fungi for the development of appropriate flavours through protease and lipase activities. Alternatively, proteases and lipases may be used for this purpose. Proteases from *Bacillus amyloliquefaciens* are used to enhance flavour in cheddar cheese.

Proteases hydrolyse proteins to produce peptides of variable sizes. Peptides having terminal acidic amino acid residues produce meaty, appetising flavours. But hydrophobic amino acid residues located non-terminally produce bitter flavours: the, flavours are the strongest in medium-sized peptides, absent in longer peptides, and decrease with a decrease in the peptide size.

Therefore, the kind and the degree of flavour in cheese can be controlled by regulating protein hydrolysis. The stronger flavours of Italian cheeses are produced by a modest lipid hydrolysis, which increases the amount of-free butyric acid. Lipolysis is brought about by lipase from M. miehei or Aspergillus niger; the. lipase is added to the milk at 30 U/l before addition of rennet.

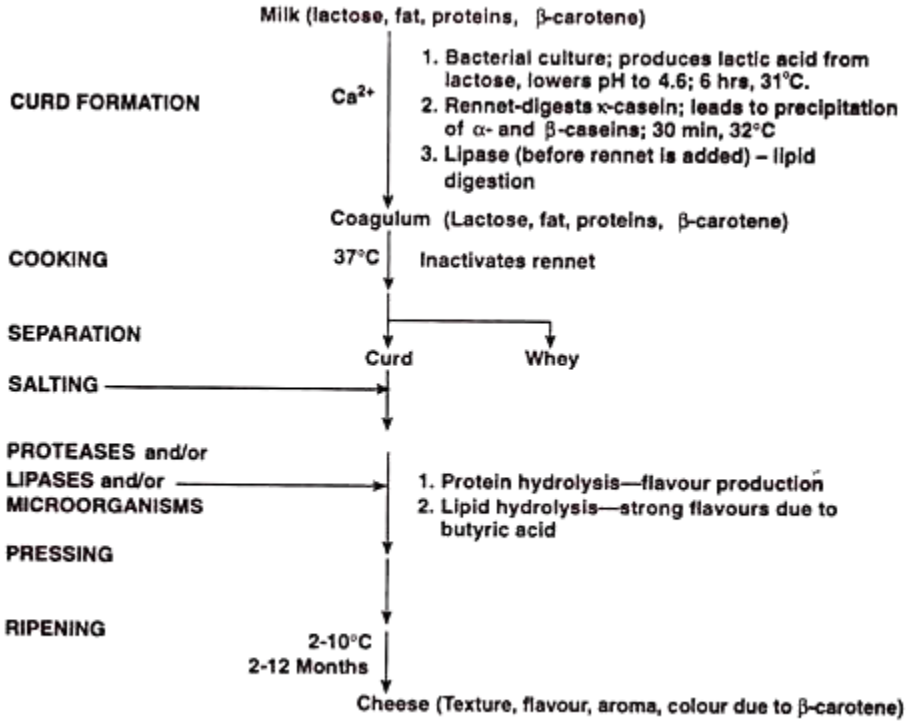


FIG. 38.1. A schematic representation of the various steps in cheese production.

Other Fermented Foods:
Some important food produced in whole or in part by microbial fermentation are pickles, sausages, etc. Different microorganisms are added to specific stages of food production to produce the desired effect. Moulds are used for the fermentation of rice to produce a variety of oriental foods.

Yeast is used for Making Bread:
Yeast is a fungus that feeds saprotrophically. The enzymes secreted by the yeast cell, digest food that contains sugar and minerals. Yeast is used to make bread. When yeast is added to raising flour and water, carbon dioxide is produced which gets trapped in the dough prepared from the flour.

The dough rises and bread is made. The flour is usually made from wheat and contains starch. Starch is the energy source for the yeast. The flour also contains a protein called gluten, which forms sticky

stretchy threads as the yeast works on the sugar. The threads trap the carbon dioxide and make the dough rise well.
Some commercial uses of yeast are shown in Table

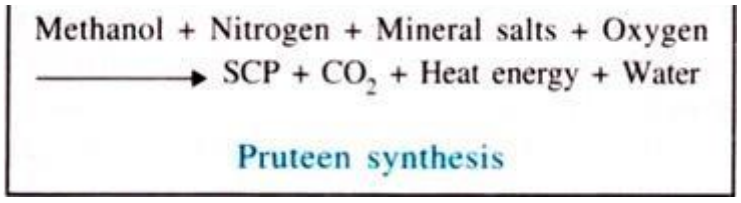
Table 4 Important commercial products of yeast.

Types of yeast	Product	Uses
Saccharomyces cerevisiae	Beer, Wine, Bread, Baker's yeast	Baking industry and brewing industry
Saccharomyces rouxii	Soy sauce	Food condiment
S. cerevisiae	Ethanol	Fuel, solvent
Eremothecium ashbyi	Riboflavin	Vitamin supplement

Baker's Yeast:
Yeast is used as leavening agent in baking since earlier times. The most commonly used species is Saccharomyces cerevisiae because of its ability to ferment sugar in the dough vigorously and to grow rapidly. The carbon dioxide used during the fermentation is responsible for the leavening or the rising of the dough. The procedure of mass production of Baker's yeast is elaborate under controlled conditions of pH, temperature conditions.
Microorganisms as Food – Single Cell Protein:
Algae, yeasts and bacteria can be grown in large quantities to yield a cell crop which is rich in protein known as single cell protein. The protein may be used for human consumption or as animal feed. It may be a useful source of minerals, vitamins, fat and carbohydrates. The composition of the different SCP depends upon the organism and the substrate on which it grows.
The advantages of using microorganisms as a food source are:

a. They grow very fast and do not need much space as conventional crops.
b. They grow on a wide range of cheap, waste products of agriculture and industry such as petroleum products, methanol, ethanol, sugar, molasses, waste from paper mills etc. The secondary advantage is that they help in recycling the materials and thereby clean up the wastes.
c. They are high yielding. In a growth medium of 1000 lb of yeast in one day, many tonnes of protein is produced. This is about 10-15 times greater than soyabean and about 25-50 times greater than corn.
d. The protein content of the cells is very high. Yeast cells have a protein content as high as 40-50%; for algae the range is 20- 40%.
e. The proteins of the microorganism contain all the essential amino acids.
f. Some microorganisms, particularly yeasts, have high vitamin content.
g. Factors, such as climate do not affect them, since they do not occupy large areas of land.

Pruteen was the first major SCP to be produced. It was produced by a bacterium, Methylophilus methylotrophus. Methanol was used as a source of energy and the temperature was maintained at 30-40°C and pH at 6.7.



Pruteen was rich in essential amino acids and has high vitamin content. It is twice as nutritious as soyabean meal and was used as an animal feed.
Some disadvantages of using SCP:

a. The high nucleic acid content causes intestinal disturbances. It can also lead to an increase in the uric acid in the blood that will eventually lead to gout. Additional processing can be done to reduce the nucleic acid content, but this would increase the cost.

b. Bacterial cells have small size and low density, which makes harvesting from the fermented medium difficult and costly.
c. The taste is not acceptable for many persons. Individual taste and customs make microorganism unattractive as a food to some individuals.

Chocolate Making:

Chocolate is prepared with the help of microbes. Chocolate comes from the seeds of cacao trees. These seeds are found in a white fleshy pod. To remove the seeds out of the pod, the pod is allowed to ferment with naturally occurring microbes that include yeasts and bacteria such as *Lactobacilli* and *Acetobacter*.

Single cell protein:

Single-cell protein (SCP) refers to edible unicellular microorganisms. The biomass or protein extract from pure or mixed cultures of algae, yeasts, fungi or bacteria may be used as an ingredient or a substitute for protein-rich foods, and is suitable for human consumption or as animal feeds.

Whereas industrial agriculture is marked by a high water footprint, high land use, biodiversity destruction, general environmental degradation and contributes to climate change by emission of a third of all greenhouse gases, production of SCP does not necessarily exhibit any of these serious drawbacks. As of today, SCP is commonly grown on agricultural waste products, and as such inherits the ecological footprint and water footprint of industrial agriculture. However, SCP may also be produced entirely independent of agricultural waste products through autotrophic growth. Thanks to the high diversity of microbial metabolism, autotrophic SCP provides several different modes of growth, versatile options of nutrients recycling, and a substantially increased efficiency compared to crops.

SCP production in India:

- National Botanical Research Institute (NBRI).
- Central Food Technological Research Institute (CFTRI).
- In CFTRI, SCP is produced from algae cultured on sewage.

Raw materials:

- Production of SCP requires micro-organisms that serve as the protein source and the substrate that is biomass on which they grow.
- There is a variety of both the sources that can be used for the production of SCP.
- The biomass used can be plant biomass or organic biomass.
- The micro-organisms used belong to the group of Algae, Fungi and Bacteria.

Micro organisms:

Micro-organisms used are fungi, yeast, algae & bacteria. The following table shows average different compositions of main groups of micro–organisms (% dry wt.).

COMPOSITON	FUNGI	ALGAE	YEAST	BACTERIA
PROTEIN	30- 40 %	40- 60 %	45- 55 %	50- 65 %
FAT	9-14 %	8-10 %	5-10 %	3-7 %
NUCLEIC ACID	7-10 %	3-8 %	6-12 %	8-12 %

A list of the micro-organisms used for SCP productionFungi

- *Aspergillus fumigatus*
- *Aspergillus niger*

- *Rhizopus cyclopium*

Yeast

- *Saccharomyces cerevisae*
- *Candida tropicalis*
- *Candida utilis*

Algae

- *Spirulina sps.*
- *Chlorella pyrenoidosa*
- *Chondrus crispus*

Bacteria

- *Pseudomonas fluroescens*
- *Lactobacillus*
- *Bacillus megaterium*

SCP production:

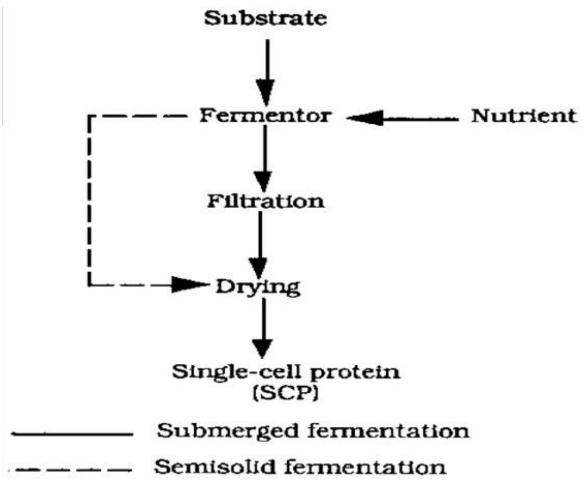
- Selection of suitable strain
- Fermentation
- Harvesting
- Post harvest treatment
- SCP processing for food

Selection of strain

- It a very critical step as the quality of protein depends totally on the microbe that is used for the production.
- Thus careful selection of the strain should be done.
- Care should be taken that the selected strain should not produce any toxic or undesirable effects in the consumer.

Fermentation

- It can be carried out in the fermentor which is equipped with aerator, thermostat, pH, etc. or in the trenches or ponds.
- Microbes are cultured in fed- batch culture.
- Engineers have developed deep lift fermentor & air lift fermentor



Harvesting

- When the colonies of microbes are fully developed, they are then harvested.
- The bulk of cells are removed from the fermentor by decantation.

Post harvest treatment

- After harvesting, the cells are subjected to a variety of processes.
- Post harvesting treatments includes steps like separation by centrifugation, washing, drying, etc.

Processing for food

It includes

1. Liberation of cell proteins by destruction of indigestible cell wall.

A. Mechanical methods

- Crushing, crumbling, grinding, pressure homogenization, etc.

B. Chemical methods

- Enzymes & salts are used to digest or disrupt the cell wall.
- Salts like NaCl, sodium dodecyl sulfate, etc. whereas nuclease enzymes are used.

C. Physical methods

- Freeze- thaw, osmotic shock, heating & drying.

2. Reduction of nucleic acid content

- Chemical & enzymatic treatments are preferred.
- Chemicals which are used includes acidified alcohol, salts, acids & alkalies.
- Use of such chemicals leads to formation of lygino-alanine which causes hypersensitivity skin reactions.
- Enzymes which are used include ribonuclease & nuclease enzymes.
- These enzymes can be used exogenously or can be induced endogenously.

Nutritional and Safety Evaluations of SCP:

1. The SCP chemical composition must be characterised in terms of protein, amino acid, nucleic acid, lipid, vitamin, etc. contents.
2. Analysis of substrate residues and toxic substances, e.g., heavy metals, mycotoxins, polycyclic hydrocarbons, etc. must be done.
3. Physical properties like density, particle size, texture, colour, storage, etc. properties should be determined.
4. Microbiological description, e.g., species, strain, should be provided, and information on contamination be also given.
5. The nutritional value should be evaluated on the target species, and other species should also be included. The products for human use will, of necessity, be evaluated over a longer period using a multistaged process.
6. Possible toxic or carcinogenic compounds must be assayed for. These compounds may have been present in the substrate, may be synthesized by the organism or produced during the processing of SCP.

Advantages of SCP:

1. The SCP processes and products offer several advantages as listed below:
2. The SCP is rich in high quality protein and is rather poor in fats, which is rather desirable.
3. They can be produced all the year round and are not dependent of the climate (except the algal processes).
4. The microbes are very fast growing and produce large quantities of SCP from relatively very small area of land.
5. They use low cost substrates and, in some cases, such substrates which are being wasted and causing pollution to the environment.
6. When the substrate used for SCP process is a source of pollution, SCP production helps reduce pollution.
7. Strains having high biomass yields and a desirable amino acid composition can be easily selected or produced by genetic engineering.

11. Biofertilizers and biopesticides in agriculture

Bio-fertilizers:

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.

(i) 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., *Azotobacter*, *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.

(ii) Free 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.

(iii) 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).

(iv) 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.

(v) 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*.

(vi) Microphos Biofertilizers:

They release phosphate from bound and insoluble states, e.g., *Bacillus polymyxa, Pseudomonas striata, Aspergillus species*.

(vii) 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.

(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.

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.

Biopesticide:

Biopesticide is a formulation made from naturally occurring substances that controls pests by non toxic mechanisms and in ecofriendly manner. Biopesticides may be derived from animals (e.g. nematodes), plants (*Chrysanthemum*, *Azadirachta*) and micro-organisms (e.g. *Bacillus thuringiensis*, *Trichoderma*, nucleopolyhedrosis virus), and include living organisms (natural enemies) etc. However, biopesticides are generally less toxic to the user and are non-target organisms, making them desirable and sustainable tools for disease management.

Advatages of biopesticides

- Inherently less harmful and less environmental load,
- Designed to affect only one specific pest or, in some cases, a few target organisms,
- Often effective in very small quantities and often decompose quickly, thereby resulting in lower exposures and largely avoiding the pollution problems.
- When used as a component of Integrated Pest Management (IPM) programs, biopesticides can contribute greatly.

Types of biopesticides

- Microbial pesticides
- Plant-incorporated-protectants (PIPs)
- Biochemical pesticides
- Botanical pesticides
- Biotic agents (parasitoids and predators)

Microbial Pesticides

Microbial pesticides are composed of microscopic living organisms (viruses, bacteria, fungi, protozoa, or nematodes) or toxin produced by these organisms. Applied as conventional insecticidal sprays, dusts, or granules. Their greatest strength is their specificity as most are essentially nontoxic and non pathogenic to animals and humans. Microbial pesticides includes insecticides, fungicides, herbicides and growth regulators of microbial origin.

Some of the important microbial pesticides

a. *Bacillus thuringiensis*

- Discovered in Japan in early 20th century and first become a commercial product in France in 1938.
- Control lepidopterous pests like American bollworm in cotton and stem borers in rice.
- When ingested by pest larvae, Bt releases toxins which damage the mid gut of the pest, eventually killing it.
- Main sources for the production of Bt preparations are the strains of the subspecies kurstaki, galeriae and dendrolimus

b. *Agrobacterium radiobacter* (Agrocin)

- *Agrobacterium radiobacter* is used to treat roots during transplanting, that checks crown gall.

- Crown gall is a disease in peaches, grapevine, roses and various plants caused by soil borne pathogen *Agrobacterium tumefaciensm*.
- The effective strains of A. radiobacter posses two important features:
  - ✓ They are able to colonize host roots to a higher population density.
  - ✓ They produce an antibiotic, agrocin, that is toxic to *A. tumefaciens*.

c. *Pseudomonas fluorescens* (Phenazine)

- This bacteria is used to control damping off caused by *Pythium* sp., *Rhizoctonia solani, Gaeumannomyces graminis*.
- It has ability to grow quickly in the rhizosphere

d. *Trichoderma*

- *Trichoderma* is a fungicide effective against soil born diseases such as root rot.
- This is also used against *Necteia galligena* that causes silver leaf disease of fruit trees by entering through pruning wounds.

e. *Metarizium anisopliae*

- It infects spittlegbugs, rhinoceros beetles.

f. *Beauveria bassiana*

- Controls Colorado potato beetle.

g. *Verticillum lecanii*:

- Controls aphids and whiteflies.

h. *Nomuraea riley*:

- Controls soybean caterpillars.

i.Baculoviruses(Bvs)

- Control lepidopterous and hymenopterous pests.
- Rod shaped, circular double stranded super coiled DNA.

Plant-incorporated-protectants (PIPs)

- Pesticidal substances that plant produce from the genetic material that has been added to the plant.
- As the pest feed on such plants they will eventually die.

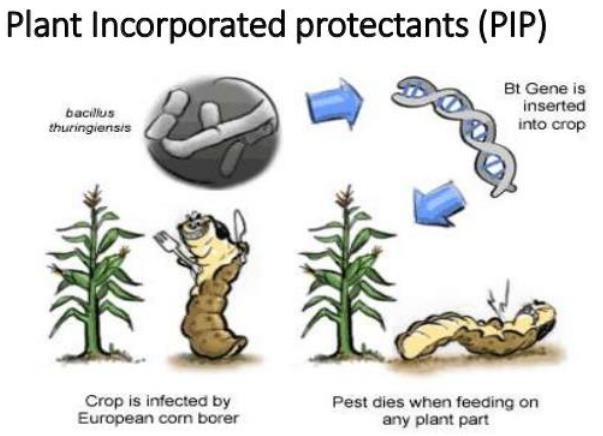


Fig. Showing the mode of action of cry protein crystal produced by genetically modified plant

Botanical pesticides:

- These are naturally occurring plant material that may be crude preparation of the plant parts ground to produce a dust or powder that can be used in full strength or dilute form in a carrier such as clay, talc
- or diatomaceous earth.
- "Azadirachtin" effects the reproductive and digestive procees of pest.
- Several plant based insecticides as nicotinoids, natural pyrethrins, rotenoids, neem products etc are used.

4. Biochemical pesticides

- They are naturally occurring substance to control pest by non-toxic mechanisms.
- Biochemical pesticides include substances as insect sex pheromones, that interfere with mating that attract insect pest to traps.
- The synthetic attractantsare used in one of four ways:
  i. As a lure in traps used to monitor pest populations;
  ii. As a lure in traps designed to "trap out" a pest population;
  iii. As a broadcast signal intended to disrupt insect mating
  iv. As an attractant in a bait containing an insecticide

Biotic agents/Natural enemies

Predators

They consume several to many prey over the course of their development, they are free living and they are usually as big as or bigger than their prey. lady beetles, rove beetles, many ground beetles, lacewings, true bugs such as *Podisus* and *Orius*, syrphid fly larvae, mantids, spiders, and mites such as *Phytoseiulus* and *Amblyseius*.

Parasitoids

- Parasitoids are almost the same size as their hosts, and their development always kills the host insect.
- An adult parasitoid deposits one or more eggs into or onto the body of a host insect or somewhere in the host's habitat.
- The larva that hatches from each egg feeds internally or externally on the host's tissues and body fluids, consuming it slowly.
- Later in development, the host dies and the parasitoid pupates inside or outside of the host's body.
- *Bathyplectes, trichogramma, encarsia, muscidifurax* etc.

Application of Biopesticides

1. Biopesticides are usually applied in a similar manner to chemical pesticides but in Environmental friendly way.
2. For effective control, microbial agent require appropriate formulation.
3. Biopesticides used to control internal seed Borne fungal pathogens.

12. Environmental biotechnology: Treatment of waste & waste water; bioremediation.

Environmental Biotechnology:

The most important topics at the threshold of the 21st century are the environment and bio-technology. Environmental biotechnology can be defined as the marriage of environmental issues with the advances in biotechnology.

It is concerned with the application of biotech-nology as an emerging technology in the con-text of environmental protection, since rapid industrialization, urbanization and other de-velopments have resulted in a threatened clean environment and depleted natural resources.

It is not a new area of interest, because some of the issues of concern are familiar examples of "old" technologies, such as: composting, wastewater treatment, etc.

In its early stage, environmental biotechnology has evolved from chemical engineering, but later, other disci-plines (biochemistry, environmental engineer-ing, environmental microbiology, molecular biology, ecology) also contribute to environ-mental biotechnology development.

The development of multiple human activities in the sector of industry, transport, agriculture, do-mestic space, etc. have amplified the pollution of air, water and soil.

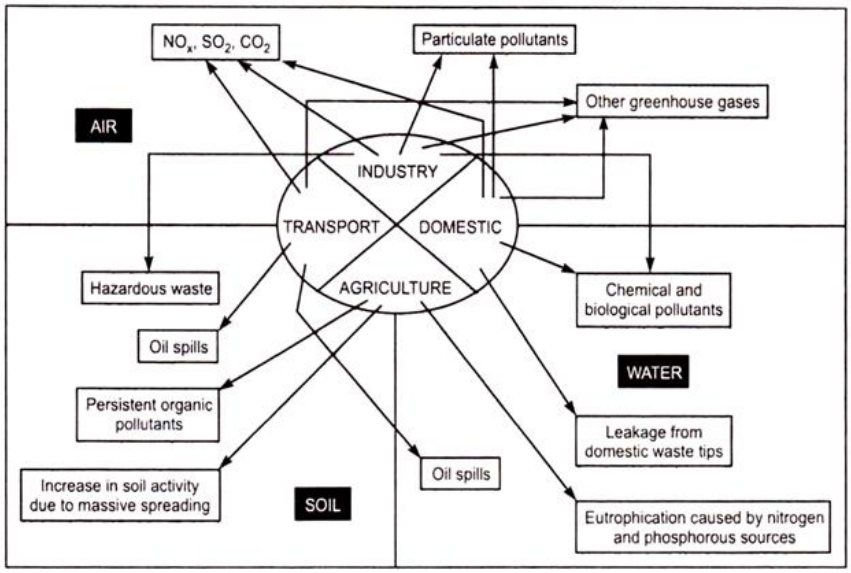


Fig. 16.1: The spider of environmental pollution due to anthropogenic activities. (Adapted from EIBE 2000)

Studies and researches demonstrated that some of these pollutants can be readily degraded or removed by means of biotechnological solutions.

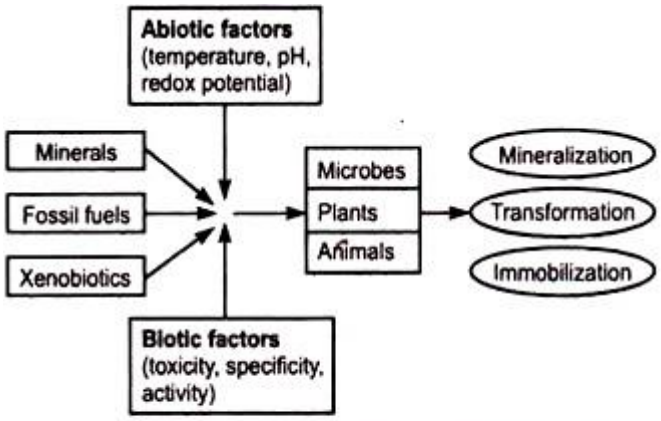


Fig. 16.2: Sources of environmental pollutants and factors that influence their removal from the environment. (Adapted from Chen *et al.*, 2005).

At least three key points are considered for environmental biotechnology. These are as fol-lows:
1. To detect the pollution and any other en-vironmental changes by means of biosensors and bio monitoring.
2. To prevent the unfavorable environmen-tal changes in the manufacturing process by substitution of traditional processes.
3. To control and remediate the emission of pollutants into the environment.
By considering all these issues, biotechnol-ogy may be regarded as a driving force for in-tegrated environmental protection by environ-mental bioremediation, waste minimization, environmental bio monitoring, bio maintenance.

Wastewater treatment:

Wastewater treatment is a process used to remove contaminants from wastewater or sewage and convert it into an effluent that can be returned to the water cycle with minimum impact on the environment, or directly reused. The latter is called water reclamation because treated wastewater can then be used for other purposes. The treatment process takes place in a wastewater treatment plant (WWTP), often referred to as a Water Resource Recovery Facility (WRRF) or a Sewage Treatment Plant (STP). Pollutants in municipal wastewater (households and small industries) are removed or broken down.

The treatment of wastewater is part of the overarching field of sanitation. Sanitation also includes the management of human waste and solid waste as well as stormwater (drainage) management. By-products from wastewater treatment plants, such as screenings, grit and sewage sludge may also be treated in a wastewater treatment plant.

The four processes are:

1. Preliminary Treatment:

As already stated, preliminary treatment involves the removal of floating materials (leaves, papers, rags) and settleable inorganic solids (sand, grit), besides oily substances (fats, oils, greases). The three major types of equipment-screeners, grit chambers, and skimming tanks, employed in preliminary screening are briefly described.

Screeners:

A screener is a device with openings (usually uniform in size) to remove the floating materials and suspended particles. The process of screening can be carried out by passing sewage through different types of screeners (with different pore sizes).

The screeners are classified as coarse, medium or fine, depending on the size of the openings. The coarse screen has larger openings (75-150 mm). The openings for medium and fine screens

respectively are 20-50 mm and less than 20 mm. Different types of screens-fixed bar screen (coarse or medium) disc type fine screen, drum type fine screen are in use.

A shredder or comminatory is a special screen that can cut and retain the floating and suspended materials.

Grit Chambers:

The heavy inorganic materials (specific gravity 2.4-2.7) like sand, ash and others can be removed by using grit chambers. This technique is based on the process of sedimentation due to gravitational forces. Grit chambers may be kept either before or after the screens. A diagrammatic representation of a typical grit chamber is depicted in.

Skimming Tanks:

Several greasy and oily materials (fats, oils, waxes, soaps etc.) from the domestic or industrial outlets find their entry into the sewage. They can be removed by using a skimming tank which is fitted with baffle walls that divide the tank. The skimming tank is divided into three compartments that are interconnected.

2. Primary Treatment:

Primary treatment is aimed at the removal of fine suspended organic solids that cannot be removed in the preliminary treatment. Primary treatment basically involves the process of sedimentation or settling. In the normal process of sewage treatment, sedimentation is usually carried out twice-once before the secondary treatment, referred to as primary sedimentation, and then after the secondary treatment is complete, a process known as secondary sedimentation. It is sometimes necessary to use chemical coagulants to facilitate or aid sedimentation, and this process is referred to as chemical precipitation or coagulation-aided sedimentation.

tanks

Principle of Sedimentation:

The solid particle of the sewage tend to settle down due to gravity. However, most of the solid particles of organic compounds remain in a suspended state in a flowing sewage. If the flow of the sewage is stopped and if it is stored in a tank referred to as sedimentation tank, the solid particles can settle down at the bottom. The process of sedimentation is influenced by several factors. These include the size, shape and specific gravity of particles, besides viscosity and flow velocity of sewage.

Types of Settling:

There are four major types of settling—discrete settling, flocculent settling, hindered or zone settling and compression. This categorization is mainly based on the tendency of the particles to interact and form solids.

The particles which do not change their size, shape and weight are referred to as discrete particles or granular particles. The use of grit in sewage may be considered as an example of discrete settling.

Flocculent settling:

The flocculent particles can change their size, shape and weight, and thus lose their identity. These particles actually coalesce during settling. Settling of bioflocs, and chemical . floes in secondary sedimentation tanks are good examples of flocculent settling.

Hindered or zone settling:

The particles as such, tend to remain in a fixed position with respect to each other. When flocculated, the whole mass of particles settle as a unit or a zone. In the hindered settling the concentration of particles increases from top to the bottom and this results in the thickening of the sludge. Zone settling is employed in conjunction with biological treatment facilities.

Compression:

Settlement of particles in the lower layers can occur by compression of the weight of the particles on the upper layers. This process facilitates sludge thickening at the bottom.

Chemical-aided Sedimentation:

It is not always possible to remove the colloidal wastes in sewage by plain sedimentation. However, addition of certain chemicals aids sedimentation, a process referred to as chemical precipitation or chemical-aided sedimentation. By this technique, about 60-80% of the suspended particles can be removed. Chemical precipitation involves three stages—coagulation, flocculation and sedimentation.

Coagulation is mainly a chemical process wherein the charged particles are destabilized (by the addition of chemical agents). On the other hand, flocculation involves the physical phenomena of aggregating the destabilized particles to finally form settleable solids (i.e. sedimentation). The chemicals used in chemical-aided sedimentation are of two types-coagulants and coagulant aids.

Coagulants:

These are the chemicals (normally positively charged) which form insoluble and gelatinous precipitates with colloidal particles (negatively charged ones present in sewage). The most commonly used coagulants in sewage treatment are alum (alluminium sulfate) iron salts (ferric sulfate, ferrous sulfate, ferric chloride), lime and soda ash (sodium carbonate), sodium silicate and sodium aluminate.

3. Secondary or Biological Treatment:

Biological treatment of sewage is required for the removal of dissolved and fine colloidal organic matter. This process involves the use of microorganisms (bacteria, algae, fungi, protozoa, rotifers, nematodes) that decompose the unstable organic matter to stable inorganic forms.

The biological treatment processes of sewage are broadly classified as aerobic, anaerobic and pond processes. Depending on the nature of the use of the microorganisms, the biological processes are categorized as suspended growth systems and attached growth systems.

Aerobic Suspended-Growth Treatment Processes:

The most important suspended-growth biological treatment systems used for the removal of organic matter are listed:

i. Activated sludge process
ii. Aerated lagoons
iii. Sequencing batch reactor
iv. Aerobic digestion.

Among these, activated sludge process is the most widely used for the secondary treatment of sewage.

Activated Sludge Process:

The activated sludge process, first developed in England in 1914, continues to be the most commonly used modern process for the biological treatment of sewage. In this method, the sewage containing organic matter with the microorganisms is aerated (by a mechanical aerator) in an aeration tank. The reactor contents are referred to as mixed liquor. Under aerobic conditions, the microorganisms metabolize the soluble and suspended organic matter. The generalized metabolic reaction is as follows.

A part of the organic matter is utilized for the synthesis of new bacterial cells while the remaining gets oxidized to CO_2 and H_2O. The newly formed microorganisms are agglomerated to form floes, technically referred to as sludge.

The separated sludge which is not in contact with organic matter becomes activated. It is separated from the settling tank, and returned to the aeration tank, and recycled. The activated sludge recycled in aeration tank serves as a seed or inoculum. The excess and waste sludge can be removed.

For efficient operation of activated sludge process, it is necessary to maintain a constant supply of O_2 which can be done by mechanical aeration or through the use of rotating paddles. Growth of protozoa in a sludge is an indication of its healthy condition. The disposal of a waste sludge is a problem. It may be used as a fertilizer in crop lands or as landfills, after drying.

Conventional activated sludge process:

In the normal treatment of sewage, the activated sludge is proceeded by primary sedimentation tank. The conventional activated sludge system consists of a separation tank, settling or sedimentation tank and sludge removal line (Fig. 57.4). The sewage after the primary treatment is introduced at the head of the tank. It is desirable to supply O_2 uniformly throughout the tank.

Activated-Sludge Process

Modified activated sludge processes:

For increasing the performance of the activated sludge system, several modifications have been done in the recent years. Most of them are directed to bring out efficient aeration. Aeration can be done by step aeration, tapered aeration, and high rate aeration by complete mixing and extended aeration.

Aerated Lagoons:

Aerated lagoons, also called as aerated ponds, are the facultative stabilization ponds wherein surface aerators are installed to overcome the bad adours (due to overload of organic materials). The

microbiological treatment of aerated ponds is comparable to the activated sludge process. The major difference is the large surface area in aerated ponds and this is more susceptible for temperature effects. It is possible to carry out continuous nitrification in aerated lagoons. This however, depends on the design and operating conditions of the pond (particularly the temperature).

Sequencing Batch Reactor:

Sequencing batch reactor (SBR) is a modification of activated sludge treatment system. The processes namely aeration and sedimentation are carried out in both the systems. The major difference is that while in the conventional activated sludge system, aeration and sedimentation occur simultaneously in separate tanks, these two processes are carried out sequentially in the same tank in SBR. Thus, the sequencing batch reactor may be regarded as fill- and-draw activated sludge process.

Aerobic Digestion:

The organic sludge's produced from various treatment processes (activated sludge treatment, trickling filter-sludge) are subjected to aerobic digestion in special reactors referred to as aerobic digesters.

Aerobic Attached — Growth Treatment Processes:

Aerobic attached-growth treatment processes are commonly used to remove the organic matter found in the sewage. These processes are also useful for the nitrification (conversion of ammonia to nitrate).

The commonly used attached-growth processes are listed:

i. Trickling filters
ii. Roughing filters
iii. Rotating biological contractors
iv. Packed bed reactors.

Among these, trickling filter is most widely used.

Anaerobic Digestion:

Anaerobic digestion is mostly useful for the stabilization of concentrated sludge's that are produced on the treatment of industrial sewage. The process of anaerobic digestion is carried out in an air tight reactor. Sludge is introduced continuously or intermittently. In the high-rate digestion system, the contents of the digester are heated and mixed completely. And it takes about 15 days for the process to be complete.

Biodegradation of organic matter of sludge (or sewage):

The biological degradation of organic matter of sludge occurs in three stages (Fig. 57.10) — hydrolysis, acidogenesis and methanogenesis.

Degradation of Organic Materials in Anaerobic Digestion

Hydrolysis:

In the enzyme-catalysed reactions, high molecular weight compounds (proteins, polysaccharides, lipids and nucleic acids) are degraded to low molecular weight compounds (amino acids, monosaccharide's, fatty acids, purines and pyrimidine's). The latter serve as substrates for energy supply and microbial growth.

Acidogenesis:

The low molecular weight compounds are converted to acidic products (propionate, butyrate, and lactate).

Methanogenesis:

This is the third and final stage and involves the production of methane and carbon dioxide, from the intermediates formed in acidogenesis. Methane gas is highly insoluble and its departure from the digester represents the stabilization of sewage or sludge.

Microorganisms to degrade organic matter of sludge (or sewage):

A consortium of anaerobic microorganisms work together for degradation of sludge (or sewage) organic matter.

They may be categorized into two types:

1. Acid-forming bacteria

These are also known as acidogens or non-methanogenic bacteria. They bring out the hydrolysis of macromolecules (e.g. carbohydrate) to simple substrates (e.g. monosaccharide's), and the latter to acids e.g. Clostridium sp, Corynebacterium sp, Lactobacillus sp, Actinomyces sp, Staphylococcus sp, Peptococcus anaerobus, Escherichia coli.

2. Methanogenic bacteria:

These bacteria, also referred to as methanogens or methane formers are responsible for the conversion of acetic acid and hydrogen to methane and carbon dioxide. The most important methanogens belong to the genera Methanobacterium, Methanobacillus, Methanococcus and Methanosarcina.

Anaerobic contact process is carried out in a specially designed reactors. The treatment process consists of mixing of sewage with recycled sludge solids and then digestion under anaerobic conditions. After the digestion is complete, the supernatant effluent is discharged and the settled sludge is recycled. Anaerobic contact process is successfully used for efficient industrial wastes with high BOD e.g. meat packing wastes.

Anaerobic Attached — Growth Treatment Processes:

There are mainly two treatment processes under the anaerobic attached—growth treatment system—anaerobic filter process and expanded bed process.

Anaerobic Filter Process:

Anaerobic filter consists of a column filled with solid media for the treatment of organic matter in sewage. In this process system, waste water (sewage) flows upwards through the column containing anaerobic bacteria. Due to the presence of solid media, the bacteria are retained in the column. This makes the treatment process more efficient.

Expanded-Bed Process:

The sewage can be treated by pumping it through a bed of inert materials (sand or coal expanded aggregates) on which the bacteria have grown and formed a film. The effluent that comes out can be recycled to maintain the flow rate.

Pond Treatment Processes:

Pond treatment processes for the treatment of sewage (containing biodegradable wastes) are carried out by specially designed and constructed ponds. These ponds, referred to as stabilization ponds, are large, shallow earthen basins. The treatment process is a natural one involving the combined use of bacteria and algae. The stabilization ponds are classified as aerobic, anaerobic and facultative ponds.

Aerobic Ponds:

The aerobic ponds, as the name indicates, maintain complete aerobic conditions. These ponds usually have a depth of about 0.5 to 1.5 feet (150 to 450 mm) and allow the penetration of light throughout the liquid depth. A second type of aerobic ponds with a depth of 5 feet (1.5 m) are also in use.

In all these ponds, oxygen is maintained through continuous atmospheric diffusion (by surface aerators or pumps), besides the production by algae grown in the pond. The aerobic stabilization ponds contain bacteria and algae in suspension. They are particularly useful for the treatment of soluble wastes.

The algae and bacteria exhibit a symbiotic and cyclic relationship in the aerobic ponds. The algae can carry out photosynthesis and release oxygen to maintain aerobic conditions in the pond. The bacteria degrade the organic matter to produce CO2 and other nutrients to be utilized by algae (Fig. 57.11). Some higher organisms like protozoa and rotifers present in the pond are responsible for the polishing of the effluent.

4. Tertiary Treatment:

Tertiary treatment or advanced treatment is sometimes needed for the removal of suspended and dissolved substances, after the conventional primary and secondary treatments. In general, the effluent of the sewage obtained after secondary treatment can be conveniently disposed without causing any nuisance.

However, tertiary treatment is needed under the following circumstances:

i. When the quality of the effluent to be discharged does not meet the standard requirements (particularly in the developed countries).

ii. When there is a necessary to reuse the sewage/ waste water (reclamation of water is quite expensive, but is required in certain situations of water shortage).

iii. For the removal of nitrogen and phosphorus compounds.

Tertiary treatment process broadly involves the removal of suspended and dissolved solids, nitrogen, phosphorus and pathogenic organisms. In the conventional hierarchy of sewage treatment, the unit operations are carried out in the order of preliminary, primary, secondary and finally tertiary treatment.

However, sometimes advanced (tertiary) treatment process may be directly carried out bypassing the other unit operations. This mainly depends on the composition of waste water and the requirements.
There are four major processes under the tertiary treatment:
1. Solids removal
2. Biological nitrogen removal
3. Biological phosphorus removal
4. Disinfection.

Solids Removal:

The techniques for the removal of suspended and dissolved solids in waste water treatment are comparable with those employed for the processing of potable (drinking) water.
The effluents obtained from secondary treatment may contain suspended solids in the size 0.1 to 100μm. The concentration of these solids is variable, and is usually 20-40 mg/l. The removal of suspended solids is carried out by granular medium (sand) filtration and micro screening. Sometimes, diatomaceous earth filters and coagulation-cum sedimentation techniques are also used.

Removal of dissolved solids:

The dissolved solids can be removed mainly by two techniques—adsorption and ion-exchange.

Adsorption by activated carbon:

Activated carbon is highly porous and provides large surface area for the adsorption of dissolved solids in the advanced treatment. The compounds that can be removed by adsorption include organic materials (herbicides, pesticides, tannins, lignin's, colour and odour producing substances), inorganic materials (toxic trace metals) and several other pollutants.

Ion-exchange for dissolved solids removal:

As the name indicates, ion-exchange involves the displacement of one ion by another. The exchange occurs between the ions of insoluble exchange material (ion-exchange materials) and the ions of different species in solution (i.e. waste water for advanced treatment).
The ion-exchange process is carried out by employing two types of ion-exchange materials— cation exchangers and anion exchangers. The synthetic resins with strong acidic (H+) and basic (OH–) functional groups serve as ion exchangers. The cation exchangers (with H+ or Na+) can replace the positively charged ions (Ca2+, Mg2+) in water by hydrogen ions. This is what is done for removing the hardness of water.

Biological Nitrogen Removal:

Decomposition products of proteins and the urea present in sewage are the major constituents of biological nitrogen. Although, nitrogen is a nutrient, its excess concentration causes eutrophication, and thus its removal is required. Biological nitrogen removal (BNR) is carried out by the methods based on the following principles.

Assimilation of nitrogen:

Since nitrogen is a nutrient, the microorganisms in the sewage can assimilate ammonia nitrogen, and grow. As some of these cells die, a portion of this ammonia nitrogen will be returned to the sewage.
Nitrification:
Ammonia nitrogen first gets oxidized to nitrite (NO–2) by the bacteria *Nitrosomonas* sp. This is followed by further oxidation nitrite (NO–2) to nitrate (NO–3) by *Nitrobacter* sp.
The bacteria involved in nitrification are auxotrophs. The nitrification process is accomplished by aerobic suspended growth and aerobic attached-growth systems. In the general practice, nitrification is carried out along with the BOD removal in the secondary treatment with suitable modifications. Trickling filters, rotating biological contactors and packed towers can be used for nitrification process.

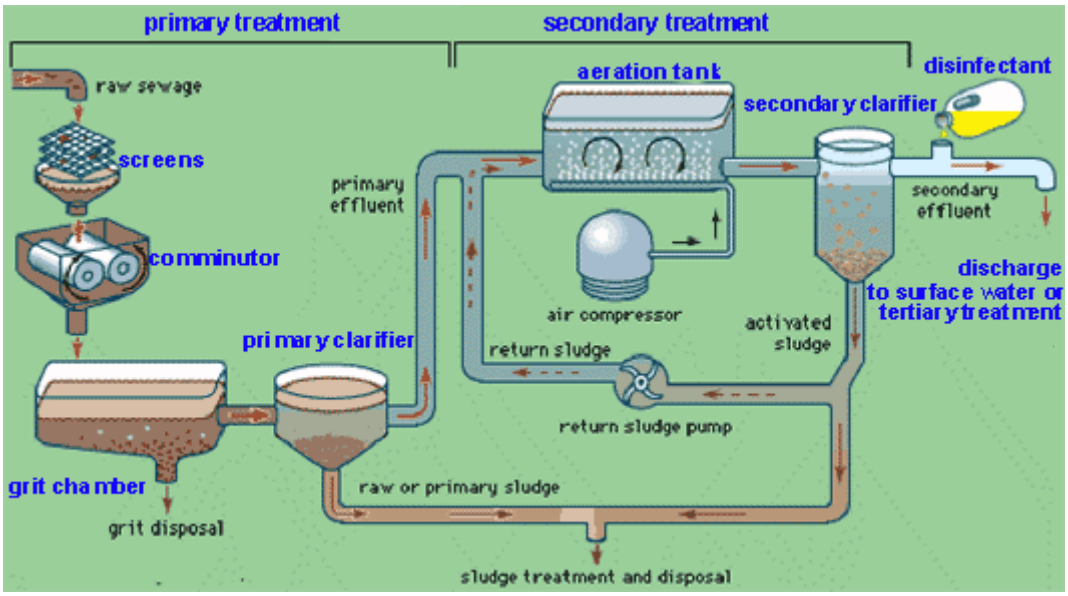
Denitrification:

The removal of nitrogen in the form of nitrate by converting to nitrogen gas is referred to as de-nitrification. This process occurs under anaerobic conditions and is brought out by certain genera of bacteria-*Aerobacter, Bacillus, Brevibacterium, Lactobacillus, Micrococcus, Pseudomonas and Spirillum.*
These bacteria are heterotrophs and require no oxygen, but the presence of organic carbon is essential. The presence of even minute quantities of O2 suppresses de-nitrification. The heterotrophic bacteria can reduce nitrate in the following stages, to finally nitrogen gas.

Biological Phosphorus Removal:
Phosphorus in the sewage is mostly present in the form of orthophosphate (PO43-), polyphosphate (P2O7) and organic bound phosphorus. In fact, phosphorus is an essential nutrient for microorganisms. Thus, during the normal secondary treatment process, 10-30% of the sewage phosphorus is utilized by the microorganisms for growth and energy purposes. Phosphorus removal from waste water is required to control eutrophication and to maintain water quality.

The process of phosphorus removal:.
The phosphorus enriched supernatant that comes out of the phosphorus stripper is treated with lime to precipitate the phosphorus. The resultant liquid supernatant can be returned to the aeration tank for further treatment.

Disinfection:
Disinfection broadly refers to the selective destruction or inactivation of disease-causing (pathogenic) organisms. In the process of disinfection, all the organisms are not destroyed. This is in contrast to sterilization which involves the destruction of all the organisms. There are several water borne diseases (typhoid, cholera, dysentery) caused by bacteria, viruses and other pathogens. The very purpose of disinfection is to control these diseases.



Agents for disinfection:
Disinfection is accomplished by using chemical and physical agents, besides mechanical and radiation means.

Chemical agents:
Chlorine and its compounds are most commonly used. The other chemicals— bromine, iodine, ozone, alcohols, phenols, heavy metals, hydrogen peroxide, alkalies and acids are sometimes employed. After chlorine (regarded as most universal disinfectant), bromine and iodine are in use. In recent years, ozone as a disinfectant is gaining importance, since it is very effective.

Physical agents:
Heat and light can be effectively used as disinfectants. Sunlight (particularly ultra-violet rays) is in fact a good disinfectant.

Mechanical means:
The pathogenic organisms can also be removed by mechanical means, during the course of waste water treatment. The processes involving screens (coarse and fine), grit chambers and sedimentation can partly remove the disease- causing organisms.

Radiation means:
The gamma rays emitted from radioisotopes can serve as effective disinfectants.
Characteristics of an ideal disinfectant:
An ideal disinfectant should possess the following characteristics:

i. Toxic to pathogens at low concentration
ii. Soluble and stable in water
iii. Non-toxic to man and higher organisms
iv. Cheap and easily available.

Disinfection with chlorine:

Chlorine is a very widely used disinfectant, as it satisfies the criteria of an ideal disinfectant. The most commonly used chlorine compounds are — chlorine gas (Cl2), calcium hypochloride [Ca(COCI2)], sodium hypochlorite (NaOCI) and chlorine dioxide (CIO2).

The disinfection efficiency of chlorine depends on the number of microorganisms in the water being treated, pH and temperature.

Sewage/Waste Water Treatment:

A conventional sewage treatment plant has the requisite operating units arranged one after another for treatment and final disposal of sewage.

Bioremediation:

Bioremediation is a treatment technology that uses biodegradation of organic contaminants through stimulation of indigenous microbial populations by providing certain amendments, such as adding oxygen, limiting nutrients, or adding exotic micro-bial species.

It uses naturally occurring or exter-nally-applied microorganisms to degrade and trans-form hazardous organic constituents into com-pound of reduced toxicity and/or availability.

Xenobiotics broadly refer to the unnatural, foreign and synthetic chemicals such as pesticide, herbicide & other organic compounds.

Types of Bioremediation

There are far more than 9 types of bioremediation, but the following are the most common ways in which it is used.

1. Phytoremediation – use of plants to remove contaminants. The plants are able to draw the contaminants into their structures and hold on to them, effectively removing them from soil or water.

2. Bioventing – blowing air through soil to increase oxygen rates in the waste. This is an effective way to neutralize certain oxygen sensitive metals or chemicals.

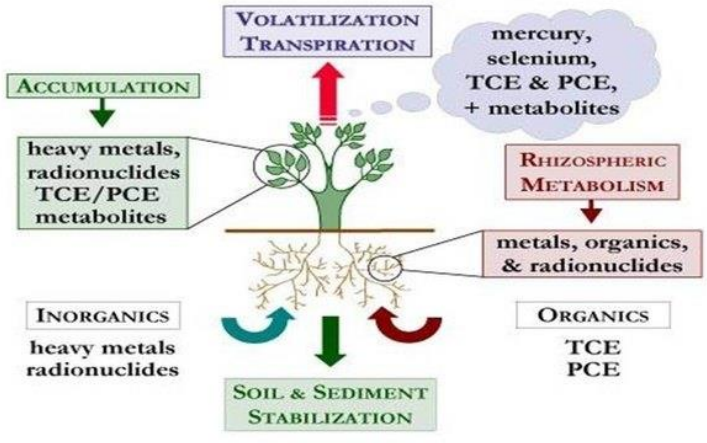
3. Bioleaching – removing metals from soil using living organisms. Certain types of organisms are draw to heavy metals and other contaminants and absorb them. One new approach was discovered when fish bones were found to attract and hold heavy metals such as lead and cadmium.

4. Landfarming – turning contaminated soil for aeration and sifting to remove contaminants, or deliberately depleting a soil of nitrogen to remove nitrogen based organisms.

5. Bioreactor – the use of specially designed containers to hold the waste while bioremediation occurs

6. Composting – containing waste so a natural decay and remediation process occurs.

7. Bioaugmentation – adding microbes and organisms to strengthen the same in waste to allow them to take over and decontaminate the area



Concept of bioremediation

8. Rhizofiltration – the use of plants to remove metals in water.

9. Biostimulation – the use of microbes designed to remove contamination applied in a medium to the waste.

There are two classes of bioremediation used. Don't confuse the class type with the actual types of bioremediation available, the classes describe the general application of the organisms. The two classes are:

In-situ – In situ refers to when contaminated waste is treated right at its point of origin. For example, there may be soil that is contaminated. Rather than remove the soil from its point of origin, it is treated right where it is. The benefit to in situ treatment is that it prevents the spread of contamination during the displacement and transport of the contaminated material.

Ex-situ – Ex situ refers to treatment that occurs after the contaminated waste has been removed to a treatment area. To use soil as the example again, the soil may be removed and transported to an area where the bioremediation may be applied. The main advantage to this is it helps to contain and control the bioremediation products, as well as making the area that was contaminated available for use.

Advantages of bioremediation

- Bioremediation is a natural process and is therefore perceived by the public.
- Bioremediation is useful for the complete destruction of a wide variety of contaminants.
- Instead of transferring contaminants from one environmental medium to another, for example, from land to water or air, the complete destruction of target pollutants is possible.
- Bioremediation can often be carried out on site, often without causing a major disruption of normal activities.
- Bioremediation can prove less expensive than other technologies that are used for cleanup of hazardous waste.

Disadvantages of bioremediation

- Bioremediation is limited to those compounds that are biodegradable. Not all compounds are susceptible to rapid and complete degradation.
- There are some concerns that the products of biodegradation may be more persistent or toxic than the parent compound.
- Biological processes are often highly specific. microbial populations, suitable environmental growth conditions, and appropriate levels of nutrients and contaminants.
- It is difficult to extrapolate (deduce) from bench and pilot-scale studies to fullscale field operations.
- Bioremediation often takes longer than other treatment options.

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13. Regulatory issues in biotechnology: Intellectual property rights (IPR): Patents, plant variety protections & geographical indications, WTO & TRIPPS, Bio safety and Food safety of GMO - a brief outline.

Intellectual property rights (IPR)

IPR is a general term covering patents, copyright, trademark, industrial designs, geographical indications, layout design of integrated circuits, undisclosed information (trade secrets) and new plant varieties.

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.

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 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.

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.

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. The number 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 electronic means;

(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 relation to 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 the computer programme:

Provided that such commercial rental does not apply in respect of computer programmes where the programme 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 two dimensional 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 such copy 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 of whether such copy has been sold or given on hire on earlier occasions;
(iii) to communicate the sound recording to the public.
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 prejudicially the interest of the owner of copyright ;
Public exhibition of infringing copies by way of trade; and
Importation 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 infringement of 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 feature of 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 the environment

• 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 the mere use of a known process, machine or apparatus, unless such known process results 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 or that 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 of known 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 form or 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

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.

2. 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.

3. 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 and the 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 or with 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 may extend 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 sold by 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', 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.

4) 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.

5) 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.

6) 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.

7) Deals with offences by companies (Sec.124) - When offence is committed by a company as well as every person in charge of and responsible to the company for the conducts of its business at the time of 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 quality seeds 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 such application;

• 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 as below:

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.

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.

Trade-Related Aspects of Intellectual Property Rights (TRIPS)

The Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS) is an international legal agreement between all the member nations of the World Trade Organization (WTO). It sets down minimum standards for the regulation by national governments of many forms of intellectual property (IP) as applied to nationals of other WTO member nations. TRIPS was negotiated at the end of the Uruguay Round of the General Agreement on Tariffs and Trade (GATT) in 1994 and is administered by the WTO.

The TRIPS agreement introduced intellectual property law into the international trading system for the first time and remains the most comprehensive international agreement on intellectual property to date. In 2001, developing countries, concerned that developed countries were insisting on an overly narrow reading of TRIPS, initiated a round of talks that resulted in the Doha Declaration. The Doha declaration is a WTO statement that clarifies the scope of TRIPS, stating for example that TRIPS can and should be interpreted in light of the goal "to promote access to medicines for all."

Specifically, TRIPS requires WTO members to provide copyright rights, covering content producers including performers, producers of sound recordings and broadcasting organizations; geographical indications, including appellations of origin; industrial designs;

integrated circuit layout-designs; patents; new plant varieties; trademarks; trade dress; and undisclosed or confidential information. TRIPS also specifies enforcement procedures, remedies, and dispute resolution procedures. Protection and enforcement of all intellectual property rights shall meet the objectives to contribute to the promotion of technological innovation and to the transfer and dissemination of technology, to the mutual advantage of producers and users of technological knowledge and in a manner conducive to social and economic welfare, and to a balance of rights and obligations.

TRIPS requires member states to provide strong protection for intellectual property rights. For example, under TRIPS:

• Copyright terms must extend at least 50 years, unless based on the life of the author. (Art. 12 and 14)

• Copyright must be granted automatically, and not based upon any "formality," such as registrations, as specified in the Berne Convention. (Art. 9)

• Computer programs must be regarded as "literary works" under copyright law and receive the same terms of protection.

• National exceptions to copyright (such as "fair use" in the United States) are constrained by the Berne three-step test

• Patents must be granted for "inventions" in all "fields of technology" provided they meet all other patentability requirements (although exceptions for certain public interests are allowed (Art. 27.2 and 27.3) and must be enforceable for at least 20 years (Art 33).

• Exceptions to exclusive rights must be limited, provided that a normal exploitation of the work (Art. 13) and normal exploitation of the patent (Art 30) is not in conflict.

• No unreasonable prejudice to the legitimate interests of the right holders of computer programs and patents is allowed.
• Legitimate interests of third parties have to be taken into account by patent rights (Art 30).
• In each state, intellectual property laws may not offer any benefits to local citizens which are not available to citizens of other TRIPS signatories under the principle of national treatment (with certain limited exceptions, Art. 3 and 5).
diseases. While looking for the global market, one has to ensure that requirements different regulatory authorities must be satisfied.

Biosafety and food safety of GMO:

As food safety is a ubiquitous public safety concern, and because tainted food is not always evident to the consumer, governments establish regulations in an attempt to assure food safety. In general, and in spite of occasional high profile exceptions, food safety regulations in technologically advanced countries are reasonably effective in protecting the public from such contaminants as E. coli or various mycotoxins.

Purpose for biosafety regulation of GM food?

If it is to provide reasonable assurance of food safety to consumers, current GM food biosafety regulations are severely flawed. Humans around the world all belong to the same species, Homo sapiens; we all share similar digestive systems and physiologies. Toxic substances in food, such as arsenic, mycotoxins and gycoalkaloids are toxic to everyone. No race or nation is immune to toxic chemicals or biological food contaminants such as *E. coli O104, Salmonella, Campylobacter, Clostridium,* or *Listeria.* With this concordance of human sensitivities, regulatory systems to assure food safety should be virtually identical worldwide, albeit recognizing regional or cultural variation in nutriment dietary exposures. But they are not. As the following chapters illustrate in this collection, biosafety regulation of GM foods varies.

The various regulation also differs remarkably in regulatory philosophy, approach, and practice. And they differ markedly in regulatory attitudes: predictably, perhaps, between industry and governments, and perhaps less predictably, as between international coalitions and their member states. This diversity of regulatory policies is unfortunate. While each jurisdiction claims sovereignty and the right to regulate as they see fit, the consequences of an international mosaic of GM safety regulations is more than merely a marketing inconvenience; it not only impedes international trade but jeopardizes public health and food safety—the antithesis of the raison d'etre of GM foods safety regulations in the first place. No state has unlimited resources to regulate everything for everything, so regulations necessarily have to be prioritized: some potentially hazardous products warrant more regulatory attention, others require less. A sensible regulatory policy maxim holds that products should receive biosafety regulatory scrutiny commensurate with degree of risk posed.

13. 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 to implement.
- 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.

- Genetically modified crops are defined as crops whose genomes have been altered in ways that 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 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.

- 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.

14. Suggested Reading

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4. Gupta, P.K. Biotechnology & Genomes, latest Ed., Rastogi Publications
5. Borer, A., Sentos, F.R. & Bowen, D.B. Understanding Biotechnology, 2003, Pearson Education
6. Ingacimuthu, S. Plant Biotechnology, 1997, Oxford & IBH
7. Walker, J.M. & Rapley, R. Molecular Biology & Biotechnology, 2000, Royal Society of Chemistry
8. Collin, H.A. and Edwards, S. Plant Cell Culture, 1998, Bios Scientific Publishers54
9. Dixon, R.A. & Gonzales, R. A. Plant Cell Culture: A Practical Approach, 1994, Oxford University Press
10. Dubey, R.C. Biotechnology, Latest Ed., S.Chand & Company Pvt. Ltd.
11. Rajdan, M.K. An Introduction to Plant Tissue Culture, Latest Ed., Oxford & IBH
12. Jha, T.B. & Ghosh, B. Plant Tissue Culture, 2003, Universities Press
13. Kar, D.K. & Halder, S. Plant Breeding, Biometry & Biotechnology, 2010, New Central Book Agency
14. 16. Slatter, A., Scott, N. & Fowler, N. Plant Biotechnology, 2003, Oxford University Press
15. Dey, K.K. Plant Tissue Culture, 1992, New Central Book Agency
16. http://www.biologydiscussion.com/
17. https://en.wikipedia.org/

15. Assignment

1. What is Golden rice?
2. What are the organizations of Vir genes?
3. What is Glyphosate? What are the different mechanisms of glyphosate function? Briefly describe mechanism of t-DNA transfer and integration.
4. How bt toxin helps to production of insecticide resistance plant.
5. What is meant by binary vector strategy?
6. What is electroporation?
7. Write a short note on direct method of gene transfer.
8. What is reporter genes?
9. What conditions do plant cells need to multiply in vitro?
10. Define embryoids?
11. Briefly describe the stages of somatic embryogenesis.
12. Mention the role of SERK and LEC genes during somatic embryogenesis.
13. What are main features doubled haploids? State its application in crop improvement.
14. How would you induce haploids?
15. Why plants produce secondary metabolites? What are the types of secondary metabolites.
16. Explain the different ways for the production of in vitro secondary metabolites,
17. What is meant by cellular totipotency?
18. Differentiate between somatic and zygotic embryogenesis.
19. Explain the rights conferred on a patentee by a compentent authority. Add a note on benefits and validity of a patent.
20. Enlist the intellectual property rights. Mention the authorities that are competent to confer such rights
21. What is IPR?
22. What is a bioreactor? How is it used to scale up multiplication of commercially important plants?
23. How is the antisense RNA technology used for production of transgenic plant varieties.
24. What is virus indexing? What is its significance in micropropagation?
25. What are transgenic crops? What are the advantages of such crops? Outline the method of obtaining such crop for a specific attribute.
26. Write notes on: Biopesticides.
27. What is bioremediation? Enlist different techniques of bioremediation.
28. Depict the classification system of fern by Sporne (1962) and Smith *et al.* (2006) by highlighting the differences between two systems
29. Distiguish between heterothallism and paraseuality.
30. What is dolipore septum?
31. How Drug enforcement help to solve forensic cases
32. What is typing? Give an overview of DNA typing.
33. Mention the role of pollen in case study. What are the advantages?
34. Name two DNA markers and mention its role in forensic botany.
35. How Diatoms help to solve forensic cases.
36. Distinguish ring porous wood and diffuse porous wood.
37. What botanical evidence can do?
38. How botanical samples are collected?
39. Describe impact of water stress on plants life .
40. Explain structure and function of HSPs in plant.
41. What is chilling injury? How do you overcome it.
42. What is hydroactive closure?
43. Explain the range of thallus structure of algae.
44. Write economic importance of jute.
45. Write the botanical characters of cotton?

46. Give the botanical name of any two fibre yielding plants.
47. Give the botanical name of any two vegetable oil yielding plants
48. What is the origin of center of wheat?
49. Write the botanical name of any two millets.
50. Name two medicinally important plant with their uses.

All the materials are self writing and collected from ebook, journals and websites.

POST GRADUATE DEGREE PROGRAMME (CBCS)
IN
BOTANY

SEMESTER - III

Course: BOHCT 3.3

(Plant Ecology, Biodiversity & Conservation)

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING
UNIVERSITY OF KALYANI
KALYANI – 741 235,
WEST BENGAL

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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 UGC Regulations, 2017 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.) Sankar Kumar Ghosh, 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.

Prof Manas Mohan Adhikary
Director
Directorate of Open and Distance Learning
University of Kalyani

SYLLABUS
COURSE – BOHCT 3.3
Plant Ecology, Biodiversity & Conservation
(Full Marks – 75)

Course	Group	Details Contents Structure		Study hour
BOHCT3.3	Plant Ecology, Biodiversity & Conservation	**Unit 1. Ecology: Introduction and Approaches**	1. Introduction to Ecology: scope and nature of plant ecology 2. Approaches in ecological studies: formulating hypothesis; theoretical ecological models; probabilistic ecological models; statistical approaches; phylogenetic approaches; remote sensing; climate diagrams	1
		Unit 2. Abiotic and biotic environment	3. Abiotic environment: variables in action; influence of abiotic environment on distribution and abundance of plants 4. Biotic environment: levels of organization of organisms in ecology; plant interactions	1
		Unit 3. Habitat and Niche	5. Habitat and Niche: concept of habitat and niche; niche width and overlap; fundamental and realized niche; competitive exclusion principle; extinction; resource partitioning; character displacement; speciati	1
		Unit 4. Population ecology	6. Population ecology: characteristics of population; population growth curves, population regulation, life history strategies (*r* and *K* selection); metapopulation, habitat fragmentation, demes, source-sink model	1
		Unit 5. Community ecology-I	7. Community ecology: concepts of community, assemblage and guilds; open and closed communities, ecotone; community continuum concept; community structure	1
		Unit 6. Community ecology-II	8. Community ecology: measures of community structure – diversity indices, similarity measures, food web analysis; succession - types, mechanisms, concept of climax	1
		Unit 7. Ecosystem ecology	9. Ecosystem ecology: concept of ecosystem, disturbance (natural and anthropogenic) and their impact on plant ecology; invasive plant species; resistance and resilience of ecosystems	1
		Unit 8. Biogeographical ecology	10. Biogeographical ecology: terrestrial ecology; wetland and freshwater ecology; coastal and marine ecology; major biogeographical zones of India	1
		Unit 9. Biodiversity	11. Biodiversity: Concept, kinds/ levels, importance, methods of study, protection from depletion; Mega - diversity and Hotspots	1
		Unit 10. Threats to Biodiversity	12. Threats to Biodiversity: Causes of threats; Concepts of rare, vulnerable, endangered and threatened plants (IUCN categories)	1

Course	Group	Details Contents Structure		Study hour
BOHCT 3.3		**Unit 11. Conservation: in-situ**	13. Conservation: Types of conservation - in-situ conservation: Biosphere Reserve, Wildlife Sanctuaries, National Parks, World Heritage Sites; Concept and types of Protected Areas Networks	1
		Unit 12. Conservation: ex-situ	14. Ex-situ conservation: principles, methods, definition, aims and activities of W.W.F., Red Data Book, MAB, CITES, Role of Botanic Gardens and Gene Banks	1

Content

COURSE – BOHCT 3.3

(Plant Ecology, Biodiversity & Conservation)

Hard Core Theory Paper **Credit: 3**

Content Structure

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1. Introduction
2. Course Objectives
3. Introduction to Ecology: scope and nature of plant ecology
4. Approaches in ecological studies: formulating hypothesis; theoretical ecological models; probabilistic ecological models; statistical approaches; phylogenetic approaches; remote sensing; climate diagrams
5. Abiotic environment: variables in action; influence of abiotic environment on distribution and abundance of plants
6. Biotic environment: levels of organization of organisms in ecology; plant interactions
7. Habitat and Niche: concept of habitat and niche; niche width and overlap; fundamental and realized niche; competitive exclusion principle; extinction; resource partitioning; character displacement; speciation
8. Population ecology: characteristics of population; population growth curves, population regulation, life history strategies (*r* and *K* selection); metapopulation, habitat fragmentation, demes, source-sink model
9. Community ecology: concepts of community, assemblage and guilds; open and closed communities, ecotone; community continuum concept; community structure; measures of community structure – diversity indices, similarity measures, food web analysis; succession - types, mechanisms, concept of climax
10. Ecosystem ecology: concept of ecosystem, disturbance (natural and anthropogenic) and their impact on plant ecology; invasive plant species; resistance and resilience of ecosystems
11. Biogeographical ecology: terrestrial ecology; wetland and freshwater ecology; coastal and marine ecology; major biogeographical zones of India
12. Biodiversity: Concept, kinds/ levels, importance, methods of study, protection from depletion; Mega - diversity and Hotspots

13. Threats to Biodiversity: Causes of threats; Concepts of rare, vulnerable, endangered and threatened plants (IUCN categories)
14. Conservation: Types of conservation - *in-situ* conservation: Biosphere Reserve, Wildlife Sanctuaries, National Parks, World Heritage Sites; Concept and types of Protected Areas Networks; *ex-situ* conservation: principles, methods, definition, aims and activities of W.W.F., Red Data Book, MAB, CITES, Role of Botanic Gardens and Gene Banks
15. Suggested Reading
16. Assignment

1. Introduction

Ecology is the branch of biology which studies the interactions among organisms and their environment. Objects of study include interactions of organisms with each other and with abiotic components of their environment. Topics of interest include the biodiversity, distribution, biomass, and populations of organisms, as well as cooperation and competition within and between species The two groups have to coexist in order to share the resources that are available within the environmental ecosystem. To understand about this mutual co relationship we need to study and understand ecology. Organism ecology: This studies how different living organisms respond to stimuli caused by physical environment.
Conservation biologists are concerned with the protection and sustainability of natural resources like air, water, land and wildlife. In post degree level in a scientific field like biology this course is needed for teaching and research.

2. Course Objectives

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

1. To know about the nature of plant ecology
2. Distinguish between abiotic and biotic environment.
3. Describe different biogeographical region of India
4. To understand population and community ecology
5. Explain types of conservation and IUCN categories

3. Introduction to Ecology: scope and nature of plant ecology

Introduction:
The word "ecology" ("**oekologie**") (coined by German scientist **Ernst Haeckel,1866**) was derived from the Greek **—oikos**" meaning "**household**" and logos meaning "science:" the "**study of the household of nature**."
Ecology: branch of science that deals with interaction between living organisms with each other and their surroundings. Ecological systems are studied at several different levels from individuals and populations to ecosystems and biosphere level. Ecology is a multi-disciplinary science, drawing on many other branches of science. Applied ecology is the practice of employing ecological principles and understanding to solve real world problems. E.g. calculating fish population, measuring environmental impact from construction or logging, building a case for the conservation of a species, and determining the most effective way to protect a species.
Ecology is the study of the interactions of living organisms with their environment. Within the discipline of ecology, researchers work at four specific levels, sometimes discretely and sometimes with overlap. These levels are organism, population, community, and ecosystem. In ecology, ecosystems are composed of dynamically-interacting parts, which include organisms, the communities they comprise, and the non-living (abiotic) components of their environment. Ecosystem processes, such as primary production, pedogenesis (the formation of soil), nutrient cycling, and various niche construction activities, regulate the flux of energy and matter through an environment. These processes are sustained by organisms with specific life-history traits. The variety of organisms, called biodiversity, which refer to the differing species, genes, and ecosystems, enhances certain ecosystem services.

Scope of Ecology:
The solution of a particular ecological problem requires several lines of approach. None of this constitutes an end in itself but each one of these makes important contribution in making the picture com-plete.

These various lines of approach towards the ecological problem can be translated as:

(a) Biotic

(b) Quantitative

(c) Climatic (both physical and chemical)

(d) Taxonomic

(e) Genetic and evolutionary.

Biotic factors are the direct outcome of the various types of activities amongst the animals. A competition for food and shelter always exists amongst the members of a community. This competition de-mands various types of activity amongst the animals.

Quantitative study includes an assessment of the population density in a given area and also an estimation of the number of members present in different communities. Information of this kind is of immense value in solving many problems like food availability and movement within a parti-cular colony.

Climatic factors include both physical and chemical conditions present in a habitat. These factors are ever changing in nature. Physical factors include mainly temperature, light and humidity. Chemical

factors include acidity or salinity that are specially present in aquatic habitat. Some animals are so sensitive that a minute climatic change becomes fatal to them. Climatic factors play an important role in the distribution of animals.

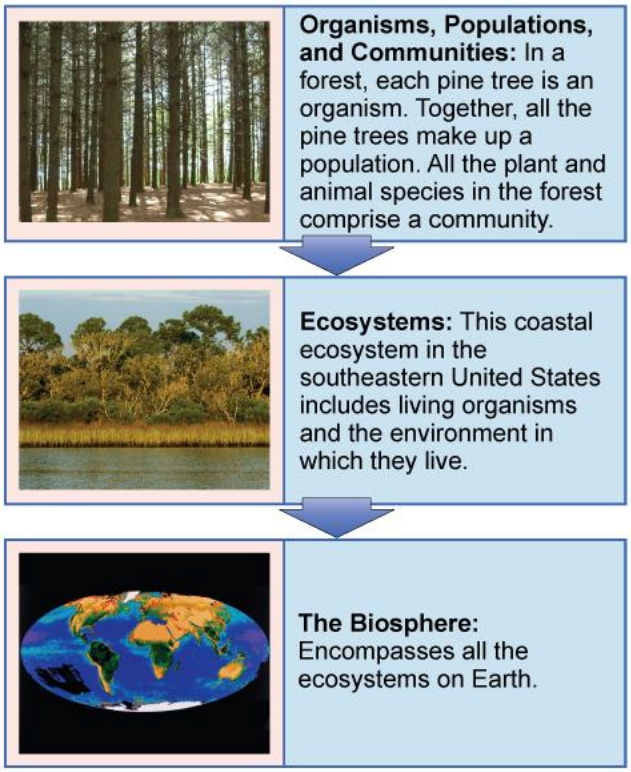
Taxonomy means classification, naming and description of organisms. A mere naming of a large number of animals of a given area, as was done earlier in ecolo-gical surveys, is meaningless without a consideration of the circumstances that enable them to live there. Thus a com-plementary observation of the various eco-logical factors together with taxonomy is emphasized in ecology.

The genetic and evolutionary aspects have taken a rightful place in ecological problems. In recent years the knowledge of heredity and the mechanism of the opera-tion of Natural Selection have increased to a considerable extent.

Evolution is no longer regarded as a thing of the past and it has been proved that evolution is a dynamic process though the progress is very slow. In certain circumstances it has become possible to detect and to measure the rate of evolution in wild population.

The above subdivisions form the back-bone of the study of ecology. The inter-relationship existing between these sub-divisions can be best understood with the help of an example. Let us assume that we want to study the ecology of a given species of edible fish inhabiting a large lake, with an object of establishing a new colony of these fishes to be started else-where.
In so doing, the first information that we need is that whether the food available in the new place is to be taken by these fishes. Our second enquiry would be to find out whether predators are pre-sent in the locality.
These two are included within the biotic factors. We will have to determine the number of fishes that are to be let loose in the new locality and the number is to be determined in such a way that they can live there without being overcrowded.
Herein lays the involvement of the quantitative aspect. We will have to study the water itself and to find out the extent of the fluctuations in its constitution such as salt content, acidity or alkalinity in order to determine the tolerance of the fishes in the changing factors.
If the first lake is a very old one and the fish in question had been isolated there for a great period of time, it is possible that a subspecies or local race might evolve there. In such cases the taxonomist might come forward and help identifying the species. Such a situation opens up a case for the Geneticists and Evolutionists to find out how and at what rate the new forms have evolved.

Levels of Ecological Study
When a discipline such as biology is studied, it is often helpful to subdivide it into smaller, related areas. For instance, cell biologists interested in cell signaling need to understand the chemistry of the signal molecules (which are usually proteins) as well as the result of cell signaling. Ecologists interested in the factors that influence the survival of an endangered species might use mathematical models to predict how current conservation efforts affect endangered organisms. To produce a sound set of management options, a conservation biologist needs to collect accurate data, including current population size, factors affecting reproduction (like physiology and behavior), habitat requirements (such as plants and soils), and potential human influences on the endangered population and its habitat (which might be derived through studies in sociology and urban ecology). Within the discipline of ecology, researchers work at four specific levels, sometimes discretely and sometimes with overlap: organism, population, community, and ecosystem.



- **Organism:** Organismal ecologists study adaptations, beneficial features arising by natural selection, that allow organisms to live in specific habitats. These adaptations can be morphological, physiological, or behavioral.
- **Population:** A population is a group of organisms of the same species that live in the same area at the same time. Population ecologists study the size, density, and structure of populations and how they change over time.
- **Community:** A biological community consists of all the populations of different species that live in a given area. Community ecologists focus on interactions between populations and how these interactions shape the community.
- **Ecosystem:** An ecosystem consists of all the biotic and abiotic factors that influence that community. Ecosystem ecologists often focus on flow of energy and recycling of nutrients.
- **Biosphere:** The biosphere is planet Earth, viewed as an ecological system. Ecologists working at the biosphere level may study global patterns—for example, climate or species distribution—interactions among ecosystems, and phenomena that affect the entire globe, such as climate change.

In essence, ecologists seek to explain:

- life processes
- interactions, interrelationships, behaviors, and adaptations of organisms
- the movement of materials and energy through living communities
- the successional development of ecosystems
- the abundance and distribution of organisms and biodiversity in the context of the environment

There are many practical applications of ecology in conservation biology, wetland management, natural resource management (agroecology, agriculture, forestry, agroforestry, fisheries), city planning (urban ecology), community health, economics, basic and applied science, and human social interaction (human ecology). Organisms and resources comprise ecosystems which, in turn, maintain biophysical feedback mechanisms that moderate processes acting on living (biotic) and nonliving (abiotic) components of the planet. Ecosystems sustain life-supporting functions and produce natural capital, such as biomass production (food, fuel, fiber and medicine), the regulation of climate, global biogeochemical cycles, water filtration, soil formation, erosion control, flood protection, and many other natural features of scientific, historical, economic, or intrinsic value.

There are also many subcategories of ecology, such as ecosystem ecology, animal ecology, and plant ecology, which look at the differences and similarities of various plants in various climates and

habitats. In addition, physiological ecology, or ecophysiology, studies the responses of the individual organism to the environment, while population ecology looks at the similarities and dissimilarities of populations and how they replace each other over time.
Finally, it is important to note that ecology is not synonymous with environment, environmentalism, natural history, or environmental science. It is also different from, though closely related to, the studies of evolutionary biology, genetics, and ethology.

Ecology is a broad science which can be subdivided into major and minor subdiscipline. The major sub-disciplines include:
Behavioral ecology, studies the ecological and evolutionary basis for animal behavior, and the roles of behavior in enabling animals to adapt to their ecological niches;
Population ecology (or autecology), deals with the dynamics of populations within species, and the interactions of these populations with environmental factors;
Community ecology (or synecology), studies the interactions between species within an ecological community;
Landscape ecology, studies the interactions between discrete elements of a landscape;
Ecosystem ecology, studies the flows of energy and matter through ecosystems;
Ecology can also be sub-divided on the basis of target groups: Animal ecology, Plant ecology, Insect ecology; or from the perspective of the studied biomes: Arctic ecology (or polar ecology), Tropical ecology, Desert ecology

Other specialized branches of ecology include:
• **Chemical ecology**, deals with the ecological role of biological chemicals used in a wide range of areas
including defense against predators and attraction of mates;
• **Systems ecology** and biogeochemistry which focus on the flow of energy and nutrients within and among
ecological units;
• **Ecophysiology**, studies the relations between a single type of organism and the factors of its environment;
• **Ecotoxicology**, looks at the ecological role of toxic chemicals (often pollutants, but also naturally occurring compounds);
• **Evolutionary ecology or Ecoevolution** which looks at evolutionary changes in the context of the populations and communities in which the organisms exist;
• **Molecular ecology**, attempts to address ecological questions at the molecular level, usually through by
looking at DNA or allozymes;
• **Paleoecology:** to understand the relationships between species in fossil assemblages, and in so doing gain
insight into the way these species might have been shaped by their interactions with other species;
• Ecology also plays important roles in many inter-disciplinary fields: ecological design and ecological
engineering, ecological economics.
• **Human ecology** and ecological anthropology social ecology, ecological health and environmental psychology.
Finally, ecology has also inspired other non-biological disciplines such as **industrial ecology, software ecology and information ecology**.

4. Approaches in ecological studies: formulating hypothesis; theoretical ecological models; probabilistic ecological models; statistical approaches; phylogenetic approaches; remote sensing; climate diagrams.

Approaches defined

Descriptive–describes organisms and their interactions within ecosystems. This is the foundation of all ecological science (explains what).

Functional–studies proximate causes, the dynamic responses of populations and communities to immediate factors of environment (answers how).

Evolutionary–considers organisms and the relationships between organisms as historical products of evolution (answers why).

Domains of Ecological Research

Domain	Topic	Domain	Topic
Single species	**Demography**	**Species interactions**	**Mutualism**
	Physiology		**Parasitism**
	Behavior	**Ecosystem**	**Food web**
	Evolution		**Climate change**
	Genetics		**Vegetation dynamics**
Community	**Biodiversity**		**Biomass**
	Structure		**Productivity**
Species Interactions	**Grazing**		**Biogeochemical**
	Predation	**Others**	**Scales & Statistics**

4.1. Formulating Hypothesis Hypothesis:

A hypothesis can be defined as a tentative explanation of the research problem, a possible outcome of the research, or an educated guess about the research outcome.

Or

It is a tentative prediction about the nature of the relationship between two or more variables.

Nature of Hypothesis:

- It can be tested – verifiable or falsifiable
- It is a prediction of consequences
- It is neither too specific nor to general
- Hypotheses are not moral or ethical questions
- It is considered valuable even if proven false

Types of Hypotheses:

NULL HYPOTHESES

Designated by: $\mathbf{H_0}$ or $\mathbf{H_N}$ **ALTERNATIVE HYPOTHESES**

Designated by: $\mathbf{H_1}$ or $\mathbf{H_A}$ **Developing Hypotheses:**

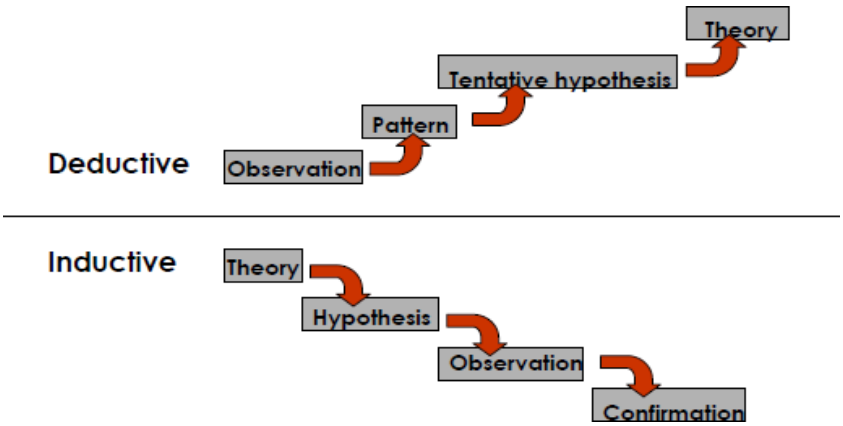
The **null hypothesis represents an assumption that has been put forward, either because it is believed to be true or because it is used to be a basis for argument, but has not been proved.**

Has serious outcome if incorrect decision is made!

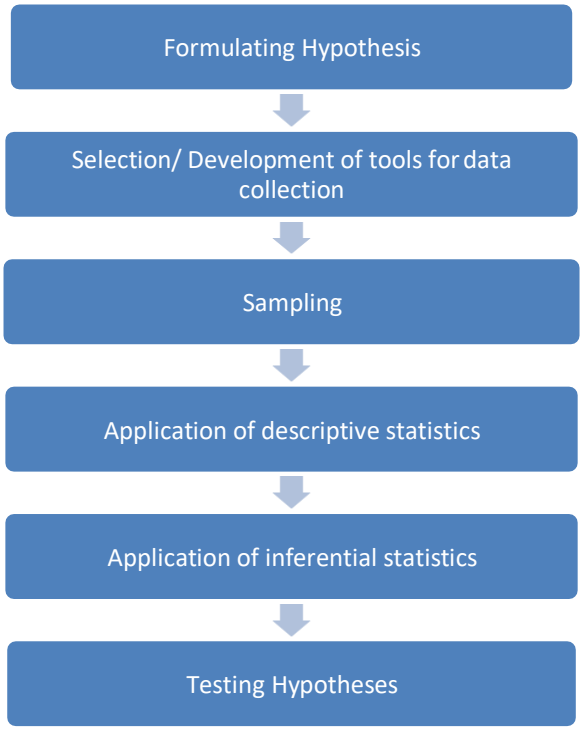
The alternative hypothesis is a statement of what a hypothesis test is set up to establish.

Opposite of Null Hypothesis. Only reached if $\mathbf{H_0}$ is rejected.

Frequently "alternative" is actual desired conclusion of the researcher!



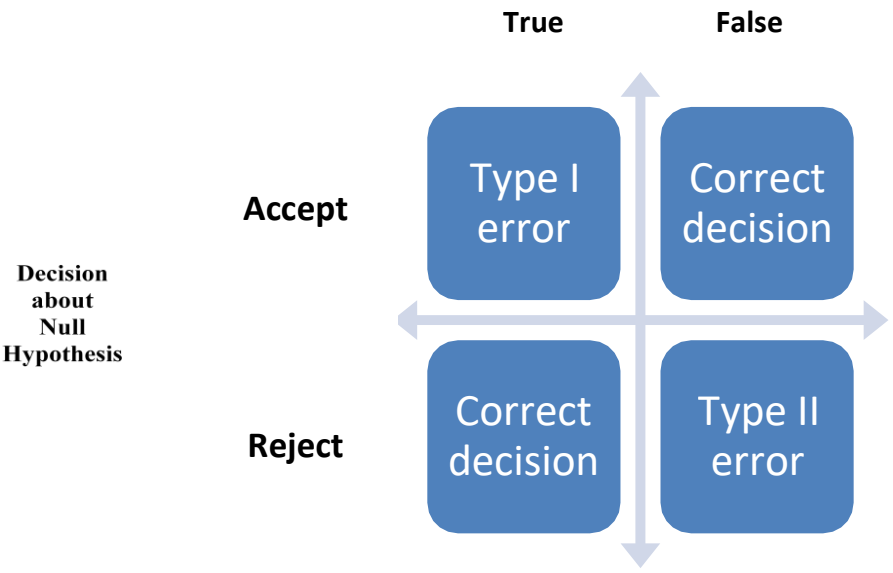
Testing Hypotheses:



Descriptive statistics: mean, median, S.D.
Nothing happened: Null hypothesis is accepted
(There is no significant relationship between X and Y)
Something happened: Alternative hypothesis is accepted (There is significant relationship between X and Y)

Errors in hypotheses testing:

Null Hypothesis



4.2. Theoretical Ecological Models

- The complexity of the natural world can make the development of null hypothesis difficult.
- It is the interconnections between the different components of nature that often give any natural system its distinct characteristics. These inter-connections are manifested in the transfer of materials, transformation of energy, feeding relationships, social interactions….
- So simple statements/questions may not lead directly to simple hypothesis, because of this many hypotheses in ecology emerge first through the development of theoretical models that explain the interactions of the variables of complex systems.
- Theoretical Ecological Models are verbal, algebraic, or graphic constructions that identify the relationships between variables of a system.
- A simple hypothesis can be considered a theoretical model in its simplest form: The fruits of genus 'A' dehisce on warm mornings following continuous rain days. This can be represented as a simple mathematical expression –

$$y = ax + b$$

Experimental Approach

- An experiment is an activity whereby natural processes are allowed to proceed under conditions that are controlled by the experimenter.
- The goal of an experiment is to make an inference about a null hypothesis, that is to determine whether it is more likely that the null hypothesis is true or false.
- If the conclusion is that the null hypothesis is false then some alternative hypothesis must be true.
- Properly designed experiments control to certain extent the possibilities of Type I and Type II errors.
- Experimental studies involve the application of treatments and the observation of responses.

4.3. Probabilistic Ecological Models

Probabilistic Ecological Models deal with the statistical probability of occurrence of certain phenomenon.
Probabilistic Models are based on the exact knowledge of the most desirable information.

By definition, there are three conditions that must be satisfied to make a prediction in Probabilistic Models –
flawless models are required to characterize the event of interest, assumptions must be honored

all model parameters must be known

Purely deterministic model has no stochastic part producing different results under stable conditions and allow reasonable extrapolation beyond the available sampling.

4.4. Statistical Approaches

Statistical approaches provide the means of understanding a process with some specified level of uncertainty. In statistics, an unknown true characteristic of a system is called a parameter. The parameters of ecological systems are usually unknowable but when the values are obtained by direct observations or measurement statistical analysis are not required. A parameter is estimated by a statistic, which is some value or index calculated from a sample of measurements of the variable that is obtained from the entire group of possible measurements of that variable.

Ideally, once the ecological question has been identified, the study is designed and the data is collected in a manner that will result in strong inferences. There are many important aspects to the collection of ecological data relating to study design and sampling method that will influence the type and strength of statistical inferences that can made: identifying the desired scope of inference, choosing appropriate observational/experimental units, choosing the types of data to collect, and establishing a robust sampling scheme (i.e., spatial and/or temporal distribution of units and method(s) of collecting the data) to ensure accurate and precise inferences.

We usually wish to make inferences about a population (statistical, not biological), which is defined as the collection of all the possible observations of interest. A biological population under consideration may or may not constitute the statistical population if, for example, the functional population extends over a broader geographic extent than the study area. We usually represent the size of the statistical population in statistical formulae as upper case N. For lots of practical reasons, we usually collect only a subset of observations from the population, and we represent the size of the sample as lower case n. Importantly, we infer characteristics of the population from the sample; e.g., estimate parameters, test hypotheses, compare models, and make predictions. Thus, the entire realm of inferential statistics applies when we seek to draw conclusions from a sample about the underlying population. Otherwise, we may be interested in or forced to merely describe the patterns in the sample without explicit inference to the population – the realm of descriptive statistics. Note, in rare cases, we may actually observe every possible entity of interest – the population – in which case simple descriptive statistics suffice to draw conclusions from since we know with exactness (to the precision of our measurement system) the characteristics of the population we are studying.

Once we have identified our ecological question, the first thing we need to do is determine what data to collect. This is one of the most important steps in the entire modeling process, because if we collect the wrong type of data, no statistical model of any kind will allow us to answer our ecological question. While there are many important considerations to this step, we need to carefully consider the number and types of variables to collect and their relationships. In ecological studies, there are several major types of data: • continuous data • counts • proportions • binary data • time at death • time series • circular data. And there are at least three major types of variables based on their relationships to each other: • independent variables • dependent variables • interdependent variables.

4.5. Phylogenetic Approaches

Ecology and population biology have been dominated by explanations rooted in processes observable today. Evolution has often been seen as rapidly bringing populations into equilibrium with local conditions, an equilibrium in which the existence of certain genotypes and phenotypes is explained through their having been the best available. In community ecology, the historical approach to hypothesis formation has been, until recently, equally negligible. Only in the biogeographical and geobotanical traditions has historical explanation continued to play a central role. While ecology has been maturing as an experimental and theoretical science, cladistics has increased the rigor of historical explanations. This rigor has made the explanations based on phylogenies more reliable and more testable than they had previously been. At the same time, their own results have led ecologists to reevaluate the role of adaptation, and they have begun to analyze comparative data with the aim of identifying historical constraints.

Community ecology and evolutionary ecology have been suffused with experimental and theoretical approaches in the last quarter century, and some of them have been making rapid progress on adaptationist assumptions. Because this progress has uncovered some patterns not readily explained by adaptation, attention has turned to techniques that can partition the effects of adaptation and constraint. The use of cladistics in comparative biology has become more widespread only during the last few years. Cladistics is a method of analysis, also known as phylogenetic systematics, that was first developed by the German entomologist Willi Hennig. Hennig devised it as an empirical method for discovering and justifying phylogenetic relationship. All species are mosaics of primitive and derived traits, and the phylogenetic relationships in a group of species can be deduced from the pattern of their shared derived traits. The method is thus based on

(1) determining which traits are relatively primitive and which are relatively derived with respect to the group being studied and

(2) grouping the various species according to their shared derived traits.

The relatively primitive traits (called plesiomorphies) are identified by using outgroup comparison - any trait found in at least one member of the group being studied that is also found in species outside the group is most parsimoniously interpreted to have evolved prior to the origin of the common ancestor of the group, and is hence plesiomorphic. The corresponding traits not present in the outgroup are then relatively derived (apomorphies). Once a collection of apomorphies has been assembled for a group, cladistic analysis groups the species according to their shared apomorphies (synapomorphies). Due to parallel and convergent evolution, not all apomorphies will conform to a single pattern, however. Contemporary cladistics uses a variety of numerical methods and computerassisted programs for discerning the pattern of synapomorphies that best fits the data at hand.

The pattern that results is a hypothesis of cladistic relationship, a cladogram, which can be interpreted as a phylogenetic tree to support historical explanations in biology. Cladograms are atemporal representations of relationship. They say nothing about the process of evolution. In contrast, phylogenetic trees are historical interpretations of cladograms. The relationship represented in one cladogram is compatible with more than one phylogeny Cladistics is, thus, a method that uses traits to investigate the systematic relationship of organisms.

Comparative biology, on the other hand, uses cladograms to investigate the systematic or temporal distribution of traits. The idea of using the comparative approach to historical explanations in ecology was widespread in the 19th century and has continued as a central theme in the European geobotanical tradition and in comparative morphology. It has become increasingly prominent in ecology. The historical approach complements the current adaptationist approach to evolutionary ecology. The main difference is the use of indirect estimates of history and the emphasis on adaptation in evolutionary ecology and the use of direct estimates of history (through phylogenetic trees) and the emphasis on constraint in historical ecology. Historical ecology is best suited to asking questions about the origins and diversification of ecological phenomena, ranging from life history traits to community structure. As a form of comparative biology, it is a method for suggesting patterns and locating possible adaptations. It is a method for discovery and descriptive explanation but it can also, indirectly, discriminate among causal explanations, e.g. through statements about the sequence of origination of traits.

4.6. Remote Sensing

Remote sensing as a tool opened up new vistas of perception of things that exist too far or on extensive spatial scales. Nowadays there is a big assortment of satellite systems actively recording information about the Earth. A wide variety of imagery is available from satellites which collect a large amount of information about the earth's surface every day. Each of the systems vary in terms of their spatial, spectral, radiometric and temporal resolution. Those characteristics play an important role in defining which applications the sensor is best suited for. Remote sensing data has shown tremendous potential for applications in various field for example in land use mapping and detection, geologic mapping, water resource applications (pollution, lake-eutrophication assessment), wetland mapping, urban and regional planning, environment inventory, natural disaster assessment or archaeological applications and other.

1. Application in Agriculture

Agriculture plays an important role in economies of countries. The production of food is important to everyone and producing food in a cost-effective manner is the goal of every farmer and an agricultural agency. The satellites have an ability to image individual fields, regions and counties on a frequent revisit cycle. Customers can receive field-based information including **crop identification, crop area determination and crop condition monitoring** (health and viability). Satellite data are employed in precision agriculture to

manage and monitor farming practices at different levels. The data can be used to farm optimization and spatially-enable management of technical operations. The images can help determine the location and extent of crop stress and then can be used to develop and implement a spot treatment plan that optimizes the use of agricultural chemicals. The major agricultural applications of remote sensing include the following:

- crop type classification
- crop condition assessment (crop monitoring, damage assessment)
- crop yield estimation
- mapping of soil characteristics
- mapping of soil type
- mapping soil erosion & erosion vulnerability
- mapping soil management practices
- compliance monitoring (farming practices)

Crop type classification

Remote sensing technology can be used to prepare maps of crop type and delineating their extent. Traditional methods of obtaining this information are census and ground surveying. The use of satellites is advantageous as it can generate a systematic and repetitive coverage of a large area and provide information about the health of the vegetation. The data of crop is needed for agricultural agencies to prepare an inventory of what was grown in certain areas and when. This information serves to predict grain crop yield, collecting crop production statistics, facilitating crop rotation records, mapping soil productivity, identification of factors influencing crop stress, assessment of crop damage and monitoring farming activity.

Crop monitoring and damage assessment

Remote sensing has a number of attributes that help in monitoring the health of crops. Remote sensing imagery also gives the required spatial overview of the land. Remote sensing can aid in identifying crops affected by conditions that are too dry or wet, affected by insect, weed or fungal infestations or weather related damage. Images can be obtained throughout the growing season to not only detect problems, but also to monitor the success of the treatment. Detecting damage and monitoring crop health requires high-resolution, multi- spectral imagery and multi-temporal imaging capabilities.

2. Application in Forest mapping

One of the basic applications is forest cover typing and species identification. Forest cover typing can consist of exploration mapping over a large area, while species inventories are highly detailed measurements of stand contents and characteristics (tree type, height, density). Using remote sensing data we can identify and delineate various forest types that would be difficult and time consuming using traditional ground surveys. Data is available at various scales and resolutions to satisfy local or regional demands. For mapping differences in forest cover we require the following:

- multi-spectral images, a very high resolution data is required to get detailed species identification;
- multi-temporal images datasets contribute phenology information of seasonal changes of different species;
- stereo photos help in the delineation and assessment of density, tree height and species;
- hyper-spectral imagery can be used to generate signatures of vegetation species and certain stresses (e.g. infestations) on trees. Hyper-spectral data offers a unique view of the forest cover, available only through remote sensing technology;
- RADAR is more useful for applications in the humid tropics because its all weather imaging capability is valuable for monitoring forest

Clear cut mapping and deforestation

One of an important global problem is deforestation. There are many implications of it: in industrialized parts of world, pollution (acid rain, soot and chemicals from factory smoke plumes) has damaged a large percentage of forested land, in tropical countries, valuable rainforest is being destroyed in an effort to clear potentially valuable agricultural and pasture land. The loss of forests increases soil erosion, river siltation and deposition, affecting the environment.

3. Application in Land cover Mapping

Land cover mapping is one of the most important and typical applications of remote sensing data. Land cover corresponds to the physical condition of the ground surface, for example, forest, grassland, concrete pavement etc., while land use reflects human activities such as the use of the land, for example, industrial zones, residential zones, agricultural fields etc. Initially the land cover classification system should be established, which is usually defined as levels and classes. The level and class should be designed in consideration of the purpose of use (national, regional or local), the spatial and spectral resolution of the remote sensing data, user's request and so on.

Land cover change detection is necessary for updating land cover maps and management of natural resources. The change is usually detected by comparison between two multi-date images, or sometimes between an old map and an updated remote sensing image.

- **seasonal change**: agricultural lands and deciduous forests change seasonally
- **annual change**: land cover or land use changes, which are real changes, for example deforested areas or newly built towns.

Information on land cover and changing land cover patterns is directly useful for determining and implementing environment policy and can be used with other data to make complex assessments (e.g. mapping erosion risks).

4.7. Climate Diagrams

Atmospheric science is often divided into three specific fields of interest:

- Aerology: It is essentially the study of the free atmosphere through its vertical extent.
- Meteorology: It deals with the motions and phenomena of the atmosphere to forecast weather and explain the processes involved.
- Climatology: It is the study of atmospheric conditions over periods of time measured in years or more.

Weather is the day-to-day state of the atmosphere, and its short-term (minutes to weeks) variation.

It describes the atmospheric conditions at a specific place at a specific point in time. Weather generally refers to day-to-day temperature and precipitation activity.

It refers to real-time measurements of atmospheric pressure, temperature, wind speed and direction, humidity, precipitation, cloud cover, and other variables.

Climate is defined as statistical weather information that describes the variation of weather at a given place for a specified interval.

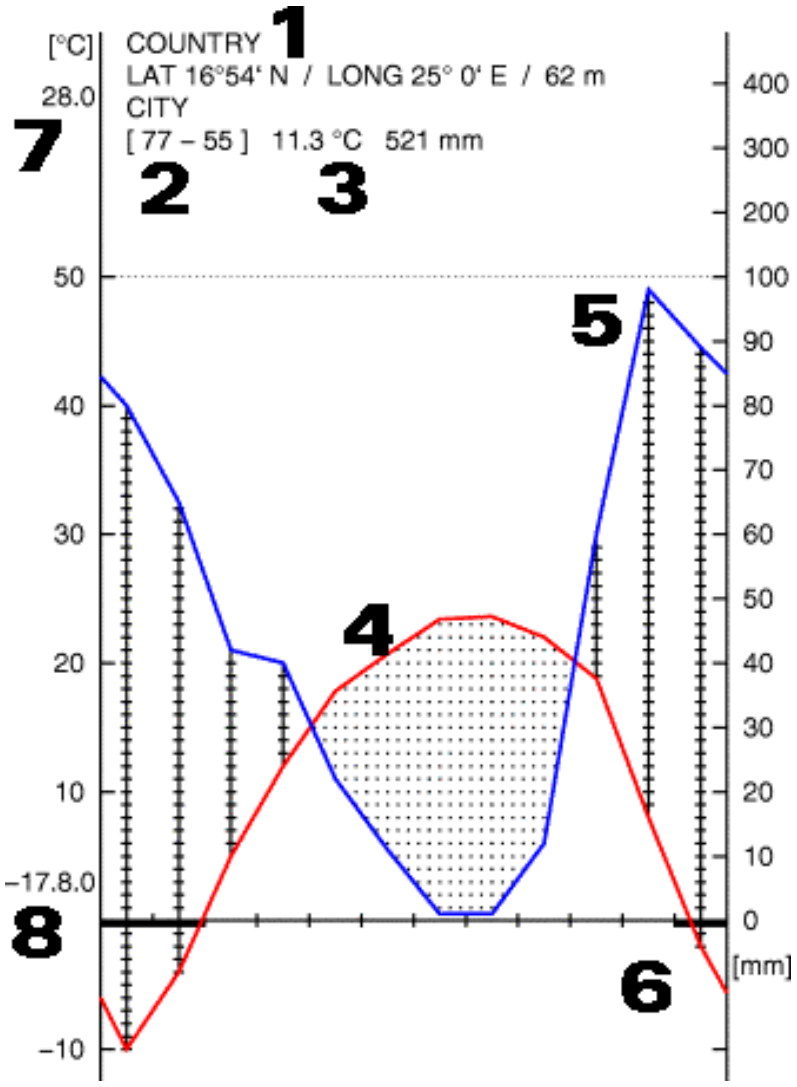
It describes the average conditions expected at a specific place at a given time. It implies aggregates of weather statistics over periods of minimum 30 years. **Elements of Climatology**

- ❖ Assessment of the effect of climate on the natural environment, agricultural production, energy supply and demand, land & water resources, transportation, human health & national security.
- ❖ Basic & applied research to improve the understanding of climatic processes - natural and human-induced.
- ❖ Social, economic and political implications of climate change.
- ❖ Methods for improving climate forecasts.
- ❖ Global data collection on continued basis.
- ❖ Measures for increasing international cooperation in climatology.

Climate Diagrams

Climate diagrams are brief summaries of average climatic variables and their time course. They have proven useful for a wide range of sciences, industry, teaching & are useful for planning and design. In

bio- and geosciences, they are used as an instrument to show the relationships between soil, vegetation, and climate. In agronomic sciences, they are used to indicate the range for crops. They indicate optimal travel schedules for the tourism industries. The diagrams display monthly averages for temp. and precipitation over a year. Each mark along the horizontal line indicates a month. The diagrams start with January in the left corner of the diagram for the northern hemisphere and with July for the southern hemisphere respectively. Thus, the astronomic summer is always shown in the middle of the diagram. 20 mm of monthly precipitation (right ordinate) equals 10°C average temperature (left ordinate). When the precipitation curve undercuts the temperature curve, the area in between them is dotted (every 2 mm) indicating dry season. When the precipitation curve supersedes the temperature curve, vertical lines are plotted for each month (with tic marks every 2 mm) indicating moist season. A very important ecological variable is frost. The diagram shows daily average minimum temperatures below zero in black bars below the horizontal line. All diagrams are designed in a uniform pattern.



1 Country name, station location and elevation, station name

2 Period of observation of temperature (77 years) and precipitation (55 years)

3 Annual average of temperature and annual precipitation sum 4 (red) Temperature curve

5 (blue) Precipitation time series 6 Indication of frost periods

7 Mean daily max. temperature of the warmest month 8 Mean daily min. temperature of the coldest month

Climate Determinants

- Air Temperature
- Barometric Pressure
- Cloud type, height and amount
- Prevailing weather
- Dew Point temperature
- Precipitation
- Sunshine
- Wind velocity & direction

Climate : Scales of Study

- MICROCLIMATE: Characterized by climate that may extend horizontally from less than 1 meter to 100 mts and vertically from the surface upto 100 metres.
- LOCAL CLIMATE: Comprises of number of microclimatic areas that make up a distinctive group and horizontal dimensions extend from100 to 10,000 mts and vertically upto 1000 mts.
- MESOCLIMATE: Ranges horizontally from 100 – 20,000 mts. And vertically upto 6,000 mts. A great variety of individual landscapes are considered.
- MACROCLIMATE: Extends horizontally for distances more than 20,000 mts and vertically to heights above 6,000 mts. Such areas can be continental in extent.

5. Abiotic environment: variables in action; influence of abiotic environment on distribution and abundance of plants

Ecological relationships are manifested in physicochemical environment. Abiotic component of ecosystem includes basic inorganic elements and compounds, such as soil, water, oxygen, calcium carbonates, phosphates and a variety of organic compounds (by-products of organic activities or death).

It also includes such physical factors and ingredients as moisture, wind currents and solar radiation. Radiant energy of sun is the only significant energy source for any ecosystem. The amount of non-living components, such as carbon, phosphorus, nitrogen, etc. that are present at any given time is known as standing state or standing quantity.

These factors include the non-living physiochemical factors of the environment. Abiotic factors are as follows:

(i) Inorganic substances: Inorganic substances like carbon, nitrogen, oxygen, water, carbon di-oxide, calcium, phosphorus and their inorganic compounds. These are available as free form or dissolved in water and may be adsorbed on the soil particles.

(ii) Organic compounds: These are carbohydrates, proteins, lipids, nuceltic acids etc.This material is present in dead organic matter. These are broken into the simple compounds by decomposers in ecosystem for recycling of matter.

(iii) Climatic factors: These are factors present in the environment such as temperature, humidity, light, wind, rainfall an atomospheric gaseous etc.

The climatic factors are as follows:

1. Temperature:

Temperature is an ecological abiotic factor. It is a form of energy and is called the thermal energy. It penetrates into each and every region of the biosphere and affects all forms of life. It influences the various stages of life activities such as growth, metabolism, reproduction, movement, distribution, behaviour, death, etc.

Temperature is usually measured in Fahrenheit or Centigrade. The biosphere obtains its thermal energy from the Sun in the form of solar radiation. It is a variable factor. It varies from place-to-place and time-to-time. It is high in the day and at night it is low. It is high at the sea level and low at high

altitudes. It is high at the equator and low in the Polar Regions. It is more in the terrestrial habitat and low in the aquatic habitat. The maximum temperature recorded on land is 85°C as in the desert and the lowest temperature is about – 70°C as in Siberia.

Temperature Fluctuations:
The temperature is high during daytime and low at night. This is called diurnal variation. The temperature on land is high at the sea level, but low at high altitudes. Approximately, an increase in altitude of 150 m results in a decrease in 1°C temperature. On land, maximum temperature is found along the equator. It gradually decreases towards the poles. Temperature varies according to the season. The temperature reaches its maximum during summer, while it is minimal during winter.
Temperature fluctuation in aquatic habitat is less than that of terrestrial habitat.

Thermal Stratification:
In lakes and ponds a gradual decrease in temperature from the surface to the bottom is seen. This leads to different layers of water with different temperatures. The arrangement of different layers based on temperature differences is called thermal stratification.

Biological Effects of Temperature:
a. Eurythermal and Stenothermal Organisms:
Organisms that can tolerate wide range of temperature fluctuations are called eurythermal organisms, e.g. man, lizard, amphibians. Those that cannot tolerate wide range of temperature fluctuations are called stenothermal organisms, e.g. corals, snails
b. Poikilothermic and Homeothermic Animals:
Animals in which the body temperature changes according to the fluctuations in the environmental temperature are called poikilothermic or cold-blooded animals or ectotherms. During cold, the body temperature also drops. For example, all animals except birds and mammals.
In birds and mammals, the body temperature remains constant and is not dependent on environment temperature. These animals are called homeotherms or warm blooded or endotherms. When the environment temperature drops the animal maintains its temperature by metabolic activities.

Effect of Temperature on Growth and Development:
Temperature affects growth and development of animals. For example, the oyster, Ostraea virginica grows to 1.4 mm when it is reared at 10°C, but when reared at 20°C it grows to 10.3 mm. Similarly, the eggs of the mackerel fish does not develop below 8°C and above 25°C. Low temperature prevents metamorphosis in salamanders and makes the animal neotenous.

Effect of Temperature on Morphology:
The morphological characters of organisms are altered by temperature. Temperature influences the size of animals and the relative proportions of the parts of the body. Three rules have been put forth to understand how the temperature influences various characteristic features.

a. Bergman's Rule:
The mammals in colder areas are larger is size than in warmer climates. This is called the Bergman's rule. For example, the penguins found in Antarctica attain a body length of 100- 200cm, whereas the penguins of equatorial Galapagos Islands are about 49cm long.

b. Allen's Rule:
According to Allen's rule, extremities of the mammals, like the tail, snout, ears and legs are relatively shorter in colder regions than in warmer regions. In the Arctic rabbit the ears are shorter, while in the Californian rabbit, the ears are longer.
The explanation in both the cases is that endothermic organisms in colder climates should have smaller surface area relative to volume across which they lose heat. Allen's rule has widespread applicability when compared to Bergman's rule because of number of factors that affect body size, though it is true at an intra-specific level.

c. Gloger's Rule:
According to Gloger's rule the animals in the tropic are darker and heavily pigmented than their counterparts of the colder and dry regions.

Effect of Temperature on Distribution:
Temperature is a limiting factor on the distribution of animals. The distribution of warm-blooded animals is not affected by temperature. But cold-blooded animals are abundant in tropical and temperate regions, and their number rapidly diminishes towards the poles.

Effect of Temperature on Plants:
a. The opening of the flowers of various plants during the day and night is often due to temperature difference between the day and night.
b. The seed of some plants (biennials) normally germinate in the spring or summer. These seeds require a cold treatment of winter. This is called vernalisation. Vernalisation can be induced in seeds artificially. This adaptation ensures that seeds do not germinate during autumn, but only after winter, when the seedlings have better chances to survive.
c. Deciduous trees lose their leaves in winter and enter into a state of dormancy, where the buds are covered for protection against the cold.
d. In the desert due to great temperature variation between day and night organisms exhibit distinct periods of activity, e.g. many cacti flower at night are pollinated by nocturnal insects. Cactus is among the most drought-resistant plants on the planet.

In a cactus:
i. Leaves are modified into spines. These spines protect the plant from animals, shade it from the Sun and also collect moisture. This also reduces transpiration.
ii. Extensive shallow root systems that are spread out just below the surface to allow the plant to absorb water immediately as it rains.
iii. Succulent stems have the ability to store water. This enables the cacti to survive in dry climate and can survive years of drought on the water collected from a single rainfall.
iv. Waxy skin to seal in moisture.
v. Cacti depend on chlorophyll in the outer tissue of stems to conduct photosynthesis for the manufacture of food.
vi. Cacti close their stomata during the day and open them at night to reduce transpiration. These plants exhibit the CAM pathway of photosynthesis.

Many other desert trees and shrubs have also adapted by eliminating leaves – replacing them with thorns, not spines, or by greatly reducing leaf size to eliminate transpiration. Many xerophytes may accumulate proline in the cells of its leaves to maintain osmotic and water potential. Chaperonins, the heat shock proteins provide physiological adaptations to plants to high temperatures. These proteins maintain the structures and avoid denaturation of other proteins.

e. Plants living in cold climates can tolerate frost conditions. When the temperature drops the plant becomes dormant and exhibits slow rate of photosynthesis and respiration. Antifreeze proteins are found in some plants which avoid chilling and frost damage by increasing their sugars and alcohols to lower the freezing point of cell fluids. This causes super cooling of the cell sap for short periods of time without causing freezing.
Structural Adaptations in a Camel;
In hot deserts, temperature is very high. To escape from the heat desert animals have the different adaptations for resistance to heat. This can be understood from the adaptations of a camel: All desert dwellers have adapted to conserve water, food and energy. The camel is one of the best survivors in the desert and it is rightly called the 'ship of the desert' because it is adapted very well to the conditions of the desert.

2. Light:

Light is the most important and indispensable physicochemical, abiotic ecological factor without which life cannot exist. Organisms get light from the Sun, Moon, stars, lightning, volcanoes and bioluminescent organisms. Among this light energy from the Sun is the most important in nearly all ecosystems. It is the energy that is used by green plants (which contain chlorophyll) during the process of photosynthesis; a process during which plants manufacture organic substances by combining inorganic substances.

The energy from the sun comprises of short, high-energy radiations to long, low energy radiations. The amount of energy in the sunrays just before entering the atmosphere is about 2 cal/cm^2/min. It is called solar constant. As the sunrays travel through the atmosphere a large amount of energy is absorbed.

Biological Effects of Light:

a. On Metabolism:

High intensity of light increases metabolic activity in animals by increasing enzyme activity.

b. On Pigmentation:

Light induces photo-chemical reactions in the formation of colour pigments called melanophores. Animals living in cave, bottom of the ocean do not possess colour.

c. Protective Coloration:

Animals develop colour patterns to conceal themselves from predators to bend with the surroundings. For example, the leaf insect, Phyllium is green in colour.

d. Colour Change in Animals:

The Chamaeleon is able to change its colour according to its background. This happens because of the distribution of the melanophores depending on the light entering the eye.

e. Vision:

Light enables organisms to see objects in the environment where it is found. Animals possess specific organs to 'see' like the eyespots in protozoa, compound eyes in insects and crustaceans, eyes in vertebrates etc. Animals that live in habitats where there is dim light have large eyes that are powerful as in the owls and loris. In animals that live in habitat where there is no light, the eyes are reduced.

f. Reproduction:

Animals are classified into the following categories according to the influence of light on reproduction:

i. Long-Day Animals:

This group of animals are sexually active when the days are long, e.g. birds.

ii. Short-Day Animals:

This group are sexually active when the days are short, e.g. sheep, deer, goats.

iii. Day-Neutral Animals:

In this group reproduction is influenced by light, e.g. man, cow.

g. Diurnal Migration:

In the oceans, planktons move to the surface in the early morning and evenings and move to the deeper parts of the ocean when there is high intensity. This movement is called diurnal migration.

h. Circadian Rhythm:

The daily rhythm in synchrony with the rotation of the earth is called circadian rhythm. This is endogenous, i.e. initiated by internal factors and is due to a biological clock present in organism. For example, many plants show rhythm of their leaves for sleep. They close or droop during night time and open at daytime. Sleeping and waking in man follow circadian rhythm.

Adaptations of Plants to Changing Light Conditions:

Light requirements of plants differ and as a result distinct layers or stratification can be observed in an ecosystem. Plants which grow well in bright sunlight are called heliophytes (Greek helios, sun) and plants which grow well in shady conditions are known as sciophytes (Greek skia, shade).

Heliophytes have a high rate of respiration and are adapted to high light intensities, while sciophytes have low rate of photosynthesis, respiration, metabolism and growth.

3. Water:
Water covers 70% of the earth's surface and is found as vapour in the atmosphere and in the soil as soil water. 97% of the water is found in the oceans and 3% is found as freshwater. Approximately 70% of freshwater is found as ice caps and glaciers, 20% as underground water while the remaining is found in lakes, streams and rivers. Water is essential for life and all organisms depend on it to survive in especially desert areas.
The Water Cycle in Nature:
Water cycles through the biosphere and is constantly exchanged between the physical and the biotic environment. The circulation of water that does not involve living organism is the global water cycle and that which involves living systems is the biological water cycle.
Adaptations of Plants in Water:
Water constitutes the hydrosphere and includes both fresh and seawater. Aquatic plants are called hydrophytes. These plants possess specialised parenchyma called aerenchyma that possesses air filled spaces in the leaves and stem. This enables the plants to float.
Adaptations of Animals in Aquatic Habitat:
A number of animals live in the aquatic medium, i.e. water. There are animals that are found exclusively in the fresh water, while there are some that are found living in the marine environment; there are some that are capable of living in both fresh and marine water. A few examples of animals that are aquatic are vertebrates like fish, mammals (whales, dolphins, seals, sea lions, etc.), invertebrates like starfish, prawns, lobsters, octopus, oysters, etc.
Adaptations in animals living in water are called aquatic adaptations and organisms living in water are called aquatic organisms. Aquatic organisms found on the surface of the water are called pelagic organisms for which they possess special adaptations. Similarly organisms living in the deep sea, called benthic animals are adapted to live in such conditions.

4. Air:
Air or atmosphere is the gaseous envelope that surrounds the lithosphere and hydrosphere. The atmosphere is a mixture of gases. Nitrogen makes up four-fifths of it and oxygen makes up a little more than one-fifth. Small quantities of other gases like argon, neon, helium, krypton, xenon, carbon dioxide, hydrogen and ozone are also found.
The most important gases used by plants and animals are oxygen, carbon dioxide and nitrogen.
a. Oxygen:
Oxygen is used by all living organisms during respiration.
b. Carbon Dioxide:
Carbon dioxide is used by green plants during photo-synthesis.
c. Nitrogen:
Nitrogen is made available to the plants by certain bacteria and through the action of lightning.
Layers of the Atmosphere:
The atmosphere is made of five or more dis¬tinct layers that differ in density, temperature, composition and properties.
a. Troposphere – 0-10 kms
b. Stratosphere – 10-40 kms
c. Mesosphere – 40-70 kms
d. Thermosphere – 70-400 kms
e. Exosphere – 400 kms and beyond

5. Wind:
Differential solar radiation in different regions of the Earth as well as rotation of the Earth causes air to move and form wind. Depending upon the velocity of the wind it is called breeze, gale, storm or hurricane. Dust storms and squall are also modified forms of wind; the former carries dust particles while the latter carries rain or snow.

Winds or air currents arise on a world-wide scale as a result of a complex interaction between hot air expanding and rising (convection) in the mid-latitudes. This has various effects on the rotation of the Earth and results in a centrifugal force which tends to lift the air at the equator. This force is known as the Coriolis force and tends to deflect winds to the left of the southern hemisphere and to the right in the northern hemisphere. Winds carry water vapour, which may condense and fall in the form of rain, snow or hail.
Wind plays a role in pollination and seed dispersal of some plants, as well as the dispersal of some animals, such as insects. Wind erosion can remove and redistribute topsoil, especially where vegetation has been reduced. Warm berg wind results in desiccation, which creates a fire hazard. If plants are exposed to strong prevailing winds are they usually smaller than those in less windy conditions.

6. Biotic environment: levels of organization of organisms in ecology; plant interactions

The biotic components include all living organisms present in the environmental system.
From nutrition point of view, the biotic components can be grouped into two basic components:
(i) Autotrophic components, and
(ii) Heterotrophic components

The autotrophic components include all green plants which fix the radiant energy of sun and manufacture food from inorganic substances. The heterotrophic components include non-green plants and all animals which take food from autotrophs.
So biotic components of an ecosystem can be described under the following three heads:
1. Producers (Autotrophic components),
2. Consumers, and
3. Decomposers or reducers and transformers

The amount of biomass at any time in an ecosystem is known as standing crop which is usually expressed as fresh weight, dry weight or as free energy in terms of calories/metre.

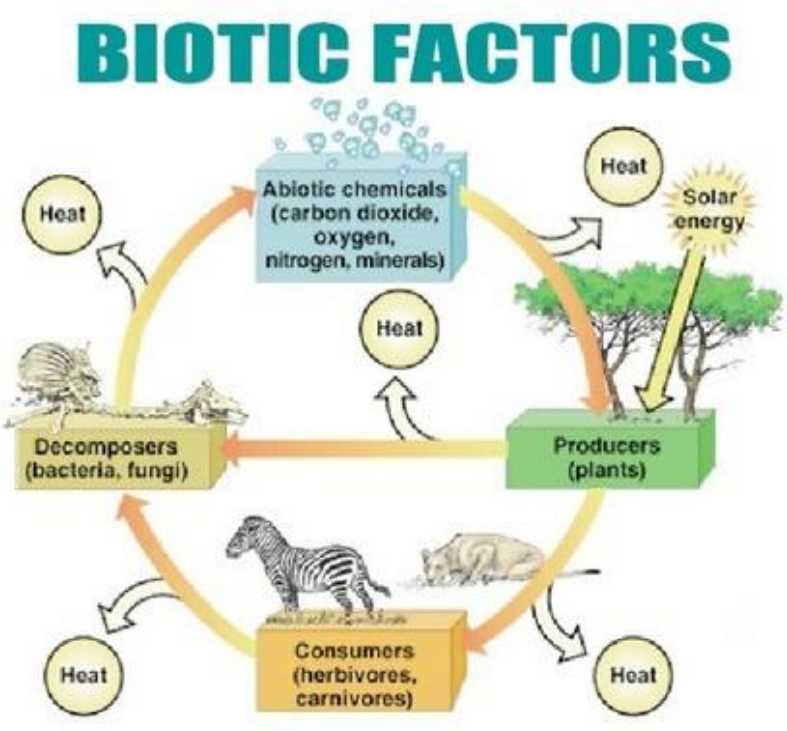
Producers (Autotrophic elements):
The producers are the autotrophic elements—chiefly green plants. They use radiant energy of sun in photosynthetic process whereby carbon dioxide is assimilated and the light energy is converted into chemical energy. The chemical energy is actually locked up in the energy rich carbon compounds. Oxygen is evolved as by-product in the photosynthesis.
This is used in respiration by all living things. Algae and other hydrophytes of a pond, grasses of the field, trees of the forests are examples of producers. Chemosynthetic bacteria and carotenoid bearing purple bacteria that also assimilate CO2 with the energy of sunlight but only in the presence of organic compounds also belong to this category.

The term producer is misleading one because in an energy context, producers produce carbohydrate and not energy. Since they convert or transduce the radiant energy into chemical form, E.J. Kormondy suggests better alternative terms ‘converters’ or ‘transducers’. Because of wide use the term producer is still retained.

Consumers:
Those living members of ecosystem which consume the food synthesized by producers are called consumers. Under this category are included all kinds of animals that are found in an ecosystem.
There are different classes or categories of consumers, such as:
(a) Consumers of the first order or primary consumers,
(b) Consumers of the second order or secondary consumers,
(c) Consumers of the third order or tertiary consumers, and
(d) Parasites, scavengers and saprobes.

(a) Primary consumers:
These are purely herbivorous animals that are dependent for their food on producers or green plants. Insects, rodents, rabbit, deer, cow, buffalo, goat are some of the common herbivores in the terrestrial ecosystem, and small crustaceans, molluscs, etc. in the aquatic habitat. Elton (1939) named herbivores of ecosystem as "key industry animals". The herbivores serve as the chief food source for carnivores.
(b) Secondary consumers:
These are carnivores and omnivores. Carnivores are flesh eating animals and the omnivores are the animals that are adapted to consume herbivores as well as plants as their food. Examples of secondary consumers are sparrow, crow, fox, wolves, dogs, cats, snakes, etc.
(c) Tertiary consumers:
These are the top carnivores which prey upon other carnivores, omnivores and herbivores. Lions, tigers, hawk, vulture, etc. are considered as tertiary or top consumers.
(d) Besides different classes of consumers, the parasites, scavengers and saprobes are also included in the consumers. The parasitic plants and animals utilize the living tissues of different plants and animals. The scavengers and saprobes utilize dead remains of animals and plants as their food.

Decomposers and transformers:
Decomposers and transformers are the living components of the ecosystem and they are fungi and bacteria. Decomposers attack the dead remains of producers and consumers and degrade the complex organic substances into simpler compounds. The simple organic matters are then attacked by another kind of bacteria, the transformers which change these organic compounds into the inorganic forms that are suitable for reuse by producers or green plants. The decomposers and transformers play very important role in maintaining the dynamic nature of ecosystems.



Effects of Biotic Factors on Vegetation:
Any activity of the living organism which may cause marked effects upon vegetation in any way is referred to as biotic effect. The biotic effect may be both direct and indirect. It may be beneficial to the plants in some respects but detrimental in other respects.The biotic effects modifying the vegetation can be discussed in the following heads:
(1) Interactions between the plants and local animals and man.
(2) Interaction among plants growing in a community.

(3) Interaction between plants and soil microorganisms.

1. Interaction between Plants and Local Animals and Man:
These can be described under the following heads:
(i) Effects of grazing and browsing by animals.
(ii) Role of animals in the pollination.
(iii) Role of animals in the dispersal of seeds and fruits.
(iv) Insects and carnivorous plants.
(v) Effects of human activities on vegetation.
(vi) Myremecophily.

(i) Effects of grazing and browsing:
Grazing means eating away of un-harvested herbs as forage by animals, as for example, eating away of grasses by goats whereas browsing refers to a similar use of shrubs or trees by animals, as for example, eating away of leaves and small twigs of *Margosa* (Neem) by camels.
The animals destroy a large part of Vegetation by grazing and browsing. Some animals prefer to graze and browse on some particular plant species they show selective grazing and browsing, e.g., sheep normally prefer forbs, horses and cattle prefer grasses and goats and deer prefer woody and leafy Parts of plant. Small annual plants become uprooted and disappear after being grazed. In browsing, taller plants such as trees and shrubs are little affected.

(ii) Role of animals in pollination:
A large number of plants depend on insects, birds and a number of animals for their pollination. These plants develop coloured flowers. The flowers possess scents, nectar, sap, edible pollens and many other characteristic structures for attracting insects towards them. Insects, birds and other pollinators visit the flowers in search of honey and edible pollens. Flowers in the families Rosaceae, Compositae, Leguminosae, Rutaceae, Umbelliferae, Euphorbiaceae, Cruciferae, Ranunculaceae are pollinated by insects.
Some plants are specialized in their pollination by particular type of animals, for example, *Rafflesia* is pollinated by elephants and birds, bilipped flowers of Salvia are pollinated by bees, entomophilous flowers of orchids, Ficus and Calotropis are pollinated characteristically by insects.
Besides insects, birds, bats and some other animals, man too is taking active part in pollinating artificially one plant with the pollen of some other plant species. The artificial pollination is being used by man for the production of high yielding and disease resistant plant varieties.

(iii) Role of animals in the dispersal of fruits and seeds:
Many animals, such as birds, bats, monkeys, act as important agents for disseminating the seeds, fruits and spores and thus they play important role in the migration of plants. The seeds of many plants are very hard. Such seeds along with fleshy parts of fruits are swallowed by animals. While passing through the elementary canals of animals hard seeds are not affected by digestive juices.
When the animals leave faecal matter, the uninjured seeds present in it germinate. Passing of seeds through the digestive tracts sometimes facilitates their germination in certain cases. The seeds of tomato, tobacco, guava and many other plants are dispersed in this way.
The hairy, spiny, hooked and sticky fruits and seeds of some plants get entangled with the bodies of birds and other animals and with the clothes of man and are brought to distant places. When the animals clean their bodies at some places the seeds are dropped there. Seeds and fruits of *Xanthium, Andropogon, Plumbago, Aegle marmelos* are dispersed in this way. Ants are good agents for transporting oily seeds and small grains of cereals.

(iv) Insects and Carnivorous plants:
Semi-autotrophic insectivorous plants, as for example, pitcher plant, *Drosera*, *Aldrovanda*, *Dionaea*, bladderwort, etc., grow in the habitats which are deficient in nitrogenous compounds. These plants have some specialized organs and mechanisms for trapping and assimilating the preys.

v) Effects of human activities on vegetation:
Man affects vegetation in the following ways:
(a) By cutting, felling and replanting the forest trees.
(b) Cultivation:
(c) Fire:

(d) Man also clears the vegetation for making houses, roads, etc.

(vi) Myremecophily:

Sometimes ants take their abode or shelter on some trees such as Mango, Litchi, Jamun, South American Acacia (*Acacia sphaerocephala*) and so on. These ants act as body guards of the plants against any disturbing agent. In lieu of this defence, the plants provide food and shelter to these ants. This phenomenon is known as myremecophily.

2. Interaction among Plants Growing in a Community:

Various plants in a community react with one another in several ways for:

(i) Water,

(ii) Essential minerals and organic compounds, and

(iii) Light and air.

The taller plants modify the habitat for the plants growing around and underneath them by casting shadow, protecting them from injuries by strong wind, by increasing the atmospheric humidity, and by determining the humus content of the soil.

The most interesting instances of interactions among plants growing in a community are as follows:

(i) Action of lianas;

(ii) Effects of some epiphytes;

(iii) Effects of parasitic plants.

(i) Action of lianas:

Lianas are woody vascular Discs plants growing on the ground, maintaining, more or less, autotrophic mode of life and growing upward taking support of some trees and other objects. The woody stems of these plants have well developed alternating vertical columns of secondary xylem and parenchymatous tissues which enable them to twist around the supporting objects.

(ii) Effects of some epiphytes:

The epiphytes grow on the leaves and stems of other plants. They are autotrophic and are dependent on other plants only for support. Epiphytes differ from parasites in not taking food from the hosts and also differ from lianas in not having any permanent connection with the soil. The examples of epiphytes may be found in the families Orchidaceae, Asclepiadaceae, Bromeliacease. Cactaceae, etc. *Dischidia*, *Tillandsia* are most common examples. Epiphytes are found in humid climates.

The two main problems for these plants are:

(a) Maximum absorption of water from the atmosphere and from the bark surface of the supporting plant and

(b) Maximum economy in the water consumption. These plants develop two types of roots, namely the aerial and clinging roots.

3. Interaction between Plants and Microorganisms:

Various kinds of bacteria, protozoa, algae, fungi, worms, nematodes and other soil microbes act as important agents which alter the physical and chemical properties of the soils and increase or decrease their fertility. These changes in the soil properties have great impact on the nature and growth of vegetation.

Very often soil microbes, such as nematodes, bacteria and fungi cause many diseases in the underground parts of plants. Viruses too cause several mosaic and other diseases in many plants, as for example, the curling of tomato leaves, mosaic patterns in papaya and lady's finger (bhindi), bean mosaic, tobacco mosaic, etc. Some microbes secrete growth stimulating substances in the soil which induce the growth of plants.

Symbiotic influence:

Some soil microbes live in close association with plants, both benefiting each other. In this association both the organisms are interdependent and they do not harm each other. This mutual relationship between two organisms is known as symbiosis and the interdependent organisms are called symbionts.

Many cases of symbiosis in plants are known. The nodulated roots of legumes contain nitrifying bacteria (*Rhizobium*) . These bacteria fix atmospheric nitrogen into nitrogenous compounds and benefit the legumes by supplying nitrogenous compounds in usable form.

Lichens also show symbiosis. These are synthetic plants in which algae and fungi live symbiotically. Generally, the algal component belongs to myxophyceae and fungal components are ascomycetous or sometimes basidiomycetous forms. In this association, algal component fixes the atmospheric nitrogen, prepares food and supplies nutrients to its fungal counterpart. Fungal component gives support to the algal component. It also saves algae from desiccation because of its sponginess and high water holding capacity.

Mycorrhizal association:

Sometimes fungi grow on the surface or inside the roots of higher plants. They are called mycorrhizae.

7. Habitat and Niche: concept of habitat and niche; niche width and overlap; fundamental and realized niche; competitive exclusion principle; extinction; resource partitioning; character displacement; speciation

Concept of habitat and niche:

Habitat: Where organisms normally live. It comprises of all the biotic and abiotic factors in the area where the organism lives.

Niche: How organisms normally live. A niche is the *role* and *position* of a species in nature. It comprises of all the biotic and abiotic factors that the organism needs to survive, stay healthy and reproduce.

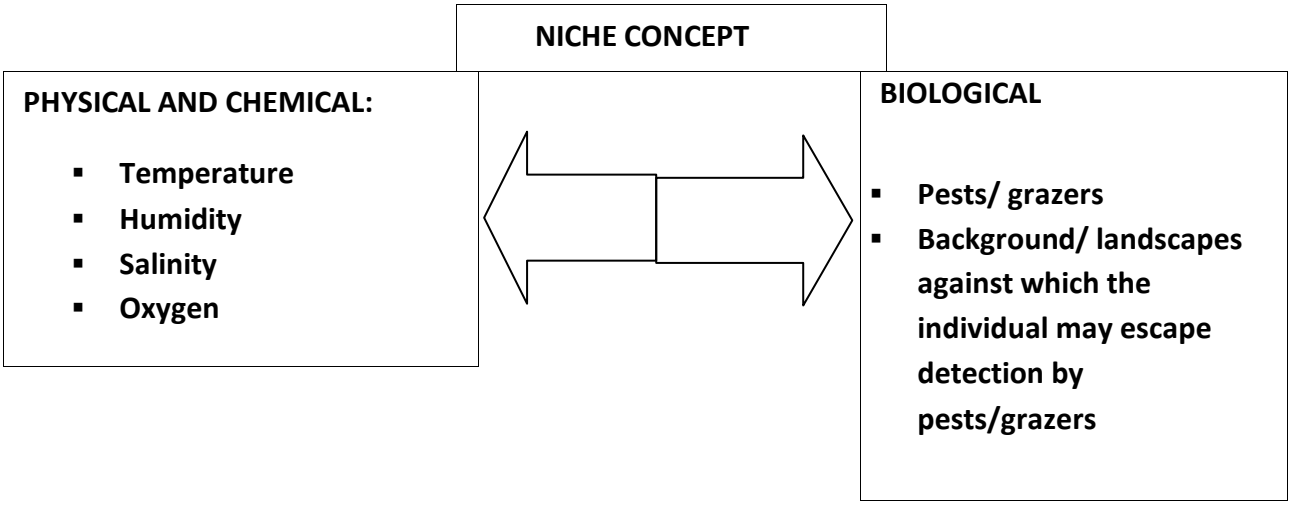
Obviously, the concepts of "niche" and "habitat" overlap, but "niche" focuses more on the organism's "role," while "habitat" focuses more on the nature of space/ place/ area that the organism occupies.

Ecologists use the term 'niche'to express the relationship of individuals or populations with all aspects of their environments, i.e., their ecological roles.Thus a 'niche' represents the range of conditions and resource qualities within which an individual or species can survive and reproduce.

NICHE CONCEPT: Hutchinson (1957) first defined the niche concept formally.

According to him-

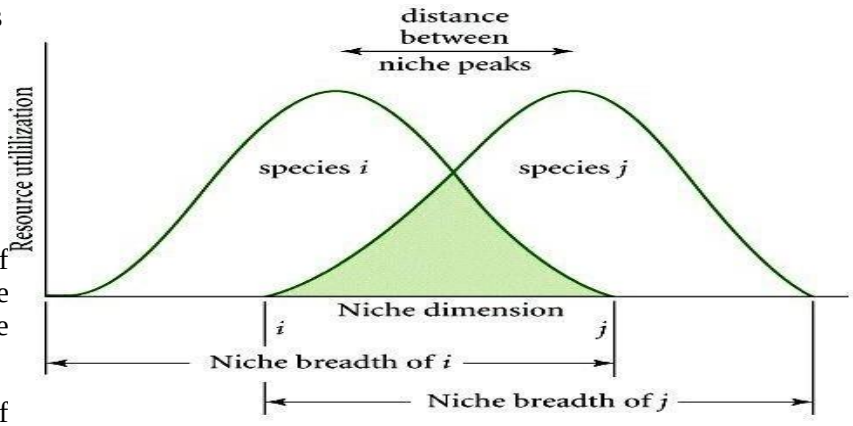
The activity range of any species can be described along every dimension of environment –



- Each of these dimensions may be thought of as a dimension in space.
- Suppose number of dimensions = n. Then niche is described in n-dimensional space.

- n-dimensional or multidimensional niche is an abstraction, also called 'niche hypervolume'; multidimensional concepts can be dealt with mathematically or statistically, and their essence depicted graphically in 3 or less dimensions.
- The niche of each species occupies a part of the n - dimensional volume that represents total resource space, or niche space, available to the community.
- So total niche space of a community is the volume into which the niches of all th species of a community fit into.The number of species in a community thus depends upon the total amount of niche space & average size of each species'niche.
- Thus studies on niche deal with factors that determine both quantities because they are part of the processes that influence patterns of biodiversity.

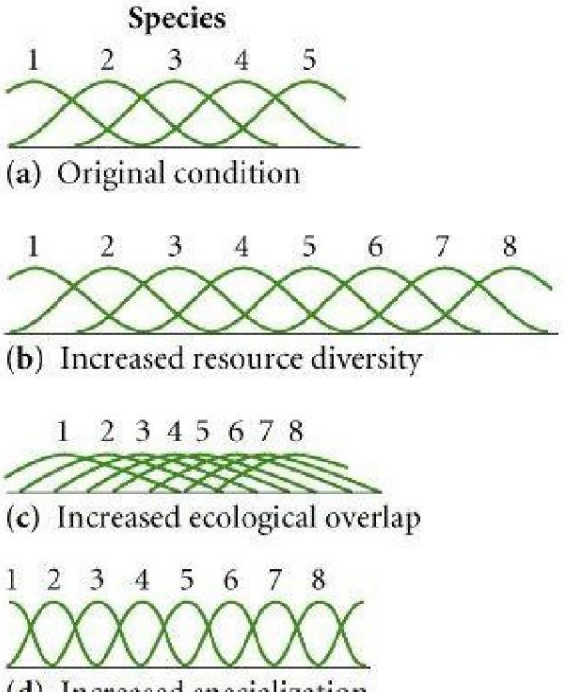
Niche width and overlap: It is difficult to identify and measure all the n-dimensions of a species'niche.We characterize niche relationships by observing the patterns of resource utilization and microhabitat preferences.



Niche breadth: The extent of the variety of resources used or the range of conditions tolerated by the individuals in the population.

Niche overlap: The extent of similarity in resource utilization or tolerance of conditions is called niche overlap.

Resource utilization along a single niche axis can be altered to accommodate more species



- Original community has a species richness of 5, with each species having same niche breadth.
- Additional resource types are added (shown with extended axis).
- The niche overlap among species increases.
- The niche breadth of the species decreases. Both 'c'& 'd'are forms of 'species
- packing'- an increase in 'S' without change in resource diversity. Species packing results in an overall reduction in productivity of the species, as each has.

Fundamental and realized niche:

Ecological Niches

The niche of a species (or an individual) refers to the ways in which it interacts with its environment, so niches are closely related to environmental tolerance curves, but niches can have behavioral dimensions (e.g method of locomotion - running, swimming, flying) as well environmental ones (e.g temperature limits).

Fundamental niche is the entire set of conditions under which an animal (population, species) can survive and reproduce itself. The niche of any organism is the role that it fills within an *ecosystem* as a response to the amount and distribution of resources, the competition present, and the way that the organism influences those same factors.

A fundamental *niche* is the full range of environmental conditions that a viable population of species can occupy and use, without any other *limiting factors* present which could constrain the population.

The fundamental niche is the potential niche that could be filled and is affected by the *life history traits* of each species and each individual organism – their dispersal ability, their tolerance to different environmental conditions, and the way in which they interact with other species.

Examples of Fundamental Niche Sparrows in a Forest

Within a forest, a population of sparrows feeds on berries that grow abundantly on bushes. The fundamental niche of the sparrows is the area where there are berries, and covers the whole of each bush as well as the forest floor, where many of the berries have fallen to the ground. However, mice, which live on the forest floor, also like to eat berries, and are quick to collect them once they have fallen. The presence of the mice causes *interspecific competition* and means that there are fewer or no berries to eat on the forest floor. The fundamental niche of the sparrows cannot be met, so they fill the *realized niche*, which is the area on the bush branches only.

The *interspecific competition* between the sparrows and the mice affects the *geographic range* of the populations; however, the individuals within the populations are also affected by *intraspecific competition*. Certain bushes within the forest may receive more light, nutrition or water than other bushes, and therefore may yield larger and more nutritious berries. Sparrows that are larger or more aggressive could claim territorial ownership over the best bushes and the actual niche of the smaller sparrows (fundamentally, covering all of the bushes in the absence of other sparrows) would be restricted to bushes with smaller, less nutritious berries.

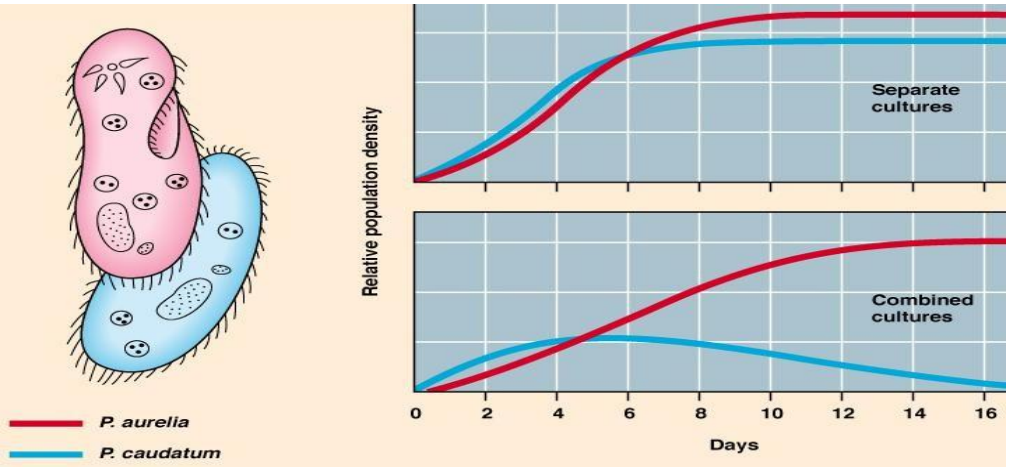
Finally, the fundamental niche of each sparrow includes access to all the berries on all the branches on a bush; the presence of kestrels in the forest, however, introduces a *limiting factor* of *predation*. Sparrows avoid the berries on the ends of branches within a bush because these areas are exposed, meaning they are more likely to be seen by kestrels.

Realized niche is the set of conditions actually used by given animal (pop, species), after interactions with other species (predation and especially competition) have been taken into account. Because of interspecific interactions, the realized niche of a species may be considerably smaller than its fundamental niche.

Sometimes FN and RN are termed precompetitive and postcompetitive niches, reflecting a traditional focus on interspecific competition's effect on niches.

Competitive Exclusion Principle:

In ecology, the competitive exclusion principle, sometimes referred to as Gause's law, is a proposition named for Georgy Gause that two species competing for the same limiting resource cannot coexist at constant population values. When one species has even the slightest advantage over another, the one with the advantage will dominate in the long term. This leads either to the extinction of the weaker competitor or to an evolutionary or behavioral shift toward a different ecological niceh. The principle has been paraphrased in the maxim "complete competitors cannot coexist"



Experimental basis

Paramecium aurelia and *Paramecium caudatum* grow well individually, but when they compete for the same resources, *P. aurelia* outcompetes *P. caudatum.*

Based on field observations, Joseph Grinnell formulated the principle of competitive exclusion in 1904: "Two species of approximately the same food habits are not likely to remain long evenly balanced in numbers in the same region. One will crowd out the other". Russian ecologist Georgy Gause formulated the law of competitive exclusion based on laboratory competition experiments using two species of *Paramecium*, *P. aurelia* and *P. caudatum*. The conditions were to add fresh water every day and input a constant flow of food. Although *P. caudatum* initially dominated, *P. aurelia* recovered and subsequently drove

P. caudatum extinct via exploitative resource competition. However, Gause was able to let the

P. caudatum survive by differing the environmental parameters (food, water). Thus, Gause's law is valid only if the ecological factors are constant.

Gause also studied competition between two species of yeast, finding that *Saccharomyces cerevisiae* consistently outcompeted *chizosa ccharSomyces* by producing a higher concentration of ethyl alcohol.

Extinction:

Extinction is an important part of the process of evolution of biodiversity and does not occur at a constant pace. It is the complete disappearance of a species from Earth. Thus, extinction is the final and irreversible event of species loss. In contrast, extirpation is the local or regional disappearance of a species from only a part of its range.

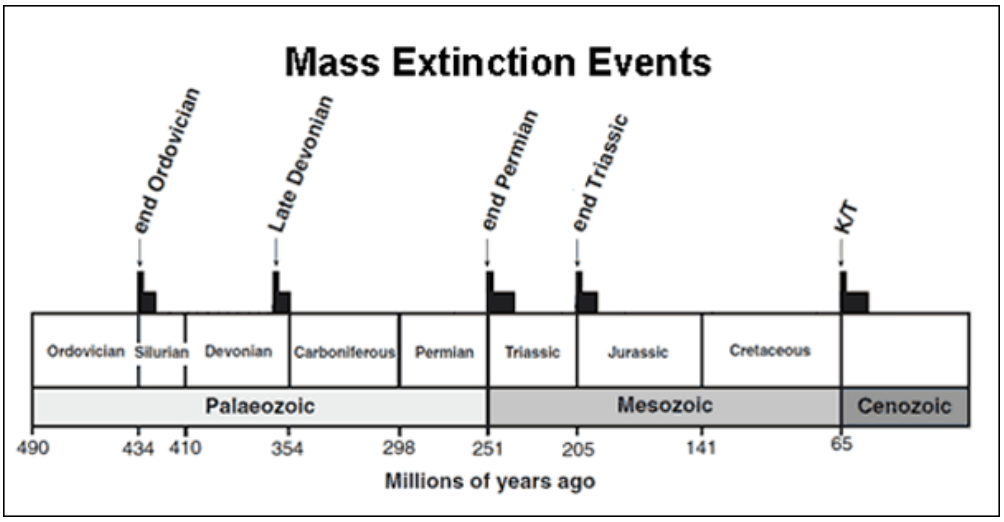
There have been at least five periods when there was a sudden increase in the rate of extinction – to at least double – affecting many different types of plants and animals.

Major Five Extinction events include:

Cretaceous-Tertiary Extinction Event (75% of all species including the dinosaurs) about 75 million years ago.

Triassic–Jurassic Extinction Event (60% of all species including most Achosaurs, Therapsids, and large Amphibians) about 205 million years ago.

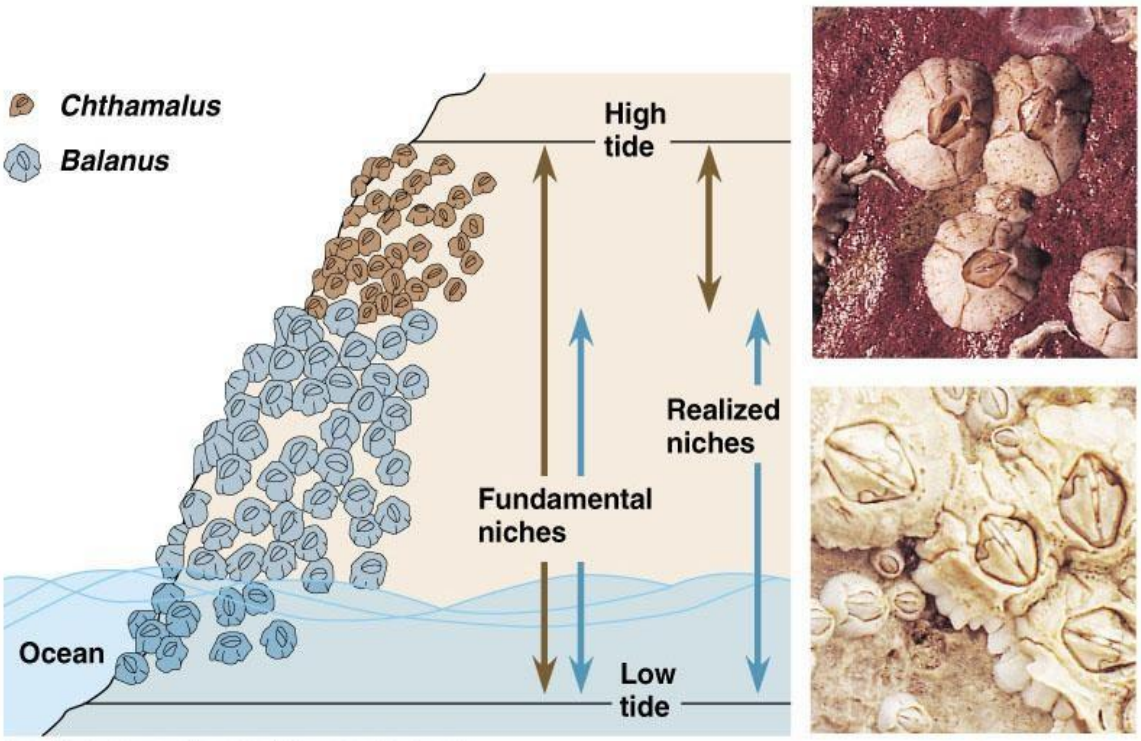
Permian–Triassic Extinction Event (96% of Aquatic Species including most of the sessile species; and 70% of land species including most Synapsids) 251 million years ago.
Late Devonian Extinction Event (70% of all species including most Brachiopods and Trilobites) 360 million years ago.
Ordovician–Silurian Extinction Event (80% of all species, mostly brachiopods, bivalves, echinoderms, bryozoans, and corals) 450 million years ago



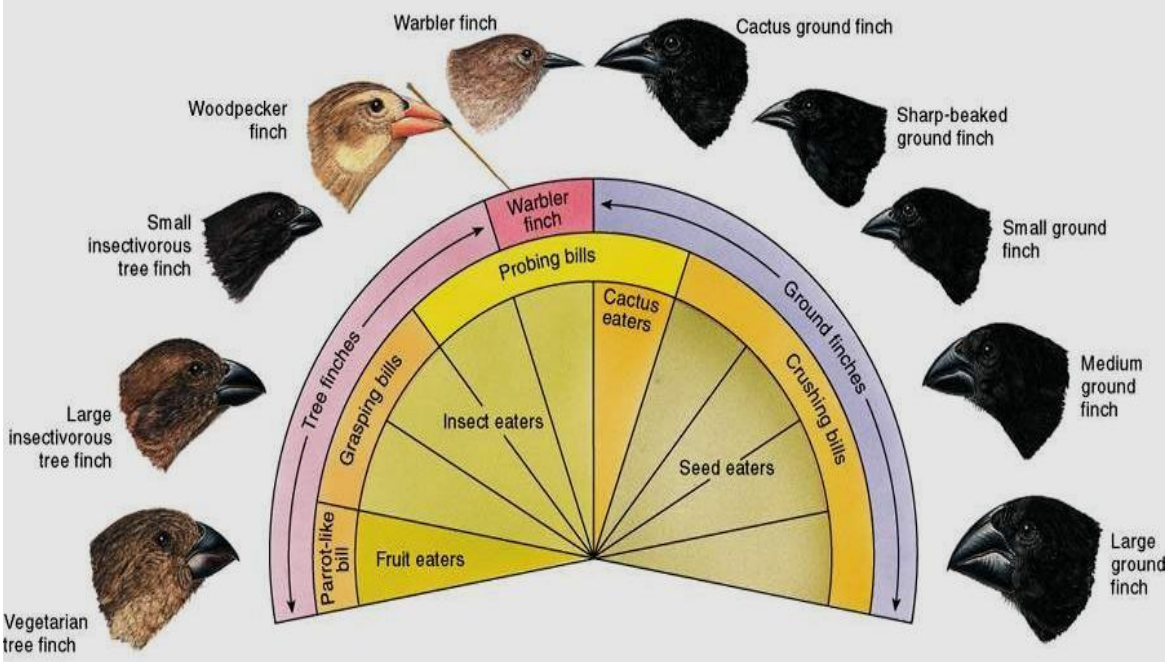
Resource Partitioning:

Natural selection favours changes among competitors to reduce the competition between them. Each species can avoid competition by using different resources than competitors this reduces niche overlap.

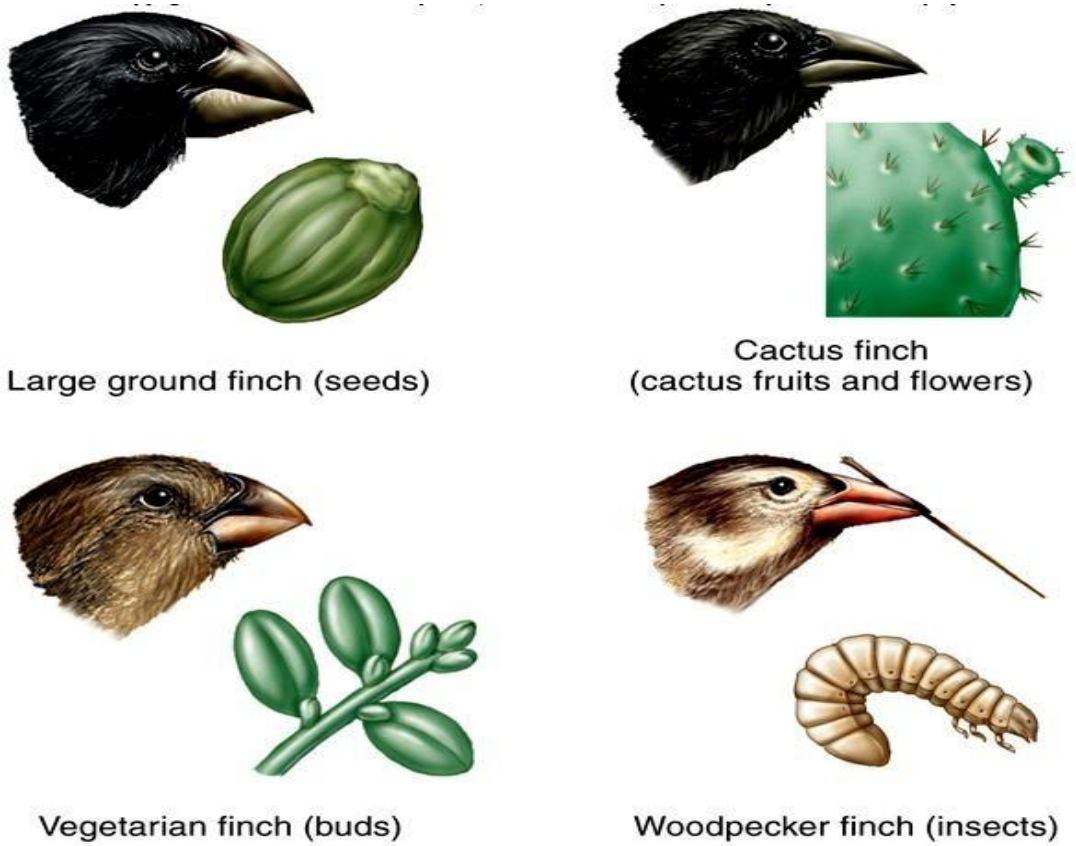
EXAMPLE 1: Connel (1961) did a classic experiment on barnacles, he showed that *Balanus* and *Chthamalus* can coexist because they differ in two traits: growth rate and vulnerability to desiccation. *Balanus*'s growth is rapid, which allows it to crush the slower-growing *Chthamalus. Balanus*, however, dies close to shore because it gets too dry during low tide. *Chthamalus* however, tolerates dry conditions. So, even though *Balanus* is a better competitor for space, the barnacles coexist because *Chthamalus* can survive in areas that *Balanus* cannot survive.



EXAMPLE 2: **Darwin's finches** are a closely related group of distinct species.All the birds are similar to each other except for the shape of their bills.Genetic differences account for the physical differences in the beaks.



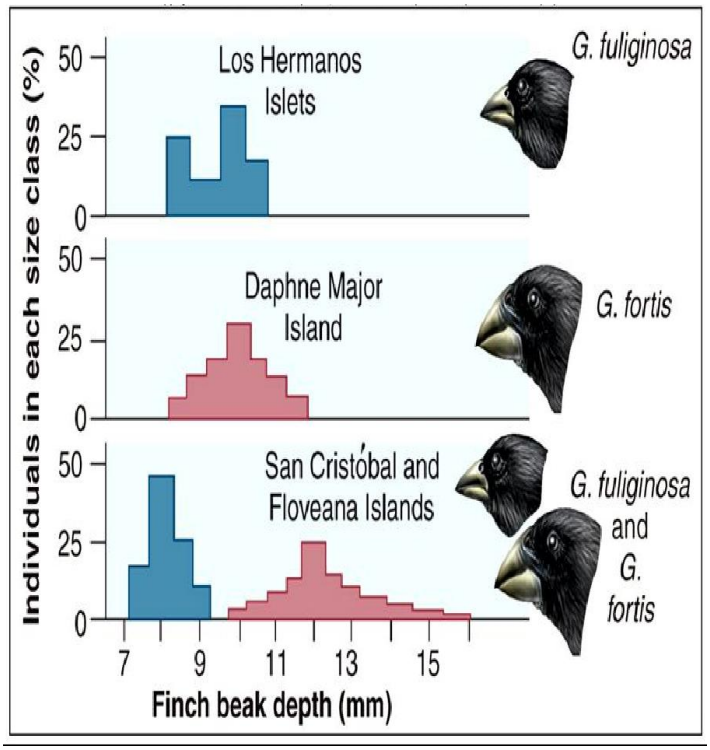
Darwin observed that, although all the finches shared a common ancestor, their beak sizes had evolved to suit their food. Darwin termed this "**descent with modification**."



Character Displacement:

Similar species evolve in such a way so as to become different from each other by enhancing their initial minor differences. Thus natural selection can result in morphological changes in species that reduce competitive effects.

Character displacement occurs as changes in competing species to reduce niche overlap species that live together show more distinctiveness than do species living separately.



Speciation:

Speciation is the formation of one or more new species from an existing species.

A species is a collection of demes. The deme is a group of populations with common gene pool.

Types of Speciation:

Speciation is of following types.

1. Allopatric Speciation (alios— other, patria— native land):

In this type of species formation, a part of the population becomes geographically isolated from the main popula-tion. The population becomes entirely separated and finally constitutes a new species. Thus geographic isolation brings about allopatric speciation. An important example of this type of speciation is formation of Darwin's finches that formed separate species in the Galapagos Islands.

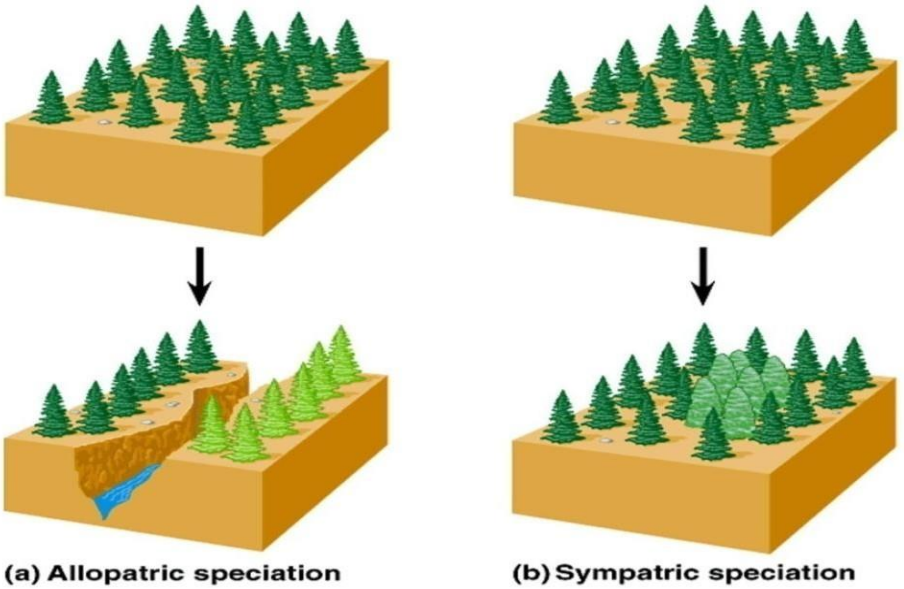
2. Peripatric Speciation:

In peripatric speciation, a subform of allopatric speciation, new species are formed in isolated, smaller peripheral populations that are prevented from exchanging genes with the main population. It is related to the concept of a founder effect, since small populations often undergo bottlenecks. Genetic drift is often proposed to play a significant role in peripatric speciation. Case studies include Mayr's investigation of bird fauna; the Australian bird Petroica multicolor; and reproductive isolation in populations of *Drosophila* subject to population bottlenecking.

3. Sympatric Speciation (sym— together, patria— native land):

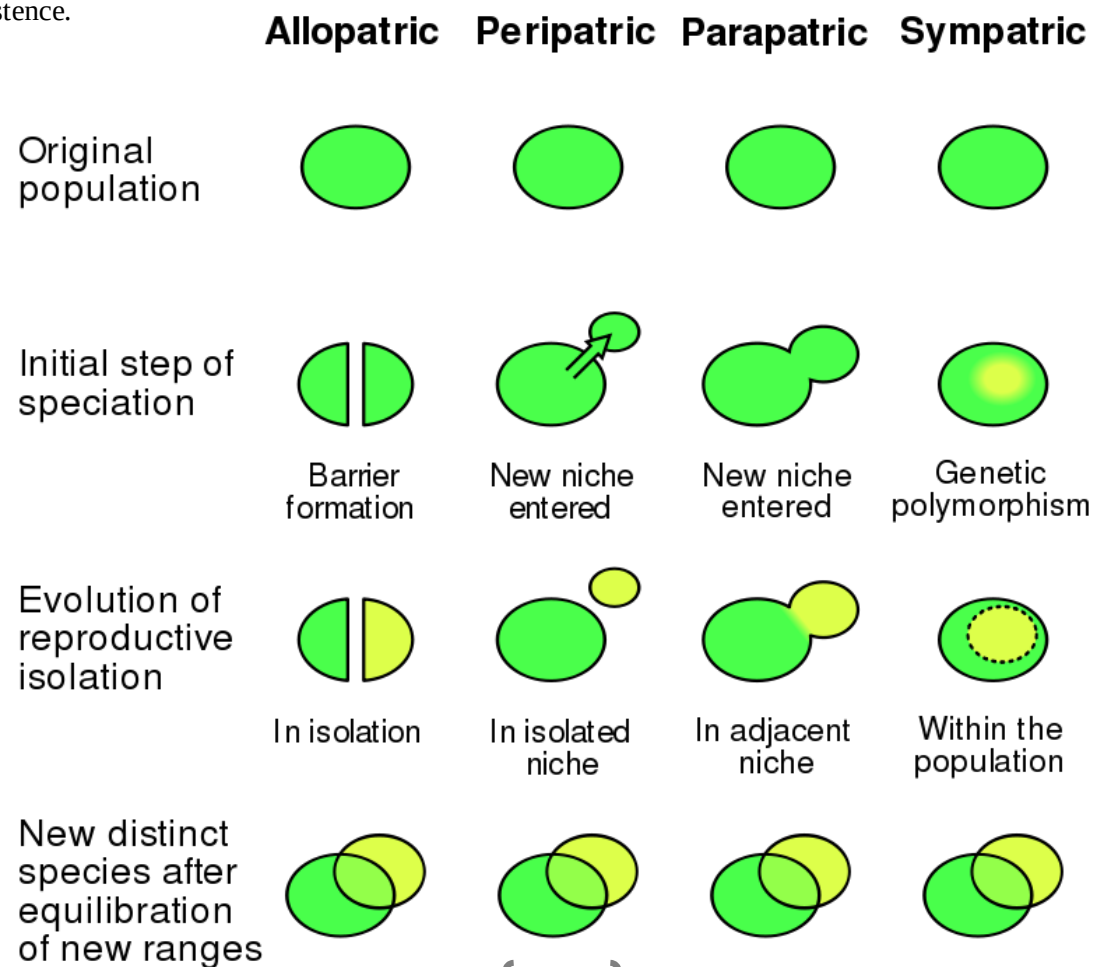
In this type of species formation, a small segment of the original population becomes isolated reproductively. As the isolating mechanism comes into force, a new subspecies emerges. In due course

of time a new species is formed. Thus sympatric speciation is the formation of species within a single population without geographical isolation. The reproductive isolation brings about sympatric speciation.



4. Parapatric Speciation:

It separates adjacent population. Parapatric speciation takes place when a population of a species enters a new niche or habitat. It occurs only at the edge of the parent species range. Although there is no physical barrier between these populations, yet the occupancy of a new niche results as a barrier to gene flow between the population of new niche. Two species are produced due to reproductive isolation from single one. Such type of speciation is found in flightless grasshoppers, smails and annual plants.Many competitors have populations that occur sympatrically or allopatrically for existence.



8. Population ecology: characteristics of population; population growth curves, population regulation, life history strategies (r and K selection); metapopulation, habitat fragmentation, demes, source-sink model.

Population Ecology:

Population ecology is a sub-field of ecology that deals with the dynamics of species populations and how these populations interact with the environment. It is the study of how the population sizes of species change over time and space. The term population ecology is often used interchangeably with population biology or population dynamics.

Term	Definition
Species population	All individuals of a species.
Metapopulation	A set of spatially disjunct populations, among which there is some immigration.
Population	A group of conspecific individuals that is demographically, genetically, or spatially disjunct from other groups of individuals.
Deme	A group of individuals more genetically similar to each other than to other individuals, usually with some degree of spatial isolation as well.
Local population	A group of individuals within an investigator-delimited area smaller than the geographic range of the species and often within a population (as defined above). A local population could be a disjunct population as well.
Subpopulation	An arbitrary spatially delimited subset of individuals from within a population (as defined above).

It is the analyses of population characteristics especially the associated dynamics, by exploring how biotic and abiotic factors influence the distribution, size and age of a population.

The studies pertaining to population ecology include -

- Intra-specific competition
- Inter-specific interactions
- Population growth
- Population regulation
- Life-history strategies

Characteristics of population:

Populations have developed separately but the life forms that contribute to populations have much in common in terms of 'population structures' and 'population processes'.

The related parameters of 'structures' and 'processes' define the characteristics of populations.

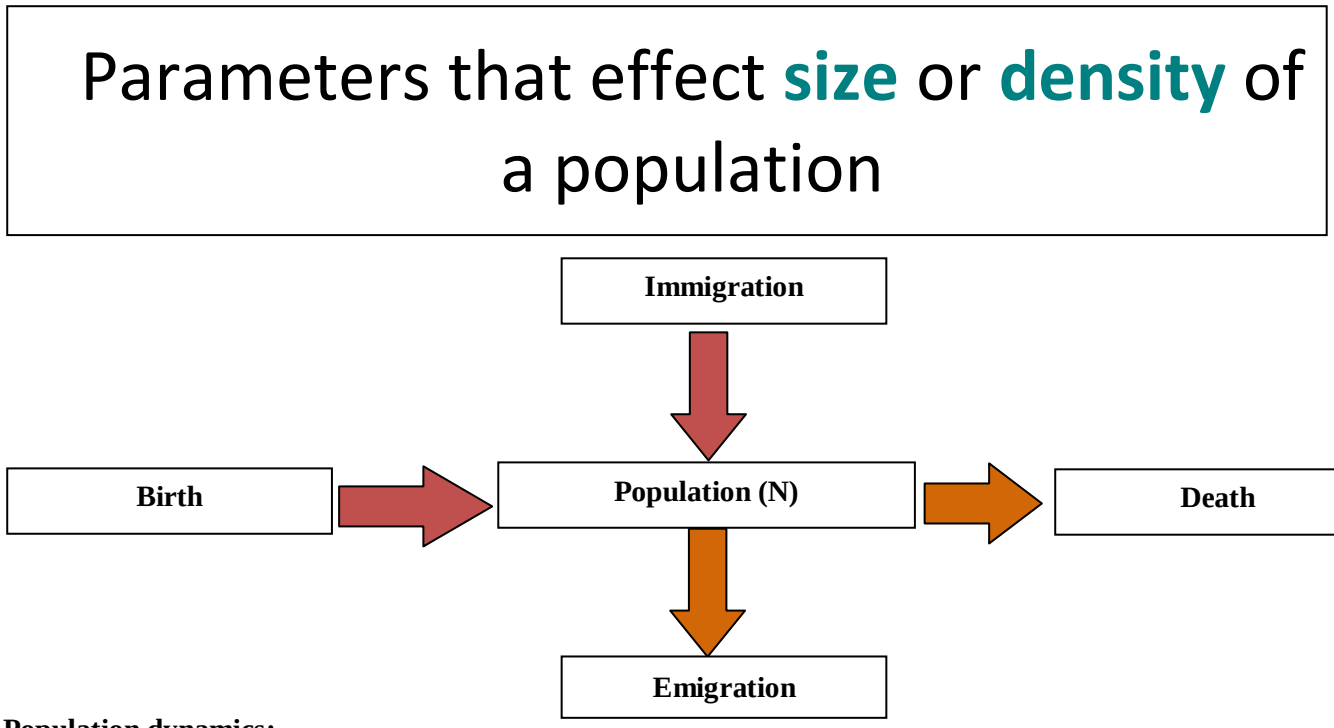
- Boundary
- Size
- Density
- Dispersion
- Survivorship Curves
- Genetic diversity
- Births & Deaths
- Immigration & Emigration

Population structure

- **Boundary**: The boundary in the form of an island, zone, state, country, is defined for a population.
- **Size**: Number of individuals in the population.
- **Density**: Number of individuals per unit area.
- **Dispersion**: How individuals are spaced within the population using measures like interspersion/ juxtaposition.
- **Life tables**: Proportion of individuals in different age
- **Survivorship curves:** Classes.
- **Genetic diversity:** Relationships between genetic diversity and population size.

Population processes:

- **Births, Deaths**
- **Immigration and Emigration**



Population dynamics:

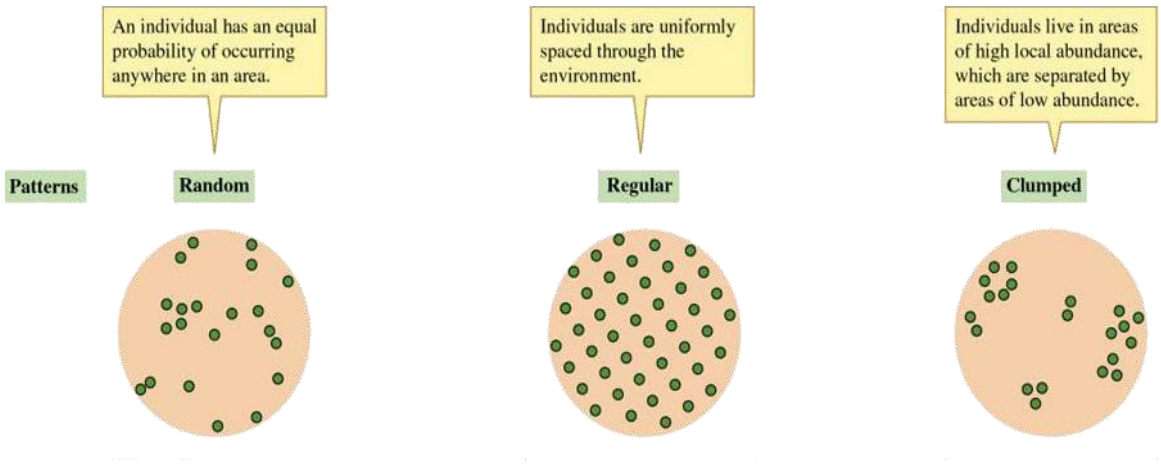
These are attributes of the populations and not of the individual plants or animals, just like population characteristics, since, populations have attributes that individuals do not have and are usually more than just the sum of properties of individuals.

1. Changes in numbers – *Reflects on whether a particular population of plants is increasing or decreasing.*
2. Spatial dispersion – *Reflects on how plants in the population are arranged spatially.*

3. Genetic composition – *Reflects on the genetic variability within a population.*

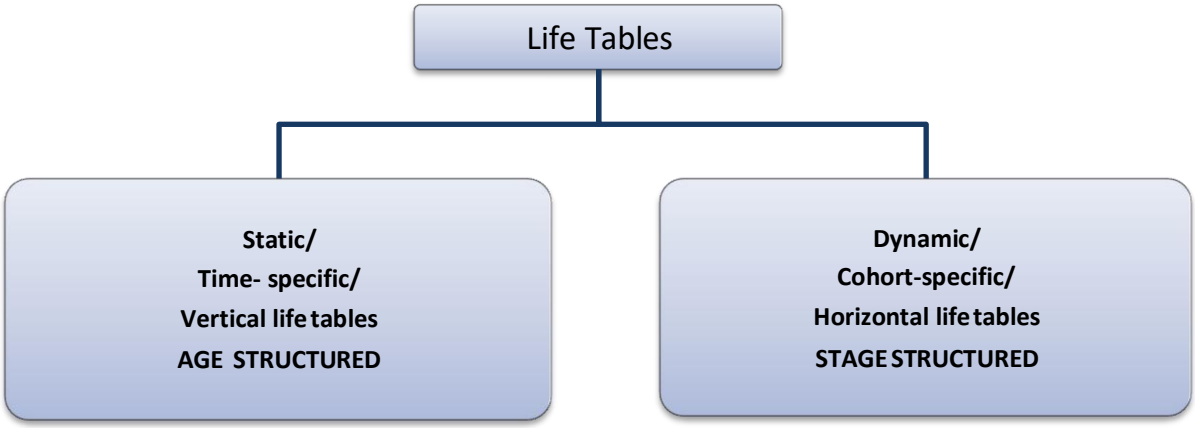
Population structure: Dispersion

1. Random
2. Regular
3. Clumped



Population structure: Life tables

Life table is a summary by age of the survivorship and fecundity of individuals in a population**.**



Population structure: Life tables

- **Elements of life tables:**

1. Age-structure of the population
   (Number of different age-classes and the number of individuals in each age class at a particular time)
2. Fecundity of the population
   (Rate at which offsprings are produced in a population

Life table variables:

Life tables provide a schedule of age-specific mortality and survival. The variables needed for constructing life tables -

1. Survivability of newborn individuals of the population to a specific age;
2. Fecundity of individuals of the population at a specific age;
3. Proportion of individuals of the population dying at a specific age;
4. Proportion of individuals of the population surviving to a specific age;
5. Expectation of further life of individuals of the population to a specific age.

- **Utility of life tables in plant population studies:**

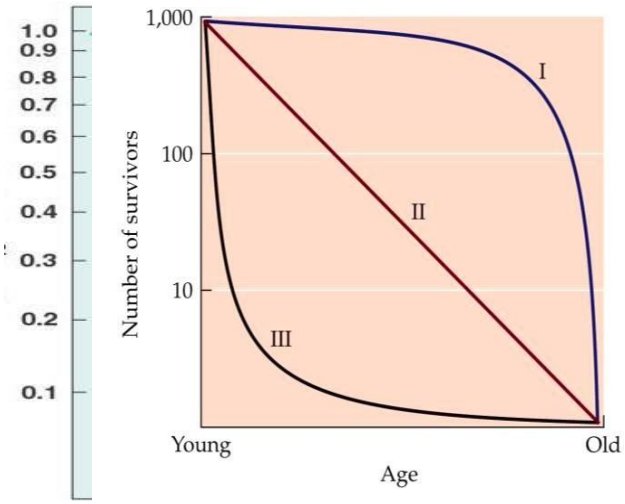
1. Seedling mortality and survival studies;
2. Population dynamics of plants;
3. Life cycles and phenological studies.

- **Types of life tables:**

Age-structured life tables are justifiable only if individuals of a population can be classified meaningfully by age alone. In plants, specially in perennials, the fate of an individual is not so much dependent on its absolute age as on its size and stage of growth. In such cases, **stage-structured life tables** are useful means of defining population dynamics.

- **Survivorship Curves:**

The time interval is on the horizontal axis and survivorship (l_x) is on the vertical axis. Survivorship Curves fall into three general idealized types. The generalized survivorship curves are idealized models to which a species can be compared. Many Survivorship Curves show components of these 3 generalized types at different times in their life histories.



Types of survivorship curves

- Type I = Individuals tend to live out their physiological life span, with high survival rate throughout their life span followed by heavy mortality at the end. Strongly convex curve (humans, other mammals, some plants).
- Type II = Survival rate does not vary with age. Straight line (birds, rodents, reptiles, small perennial plants).
- Type III = Mortality rate extremely high in early life. Concave curve (oysters, fish, most invertebrates & tree species).

Population structure: Genetic diversity

- Small populations support less genetic variations;
- Reduced genetic diversity influence the ability of a population to adapt itself to new diseases,

new predators or changes in the physical environment or climate change adaptations;

- Two mechanisms operating in a population can lead to such situations, *i.e.*, reduced genetic diversity:
- Genetic drift
- Inbreeding

Genetic drift: Along with natural selection, mutation, & migration is one of the basic mechanisms of evolution.

- A random process occurring in a population, leading to reduction in the genetic diversity within the population.
- This reduction in the frequency of alleles within the gene pool is called genetic drift.
- It occurs in all populations and represents a mechanism of evolution, although the rate of genetic drift is faster in small, isolated populations.
- On a time scale, some genes become homozygous for one allele in a population and if the gene involved in such a situation happens to be maladaptive, the small population may become extinct.

Population Process: Growth

- How the number of individuals in a population increases or decreases with time.
- Controlled by the processes of births & deaths and rates of immigration & emigration.
- Populations in which immigration & emigration occur & have significant effect on population growth are called **open populations**.
- Populations in which immigration & emigration does not occur or does not have significant effect on population growth are called **closed populations.**

➢ **Population Models**

	Discrete reproduction	Continuous reproduction
Non over lapping generations	Annual plants	Bacteria
Overlapping generations	Higher plants	Human beings

Population process dynamics:

Change in population size (ΔN) is defined by the following equation:

$$\Delta N = B + I - D - E$$

or

$$N_{t+1} = N_t + \Delta N$$

$$N_{t+1} = N_t + (B + I) - (D + E)$$

N_t is the population size at time t

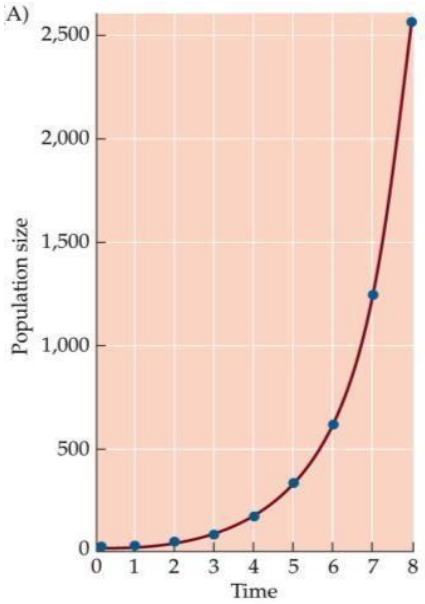
N_{t+1} is the population size one time period later, at time $t+1$ B is the number of new individuals born between t and $t+1$ D is the number of individuals which die between t and $t+1$

I & E are the numbers of immigrants & emigrants during the same period.

Exponential population growth

In the case of J-shaped growth form, the population grows exponentially, and after attaining the peak value, the population may abruptly crash. This increase in population is continued till large amount of food materials exist in the habitat.

After some time, due to increase in population size, food supply in the habitat becomes limited which ultimately results in decrease in population size. For example, many insect populations show explosive increase in numbers during the rainy season, followed by their disappearance at the end of the season.He also gave the estimate of an elephant population that after 500 years there would be nearly 16 thousand x elephants alive, who have descended from the first pair.



Exponential Population Growth

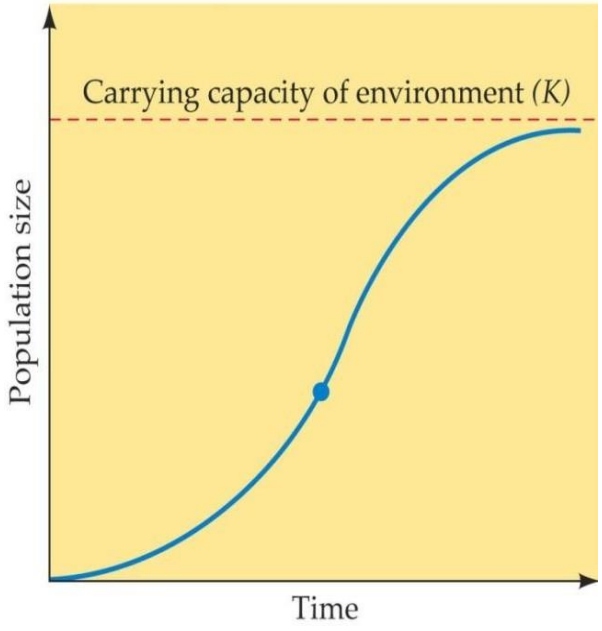
$$\frac{dN}{dt} = rN$$

or $Nt = N_0e^{rt}$

Logistic population growth

When a few organisms are introduced in an area, the population increase is very slow in the beginning, i.e., positive acceleration phase or lag phase, in the middle phase, the population increase becomes very rapid, i.e., logarithmic phase, and finally in the last phase the population increase is slowed down, i.e., negative acceleration phase, until an equilibrium is attained around which the population size fluctuates according to variability of environment.

The level beyond which no major increase can occur is referred to as saturation level or carrying capacity (K). In the last phase the new organisms are almost equal to the number of dying individuals and thus there is no more increase in population size.



The **logistic model of population growth** incorporates the concept of carrying capacity (K).

$$\frac{\Delta N}{\Delta t} = r\left(\frac{K-N}{K}\right)N$$

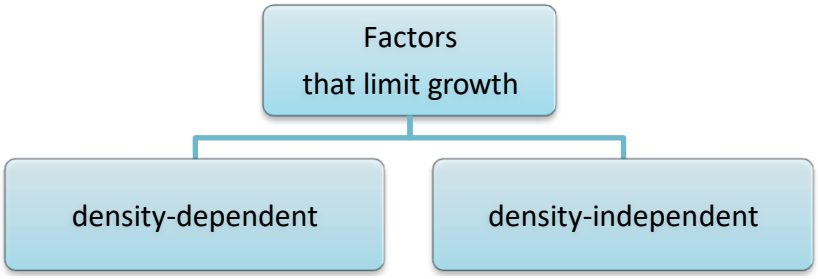
(K – N) = # of individuals a population can accommodate

Logistic Growth Rate (S-shaped curve)

Rate of population growth slows as the population size approaches carrying capacity (K), leveling to a constant level.

Population regulation

- Logistic equations have been successful in describing growth of populations in the laboratory and natural environments.
- It suggests that factors that limit growth exert stronger effects on mortality and fecundity as a population grows



- ❑ Density-dependent Factors: Factors whose effect increase with crowding
    1. Limitations in food supplies
    2. Limitations in space occupancy
    3. Effect of predators/ grazers
    4. Effect of parasites/ pests
    5. Effect of diseases/ epidemics

These effects are felt more strongly when the population is more crowded than in sparse populations and are responsible for bringing populations under control.

- ❑ Density-independent Factors: Factors that are unaffected by population size
    1. Temperature
    2. Precipitation
    3. Catastrophic events
    4. Extreme climate events

These factors may influence the exponential growth rate of a population, but they do not regulate the size that the population will attain in the environment

Density vagueness:

- ❑ **Demographic stochasticity**: variations in birth & death and
- ❑ **Environmental stochasticity**: variations in environmental conditions may obscure the effects of density dependence, giving rise to a situation called '**density vagueness**'.
  As a result of this obscurity the concept of population equilibrium raises doubt.

So it is likely that a combined effect/ interaction of both factors (density-dependent & density-independent) regulate population levels and can cause complex population dynamics.

Life history strategies:

- r & K selection strategy
- Grime's Triangular Model

Dobzhansky (1950) proposed that natural selection in the tropics operates in a fundamentally different way than it does in temperate zones. He argued that much of the mortality in the temperate zones is relatively independent of the genotype of the organism concerned & has little to do with the size of the population. (eg. mass winter kills of many fish and bird species). But in the tropics where environment is more constant, most mortality is more directed generally favouring individuals with better competitive abilities.

Accordingly it was derived that in the temperate zones selection often favours high fecundity and rapid development, whereas in the tropics lower fecundity and slower development could increase competitive ability.

One of the ways of classifying **environments** or **species habitats** and also **evolutionary strategies** relates to their **variability in time**

1. **those that are short-lived**
2. ***those that are long-lived***

The ecologists Robert Mac Arthur & E. O. Wilson used this dichotomy and Dobzhansky's inference to develop the concept of ***r*- and *K*- selection,** though these are clearly not found to be restricted to tropical or temperate zones as was thought earlier.

The initials ***r*** and ***K*** are derived from the logistic equation for describing actual rate of growth of populations.

K refers to the carrying capacity

r refers to the maximal intrinsic rate of natural increase [r_{max}]

$$\frac{\Delta N}{\Delta t} = r\left(\frac{K-N}{K}\right)N$$

The theory of ***r*- and *K*- selection** predicts that species adapted to these two different environments will differ in life history traits such as size, fecundity, age at first reproduction, no. of reproductive events and total life span. These parameters taken together reflect upon the **evolutionary strategies** of populations.

Life history strategies as evolutionary strategies:

Different organisms have evolved different sets of traits related to population dynamics. Traits that affect an organism's schedule of reproduction and survival make up its **life history**

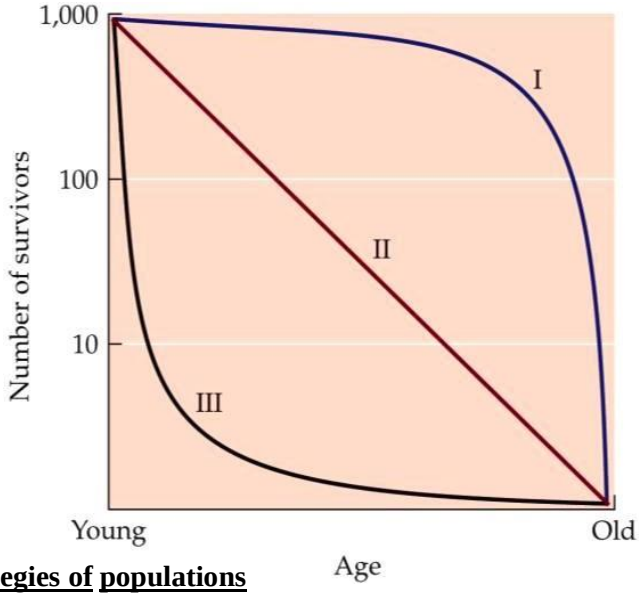
- **Semelparous:**
- Reproduction occurs once **(semelparity)**
- It occurs after one season
- Death occurs after reproduction
- Many small seeds are produced
- Favored in unstable environment

- **Iteroparous**
- Reproduction occurs more than once **(iteroparity)**
- It occurs after many seasons
- Larger seeds are produced (few well-provisioned seeds have higher survival rates)
- Favored in stable environments

Survivorship curves as indicators of evolutionary strategies:

The survivorship curves indicate the evolutionary strategies of species & how nos. in population are maintained

- Individuals in populations with curves like 'I' & 'II' usually have few offsprings. These offsprings are well cared for so that their chance of survival is high. (eg. birds, rodents, humans, some plants, etc.)
- Individuals in populations with curves like 'III' usually have large number of offsprings, most of which die before they reach maturity. (eg. many plant species, fungi, fish, amphibians, etc.)



Evolutionary strategies of populations

➢ *r*- & *K*-selection as an evolutionary strategy:

Another way of classifying evolutionary strategies was suggested by MacArthur & Wilson (1967).

They applied the terms ***r-selected*** and ***K-selected*** to populations classifying them accordingly as different ecological strategies that populations adopt.

- An *r*-selected population is one in which the maximum rate of increase (r) is important.A *K*-selected population is associated with a steady carrying capacity (K).
- An *r*-selected population can take advantage of a favorable situation by having the ability to increase population size rapidly.A *K*-selected population is less capable of taking advantage of such opportunities for increasing population size.
- An *r*-selected population can produce many offsprings, which under normal circumstances die before reaching maturity but may survive if circumstances change. Thus being associated with Type III survivorship curve.A *K*-selected population produces few, well cared offsprings, are more stable and unlikely to suffer high mortality rates. Thus being associated with Type I and Type II survivorship curves

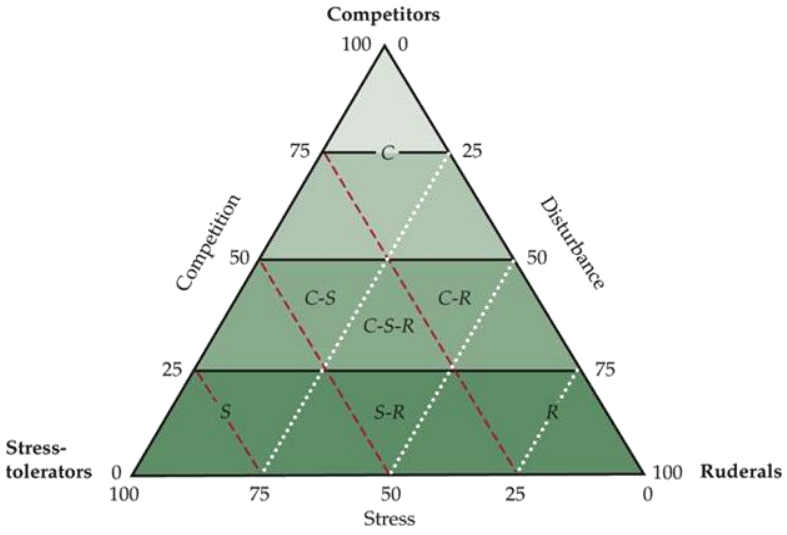
Some correlates of *r*- & *K*-selection - Pianka (1970)

	***r*-selection**	***K*-selection**
Climate	Variable and unpredictable; uncertain	Fairly constant or predictable; more certain
Mortality	Often catastrophic, nondirected, density independent	More directed, density dependent
Survivorship	Often Type III	Usually Types I and II
Population size	Variable in time, nonequilibrium; usually well below carrying capacity of environment; unsaturated communities or portions thereof; ecologic vacuums; recolonization each year	Fairly constant in time, equilibrium; at or near carrying capacity of the environment; saturated communities; no recolonization necessary
Intra- and interspecific competition	Variable, often lax	Usually keen
Selection favors	1. Rapid development 2. High maximal rate of increase, r_{max} 3. Early reproduction 4. Small body size 5. Single reproduction 6. Many small offspring	1. Slower development 2. Greater competitive ability 3. Delayed reproduction 4. Larger body size 5. Repeated reproduction 6. Fewer, larger progeny
Length of life	Short, usually less than a year	Longer, usually more than a year
Leads to	Productivity	Efficiency
Stage in succession	Early	Late, climax

The continuum concept of *r*- & *K*-selection

- The two classes *r*- & *K*-selection are actually the extreme ends of a continuum of adopted strategies of organisms (Pianka, 1970).
- Each end is associated with a whole group of characteristics of life which fit together into a particular evolutionary strategy.*r*- & *K*-selection are also known as density- independent and density-dependent selection.
- No organism is completely *r*-selected or completely *K*-selected, rather all must reach some compromise between these two extremes. An organism can be considered as an "*r*-strategist" or a "*K*-strategist" only relative to some other organism; thus statements about *r* and *K* selection are invariably comparative
- Eg. Cats and dogs are *r*-selected compared to humans, but *K*-selected compared to mice and rats. Mice and rats, in turn, are *K*-selected compared to most insects.
- So there exists a ***r-K* selection continuum** and an organism's position along it in a particular environment at a given time can thus be defined.
- Also, in ecological systems it is highly probable that populations are constantly undergoing *r*- and *K*-selection. Their position on the r-K continuum depends on the strength of selection pressures and where they can balance.
- If some of the populations enter new conditions suitable to a more r- or more K- oriented strategy than a new balance will set up.

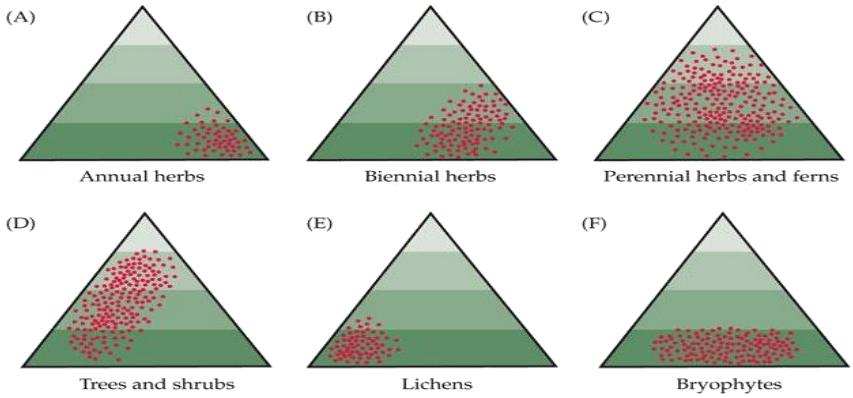
<u>Life history strategies based on Grime's Triangular Model</u>



- Characteristics of competitive, stress-tolerant & ruderal plants based on Grime'sTriangular Model

	Competitive	Stress-tolerant	Ruderal
Growth forms	Perennial herbs, shrubs, or trees	Lichens, perennial herbs, shrubs, and trees	Annuals
Seed production	Small	Small	Large
Maximum potential growth rate	Rapid	Slow	Rapid
Leaf litter	Abundant, often persistent	Little, often persistent	Little, not persistent
Leaf longevity	Short	Long	Short
Flowering phenology	Flowering near time of maximum productivity	No pattern	Flowering at end of favorable period
Vegetative phenology	Leaf production coincides with maximum productivity	Evergreens; various patterns	Brief period of leaf production at time of maximum productivity
Life span	Long	Long	Short

Source: Grime 1977.



- Habitat fragmentation describes a state (or a process) of discontinuities (fragments) within the preferred living area (habitat) of a species.
- The classical concept of population ecology is that of a single, large and homogeneous population, but now it is widely recognised that most populations are fragmented and heterogeneous.
- Thus the process of fragmentation has significant implications for ecological and evolutionary processes . Habitat fragmentation involves alteration of habitat resulting in spatial separation of habitat units from a previous state of greater continuity.
- This phenomenon occurs naturally on a geologic time-scale or in unusual and catastrophic events.

Man brings about habitat fragmentation chiefly from agricultural land conversion, urbanization, pollution, deforestation and introduction of alien species.

Prior to the dominance of mankind, long term changes brought about by geologic processes or climate oscillations contributed to habitat fragmentation.

Important natural phenomena that cause fragmentation:

1. Glacial advances
2. Volcanic activity
3. Geologic faults & tectonic movement
4. Mass land slumping
5. Major sea level rise
6. Climate oscillation

Each of these actions has the potential to create irreversible effective isolation of previously connected habitat units

Anthropogenic factors leading to habitat fragmentation:

1. Land conversion for agriculture / aquaculture
2. Urbanization
3. Pollution
4. Deforestation
5. Introduction of exotic species
6. Human caused wildfires

- **<u>Ecology of fragmented habitats</u> Spatial structure:**

Existence of discrete, localised patches of preferred habitat separated by a matrix of non- preferred habitat.

Local demography:

Small patches are more likely to go extinct and are more variable than large populations.

Connectivity:

Patches are separated by a matrix of non-preferred habitat putting limits on dispersal abilities.

Interactions among Populations:

Plants and animals exhibit a wide range of relationships. Individuals of one species interact with the other individuals of the same species or with those of other species.

Various types of interactions are as follows:

1. Neutralism:

When the presence of one species appears to have no effect on the second species (i.e., no interaction), it is a state of neutralism.

2. Commensalism:

It is one-sided relationship between two species in which one species is benefited but the other is neither benefited nor harmed. Some epiphytes, as for example orchids, are the best examples. Epiphytes depend upon the other trees for support and nutrients. They manufacture their own food but do not help supporting plant in any way.

Table 1.: Population Interactions

Species A	Species B	Name of Interaction
+	+	*Mutualism*
–	–	*Competition*
+	–	*Predation*
+	–	*Parasitism*
+	0	*Commensalism*
–	0	*Amensalism*

3. Proto-cooperation:

It is less extreme type of interaction in which two species interact favorably with each other, though both of them are able to survive separately.

4. Mutualism:

It is an obligatory interaction that is beneficial to both species. The term symbiosis has also been used for this relationship. Mutualism is best demonstrated in lichens. The lichen is composed of two components—alga and a fungus. The fungus supports the alga while the alga supplies food to fungus. Green Hydra presents another example of mutualism. This animal has green photosynthetic alga in the protective ectoderm. The alga gives off oxygen benefitting the animal which, in turn, supplies CO_2 and N_2 to the plant. Root nodules of legumes containing Rhizobium leguminasarum bacterium provide another example of mutualism in which there is reciprocal beneficial relationship between the root and bacteria.

5. Amensalism:

In this type of interaction between the two species, one species is harmed or inhibited and the other is neither benefitted nor harmed by the association. Many algae produce extracellular toxic metabolites which inhibit the growth of other algae species. Chlorella vulgaris (green alga) produces chlorellin which is toxic to other species of algae.

6. Parasitism:

When two organisms live together in which one derives nourishment at the expense of the other, the condition is called parasitism. In the parasitic association, the species which provides nourishment and support is called the host and the one which gets support and nourishment is called the parasite. Several species of plants and animals form parasitic associations with other organisms. A parasite usually parasitizes a host which is larger in body size than it and ordinarily it does not kill the host, at least until it has completed its reproductive cycle.

7. Cannibalism:

This type of interaction is limited within a species in which the bigger individuals kill and feed on the smaller ones. It is a natural method of population control.

8. Predation:

In this type of association and interaction one species (predator) kills and feeds on second species (prey). Predation is important process in the community dynamics. Predator is always larger than the prey. From population ecology point of view predation is the action and reaction in the transfer of energy from one trophic level to the other. It represents a direct and complex interaction between two or more species of eaters and eaten.

9. Competition:

When in the association of two or more species each species is adversely affected by the presence of the other species in respect of food, shelter, space, light, etc., this phenomenon is termed competition.

It is of two types:

(i) Intraspecific competition:

When competition occurs between the individuals of the same species and their requirements are common, the process is called intraspecific competition.

(ii) Interspecific competition:

In this type of competition, the individuals of different species compete for common materials and conditions.

- **Population & metapopulation**

Population: A population is a group of organisms of the same species occupying a given space at the same time with the mature individuals capable of inter-breeding.

Metapopulation: Coined by Richard Levins in 1970 to describe a population consisting of many local populations - population of populations. A metapopulation is a network of populations with scope of occasional movement between them.

Deme: A population which is part of a metapopulation.

Application of the concept of metapopulation

Very few ecological concepts have received more attention and generated more theoretical and empirical research in recent years than the metapopulation concept. It is a valuable framework in studies concerning the following among many others -

- Population dynamics of subdivided populations;
- Conservation of threatened & endangered species;
- Dynamics of prey-predator systems;
- Biological control of pest organisms;
- Competition among plants and animals;
  Evolution of virulence in host-parasite interactions

Conditions that define metapopulations

Although many populations exhibit a patchy spatial distribution there are four necessary conditions to be met for the concept of metapopulation to be applicable:

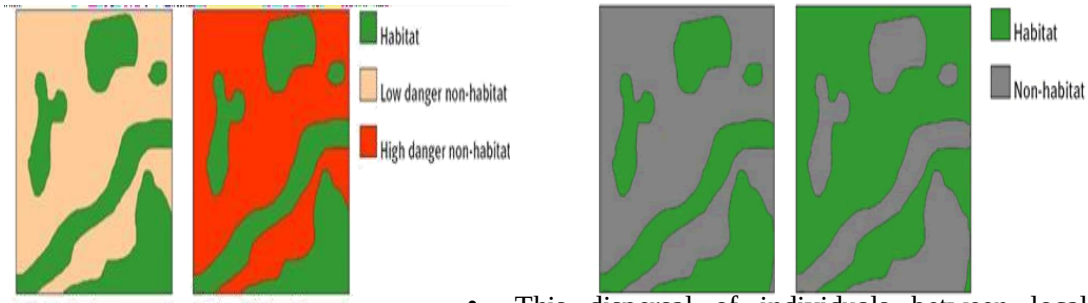
1. The suitable habitat occurs in discrete patches that may be occupied by local breeding populations.
2. Even the largest populations have a substantial risk of extinction.
3. Habitat patches should not be too isolated to prevent recolonization after local extinction.
4. The dynamics of local populations are not synchronized.

The dynamics of metapopulation differs from population dynamics as it is governed by two sets of processes operating at two distinctive spatial scales:

a. Local or within-patch scale
b. Metapopulation or regional scale

Colonization and dispersal

- The process of colonization involves the movement of individuals from occupied patches (existing local populations) to unoccupied patches to form new local populations.
- Individuals moving from one patch to another typically move across habitat types that are not suitable for their feeding and breeding activities and often with substantial risk of failing to locate another suitable habitat patch in which to settle.



- This dispersal of individuals between local populations is a key feature of metapopulation dynamics. If no individuals move between habitat patches the local populations act independently.
- If the movement of individuals between local populations is sufficiently high, then the local populations will function as a single large population.
- In such a situation, the dynamics of the various local populations may be synchronized and equally susceptible to factors that can lead to possible extinction.
- At intermediate levels of dispersal, a dynamic emerges where the processes of local extinction and recolonization achieve some balance and where the metapopulation exists as a shifting mosaic of occupied and unoccupied habitat patches.
- Metapopulation concept is thus closely linked with the processes of
    - Population turnover
    - Extinction and establishment of new populations
    - And the study of metapopulation dynamics is essentially the study of the conditions under which these two processes are in balance.

Theory of island biogeography:

- In 1967, ecologists Robert MacArthur and E.O. Wilson, coined the Theory of Island Biogeography.
- This theory attempted to predict the number of species that would exist on a newly created island.
- It also explained how distance and area combine to regulate the balance between immigration and extinction in an island population.
- Immigration is the appearance of a species in a community.
- Extinction is the disappearance of a species from a community.
- This relationship is known as "species turnover", and it states that the equilibrium value for the island is proportional to the number of immigrants that come to the island, and the loss of individuals due to emigration and extinction
- E.O. Wilson and R. MacArthur did several experiments and made predictions related to the Theory of Island Biogeography.

(1) species richness tends toward an equilibrium value and

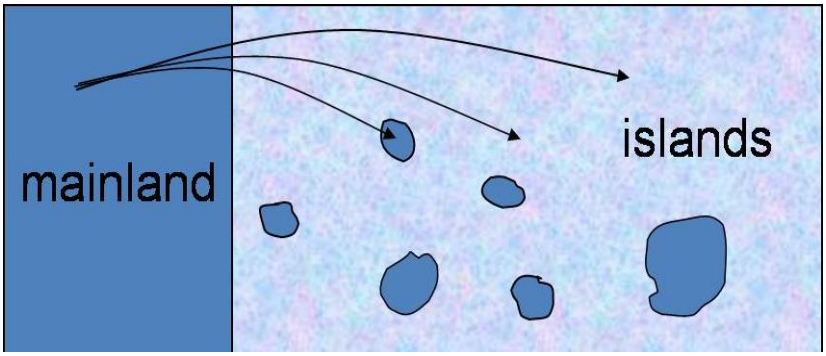
(2) the equilibrium value is the result of immigration, but emigration and extinction may

also occur.

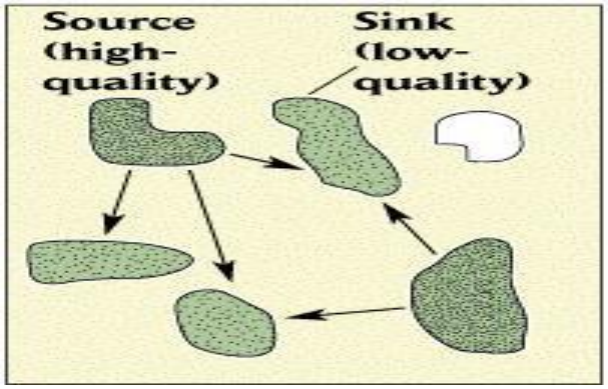
- The equilibrium value (equilibrium diversity value) of an island depends on the area of the island- the larger the area the more resources there are on the island.
- Smaller islands support smaller populations, andsmaller populations are more likely to become extinct.
- Here the Target Effect comes in play. The Target Effect says that larger islands have higher immigration rates because they are a bigger target.

❑ **Salient features of mainland-island metapopulation**

- Network of populations located within dispersal distance from a very large habitat patch where the local population never goes extinct (hence, metapopulations do not become extinct).
- Island populations would go extinct without immigrants from mainland population.
- Danger is in severing islands from mainland populations.



Salient features of source - Sink model



The **source-sink model** of metapopulation dynamics recognizes *differences in the quality* of suitable habitat patches.

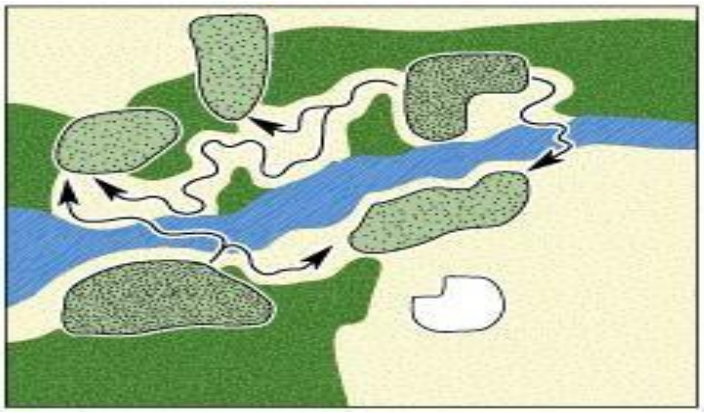
– in **source patches**, where resources are abundant

- individuals produce more offsprings than needed to replace themselves, i.e., positive population growth
- surplus offspring disperse to other patches (net export)

– in **sink patches**, where resources are low

- populations are maintained by immigration of individuals from elsewhere (net importers)
- Negative growth rate without immigration.

Landscape model



- The landscape model considers effects of differences in habitat quality within the habitat matrix.
- The quality of a habitat patch can be affected by the nature of the surrounding matrix
- Quality is enhanced by presence of resources, such as nesting materials or pollinators

– Quality is reduced by presence of predators or disease organisms

– So local extinction rate is a function of both patch suitability and immediate surroundings

- Some matrix habitats are more easily traversed than others

– So, local colonization rate is determined not just by proximity of other populations and connectivity but also by other features of the surrounding matrix that may facilitate or impede movement for particular species

– Thus, study of individual movement is key. Such studies allow us to identify barriers to movement and consequently, assess the quality of matrix habitat.

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9. Community ecology: concepts of community, assemblage and guilds; open and closed communities, ecotone; community continuum concept; community structure; measures of community structure – diversity indices, similarity measures, food web analysis; succession - types, mechanisms, concept of climax

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A plant community is a collection or association of plant species within a designated geographical unit, which forms a relatively uniform patch, distinguishable from neighboring patches of different vegetation types. The components of each plant community are influenced by soil type, topography, climate and human disturbance. In many cases there are several soil types within a given phytocoenosis.

A plant community can be described floristically (the species it contains) and/or physiognomically (its physical structure). For example, a forest (a community of trees) includes the overstory, or upper tree layer of the canopy, as well as the understory, further subdivided into the shrub layer, herbaceous layer, and sometimes also moss layer. In some cases of complex forests there is also a well-defined lower tree layer. A plant community is similar in concept to a vegetation type, with the former having more of an emphasis on the ecological association of species within it, and the latter on overall appearance by which it is readily recognized by a layperson.

Characteristics of Biotic Community:

Each biotic community consists of very diverse organisms belonging to different kingdoms of living things. The number of species and abundance of population in communities also vary greatly. The organisms in a community depend upon each other as well as upon the non-living environment for food, shelter and reproduction.

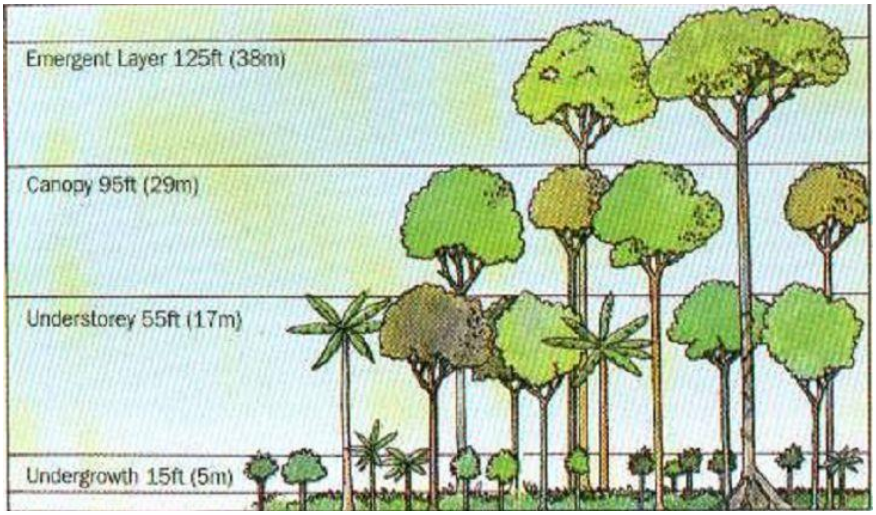
Species Composition:
The kinds of plants and other organisms present in a community indicate its species composition, which differs from one community to another. Sometimes, in the same community, there may be seasonal variation in plant species.
Each species of community has got definite range of tolerance towards the physical and biological environmental conditions of the habitat. The range of environment a species can tolerate is called its ecological amplitude. The nature of community of a particular habitat is determined by the species, and physical and biotic influences prevailing in the locale of community.

Dominance:
A biotic community may have major categories of growth forms, such as trees, shrubs, herbs and mosses. Out of hundreds of species present in the community, relatively only a few exert a major controlling influence due to their large size, numbers of activities.
The phenomenon is called dominance. "**Dominant species are those which are highly successful ecologically and which determine to a considerable extent the conditions under which the associated species must grow."**
The dominance in the community may be the result of co-action between two or more species. Different communities are generally recognized and named on the basis of dominant species occurring in them. For example, a forest community in which pine trees are dominant is called pine forest.
Grassland represents a community which has grass species dominating over the other herbs. Sometimes, communities are named after environmental factors, such as desert community, marine community, mangrove vegetation, etc.

Physiognomy:
General appearance of vegetation is referred to as physiognomy. It constitutes general stature, shape and life-forms of the species comprising the vegetation and actually the classification of vegetation types has been done on the basis of physiognomy.
The species of a community can be grouped into several life-forms on the basis of general appearance and growth. The physiognomy is the total effect created by the combination of vertical structure and architecture of dominant species of vegetation.
For example, the high physiognomy of a forest differs distinctly from a low physiognomy of a grassland. However, several communities though possess similar physiognomy, yet differ markedly on the basis of species composition and dominants, e.g., different types of forests.

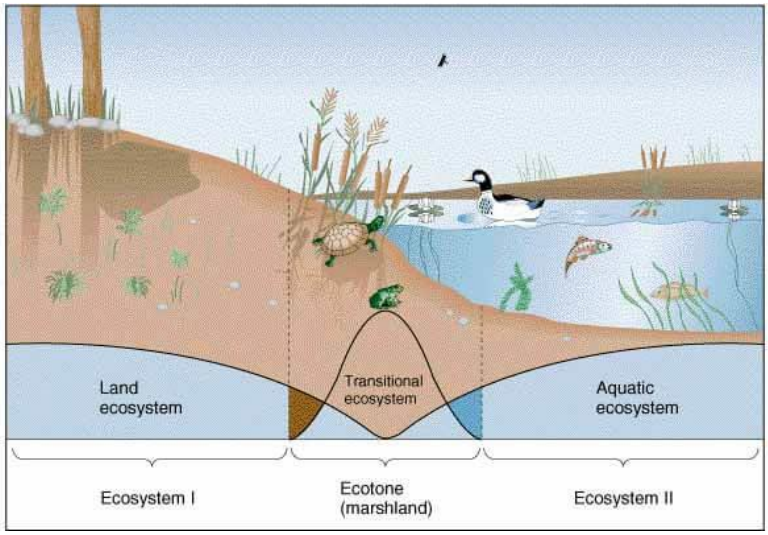
Stratification:
Every biotic community has a vertical layering or stratification of organisms or environmental conditions. A number of examples can be cited to support the concept of community stratification from different habitats.
In grassland community three strata, namely:
(a) Subterranean,
(b) Floor and
(c) Herbaceous may be recognized.
The subterranean stratum contains the roots of the principal vegetation and provides permanent shelter to bacteria, fungi, protozoans, nematodes, earthworms, ringworms and several other invertebrates. The floor stratum consists of basal parts of the vegetation, including the rhizomes of grass plants.
In this stratum, generally the insects, spiders, reptiles and rodents are present. The herbaceous stratum of grassland community is represented by upper parts of grasses and herbs. Several types of insects, birds and grazing mammals are included in this stratum.

In a forest community, five vertical strata are present. They are:
(a) Overstorey stratum,
(b) Understorey stratum,
(c) Transgressive stratum,
(d) Seedling stratum, and
(e) Subterranean stratum.
In pond community, vertical stratification is very little. However, in deep ponds and lakes three strata:
(a) Littoral zone,
(b) Limnetic zone and
(c) Profundal zone can be recognized.



Species Diversity:
The biotic community is a natural assemblage of a large number of plant and animal species in an area. However, in any particular habitat there is no considerable variation in environmental condition, the plants growing together in a community show unique uniformity in their behaviour.
Vegetation, therefore, is reflection of a climate and, in general, widely separated areas having similar climate have similar aspects of landscape.
Some communities, for example, tropical rain forest and coral reef community, show high species diversity with many different kinds of species living at each trophic level.
Some community areas have limits but more often the community boundaries are hard to define. A clearly distinguished area or a type of area with uniform habitat conditions and supporting characteristic type of vegetation is termed biotype.

Ecotone and Edge Effect:
The zone where two or more different communities meet and integrate, is called transition zone or ecotone. This zone of integration may be narrow or wide, local (e.g., a zone between field and a forest) or regional (e.g., the transition between forest and grass land). Ecotone contains few species from both communities. The total number of species is often greater in the ecotone than in the adjoining communities.



The ecotone or transition zone exhibits a shift in dominance of the conspicuous species of both sides. It may also include a number of highly adaptable species that tend to colonize such transitional areas. Because of this, the variety (i.e., species diversity) and density of life is often greatest in such areas.
This potential for the ecotone to act as a habitat for species found in neither major community is called edge effect. Thus the tendency of increased variety and density of some organisms at the community border is known as edge effect.
The organisms that occur primarily, or most abundantly, or spend the greatest amount of their time in junctions between communities are called edge species. A common example of the edge effect in

action can be seen in those species of owl that live in or near ecotones between forests and grasslands. They depend on forest trees for nesting and do their hunting in the grassland, where they depend on field rodents for food.

Keystone Species:
The species, which have much greater influence on community characteristics, relative to their low abundance or biomass, are known as keystone species. Such species play a vital role in controlling the relative abundance of other species.
When keystone species is removed, it causes serious disruption in the functioning of the community. For example, in the tropical rain forests, the different species of figs are the keystone species as they produce large quantity of fruits. During the time of food scarcity, these fruits are consumed by monkeys, bats, birds, etc. Thus, by protecting the fig trees, the animals dependent on them are also conserved.

Link Species:
However, only a few species act as keystone species, while others act as link species. For example, mycorrhizal fungi in soil are critical link species as they establish essential links in the absorption of nutrients from the soil and other organic substances.
Some critical link species are responsible for providing food to the network species, while others act as pollinators of flowers, and some act as agents for dispersal of seeds and fruits. Tropical rain forests are rich in critical link species due to high degree of animal dependent pollination and dispersal.

1. Analytic Characters:
They are directly observed or measured in sample plots. They include kinds and number of species, distribution of individuals, number of individuals, height of plants, etc.
2. Synthetic Characters:
They are derived from the measurements of analytic characters and utilise data obtained in the analysis of a number of stands.

Analytical characters are of two types:
(i) Qualitative:
They are based on non-quantitative observations, e.g., species composition and stratification of vegetation. They are expressed only in qualitative way.
(ii) Quantitative:
They are expressed in quantitative terms. They are measured. The major quantitative characters include frequency, diversity, cover, biomass, leaf size, abundance, dominance, etc.

They are as follows
Frequency:
This is based on percentage of sample plots in which a species is present, indicating its dispersion in space.
This frequency of each species is calculated as follows:
Frequency percentage = number of sampling units in which that species occurred / number of sampling units studied X 100
Diversity:
This is denoted by number of individuals per unit area, indicating the relative abundance of a species.
Cover and Basal Area:
This is percentage land area occupied by a species, indicating the influence zone of a species. Although sometimes used in general sense for the area occupied by a plant, (which may be the herbage cover or the cover of basal area), it is generally used for above ground parts.
Thus, cover or herbage cover signifies primarily the area of the ground occupied by the above ground parts of plants, such as leaves, stems and inflorescences as viewed from above.

However, basal area refers to the ground actually penetrated by the stems and is readily seen when the leaves and stems are clipped at the ground surface. It is one of the chief characteristics to determine dominance. It is measured either at 2.5 cm above ground or actually on the ground level.

Biomass:

This expresses quantity of living materials per unit area, indicating the growth of a species. Thus, biomass is the standing crop expressed in terms of weight (i.e., organism mass) of the living matter present. The amount of living material, present in a component population at any time, is known as the standing crop, which may be expressed in terms of weight per unit area.

Leaf Area:

The percentages of species having different leaf sizes, indicating the adaptation of the vegetation to the prevailing environment. As the leaves are essential part and are very much affected by climate condition, their shapes and sizes have been taken as important criteria in determination of quantitative characters.

Density:

Density represents the numerical strength of a species in the community. The number of individuals of that species in any unit area is its density. This gives an idea of degree of competition.

It is calculated as follows:

Density = Number of individuals of the species in all the sampling unit/Total number of sampling units studied

The value thus obtained is then expressed as number of individuals per unit area.

Abundance:

This is the number of individuals of any species per sampling unit of occurrence.

It is calculated as follows:

Abundance =Total number of individuals of the species in all the sampling units/Number of sampling units studied

Synthetic Characters:

These are determined after computing the data on the quantitative and quantitative characters of the community. For comparing the vegetation of different areas, community comparison needs the calculation of their synthetic characters. These are determined in terms of presence and Constance, fidelity, etc.

Presence and Constance:

It expresses the extent of occurrence of the individuals of a particular species in the community.

Fidelity:

This is the degree with which a species is restricted in distribution to one kind of community. Such species are sometimes known as indicators.

Dominance:

Here, the dominance is expressed in synthetic form. On the basis of density, frequency and dominance (cover) values; there has been proposed idea of Importance Value Index (IVI). IVI of a species in the community give the idea of its relative importance. For IVI, values of Relative density.

Relative frequency and Relative dominance (cover basis) are obtained as follows:

Relative density = Density of the species x 100/Total density of all the species

Relative Frequency = Frequency of the species x 100/Total frequency of all the species

Relative dominance (cover) = Dominance (cover) of the species x 100/Total dominance (cover) of all the species

Now for IVI, three values are added. IVI values of different species are then arranged in decreasing order.

Other Synthetic Characters:

In addition to above mentioned characters, there are some other synthetic characters. They are quite useful in comparative studies on communities. Such characters include, interspecific association and association index, index of similarity, dominance index, species diversity and diversity index, etc.

Life Forms:

Life forms better known as Raunkiaer's life forms or Botanical Life Forms were proposed in 1934 by a Danish botanist Christen C. Raunkiaer. According to Raunkiaer, in a community it is very important to know how a plant survives during unfavourable conditions. He took the criterion of protection of perennating buds during adverse conditions as an adaptation of plant to climate. Accordingly he proposed a system known as Raunkiaer's system in which plants were categorized into various life forms based on the position of their budsduring seasons of unfavourable conditions (too much cold or too much hot). Raunkiaer considered five major types of life forms viz. Phanerophytes, Chamaephytes, Hemicryptophytes, Cryptophytes, and Therophytes.

1. Phanerophytes (Phanero – visible; phytes – plants; plants where buds are visible): These are those plants whose buds are situated high up on the plant on the top of the shoots. These are either naked or covered with scales. Phanerophytes are very common in tropical areas and their number decreases towards temperate and polar areas. Based on the height of trees, phanerophytes are further divided into 4 categories:

a) Mega-phanerophytes – trees taller than 30 m
b) Meso-phanerophytes – trees between 8 – 30 m
c) Micro-phanerophytes – trees between 2 – 8 m height
d) Nano-phanerophytes – shrubs shorter than 2 m but more than 25 cm

2. Chamaephytes: These are those plants whose buds are close to ground or maximum up to 25 cm. These plants are found in colder regions at high altitudes or latitudes, e.g. Temperate America. During the growing season, sometimes the aerial parts of chamaephytes die and cover the buds. Fresh growth occurs during the onset of favourable season.

3. Hemicryptophytes: These are also found in the cold regions where buds remain covered under surface soil (but not deep-seated), and are protected. These include annual (plants which complete their life cycle in one year or one season) or biennial (which complete their life cycle in 2 years or 2 seasons) herbs.

4. Cryptophytes: These are also known as Geophytes. In these plants, the buds remain buried under soil such as bulbs and rhizomes. Such plants are mostly found in the arid regions of the world.

5. Therophytes: These are the ephemerals or seasonal plants that complete their life cycle quickly under favourable conditions and during the rest of the unfavourable conditions remain in the form of seeds.

Besides these five major categories, Raunkiaer also identified epiphytes (plant growing on or attached to other plants) as a separate category of life forms. Additionally, he also divided cryprophytes into three subtypes: geophytes (plants buried in soil with subterranean or perennating buds), hydrophytes (plants submerged or floating in aquatic systems with perennating buds inside water), and halophytes (plants in marshy swampy areas with high salt concentrations).

Guilds:

A guild (or ecological guild) is any group of species that exploit the same resources, or that exploit different resources in related ways. It is not necessary that the species within a guild occupy the same, or even similar, ecological niches. An ecological niche is defined as the role an organism plays in its community, i.e. decomposer, primary producer, etc. Guilds are defined according to the locations, attributes, or activities of their component species. For example, the mode of acquiring nutrients, the mobility, and the habitat zones that the species occupy or exploit can be used to define a guild. The number of guilds occupying an ecosystem is termed its disparity. Members of a guild within a given ecosystem could be competing for resources, such as space or light, while cooperating in resisting wind stresses, attracting pollinators, or detecting predators, such as happens among savannah-dwelling antelope and zebra.

Open and Closed community:

Henry Allan Gleason (1882–1975) was an American ecologist, botanist, and taxonomist, known for his endorsement of the individualistic or open community concept of ecological succession, and his opposition to Frederic Clements's concept of the climax state of an ecosystem.

A closed community intentionally limits links with outsiders and outside communities. Closed communities may be of a religious, ethnic, or political nature. Governance of closed societies varies. Typically, members of closed communities are either born into the community or are accepted into it.

The opposite of a closed community is an open community, which maintains social relations with external communities.

Frederic Clements was an American ecologist and pioneer who studied vegetation formation and development, he created the idea that plants are supposed to birth, grow/mature, and decay. Their life cycle is similar to that of a human being. Clements also tested a theory known as "climax community"

Diversity indices:
A diversity index (also called phylogenetic indices or phylogenetic metrics) is a quantitative measure that reflects how many different types (such as species) there are in a dataset (a community) and that can simultaneously take into account the phylogenetic relations among the individuals distributed among those types, such as richness, divergence or evenness.

Species Richness
The simplest way to describe a community is to list the species in it.
Species richness (S) is the number of species on that list, and is most often used as the first pass estimate of diversity for a community.
How would one generate such a list? A simple and widely used method is to define the boundaries of the community and then walk through it seasonally, noting all the species you encounter. This is what we call a flora.

Simpson's Index
Simpson's Index is considered a dominance index because it weights towards the abundance of the most common species.
Simpson's Index gives the probability of any two individuals drawn at random from an infinitely large community belonging to different species.
For example, the probability of two trees, picked at random from a tropical rainforest being of the same species would be relatively low, whereas in boreal forest in Canada it would be relatively high.

The range is from 0 to 1, where:
High scores (close to 1) indicate high diversity.
Low scores (close to 0) indicate low diversity.

The bias corrected form of Simpson's Index is:

$$D_S = \sum_{i=1}^{S} \frac{\left(n_i \left(n_i - 1\right)\right)}{\left(N\left(N-1\right)\right)}$$

Where, ni is the number of individuals in the ith species.
Since Ds and diversity are negatively related, Simpson's index is usually expressed as the reciprocal (1-D) so that as the index goes up, so does diversity

Simpson's Index

A worked example for 201 trees of 5 species assessed in several quadrats:

Tree spp.	No. Individuals
A	100
B	50
C	30
D	20
E	1
Total	201

$$D_S = \left(\frac{100\times99}{201\times200}\right)+\left(\frac{50\times49}{201\times200}\right)+\ldots\left(\frac{1\times0}{201\times200}\right)= 0.338$$

Then $1/D = 1/0.388 = 2.96$

Shannon-Weiner Index

The Shannon-Weiner Index belongs to a subset of indices that maintain that diversity can be measured much like the information contained in a code or message (hence the name information index).

The rationale is that if we know a letter in a message, we can know the uncertainty of the next letter in a coded message (i.e., the next species to be found in a community).

The uncertainty is measured as H', the Shannon Index. A message coded bbbbbb has low uncertainty (H' = 0).

The Shannon Index assumes that all species are represented in a sample and that the sample was obtained randomly: Si i

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

Where, pi is the proportion of individuals found in the ith species and ln is the natural logarithm.

	Species	Abund	p_i	$p_i \ln p_i$
	A	50	0.5	-0.347
	B	30	0.3	-0.361
	C	10	0.1	-0.230
	D	9	0.09	-0.217
	E	1	0.01	-0.046
Total	5	100	1.00	-1.201

H' = 1.201

Similarity Indices - measure similarity between communities based on species composition.

Binary Similarity Coefficients

The simplest similarity measures deal only with presence-absence data. The basic data for calculating binary (or association) coefficients is a 2x2 table:

		Sample A	
		No. of species present	No. of species absent
Sample B	No. of species present	*a*	*b*
	No. of species absent	*c*	*d*

where:

a = Number of species in sample A and sample B (joint occurences)
b = Number of species in sample B but not in sample A
c = Number of species in sample A but not in sample B
d = Number of species absent in both samples (zero-zero matches)

Jaccard coefficient:

$$S_j = \frac{a}{a + b + c}$$

Where,

S_j = Jaccard similarity coefficient,

a = number of species common to (shared by) quadrats,
b = number of species unique to the first quadrat, and
c = number of species unique to the second quadrat

Sørensen coefficient:

$$S_S = \frac{2a}{2a + b + c}$$

Where, S_S = Sorensen's similarity coefficient
This index can also be modified to a coefficient of dissimilarity by taking its inverse:
Sorensen's dissimilarity coefficient 1 - Ss
This coefficient weights matches in species composition between the two samples more heavily than mismatches. Whether or not one thinks this weighting is desirable will depend on the quality of the data. If many species are present in a community but not present in a sample from that community, it may be useful to use Sorensen's coefficient rather than Jaccard's. But the Sorensen and Jaccard coefficients are very closely correlated.

Simple Matching Coefficient:

This is the simplest coefficient for binary data that makes use of negative matches as well as positive matches. It is not used very frequently because for most data sets negative matches are not biologically meaningful.

$$S_{SM} = \frac{a+d}{a+b+c+d}$$

where S_{SM} = Simple matching similarity coefficient

Succession

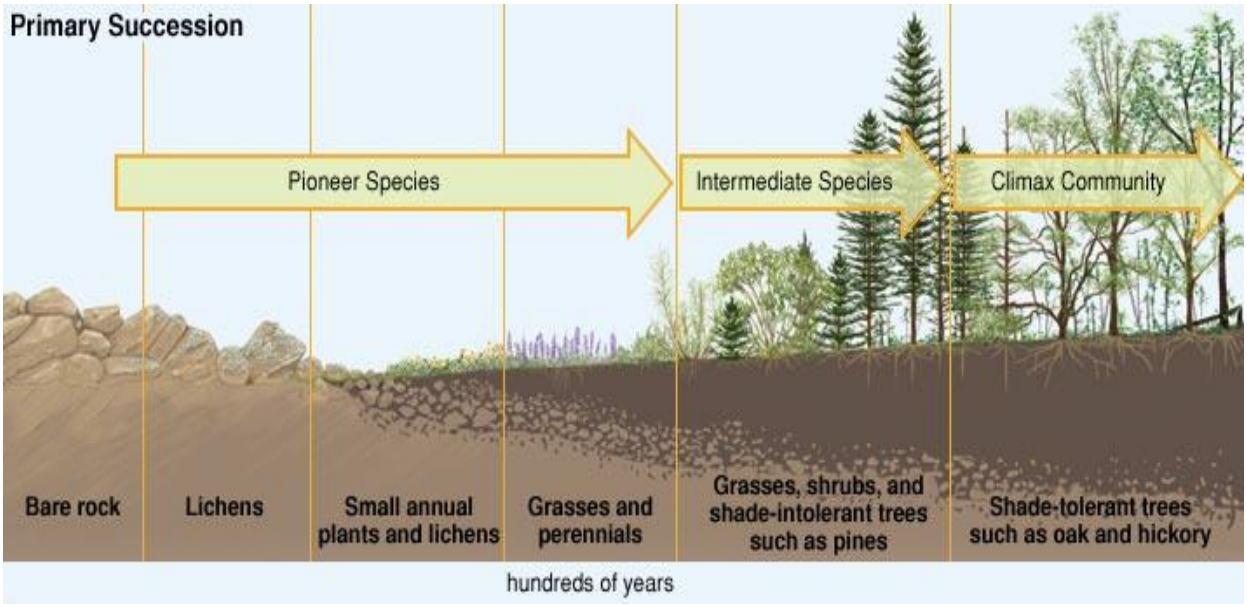
Ecological succession is the process of change in the species structure of an ecological community over time. The time scale can be decades (for example, after a wildfire), or even millions of years after a mass extinction.
The community begins with relatively few pioneering plants and animals and develops through increasing complexity until it becomes stable or self-perpetuating as a climax community. The "engine" of succession, the cause of ecosystem change, is the impact of established species upon their own environments. A consequence of living is the sometimes subtle and sometimes overt alteration of one's own environment.
It is a phenomenon or process by which an ecological community undergoes more or less orderly and predictable changes following a disturbance or the initial colonization of a new habitat. Succession may be initiated either by formation of new, unoccupied habitat, such as from a lava flow or a severe landslide, or by some form of disturbance of a community, such as from a fire, severe wind throw, or logging. Succession that begins in new habitats, uninfluenced by pre-existing communities is called primary succession, whereas succession that follows disruption of a pre-existing community is called secondary succession.

Primary, secondary and cyclic succession

Successional dynamics beginning with colonization of an area that has not been previously occupied by an ecological community, such as newly exposed rock or sand surfaces, lava flows, newly exposed glacial tills, etc., are referred to as primary succession. The stages of primary succession include pioneer plants (lichens and mosses), grassy stage, smaller shrubs, and trees. Animals begin to return when there is food there for them to eat. When it is a fully functioning ecosystem, it has reached the climax community stage.

Successional dynamics following severe disturbance or removal of a pre-existing community are called secondary succession. Dynamics in secondary succession are strongly influenced by pre-disturbance conditions, including soil development, seed banks, remaining organic matter, and residual living organisms. Because of residual fertility and pre-existing organisms, community change in early stages of secondary succession can be relatively rapid. In a fragmented old field habitat created in eastern Kansas, woody plants "colonized more rapidly (per unit area) on large and nearby patches.

Secondary succession is much more commonly observed and studied than primary succession. Particularly common types of secondary succession include responses to natural disturbances such as fire, flood, and severe winds, and to human-caused disturbances such as logging and agriculture. As an example, secondary succession has been occurring in Shenandoah National Park following the 1995 flood of the Mormon River, which destroyed plant and animal life. Today, plant and animal species are beginning to return.



Causes of plant succession:

Autogenic succession can be brought by changes in the soil caused by the organisms there. These changes include accumulation of organic matter in litter or humic layer, alteration of soil nutrients, or change in the pH of soil due to the plants growing there. The structure of the plants themselves can also alter the community. For example, when larger species like trees mature, they produce shade on to the developing forest floor that tends to exclude light- requiring species. Shade-tolerant species will invade the area.

Allogenic succession is caused by external environmental influences and not by the vegetation. For example, soil changes due to erosion, leaching or the deposition of silt and clays can alter the nutrient content and water relationships in the ecosystems. Animals also play an important role in allogenic changes as they are pollinators, seed dispersers and herbivores. They can also increase nutrient content of the soil in certain areas, or shift soil about (as termites, ants, and moles do) creating patches in the habitat. This may create regeneration sites that favor certain species.

Autotrophic and Heterotrophic Succession: A succession in which green plants or the autotrophs dominate, so that there is a continuous energy flow during the whole process, is known as Autotrophic succession. In contrast, succession wherein heterotrophs such as bacteria, fungi, actinomycetes or even animals dominate and there is a continuous decline in the energy flow is known as Heterotrophic succession.

Further, based on the nature of environment (primarily moisture conditions) at the start of the succession, the succession can be of following types:

Hydrarch or Hydrosere: If the succession begins from a water body, it is termed as Hydrosere or Hydrarch. The water body could be lake, pond, stream, bog or even the swampy area.
Xerosere or Xerarch: In this case, the succession begins from the dry conditions with very little moisture content, for example, a desert area, sandy areas, rocks etc. If specifically the succession starts from the rocky areas, it is known as Lithosere, whereas if it starts from a sand dune it is known as Psammosere.
Mesosere or Mesarch: If the succession starts in an area with adequate moisture conditions and temperature, it is known as mesosere or mesarch.
Halosere: Halosere is a type of succession occurring in a saline area i.e. where concentration of salt in the substratum is very high.

Process of Hydrosere
A hydrosere, also known as hydrarch, starts from a water body like pond, lake or pool that is gradually convertedinto a forest through an orderly process. The various plant communities come in sequence during this succession are grouped into seven main stages as follows:

1. Phytoplankton stage (pioneer stage)
↓
2. Rooted Submerged stage (seral stage 1)
↓
3. Rooted Floating stage (seral stage 2)
↓
4. Reed Swamp stage (seral stage 3)
↓
5. Sedge-Meadow stage (seral stage 4)
↓
6. Woodland stage (seral stage 5)
↓
7. Forest stage (climax stage)

Process of Lithosere, a type of Xerosere
This type of succession starts from a rock, which is in un-weathered state and lacks any organic matter or moisture content. At this stage, only the simple plants like lichens can colonize. The lichens are then followed by various other plants that contribute towards building up of the soil substratum congenial for supporting a mature forest community. Various plant communities that colonize in succession one after the other are summarized as under:

1. Crustose lichen stage (pioneer stage)
↓
2. Foliose lichen stage (seral stage 1)
↓
3. Moss stage (seral stage 2)
↓
4. Herb stage (seral stage 3)
↓
5. Shrub stage (seral stage 4)
↓
6. Forest stage (Climax stage)

General process of succession:
The entire process of primary autotrophic succession is completed through a series of sequential steps followed by one after another.

The different sequential steps may be outlined as below:

(1) Nudation:

It is a process of developing a bare area without any form of life for the arrival of new species. The causes of nudation may be:

(a) Topographic:

The existing community may disappear due to soil erosion (by gravity, water or wind), land slide, volcanic activity etc.

(b) Climatic:

The existing community may be destroyed due to storm, fire, frost, drought.

(c) Biotic:

The community may also be destroyed by anthropogenic activities like destruction of forest, destruction of grass land etc. Besides, diseases induced by bacteria and virus can also destroy the population.

(2) Invasion:

The successful establishment of a species in a bare area is called as invasion. This process of establishment is completed in three successive steps:

(a) Migration (dispersal):

The seeds, spores or other progagules of the species are brought to the bare area by the agents like air, water etc.

(b) Ecesis (Establishment):

The process of successful establishment (germination and growth) of the species in the new area as a result of adjustment with the prevailing conditions is known as ecesis.

(c) Aggregation:

After ecesis, the individuals of species increase their number by reproduction and thus, are aggregated in a particular area.

(3) Competition and Coaction:

As the species aggregate at a limited space, there happens competition (inter as well as intra specific) mainly for space and nutrition. Secondly the life process of one individual is affected by the surrounding species in various ways which is known as coaction. The species which are found unable to compete with others in the existing environment get discarded.

(4) Reaction:

The species present in an environment constantly interact with it there by causing its modification. The mechanism of the modification of the environment through the influence of living organisms on it, is known as reaction.

Reaction induces changes in soil composition, water content and light organisms on it and is known as reaction. Reaction induces changes in soil composition, water content, light conditon, temperature etc. Due to drastic modifications of the environment/ it may not be suitable for the existing community.

Hence, the existing community may be replaced by another community. The whole sequence of communities that substitute one another in the given area is known as sere and the various communities constituting the sere are known as seral communities or seral stages.

(5) Stablisation (Climax):

At last a final or terminal community is established. Which is stabilized for a longer period of time and which can maintain an equilibrium with the environment of that area. This community is known as climax community and the stage is as climax stage.

Concept of Climax community:

In ecology, climax community, is a historic term for a biological community of plants, animals, and fungi which, through the process of ecological succession in the development of vegetation in an area over time, have reached a steady state. This equilibrium was thought to occur because the climax community is composed of species best adapted to average conditions in that area. The term is sometimes also applied in soil development. Nevertheless, it has been found that a "steady state" is more apparent than real, particularly if long-enough periods of time are taken into consideration. Notwithstanding, it remains a useful concept.

The idea of a single climatic climax, which is defined in relation to regional climate, originated with Frederic Clements in the early 1900s. The first analysis of succession as leading to something like a climax was written by Henry Cowles in 1899, but it was Clements who used the term "climax" to describe the idealized endpoint of succession.

Characteristics

- The vegetation is tolerant of environmental conditions.
- It has a wide diversity of species, a well-drained spatial structure, and complex food chains.
- The climax ecosystem is balanced. There is equilibrium between gross primary production and total respiration, between energy used from sunlight and energy released by decomposition, between uptake of nutrients from the soil and the return of nutrient by litter fall to the soil.
- Individuals in the climax stage are replaced by others of the same kind. Thus the species composition maintains equilibrium.

It is an index of the climate of the area. The life or growth forms indicate the climatic type.

Types of climax

Climatic Climax

If there is only a single climax and the development of climax community is controlled by the climate of the region, it is termed as climatic climax. For example, development of Maple-beech climax community over moist soil. Climatic climax is theoretical and develops where physical conditions of the substrate are not so extreme as to modify the effects of the prevailing regional climate.

Edaphic Climax

When there are more than one climax communities in the region, modified by local conditions of the substrate such as soil moisture, soil nutrients, topography, slope exposure, fire, and animal activity, it is called edaphic climax. Succession ends in an edaphic climax where topography, soil, water, fire, or other disturbances are such that a climatic climax cannot develop.

Catastrophic Climax

Climax vegetation vulnerable to a catastrophic event such as a wildfire. For example, in California, chaparral vegetation is the final vegetation. The wildfire removes the mature vegetation and decomposers. A rapid development of herbaceous vegetation follows until the shrub dominance is re-established. This is known as catastrophic climax.

Disclimax

When a stable community, which is not the climatic or edaphic climax for the given site, is maintained by man or his domestic animals, it is designated as Disclimax (disturbance climax) or anthropogenic subclimax (man-generated). For example, overgrazing by stock may produce a desert community of bushes and cacti where the local climate actually would allow grassland to maintain itself.

Subclimax

The prolonged stage in succession just preceding the climatic climax is subclimax.

Preclimax and Postclimax

In certain areas different climax communities develop under similar climatic conditions. If the community has life forms lower than those in the expected climatic climax, it is called preclimax; a community that has life forms higher than those in the expected climatic climax is postclimax. Preclimax strips develop in less moist and hotter areas, whereas Postclimax strands develop in more moist and cooler areas than that of surrounding climate.

Theories

There are three schools of interpretations explaining the climax concept:

- Monoclimax or Climatic Climax Theory was advanced by Clements (1916) and recognizes only one climax whose characteristics are determined solely by climate (climatic climax). The processes of succession and modification of environment overcome the effects of differences in topography, parent material of the soil, and other factors. The whole area would be covered with uniform plant community. Communities other than the climax are related to it, and are recognized as subclimax, postclimax and disclimax.

- ❖ Polyclimax Theory was advanced by Tansley (1935). It proposes that the climax vegetation of a region consists of more than one vegetation climaxes controlled by soil moisture, soil nutrients, topography, slope exposure, fire, and animal activity.
- ❖ Climax Pattern Theory was proposed by Whittaker (1953). The climax pattern theory recognizes a variety of climaxes governed by responses of species populations to biotic and abiotic conditions. According to this theory the total environment of the ecosystem determines the composition, species structure, and balance of a climax community. The environment includes the species responses to moisture, temperature, and nutrients, their biotic relationships, availability of flora and fauna to colonize the area, chance dispersal of seeds and animals, soils, climate, and disturbance such as fire and wind. The nature of climax vegetation will change as the environment changes. The climax community represents a pattern of populations that corresponds to and changes with the pattern of environment. The central and most widespread community is the climatic climax.

The theory of alternative stable states suggests there is not one end point but many which transition between each other over ecological time.

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10. Ecosystem ecology: concept of ecosystem, disturbance (natural and anthropogenic) and their impact on plant ecology; invasive plant species; resistance and resilience of ecosystems

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Ecosystem:

Ecology deals with the study of interactions between living organisms and their physical environment. Now ecology is defined as the study of ecosystems. The term ecosystem was proposed by A.C. Tansley in 1935 where ecoimplies the environment and system denotes an interacting, interdependent, integrated complex.

Ecosystem may be defined as the system resulting from the integration of all living and non-living factors of the environment. Thus any structural and functional unit of biosphere where the organisms interact with the physical environment so that a flow of energy leads to clearly defined trophic structure, biotic diversity and material cycle (i.e., exchange of materials between living and non-living components) within the system is known as an ecological system or ecosystem.

Earth is a giant ecosystem where abiotic and biotic components are constantly acting and reacting with each other bringing structural and functional changes in it. This vast ecosystem-biosphere is subdivided into units of smaller ecosystems such as terrestrial and aquatic ecosystems.

These systems may be freely exchanging energy and matter from outside—an open ecosystem or may be isolated from outside—a closed ecosystem.

An ecosystem is normally an open system with a continuous but variable influx and loss of material and energy. It is a basic, functional unit with no limits of boundaries.

Thus an ecosystem represents the highest level of ecological integration which is energy based and this functional unit is capable of energy transformation, accumulation and circulation. Its main function in ecological sense is to emphasize obligatory relationships, interdependence and casual relations.

Classification of Ecosystems

1. Natural Ecosystems:

These systems operate by themselves under natural conditions without any major interference by man. These are further divided into following ecosystems:

(i) Terrestrial ecosystem includes forests, grasslands and deserts etc.

(ii) Aquatic ecosystem may be further distinguished as

(a) Fresh water which may be lotic (running water as springs, streams or rivers) or lentic (standing water as lakes, ponds, pools, ditches, puddles, swamps etc.).

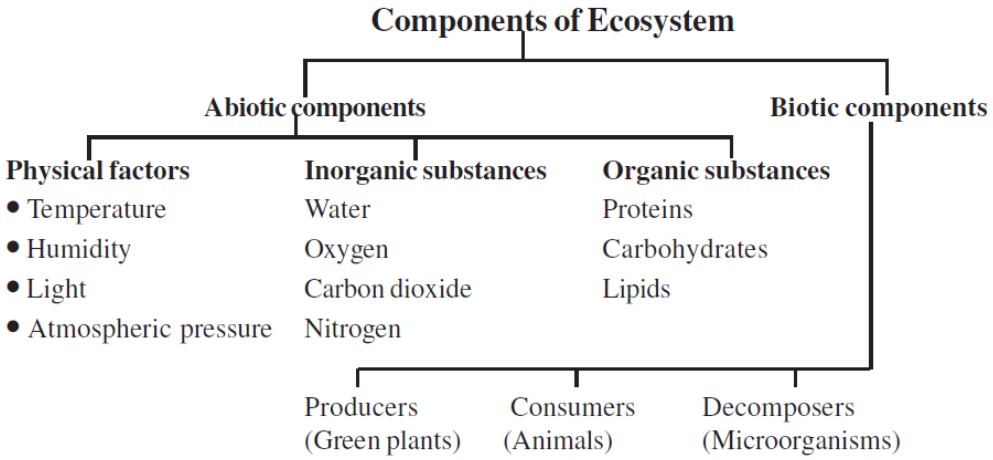
(b) Marine water such as oceans (deep bodies) or seas or estuaries (shallow ones).

2. Artificial (Man-engineered) Ecosystems:

These are maintained artificially by man where, by addition of energy and planned manipulations, natural balance is disturbed regularly. Crop, urban, industrial, space and control of biotic community as well as the physico-chemical environment are man-engineered ecosystems.

3. **Space Ecosystem** is also recognised as one of ecosystems and play a very important role in human life.The common features of all ecosystems — terrestrial, aquatic and agricultural are the interactions of the autotrophic and heterotrophic components.

Components of Ecosystem:
An ecosystem has two major components—biotic and abiotic.
(A) Biotic (Living) Components:
Plants, animals and micro-organisms having different nutritional behaviour constitute the biotic components of an ecosystem.



1. Producers (Autotrophs):
Producers are mainly chlorophyll bearing green plants (photo autotrophs) which can synthesize their food in presence of sunlight making use of CO_2 and water through the process of photosynthesis. Since plants convert solar energy into chemical energy so they must be better called converters or transducers. Chemosynthetic organisms or chemo-autotrophs can also synthesize some organic matter by the oxidation of certain chemicals in absence of sunlight.
2. Consumers (or Heterotrophs or Phagotrophs):
Consumers consume the matter built up by the producers. They utilise, rearrange and decompose complex materials.
Consumers are of the following types:
(i) Herbivores:
They feed directly on producers and hence are known as primary consumers, e.g., rabbit, deer, cattle, insects etc. Elton (1927) called herbivores as key industry animals because they convert plants into animal materials.
(ii) Carnivores (Meat eaters):
They feed on other consumers. If they feed on herbivores, they are called secondary consumers (e.g., frog, birds, cat) and if they prey on other carnivores (snake, peacock), they are known as tertiary carnivores/consumers. Lion, tiger etc. which cannot be preyed are called top carnivores since they occupy top position in the food chain.
(iii) Omnivores:
They feed both on plants and animals, e.g., rat, fox, birds and man.
(iv) Detritivores (Detritus feeders or saprotrophs):
They feed on partially decomposed matter such as termites, ants, crabs, earthworms etc.
3. Decomposers (or Micro-consumers):

Decomposers are saprophytic (osmotrophs) micro-organisms such as bacteria, actinomycetes and fungi. They derive their nutrition by breaking down complex organic compounds and release inorganic nutrients into environment, making them available again to producers.
The biotic components of any ecosystem may be thought of as the functional kingdom of nature, since they are based on the type of nutrition and the energy source used. The entire earth is considered as an ecosystem which is referred to as biosphere or ecosphere.

(B) Abiotic (Non-living) Components:

Structurally abiotic components include:

1. Climatic regime:

Precipitation, temperature, sunlight, intensity of solar flux, wind etc. have a strong influence on the ecosystem.

2. Inorganic substances:

These are C, N, H, O, P, S involved in material cycles. The amount of these substances present in an ecosystem is known as standing state or standing quality.

3. Organic Substances:

Carbohydrates, proteins, lipids and humic substances link the abiotic components with the biotic components. All the biotic and abiotic components of an ecosystem are influenced by each other and are linked together through energy flow and matter cycling.

Functions of ecosystem

Ecosystems are complex dynamic system. They perform certain functions. These are:-
(i) Energy flow through food chain
(ii) Nutrient cycling (biogeochemical cycles)
(iii) Ecological succession or ecosystem development
(iv) Homeostasis (or cybernetic) or feedback control mechanisms

Ponds, lakes, meadows, marshlands, grasslands, deserts and forests are examples of natural ecosystem. Many of you have seen an aquarium; a garden or a lawn etc. in your neighbourhood. These are man made ecosystem.
Ecosystem is the functional unit of nature where living organisms interact among themselves and also with the surroundings physical environment.
There are two basic categories of ecosystem, namely the terrestrial and the aquatic.
Terrestrial ecosystem – forest, grassland, desert etc.
Aquatic ecosystem – ponds, lake, river, estuary etc.

Pond as an example of an ecosystem

A pond is an example of a complete, closed and an independent ecosystem. It is convenient to study its basic structure and functions. It works on solar energy and maintains its biotic community in equilibrium. If you collect a glass full of pond water or a scoop full of pond bottom mud, it consists of a mixture of plants, animals, inorganic and organic materials. Following components are found in a pond ecosystem.

(a) Abiotic components

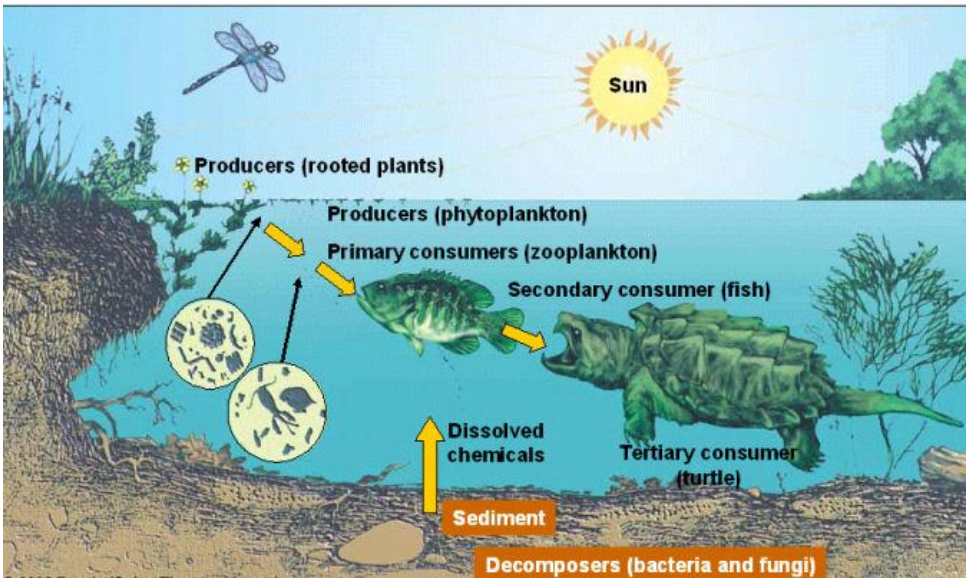
(i) Light: Solar radiation provides energy that controls the entire system. Penetration of light depends on transparency of water, amount of dissolved or suspended particles in water and the number of plankton. On the basis of extent of penetration of light a pond can be divided into euphotic (eu=true,photic=light), mesophotic and aphotic zones. Plenty of light is available to plants and animals in euphotic zone. No light is available in the aphotic zone.

ii) Inorganic substances: These are water, carbon, nitrogen, phosphorus, calcium and a few other elements like sulphur depending on the location of the pond. The inorganic substances like O_2 and CO_2 are in dissolved state in water. All plants and animals depend on water for their food and exchange of gases- nitrogen, phosphorus, sulphur and other inorganic salts are held in reserve in bottom sediment and inside the living organisms. A very small fraction may be in the dissolved state.

(iii) Organic compounds: the commonly found organic matters in the pond are amino acids and humic acids and the breakdown products of dead animals and plants. They are partly dissolved in water and partly suspended in water.

(b) Biotic components

(i) Producers or autotrophs: synthesize food for all the heterotrophs of the pond. They can be categorized into two groups:-
(a) Floating microorganisms and plants
(b) Rooted plants
(a) Floating microorganisms (green) and plants are called phytoplankton ("phyto"- plants, "plankton" –floating). They are microscopic organisms. Sometimes they are so abundant in pond that they make it look green in colour e.g. *Spirogyra*, *Ulothrix*, *Cladophora*, Diatoms, *Volvox*.
(b) Rooted plants: These are arranged in concentric zones from periphery to the deeper layers. Three distinct zones of aquatic plants can be seen with increasing deapth of water in the following order:
i) Zone of emergent vegetation: . eg. *Typha*, *Bulrushes* and *Sagittaria*
ii) Zone of rooted vegetation with floating leaves . eg. *Nymphaea*
iii) Zone of submergent vegetation: eg. All pond weeds like *Hydrilla* , *Rupia*, musk grass etc.
(ii) Consumers/Heterotrophs are animals which feed directly or indirectly on autotrophs eg. Tadpole, snails, sunfish, bass etc.
Pond animals can be classified into the following groups
(a) Zooplanktons are floating animals. *Cyclops, Cypris*
(b) Nektons are the animals that can swim and navigate at will. Eg. fishes
(c) Benthic animals are the bottom dwellers: beetle, mites, mollusks and some crustaceans.
(iii) Decomposers: They are distributed through out the entire in the whole pond but in the sediment most abundant. There are bacteria and fungi. (*Rhizopus*, *Penicillium*, *Curvularia* ,*Cladosporium*) found at the bottom of the pond.



Measuring Production
The bulk of any ecosystem is plants compared to which only a small fraction is animal life. Therefore, measurement of energy of an ecosystem primarily involves plants. And because plants represent the first or primary trophic level, plant production is measured as **Gross Primary Production**, which is equivalent to the energy fixed during photosynthesis.
Net Primary Production measures the amount of biomass accumulated by the plant and is measured as gross primary production minus the energy the plant loses through respiration.Net Primary Production is measured as the weight of living plant material, including leaves, stems and roots.
Productivity: The rate of biomass production. [Unit: g –2 yr –1 or (kcal m – 2) yr –1]
Gross primary productivity (GPP): The rate of production of organic matter during photosynthesis.
Net primary productivity (NPP): Gross primary productivity minus respiration losses (R).

$$GPP - R = NPP$$

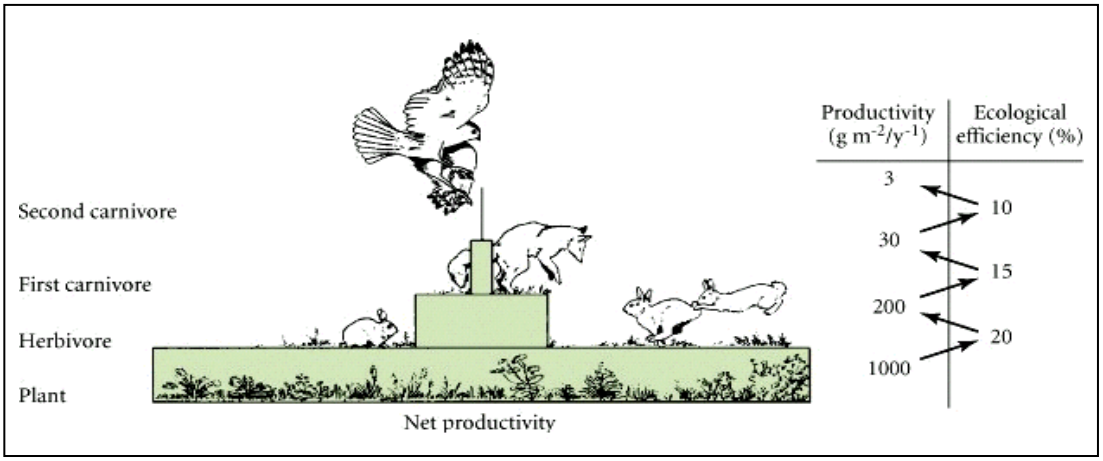
Secondary productivity: The rate of formation of new organic matter by consumers.
Primary productivity depends upon-

- type of plant species inhabiting a particular area

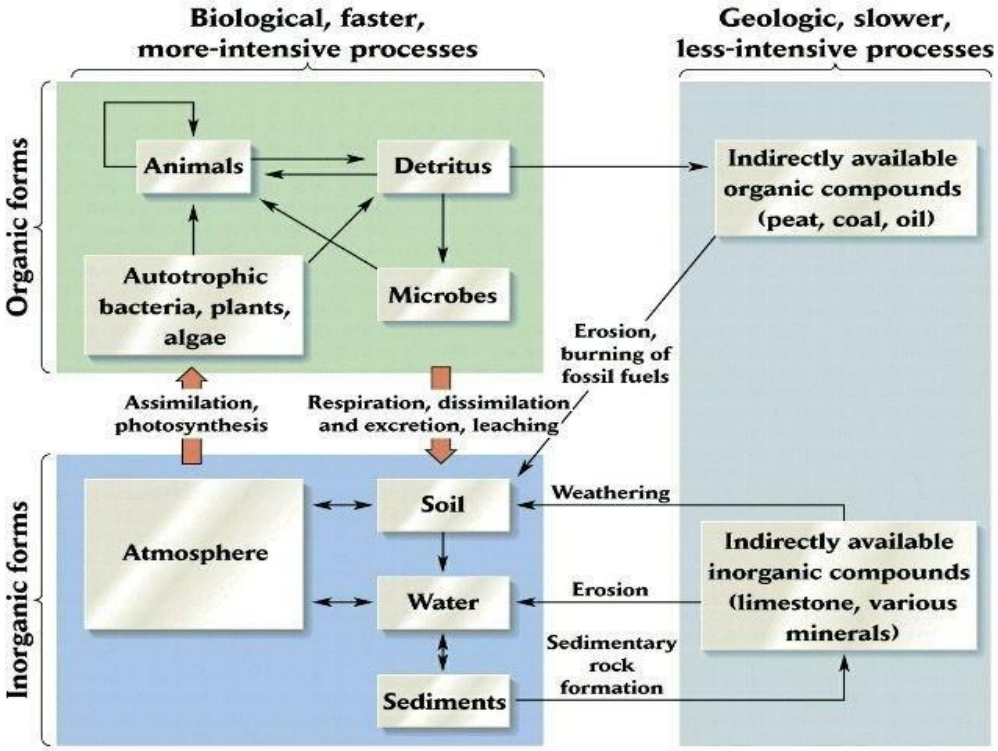
- photosynthetic capacity of plants
- nutrient availability

Patterns in Primary Production

- ❖ With each link in a food chain, a great deal of energy is dissipated before it can be consumed by organisms feeding at the next higher trophiclevel.
- ❖ As Lindeman (1942) pointed –the amount of energy reaching each trophic level depends on the net primary production at the base of the food chain and the efficiencies with which animals convert food energy into their own biomass energy through growth and reproduction at each higher trophiclevel.
- ❖ •Of the light energy assimilated by photosynthesis, plants use between 15 to 70% for their own maintenance, thereby making thisportion unavailable to the consumers.
- ❖ Herbivores and carnivores are more active than plants and expend correspondingly more of their assimilated energy on maintenance.
- ❖ As a result, the productivity of each trophic level is typically only 5-20% that of the level below it.
- ❖ This percentage of energy transferred from one trophic level to the next is called
- ❖ ecological efficiency or food chain efficiency between two trophic levels



Pathways of Elements in the Ecosystem –a generalization



Energy Flow in an Ecosystem:
Energy has been defined as the capacity to do work. Energy exists in two forms potential and kinetic. Potential energy is the energy at rest {i.e., stored energy) capable of performing work. Kinetic energy is the energy of motion (free energy).
It results in work performance at the expense of potential energy. Conversion of potential energy into kinetic energy involves the imparting of motion.
The source of energy required by all living organisms is the chemical energy of their food. The chemical energy is obtained by the conversion of the radiant energy of sun.
The radiant energy is in the form of electromagnetic waves which are released from the sun during the transmutation of hydrogen to helium. The chemical energy stored in the food of living organisms is converted into potential energy by the arrangement of the constituent atoms of food in a particular manner. In any ecosystem there should be unidirectional flow of energy.
This energy flow is based on two important Laws of Thermodynamics which are as follows:
(1) The first law of Thermodynamics:
It states that the amount of energy in the universe is constant. It may change from one form to another, but it can neither be created nor destroyed. Light energy can be neither created nor destroyed as it passes through the atmosphere. It may, however, be transformed into another type of energy, such as chemical energy or heat energy. These forms of energy cannot be transformed into electromagnetic radiation.
(2) The second law of Thermodynamics:
It states that non-random energy (mechanical, chemical, radiant energy) cannot be changed without some degradation into heat energy. The change of energy from one form to another takes place in such a way that a part of energy assumes waste form (heat energy). In this way, after transformation the capacity of energy to perform work is decreased. Thus, energy flows from higher to lower level.
Main source of energy is sun. Approximately 57% of sun energy is absorbed in the atmosphere and scattered in the space. Some 35% is spent to heat water and land areas and to evaporate water. Of the approximately 8% of light energy striking plant surface, 10% to 15% is reflected, 5% is transmitted and 80 to 85% is absorbed; and an average of only 2% (0.5 to 3.5%) of the total light energy striking on a leaf is used in photosynthesis and rest is transformed into heat energy.
Energy flow in Ecosystems:
Living organisms can use energy in two forms radiant and fixed energy. Radiant energy is in the form of electromagnetic waves, such as light. Fixed energy is potential chemical energy bound in various organic substances which can be broken down in order to release their energy content.
Organisms that can fix radiant energy utilizing inorganic substances to produce organic molecules are called autotrophs. Organisms that cannot obtain energy from abiotic source but depend on energy-rich organic molecules synthesized by autotrophs are called heterotrophs. Those which obtain energy from living organisms are called consumers and those which obtain energy from dead organisms are called decomposers.

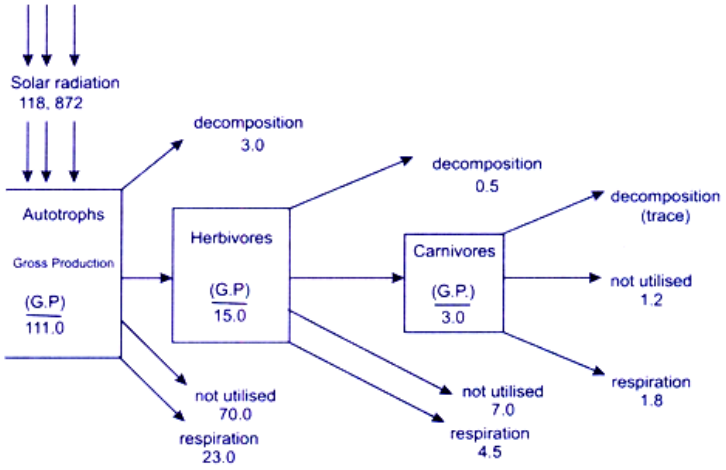


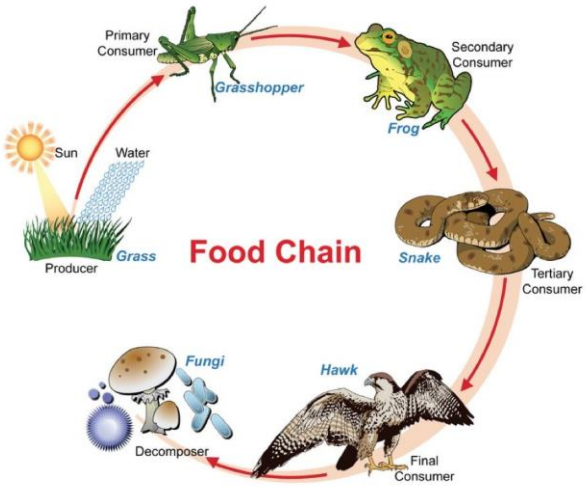
Fig. 1.3 Energy flow diagram for a lake (freshwater ecosystem) in g cal/cm2/yr

Flow of Energy at different Levels of Ecosystem

When the light energy falls on the green surfaces of plants, a part of it is transformed into chemical energy which is stored in various organic products in the plants. When the herbivores consume plants as food and convert chemical energy accumulated in plant products into kinetic energy, degradation of energy will occur through its conversion into heat. When herbivores are consumed by carnivores of the first order (secondary consumers) further degradation will occur. Similarly, when primary carnivores are consumed by top carnivores, again energy will be degraded.

Food chain

A food chain may be defined as the transfer of energy and nutrients from the source in plants through a series of organisms with repeated processes of eating and being eaten. For example, the marsh vegetation is eaten by the grasshopper the grasshopper is consumed by shrew, the shrew by the marsh hawk or owl.



Thus, a relationship is established:

Marsh grass → Grass hopper → Shrew → Marsh hawk

It is estimated that only about 10 per cent of the potential energy available at the previous trophic level is being available to an organism. The efficiency of a food chain is, therefore, dependent on the number of trophic levels or links in a food chain. The shorter the food chain, the more is the amount of energy available to the last trophic levels in an eco-system.

In all the eco-systems, energy moves as per the direction given below:

Sun → Producer → Consumer → Decomposer

Sun → Autotroph → Heterotroph.

Food chains are of three types:

(a) Grazing or Predator food chain.

(b) Parasitic food chain.

(c) Saprophytic or detritus food chain.

(a) Grazing or Pasturing Food Chain:

This food chain starts from green plants (producers), passes through the herbivore (primary consumers) and ends with carnivore (secondary or tertiary consumers).

The total energy assimilated by green plants is subjected to three important processes:

(i) It may be oxidised in respiration.

(ii) It may die and decay.

(iii) It may be eaten by herbivorous animals.

Like green plants, the disposition of energy in herbivores takes place by either respiration or decay of organic matter by microbes or consumption by carnivore. The primary carnivores eat herbivore and secondary carnivore eat primary carnivore. The total energy in herbivores takes by decay of organic matter by microbes the primary carnivore eat herbivore eat primary carnivore.

The total energy assimilated by primary carnivore is derived entirely from the herbivore and its disposition in to respiration, decay and further consumption by other carnivore is entirely similar to that of herbivore. Thus, the grazing food chain is more effective or efficient as most of the primary production is passed on through different trophic levels and only a small fraction goes to the decomposer system.

A grazing food chain can be described in terms of trophic levels as shown below:

Autotroph→ Herbivore→ Primary Carnivore →Secondary Carnivore→ Tertiary Carnivore → Decomposer etc.

(b) Parasitic rood chain:

This food chain starts from herbivore but food energy passes from larger to smaller organism without outright killing as in case of predator. Hence, the larger animals are considered to be the hosts and the smaller animals which fulfill their nutritional requirements from the hosts are considered as parasites.

(c) Saprophytic or Detritus Food chain:

In this food chain, the dead organic matter or organic wastes (metabolic wastes and extrudates) of eco-system go to the micro-organisms and finally to detritus feeding organiss known as detrivore. The energy stored in detritus serves as a source of energy for detrivore. This type of food chain is less efficient as the major portion of energy is lost to the eco-system without being properly used.

Food Chain of Different Eco-Systems:

Grassland eco system:

Grass →Grasshopper →Toad→ Snake
Grass→ Rat→ Snake→ Hawk
Grass→ Goat→ Man→ Tiger

Forest eco-system:

Plant→ Deer→ Lion
Plant→ Deer→ Tiger
Plant→ Goat→ Tiger

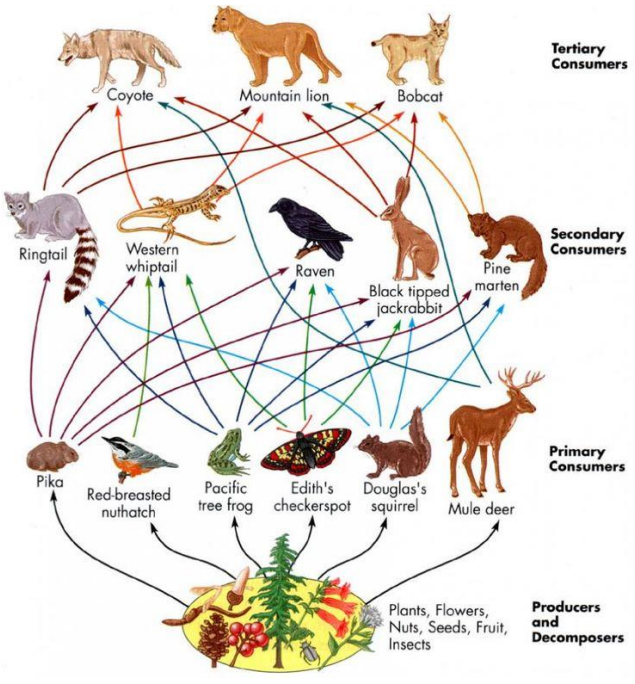
Pond eco-system:

Phytoplankton→ Zooplanktons→ Small fish→ Big fish

Food Web:

Food chains are not always simple and isolated but are interconnected with one another. The interlocking pattern of food chains or a matrix of food chains, with all sorts of short circuits and connections is often called the food web or food net. However, in any food web all the species not equally important and many could be removed without seriously affecting the more important species.

Thus, food web is a simplified representation of the complex interrelationships of the population of plants and animals which exist in a community. The basic operational principle in a food web is that each species is dependent upon at least one other species, and the numbers of each link species must be sufficient for their continued existence. If these conditions are maintained, the web will exist in an ecological nutritional equilibrium.



Ecological pyramid

Ecological pyramids are the graphic representations of trophic levels in an ecosystem. They are pyramidal in shape and they are of three types: The producers make the base of the pyramid and the subsequent tiers of the pyramid represent herbivore, carnivore and top carnivore levels.

(1) Pyramid of number: This represents the number of organisms at each trophic level. For example in a grassland the number of grasses is more than the number of herbivores that feed on them and the number of herbivores is more than the number of carnivores. In some instances the pyramid of number may be inverted, i.e herbivores are more than primary producers as you may observe that many caterpillars and insects feed on a single tree.

(2) Pyramid of biomass: This represents the total standing crop biomass at each trophic level. Standing crop biomass is the amount of the living matter at any given time. It is expressed as gm/unit area or kilo cal/unit area. In most of the terrestrial ecosystems the pyramid of biomass is upright. However, in case of aquatic ecosystems the pyramid of biomass may be inverted e.g. in a pond

phytoplankton are the main producers, they have very short life cycles and a rapid turn over rate (i.e. they are rapidly replaced by new plants). Therefore, their total biomass at any given time is less than the biomass of herbivores supported by them.

(3) Pyramid of energy: This pyramid represents the total amount of energy at each trophic level. Energy is expressed in terms of rate such as kcal/unit area /unit time or cal/unit area/unit time.eg. in a lake autotroph energy is 20810 kcal/m/year. Energy pyramids are never inverted.

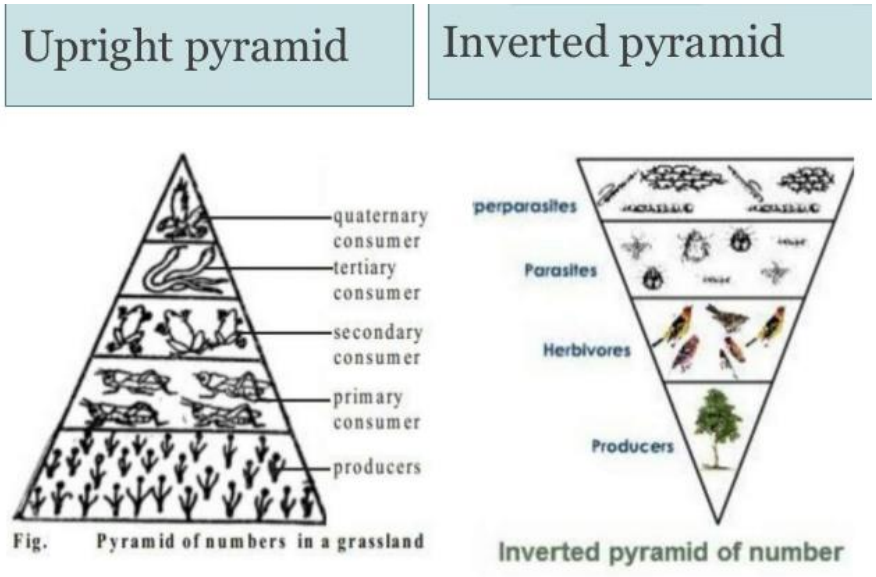


Fig. Pyramid of numbers in a grassland

Quantifying energy flow

To quantify the flux of energy through the ecosystem it is necessary to determine the processes involve and quantify them. The processes include – consumption, ingestion, assimilation, respiration and production**. Ecological Efficiency** is a product of **Consumption efficiency**, **Assimilation efficiency** and the **Production Efficiency**.

Of the food ingested by a consumer (I), a portion isassimilated across the gut wall (A), and the remainder isexpelled aswaste (W). Of the energy that is assimilated some is used in respiration (R) and the remainder goes into production (P), which includes growth and reproduction. The ratio of assimilation to ingestion (A/I) is called assimilation efficiency. It is a measure of the efficiency with which the consumer extractsenergy from its food.

Food Chains–Quantifying energy flow

- The ratio of production to assimilation (P/A) is called **production efficiency**. It is a measure of the efficiency with which the consumer incorporates assimilated energy into secondary production.
- The energy available to a given trophic level (n) is the production of the next lower level (n - 1).
- The **consumption efficiency** is the ratio of ingestion to production (I_n/P_{n-1}), it defines the amount of available energy beingconsumed.

- Application of efficiency values for each trophic level gives an estimate of the flow of energy through the whole ecosystem.

TABLE 10-3 Definitions of several energetic efficiencies

$$\text{Exploitation efficiency} = \frac{\text{Ingestion of food}}{\text{Prey production}}$$

$$\text{Assimilation efficiency} = \frac{\text{Assimilation}}{\text{Ingestion}}$$

$$\text{Net production efficiency} = \frac{\text{Production (growth and reproduction)}}{\text{Assimilation}}$$

$$\text{Gross production efficiency} = \text{Assimilation efficiency} \times \text{Net production efficiency} = \frac{\text{Production}}{\text{Ingestion}}$$

$$\text{Ecological efficiency} = \text{Exploitation efficiency} \times \text{Assimilation efficiency} \times \text{Net production efficiency} = \frac{\text{Consumer production}}{\text{Prey production}}$$

Disturbance (natural and anthropogenic) and their impact on plant ecology

- Ecological disturbance is an event or force, of abiotic or biotic origin, that brings about either mortality of organisms or changes in their spatial patterning in the ecosystems they inhabit.
- It is a relatively discrete event such as fire, windstorm, flood, extremely cold temperature, drought or epidemic that disrupts an ecosystem by influencing community structure & function.
- Disturbance plays a significant role in shaping the structure of individual populations and the character of whole ecosystems.
- Disturbances, both create and are influenced by patterns on the landscape

Characteristics of Disturbance

Disturbances have spatial and temporal characteristics:

- Small-scale disturbances make gaps in the substrate or vegetation, creating patches of different composition or succession stages.
  - Large-scale disturbances favor opportunistic species.
  - Severe disturbances can replace a community altogether from an ecosystem.
- Frequent disturbances can eliminate certain species from the ecosystem.
- Disturbance Regime -Disturbance regime of a vegetation system is the sum of all disturbances affecting the system. It can be characterized by several parameters:

1.	Kind	Kind refers to the type of disturbances, which vary with climate, topography, substrate and biota.
2.	Spatial parameters	Spatial parameters are the area, shape & spatial distribution of patches created by disturbances

3.	Temporal parameters	Temporal parameters are the frequency, return interval, cycle, and rotation period of disturbances.
4.	Specificity	Specificity is correlation between a type of disturbance & specific characteristics of disturbed sites, such as species, size class, seral stage and location.
5.	Magnitude	Magnitude includes the intensity (the physical force per event per area per time) and severity (the impact on organisms and ecosystem structure and composition) of disturbances, and generates patch variations, internal heterogeneity and biological legacies
6.	Synergism	Synergisms are the interactions among different kinds of disturbances.

Invasive Plant Species

Species have been and are being introduced to regions outside their historic (post- glacial) native range.

Species are being transported to new locations at up to 10,000 timesgreater rate than by natural dispersal.

Such species are also called: exotic species, non- indigenousspecies, non-native species, introduced species, colonizingspecies.

There is a general agreement now to the term 'invasive alien species', meaning non-native species introduced to new areas where they are found to cause problems.

Species that are rapidly expanding outside their native range.Usually exotics far from their native habitats, often other continents. In most cases they have not dispersed naturally, but have been brought either purposefully or in advertently to a new habitat. Large proportion of the plants that have become seriously invasive were deliberately introduced, planted and cultivated by people. Species invasions affect plant communities in two ways: Through the effects of the spread of non-native plants on native plant species, communities and ecosystems. Through the effects of invasive insect herbivores and pathogens on native plant species. A plant becomes potentially invasive when it is capable of

i. Reproduction at younger ages
ii. Has smaller seeds
iii. Produces large seed crops at shorter interval

What influences success of invasion?

Species Characteristics

- high fecundity
- small body size
- vegetative or asexual reproduction
- high genetic diversity
- high phenotypic plasticity
- broad native range
- abundant in native range
- physiological tolerance
- habitat generalist
- human commensal
- loss of natural enemies
- invasional meltdown

Generalizations Regarding Habitat Invasibility

- climatically matched
- disturbed
- low diversity
- absence of predators
- presence of vacant niches
- low connectance of food web
- nutrient rich (plants)

Vulnerability of Communities to Invasions

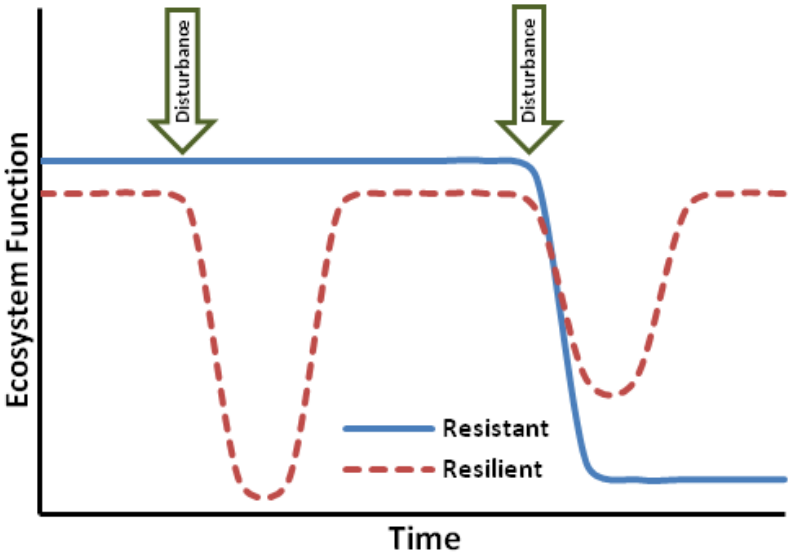
- Some communities are vulnerable to invasions while others can resist invasions.
- Invaders are most likely to become established in disturbed and species-poor communities.
- Disturbance may be a critical factor in promoting invasions but there exist many conflicting evidences against uniformity in its role: since it includes more than a single factor like changes in resource availability, competitive interactions, microclimates, predator-prey relationships.
- Invasive species usually grow vigorously beyond their own native ranges, an explanation being that the new range is devoid of its natural enemies(competitors, predatorsand pathogens).
- Variations in resource availability has also been proposed to be an underlying cause for positive interactions between native and invasive species **diversity.**

Resistance and Resilience of Ecosystems

Resistant system

The black spruce forest of Alaska burns easily in the summer. Afterwards there is a secondary succession. There are immediate physical changes. The blackened soil, without its insulating layers of moss and litter, warms up and the permafrost retreats. Nutrients in previously frozen areas are now available. Black spruce growsfrom seed, but slowly. In the meantime, windblown birch and poplar establish and thrive in the nutrient-rich, warmer soil. Slowly, spruce and moss grow and the litter layer redevelops. The soil becomes colder again and once thebirch and poplar die they are not replaced.

Moss and spruce litter decompose very slowly. The litter layer thickens; the permafrost rises and nutrients become bound in the un-decomposed material.



Resilient system

The taiga therefore has low resistance to fire: it burns easily. But the original system is soon restored after the fire. It recovers, it has high resilience. Although, it is resilient in the face of fire, taiga is however not resilient to any other kind of disturbance. If the spruce trees were felled and removed, there would be no cones from which new seeds could establish. The spruce forest might then never recover.

- Rain forest doesnot burn easily, because it doesn't dry out as the taiga does in summer. It is therefore resistant to fire, and the only large-scale firesare those caused by humans. However, once burned, it showslow resilience, particularly if burned on slopes, where nutrient leaching is much faster than on level ground. Low resilience means that theoriginal system may never recover.The extent of resistance and resilience therefore depends on both the natureof the ecosystem, and the type of disturbance.

- Ecosystems that show a high degree of stability may have different combinationsof resistance and resilience.
- Research has shown that species diversity is often the key to both ecosystem resistance and resilience.An ecosystem rich in biodiversity will likely be more stable than one whose biodiversity is low.
- Changing environmental conditionscan cause the decline of local biodiversity. If this happens, an ecosystem's resistance and/or resilience may decline. The end result isthat the ecosystem loses stability.
- Ecosystems that are less stable may not be able to respond to a normal environmental disturbance, which may damage ecosystem structure, ecosystem function, or both.

11. Biogeographical ecology: terrestrial ecology; wetland and freshwater ecology; coastal and marine ecology; major biogeographical zones of India

Terrestrial Ecosystem:

The concept of ecosystem includes the abiotic (or non-living) and biotic (or living) portions of an area as well as the interactions between the two. Matter and energy flow between the abiotic and biotic components of the ecosystem. Abiotic factors influencing an ecosystem include temperature, precipitation, elevation and soil type. Scientists divide ecosystems into terrestrial and non-terrestrial. Ecosystems may be further classified by their geographical region and dominant plant type Six primary terrestrial ecosystems exist:

- ❖ Tundra,
- ❖ Taiga,
- ❖ Temperate deciduous forest,
- ❖ Tropical rain forest,
- ❖ Grassland; and
- ❖ Desert.

Tundra:

An ecosystem situated near the North Pole in the Arctic Circle; thus, the coldest and driest of all terrestrial ecosystems. Winters are extremely cold with temperatures typically below -34° C. The summers last only about two months and the temperatures are still very cold ranging from 3° to 12° C. The word tundra comes from the Finnish word tunturia which means treeless land. There are two types of tundra – arctic tundra and alpine tundra. The arctic tundra is located within the Arctic Circle while the alpine tundra is the area high in the mountains above trees. The largest animal that lives in the tundra ecosystem is the **polar bear**. The herbivores are the arctic fox, arctic wolf, bobcat and snowy owl. Polar bears live along coastal areas, and prey on seals.

There are no upright trees on the tundra. Only trees such as dwarf willows and birches, which grow low to the ground, can escape the drying effect of the wind which upright trees would experience. This biome consists mainly of mosses, grasses, sedges, lichens and some shrubs. Seasonal thawing of the frozen soil occurs only up-to a few centimetres depth, which permits the growth of shallow rooted plants.

Taiga:

The largest terrestrial ecosystem and extends across Europe, North America and Asia. It is also known as coniferous forest or boreal forest. It has short, wet summers and long, cold winters. It gets plenty of snow during the winter and plenty of rainfall during the summer.

Taiga

This terrestrial environment has long summer days and very short winter days. Animals found in the taiga include lynx, moose, wolves, bears and burrowing rodents.

Temperate deciduous forest:

It experiences all four seasonswinter, spring, summer, and fall. The four seasons are easily recognizable and each lasts about 3 months. It is located in the United States, Canada, Europe, China, Japan and some parts of Russia. Its soil is very fertile and rich with nutrients.

Common deciduous trees are the hardwoods such as beech, maple, oak, hickory and walnut. They are broad-leaved trees. The trees shed their leaves in the late fall so the biome has an entirely different appearance in the winter than in the summer. The leaves of deciduous trees change color in the fall because the plant or tree stops producing chlorophyll which is what gives them their green color.

The fallen leaves provide food for a large variety of consumer and decomposer populations, such as millipedes, snails and fungi living in or on the soil. The temperate deciduous forest produces flowers, fruits and seeds of many types which provide a variety of food for animals.

The common herbivores of this biome are deer, chipmunks, squirrels, rabbits and beavers. Tree-dwelling birds are abundant in number and diversity. Important predators are—black bears, bobcats, and foxes. Predatory birds are also found, such as hawks, owls and eagles. The cold-blooded or ectothermic animals, such as snakes, lizards, frogs, and salamanders are also common.

Tropical rain forest:

This forest is situated in the equatorial regions having the annual rainfall more than 140 cm. However, the tropical rain forest makes an important biome across the earth as a whole. This biome is found in Central America, the Amazon Basin, Orinocon Basin of South America, Central Africa, India and Southeast Asia.

Tropical rain forests have high rainfall, high temperature all year, and a great variety of vegetation. Plant life is highly diverse reaching up-to a framework of 200 species of trees per hectare. The warm, humid climate supports broad- leaved evergreen plants showing peculiar stratification into an upper storey and two or three understoreys.

The tallest trees make an open canopy, but the understoreyed plants block most of the light from the jungle floor. The climbers and lianas reach the highest level of the trees in search of light.

An enormous variety of animals lives in the rain forest, such as insects, lizards, snakes, monkeys and colorful birds. The ant eaters, bats, large carnivorous animals, and a variety of fish in the rivers are quite common. About 70-80 per cent of the known insects are found in tropical rain forests. Such rich animal diversity is linked to plant-animal interaction for pollination and dispersal of fruits and seeds.

Amazon Rainforest

Grassland:

Grasslands are also known as prairies and savannas. Grasslands are normally situated between a forest and a desert. In fact, grasslands surround every desert in Asia. Twenty-five percent of the Earth is covered by the grassland ecosystem. There is grassland on each continent with the exception of Antarctica.

Tropical and temperate are the two kinds of grasslands. Tropical grasslands experience warm weather all year long while temperate grasslands are warm part of the year and very cold during the other part. Grasslands are perfect for cropping and pasturing because its soil runs deep and is extremely fertile.

Tropical grasslands are located in the Southern Hemisphere while temperate grasslands are located in the Northern Hemisphere.

The dominant plant species comprise short and tall grasses. In tall-grasses prairies in the United States, important grasses are tall bluestem, Indian grass and slough grass. Short-grass prairies generally have blue grama grass, mesquite grass and bluegrass. Many grasses have long, well-developed root systems which enable them to survive limited rainfall and the effects of fire.

Animals that you can expect to find in a grassland ecosystem are zebras, lions, wolves, prairie dogs, and foxes.The grasslands in the United States are known as prairies and are considered to be temperate grasslands.

A grassland west of Coalinga, California

Colorado prairie

Desert:

Deserts cover about 20% of the Earth. The Sahara Desert is the largest desert in the desert ecosystem. It covers over 300 million square miles. Many desert animals tend to be nocturnal, sleeping during the day and coming out at night when the temperatures are more tolerable.

The desert biome is characterised by its very low rainfall, which is usually 25 cm per year or less. Most of this limited moisture comes as short, hard showers. Primarily the deserts of the world are located in the south-west U.S.A., Mexico, Chile, Peru, North Africa (Sahara desert), Asia (Tibet Gobi Thar) and central Western Australia. Deserts generally have hot days and cold nights, and they often have high winds.

The reason for the difference of temperature between day and night is due to the lack of water vapour in the air. Deserts are characterised by scanty flora and fauna. Desert organisms must meet some initial requirements if they are to survive. The plants must be able to obtain and conserve water.

In order to meet these requirements, many adaptations have been made by desert plants. Such adaptations are—reduced leaf surface area, which reduces evaporation from the plants, loss of leaves during long dry spell; small hairs on the leaf surfaces, and the ability to store large amount of water.

The examples of important desert plants are—yuccas, acacias, euphorbias, cacti, many other succulents and hardy grasses. Many of the small plants are annuals.

Animals also must meet the requirements of heat, cold and limited water. Many desert animals are nocturnal in habit, and are active mainly at night. Many reptiles and small mammals burrow to get away from the intense heat of midday. The other common desert animals are the herbivorous kangaroo, rat, ground squirrel, and jack rabbit.

The important predators are—coyotes, badgers, kit fox, eagles, hawks, falcons and owls. Ants, locusts, wasps, scorpions, spiders, insect-eating birds, such as swifts and swallows, seed-eating quails, doves and various cats are other common desert animals.

Sahara Desert

Freshwater ecosystems:

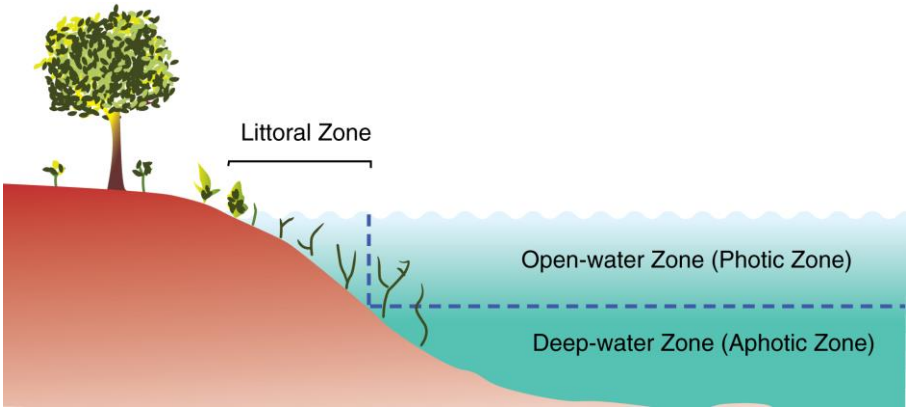
Freshwater ecosystems are a subset of Earth's aquatic ecosystems. They include lakes and ponds, rivers, streams, springs, bogs, and wetlands. They can be contrasted with marine ecosystems, which have a larger salt content. Freshwater habitats can be classified by different factors, including temperature, light penetration, nutrients, and vegetation.

Freshwater ecosystems can be divided into lentic ecosystems (still water) and lotic ecosystems (flowing water).

Limnology (and its branch freshwater biology) is a study about freshwater ecosystems. It is a part of hydrobiology.

Original attempts to understand and monitor freshwater ecosystems were spurred on by threats to human health (ex. *Cholera* outbreaks due to sewage contamination). Early monitoring focused on chemical indicators, then bacteria, and finally algae, fungi and protozoa. A new type of monitoring

involves quantifying differing groups of organisms (macroinvertebrates, macrophytes and fish) and measuring the stream conditions associated with them.
Freshwater ecosystems cover 0.78% of the Earth's surface and inhabit 0.009% of its total water. They generate nearly 3% of its net primary production. Freshwater ecosystems contain 41% of the world's known fish species.
There are three basic types of freshwater ecosystems:
Lentic: slow moving water, including pools, ponds, and lakes.
Lotic: faster moving water, for example streams and rivers.
Wetlands: areas where the soil is saturated or inundated for at least part of the time.
Lentic:
Lake ecosystems can be divided into zones. One common system divides lakes into three zones . The first, **the littoral zone**, is the shallow zone near the shore. This is where rooted wetland plants occur. The offshore is divided into two further zones, an open water zone and a deep water zone. In the open water zone (or photic zone) sunlight supports photosynthetic algae, and the species that feed upon them. In the deep water zone, sunlight is not available and the food web is based on detritus entering from the **littoral and photic zones**. Some systems use other names. The off shore areas may be called the **pelagic zone**, the photic zone may be called the limnetic zone and the aphotic zone may be called the **profundal zone**. Inland from the littoral zone one can also frequently identify a riparian zone which has plants still affected by the presence of the lake—this can include effects from windfalls, spring flooding, and winter ice damage. The production of the lake as a whole is the result of production from plants growing in the littoral zone, combined with production from plankton growing in the open water.



Wetlands can be part of the lentic system, as they form naturally along most lake shores, the width of the wetland and littoral zone being dependent upon the slope of the shoreline and the amount of natural change in water levels, within and among years. Often dead trees accumulate in this zone, either from windfalls on the shore or logs transported to the site during floods. This woody debris provides important habitat for fish and nesting birds, as well as protecting shorelines from erosion.
Two important subclasses of lakes are ponds, which typically are small lakes that intergrade with wetlands, and water reservoirs. Over long periods of time, lakes, or bays within them, may gradually become enriched by nutrients and slowly fill in with organic sediments, a process called succession. When humans use the watershed, the volumes of sediment entering the lake can accelerate this process. The addition of sediments and nutrients to a lake is known as eutrophication.
Ponds
Ponds are small bodies of freshwater with shallow and still water, marsh, and aquatic plants. They can be further divided into four zones: vegetation zone, open water, bottom mud and surface film. The size and depth of ponds often varies greatly with the time of year; many ponds are produced by spring flooding from rivers. Food webs are based both on free-floating algae and upon aquatic plants. There is usually a diverse array of aquatic life, with a few examples including algae, snails, fish, beetles, water bugs, frogs, turtles, otters and muskrats. Top predators may include large fish, herons, or alligators. Since fish are a major predator upon amphibian larvae, ponds that dry up each year, thereby killing resident fish, provide important refugia for amphibian breeding. Ponds that dry up completely each year are often known as vernal pools. Some ponds are produced by animal activity, including alligator holes and beaver ponds, and these add important diversity to landscapes.

Lotic:

The major zones in river ecosystems are determined by the river bed's gradient or by the velocity of the current. Faster moving turbulent water typically contains greater concentrations of dissolved oxygen, which supports greater biodiversity than the slow moving water of pools. These distinctions form the basis for the division of rivers into upland and lowland rivers. The food base of streams within riparian forests is mostly derived from the trees, but wider streams and those that lack a canopy derive the majority of their food base from algae. Anadromous fish are also an important source of nutrients. Environmental threats to rivers include loss of water, dams, chemical pollution and introduced species. A dam produces negative effects that continue down the watershed. The most important negative effects are the reduction of spring flooding, which damages wetlands, and the retention of sediment, which leads to loss of deltaic wetlands.

Wetlands

Wetlands, as the name signify, are wet ground, rather than standing water (aquatic habitat). Wetlands develop an organic soil profile with waterlogged soil. The saturation of soil with water, possess a problem for plants as anaerobic conditions are produced with little or no oxygen availability.

According to Ramsar Convention (1971) wetlands are "areas of marsh, fen, peat-land or water, whether natural or artificial, perma-nent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water, the depth of which at low tide does not exceed six meters."

Wetlands are divided according to the source and nature of the water which maintains it and thus can be broadly divided into the following four types:

i. Marine wetland ecosystems
ii. Flood land ecosystems
iii. Swamp and marsh ecosystems
iv. Bog ecosystems.

i. Marine Wetland Ecosystems:

a. Mangrove swamps:

Mangrove swamps are found along the coastlines in the tropical and subtropical regions. This area is flooded during every high tide, with marine or brackish waters. These coastal wetlands are densely vegetated with thickets of man-grove trees. About 70 species of mangroves are found all over the world.

The most important genera being Rhizophora, Sonneratia and Avicennia. Mangrove vegetations are well-adapted to the salty conditions of the intertidal zone as they have mechanisms to prevent high concentrations of salt from entering the roots and can excrete the excess salt from their leaves and even can drop their leaves.

A number of animal life has been wit-nessed within the mangrove such as fiddler crabs (*Uca* sp.), mudskippers (*Periophthalamus* sp.). A number of fishes are washed into this area by the tides which attract a number of birds. There are many detritivores and some major carnivores like alligators, crocodiles, tigers (*Panthera tigris*) etc.

b. Salt marshes:

Mangrove forests do not grow in higher latitudes, as the trees cannot tolerate cold weather. In such places, salt marshes grow. The salt marshes like the mangrove swamps are under the influence of sea, where salt water enters at the time of high tide. Salt marshes are made up of patches of low vegetation separated by tidal creeks and tend to develop in sheltered intertidal regions where wave action is not too strong.

The vegetation in salt marshes is domi-nated by grasses such as *Spartma* and rushes (*juncus* sp.). The lower lying areas where pools have formed, having high salt concen-tration, a very succulent plant, called glass wort (*Salicornia europaea*) grows. During high rainfall, the salt are washed away from the marsh areas and are colonised by different types of plant species such as sea lavender (*Limonium* sp.) and arrow grass (*Triglochin* sp.).

ii. Flood-Land Ecosystems:

This group of wetland ecosystem obtains their water from rivers. During rainy season, the river overflows its banks and the flood-lands are flooded deeply with water. Being seasonal they may dry

out during summer. Flood-lands occur in lowland flat-bottomed valley through which a river meanders.

Normally a mature river follows a channel through which the river flow builds up at the side into sandy banks called levees. The valley gets flooded when the river overflows. There are permanent wet-land areas which form when the river takes a new path on the valley floor, called oxbow lake. A flood plain may have seve-ral oxbow lakes in various stages of infill.

As the river floods the valley, clay, silt and mineral are carried and deposited on the flood plain. The larger particles settles on the bank to form levees, while the smaller parti-cles are carried further away and are left behind as the water recedes. This makes the soil of the flooded plain rich in nutrient.

In temperate flood-land the natural vege-tation is mixed deciduous forest. In tempe-rate North America, the areas flood all year round, have small number of specialized swamp trees like swamp cypress (*Taxodium distichum*) and by tupels (*Nyssa aquatica*).

In areas where the river water floods for more than half the year, the above species along with specialist oaks (*Quercus* sp.) and ashes (*Fraxinus* sp.) are found. In areas where flooding occurs less than 50%, the swamp species disappears and larger variety of oaks and elms (*Ulmus* sp.) take their place. These mixed woodlands are invaded by rich fauna of amphibians, reptiles, birds and mammals.

iii. Swamp and Marsh Ecosystems:

These wetlands are found in areas where water runs off the surrounding land or drains and collects or where ground water lies close to the surface or where such areas are fed by rivers and streams. Such areas are flooded all-round the year and are variable in size and form, depth of soil and plant community structure. In those areas where trees are the dominant vegetation, it forms swamps, while those which have large open areas of grasses and reeds form marshes.

a. Swamps:

Swamps are wooded wet-lands, dominated by trees that are similar to those found in the very wettest areas of floo-ded river valleys. The most famous swamp areas occur in Florida (USA). The dominant free species are the swamp cypress (*Taxodium* sp.) and water tupelo. Several species in the swamp have knee roots that protrude out of the water.

These pneumatophores may act just like the ones present in man-grove swamps. Carbon dioxide has been reported to leave these knee roots. However, there is no report that oxygen is taken in. Swamp trees produce seeds but unlike the mangrove trees, they germinate and grow in dry conditions only. This means that vege-tation of swamp trees can only take place when the swamp temporarily dries.

b. Marshes:

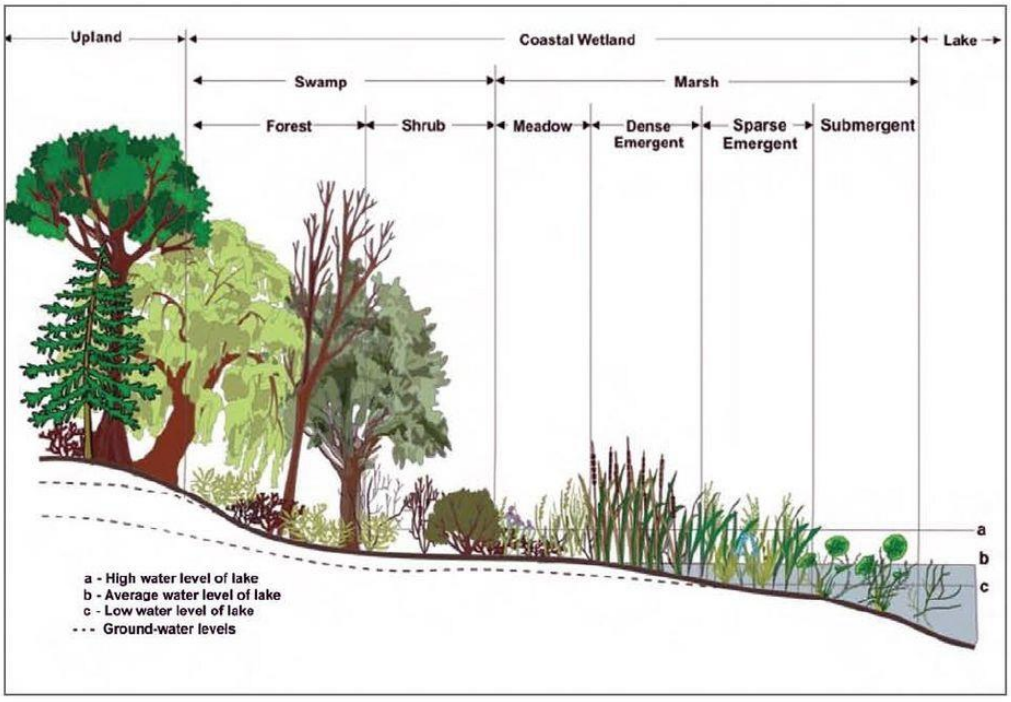
Marsh is dominated by graminoids (grass like plants) that include not only grasses (Poaceae) but may also be cattails, sedges (Cyperaceae), rushes or other plants with more or less grass like leaves. Marshes are common in temperate zones and may occur around a lake or pond or along-side a river or away from any water bodies in areas areas where the water table is high.

The movement of ground water runoff and the addition of stream water makes the marshes nutrient rich and the pH is usually near neutral to slightly alkaline. The depth of the standing water in marshes may be up to a couple of metres or generally lesser. At greater water depths only floating leaved or sub-merged vegetations are found.

The species which live in marshes often have sharp-edged or tough leaves (*Cladium, Phragmites* etc.). These tough leaves have probably evolved to deter large herbivores like swamp rhinoceros and straight tusked elephants in the Pliocene and Pleistocene.

These animals have become extinct but the tough grass species have been left relatively untouched. In marshes where the soil surface becomes dry by the middle of summer, the types of vegetation generally found are the sedges in the genus *Carex*.

Marshes are important breeding sites for frogs and toads. Bird populations are high about 300 to 400 pairs per 40 hectare. Some important species are rails, bitterns, ducks, marsh wren, red-winged black bird and swamp sparrow. Sand hill cranes feeds in drier areas but build their nest in marshes. The mammalian fauna includes musk rat and mink.



iv. Bog Ecosystems:

Bog are wetlands that receive water only from rainfall (not from streams, rivers or ground-waters) and this sole source of water greatly affects its nutrient content. Unlike river and ground water, rainwater has very little nutrients. Moreover, whatever nutrients may be present get leached as water is drained through the soil profile.

Bog is characterised by the accumulation of peat. Peat filled depressions are common. The dominant species in bogs are mosses in the genus *Sphagnum* . Different species of Sphagnum are found in bogs, each with slightly different water requirements.

Sphagnum bog develops where rainfall is high and temperature is cool. Bogs generally occur in the temperate and boreal regions. The bog may slowly grow up to form a huge dome of peat called raised bog . In areas where rainfall is high (about 1,000 mm a year), blanket bog develops.

Fen:

Fen is an "alkaline bog", with a pH which is slightly alkaline. So fens are mineral-rich peat-land. Fens usually occur at the base of slopes and are minerotrophic (mineral fed).

Fens do not have Sphagnum, although other bog plants like pitcher plant may be prominent. Sedges are usually common and the flora is rich in bogs. Some characteristic fen plants are swamp milkweed, marsh bell- flower, Kalm's lobelia, grass-of-parnassus, Ridell's goldenrod, dwarf birch and shrubby cinquefoil.

Sometimes tamarck invades fen and may be replaced by white cedar. Cedar swamps have few distinctive bird species and are a favoured winter habitat for white-tailed deer.

Coastal and Marine Ecology:

Marine Ecology is the scientific study of marine-life habitat, populations, and interactions among organisms and the surrounding environment including their abiotic (non-living physical and chemical factors that affect the ability of organisms to survive and reproduce) and biotic factors (living things or the materials that directly or indirectly affect an organism in its environment).

Marine ecology is a subset of the study of marine biology and includes observations at the biochemical, cellular, individual, and community levels as well as the study of marine ecosystems and the biosphere.

What is Marine Ecosystem?

- Marine ecosystems are among the largest of Earth's aquatic ecosystems.
- Examples include salt marshes, intertidal zones, estuaries, lagoons, mangroves, coral reefs, the deep sea, and the sea floor.

- Marine waters cover two-thirds of the surface of the Earth. Such places are considered ecosystems because the plant life supports the animal life and vice versa.. Marine ecosystems are essential for the overall health of both marine and terrestrial environments.
- Marine ecosystems are essential for the overall health of both marine and terrestrial environments.
- Marine ecosystems are located mainly in **coastal areas and in the open ocean**.
- Organisms that live in coastal areas adapt to changes in **water level and salinity**.
- Organisms that live in the open ocean adapt to changes in **temperature and the amount of sunlight and nutrients available**.

Types of Marine Ecosystem:

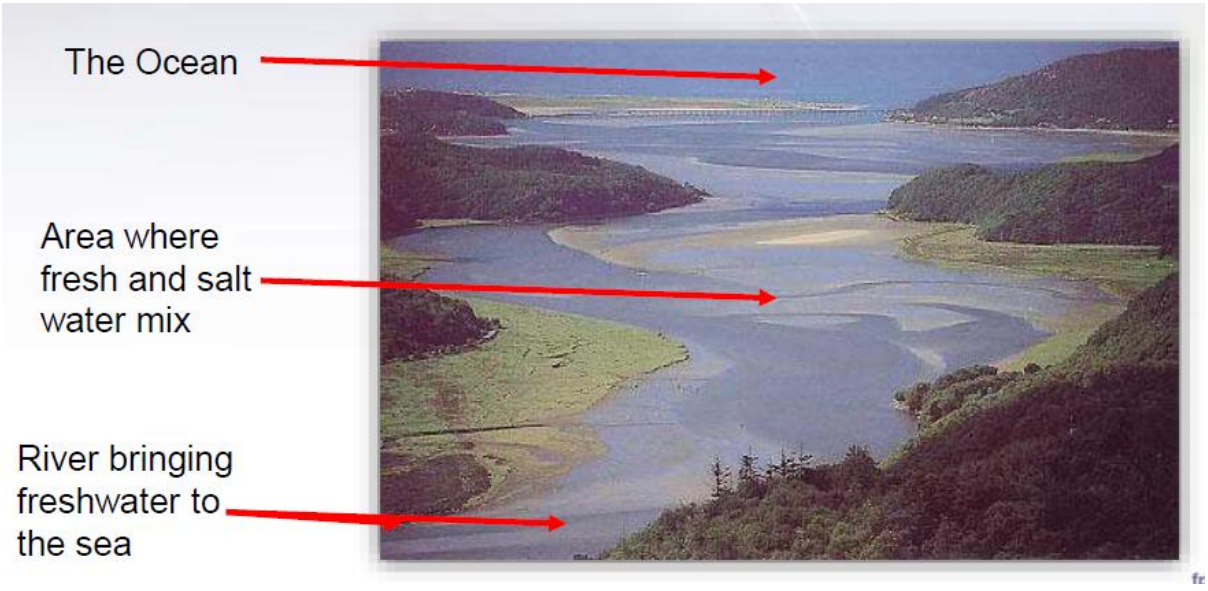
Coastal Wetlands:

Coastal land areas that are covered by salt water for all or part of the time are known as coastal wetlands. Coastal wetlands provide habitat and nesting areas for many fish and wildlife. They also absorb excess rain, which protects them from flooding, they filter out pollutants and sediments, and they provide recreational areas for boating, fishing, and hunting.

Estuaries:

An estuary is an area where fresh water from rivers mixes with salt water from the ocean. As the two bodies meet, currents form and cause mineral rich mud with many nutrients to fall to the bottom making in available to producers. Estuaries are very productive because they constantly receive nutrients from the river and ocean while the surrounding land protects the estuaries from the harsh force of ocean waves. Water is brackish: a mixture of freshwater and saltwater.

Very nutrient rich ecosystems lead to high productivity and high biodiversity. Fast-moving rivers and waves carry nutrient-rich particles. Sediment settles out in the estuary when the water slows down. It accumulates on the bottom.



Plants and Animals of Estuaries:

Estuaries support many marine organisms because they receive plenty of light for photosynthesis and plenty of nutrients for plants and animals. The light and nutrients support large populations of rooted plants as well as plankton. Plankton in turn provides food for fish, which can then be eaten by larger animals such as dolphins. Oysters and clams live anchored to rocks and feed by filtering plankton from the water. Organisms that live in estuaries are able to tolerate variations in salinity because the salt content of the water varies as fresh water and salt water mix when tides go in and out. Estuaries also provide protected harbors, access to the ocean, and connection to rivers. As a result, many of the largest ports have been built on estuaries. Six of the ten largest urban areas, including New York have been built on estuaries.

Salt marsh

Salt marshes are defined as "coastal wetlands that are flooded and drained by salt water brought in by the tides".These marshy grounds are able to prevent flooding as well as help maintain water quality by absorbing rainwater and runoff that comes through the area

- **Salt marshes** are maritime habitats characterized by grasses, sedges, and other plants that have adapted to continual, periodic flooding and are found primarily throughout the temperate and subarctic regions.
- The salt marsh supports a community of clams, fish, aquatic birds, crabs, and shrimp.
- Salt marshes, like other wetlands, also **absorb pollutants** to help protect inland areas.

Mangrove swamps:

Mangrove swamps are tropical or subtropical marine swamps that are characterized by the abundance of low to tall mangrove trees. The swamps help protect the coastline from erosion and reduce the damage from storms. They also provide a home for about 2,000 animal species. Mangrove swamps have been filled with waste and destroyed in many parts of the world.

Rocky and Sandy Shores

Rocky shores have many more plants and animals than sandy shores do because the rocks provide anchorage for seaweed that animals can live on. Sandy shores dry out when the tide goes out, and many organisms that live between sand grains eat the plankton left stranded on the sand. A barrier island is a long ridge of sand or Narrow Island that lies parallel to the shore and helps protect the mainland.

Coral reefs:

Coral reefs are limestone ridges found in tropical climates and composed of coral fragments that are deposited around organic remains. Thousands of species of plants and animals live in the cracks and crevices of coral reefs, which makes coral reefs among the most diverse ecosystems on Earth. Corals are predators that use stinging tentacles to capture small animals, such as zooplankton, that float or swim close to the reef. Coral reefs form in clear, warm coastal waters of the tropics and subtropics. These stunningly beautiful natural wonders are

among the world's oldest , most diverse, and most productive ecosystems. In terms of biodiversity, they are the marine equivalents of tropical rain forest.
Require two things: warm temperatures and sunlight Found between 30°N and 30°S of the equator.
Corals are animals that belong to the phylum Cnidaria, the stinging-celled animals such as jellyfish and hydra.

Coral polyps resemble small sea anemones with tentacles that can sting and paralyze prey.
Coral polyps slowly build reefs by secreting a protective crust of limestone ($CaCO_3$) around their soft bodies. When the polyps die, their empty crust remains behind as a platform for more reef growth. The resulting elaborate network of crevices, ledges and holes serves as $CaCO_3$ condominiums for a variety of marine animals.
Corals live in a symbiotic relationship with algae called zooxanthellae; the algae are photosynthetic and give the coral food and oxygen; the algae get carbon dioxide and nutrients from the nitrogenous wastes of the coral.
Although coral reefs only occupy about 0.1% of the world's ocean area, they provide numerous ecological and economic services:
1. They help moderate the Earth's temperature by removing carbon dioxide from the atmosphere.
2. 2. Act as natural barriers that help protect 15% of the world's coastline.
3. They provide habitat for as many as 1 million species
4. Molecules derived have been used to develop antibiotics and drugs for certain cancers and HIV.

Coral reefs destruction:
Coral reefs are vulnerable to damage because they grow slowly and disrupted easily.
1. Coral bleaching – when temperatures go above normal, the zooxanthellae (algae) in the coral can be rejected, the coral turns a whitish color and dies .Temperature should only range to 18-30 degrees Celsius.
2. Water Acidity – increasing levels of Carbon dioxide in the atmosphere and ocean which makes polyps harder to create $CaCO_3$
3. Dynamite Fishing
4. Moro Ami
5. Land Development/ Reclamation Activities
6. Loss of Mangrove Forest
7. Fish and Coral Trade
8. Exposure to UV radiation due to Ozone Depletion.
9. Deep Sea Bottom Trawling

Lagoons:
The National Geographic Society defines lagoons as a "shallow body of water protected from a larger body of water (usually the ocean) by sandbars, barrier islands, or coral reefs." There are two different types of lagoons: coastal lagoons and atoll lagoons.
Intertidal zones:
Intertidal zones are the areas that are visible during low tide and covered up by saltwater during high tide. In these zones simple organisms can be found in tide pools. These areas also have a higher salinity because salt is left poration has occurred.
Deep sea and sea floor

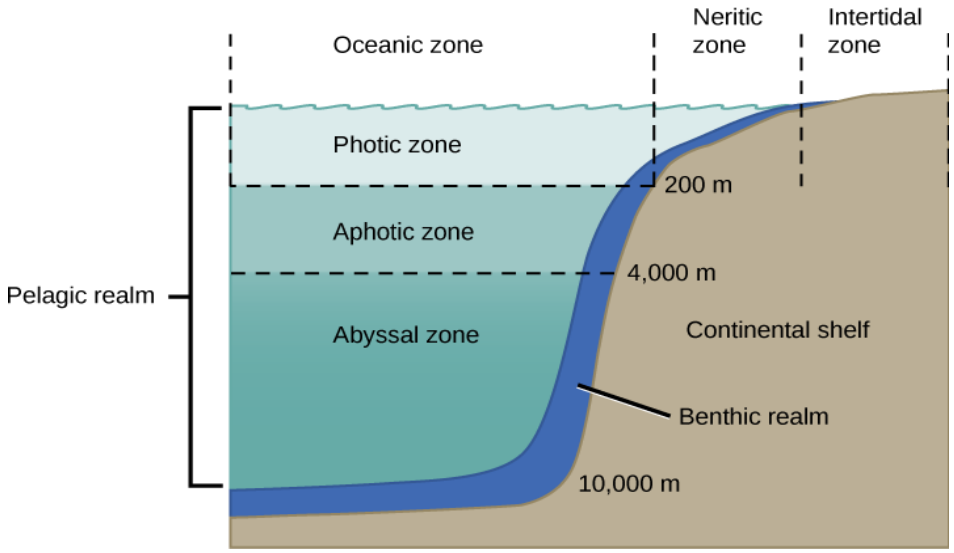
The deep sea conquers up to 95% of the space occupied by living organisms. Combined with the sea floor (or benthic zone), these two areas have yet to be fully explored and have their organisms documented making these ecosystems some of the hardest to understand by scientists

Oceans represent the largest and the most diverse type of ecosystem.Oceans can be divided into numerous regions dependimg on the physical and biological conditions.Oceans environment is generally specified on the basis of light penetration [photic and aphotic zones], distance from shore and water-depth [littoral and intertidal, neritic and oceanic zones] and whether it is open water [pelagic zone neither close to the bottom nor near the shore] or bottom [benthic and abyssal zones].

- **The Littoral Zone** [Or Inter-Tidal Zone] is the shoreline between land and open sea.
- **The Neritic Zone** is a continental shelf ,extending outward to its edge to a water depth of about 200m.
- The open ocean is called the **Pelagic Zone** and the area underneath the pelagic zone is called the **Benthic Zone**, or deep-sea. The deepest part of the ocean is called **Abyssal Zone**.

The Pelagic zone which includes all open ocean categories can be subdivided into further regions categorized by light abundance:

- To about 200m is the **Epipelagic Zone** [Photic Zone].This is the region where the photosynthesis most commonly occurs and therefore contains the largest biodiversity in the ocean.
- From 200m to 1000m is the **Mesopelagic Zone**, where little light penetrates.
- Below the mesopelagic zone is the **Bathypelagic Zone**, where darkness is virtually complete.



Plants and Animals of Oceans:

In the open ocean, phytoplankton grows only in areas where there is enough light and nutrients, resulting in one of the least productive of all ecosystems. The sea's smallest herbivores are zooplankton, including jellyfish and tiny shrimp, which live near the surface with the phytoplankton they eat. Fish feed on the plankton as do marine mammals such as whales. The depths of the ocean are very dark, so most food at the ocean floor consists of dead organisms that fall from the surface. Decomposers, filter feeders, and the organisms that eat them live in the deep areas of the ocean.

Overall, the type of organisms that may be found in the layers of the ocean at various depths is dependent on available sunlight.

Major Biogeographical Zones of India

Biogeography is the study of the distribution of species and ecosystems in geographic spaceand through geological time. Organisms and biological communities often vary in a regular fashion along geographic gradients of latitude, elevation, isolation and habitat area.

Phytogeography is the branch of biogeography that studies the distribution of plants.

Biogeographic classification of India is the division of India according to biogeographic characteristics. Biogeography is the study of the distribution of species (biology), organisms, and ecosystems in geographic space and through geological time. There are ten biogeographic zones in India

Sl. No.	Bio-geographic zones	Biotic province
1	Trans-Himalaya	Ladakh mountains, Tibetan Plataeu
2	Himalaya	North-West, West, Central & East Himalayas
3	Desert	Thar, Kutch
4	Semi-arid	Punjab plains, Gujarat Rajputana
5	Western Ghats	Malabar plains, Western Ghats
6	Deccan Peninsula	Central Highlands, Chotta Nagpur, Eastern Highlands, Central Plateau, Deccan South
7	Gangetic Plains	Upper & Lower Gangetic Plains
8	Coast	West & East Coast, Lakshadweep
9	North-East	Brahmaputra Valley, North East Hills
10	Islands	Andaman & Nicobar Islands

1. The Trans-Himalayan Region:
The Himalayan ranges immediately north of the Great Himalayan range are called the Trans-Himalayas. This area is very cold and arid (4,500 Œ 6,000 mts. above msl). The Trans-Himalayan region with its sparse vegetation has the richest wild sheep and goat community in the world. The snow leopard is found here, as is the migratory black-necked crane.The only vegetation is a sparse alpine steppe. Extensive areas consist of bare rock and glaciers

2. The Himalayan Region:
The extremely high altitude gradient results in the tremendous biodiversity of the Himalayan region. Flora and fauna vary according to both altitude and climatic conditions: tropical rainforests in the Eastern Himalayas and dense subtropical and alpine forests in the Central and Western Himalayas. The forests are very dense with extensive growth of grass and evergreen tall trees. Oak, chestnut,

conifer, ash, pine, deodar are abundant in Himalayas. There is no vegetation above the snowline. Several interesting animals live in the Himalayan ranges. Chief species include wild sheep, mountain goats, ibex, shrew, and tapir. Panda and snow leopard are also found here. The lower levels of the mountain range support many types of orchids. On the eastern slopes, rhododendrons grow to tree height.

3. The Indian Desert:

The natural vegetation consists of tropical thorn forests and tropical dry deciduous forests, sandy deserts with seasonal salt marshes are found in the Kutch region. Typical shrubs are found growing on sand dunes. Sewan grass covers extensive areas.

4. The Semi-Arid Region:

The semi-arid region in the west of India includes the arid desert areas of Thar and Rajasthan extending to the Gulf of Kutch and Cambay and the whole Kathiawar peninsula. The natural vegetation consists of tropical thorn forests and tropical dry deciduous forests, moisture forests (extreme north) and also mangroves. Thorny shrubs, grasses and some bamboos are present in some regions. The sandy plains have a few scattered trees of *Acacia* and *Prosopis*. A few species of xerophytic herbs and some ephemeral herbs are found in this semi-arid tract. Birds, jackals, leopards, eagles, snakes, fox, buffaloes are found in this region.The rocky habitats are covered by bushes of Euphorbia while species of Salvadora and Tamarix occur mainly near saline depressions.

5. The Western Ghats:

The mountains along the west coast of peninsular India are the Western Ghats, which constitute one of the unique biological regions of the world. The Western Ghats extend from the southern tip of the peninsula (8°N) northwards about 1600 km to the mouth of the river Tapti (21°N).They cover only 5% of India's land surface but are home to more than about 4,000 of the country's plant species of which 1800 are endemic. The monsoon forests occur both on western margins of the ghats and on the eastern side where there is less rainfall. This zone displays diversity of forests from evergreen to dry deciduous.

The mountains rise to average altitudes between 900 and 1500 m above sea level, intercepting monsoon winds from the southwest and creating a rain shadow in the region to their East.

The varied climate and diverse topography create a wide array of habitats that support unique sets of plant and animal species. Apart from biological diversity, the region boasts of high levels of cultural diversity, as many indigenous people inhabit its forests.

The Western Ghats are amongst the 25 biodiversity hot-spots recognized globally. These hills are known for their high levels of endemism expressed at both higher and lower taxonomic levels. Most of the Western Ghat endemic plants are associated with evergreen forests.

The region also shares several plant species with Sri Lanka. The higher altitude forests were, if at all, sparsely populated with tribal people. Rice cultivation in the fertile valley proceeded gardens of early commercial crops like areca nut and pepper. The original vegetation of the ill-drained valley bottoms with sluggish streams in elevations below 100m would be often a special formation, the Myristica swamp.

Expansion of traditional agriculture and the spread of particularly rubber, tea, coffee and forest tree plantations would have wiped out large pockets of primary forests in valleys. The Western Ghats are well known for harboring 14 endemic species of caecilians (i.e., legless amphibians) out of 15 recorded from the region so far.

6. The Deccan Peninsula:

The Deccan Peninsula is a large area of raised land covering about 43% of India's total land surface. It is bound by the Satpura range on the north, Western Ghats on the west and Eastern Ghats on the east. The elevation of the plateau varies from 900 mts. in the west to 300 mts. in the east. There are four major rivers that support the wetlands of this region which have fertile black and red soil. Large parts are covered by tropical forests. Tropical dry deciduous forests occur in northern, central & southern part of the plateau. The eastern part of the plateau in Andhra Pradesh, Madhya Pradesh & Orissa have moist deciduous forests.

7. The Gangetic Plain:

The Gangetic plain is one of India's most fertile regions. The soil of this region is formed by the alluvial deposits of the Ganges and its tributaries. The Gangetic plains stretching from western Uttar

Pradesh to Bihar & West Bengal are mostly under agriculture. The large forest area is under tropical dry deciduous forest and the southeastern end of the Gangetic plain merges with the littoral and mangroves regions of the Sunderbans.

Topographic uniformity, except in the arid Western Rajasthan is a common feature throughout these plains. The plain supports some of the highest population densities depending upon purely agro-based economy in some of these areas. The trees belonging to these forests are teak, sal, shisham, mahua, khair etc.

8. The Coastal Region:

India has a coastline extending over 7,516. 4 km. The Indian coasts vary in their characteristics and structures. The west coast is narrow except around the Gulf of Cambay and the Gulf of Kutch. In the extreme south, however, it is somewhat wider along the south Sahyadri.

The backwaters are the characteristic features of this coast. The east coast plains, in contrast are broader due to depositional activities of the east-flowing rivers owing to the change in their base levels.

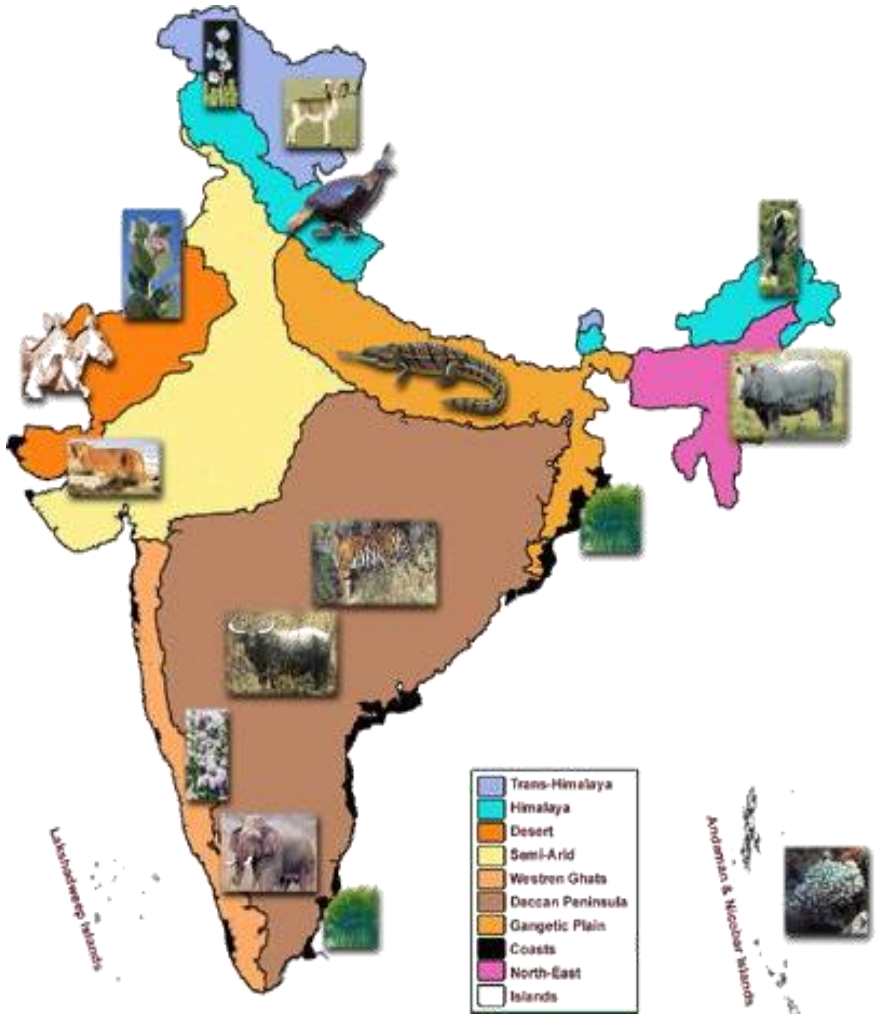
Extensive deltas of the, Godavari, Krishna and Kaveri are the characteristic features of this coast. Mangrove vegetation is characteristic of estuarine tracts along the coast for instance, at Ratnagiri in Maharashtra

Larger parts of the coastal plains are covered by fertile soils on which different crops are grown. Rice is the main crop of these areas. Coconut trees grow all along the coast.

Coconut and rubber are the main vegetation of coastal area. The main states of coastal areas are Gujarat, Maharashtra, Goa, Karnataka, Kerala, West Bengal, Odisha, Andra pradesh, Tamil nadu and Puducherry. Animal species include dugong, dolphins, crocodiles and avifauna

9. The North-East:

Biological resources are rich in this zone. The tropical vegetation of the northeast is rich in evergreen & semi-evergreen rain forests, moist deciduous monsoon forests, swamps and grasslands.



10. The Indian Islands:
It is a group of 325 islands: Andaman to the north and Nicobar to the south. Rainfall is heavy, with both Northeast and Southwest monsoons. Many unique plants and animals are found here. About 2,200 species of angiosperms are found here of which 200 are endemic. The Andaman & Nicobar Islands have tropical evergreen forests and tropical semi-evergreen forests as well as moist deciduous forests, littoral and mangrove forests.

12. Biodiversity: Concept, kinds/ levels, importance, methods of study, protection from depletion; Mega - diversity and Hotspots

What is biodiversity?
The term biological diversity or biodiversity refers to the variety of life forms and habitats found in a defined area.
UNEP (1992) defines it"**as the variety and variability of all animals, plants and micro-organisms and the ecological complexes of which they are a part"**.
Biodiversity is defined as "the intrinsically-inbuilt plus the externally-imposed variability in and among living organisms existing in terrestrial, marine and other ecosystem at a specific period of time".
The term biodiversity was coined by **W.G. Rosen (1985)**.
Diversity characterizes most living organisms, the our earth supports something like 5 to 10 million species of plants and animals (IUCN, 1980) which have been the result of 3 billion years of evolution involving mutation, recombination and natural selection.
Article 2 of the **CBD defines** "Biological diversity means the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes biological diversity within species and ecosystems".
• "Biodiversity includes assemblages of plant, animals and micro-organisms, their genetic variability expressed and populations, their habitats, ecosystems and natural areas, the mosaic of which constitutes the landscape which gives the richness to the natural environment" (Denny, 1997).
• For the assessment of global biodiversity, it is defined as the total diversity and variability of living organisms and of the systems of which they are a part. This covers the total range of variation and variability among systems and organisms at the bioregional, landscapes, ecosystem, habitat (levels), and organismal level down to species, populations, individuals and genes (genetic diversity) [Heywood, 1995].
According to Edward Wilson "Biodiversity is the combined diversity at all the levels of biological organization."
Biodiversity is an umbrella term covering diversity at genetic, species and ecosystem level.
The convention on Biological Diversity defines biodiversity as "The variability among living organisms form all sources including, interalia terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are a part; this includes diversity within species, between species and of ecosystems."
History of Biodiversity
The term 'diversity' is not new, rather has a long history, but 'biological diversity' came into use in scientific literature only in the 1980s. The term was first coined by Lovejoy who, however, did not provide any formal definition to it, but considered it as only the number of species (Lovejoy, 1980). Rosen in 1985 used the term 'biodiversity' in the first planning conference of the 'National Forum on Biodiversity', Washington D. C., on Sept. 1986. Wilson (1988) edited the proceedings of the conference titled Biodiversity, and this popularized the concept.
Convention on Biological Diversity in June 1992, constituted a historical commitment by all (many) nations of the world. For the first time, biodiversity was comprehensively addresses in this global treaty. At the same time the genetic diversity was considered and conservation of biodiversity was accepted as the common concern for the cause of human welfare (Gatson, 1998).

How Many Species are there on Earth and How Many in India?

Since there are published records of all the species discovered and named, we know how many species in all have been recorded so far, but it is not easy to answer the question of how many species there are on earth. According to the IUCN (2004), the total number of plant and animal species described so far is slightly more than 1.5 million, but we have no clear idea of how many species are yet to be discovered and described. Some extreme estimates range from 20 to 50 million, but a more conservative and scientifically sound estimate made by Robert May places the global species diversity at about 7 million.

Although India has only 2.4 per cent of the world's land area, its share of the global species diversity is an impressive 8.1 per cent. That is what makes our country one of the 12 mega diversity countries of the world. Nearly 45,000 species of plants and twice as many of animals have been recorded from India. How many living species are actually there waiting to be discovered and named? If we accept Robert May's global estimates, only 22 per cent of the total species have been recorded so far.

Applying this proportion to India's diversity figures, we estimate that there are probably more than 1,00,000 plant species and more than 3,00, 000 animal species yet to be discovered and described.

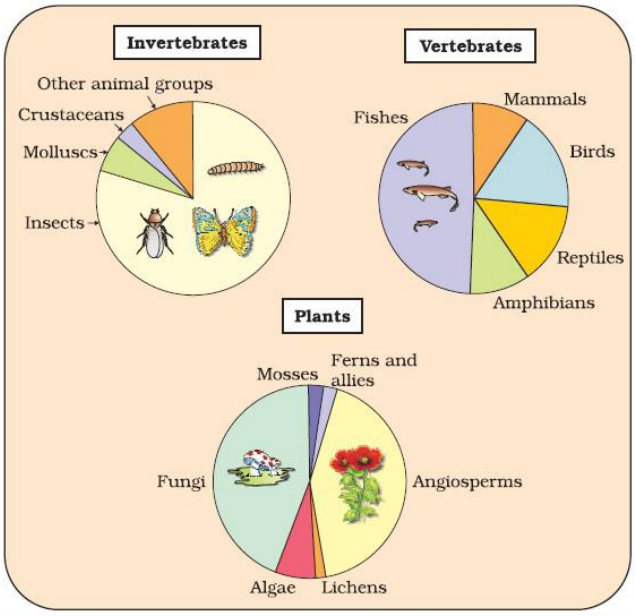


Figure 15.1 Representing global biodiversity: proportionate number of species of major taxa of plants, invertebrates and vertebrates

Table 15.1. Distribution of species in some major groups of flora and fauna in India.

Group-wise species Distribution			
Plants	**Number**	**Animals**	**Number**
1. Bacteria	850	8. Lower groups	9979
2. Fungi	23,000	9. Mollusca	5042
3. Algae	2500	10. Arthropoda	57,525
4. Bryophytes	2564	11. Pisces (Fishes)	2546
		12. Amphibia	428
5. Pteridophytes	1022	13. Reptiles	1228
6. Gymnosperms	64	14. Birds	204
7. Angiosperms	15,000	15. Mammals	372

Levels of biodiversity

Biodiversity is commonly considered at three different levels:

1. Within species (intraspecific) diversity; usually measured in terms of genetic differences between individuals or populations.
2. Species (interspecific) diversity, measured as a combination of number and evenness of abundance of species.

3. Community or ecosystem diversity, measured as the number of different species assemblages.
Biodiversity, therefore, is usually considered at three hierarchical levels i.e. **Genetic, Species and Community and Ecosystem levels**.

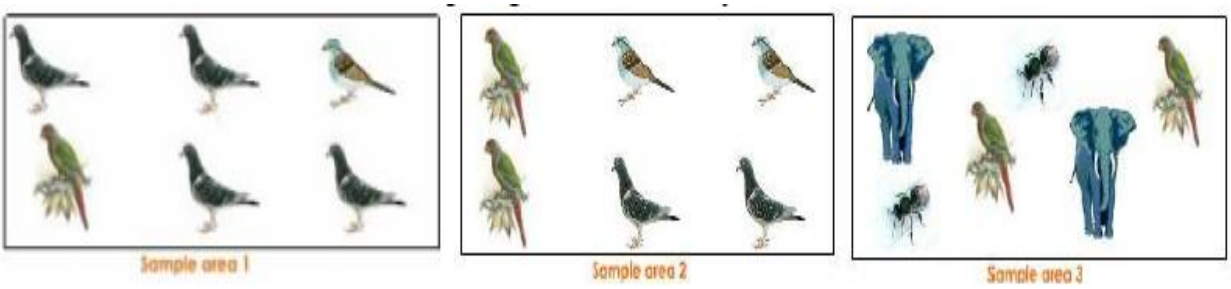
1. Genetic diversity:

Genetic diversity refers to any variation in the nucleotides, genes, chromosomes, or whole genomes of organisms. This is the "fundamental currency of diversity" (Williams and Humphries, 1996) and the basis for all other organismal diversity.

- Genetic diversity is the sum total of genetic information, contained in the genes of individuals of plants, animals and microorganisms that inhabit the earth.
- It is needed by any species in order to maintain reproductive vitality, resistance to disease and the ability to adapt to changing conditions.
- It enables a population to adapt to its environment and to respond to natural selection.
- The amount of genetic variation is the basis of speciation.
- Genetic diversity within a species often increases with environmental variability.
- Such genetic variability has made it possible to produce new breed of crops plants and domestic animals, and in the world allowed species to adapt to changing conditions.

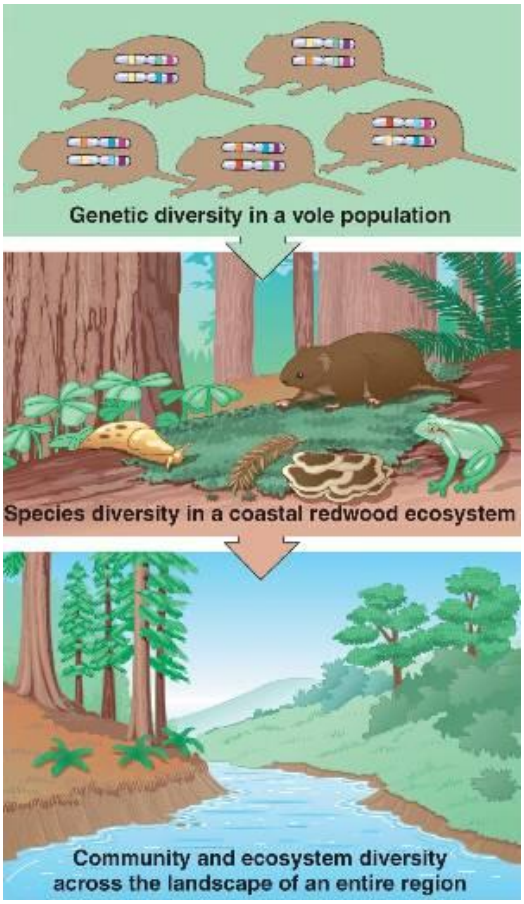
2. Species diversity:

Species diversity refers to variety of species in a region. Number of species per unit area is called species richness. Eveness or equitability differs due to difference in number of individuals in an area.
With increase in area, number of species increase. Usually, species diversity increases, if species richness is higher. Somehow, number of individuals among species may differ. This may lead to differences in evenness or equatibility. This also results to change in diversity. Some examples are as in the following figure:
Suppose in an area -1, there are three species of bird. Out of them two species bear only one bird each. Third species has four birds.
In area -2, there are 3 species each having 2 birds. This area represents greater evenness. It is more diverse than area.
In area-3, an insect, a mammal and a bird is also present



Ecological (Ecosystem) Diversity:

The diversity of ecological complexes or biotic communities found in a given area. Indian ecosystem diversity is described at each of the three levels (biogeographical region, biotic province and biome). Largest of identified ecosystems has been the biogeographical zone. The vast area covered by biogeographical zone contains a wide diversity of smaller units called biotic provinces.Finally, within each biotic province, various kinds of biomes are distinguished. Biome classification broadly follows commonly used terminology, distinguishing between forests, grasslands, wetlands, deserts, and other such ecosystems on the basis of their physical appearance and dominant biotic or abiotic element. The enormous range of terrestrial and aquatic environments on earth has been classified into number of ecosystems.Few examples are:(i) Tropical rain forests,(ii) Grasslands, and (iii) Wetlands.Ecosystems differ not only in the species composition of their communities but also in their physical structures (including the structures created by organisms). Some of the world's richest habitats are tropical moist forests. Although they cover only 7% of the world's surface, these areas contain atleast 50%, and possibly upto 90% of all plant and animal species.



Measuring Biodiversity:

There are various mathematical ways of measuring biodiversity, which calculate the number of species diversity in different regions. The measure of diversity of species is also known as species richness.

These are as follows:

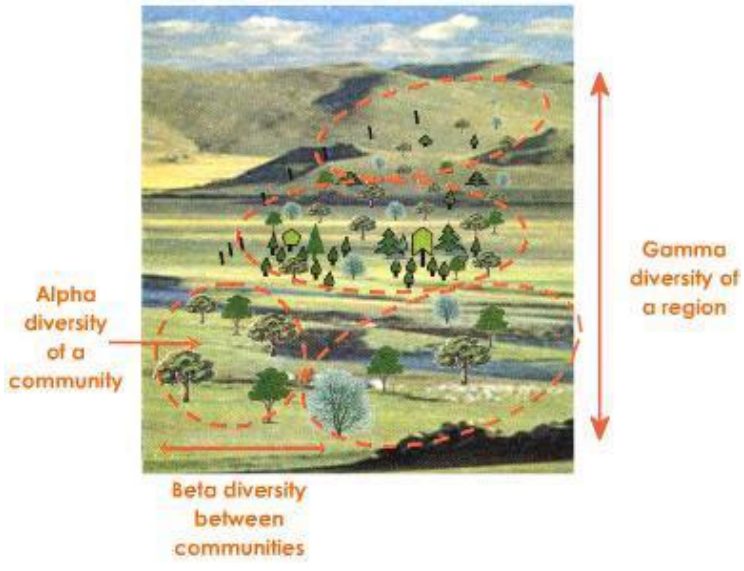
a. Alpha Diversity (with in community diversity):

It represents number of species in a given habitat. It represents the diversity of organisms sharing the same community/habitat. A combination of equitability/evenness and species richness is used to know that diversity prevalent within community or habitat.

Differences can be observed in species composition of communities along different environmental ranges like moisture gradient, altitudinal gradients, etc. Beta diversity will be always high with increase in heterogeneity in habitats in a region or with higher dissimilarity found between communities.

b. Beta Diversity (between community diversity):

The rate of turnover or replacement of species while moving from one habitat to another within a given geographical area. For instance the differences in species composition between a coral reef and the adjoining intertidal zone would be termed as beta diversity.



c. Gamma Diversity:

This term is used for the rate of turnover or replacement of species between similar habitats in different geographical areas. For example, the differences in species;

composition between the coral reef in the Gulf of Kutch and in the Andaman Islands would be called as gamma diversity. Diversity of habitats is the total landscape or geographical area is called gamma diversity.

Importance:

The biodiversity found on Earth today is the result of approximately 3.5 billion years of evolution. Until the emergence of humans, the earth supported more biodiversity than any other period in geological history. However, since the dominance of humans, biodiversity has begun a rapid decline, with one species after another suffering extinction.

The maintenance of biodiversity is important for the following reasons:

1. Ecological stability

 Each species performs a particular function within an ecosystem. They can capture and store energy, produce organic material, decompose organic material, help to cycle water and nutrients throughout the ecosystem, control erosion or pests, fix atmospheric gases, or help regulate climate.

 Ecosystems provide support of production and services without which humans could not survive. These include soil fertility, pollinators of plants, predators, decomposition of wastes, purification of the air and water, stabilisation and moderation of the climate, decrease of flooding, drought and other environmental disasters.

 Research show that the more diverse an ecosystem the better it can withstand environmental stress and the more productive it is. The loss of a species thus decreases the ability of the system to maintain itself or to recover in case of damage. There are very complex mechanisms underlying these ecological effects.

2. Economic benefits to humans

- For all humans, biodiversity is first a resource for daily life. Such 'crop diversity' is also called agrobiodiversity.
- Most people see biodiversity as a reservoir of resources to be drawn upon for the manufacture of food, pharmaceutical, and cosmetic products. Thus resource shortages may be related to the erosion of the biodiversity.

Some of the important economic commodities that biodiversity supplies to humankind are:

i. **FOOD :** crops, livestock, forestry, and fish

ii. **MEDICATION:** Wild plant species have been used for medicinal purposes since before the beginning of recorded history. For example, quinine (Used to treat malaria) comes from the bark of the Amazonian tree Cinchona tree; digitalis from the Foxglove plant (chronic heart trouble), and morphine from the Poppy plant (pain relief).

According the National Cancer Institute of the USA, over 70 % of the promising anti-cancer drugs come from plants in the tropical rainforests. Animal may also play a role, in particular in research. It is estimated that of the 250,000 known plant species, only 5,000 have been researched for possible medical applications.

iii. INDUSTRY: fibres for clothing, wood for shelter and warmth. Biodiversity may be a source of energy (such as biomass). Other industrial products are oils, lubricants, perfumes, fragrances, dyes, paper, waxes, rubber, latexes, resins, poisons and cork can all be derived from various plant species. Supplies from animal origin are wool, silk, fur, leather, lubricants, waxes. Animals may also be used as a mode of transportation.

iv. TOURISM & RECREATION: biodiversity is a source of economical wealth for many areas, such as many parks and forests, where wild nature and animals are a source of beauty and joy for many people. Ecotourism in particular, is a growing outdoor recreational activity.

3. Ethical reasons

The role of biodiversity is to be a mirror of our relationships with the other living species, an ethical view with rights, duties, and education. If humans consider species have a right to exist, they cannot cause voluntarily their extinction. Besides, biodiversity is also part of many cultures' spiritual heritage.

Methods of study

Biodiversity assessments are considered important because measures of biodiversity are frequently interpreted as indicators of the wellbeing of ecological systems. Moreover, the patterns of spatial and

temporal variations in diversity of different forms have and still continue to stimulate the minds of ecologists to discover, speculate, hypothesize and analyse information regarding different systems of interest. This has led to devising a huge range of indices and models for measuring diversity, which should be used with some caution in that they should be used only after their suitability has been evaluated under different habitats and situations.

Biodiversity assessments can generally follow certain approaches that describe biodiversity in the way it has been defined by the Convention on Biological Diversity, to incorporate the diversity of ecosystems, diversity between species and within species. To name a few such approaches in biodiversity studies - habitat analysis, taxonomic approach, approaches that reflect on the dynamics of populations, associations and communities of the algae using different indices that can measure their diversity, dominance and similarities, metapopulation approaches that can define the populations' viability, dispersal and establishment dynamics, along with the scope of finding suitable but unoccupied habitats, and finally molecular and genomic approaches. Such assessments emphasize upon the species as the functioning entities and the categories by which all such diversity related information can be organized and retrieved. The methods to obtain reliable measures of species richness recognize sampling and identification of species as the essential baseline for understanding their diversity and it goes without saying that properly conducted inventories will remain as the core of future endeavours. Few such approaches and indices of measurements that yield important and interesting results have been discussed here.

Habitat analysis

A study of the places in which different forms of organisms grow yield interesting sets of results. The habitat is described by geographic, physical, chemical and biotic characteristics. The overall habitat of many plant forms within a specific geographical or climatic unit is its macrohabitat. This can be further classified into smaller units called microhabitats. Habitat at both macro and microlevels need to be treated as multidimensional biophysical entities including the dimensions of temporal, spatial, physical-chemical and biotic as important measures. Such studies find many applications in the assessment of environmental impacts, vulnerability assessment, management of the desired species, preparation of inventories that can serve as baseline data for future studies, reflections on invasive species and habitat reclamation.

Diversity of habitat is in fact a partial measure of the diversity of species in a community. In general, it has been found that in both terrestrial and aquatic habitats diversity of species can be strongly correlated with the structural diversity of the habitat. Analysis of any habitat can yield adequate information only when it is executed over a considerable temporal and spatial scale. The initial analysis should deal with an account of the general macrohabitat description about the geographic, climatic, geologic and biotic factors most important to the inhabiting plant assemblage(s). A detailed analysis of some specific habitat components can follow, such as information regarding weather along with water and soil quality. Finally a microhabitat study of specific plant assemblage(s) can be designed to evaluate the environmental factors that influence the presence or absence of one or more plant species that contribute towards the biodiversity of the plants specific to the location. The indices for such assessments can be chosen amongst the different habitat diversity indices and habitat rating indices depending on suitability of the specific index and with the objective of own study in mind.

Populations, Associations and Communities

A large number of basic measurements exist that are found suitable in describing populations and communities. The best known include measures of density, frequency, coverage and biomass. The other important measures that can be derived from such basic information include the likes of population distribution, species diversity and productivity. The census method among such general studies gives an account of the total count of all individuals in a population.

Sample collections for such studies involve quadrat analysis. Sampling should be done with the objective of the study in mind or on a more general note whether the intention is to execute a qualitative or quantitative analysis.

Given the availability of large number of indices available for analysing such studies, it is often difficult to decide which among these poses as the best method of measuring diversity. One must initially assess the diversity measures to test their applicability and performance with one's own data.

The selection procedure of the index to be used can further be strengthened by examining whether it fulfils the criteria of being able to discriminate between different habitats, whether it is proofed against its dependence on the available sample size, what component of diversity the researcher wishes to measure, and whether the index is widely in use and understood.

Biodiversity Assessment Techniques

Biodiversity is a broad term, and since the adoption of the Convention of Biological Diversity it is commonly defined in terms of three different components: intra-specific genes (genetic diversity), inter-specific species (species diversity) and ecosystems (ecological diversity), each of which have structural, compositional, and functional attributes. Given this complexity and the lack of knowledge on all three components, identifying, measuring and monitoring all three levels exhaustively proof to be impossible. An array of international and national initiatives has, therefore, sought to overcome this problem by trying to come up with simplified, yet significant methodologies of biodiversity assessments. One way is the identification of indicators – a subset of attributes that could serve as surrogates for total biodiversity. These were developed for various levels – national, regional and stand level. Indicators of biodiversity can be divided into two broad groups:

i) biological or taxon based indicators, particularly indicator species and guilds and

ii) structure-based indicators – stand and landscape level (spatial) features such as stand structure complexity, plant species composition and connectivity and heterogeneity

The selection of indicators differs for biodiversity monitoring and biodiversity inventory. Various criteria have been developed for the selection of indicators taking into account biological as well as logistical aspects. Relatively well-known taxa such as higher vertebrates (eg. mammals, birds), certain invertebrate groups (eg. butterflies, ants, land snails) and higher plants (particularly trees) are commonly used as indicator groups. Threatened species, endemic species, and economically or socially important species are often chosen as priorities for data collection. As much as it is impossible to assess each single element of biodiversity, it is also impossible in practice to inventorize every site. The knowledge of species habitat requirements, coupled with baseline data on climate, altitude, soil type, or vegetation cover, is used to predict their occurrence in areas not inventoried. Geographic information systems (GIS) are commonly used in biodiversity inventory techniques.

Some selected biodiversity assessment techniques include:

1.1. Baseline studies

The techniques described under this section are used for collecting general data on biodiversity mainly within the frame of national biodiversity conservation planning. They include biodiversity inventories, the identification and prioritization of areas rich in biodiversity as well as gap analysis. The techniques vary from using an advanced and highly technical approach (e.g. REA) to simpler ground-based inventories (e.g. RBA).

Rapid Biodiversity Assessment (RBA)

A very rapid, cheap and attractive method to assess the **relative biodiversity value of different sites**, provided they are assessed using the same indicator groups of species. RBA is based on the premise that certain aspects of biological diversity can be quantified without knowing the scientific names of the species involved. The main characteristic of RBA is the minimization of the formal taxonomic content in the classification and identification of organisms.

Data are gathered on certain groups of organisms. Several groups, chosen as good "predictor sets" of biodiversity are needed at each location inventoried. Appropriate groups are ones that are relatively abundant, have high species richness, contain many specialist species, are easy to sample, and have taxonomic traits amenable to RBA methods. RBAs focus on invertebrate groups, such as butterflies, ants, termites, certain beetle families, grasshoppers and spiders.

Rapid Assessment Program (RAP)

The RAP conducts preliminary assessments of the biological value of poorly known areas. RAP teams usually consist of experts in taxonomically well-known groups such as higher vertebrates (e.g., birds and mammals) and vascular plants, so that the ready identification of organisms to the species level is possible. The biological value of an area can be characterised by species richness, degree of species endemism (i.e., percentage of species that are found nowhere else), the uniqueness of the ecosystem, and the magnitude of the threat of extinction. RAPs are undertaken by identifying potentially rich

sites from satellite images/aerial reconnaissance, and then sending in ground teams to conduct field-survey transects. Such field trips last from two to eight weeks, depending on the remoteness of the terrain.

Rapid Biological Inventories (RBI)

"The goal of rapid biological inventories is to catalyze effective action for conservation in threatened regions of high biological diversity and uniqueness. During rapid biological inventories, which typically take a month, scientific teams focus primarily on groups of organisms that indicate habitat type and condition and that can be surveyed quickly and accurately. These inventories do not attempt to produce an exhaustive list of species or higher taxa. Rather, the rapid surveys identify the important biological communities in the site or region of interest and determine whether these communities are of outstanding quality and significance in a regional or global context. The rapid biological inventory teams use protocols that are specific to the organism groups under study and which are often modified to meet the demands of a particular expedition.

Rapid Ecological Assessment (REA)

Rapid Ecological Assessment is a technique used to assess biodiversity in large, poorly- studied, or exceptionally biodiverse areas. The REA process consists of a series of increasingly refined analyses, with each level further defining sites of high conservation interest. The levels involved are satellite observation; airborne remote sensing; aerial reconnaissance; and field inventory. Analysis of satellite images is used to produce maps of eco-regions, land cover and priority areas; while integration with data from airborne sensors and aerial reconnaissance produces more detailed maps, extended to cover vegetation types and ecological communities. These are used to direct the cost-effective acquisition of biological and ecological data through stratified field sampling. Such data are used to identify priority sites. Spatially-referenced information is managed by GIS, allowing easy data handling and generation of maps.

Gap Analysis

The technique "Gap Analysis" is essentially a coarse-filter approach to biodiversity conservation. It is used to identify gaps in the representation of biodiversity within protected areas. The goal is to ensure that all ecosystems and areas rich in species diversity are adequately represented in protected areas. Gaps in the protection of biodiversity are identified by superimposing three digital layers in a Geographical Information System (GIS), namely maps of vegetation types, species distributions and land management use. A combination of all three layers can be used to identify individual species, species-rich areas and vegetation types that are either not represented at all or are under-represented in existing reserves. In practice, vegetation, common terrestrial vertebrate species, and endangered species are used as surrogates to represent overall biodiversity.

1.2. Monitoring and Modeling Techniques Biodiversity Monitoring Techniques

The initial phase in biodiversity surveys is estimating diversity at one point in time and location (in other words, knowing what species or communities are present). The second phase, monitoring biodiversity, is estimating diversity at the same location at more than one time period for drawing inference about change. Wilson, et al. (1996) identified attributes of biodiversity that can be assessed at each level of ecological organization. At the landscape level, attributes that could be monitored include the identity, distribution, and proportions of each type of habitat, and the distribution of species within those habitats. At the ecosystem level, richness, evenness, and diversity of species, guilds, and communities are important. At the species level, abundance, density, and biomass of each population may be of interest. And, at the genetic level, genetic diversity of individual organisms within a population is important. It is best to assess and interpret biodiversity across all these levels of organization by using various approaches at several spatial and temporal scales.

Identifying monitoring questions is a critical and difficult step. It could be accomplished through an interdisciplinary process with experts knowledgeable of the issues at the appropriate level (e.g., landscape, ecosystem, species, genetic, etc.) and should be considered an iterative process that is adapted as new information becomes available.

Monitoring questions can be derived from information available in watershed analysis, late-successional reserve (LSR) assessments, or regional assessments. The monitoring questions are ranked as low, moderate, and high priority. Ultimately, management must determine which

monitoring questions should be addressed. Methods selected for monitoring biodiversity depend on management objectives. A management objective of maintaining species viability would involve different monitoring methods than an objective of restoring inherent disturbance regimes. Selecting the appropriate biodiversity monitoring approach includes identifying methods that will provide answers to specific monitoring questions. A wide range of methods are available, and selection of methods would be made based on costs, available resources, and statistical constraints. It might be helpful, if not absolutely necessary, to consult a statistician at this stage to determine sampling sizes, strategies, and statistical power. Periodically, data collected from monitoring should be analyzed and integrated into management strategies based on the knowledge gained. If monitoring reveals that adjustments need to be made in management strategies, then this becomes a decision. Several examples of monitoring biological diversity at each level of ecological organization have been described below:

Landscape Monitoring: A landscape has been defined as a land area with groups of plant communities or ecosystems forming an ecological unit with distinguishable structure, function, geomorphology, and disturbance regimes. Landscape diversity is the number of ecosystems, or combinations of ecosystems, and types of interactions and disturbances present within a given landscape. The relevance of landscape structure to biodiversity has been established through ecological studies. Landscape features such as patch size, heterogeneity, connectivity, etc., have major implications to species composition, distribution, and viability. Because of this, it may be important that managers monitor elements of biodiversity at the landscape scale to meet species viability requirements. There exist several approaches to assessing biological diversity at a landscape scale. Each of these approaches relies on the use of geographic information systems (GISs) and requires mapped vegetation and other layers that can be analyzed with GIS technologies. Landscape pattern measurements, or metrics, can be classified into three categories: patch, class, and landscape. The various metrics in use include Patch metrics, Class metrics and Landscape pattern metrics.

Patch metrics describe the attributes of individual patches of vegetation. The size, shape, edge, or nearest-neighbor relations of individual patches are measured.

Class metrics describe those same patch attributes as the mean, minimum, maximum, or variance for a class of mapped landscape attributes (e.g., late-successional forest).

Landscape pattern metrics describe these and other attributes for all landscape classes combined without distinction between different classes. For example, mean patch size might be measured for all patches in a landscape, instead of for just one vegetation type (class).

Community Monitoring: A community comprises the populations of some or all species coexisting at a site. An ecosystem includes the abiotic aspects of the environment and the biotic community. Monitoring at this level is important to the maintenance of ecosystem functions and integrity that have been identified as a main theme of ecosystem management. Land managers may be interested in monitoring communities or ecosystems to determine if current management strategies meet legal and social obligations to sustain the health, diversity, and productivity of ecosystems.

Monitoring methods include by measuring the number and relative abundance of species in a community or ecosystem, often referred to as species diversity. Species diversity is a function of the number of species present (richness) and the evenness or equitability (relative abundance) of each. Although species diversity and species richness are often positively correlated, situations do exist in which increases in species diversity are accompanied by decreases in species richness. Care should be taken therefore, when only species richness (counts of the number of species) is used to evaluate biodiversity. On the other hand, species diversity indices also should be carefully used because it may be hard to interpret differences in species composition at different sites. For example, two sites may have similar indices of diversity but have entirely different species composition. One site may be primarily exotic species, whereas the other mainly native or endemic species.

Guild Monitoring: Some investigators have taken a different viewpoint by lumping species into functional groups or guilds. Many approaches for grouping species based on habitat or behavioral similarities and their potential problems have been discussed in the literature. An approach to the same is in which species are grouped into guilds based on their function in the ecosystem, and then the relative importance of each guild is considered based on how a change in their abundance affects ecosystem and community processes.

Population Monitoring: A population is defined as all individuals of one species occupying a defined area and usually isolated to some degree from other similar groups. A species is generally defined as a group of organisms formally recognized as distinct from other groups. Monitoring at this level may have the most relevance to meeting the species or population viability objectives. For example, land managers may decide to monitor a species or population in order to measure trends. This would be important to determine if management strategies maintain population viability. Monitoring methods at this level are sufficiently enriched as most monitoring of biodiversity has occurred at the population- species level. Deciding which species or population to monitor has received considerable discussion, and no single approach is without pitfalls.

Five categories of species may be selected for monitoring. These include (1) ecological indicators—species that signal the effects of perturbations on a number of other species with similar habitat requirements; (2) keystones—species on which the diversity of a large part of a community depends; (3) umbrella species—species with large area requirements, which if conserved, many other species also would be conserved; (4) flagship species — popular, charismatic species; and (5) vulnerable species —species that are rare, genetically impoverished, or for some reason prone to extinction. When determining which species are best to monitor as bioindicators, it is appropriate to consider species of invertebrates, fungi, lichens, and amphibians, as well as vertebrates and vascular plants.

Monitoring a species or population may include counting of individuals but most often involves the monitoring of habitat that is used by or is important to a species. It has been pointed out that monitoring habitat variables does not alleviate the need to monitor populations because the presence of habitat is no guarantee that the species is present. Conversely, monitoring only population variables could be misleading because someindividuals may occur in areas of marginal habitat. The most reliable approach would include monitoring both habitat and population variables.

Population Viability Analysis: (PVA) estimates what conditions are necessary for a population to persist for a given period of time in a given place. The PVA allows for a prediction of the possible trend of a population and can provide insights into why a population may be decreasing. One of the disadvantages of a quantitative PVA is the rigorous data set that is needed to complete the analysis. These data are often expensive to obtain and require several years or decades of study. Because of the need to have such a rigorous and extensive data set, PVA may be too cumbersome to use to monitor biodiversity. When such data are available, however, PVA may be useful in establishing baseline population information to predict how management actions might effect viability. Recently, a new approach has been used to develop a qualitative PVA relying on the professional judgement of scientists familiar with a species or group of species. This qualitative approach has the advantage of not requiring such a rigorous data set, yet still meets the essential criterion of a PVA, to provide an estimate of the likelihood that a population will persist over a given time period.

Genetic Monitoring: Genetic diversity refers to the breadth of genetic variation within and among individual populations and species. Genetic variation is essential for the long-term survival of endangered species, especially those that occur in fast-changing or harsh environments. Genetic diversity is a necessary prerequisite for future adaptive change or evolution, and presumably, populations and species that lack genetic variation are at greater risk of extinction. Land managers may decide to monitor genetic diversity to determine if management strategies are meeting the requirements for species viability. Monitoring methods include determining which population or species should be monitored for genetic diversity would be identified when specific monitoring questions are developed. Most of the time, however, the resources available in which to conduct genetic diversity studies will be the limiting factor in their application. The following criteria has been recommended in the selection of populations or species for this level of monitoring:

(1) Species or populations that are limited in their numbers and distribution (e.g., endangered, threatened, and candidate species);

(2) Populations that are naturally fragmented or have become fragmented as a result of human activities and the likelihood of genetic interchange among component populations is low;

(3) Populations that are on the edge of a species range, and

(4) Species that naturally occur at low densities but may have wide distribution (e.g., large carnivores).

Morphological Monitoring: The measurement of morphological variation is the most easily obtained indicator of genetic diversity. Morphological measurements often can be obtained in the field or from field specimens, not requiring laboratory studies. Another advantage is that morphological characters may be ecologically adaptive, meaning they are good indicators of genetic variation, local differentiation, or ecotypes. This method is often the most realistic when the biochemical analysis are impractical. Perhaps the greatest disadvantage is the assumption that morphological variation is a reliable indicator of underlying genetic variation. This assumption can be difficult to validate unless it is done in conjunction with allozyme or DNA analysis.

New Approaches to Biodiversity

Assessment People's Biodiversity Register (PBR) exercise

The evolution of human societies over several millennia is closely related to plants and animals. The domestication of crop plants and farm animals about 12000 years ago revolutionized the human civilization by creating more stabilized societies. The early historic and medieval period gradually reduced human interaction with the wild plants and animals. The development of modern science and technologies during the industrial and post- industrial period did not do away with our link to nature. Different groups of people continue to depend on natural resources at varying scales. Some draw resources from across continents while others within a country or a region. There are also people continue to depend on locally available biodiversity and bio-resources for their livelihoods. Such population who are directly dependent on local biological resources have, through their keen sense of observation, practices, and experimentation developed and established a body of knowledge that is passed on from generation to generation. Some are widespread traditional knowledge like cultivation practices; others are highly specialized such as bone setting or jaundice, which are generally passed only to close members of the family.

India is land of biological and cultural diversity. It is one of the ten-mega bio-diverse countries of the world. It also the home of a large number of tribal groups, pursing different kinds of nature based livelihoods. In addition, a large number of farming and fishing communities and nomadic groups possess traditional knowledge of varying degrees. The development of modern science and technologies notably biotechnology and information technologies have increased the value of biodiversity and associated knowledge including traditional knowledge (TK) .The growing importance of biodiversity, bio-resources and associated knowledge is fairly well understood. The first step towards conservation or sustainable utilization of biodiversity is its documentation. Biodiversity and associated knowledge is found in different ecosystems, under different legal management regimes and hence the results and manner of documentation will also differ.

The preparation of People's Biodiversity Registers are an initiative to document people's knowledge regarding biodiversity resources of their areas pertaining to status, use, history, changing trends, forces driving such changes, the gainers and losers in these processes and also the local people's perceptions of how these resources should be managed. These documents are capable of bringing together important locality specific information on bioresources and the ecological processes affecting them. These exercises are capable of leading to recognition and promotion of conservation oriented local practices and help mobilize and motivate communities to prudently manage local biodiversity resources. As such, these registers have an important role to play in promoting conservation, sustainable use and equitable sharing of benefits of biodiversity resources in future. Thus the process of preparation of PBRs and the resultant documents are of immense importance in promoting more sustainable, flexible, participatory systems of management and in ensuring a proper access and benefit sharing from economic use of the living resources to the local communities.

The Biological Diversity Act, 2002 (No. 18 of 2003) was notified by Government of India on 5th February, 2003. The Act extends to the whole of India and reaffirms the sovereign rights of the state over its biological resources. Subsequently the government of India published Biological diversity Rules, 2004 (15th April, 2004). The Rules under section 22 states that 'every local body shall constitute a Biodiversity Management Committee (BMC's) within its area of jurisdiction'.

People's Biodiversity Registers and the role of the Biodiversity Management Committee: The mandate of the Biodiversity Management Committee has been clearly highlighted in the Biodiversity Rules 2002 as follows:

- The main function of the BMC is to prepare People's Biodiversity Register in consultation with the local people. The Register shall contain comprehensive information on availability and knowledge of local biological resources, their medicinal or any other use or any other
- The other functions of the BMC are to advice on any matter referred to it by the State Biodiversity Board or National Biodiversity Authority for granting approval, to maintain data about the local vaids and practitioners using the biological resources.
- The National Biodiversity Authority shall take steps to specify the form of the People's Biodiversity Registers, and the particulars it shall contain and the format for electronic database.
- The National Biodiversity Authority and the State Biodiversity Boards shall provide guidance and technical support to the Biodiversity Management Committees for preparing People's Biodiversity Registers.
- The People's Biodiversity Registers shall be maintained and validated by the Biodiversity Management Committees.

People's Biodiversity Registers and Role of the Technical Support Group (TSG): The Technical Support Group (TSG) consists of experts drawn from various disciplines and line departments, universities, research institutes, colleges and schools and non-governmental organizations. The Technical Support Group provides technical inputs and advice to the BMCs on identification of plants and animals, monitors and evaluates the PBR exercise, examines confidential information and advices on legal protection, maintains a database of local and external experts on biodiversity.

People's Biodiversity Registers and the role of State Biodiversity Board (SBB): The State Biodiversity Board (SBB) provides necessary training to the Technical Support Group (TSG) of the district and enable smooth functioning and aid in networking for creation and maintenance of People's Biodiversity Registers (PBRs).

People's Biodiversity Registers and the role of National Biodiversity Authority (NBA): The National Biodiversity Authority provides guidance and technical support to the Biodiversity Management Committee (BMC) for preparing People's Biodiversity Register

The present manual guidelines have been drafted by the National Biodiversity Authority taking into consideration different ecosystems and include rural urban and protected areas. The guidelines may be customized and further information may be added to enrich the effort. It is important to keep in mind some of the issues related to PBRs:

- It is to be undertaken in a participatory mode involving varying sections of village society.
- While documenting, the knowledge and views of both genders are to be recorded.
- Information provided by people need to be collated, analysed and crosschecked by the members of the Technical Support Group (TSG) before documentation.
- The PBR is an important base document in the legal arena as evidence of prior knowledge and hence careful documentation is necessary.
- The document should be endorsed by the BMC and later publicized in the Gram Sabha / Gram Panchayat / Panchayat Samiti. The document can be a very useful tool in the management and sustainable use of diversity. The document can also be a very useful teaching tool for teaching environmental studies at schools, colleges and university level.
- The document should be periodically updated with additional and new information as and when generate.

The PBR Process: The preparation of People's Biodiversity Registers (PBRs) involves the active support and cooperation of a large number of people who need to share their common as well as specialized knowledge. One of the first steps for preparing a PBR is to organize a group meeting to explain the objectives and purpose of the exercise. Different social groups in the village need to be identified for purpose of data collection from those groups. In an urban situation, spots where biodiversity are important need to be identified for the purpose of the study and documentation. The documentation process includes information gathered from individuals through detailed questionnaire, focused group discussion with persons having knowledge and published secondary information.

Documentation of Traditional Knowledge (TK) related to biodiversity: Documentation of knowledge of individuals with regard to biodiversity and its uses is an important part of PBR. Every effort should be made to identify the persons with proven knowledge of local biodiversity; special

attention should be given to the elderly persons who can also provide information on the biodiversity which was available in the past but no longer seen at present. In some cases focus group discussion may be held for the purpose of documentation.

PBR Methodology: The PBR is a participatory process requiring intensive and extensive consultation with the people. The objectives and purpose is to be explained in a group meeting in the presence of all sections of people in the Panchayat, members of the BMC, students, knowledgeable individuals and all those interested in the effort. Documentation includes photographs (including digital images), drawings, audio and video recordings and other records like printed material.

Process in PBR Preparation:

Step 1 Formation of Biodiversity Management Committee (BMC)

Step 2 Sensitization of the public about the study, survey and possible management

Step 3 Training of members in identification and collection of data on biological resources and traditional knowledge

Step 4 Collection of data. Data collections includes review of literature on the natural resources of the districts, Participatory Rural Appraisal (PRAs) at village level, housel hold interviews, individual interviews with village leaders and knowledgeable individuals, household heads, key actors of the Panchayat Raj Institutions and NGOs and direct field observations.

Step 5 Analysis and validation of data in consultation with technical support group and BMC

Step 6 Preparation of People's Biodiversity Register (PBR).

Step 7 Computerization of information and resources.

Protection from depletion

Biodiversity is being depleted by the loss of habitat, fragmentation of habitat, over exploitation of resources, human sponsored ecosystems, climatic changes, pollution invasive exotic spices, diseases, shifting cultivation, poaching of wild life etc.

Since the human beings are enjoying all the benefits from biodiversity, they should take proper care for the preservation of biodiversity in all its form and good health for the future generation i.e., the human being should prevent the degradation and destruction of the habitats thereby maintaining the biodiversity at its optimum level.

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. In other words, conservation of bio-diversity is the proper management of the biosphere by human beings in such a way that it gives maximum benefits for the present generation and also develops its potential so as to meet the needs of the future generations.

Mainly the conservation of biodiversity has three basic objectives:

(a) To maintain essential ecological processes and life supporting systems.

(b) To preserve the diversity of species.

(c) To make sustainable utilisation of species and ecosystems.

Strategies for Conservation of Biodiversity:

The following strategies should be undertaken in order to conserve biodiversity:

(1) All the possible varieties (old or new) of food, forage and timber plants, live stock, agriculture animals and microbes should be conserved.

(2) All the economically important organisms in protected areas should be identified and conserved.

(3) Critical habitats for each species should be identified and safeguarded.

(4) Priority should be given to preserve unique ecosystems.

(5) There should be sustainable utilisation of resources.

(6) International trade in wild life should be highly regulated.

(7) The poaching and hunting of wildlife should be prevented as far as practicable.

(8) Care should be taken for the development of reserves and protected areas.

(9) Efforts should be made to reduce the level of pollutants in the environment.

(10) Public awareness should be created regarding biodiversity and its importance for the living organisms.

(11) Priority should be given in wildlife conservation programme to endangered species over vulnerable species and to vulnerable species over rare species.

(12) The habitats of migratory birds should be protected by bilateral and multilateral agreement.
(13) The over exploitation of useful products of wild life should be prevented.
(14) The useful animals, plants and their wild relatives should be protected both in their natural habitat (in-situ) and in zoological botanical gardens (ex-situ)
(15) Efforts should be made for setting up of National parks and wild life sanctuaries to safeguard the genetic diversity and their continuing evolution.
(16) Environmental laws should be strictly followed.

Megadiversity and Hotspots
The term megadiverse country refers to any one of a group of nations that harbour the majority of Earth's species and high numbers of endemic species. Conservation International identified 17 megadiverse countries in 1998. Many of them are located in, or partially in, tropical or subtropical region.
The 17 megadiverse countries are : Australia, Brazil, China, Colombia, Democratic Republic of the Congo, Ecuador, India, Indonesia, Madagascar, Malaysia, Mexico,Venezuela, Papua, New Guinea, Peru, Philippines, South Africa, United States .

India as a megadiverse country:
India has tremendous biodiversity, genetic as well as of species and ecosystems. It contains over 7 per cent of the world's biodiversity on 2.5 per cent of the Earth's surface. This diversity can be attributed to the vast variety of landforms and climates resulting in habitats ranging from tropical to temperate, and from alpine to desert.

- India is one of the world's 'mega diversity' countries. It is ranked ninth in the world in terms of higher plant species richness. At the ecosystem level, India is also well-endowed, with ten distinct biogeographic zones.
- It also contains two of the world's 25 biodiversity hotspots, because of their extraordinarily high levels of species-richness and endemicity, and threatened status.
- India is considered to be the centre of origin for the following crop species pigeon pea, egg plant cucumber, possibly cotton and sesame. But for millennia, numerous other crop species have been introduced to India and adapted to localised conditions.The country has become an important centre of diversity of a great many domesticated species, including various cereals, millets, legumes, vegetables, temperate and tropical fruits, fibre crops, medicinal and aromatic plants.

- The number of plant species in India is estimated to be over 45,523 representing about 11.8 per cent of the world's flora. These include over 17,500 flowering plants of which 4,950 species are endemic to the country.
- India's faunal wealth is equally diverse. The total number of animal species is estimated at 91,307, representing about 7.46 per cent of the world's fauna. India's known animal diversity includes about 8,61,696 insects, 21,723 fish, 240 amphibians, 460 reptiles, 1,232 birds and 397 mammals. In also includes about 86,413 invertebrates. Among amphibians found in India, 62% are unique to this country. Among lizards, of the 153 species recorded, 50% are endemic. High endemism has also been recorded for various groups of insects, marine worms, centipedes, mayflies and fresh water sponges.
- The ancient practice of domesticating animals has resulted in India's diverse livestock, poultry and other animal breeds. India has 26 breeds of cattle, 40 breeds of sheep, 20 breeds of goats, 8 breeds of camels, 6 breeds of horses, 2 breeds of donkeys and 18 breeds poultry birds. India also contains vast microbial diversity
- Although exact numbers of viruses, microscopic algae and other microscopic organisms are not known. India has at least 850 species of bacteria and virus, also 12,500 of fungi.
- It is estimated that 32% of Indian plants are endemic to the country and found nowhere else in the world. Among the plant species the flowering plants have a much higher degree of endemism, a third of these are not found elsewhere in the world.

Biodiversity Hotspots:

A biodiversity hotspot is a biogeographic region with a significant reservoir of biodiversity that is under threat from humans. A hotspot is an area which faces serious threat from human activities and supports a unique biodiversity (endemic, threatened, rare species) with representatives of evolutionary of speciation and extinction.

The concept of biodiversity was given by Norman Myers (1988).

To qualify as a biodiversity hotspots on Myers 2000 edition of the hotspot map, a region must meet two strict criteria:

1. It must contain at-least 0.5% or 1500 species of vascular plants of the world.
2. It has to have lost at least 70% of its primary vegetation.

Myers originally recognised 25 hotspots but recently the Conservation International has added 9 more biodiversity hotspots which make the present number to 34. These sites support nearly 60% of the world's plant, bird, mammal, reptile, and amphibian species, with a very high share of endemic species.

BIODIVERSITY HOTSPOTS IN INDIA

1. Himalaya: Includes the entire Indian Himalayan region (and that falling in Pakistan, Tibet, Nepal, Bhutan, China and Myanmar)

2. Indo-Burma: Includes entire North-eastern India, except Assam and Andaman group of Islands (and Myanmar, Thailand, Vietnam, Laos, Cambodia and southern China)

3. Sundalands: Includes Nicobar group of Islands (and Indonesia, Malaysia, Singapore, Brunei, Philippines)

4. Western Ghats and Sri Lanka: Includes entire Western Ghats (and Sri Lanka)

The hotspots are rich in floral wealth, reptiles, amphibians, mammals and also in their endem

1. The Western Ghats:

About the region:

The Western Ghats are a chain of hills that run along the western edge of peninsular India. They are also known as Sahyadri Mountains. They receive high rainfall. It

run parallel to the west coast of India and constitute more than 1600 km strip of forests in the states of Maharashtra, Goa, Karnataka, Tamil Nadu and Kerala.

Flora:

These regions have moist deciduous forest and rain forest. The region shows high species diversity as well as high levels of endemism. There are over 6000 vascular plants belonging to over 2500 genera in this hotspot, of which over 3000 are endemic.

Much of the world's spices such as black pepper and cardamom have their origins in the Western Ghats. Many economically important plants such as banana, rice, ginger etc. have spread to other parts of the country from here.

Fauna:

Nearly 77% of the amphibians and 62% of the reptile species found here are found nowhere else. The region also harbours over 450 bird species, about 140 mammalian species, 260 reptiles and 175 amphibians. Over 60% of the reptiles and amphibians are completely endemic to the hotspot. Remarkable as this diversity is, it is severely threatened.

2. The Eastern Himalayas:

About the region:

The Eastern Himalayas is the region encompassing Bhutan, northeastern India, and southern, central, and eastern Nepal. The region is geologically young and shows high altitudinal variation. Together, the Himalayan mountain system is the world's highest, and home to the world's highest peaks, which include Mount Everest and K2.

Flora:

There are an estimated 10,000 species of plants in the Himalayas, of which one-third are endemic and found nowhere else in the world. Five families —Tetracentraceae, Hamamelidaceae, Circaesteraceae, Butomaceae and Stachyuraceae — are completely endemic to this region.

Many plant species are found even in the highest reaches of the Himalayan Mountains, For example, a plant species Ermania himalayensis was found at an altitude of 6300 metres in northwestern Himalayas.

Fauna:

Few threatened endemic bird species such as the Himalayan Quail, Cheer pheasant. Western tragopans are found here, along with some of Asia's largest and most endangered birds such as the Himalayan vulture and White-bellied heron.

The Eastern Himalayan hotspot has nearly 163 globally threatened species including the One-horned Rhinoceros (Rhinoceros unicornis), the Wild Asian Water buffalo (Bubalus bubalis) and in all 45 mammals, 50 birds, 17 reptiles, 12 amphibians, 3 invertebrate and 36 plant species. The Relict Dragonfly (Epiophlebia laidlawi) is an endangered species found here with the only other species in the genus being found in Japan.

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13. Threats to Biodiversity: Causes of threats; Concepts of rare, vulnerable, endangered and threatened plants (IUCN categories).

Biodiversity is considered as a reservoir of resources to be used for the manufacture of food, medicine, industrial products, etc. But with an increased demand of rapid population growth, biodiversity is gradually depleting. A number of plants" and animal species have already become extinct and many are endangered.

Causes of threats:

Habitat Loss:

The natural habitat may be destroyed by man for his settlement, agriculture, mining, industries, highway construction, dam building etc.

As a consequence, the species must adapt to the changes in the environment, move elsewhere or may succumb to predation, starvation or disease and eventually die. Several rare butterfly species are facing extinction due to habitat destruction in the Western Ghats. Of the 370 butterfly species available in the Ghats, around 70 are at the brink of extinction.

Habitat fragmentation:

Habitat fragmentation may be defined as an "unnatural detaching or separation of expansive tracts of habitats into spatially segregated fragments" that are too limited to maintain their different species for an infinite future.

This phenomenon was observed as early as 1885 when de Candolle noticed that 'the break-up of a landmass into smaller units would necessarily lead to the extinction or local extermination of one or more species and the differential preservation of others'.

Habitat fragmentation is one of the most serious causes of erosion of biodiversity. Fragmentation leads to artificially created 'terrestrial islands'. Such fragments experience microclimatic effects markedly different from those that existed in the large tracks of habitats before fragmentation. Air temperature at the edges of fragments can be significantly higher than that found in the interior; light can penetrate deep into the edge, thereby affecting the growth of existing species. Fragmentation promotes the migration and colonization of alien species. Such substantial and continuous colonization, profoundly affect the survival of native species.

The most serious effect of fragmentation is segregation of larger populations of a species into more than one smaller population. There is considerable evidence that the number of species in a fragmented habitat will decrease over time, although the probable rates at which it will happen are variable. In fact, actual data on rain forests show that forest fragments have lower species richness and fewer populations compared with continuous undisturbed forests.

An example of loss of biodiversity as the result of the fragmentation is that of the Western forest of Ecuador, which were largely undisturbed till 1960, where newly constructed network of roads led to rapid human settlements and clearance of much of the forest area, have been fragmented into small patches of one to few square kilometers.

Deforestation:

Forest ecosystems contain as much as 80 percent of the world's terrestrial biodiversity and provide wood fiber and biomass energy as well as critical components of the global cycles of water, energy and nutrient. Forest ecosystems are being cleared and degraded in many parts of the world.

Current projections suggest that demand for wood will roughly double over the next 50 years, which will make increasing use of sustainable forest practices more difficult. In addition to threats to biodiversity and potential shortages in the supply of forest products, the degradation of forests represents an enormous potential source of green house gas emissions.

Forest ecosystems contain about three times the amount of carbon currently present in the atmosphere and about one-third of this carbon is stored above ground in trees and other vegetation and two-third is stored in the soil.

Invasive Species:

Invasive species are 'alien' or 'exotic' species which are introduced accidentally or intentionally by human. These species become established in their new environment and spread unchecked, threatening the local biodiversity. These invasive alien species have been identified as the second greatest threat to biodiversity after habitat loss.

• Invasion by exotic species

Prominent examples are the spread of the Peruvian thorny *Prosopis juliflorain in* the dry parts of northern India where it replaced native species such as *Acacia nilotica* and the spread of the South American flowering bush *Lantana camara* in the sub-Himalayan belt.

Overexploitation for Commercialization:

Over-exploitation of resources has coasted more environmental degradation than earning. For example; shrimp farming in India, Thailand, Ecuador and Indonesia results in Wetland destruction, pollution of coastal waters and degradation of coastal fisheries. Scientific studies have concluded that cost of environmental degradation resulting from shrimp farming was costing more than the earning through shrimp exports.

Pollution:

Pollution is a major threat to biodiversity, and one of the most difficult problems to overcome; Pollutants do not recognize international boundaries. For example, agricultural run-off, which contains a variety of fertilizers and pesticides, may seep into ground water and rivers before ending up in the ocean. Atmospheric pollutants drift with prevailing air currents and are deposited far from their original source.

Global Climate Change:

Many climatologists believe that the greenhouse effect is likely to raise world temperatures by about 2°C by 2030, meaning that sea levels will rise by around 30-50 cm by this time. Global warming, coupled with human population growth and accelerating rates of resource use will bring further losses in biological diversity. Vast areas of the world will be inundated causing loss of human life as well as ecosystems.

- ecosystem encroachment (inundation of shoreline ecosystems & drowning of coral reefs from sea level rise, sand dune encroachment from desertification)
- changes in geochemical regimes (ocean acidification, changes in atmospheric CO2 affecting plant growth, loss of sediment leading to broad-scale subsidence)
- changes in temperature regimes (heat waves, cold spells, oceanic temperature changes, melting of glaciers/sea ice)
- changes in precipitation & hydrological regimes (droughts, rain timing, loss of snowcover, increased severity of floods)

- ❖ severe weather events (thunderstorms, tropical storms, hurricanes, cyclones, tornadoes, hailstorms, ice storms or blizzards, dust storms, erosion of beaches during storms)

Population Growth and Over-consumption:

From a population of one billion at the beginning of the 19th century, our species now numbers more than six billion people. Such rapid population growth has meant a rapid growth in the exploitation of natural resources— water, foods and minerals. Although there is evidence that our population growth rate is beginning to slow down, it is clear that the exploitation of natural resources is currently not sustainable. Added to this is the fact that 25 per cent of the population consumes about 75 per cent of the world's natural resources. This problem of over-consumption is one part of the broader issue of unsustainable use.

Illegal Wildlife Trade:

The international trade in wild plants and animals is enormous. Live animals are taken for the pet trade, or their parts exported for medicines or food. Plants are also taken from the wild for their horticultural or medicinal value.

Species extinction:

Extinction is a natural process. The geological record indicates that many hundreds of thousands of plant and animal species have disappeared over the eras as they have failed to adapt to changing conditions. Recent findings however indicate that the current rate of species extinction is at least a hundred to a thousand times higher than the natural rate.

IUCN:

The International Union for Conservation of Nature (IUCN; officially International Union for Conservation of Nature and Natural Resources) is an international organization working in the field of nature conservation and sustainable use of natural resources. It is involved in data gathering and analysis, research, field projects, advocacy, and education. IUCN's mission is to "influence, encourage and assist societies throughout the world to conserve nature and to ensure that any use of natural resources is equitable and ecologically sustainable".

Over the past decades, IUCN has widened its focus beyond conservation ecology and now incorporates issues related to sustainable development in its projects. IUCN does not itself aim to mobilize the public in support of nature conservation. It tries to influence the actions of governments, business and other stakeholders by providing information and advice, and through building partnerships. The organization is best known to the wider public for compiling and publishing the IUCN Red List of Threatened Species, which assesses the conservation status of species worldwide.

IUCN has a membership of over 1400 governmental and non-governmental organizations. Some 16,000 scientists and experts participate in the work of IUCN commissions on a voluntary basis. It employs approximately 1000 full-time staff in more than 50 countries. Its headquarters are in Gland, Switzerland.

IUCN has observer and consultative status at the United Nations and plays a role in the implementation of several international conventions on nature conservation and biodiversity. It was involved in establishing the World Wide Fund for Nature and the World Conservation Monitoring Centre. In the past, IUCN has been criticized for placing the interests of nature over those of indigenous peoples. In recent years, its closer relations with the business sector have caused controversy.

IUCN was established in 1948. It was previously called the International Union for the Protection of Nature (1948–1956) and the World Conservation Union (1990–2008).

Current work

IUCN Programme 2017–2020

According to its website, IUCN works on the following themes: business, climate change, economics, ecosystems, environmental law, forest conservation, gender, global policy, marine and polar, protected areas, science and knowledge, social policy, species, water and world heritage.

IUCN works on the basis of four-year programs, determined by the membership. In the IUCN Programme for 2017–2020 conserving nature and biodiversity is linked to sustainable development and poverty reduction. IUCN states that it aims to have a solid factual base for its work and takes into account the knowledge held by indigenous groups and other traditional users of natural resources.

The IUCN Programme 2017–2020 identifies three priority areas:
1. Valuing and conserving nature.
2. Promoting and supporting effective and equitable governance of natural resources
3. Deploying Nature Based Solutions to address societal challenges including climate change, food security and economic and social development.
IUCN does not itself aim to directly mobilize the general public. Education has been part of IUCN's work program since the early days but the focus is on stakeholder involvement and strategic communication rather than mass-campaigns.

Habitats and species

IUCN runs field projects for habitat and species conservation around the world. It produces the IUCN Red List of Threatened Species and the IUCN Red List of Ecosystems. The IUCN Red List of Ecosystems is applicable at local, national, regional and global levels.
IUCN' stated goal is to expand the global network of national parks and other protected areas and promote good management of such areas. In particular, it focuses on greater protection of the oceans and marine habitats.

Business partnerships

IUCN has a growing program of partnerships with the corporate sector on a regional, national, and international level to promote sustainable use of natural resources.

National and international policy

On the national level, IUCN helps governments prepare national biodiversity policies. Internationally, IUCN provides advice to environmental conventions such as the Convention on Biological Diversity, CITES and the Framework Convention on Climate Change. It advises UNESCO on natural world heritage.
It has a formally accredited permanent observer mission to the United Nations.
IUCN has official relations with the multiple other international bodies.

IUCN in India:

- India a mega diverse country accounts for 7-8% of all recorded species.
- Over 45,968 species of plants and 91,364 species of animals.
- of 34 globally identified biodiversity hotspots: The Himalayas, the Western Ghats, the North-East, and the Nicobar Islands in India.
- India became a State Member of IUCN in 1969, through the Ministry of Environment Forest and Climate Change (MoEFCC).
- The IUCN India Country Office was established in 2007 in New Delhi.

Red data list:

- **The IUCN Red list of threatened species is also known as the IUCN Red list or Red data list.**
- IUCN Red list was founded in **1964**, is the world's most comprehensive inventory of the global conservation status of biological species.
- The IUCN is the world's main authority on the conservation status of species.
- A series of regional red lists are produced by countries or organizations, which assess the risk of extinction
- The IUCN Red list is set upon precise criteria to evaluate the extinction risk of thousands of species and subspecies.

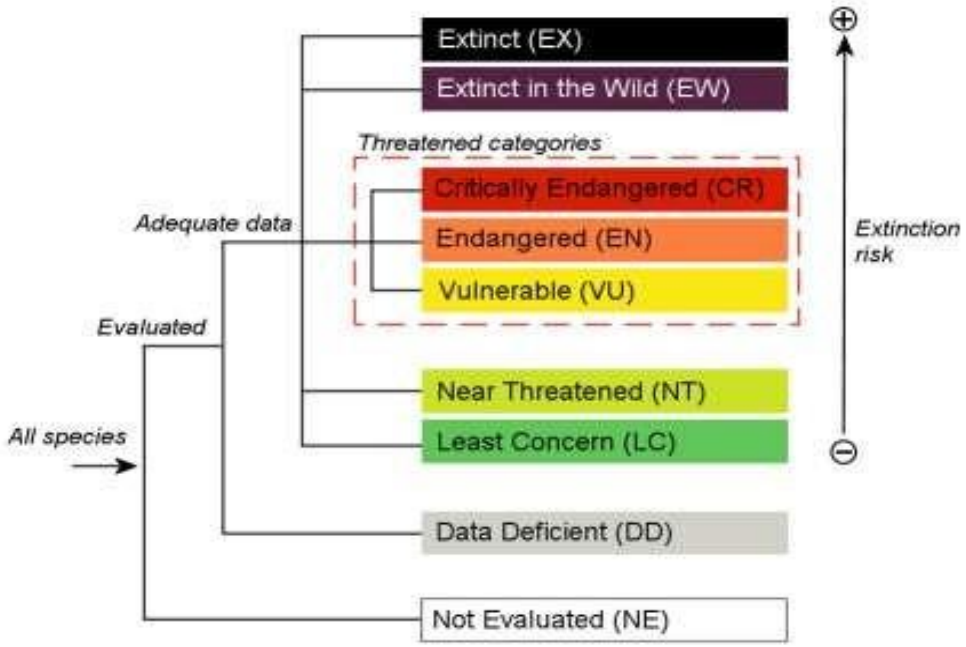
According to IUCN (1996), the formally stated goals of the Red List are

(1) To provide scientifically based information on the status of species and subspecies at a global level.
(2) To draw attention toward the importance of threatened biodiversity.
(3) To provide information to guide actions to conserve biological diversity.

Category	Total no. of Species in world
Extinct	868
Extinct in the wild	78
Critically endangered	5176
Endangered	7705
Vulnerable	11654
Near threatened	4406
Least concern	36952
Total	**82065**

IUCN Red list Categories:

Species are classified by the IUCN Red list into nine groups, set through criteria such as rate of decline, population size, area of geographic distribution, and degree of population and distribution fragmentation.



Extinct:

- A species is Extinct when the last existing member dies.
- The moment of extinction is generally considered to be the death of the last individual of the species.
- Total 868 species Extinct in the world.

Extinct in the Wild:

- "Extinct in the Wild" (EW) Species listed under this status by IUCN are not known to have any living specimens in the wild, and are maintained only in zoos or other artificial environments.

- ❖ When possible, modern zoological institutions try to maintain a viable population for species preservation and possible future reintroduction to the wild, through use of carefully planned breeding programs.
- ❖ Total **78** species Extinct in the wild in world.

Critically endangered:

- ❖ A Critically endangered (cr) species is one which has been categorized by the IUCN as **facing a very high risk of extinction in the wild.**
- ❖ Total **5176** species critically endangered in the world.
- ❖ As of September 2016, the IUCN lists **455** Critically endangered fish species
- ❖ **The important criteria are :**

  (*a*) an observed estimated, inferred or suspected reduction of at least **80 %** over at least **10 year or 3 generation**.

  (*b*) population estimated to number less than **250** mature individuals.

Endangered:

- ❖ A taxon is endangered when it is not critically endangered but it is facing a very **high risk of extinction in the wild in a near future.**
- ❖ **7705** species are endangered worldwide.

The important criteria are:

(a) an observed, estimated , inferred or suspected reduction of at least **50 %** over at least 10 years or 3 generation.

(b) population estimated to number less than **2,500** mature individuals

Vulnerable:

- A taxon is vulnerable when it is not critically endangered or endangered but is facing a high risk of **extinction in the wild in the medium term future .**
- Total 11654 species are Vulnerable world wide.
- **The important criteria are :**

(a) an observed ,estimated, inferred, or suspected reduction of at least **20 %** over at least 10 year or 3 generation.

(b) population estimated to number less than 10,000 mature individual.

Rare:

Taxa which are not presently endangered or vulnerable but can become Rare because of small population usually located in restricted scattered over a more extensive range .

Lower Risk:

Taxa included in the lower risk category can be separated in to three sub categories :

1) Conservation dependent (lr-cd) : Taxa which would qualify for threatened categories within five year it is known as conservation dependent.

2) Near threatened (lr-nt) : Taxa which do not qualify for conservation dependent, but which are close to qualifying for vulnerable.

3) Least concern (lr-lc) : Taxa which do not qualify for conservation dependent or near threatened.

Data deficient:

- ❖ Known, but appropriate data on abundance and /or distribution is lacking.
- ❖ Listing a taxon is data deficient (dd) when there is inadequate information to make a direct or indirect assessment of its of extinction based on its distribution and / or population status.

Not evaluated (NE): A taxon is not evaluated (ne), when it has not yet been assessed against the criteria.

14. Conservation: Types of conservation - in-situ conservation: Biosphere Reserve, Wildlife Sanctuaries, National Parks, World Heritage Sites; Concept and types of Protected Areas Networks; ex-situ conservation: principles, methods, definition, aims and activities of W.W.F., Red Data Book, MAB, CITES, Role of Botanic Gardens and Gene Banks

Conservation:

Conservation is the protection, preservation, management, or restoration of. There is an urgent need, not only to manage and conserve the biotic wealth, but also restore the degraded ecosystems.

Humans have been directly or indirectly dependent on biodiversity for sustenance to a considerable extent. However, increasing population pressure and developmental activities have led to large scale depletion of the natural resources.

Conservation is the protection, preservation, management, or restoration of wildlife and natural resources such as forests and water. Through the conservation of biodiversity and the survival of many species and habitats which are threatened due to human activities can be ensured. There is an urgent need, not only to manage and conserve the biotic wealth, but also restore the degraded ecosystems.

Mainly the conservation of biodiversity has three basic objectives:

(a) To maintain essential ecological processes and life supporting systems.

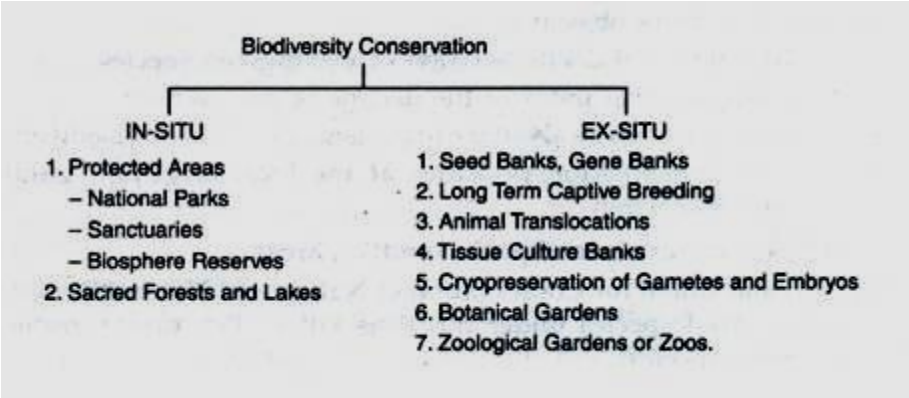
(b) To preserve the diversity of species.

(c) To make sustainable utilisation of species and ecosystems.

Types of Conservation:

Conservation can broadly be divided into two types:

1. In-situ conservation
2. Ex-situ conservation



1. In-Situ Conservation Strategies:

In-situ or on site conservation is conservation of wild animals and plants in their natural habitat. The aim of in-situ conservation is to allow the population to maintain or perpetuate itself within the community environment, to which it is adapted. In-situ conservation is the ideal method of conserving wild plant genetic resources. In-situ conservation of plant genetic resources presents a number of advantages as compared to ex-situ conservation.

Advantages of In-Situ Conservation of Plant Resources:

a. It enables the conservation of a large range of potentially interesting alleles.

b. This method is especially suitable for species, which cannot be established or regenerated outside the natural habitats.

c. This method allows natural evolution to continue because of the existence of variation.

d. It facilitates research on species in their natural habitats.

e. It assures protection of other species that are dependent on the species under consideration.

Methods of In-Situ Conservation:

In-situ conservation is done by providing protection to biodiversity rich areas through a network of protected areas. In India, the protected areas are of the following kinds – national parks, wildlife sanctuaries, biosphere reserves and ecologically fragile and sensitive areas. A protected area network

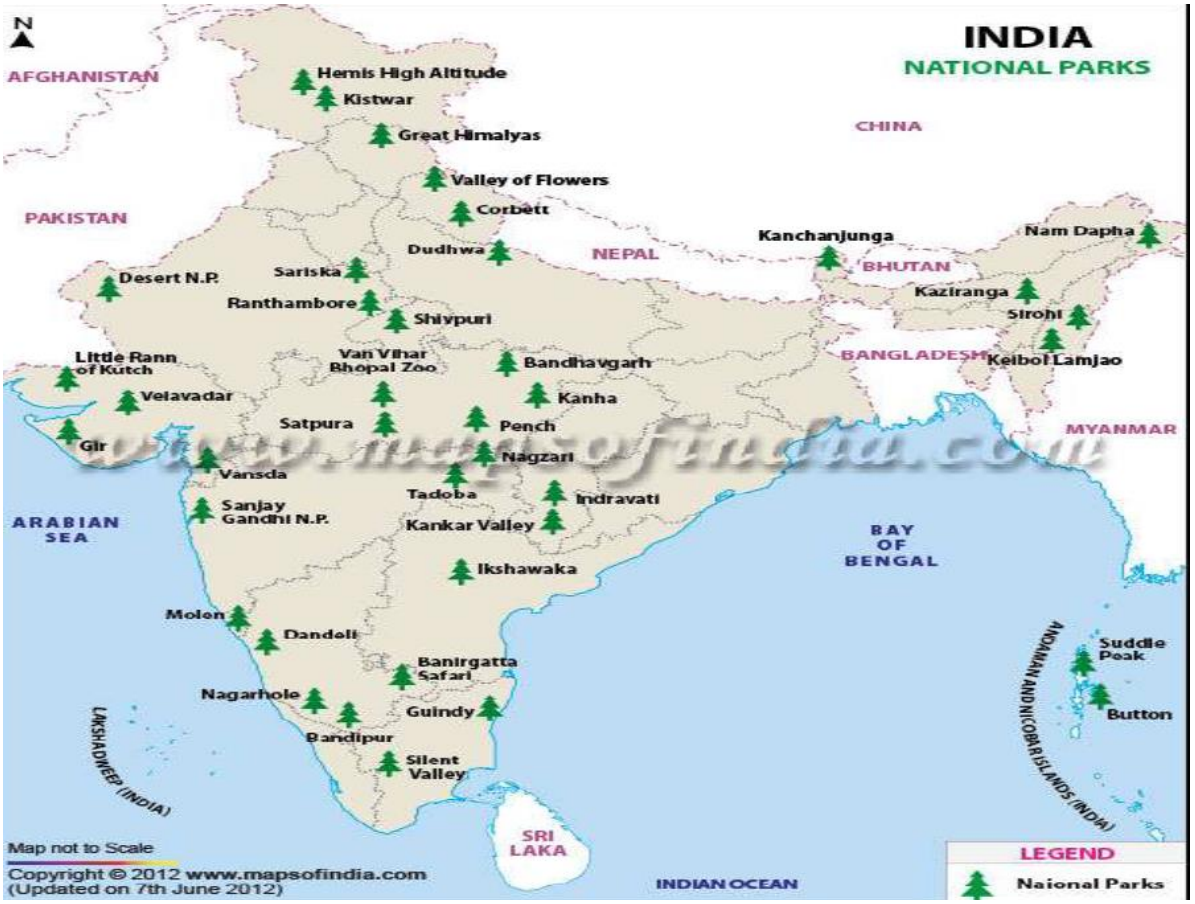
of 85 national parks and 448 wildlife sanctuaries has been created. The results of this network have been significant in restoring viable population of large mammals such as tiger, lion, rhinoceros, crocodiles and elephants.
Eco-development programmes involving local communities have been initiated recently for sustained conservation of ecosystems. The economic needs of the local communities are taken care under this programme through provision of alternative sources of income and a steady availability of forest and related products.
Programmes have also been launched for scientific management and wise use of wetlands, mangroves and coral reef ecosystems. Twenty-one wetlands and mangrove areas and four coral reef areas have been identified for intensive conservation and management purposes.
Six significant wetlands of India have been declared as 'Ramsar Sites' under the Ramsar Convention. Under the World Heritage Convention, five natural sites have been declared as 'World Heritage Sites'.
In India following types of natural habitats are being maintained:
1. National parks
2. Wildlife sanctuaries
3. Biosphere reserves
INDIA has over 600 protected areas, which includes over 90 national parks, over 500 animal sanctuaries and 15 biosphere reserves.

National Park:
A national park is a reserve of land, usually owned by a national government. It is a tract of land, which is declared public property to preserve and develop for the purpose of recreation and culture. It is protected from human development activities and pollution. National parks are protected areas of IUCN category II.

- There are 10 existing national parks in India covering an area of 38,024.10 km2, which is 1.16% of the geographical area of the country.
- Yellowstone National Park in California was established as the world's first protected area.
- The first national park in India was Hailey National Park, now known as Jim Corbett National Park, established in the year 1935.
- Largest National Park-Northeast Greenland National park,1979

Sl. No.	Name	State	Established	Area (in km^2)
1.	Corbett National Park	Uttarakhand	1921	1318.5
2.	Dudhwa National Park	Uttar Pradesh	1977	490.29
3.	Gir National Park	Gujarat	1965	258.71
4.	Kanha National Park	Madhya Pradesh	1955	940
5.	Kanger Ghati National Park (Kanger Valley)	Chhattisgarh	1982	200
6.	Kaziranga National Park	Assam	1974	471.71

7.	Nanda Devi National Park	Uttarakhand	1982	630.33
8.	Sariska National Park	Rajasthan	1955	866
9.	Silent Valley National Park	Kerala	1980	237
10.	Sundarbans National Park	West Bengal	1984	1330.12



Biosphere reserves:

Biosphere reserves are a special category of protected areas of land and/or coastal environments, wherein people are an integral component of the system.

The biosphere reserves are representative examples of natural biomes and contain unique biological communities.

The concept of Biosphere Reserves was launched in 1975 as a, part of UNESCO's 'Man and Biosphere Programme, dealing with the conservation of ecosystems and the genetic resources contained therein.

Till May 2002, there were 408 biosphere reserves dispersed in 94 countries.

In India, thirteen biodiversity rich areas have been designated as Biosphere Reserves applying the diversity and genetic integrity of plants, animals and microorganisms.

The biosphere reserve has concentric areas zoned for different use.

a. The **core zone** is the innermost zone devoted to preserve biodiversity with no human interference.
b. Around the core zone there is the **buffer zone** in which some settlement and resource use is allowed. In this area, variety of educational programmes and research activities are carried out, such as identifica-tion of endangered species, artificial propagation of species, and application of tissue culture techniques to enable rapid multiplication of threatened species.
c. The outermost zone is the **transition zone** where sustainable development activities are permitted. This is an area of interaction between the biosphere reserve management and the local people. Here activities such as forestry, recreation, cropping, etc. are permitted.
These reserves aim at conserving the biological diversity and genetic integrity of plants, animals and microorganisms in their totality as part of the natural ecosystems. There are approximately 400 biosphere reserves in 94 countries. The list of biosphere reserves in India is given in Table 6.

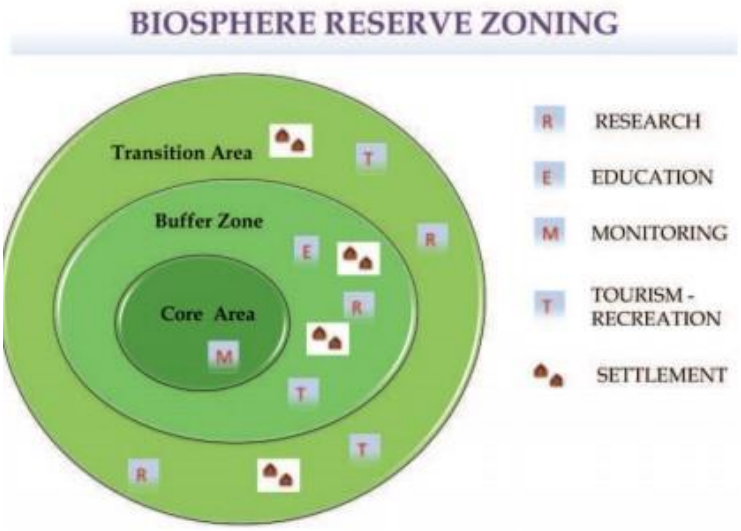


Table 6 List of biosphere reserves in India.

S. No.	Reserve	Location
1.	Great Nicobar	Andaman and Nicobar Islands
2.	Gulf of Mannar	Tamil Nadu
3.	Kanha	Madhya Pradesh
4.	Kaziranga	Assam
5.	Manas	Assam
6.	Namdapha	Arunachal Pradesh
7.	Nanda Devi	Uttar Pradesh
8.	Niligris	Tamil Nadu, Kerala and Karnataka
9.	Nokrek Tura	Meghalaya
10.	Rann of Kutch	Gujarat
11.	Sunderbans	West Bengal
12.	Thar Desert	Rajasthan
13.	Valley of flowers	Uttar Pradesh

The main functions of biosphere reserves are:
(i) Conservation:
To conserve the ecosystems, a biosphere reserve programme is being implemented, for example, conservation of landscapes, species and genetic resources. It also encourages traditional resource use.
(ii) Eco-Development:
The concept of eco-development integrates the ecological and economic parameters for sustained conservation of ecosystems by involving the local people with the maintenance of earmarked regions. Biosphere reserves are also used to promote economic development which is culturally, socially and ecologically sustainable.
(iii) Scientific Research Programme:
Programmes have also been launched for scientific management and wise use of fragile ecosystem. Specific programmes for management and conservation of wetlands, mangroves and coral reef systems are also being implemented.

Under this programme, 21 wetlands, 15 mangrove areas and 4 coral reef areas have been identified for management. National and sub-national level committees oversee and guide these programmes to ensure strong policy and strategic support.

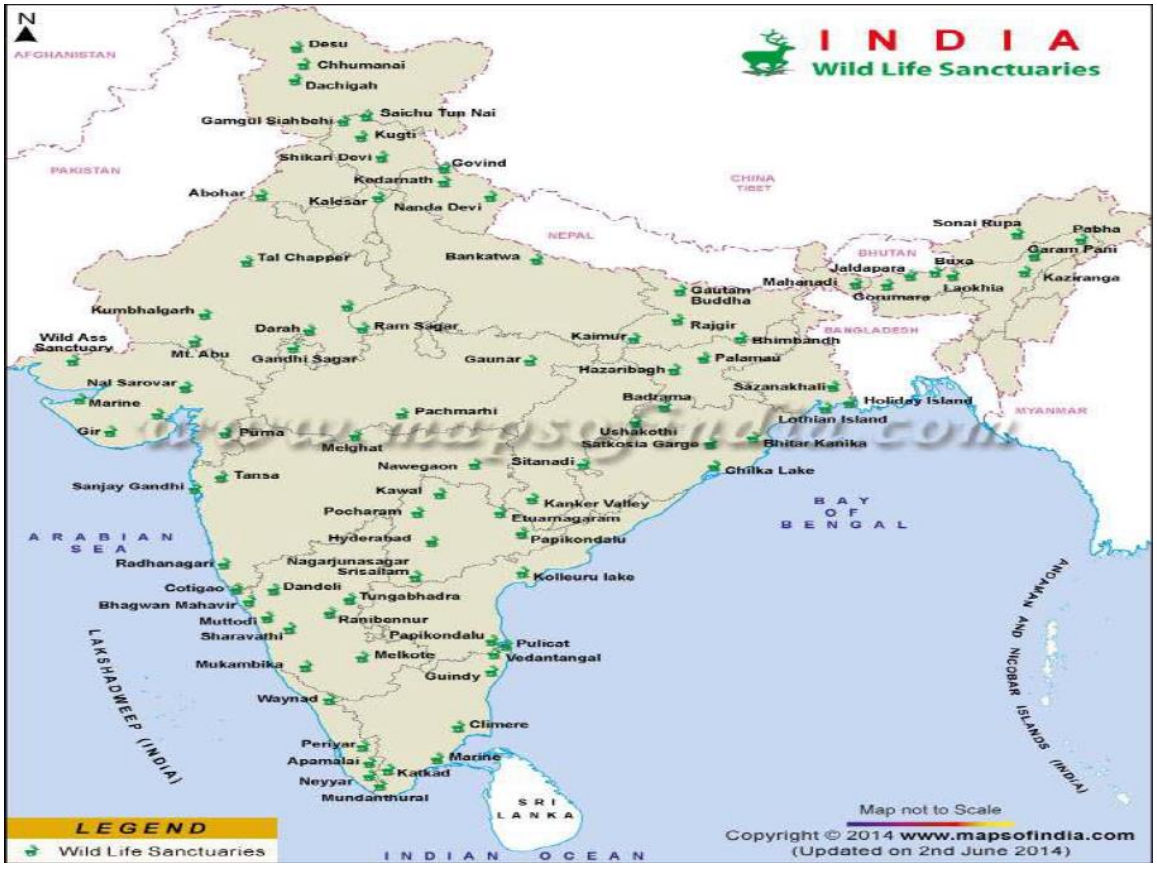
Wildlife Sanctuaries:

A sanctuary is a protected area which is reserved for the conservation of only animals and human activities like harvesting of timber, collecting minor forest products and private ownership rights are allowed as long as they do not interfere with well-being of animals. Boundaries of sanctuaries are not well defined and controlled biotic interference is permitted, e.g., tourist activity.

Wildlife sanctuaries of India

- IUCN category IV
- There are 515 wildlife sanctuaries in India
- 41 tiger reserves, governed by Project Tiger
- Largest sanctuary: Nagarjun sagar - srisailam tiger reserve (A.P,Telengana)

S.No.	Name	State	Established	Area (in km^2)
1.	Ghana Bird Sanctuary	Rajasthan	1982	28.73
2.	Hazaribag Wildlife Sanctuary	Jharkhand	1954	183.89
3.	Mudumalai Wildlife Sanctuary	Tamil Nadu	1940	321.55
4.	Jaldapara Wildlife Sanctuary	West Bengal	2012	216
5.	Mount Abu Wildlife Sanctuary	Rajasthan	1960	288.84
6.	Anamalai Wildlife Sanctuary (Indira Gandhi Wildlife Sanctuary and National Park)	Tamil Nadu	1989	117.10



World Heritage Site:

A World Heritage Site is a landmark or area which is chosen by the **United Nations Educational, Scientific and Cultural Organisation (UNESCO)** as having cultural, historical, scientific or other form of significance, and is legally protected by international treaties. The sites are judged important to the collective interests of humanity.

To be selected, a World Heritage Site must be an already-classified landmark, unique in some respect as a geographically and historically identifiable place having special cultural or physical significance (such as an ancient ruin or historical structure, building, city, complex, desert, forest, island, lake, monument, mountain, or wilderness area). It may signify a remarkable accomplishment of humanity, and serve as evidence of our intellectual history on the planet.

The sites are intended for practical conservation for posterity, which otherwise would be subject to risk from human or animal trespassing, unmonitored/uncontrolled/unrestricted access, or threat from local administrative negligence. Sites are demarcated by UNESCO as protected zones. The list is maintained by the international World Heritage Program administered by the UNESCO World Heritage Committee, composed of 21 "states parties" that are elected by their General Assembly.

The programme catalogues, names, and conserves sites of outstanding cultural or natural importance to the common culture and heritage of humanity. Under certain conditions, listed sites can obtain funds from the World Heritage Fund. The program began with the Convention Concerning the Protection of the World's Cultural and Natural Heritage, which was adopted by the General Conference of UNESCO on 16 November 1972. Since then, 193 state parties have ratified the convention, making it one of the most widely recognized international agreements and the world's most popular cultural program.

As of July 2019, a total of 1,121 World Heritage Sites (869 cultural, 213 natural, and 39 mixed properties) exist across 167 countries. China and Italy, both with 55 sites, have the most of any country, followed by Spain (48), Germany (46), France (45), India (38), and Mexico (35).

World Heritage Sites in India:

According to UNESCO World Heritage Sites located in India are 37, as of June 2018, the sixth most of any country.

Some e.g.: Ajanta cave, Maharastra; Agra Fort, Uttar Pradesh, Taj Mahal, Uttar Pradesh.

Ramsar Sites:

Six internationally significant wetlands of India have been declared as Ramsar Sites under the Ramsar Convention. To focus attention on urban wetlands threatened by pollution and other anthropogenic activities, state Governments were requested to identify lakes that could be include the National Lake Conservation Plan (NLCP).

Protected area:

A clearly defined geographical space, recognised, dedicated and managed through local or other effective means,to achieve the long term conservation of nature with associated ecosystem series and cultural values. **:(IUCN World Commission,2008)**

Protected areas – national parks, wilderness areas, community conserved areas, nature reserves and so on – are a mainstay of biodiversity conservation, while also contributing to people's livelihoods, particularly at the local level. Protected areas are at the core of efforts towards conserving nature and the services it provides us – food, clean water supply, medicines and protection from the impacts of natural disasters. Their role in helping mitigate and adapt to climate change is also increasingly recognized; it has been estimated that the global network of protected areas stores at least 15% of terrestrial carbon.

Helping countries and communities designate and manage systems of protected areas on land and in the oceans, is one of IUCN's main areas of expertise. Together with species conservation, this has been a key focus of attention of IUCN's work and of a vast majority of IUCN Member organizations. Effectively managed systems of protected areas have been recognized as critical instruments in achieving the objectives of the Convention on Biological Diversity and the Sustainable Development Goals.

IUCN Protected Area Management Categories:

Through its World Commission on Protected Areas (WCPA), the IUCN has developed six Protected Area Management Categories that define protected areas according to their management objectives, which are internationally recognised by various national governments and the United Nations.The categories provide international standards for defining protected areas and encourage conservation planning according to their management aims.

IUCN Protected Area Management Categories:

Category Ia — Strict Nature Reserve
Category Ib — Wilderness Area
Category II — National Park
Category III — Natural Monument or Feature
Category IV — Habitat/Species Management Area
Category V — Protected Landscape/Seascape
Category VI – Protected Area with sustainable use of natural resources

Protected area of India

India is one of the 17 mega diverse countries of the world.India is home to World's largest wild tiger population and has got unique assemblage of globally important endangered species like Asiatic lion,asian elephant,one horned rhinoceros,gangetic river dolphin,snow leopard,kashmir stag,gharial,great indian bustard etc.

A National Board for Wildlife(NBWL),chaired by the PM of India provides for policy frameworkfor wildlife conservation in the country.the National Wildlife Action Plan(2002-2016) has been adopted;emphasizing the peoples' participation & their support for wildlife conservation.

The main benefits of protected areas are:

a. To maintain viable populations of all native species and subspecies.
b. To maintain the number and distribution of communities and habitats. Conservation of the genetic diversity of all the existing species.
c. To prevent human caused introductions of alien species.
d. To make it possible for species and habitats and shift in response to environmental changes.

Ex-situ conservation:

Ex-situ conservation literally means, "off-site conservation". It is the process of protecting an endangered species of plant or animal by removing part of the population from a threatened habitat and placing it in a new location, which may be a wild area or within the care of humans. While ex-situ

conservation comprises some of the oldest and best known conservation methods, it also involves newer, sometimes controversial laboratory methods.

Ex situ conservation, using sample populations, is done through establishment of gene banks, whih include genetic resources centres, zoo's, botanical gardens, cultue collections etc.

Advantanges of Ex situ conservation:

The conservation of biodiversity can be achieved through an integrated approach balancing in situ and ex situ conservation strategies. The preservation of species in situ offers all the advantages of allowing natural selection to act, which cannot be recreated ex situ. The maintenance of viable and self-sustainable populations of wild species in their natural state represents the ultimate goal, but habitat destruction is inevitable and endangered species need to be preserved before they become extinct. Ex situ conservation provide the opportunity to study the biology of, and understand the threats to, endangered species in order to eventually consider successful species recovery programmes, which would include restoration and reintroduction. It also has the advantage of preserving plant material and making it available for research purposes, without damaging the natural populations. Their conservation ex situ is therefore complementary to in situ conservation and can act as an "insurance policy" when species are threatened in their natural habitats It is the process of protecting an endangered species of plant or animal by removing part of the population from a threatened habitat and placing it in a new location, which may be a wild area or within the care of humans.

Ex-situ conservation has several purposes:

- Rescue threatened germplasm.
- Produce material for conservation biology research.
- Bulk up germplasm for storage in various forms of ex situ facility.
- Supply material for various purposes to remove or reduce pressure from wild collecting.
- Grow those species with recalcitrant seeds that cannot be maintained in a seed store.
- Make available material for conservation education and display.
- Produce material for reintroduction, reinforcement, habitat restoration and management.

Some important areas under these conservation are:

Gene Banks:

Plant genetic resources gene banks store, maintain and reproduce living samples of the world's huge diversity of crop varieties and their wild relatives. They ensure that the varieties and landraces of the crops and their wild relatives that underpin our food supply are both secure in the long term and available for use by farmers, plant breeders and researchers.

Gene banks conserve genetic resources. The most fundamental activity in a gene bank is to treat a new sample in a way that will prolong its viability as long as possible while ensuring its quality. The samples (or accessions as they are called) are monitored to ensure that they are not losing viability. A cornerstone of gene bank operations is the reproduction-called regeneration-of its plant material. Plant samples must periodically be grown out, regenerated, and new seed harvested because, even under the best of conservation conditions, samples will eventually die.

To conserve and regenerate genetic resources, gene banks first must collect genetic resources. But gene banks aren't built just to conserve genetic resources; they are intended to ensure that these resources are used, whether it is in farmers' fields, breeding programmes or in research institutions. This means making sure the collections are properly characterized and documented; and that the documentation is available to those who need it. The information systems used by gene banks are becoming increasingly important tools for researchers and breeders seeking data on the distribution of crops and their wild relatives.

Purpose of Gene Banks

- Physical facilities for maintaining collections of live plant materials – domesticated cultivated plants – wild plant species (crop wild relatives and other wild plant species useful for food and agriculture and other end uses) – entire plants, seeds, pollen, embryos, meristems, cells, or DNA, depending on the biology of the species
- It is a facility for maintaining crop diversity.
- Usually this diversity is in the form of seeds, stored and conserved in a frozen state.

- Some gene banks use normal household freezers for this purpose.
- The ideal temperature is between -10and -20.
- Each different type is stored in its own container.
- Such as a bottle, a can or a sealed aluminum foil package.

Activities in Gene Bank

The main activities in the development and management of a gene bank include:

- **Collecting and Acquisition** – assembling the collection
- **Processing –** assessing the quantity, viability, health of samples
- and preparation for storage
- **Storage –** in a cold store, laboratory or in the field
- **Regeneration and Multiplication –** periodically rejuvenating and increasing the material
- **Characterization and Evaluation**
- **Documentation, Inventory –** maintaining and making available detailed records on each sample
- **Distribution –** of clean, disease-free seeds, or other planting material, to requestors

Seed Gene Bank:

- A place where germplasm is conserved in the form of seeds is called seed gene bank.
- Seeds are very convenient for storage because they occupy smaller space than whole plants.
- However, seeds of all crops can not be stored at low temperature in the seed banks.
- The germplasm of only orthodox species can be conserved in seed banks.
- In the seed banks, there are three types of conservation, viz.
  1) Short term,
  2) Medium term and
  3) Long term.
- Base collections are conserved for long term (50 years or more) at 18 or 20°C.
- Active collections are stored for short term (3-5 years) at 5-10°C.

Pollen Bank:

Pollen preservation may be useful for base collections of species that do not produce orthodox seeds.

- It requires little space but some cytoplasmic genes would be lost.
- Like seeds, pollen can be divided into desiccation tolerant and intolerant.
- However, information about storage characteristics of pollen from wild species is fragmentary, existing mainly for some crop relatives and for medicinal and forest species (Eberhart, Roos & Towill, 1991).

DNA Bank:

The creation of a network of DNA banks is ex situ conservation and more precisely germplasm collections can allow large quantities of genetic resources (genes, DNA) to be stored quickly and at low cost and could act as an insurance policy against rapid loss of the world's gene pool.

- It could be used in molecular phylogenetic and systematics of extinct taxa and genes can be distributed via the polypeptide chain reaction (PCR) using primers supplied by the users (Adams et al., 1994).
- DNA samples have been mainly used for bio-prospecting and assessment of biodiversity studies.
- Its use in conservation is limited as whole plants cannot be reconstituted from DNA but the genetic material can be introduced to other genotypes for plant breeding and enhancement purposes.

Botanical gardens:

Botanical gardens and zoos are the most conventional methods of ex-situ conservation, all of which house whole, protected specimens for breeding and reintroduction into the wild when necessary and possible. These facilities provide not only housing and care for specimens of endangered species, but also have an educational value. They inform the public of the threatened status of endangered species and of those factors which cause the threat, with the hope of creating public interest in stopping and reversing those factors which jeopardize a species' survival in the first place. They are the most publicly visited ex-situ conservation sites.

The history of botanic gardens can be traced as far back as the Hanging Gardens of Babylon, built by Nebuchadnezzar in 570 BC as a gift to his wife. Early botanic gardens were designed mainly for the purpose of recreation. By the 16th Century, however, they had also become important centers for research. They promoted the study of taxonomy and became a focal point for the study of aromatic and medicinal plants. More recently, they have taken on significant conservation responsibilities and they often have conservation facilities, such as seed banks and tissue culture units.

Botanical gardens hold living collections. Indeed botanical garden conservation could be considered as field gene bank or seed gene bank or both, depending on the conservation method being used. However, they tend to focus their conservation efforts on wild, ornamental, rare and endangered species. Most of the germplasm conserved in botanical gardens do not belong to the plant genetic resources for food and agriculture.

A botanic garden which wishes to start a small seed bank /gene bank would be advised to start with collecting germplasm that is very well documented from their living plant collection. This would allow them to experiment with a wide range of species and find suitable facilities and techniques for their particular needs. Once the set up is organised and functional, it would be advisable to collect accessions directly from the wild in order to distribute a wider genetic variability and to reduce the effect of domestication on the genetic make up of the accessions.

Last two hundred years, efforts of botanic gardens in collecting plant material, and the great efforts on crop germplasm collection during the 1970s and the 1980s, there are a large number of gene banks and germplasm collections around the world. According to the FAO and World Information & Early Warning System (WIEWS) database, it is estimated that there are now more than 2000 botanic gardens known around the world in over 150 countries. Together, they maintain more than 6 million accessions in their living collections and 142 million herbaria specimens in the botanic garden herbaria. 60% of the total numbers of accessions are known to be stored in medium-term or long-term facilities, 8% in short-term facilities and 10% in field gene banks, in vitro and under cryopreservation. Clearly, seed storage is the predominant form of plant genetic resource conservation, accounting for about 90% of the total accessions held ex situ.

It is very difficult to give an estimate of the type of collections stored around the world as such information is known for only a third of the accessions in the WIEWS database. However, it has been estimated that 48% of all accessions are advanced cultivars or breeders' lines, while over a third are landraces or old cultivars and about 15% are wild or weedy plants or crop relatives.

Some famous botanic gardens/ research centers/ institutes

International

1**. Royal Botanical Garden Kew, England :** Largest botanical garden in world and its herbarium is also largest in world, having 6 million specimen.

2. **CIAT :** International Center for Tropical Agriculture located at Palmira, Columbia

3. ICARDA : International Center for Agriculture Research in Dry Areas located at Allepo, Syria

4. ICRISAT : International Center for Agriculture Research for Semi Arid Tropics located at Patencheru, (Hyderabad) India.

5. IRRI : International Rice Research Institute located at Manila, Philippines.

National

1. **Indian Botanical Garden, Calcutta :** Largest Botanical Garden in India and its herbarium is largest in India, having 1 million specimen

2.NBRI : National Botanical Research Institute located at Lucknow (UP) formally known as National Botanical Garden.

3. BSI : Botanical Survey of India started working in 1890 and is connected with plant exploration and writing up of regional floras and also preparation of flora of India.

4. IARI : Indian Agricultural Research Institute or Pusa Institute located at New Delhi. It was initially established at village Pusa in Darbhanga District of Bihar in 1905 under the name Imperial Agricultural Research Institute. After a severe earthquake, this institute was shifted to New Delhi in 1936 under the same name. But after independence, it was renamed as Indian Agricultural Research Institute.

5. FRI : Forest Research Institute located at Dehradun (Uttarakhand), established in 1906 under name Imperial Forest Research Institute (IFRI), but after independence, name was changed to FI. This

institute is connected with researchers on different aspects of forest trees and also provides training to forest officer.

Royal Botanical Garden

Acharya Jagadish Chandra Bose Botanical Garden

Role of Botanical Garden:

Many of the functions of botanical gardens have already been discussed in the sections above, which emphasise the scientific underpinning of botanical gardens with their focus on research, education and conservation. However, as multifaceted organisations, all sites have their own special interests. In a remarkable paper on the role of botanical gardens, Ferdinand Mueller (1825–1896), the director of the Royal Botanic Gardens, Melbourne (1852–1873), stated, "in all cases the objects [of a botanical garden] must be mainly scientific and predominantly instructive". He then detailed many of the objectives being pursued by the world's botanical gardens in the middle of the 19th century, when European gardens were at their height. Many of these are listed below to give a sense of the scope of botanical gardens' activities at that time, and the ways in which they differed from parks or what he called "public pleasure gardens".

- availability of plants for scientific research
- display of plant diversity in form and use
- display of plants of particular regions (including local)
- plants sometimes grown within their particular families
- plants grown for their seed or rarity
- major timber (American English: lumber) trees
- plants of economic significance
- glasshouse plants of different climates
- all plants accurately labelled
- records kept of plants and their performance
- catalogues of holdings published periodically
- research facilities utilising the living collections
- studies in plant taxonomy
- examples of different vegetation types
- student education
- a herbarium
- selection and introduction of ornamental and other plants to commerce
- studies of plant chemistry (phytochemistry)
- report on the effects of plants on livestock
- at least one collector maintained doing field work

Botanical gardens must find a compromise between the need for peace and seclusion, while at the same time satisfying the public need for information and visitor services that include restaurants, information centres and sales areas that bring with them rubbish, noise, and hyperactivity. Attractive landscaping and planting design sometimes compete with scientific interests — with science now

often taking second place. Some gardens are now heritage landscapes that are subject to constant demand for new exhibits and exemplary environmental management.
Many gardens now have plant shops selling flowers, herbs, and vegetable seedlings suitable for transplanting; many, like the UBC Botanical Garden and Centre for Plant Research and the Chicago Botanic Garden, have plant-breeding programs and introduce new plants to the horticultural trade.

Cryopreservation:

In this system stability is imposed by ultra low temperature and storage is at, or close to -1960C using liquid Nitrogen (or the vapour immediately above it), as practical and convenient oxygen. At such temperature normal cellular chemical reactions do not occur as energy level are too low to allow sufficient molecular motion to complete the reaction. Water exists either in a crystalline or glassy state under these conditions and such high viscosity (> 1013 poises) that rates of diffusion are insignificant over time spans measured at least as decades. The majority of the chemical changes that might occur in a cell are therefore; effectively prevented and so the cell is stabilized the maximum extent that is practically possible.
Unfortunately, that is not to say that biological material successfully cooled to -1960C is in state of complete suspended animation. Certain type of chemical reaction can still occur at these temperatures, such as the formation of the free radicals and macromolecular damage due to ionizing radiation. The only real threat to genetic stability comes from such reactions, especially those that damage nucleic acids. Any damage that does occur will necessarily be cumulative as enzyme repair mechanisms are also totally inhibited at these low temperatures.
While there are as yet few quantitative data on genetic stability at ultra low temperature for higher organisms, studied by Ashwood, Smith and Grant (1977) indicate that to reach a D10 level (where D10 is the readiation dose resulting in 90% mortality of the population) a frozen cell population would have to be exposed to back ground radiation for some 32,000 years. It is also noteworthy that dimethyl sulphoxide, probably the most commonly used cryoprotectant in-vitro preservation and may aid in radiation damage.
The potential of conservation system for in-vitro material based upon cryogenic storage is therefore, clear and the technique has become relatively widely used.

Field Gene banks:

Field gene banks or living collections are the main conservation strategy for long-lived perennials, recalcitrant species and vegetatively propagated species. Their main limitation is that they take a great deal of space and are difficult to maintain and protect from natural disasters. They are susceptible to the spread of diseases and may suffer from neglect. Furthermore, out-breeders require controlled pollination for regeneration from seed. In many circumstances they are the only available option for the conservation of important germplasm. When displayed, the plants have an important educational value and can easily be accessed for research purposes.
The conservation of germplasm in field gene banks involves the collecting of materials and planting in the orchard or field in another location. Field gene banks have traditionally been used for perennial plants, including:

- Species producing recalcitrant seeds;
- Species producing little or no seeds;
- Species that are preferably stored as clonal material; and
- Species that have a long life cycle to generate breeding and/or planting material.

Field gene banks are commonly used for such species as cocoa, rubber, coconut, coffee, sugarcane, banana, tuber crops, tropical and temperate fruits, vegetatively propagated crops, such as wild onion and garlic, and forage grasses.

Tissue culture bank:
Cryopreservation of disease free meristems is very helpful. Long term culture of excised roots and shoots are maintained. Meristem culture is very popular in plant propagation as it's a virus and disease free method of multiplication.

Captive breeding:
Captive breeding is the process of maintaining plants or animals in controlled environments, such as wildlife reserves, zoos, botanic gardens, and other conservation facilities. It is sometimes employed to help species that are being threatened by human activities such as habitat loss, fragmentation, over hunting or fishing, pollution, predation, disease, and parasitism. In some cases a captive breeding program can save a species from extinction, but for success, breeders must consider many factors—including genetic, ecological, behavioral, and ethical issues. Most successful attempts involve the cooperation and coordination of many institutions.

Long term captive breeding:
The method involves capture, maintenance and captive breeding on long term basis of individuals of the endangered species which have lost their habitat permanently or certain highly unfavorable conditions are present in their habitat.

Animal Translocation:
Release of animals in a new locality which come from anywhere else.
Translocation is carried in following cases:
1. When a species on which an animal is dependent becomes rare.
2. When a species is endemic or restricted to a particular area.
3. Due to habit destruction and unfavorable environment conditions.
4. Increase in population in an area.

Zoological Gardens:
In zoos wild animals are maintained in captivity and conservation of wild animals (rare, endangered species). The oldest zoo, the Schonbrumm zoo which exists today also, was established in VIENNA in 1759.
In India, the 1st zoo came into existence at BARRACKPORE in 1800. In world there are about 800 zoos. Such zoos have about 3000 species of vertebrates. Some zoos have undertaken captive breeding programmes.

7 major organization involved in consrvation of biodiversity:

- **IUCN** (International Union for Conservation of Nature and Natural Resources)
- **CITES** (Convention on International Trade in Endangered Species of Wild Fauna and Flora, also known as the Washington Convention)
- **SSC** (Species Survival Commission)
- **CBD** (Convention on Biological Diversity)
- **TRAFFICS** (Trade Records Analysis of Flora and Fauna in Commerce)
- **WWF** (World Wide Fund for Nature)
- **WCMC** (World Conservation Monitoring Centre)

WWF (World Wide Fund for Nature):
The World Wide Fund for Nature (WWF) is an international non-governmental organization founded in 1961, working in the field of the wilderness preservation, and the reduction of human impact on the environment. It was formerly named the World Wildlife Fund, which remains its official name in Canada and the United States.
WWF is the world's largest conservation organization with over five million supporters worldwide, working in more than 100 countries, supporting around 1,300 conservation and environmental projects. They have invested over $1 billion in more than 12,000 conservation initiatives since 1995. WWF is a foundation with 55% of funding from individuals and bequests, 19% from government sources (such as the World Bank, DFID, USAID) and 8% from corporations in 2014.
WWF aims to "stop the degradation of the planet's natural environment and to build a future in which humans live in harmony with nature." The Living Planet Report is published every two years by WWF since 1998; it is based on a Living Planet Index and ecological footprint calculation. In addition, WWF has launched several notable worldwide campaigns including Earth Hour and Debt-

for-Nature Swap, and its current work is organized around these six areas: food, climate, freshwater, wildlife, forests, and oceans.

WWF has received criticism for its alleged corporate ties. It has also been accused of supporting paramilitary groups tasked with stopping poaching who are responsible for numerous human rights abuses.

Being famous as the onlypanda residing in the Western world at that time,its uniquely recognisable physical features and status as an endangered species were seen as ideal to serve the organization's need for a strong recognisable symbol that would overcome all language barriers.

LOGO

WWF's giant panda logo originated from a panda named *Chi Chi* that had been transferred from Beijing Zoo to London Zoo in 1958

Who works with WWF?

In carrying out its work, WWF cooperates with many partners, including UN organizations, IUCN, and development agencies such as USAID and the World Bank. WWF also works with business & industry partners.

WWF's mission:

- WWF's mission is to stop the degradation of the planet's natural environment and to build a future in which humans live in harmony with nature by,
- Conserving the world's biological diversity.
- Ensuring that the use of renewable natural resources is sustainable.
- Promoting the reduction of pollution and wasteful consumption.
- WWF focuses their efforts at multiple levels, starting with wildlife, habitats and local communities and expanding up through governments and global networks.

What does WWF do?

WWF's mission is to stop the degradation of our planet's natural environment, and build a future in which humans live in harmony with nature.

In order to achieve this mission, WWF focuses its efforts on two broad areas:

- Biodiversity
- Footprint

The first is to ensure that the earth's web of life - biodiversity - stays healthy and vibrant for generations to come. We are strategically focusing on conserving critical places and critical species that are particularly important for the conservation of our earth's rich biodiversity.

The second, is to reduce the negative impacts of human activity - ourecological footprint. We are working to ensure that the natural resources required for life -land, water, air - are managed sustainably and equitably.

2050 Biodiversity Goal

By 2050, the integrity of the most outstanding natural places on Earth is conserved, contributing to a more secure and sustainable future for all

2050 Footprint Goal

By 2050, humanity's global footprint stays within the Earth's capacity to sustain life and the natural resources of our planet are shared equitably.

Man and the Biosphere Programme (MAB):

Man and the Biosphere Programme (MAB) is an intergovernmental scientific programme, launched in 1971 by UNESCO, which aims to establish a scientific basis for the improvement of relationships between people and their environments.

MAB's work engages fully with the international development agenda—specially with the Sustainable Development Goals and the Post 2015 Development Agenda—and addresses challenges linked to scientific, environmental, societal and development issues in diverse ecosystems; from mountain regions to marine, coastal and island areas; from tropical forests to drylands and urban areas. MAB combines the natural and social sciences, economics and education to improve human livelihoods and

the equitable sharing of benefits, and to safeguard natural and managed ecosystems, thus promoting innovative approaches to economic development that are socially and culturally appropriate, and environmentally sustainable.

The MAB programme provides a unique platform for cooperation on research and development, capacity-building and networking to share information, knowledge and experience on three interlinked issues: biodiversity loss, climate change and sustainable development. It contributes not only to better understanding of the environment, but also promotes greater involvement of science and scientists in policy development concerning the wise use of biological diversity.

As of December 2018, 686 biosphere reserves in 122 countries, including 20 transboundary sites, have been included in the World Network of Biosphere Reserves. Today, all MAB reserves form the World Network of Biosphere Reserves (WNBR) which serves three different functions:

1. Conservation - to contribute to the conservation of landscapes, ecosystems, species and genetic variation;

2. Development - to foster economic and human development which is socially, culturally and ecologically sustainable; and

3. Logistic - to provide support for research, monitoring, education and information exchange related to local, national and global issues of conservation and development.

Fundamentally, the network of MAB Reserves aims to be an international tool to develop and implement sustainable development and to contribute towards the Millennium Development Goals. Physically, each MAB reserve should contain three zones: one or more core zones which are legally protected; and clearly identified buffer and transition zones. The transition zone may be referred to as an area of co-operation.

Supported by:

The United Nations Educational, Scientific and Cultural Organization (UNESCO) Man and the Biosphere (MAB) Programme.

Year of creation:

1971 (launch of MAB programme). The first MAB reserves were designated in 1976.

Coverage

Global network of 631 marine and terrestrial sites covering 119 countries, including 14 transboundary sites (Year: 2014).

Criteria:

According to the 1995 Statutory Framework for the World Network of Biosphere Reserves, for an area to be qualified for designation as a MAB reserve it should:

1. Encompass a mosaic of ecological systems representative of major biogeographic regions, including a gradation of human interventions;
2. Be of significance for biological diversity conservation;
3. Provide an opportunity to explore and demonstrate approaches to sustainable development on a regional scale;
4. Have an appropriate size to serve the three functions of biosphere reserves;
5. Include appropriate zonation of core area(s),buffer zone(s) and an outer transition area;
6. Provide organisational arrangements for the involvement and participation of a suitable range of inter alia public authorities, local communities and private interests in the design and carrying out the functions of a biosphere reserve; and
7. Make provisions for a) mechanisms to manage human use and activities in the buffer zone(s), b) a management policy or plan for the area as a biosphere reserve, c) a designated authority or mechanism to implement this policy or plan, and d) programmes for research, monitoring, education or training.

CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora):

CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora, also known as the Washington Convention) is a multilateral treaty to protect endangered plants and animals. It was drafted as a result of a resolution adopted in 1963 at a meeting of members of the International Union for Conservation of Nature (IUCN). The convention was opened for signature in 1973 and CITES entered into force on 1 July 1975. Its aim is to ensure that international trade in

specimens of wild animals and plants does not threaten the survival of the species in the wild, and it accords varying degrees of protection to more than 35,000 species of animals and plants. In order to ensure that the General Agreement on Tariffs and Trade (GATT) was not violated, the Secretariat of GATT was consulted during the drafting process.
As of 2018, Secretary-General of the CITES Secretariat is Ivonne Higuero.

CITES coverage & scope:

• 179 member countries
• Regulates international trade of 35,000+ listed species (live, dead, parts and derivatives)

Value of CITES trade

• Record of 13 million trade transactions
• Globally valued in billions of US Dollars

Examples:

– Queen conch: $ 60 MM/year
– Python skins = $ 1 BN/year
– Bigleaf mahogany = $ 33 MM/year

CITES Appendix:

CITES Appendix I

• Species threatened with extinction, which are or may be affected by trade
• International (commercial) trade in wild specimens is generally prohibited
• 3% of all listings (Conference of the Parties to decide)

CITES Appendix II

• Species not necessarily currently threatened with extinction, but may become so unless trade is strictly regulated to avoid utilization incompatible with their survival
• Also, species that resemble species already included in Appendix II
• International (commercial) trade is permitted but regulated
• 96% of all listings (Conference of the Parties to decide)

CITES Appendix III

• Species for which a country is asking Parties to help with its protection
• International trade is permitted but regulated (less restrictive than Appendix II)
• 1% of CITES trade (no CoP decision needed)

15. Let's sum up

- Ecology: branch of science that deals with interaction between living organisms with each other and their surroundings. Ecological systems are studied at several different levels from individuals and populations to ecosystems and biosphere level.
- Within the discipline of ecology, researchers work at four specific levels, sometimes discretely and sometimes with overlap: organism, population, community, and ecosystem.
- There are many practical applications of ecology in conservation biology, wetland management, natural resource, city planning, community health, economics, basic and applied science, and human social interaction.
- Descriptive describes organisms and their interactions within ecosystems. This is the foundation of all ecological science. Functional studies proximate causes, the dynamic responses of populations and communities to immediate factors of environment. Evolutionary considers organisms and the relationships between organisms as historical products of evolution.

- A hypothesis can be defined as a tentative explanation of the research problem, a possible outcome of the research, or an educated guess about the research outcome.
- Probabilistic Ecological Models deal with the statistical probability of occurrence of certain phenomenon. Probabilistic Models are based on the exact knowledge of the most desirable information.
- Statistical approaches provide the means of understanding a process with some specified level of uncertainty. In statistics, an unknown true characteristic of a system is called a parameter.
- Remote sensing as a tool opened up new vistas of perception of things that exist too far or on extensive spatial scales. Nowadays there is a big assortment of satellite systems actively recording information about the Earth.
- Climate diagrams are brief summaries of average climatic variables and their time course. They have proven useful for a wide range of sciences, industry, teaching & are useful for planning and design.
- Abiotic factors are the non-living parts of an environment. These include things such as sunlight, temperature, wind, water, soil and naturally occurring events such as storms, fires and volcanic eruptions. Biotic factors are the living parts of an environment, such as plants, animals and micro-organisms.
- Habitat is a set of the place of environmental conditions in which particular organism lives and adapt the situation accordingly. A niche is nothing but an idea or role played by organisms that how they can live in an environment including their diet, shelter, etc. Mainly niche is concerned with the factor of gaining energy by organisms and supplying it to other, in the ecosystem.
- Population ecology is a sub-field of ecology that deals with the dynamics of species populations and how these populations interact with the environment. It is the study of how the population sizes of species change over time and space. Some of the most important characteristics of population are population density, natality, mortality, population growth and age distribution of population.
- A plant community is a collection or association of plant species within a designated geographical unit, which forms a relatively uniform patch, distinguishable from neighboring patches of different vegetation types. The components of each plant community are influenced by soil type, topography, climate and human disturbance.
- Each biotic community consists of very diverse organisms belonging to different kingdoms of living things. The number of species and abundance of population in communities also vary greatly. The organisms in a community depend upon each other as well as upon the non-living environment for food, shelter and reproduction.
- A diversity index (also called phylogenetic indices or phylogenetic metrics) is a quantitative measure that reflects how many different types (such as species) there are in a dataset (a community) and that can simultaneously take into account the phylogenetic relations among the individuals distributed among those types, such as richness, divergence or evenness.
- Ecological succession is the process of change in the species structure of an ecological community over time. The time scale can be decades, or even millions of years after a mass extinction. The community begins with relatively few pioneering plants and animals and develops through increasing complexity until it becomes stable or self-perpetuating as a climax community.

- Ecosystem may be defined as the system resulting from the integration of all living and non-living factors of the environment. An ecosystem has two major components; biotic and abiotic

- The bulk of any ecosystem is plants compared to which only a small fraction is animal life. Therefore, measurement of energy of an ecosystem primarily involves plants. And because plants represent the first or primary trophic level, plant production is measured as Gross Primary Production, which is equivalent to the energy fixed during photosynthesis.

- A food chain may be defined as the transfer of energy and nutrients from the source in plants through a series of organisms with repeated processes of eating and being eaten. Food chains are not always simple and isolated but are interconnected with one another. The interlocking pattern of food chains or a matrix of food chains, with all sorts of short circuits and connections is often called the food web or food net.

- Ecological pyramids are the graphic representations of trophic levels in an ecosystem. They are pyramidal in shape and they are of three types.

- Scientists divide ecosystems into terrestrial and non-terrestrial. Ecosystems may be further classified by their geographical region and dominant plant type six primary terrestrial ecosystems exist: Tundra, Taiga, Temperate deciduous forest, Tropical rain forest, Grassland; and Desert.

- Freshwater ecosystems are a subset of Earth's aquatic ecosystems. They include lakes and ponds, rivers, streams, springs, bogs, and wetlands. They can be contrasted with marine ecosystems, which have a larger salt content. Freshwater habitats can be classified by different factors, including temperature, light penetration, nutrients, and vegetation.

- Biogeography is the study of the distribution of species and ecosystems in geographic spaceand through geological time. Organisms and biological communities often vary in a regular fashion along geographic gradients of latitude, elevation, isolation and habitat area.

- Biodiversity is defined as the intrinsically-inbuilt plus the externally-imposed variability in and among living organisms existing in terrestrial, marine and other ecosystem at a specific period of time.

- The term megadiverse country refers to any one of a group of nations that harbour the majority of Earth's species and high numbers of endemic species. Conservation International identified 17 megadiverse countries in 1998. A biodiversity hotspot is a biogeographic region with a significant reservoir of biodiversity that is under threat from humans.

- Biodiversity is considered as a reservoir of resources to be used for the manufacture of food, medicine, industrial products, etc. But with an increased demand of rapid population growth, biodiversity is gradually depleting.

- The IUCN Red list of threatened species is also known as the IUCN Red list or Red data list. Species are classified by the IUCN Red list into nine groups, set through criteria such as rate of decline, population size, area of geographic distribution, and degree of population and distribution fragmentation.

- 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. Conservation can broadly be divided into two types in-situ conservation and ex-situ conservation.

- Botanical gardens and zoos are the most conventional methods of ex-situ conservation, all of which house whole, protected specimens for breeding and reintroduction into the wild when

necessary and possible. These facilities provide not only housing and care for specimens of endangered species, but also have an educational value.

- WWF is an international non-governmental organization founded in 1961, working in the field of the wilderness preservation, and the reduction of human impact on the environment. It was formerly named the World Wildlife Fund, which remains its official name in Canada and the United States.
- MAB is an intergovernmental scientific programme, launched in 1971 by UNESCO, which aims to establish a scientific basis for the improvement of relationships between people and their environments.
- CITES is a multilateral treaty to protect endangered plants and animals. It was drafted as a result of a resolution adopted in 1963 at a meeting of members of the IUCN.

14. Suggested Reading

1. Odum, E.P. (2005). Fundamentals of ecology. Cengage Learning India Pvt. Ltd., New Delhi. 5th edition.
2. Sharma, P.D. (2010). Ecology and Environment. Rastogi Publications, Meerut, India. 8th edition.
3. Shukla, R.S. & Chandel, P.S. Plant Ecology, Latest Ed., S. Chandel and Co.
4. Singh, J.S., Singh, S.P., Gupta, S. (2006). Ecology Environment and Resource Conservation. Anamaya Publications, New Delhi, India.
5. Wilkinson, D.M. (2007). Fundamental Processes in Ecology: An Earth Systems Approach. Oxford University Press. U.S.A.
6. Kormondy, E.J. (1996). Concepts of ecology. PHI Learning Pvt. Ltd., Delhi, India. 4th edition.
7. Chapman, J. L. & Reiss, M. J. 1999. Ecology Principles and Applications. Cambridge University Press, U.K.
8. Krishnamurthy, K.V. An Advanced Text Book on Biodiversity, 2003, Oxford & IBH Publishing Co. Ltd. Coyle.
9. http://www.biologydiscussion.com/
10. https://en.wikipedia.org/

15. Assignment

1. What is ecotone?
2. Who coined the term ecology?
3. Write about biotic factor of ecosystem.
4. Define character displacement.
5. Write the difference between Niche Width and Overlap?
6. Define deforestation.
7. What is net primary productivity?
8. Write a short note on remote sensing.
9. What is meant by climate diagram?
10. How fundamental niche and realized niche can be differentiated to each other?
11. Give examples of inverted pyramids.
12. Write short notes on any two:
    (a) Ecological pyramid
    (b) Food web
13. Name a few biotic and abitoic components of an ecosystem.
14. Explain the analytic characters of community.
15. What is key stone species?
16. What are the different zone of Biosphere reserves? Mention its role in ex-situ conservation.
17. Give an account of general process of succession in nature.

18. What are the different types of speciation
19. Distinguish between allopatric and sympatric speciation.
20. What is guilds?
21. What is meant by carrying capacity?
22. Explain different characteristics of population ecology.
23. Differentiate between r and k-selection.
24. Give an account on survivorship curves.
25. What is physiognomy?
26. Describe the normal biological spectrum of Raunkiaer based on the position of buds.
27. What is succession? Describe the causes, trends and basic types of succession.
28. Give an account of general process of succession in nature.
29. Differentiate between autogenic and allogenic succession.
30. What is species diversity?
31. Write a short note on 'hot spots in India'.
32. Describe values and threats of biodiversity.
33. India has a rich biodiversity status. Explain?
34. What are the strategies of ex-situ conservation?
35. What are the appendices of CITES?
36. Explain different categories of IUCN with suitable diagram
37. When did oldest and recent mass extinction events occur in geological history?
38. What is meant by cryopreservation?
39. Explain different strategies of in-situ conservation with example.
40. Discuss about major biogeographical region of India.

All the materials are self writing and collected from ebook, journals and websites.

POST GRADUATE DEGREE PROGRAMME
(CBCS)
IN
BOTANY

SEMESTER - III

Course: BOSCT 3.1

(Environmental Biology)

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING
UNIVERSITY OF KALYANI
KALYANI – 741 235,
WEST BENGAL

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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 UGC Regulations, 2017 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.) Sankar Kumar Ghosh, 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.

Prof Manas Mohan Adhikary
Director
Directorate of Open and Distance Learning
University of Kalyani

SYLLABUS
COURSE – BOSCT3.1
Environmental Biology

(Full Marks – 75)

Course	Group	Details Contents Structure		Study hour
BOSCT3.1	Environmental Biology	**Unit 1. Introduction and The biosphere**	An introduction to environmental biology. The biosphere- The terrestrial biomes and the aquatic biomes.	1
		Unit 2. Basic ecological concepts and Biogeochemical cycles	Basic ecological concepts and processes- Energy in ecosystem, food chain and food web. Biogeochemical cycles- Concept, gaseous and sedimentary cycle, nitrogen, Sulphur and phosphorous cycles.	1
		Unit 3. Environmental pollution and Air pollution	Environmental pollution- Pollution and pollutant- Concept, definition and characteristics. Air pollution- Source and types of air pollutant and their chemistry, photochemical reactions, green house and global warming, O3 depletion, acid rain, air pollutant in India	1
		Unit 4. Water pollution and Electronic waste	Water pollution- Source and type of water pollution, effect of water pollution on ecosystem, heavy metals and their effect on biota, nuclear pollution and thermal pollution. Electronic waste (e waste), sources and types, constituents of e waste, recycling of e waste, impact of e waste on environment and its management.	1
		Unit 5. Soil pollution and Pesticides	Soil pollution- Sources and classes of soil pollutants and their environmental effects, solid waste-pollution and disposal problems, waste- effect disposal and management. Pesticides, classification, chemical pollution, effect on living organisms.	1
		Unit 6. Environmental toxicology, laws and policies	Environmental toxicology- Principles and mechanisms. Environmental laws and policies.	1

Content

Environmental Biology

Soft Core Theory Paper **Credits: 2**

Content Structure:

1. Introduction
2. Objectives
3. An introduction to environmental biology.
4. The biosphere- The terrestrial biomes and the aquatic biomes.
5. Basic ecological concepts and processes- Energy in ecosystem, food chain and food web.
6. Biogeochemical cycles- Concept, gaseous and sedimentary cycle, nitrogen, Sulphur and phosphorous cycles.
7. Environmental pollution- Pollution and pollutant- Concept, definition and characteristics
   a. Air pollution- Source and types of air pollutant and their chemistry, photochemical reactions, green house and global warming, O3 depletion, acid rain, air pollutant in India
   b. Water pollution- Source and type of water pollution, effect of water pollution on ecosystem, heavy metals and their effect on biota, nuclear pollution and thermal pollution
   c. Electronic waste (e waste), sources and types, constituents of e waste, recycling ofe waste, impact of e waste on environment and its management
   d. Soil pollution- Sources and classes of soil pollutants and their environmental effects, solid waste-pollution and disposal problems, waste- effect disposal and management
   e. Pesticides, classification, chemical pollution, effect on living organisms.
8. Environmental toxicology- Principles and mechanisms.
9. Environmental laws and policies.
10. Suggested reading
11. Assignment

1. Introduction

Environmental Biology is increasingly gaining attention, especially in these environmentally- conscience times. Malaysia enjoys the luxury of a rich biodiversity in a relatively small area. Its effective preservation and conservation requires a strong understanding and appreciation of the environment. Students are trained in fundamental and practical aspects of ecology and the environment. The interdisciplinary field of environmental biology focuses on the relationships among plants, animals and their surroundings, including their responses to environmental stimuli. Environmental biology is closely linked to and often coupled with evolutionary biology, since both involve an exploration of how organisms adapt to changing conditions.
Environmental biologists may specialize in a single ecosystem, such as wetlands or forests, or in the human-wildlife interface created through development, agriculture and other man- made systems. Environmental biologists often work to preserve natural landscapes and biodiversity, protect wildlife populations and reverse

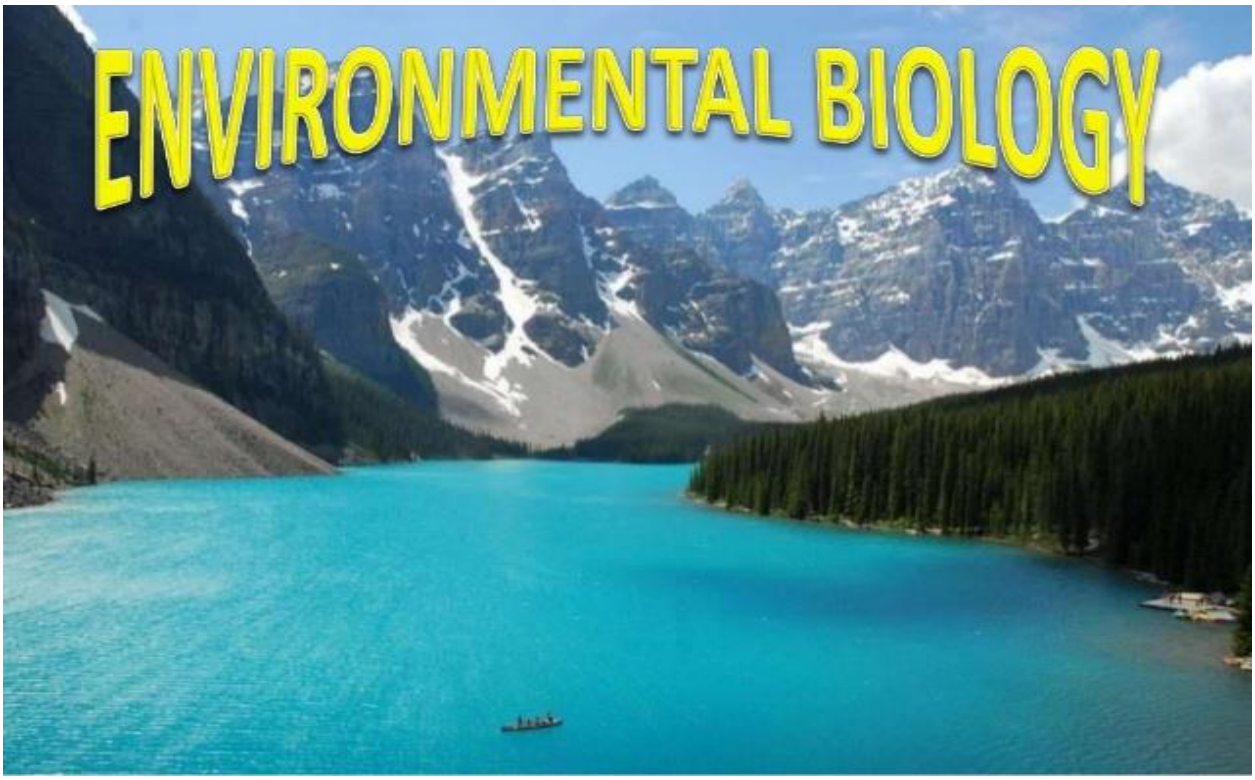
ecosystem degradation.

2. Course Objectives

A student who has completed the course should have solid knowledge of:

1. Explain the ideas of environmental biology
2. Differentiate between terrestrial and aquatic biome
3. To understand about environmental pollution
4. Gathering knowledge about energy in ecosystem, food chain and food web.
5. Describe principles and mechanisms of environmental toxicology
6. To know about environmental laws and policies

3. An Introduction to Environmental Biology



What is Ecology :

This book is about ecology and conservation. Ecology is the study of organisms in their natural surroundings. The word ecology comes from two Greek words – oikos meaning home and logos meaning understanding. So ecology is all about understanding the homes of animals, plants and other organisms. The surroundings of an organism are known as its environment.

Environments consist of many components including both physical features, such as climate and soil type, and biological features, such as predators and prey. The term environmental biology has wider connotations than ecology because it includes the study of humans in the environment, so you will find such subjects as agriculture, pollution and the unnatural surroundings we create in this book too. Understanding the ecology of an area is like trying to put together a gigantic, multidimensional jigsaw. Some pieces are the individual species in the area. In an oak wood, for example (figure 1.1), the species might include bluebells, oak trees, earthworms, snails, hedgehogs, wood ants and tawny owls. Other pieces in the jigsaw are the important aspects of the physical environment, for example the pH of the rainwater, the total amount that falls in a year, how it is distributed throughout the seasons, and significant information about the temperature, sunlight and soil type. The jigsaw pieces interlock with one another in numerous, subtle ways. In many ways ecology is a relatively new science. Indeed, the word was only coined by the German biologist Ernst Haeckel in 1869, fully ten years after Charles Darwin published his theory of natural selection. Yet, in little over a century ecology has grown to become one of the most important disciplines within biology. Like all branches of science, it has its own language. This includes the terms habitat, population, community and ecosystem.

A habitat is the place where an organism lives. The word is Latin and literally means ‘it dwells’. Actually, organisms from a single species can live in a number of habitats. For example, the common rat (Rattus norvegicus) is typically found associated with farms, refuse tips, sewers and warehouses. However, it also occurs in hedgerows close to cereal crops or sugar beet, and in salt marshes. On islands (e.g. the Isle of Man, Rhum and Lundy) rats also occupy grassland and the sea shore. With small organisms, especially those living in a restricted area such as in the soil or on a single plant or animal, it is worth being more precise about exactly where they live.

The term microhabitat – 'a small habitat' – is used to describe this. A single habitat may have many microhabitats. For example, if you are an insect living on an oak tree, life is very different depending on whether you live on the upper surface of the leaves, the lower surface of the leaves or inside them. It is even more different if you live under the bark, next to the roots or inside an acorn. Each of these different places is a microhabitat. A niche is a complete description of how the organism relates to its physical and biological environment. Just as in a jigsaw puzzle each piece has its own unique shape and pattern, and only fits in one place, so each species has a unique niche – the way it fits into its environment.

Consider a particular species, the grey heron (*Ardea cinerea*). Its habitats are water meadows, rivers, lakes and the sea shore. A complete account of its niche would include a description both of its physical environment (such as the type of water it needs, the temperature range in which it can survive and reproduce) and of its biological environment (such as the prey it eats, its competitors and the vegetation it needs for its nest). It is difficult to provide a quantitative description of an organism's niche.

Polioptila caerulea, a North American bird. This is an insectivore and the horizontal axis shows the length of the insects on which it feeds. The vertical axis shows the height above ground at which it forages. The contour lines with numbers indicate the frequency with which the birds feed at a particular height and on a particular length of prey. You can see that the birds concentrate on prey 4 mm in length, which they catch about 3–6 m off the ground. However, there are many other aspects to an organism's niche in addition to its feeding niche. In theory, other axes could be added at rightangles to those. Temperature could be shown on a third axis, risk of predation at different times of the year on a fourth, height above ground of the bird's nest on a fifth, and so on. In practice, though, no more than two or three axes can be shown on a graph.

Computers, however, can store and compute data for many more. The ecological principle that each species has its own unique niche and that no two species can coexist if they occupy the same niche is known as Gause's competitive exclusion principle. The biologist G. F. Gause gets the credit because of his research on single-celled ciliates in the genus Paramecium. A population is a group of individuals within a species that have the opportunity to breed with one another because they live in the same area at the same time. It follows from this definition that individuals from two different species cannot belong to the same population. This is because, with occasional exceptions, species are reproductively isolated from one another. Tawny owls do not breed with short-eared owls, for example. Most species are divided into many populations that are geographically separated. Bluebells in one wood, for example, will belong to a different population from the bluebells in another wood several kilometres away. Indeed, in a large wood there may be several populations of bluebells, though the boundaries between populations may be somewhat arbitrary.

A community is an association of species that live together in some common environment or habitat. Most communities are composed of a mixture of prokaryotes, protoctists, fungi, plants and animals. The organisms in a community interact with one another in all sorts of ways. For a start, there will be feeding relationships. In most communities, autotrophs (also known as producers and comprising green plants, photosynthetic algae, photosynthetic bacteria and chemosynthetic bacteria) provide food for herbivores (also known as primary consumers). In turn, herbivores are eaten by first-level carnivores (also known as secondary consumers), and these may be eaten by second-level carnivores (or tertiary consumers). Eventually organisms die and their remains are broken down by decomposers. These feeding relationships can be represented by food chains or by food webs that show the interrelationships between the various food chains in a community. The species in a community also interact with one another in other ways. They may rely on one another for reproduction, as is the case in insect pollinated plants. Or one species may act as a home for another, as a humpback whale carries barnacles. Or the interaction may be more subtle – all the species in a wood land, for example, rely on the activities of the various soil organisms which recycle nutrients.

The term 'community' is a valuable one in ecology. However, in 1935 Sir Arthur Tansley invented the term ecosystem because he realised that the organisms that make up a community cannot realistically be considered independently of their physical environment. The term ecosystem, therefore, applies to a community of organisms and its associated physical environment. There is one other feature of ecosystems and their associated communities worth stressing. This is that ecosystems are dynamic. Indeed, some ecosystems change as new species invade and others die out. A grassland invaded by shrubs and trees will change gradually as scrubland and then woodland develops. In a mature ecosystem, such as oak woodland, the population sizes and activities of the different species will alter from season to season and year to year. The bluebells flower so beautifully in spring, but by late summer they have set seed, the leaves have died back and the bluebell bulbs are ready to lie dormant until the next spring.

Humans in the environment

We have given ourselves a Latin binomial, Homo sapiens, just like all the other species we have classified. However, it is obvious that the impact humans have on the environment is unlike that of any other species. Ancient humans evolved in Africa and migrated out into Asia and Europe a million or more years ago. A second wave of migration of modern humans spread out of Africa about 130 000 years ago resulting in the colonisation of every continent. Before humans evolved, of course, all the communities in the world were natural. In Britain, natural vegetation during the Ice Ages was treeless Arctic grassland called tundra; during warmer interglacials, after the ice sheets melted, trees invaded. In the south, forests of oaks, ash, lime and hornbeam grew; in the Scottish highlands, the main vegetation was Scots pine conifer forest. These mature forests are called the climax vegetation, but such vegetation is now rare due to human activities.

Humans learned to make and use fire early in their history, about half a million years ago – very useful during Ice Ages! Before this, only lightning started wildfires that had the potential to damage vegetation. About 10 000 years ago humans also began to change the natural vegetation by cultivating crops. Animals were domesticated at about the same time. Captive animals graze areas of vegetation in much greater densities than natural animal populations do. The practice of burning and grazing led to the vegetation in many areas of the world developing into grasslands.

As human populations grew, their dwellings – in villages, towns and then cities – also restructured or even destroyed the vegetation. As humans are animals, human population biology might be expected to follow the same rules as those of other animal populations (figure 1.3a and Biology 2, chapter 3). In other animal species, the population initially grows at a rate of increase related to the reproductive rate of the species. Plotting the log of the numbers of individuals in the population against a linear plot of time gives a straight line (figure 1.3b). Eventually there will be competition for resources that are in limited supply. This competition is intraspecific because it occurs between individuals belonging to the one species. The result of this increasing competition is that the population growth slows down. Eventually the population should reach the maximum size that the environment can sustain, a figure known as the environment's carrying capacity. The population may overshoot the numbers the environment can support, but will then fall to stabilise at the carrying capacity.

However, human population biology is more complex, and seems to have gone through different phases of growth. As you can see from figure 1.4, anthropologists and archaeologists think that the world's human population was stable, or only rising very slowly, up to about 10 000 years ago and that it was rather small – somewhere between 5 and 10 million. Archaeological evidence indicates that about 10 000 years ago the

population started to rise more rapidly; there was a change in the rate of increase of population and in the carrying capacity. This reflects the change from mobile gatherer-hunter societies towards a more stationary agricultural lifestyle and the gradual development of the first towns and cities. There was another change in about 1750 with the onset of the industrial revolution. Since then world population has continued to rise sharply. On 12th October 1999, the world population officially reached 6 billion. That's six thousand million of us. Every day, the number increases by about 250 000. In other words, each day a quarter of a million more people are born than die. We in the West are used to thinking that this is a problem of developing countries. It is true that most industrialised countries, such as the UK, the USA and France, have population growth rates that are low compared to those in other countries. Bangladesh's population, for example, is growing 12 times faster than that of the UK. Yet the average person in the industrialised world uses about 60 times more resources than someone in the developing world. What is the carrying capacity of the UK for people? The current population of the UK is 59 million, but we have to import a large proportion of our food.

Under intensive cultivation, agricultural self-sufficiency could support around 41 million people. In other words, given a population of 41 million we should be able to provide all our nutritional needs, provided we carried on farming intensively using fertilisers and pesticides. A less intensive use of our land, which might prevent the net loss of soil through soil erosion, would probably mean a population of, at most, 35 million. So the carrying capacity of the UK estimated from food supply may be somewhere between 35 and 41 million people.

However, if we had to rely on renewable energy sources (wind, solar, tidal, wave and geothermal) rather than on fossil fuels (coal, gas, oil and peat) or nuclear power we would probably have to reduce our population to 15–20 million. Such a reduction may seem far-fetched, though it is interesting to note that only immigration is preventing the populations of many industrialized Western European countries from falling. It has been argued that the quality of life would be much better in the UK if there were only half or a third the number of people there are today. Imagine if this were the case. There would be less pollution, more room for wildlife and no more getting stuck in traffic jams.

The effects of human activity Agriculture

Britain during the last Ice Age was treeless. Scotland and northern England were covered in a great ice sheet and the south of England was a cold, windswept landscape. After the climate warmed and the ice melted, trees colonized the area from southern and eastern Europe and dense woodland developed. At lower latitudes, in the tropics, the climate became warmer and wetter and tropical forests flourished. The 'magic' date of 10 000 years ago, when plants and animals started to be domesticated and the human population began to rise significantly marks the end of the last Ice Age and the start of the warm period we live in today. Past warm periods, the interglacial, lasted about 12 000–15 000 years.

We do not yet know if we are living in an interglacial, as we do not know if there is another glacial coming, when global temperatures will fall and the ice caps expand over Britain. At the moment humans are more concerned with global warming than global cooling. In the Near East 10 000 years ago a quiet revolution was about to take place. Archaeological excavations have revealed villages with evidence of early cereal crops and herded sheep and goats: farming had begun. As farming spread, human lifestyles changed and population densities increased (see pages 3–5). Gradually the natural vegetation of many areas was modified and replaced due to the action of farmers grazing their animals and planting crops. Agriculture seems to have first made an impact in Britain between 6000 and 5000 years ago. The gradual replacement of natural vegetation as a result of cultivation occurred throughout the next 3000 years with the introduction of ploughs, then better ploughs, and the increasing use of animals for ploughing and transport, milk and wool. By the time William I had the census taken which is recorded in the Domesday Book of 1086, only about 15% of England retained its original woodland. Intensive farming is now the normal method of food production in most of Europe and North America. Some of the biosocial consequences of modern agricultural practices are discussed in chapter 2. Not every culture farms intensively. There are some groups, including the many small tribes of the Amazon basin and the Inuit of the Arctic, who still live in ways similar to those of our ancestral gatherer-hunters. Some, like the Dinka and Maasai in Africa, and nomads in the Middle East and Mongolia, herd animals, be they cattle, goats, camels or horses. Many groups in South America, India, Africa and elsewhere grow mixtures of crops local to their area such as maize, cassava, sorghum, rice, vegetables and fruits, to meet their immediate needs.

Whatever way of life a group has, whether gatherer-hunter or of industrial complexity, it is important that their way of life is sustainable. Hunters must never overhunt their prey, gatherers must leave enough seed for the next harvest, herds must not damage their grazing land beyond recovery, villagers must not take all the trees for firewood, intensive farming must not lead to soil erosion and dustbowl creation, and industry must not pollute the land, rivers or seas beyond repair. You can judge for yourself just how sustainable many human activities are as you read the rest of this book.

Pollution

Almost any substance can become a pollutant if it occurs in the wrong place, in the wrong concentration or at the wrong time. Hence fertilizers are excellent substances for increasing crop yields in intensive agricultural systems, but the same fertilizers running off the land into a river can pollute the water and cause the death of organisms in the natural ecosystem. Farming pollutants include fertilizers, pesticides and animal waste.

Our domestic lifestyle produces distinctive pollutants too – domestic refuse, car exhaust fumes and chlorofluorocarbons (CFCs) from refrigerators and aerosol sprays. Pollution from industrial processes has been around longer than you might think. Metal extraction was known as early as 6000 years ago and metal pollution has been found deep in the ice of Greenland, which is about 4000 years old! The Romans were determined polluters. They burned coal in their underfloor heating systems and smelted all sorts of heavy metal ores to extract lead, copper, silver and zinc. Smelting ores in open fires and crude furnaces was an inefficient process that produced considerable atmospheric pollution. Although huge quantities of metals are now smelted compared with Roman times, luckily for us the methods of extraction have improved. Metal extraction is more efficient, so more metal is extracted even from poorer grade ores and less contaminating metal escapes during the process. Pollution is, however, still a big problem in the world.

Threats to biodiversity

Biodiversity is a much-heard word these days, although it was probably first used as recently as 1985. It is really shorthand for biological diversity. Biological diversity can be measured at all sorts of levels: the diversity of ecosystems in a region, the number of species in each ecosystem, and the genetic diversity within the populations of each species. Biodiversity includes all these levels of complexity and can be assessed on a local, national or global scale. Ecologists and conservationists are very concerned about the threatened and actual loss of global biodiversity.

The activities of humans over the last 100 000 years have severely compromised biodiversity. Hunting large animals for food probably led to the extinction of species such as mammoths and giant, flightless birds. Clearing natural vegetation for farmland and dwelling space and the polluting of soil, sea and atmosphere have all had the effect of reducing biodiversity.

We still have no certain idea how many species there are in the world or what many of those species are. Estimates vary a lot, but there may be as many as 15 million species, of which eight million are insects. By far the most diverse places on land (we are not so sure about the sea as the very deep oceans are mostly unexplored) are the tropical rainforests and the largest, in the Amazon basin of South America, is the richest of all. We have still not studied the rainforests enough to know exactly what is there, or how their ecosystems function. Yet it is these forests that are disappearing at an alarming rate, cleared for subsistence farming, for cattle ranching, for timber extraction, for mining, for access, and by accidental burning.

Monitoring the environment Ecology is in many ways the most complicated of all the biological sciences. Ecologists have to know something about the structure, physiology and behaviour of organisms before they can begin to understand how such organisms interact with one another and with the physical environment. For these reasons, ecology is increasingly an experimental science. Ecologists constantly need to test their predictions either in natural environments, in seminatural experiments out in the field, or in artificial, simplified laboratory experiments. Theories can be of great value in ecology, but they must always be tested against reality, and this is where practical ecology is so important. We can only gain an understanding about the ecosystems around us by getting information about them through practical ecology.

However, whole ecosystems are often far too complex to understand all in one go. It is easier to begin by choosing one or two species, or a small area of habitat, to study in detail. Practical ecology involves making observations, taking measurements and sometimes testing ideas by experimentation. Because there is so much to study in the environment and because the environment may change considerably in the next few decades (due to global warming and pollution) anyone can do valuable research. Carefully designed and long-term observations can be of great value. Just recording when the first bluebells start to flower and the first tree leaves appear, how often late frosts damage leaves or when frogs spawn each year could be important records of the effects of changing climate. As Oliver Rackham, a leading expert on ancient woodland, put it: ‘I often lament the observations which I would have begun if I had known in the 1960s what were to be the ecological problems of the 1990s ... I would urge conservation trusts to be more active in long-term research, experimentation and maintaining archives. Photographing from fixed points, recording permanently marked plots or transects, or following the fate of marked individual plants.

4. The Biosphere - The Terrestrial Biomes and the Aquatic Biomes

Biosphere, relatively thin life-supporting stratum of Earth's surface, extending from a few kilometres into the atmosphere to the deep-sea vents of the ocean. The biosphere is a global ecosystem composed of living organisms (biota) and the abiotic (nonliving) factors from which they derive energy and nutrients.

Before the coming of life, Earth was a bleak place, a rocky globe with shallow seas and a thin band of gases—largely carbon dioxide, carbon monoxide, molecular nitrogen, hydrogen sulfide, and water vapour. It was a hostile and barren planet. This strictly inorganic state of the Earth is called the geosphere; it consists of the lithosphere (the rock and soil), the hydrosphere (the water), and the atmosphere (the air). Energy from the Sun relentlessly bombarded the surface of the primitive Earth, and in time-millions of years-chemical and physical actions produced the first evidence of life: formless, jellylike blobs that could collect energy from the environment and produce more of their own kind. This generation of life in the thin outer layer of the geosphere established what is called the biosphere, the "zone of life," an energy-diverting skin that uses the matter of the Earth to make living substance.

The biosphere is a system characterized by the continuous cycling of matter and an accompanying flow of solar energy in which certain large molecules and cells are self-reproducing. Water is a major predisposing factor, for all life depends on it. The elements carbon, hydrogen, nitrogen, oxygen, phosphorus, and sulfur, when combined as proteins, lipids, carbohydrates, and nucleic acids, provide the building blocks, the fuel, and the direction for the creation of life. Energy flow is required to maintain the structure of organisms by the formation and splitting of phosphate bonds. Organisms are cellular in nature and always contain some sort of enclosing membrane structure, and all have nucleic acids that store and transmit genetic information.

All life on Earth depends ultimately upon green plants, as well as upon water. Plants utilize sunlight in a process called photosynthesis to produce the food upon which animals feed and to provide, as a by-product, oxygen, which most animals require for respiration. At first, the oceans and the lands were teeming with large numbers of a few kinds of simple single-celled organisms, but slowly plants and animals of increasing complexity evolved. Interrelationships developed so that certain plants grew in association with certain other plants, and animals associated with the plants and with one another to form communities of organisms, including those of forests, grasslands, deserts, dunes, bogs, rivers, and lakes. Living communities and their nonliving environment are inseparably interrelated and constantly interact upon each other. For convenience, any segment of the landscape that includes the biotic and abiotic components is called an ecosystem. A lake is an ecosystem when it is considered in totality as not just water but also nutrients, climate, and all of the life contained within it. A given forest, meadow, or river is likewise an ecosystem. One ecosystem grades into another along zones termed ecotones, where a mixture of plant and animal species from the two ecosystems occurs. A forest considered as an ecosystem is not simply a stand of trees but is a complex of soil, air, and water, of climate and minerals, of bacteria, viruses, fungi, grasses, herbs, and trees, of insects, reptiles, amphibians, birds, and mammals.

Stated another way, the abiotic, or nonliving, portion of each ecosystem in the biosphere includes the flow of energy, nutrients, water, and gases and the concentrations of organic and inorganic substances in the environment. The biotic, or living, portion includes three general categories of organisms based on their methods of acquiring energy: the primary producers, largely green plants; the consumers, which include all the animals; and the decomposers, which include the microorganisms that break down the remains of plants and animals into simpler components for recycling in the biosphere. Aquatic ecosystems are those involving marine environments and freshwater environments on the land. Terrestrial ecosystems are those based on major vegetational types, such as forest, grassland, desert, and tundra. Particular kinds of animals are associated with each such plant province.

Ecosystems may be further subdivided into smaller biotic units called communities. Examples of communities include the organisms in a stand of pine trees, on a coral reef, and in a cave, a valley, a lake, or a stream. The major consideration in the community is the living component, the organisms; the abiotic factors of the environment are excluded.

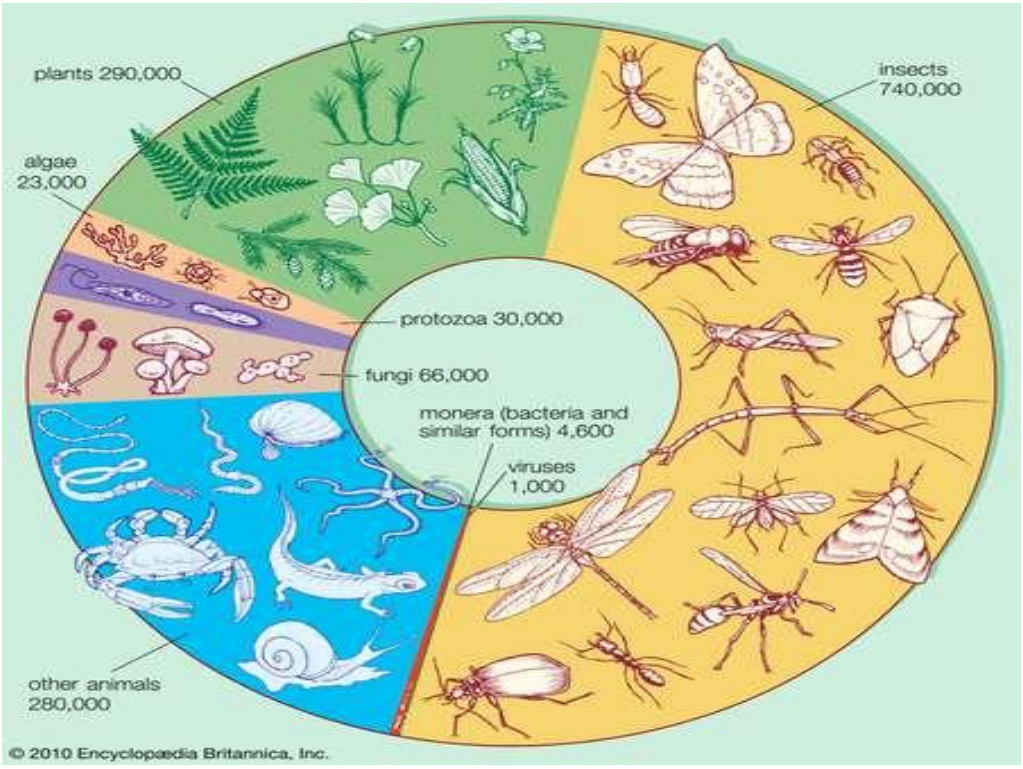
A community is a collection of species populations. In a stand of pines, there may be many species of insects, of birds, of mammals, each a separate breeding unit but each dependent on the others for its continued existence. A species, furthermore, is composed of individuals, single functioning units identifiable as organisms. Beyond this level, the units of the biosphere are those of the organism: organ systems composed of organs, organs of tissues, tissues of cells, cells of molecules, and molecules of atomic elements and energy. The progression, therefore, proceeding upward from atoms and energy, is toward fewer units, larger and more

complex in pattern, at each successive level.

This article focuses on the makeup of the biosphere and examines the relationships between its principal components, including man. The characteristics and dynamics of biological populations and communities are dealt with, as are the interactions that constitute the primary stabilizing links among the constituent organisms. Due attention is also given to the distribution patterns of these biotic units and to the processes that produced such patterns. The major aquatic and terrestrial ecosystems of the Earth are treated in some detail. Other points include energy transformations and transfers within the biosphere and the cyclic flow of materials needed for life. For the development, methodology, and applications of the study of interrelations of organisms with their environment and each other, *see* ecology. Further treatment of the various aquatic and terrestrial environments is provided in ocean, lake, river, continental landform, Arctic, and Antarctica. For a discussion of the origin of life on Earth and the varieties of and commonalities among organisms, *see* life and Earth, pregeologic history of. The characteristics and classifications of living organisms are covered in detail in algae, amphibian, angiosperm, animal, annelid, arachnid, arthropod, aschelminth, bacteria, bird, bryophyte, c hordate, cnidarian, crustacean, dinosaur, echinoderm, fern, fish, flatworm, fungus, gymnosperm, insect, lampsh ell, mammal, mollusk, moss,animal, plant, protist, protozoa, reptile, sponge, and virus.

The Diversity of Life

The biosphere supports between 3 and 30 million species of plants, animals, fungi, single-celled prokaryotes such as bacteria, and single-celled eukaryotes such as protozoans. Of this total, only about 1.4 million species have been named so far, and fewer than 1 percent have been studied for their ecological relationships and their role in ecosystems. A little more than half the named species are insects, which dominate terrestrial and freshwater communities worldwide; the laboratories of systematists are filled with insect species yet to be named and described. Hence, the relationships of organisms to their environments and the roles that species play in the biosphere are only beginning to be understood.



Estimated number of known living species. The majority of species are still unknown *i.e.*, yet to be described by taxonomists.

The importance of the biosphere:

The continued functioning of the biosphere is dependent not only on the maintenance of the intimate interactions among the myriad species within local communities but also on the looser yet crucial interactions of all species and communities around the globe. The Earth is blanketed with so many species and so many different kinds of biological communities because populations have been able to adapt to almost any kind of environment on Earth through natural selection. Life-forms have evolved that are able to survive in the ocean depths, the frigid conditions of Antarctica, and the near-boiling temperatures of geysers. The great richness of adaptations found among different populations and species of living organisms is the Earth's

greatest resource. It is a richness that has evolved over millions of years and is irreplaceable.
It is therefore startling to realize that our inventory of the Earth's diversity is still so incomplete that the total number of living species cannot be estimated more closely than between 3 and 30 million species. Decades of continuous research must be carried out by systematists, ecologists, and geneticists before the inventory of biodiversity provides a more accurate count. The research has been slow. Only recently, as the extinction rate of species has been increasing rapidly, have societies begun to realize the interdependence of species. To sustain life on Earth, more than the few animal and plant species used by humans must be preserved. The flow of energy and the cycling of nutrients through ecosystems, the regulation of populations, and the stability of biological communities, all of which support the continued maintenance of life, rely on the diversity of species, their adaptations to local physical conditions, and their coevolved relationships.
Despite the limited scientific knowledge of most species, ecological studies during the 20th century made great headway in unraveling the mechanisms by which organisms coevolve with one another and adapt to their physical environment, thereby shaping the biosphere. Each new decade has produced a steady stream of studies showing that the biological and physical elements of the Earth are more interconnected than had been previously thought. Those studies also have shown that often the most seemingly insignificant species are crucial to the stability of communities and ecosystems. Many seemingly obscure species are at risk worldwide of being dismissed as unimportant. The effect that the loss of species will have on ecosystems is appreciated only by understanding the relationships between organisms and their environments and by studying the ecological and evolutionary processes operating within ecosystems.
The need to understand how the biosphere functions has never been greater. When human population levels were low and technological abilities crude, societies' impact on the biosphere was relatively small. The increase in human population levels and the harvesting of more of the Earth's natural resources has altered this situation, especially in recent decades. Human activities are causing major alterations to the patterns of energy flow and nutrient cycling through ecosystems, and these activities are eliminating populations and species that have not even been described but which might have been of central importance to the maintenance of ecosystems.
The biologist Edward O. Wilson, who coined the term *biodiversity*, estimated conservatively that in the late 20th century at least 27,000 species were becoming extinct each year. The majority of these were small tropical organisms. The impact that this freshet of extinctions would have on the biosphere is akin to receiving a box of engine parts and discarding a portion of them before reading the directions, assuming that their absence will have no negative repercussions on the running of the engine. The following sections describe how many of the biological and physical parts fit together to make the engine of the biosphere run and why many seemingly obscure species are important to the long-term functioning of the biosphere.

AQUATIC BIOME:

Water is the common link among the five biomes and it makes up the largest part of the biosphere, covering nearly 75% of the Earth's surface. Aquatic regions house numerous species of plants and animals, both large and small. In fact, this is where life began billions of years ago when amino acids first started to come together. Without water, most life forms would be unable to sustain themselves and the Earth would be a barren, desert-like place. Although water temperatures can vary widely, aquatic areas tend to be more humid and the air temperature on the cooler side.

The aquatic biome can be broken down into two basic regions, freshwater (i.e, ponds and rivers) and marine (i.e, oceans and estuaries).

Freshwater Regions

Freshwater is defined as having a low salt concentration—usually less than 1%. Plants and animals in freshwater regions are adjusted to the low salt content and would not be able to survive in areas of high salt concentration (i.e, ocean). There are different types of freshwater regions: ponds and lakes, streams and rivers, and wetlands. The following sections describe the characteristics of these three freshwater zones.

Ponds and Lakes-

These regions range in size from just a few square meters to thousands of square kilometers. Scattered throughout the earth, several are remnants from the Pleistocene glaciation. Many ponds are seasonal, lasting just a couple of months (such as sessile pools) while lakes may exist for hundreds of years or more. Ponds and lakes may have limited species diversity since they are often isolated from one another and from other water sources like rivers and oceans. Lakes and ponds are divided into three different "zones" which are usually determined by depth and distance from the shoreline.

The topmost zone near the shore of a lake or pond is the *littoral zone*. This zone is the warmest since it is shallow and can absorb more of the Sun's heat. It sustains a fairly diverse community, which can include several species of algae (like diatoms), rooted and floating aquatic plants, grazing snails, clams, insects, crustaceans, fishes, and amphibians. In the case of the insects, such as dragonflies and midges, only the egg and larvae stages are found in this zone. The vegetation and animals living in the littoral zone are food for other creatures such as turtles, snakes, and ducks.

The near-surface open water surrounded by the littoral zone is the *limnetic zone*. The limnetic zone is well-lighted (like the littoral zone) and is dominated by plankton, both phytoplankton and zooplankton. Plankton are small organisms that play a crucial role in the food chain. Without aquatic plankton, there would be few living organisms in the world, and certainly no humans. A variety of freshwater fish also occupy this zone.

Plankton have short life spans—when they die, they fall into the deep-water part of the lake/pond, the *profundal zone*. This zone is much colder and denser than the other two. Little light penetrates all the way through the limnetic zone into the profundal zone. The fauna are heterotrophs, meaning that they eat dead organisms and use oxygen for cellular respiration.

Temperature varies in ponds and lakes seasonally. During the summer, the temperature can range from 4° C near the bottom to 22° C at the top. During the winter, the temperature at the bottom can be 4° C while the top is 0° C (ice). In between the two layers, there is a narrow zone called the thermocline where the temperature of the water changes rapidly. During the spring and fall seasons, there is a mixing of the top and bottom layers, usually due to winds, which results in a uniform water temperature of around 4° C. This mixing also circulates oxygen throughout the lake. Of course there are many lakes and ponds that do not freeze during the winter, thus the top layer would be a little warmer.

Streams and river-

These are bodies of flowing water moving in one direction. Streams and rivers can be found everywhere-they get their starts at headwaters, which may be springs, snowmelt or even lakes, and then travel all the way to their mouths, usually another water channel or the ocean. The characteristics of a river or stream change during the journey from the source to the mouth. The temperature is cooler at the source than it is at the mouth. The water is also clearer, has higher oxygen levels, and freshwater fish such as trout and heterotrophs can be found there. Towards the middle part of the stream/river, the width increases, as does species diversity—numerous aquatic green plants and algae can be found. Toward the mouth of the river/stream, the water becomes murky from all the sediments that it has picked up upstream, decreasing the amount of light that can penetrate through the water. Since there is less light, there is less diversity of flora, and because of the lower oxygen levels, fish that require less oxygen, such as catfish and carp, can be found.

Wetlands:
Wetlands are areas of standing water that support aquatic plants. Marshes, swamps, and bogs are all considered wetlands. Plant species adapted to the very moist and humid conditions are called hydrophytes. These include pond lilies, cattails, sedges, tamarack, and black spruce. Marsh flora also include such species as cypress and gum. Wetlands have the highest species diversity of all ecosystems. Many species of amphibians, reptiles, birds (such as ducks and waders), and furbearers can be found in the wetlands. Wetlands are not considered freshwater ecosystems as there are some, such as salt marshes, that have high salt concentrations—these support different species of animals, such as shrimp, shellfish, and various grasses.

Marine Regions:

Marine regions cover about three-fourths of the Earth's surface and include oceans, coral reefs, and estuaries. Marine algae supply much of the world's oxygen supply and take in a huge amount of atmospheric carbon dioxide. The evaporation of the seawater provides rainwater for the land.

Oceans
The largest of all the ecosystems, oceans are very large bodies of water that dominate the Earth's surface. Like ponds and lakes, the ocean regions are separated into separate zones: intertidal, pelagic, abyssal, and benthic. All four zones have a great diversity of species. Some say that the ocean contains the richest diversity of species even though it contains fewer species than there are on land.

The *intertidal zone* is where the ocean meets the land—sometimes it is submerged and at other times exposed, as waves and tides come in and out. Because of this, the communities are constantly changing. On rocky coasts, the zone is stratified vertically. Where only the highest tides reach, there are only a few species of algae and mollusks. In those areas usually submerged during high tide, there is a more diverse array of algae and small animals, such as herbivorous snails, crabs, sea stars, and small fishes. At the bottom of the intertidal zone, which is only exposed during the lowest tides, many invertebrates, fishes, and seaweed can be found. The intertidal zone on sandier shores is not as stratified as in the rocky areas. Waves keep mud and sand constantly moving, thus very few algae and plants can establish themselves—the fauna include worms, clams, predatory crustaceans, crabs, and shorebirds.

The *pelagic zone* includes those waters further from the land, basically the open ocean. The pelagic zone is generally cold though it is hard to give a general temperature range since, just like ponds and lakes, there is thermal stratification with a constant mixing of warm and cold ocean currents. The flora in the pelagic zone include surface seaweeds. The fauna include many species of fish and some mammals, such as whales and dolphins. Many feed on the abundant plankton.

The *benthic zone* is the area below the pelagic zone, but does not include the very deepest parts of the ocean (see *abyssal zone* below). The bottom of the zone consists of sand, slit, and/or dead organisms. Here temperature decreases as depth increases toward the abyssal zone, since light cannot penetrate through the deeper water. Flora are represented primarily by seaweed while the fauna, since it is very nutrient-rich, include all sorts of bacteria, fungi, sponges, sea anemones, worms, sea stars, and fishes.

The deep ocean is the *abyssal zone*. The water in this region is very cold (3°C), highly pressured, high in oxygen content, but low in nutritional content. The abyssal zone supports many species of invertebrates and fishes. Mid-ocean ridges (spreading zones between tectonic plates), often with hydrothermal vents, are found in the abyssal zones along the ocean floors. Chemosynthetic bacteria thrive near these vents because of the large amounts of hydrogen sulfide and other minerals they emit. These bacteria are thus the start of the food web as they are eaten by invertebrates and fishes.

Coral Reefs

Coral reefs are widely distributed in warm shallow waters. They can be found as barriers along continents (e.g., the Great Barrier Reef off Australia), fringing islands, and atolls. Naturally, the dominant organisms in coral reefs are corals. Corals are interesting since they consist of both algae (zooanthellae) and tissues of animal polyp. Since reef waters tend to be nutritionally poor, corals obtain nutrients through the algae via photosynthesis and also by extending tentacles to obtain plankton from the water. Besides corals, the fauna include several species of microorganisms,

invertebrates, fishes, sea urchins, octopuses, and sea stars.

Estuaries

Estuaries are areas where freshwater streams or rivers merge with the ocean. This mixing of waters with such different salt concentrations creates a very interesting and unique ecosystem. Microflora like algae, and macroflora, such as seaweeds, marsh grasses, and mangrove trees (only in the tropics), can be found here. Estuaries support a diverse fauna, including a variety of worms, oysters, crabs, and waterfowl.

TERRESTRIAL BIOME

Differences in temperature or precipitation determine the types of plants that grow in a given area. Generally speaking, height, density, and species diversity decreases from warm, wet climates to cool, dry climates. Raunkiaer (1934) classified plant life forms based on traits that varied with climate. One such system was based on the location of the perennating organ (Table 1). These are tissues that give rise to new growth the following season, and are therefore sensitive to climatic conditions. The relative proportions of different life forms vary with climate. In fact, life form spectra are more alike in similar climates on different continents than they are in different climates on the same continent (Figure 3). Regions of similar climate and dominant plant types are called biomes. This chapter describes some of the major terrestrial biomes in the world; tropical forests, savannas, deserts, temperate grasslands, temperate deciduous forests, Mediterranean scrub, coniferous forests, and tundra (Figure 4).

Table 1: Raunkiaer life form classification system based on location of the perennating bud

Raunkiaer life form classification system based on location of the perennating bud.		
Life form	Location of perennating tissue	Plant types
Phanerophyte	*>0.5 m*	Trees and tall shrubs
Chamaephyte	0 - 0.5 m	Small shrubs and herbs
Hemicryptophyte	Soil surface	Prostrate shrubs or herbaceous plants that dieback each year
Cryptophyte	In the soil	Rhizomatous grasses or bulb forming herbs
Therophyte	Seed	Annuals

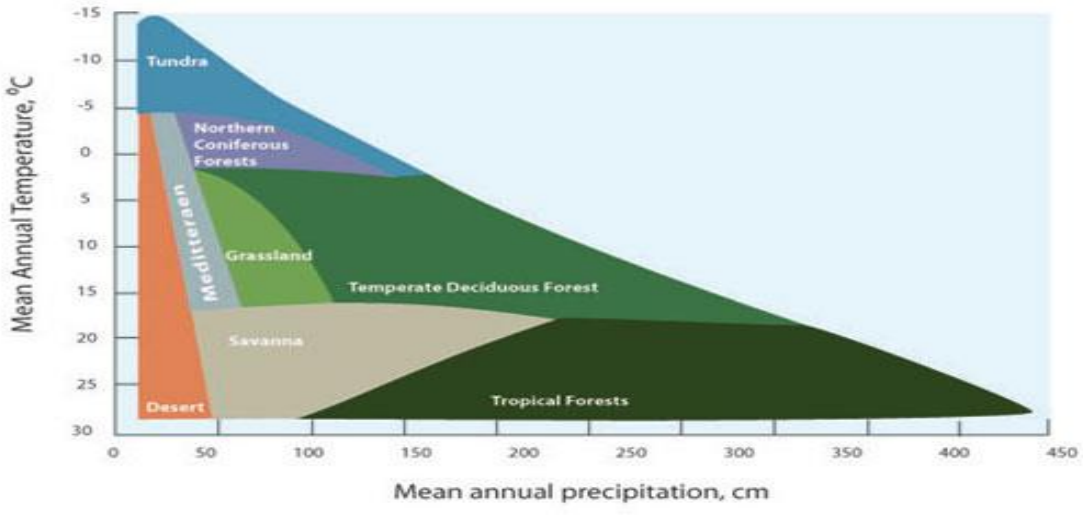
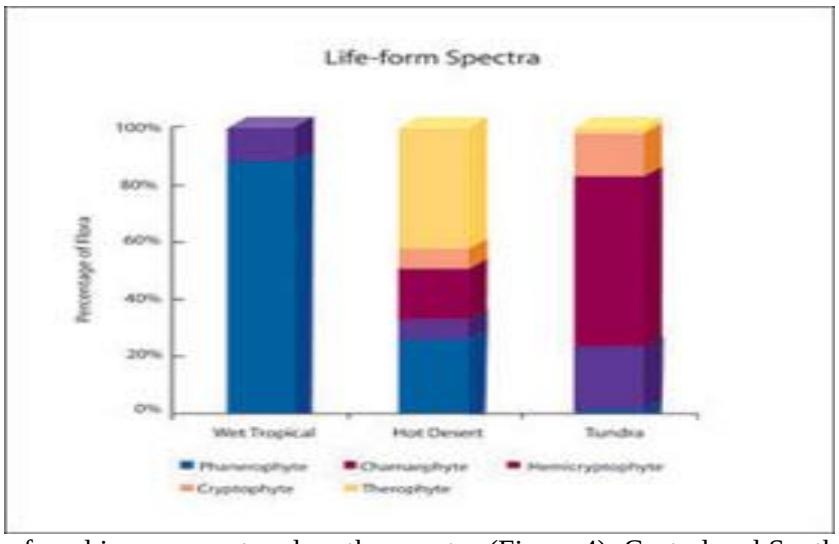


Figure 1: The distribution of vegetation types as a function of mean annual temperature and precipitation.

Tropical Forest Biomes:

Figure 2: Life-form spectra in different climates

Raunkiar classified plant life forms on traits that varied with climate, such as the perennating organ, or tissues that give rise to new growth the following season.



Tropical forests are found in areas centered on the equator (Figure 4). Central and South America possess half of the world's tropical forests. Climate in these biomes shows little seasonal variation (Figure 5), with high yearly rainfall and relatively constant, warm temperatures. The dominant plants are phanerophytes - trees, lianas, and epiphytes. Tropical rainforests have an emergent layer of tall trees over 40 m tall, an over story of trees up to 30 m tall, a sub-canopy layer of trees and tall shrubs, and a ground layer of herbaceous vegetation. Tropical forests have the highest biodiversity and primary productivity of any of the terrestrial biomes. Net primary productivity ranges from 2–3 kg m^{-2} y^{-1} or higher. This high productivity is sustained despite heavily leached, nutrient poor soils, because of the high decomposition rates possible in moist, warm conditions. Litter decomposes rapidly, and rapid nutrient uptake is facilitated by mycorrhizae, which are fungal mutualists associated with plant roots.

The tropical forest biome is estimated to contain over half of the terrestrial species on Earth. Approximately 170,000 of the 250,000 described species of vascular plants occur in tropical biomes. As many as 1,209 butterfly species have been documented in 55 square kilometers of the Tambopata Reserve in southeastern Peru, compared to 380 butterfly species in Europe and North Africa combined.

The tropical forest biome is composed of several different sub-biomes, including evergreen rainforest, seasonal deciduous forest, tropical cloud forest, and mangrove forest. These sub-biomes develop due to changes in seasonal patterns of rainfall, elevation and/or substrate.

Savanna Biomes:

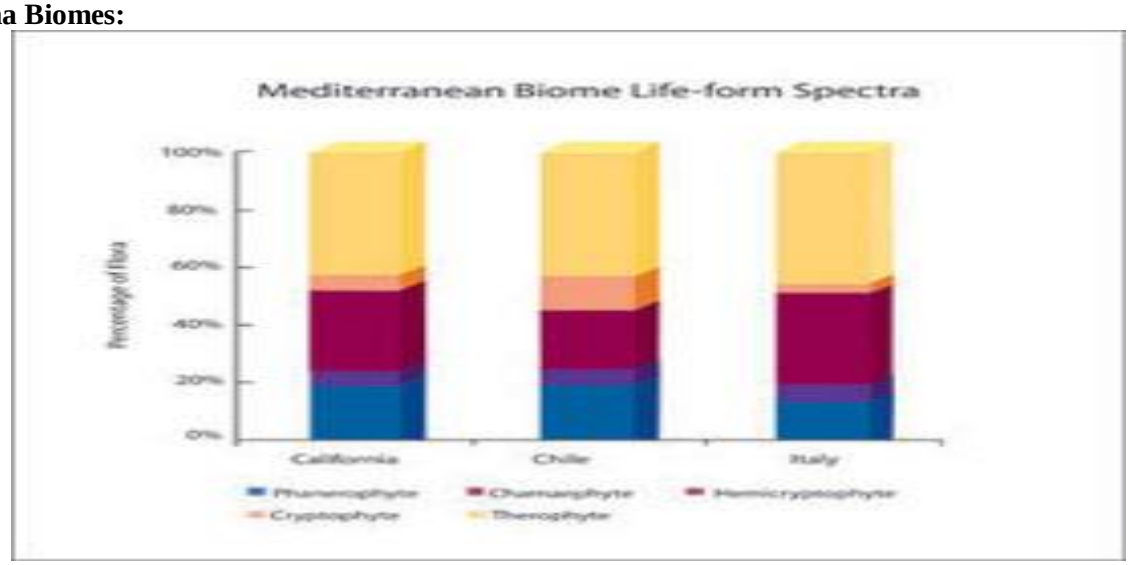


Figure 3: Life-form spectra in similar Mediterranean type climates on different continents

Located north and south of tropical forest biomes are savannas (Figure 4), with lower yearly rainfall and longer dry seasons (Figure 6). These biomes are dominated by a mix of grasses and small trees. Savannas cover 60% of Africa and represent a transition from tropical forests to deserts. Trees in savannas are usually drought deciduous. Several savanna types associated with differing rainfall patterns, height of the water table and soil depth can be distinguished by their relative abundance of trees and grass.

Repetitive dry season fires have occurred in the African savanna over the last 50,000 years. Fire plays a major role in the balance between trees and grasses in savannas. With long periods between fires, tree and shrub populations increase. Fires release nutrients tied up in dead plant litter. Soil provides a good thermal insulator, so seeds and below ground rhizomes of grasses are usually protected from damage. Net primary productivity ranges from 400–600 g m^{-2} yr^{-1}, but varies depending upon local conditions such as soil depth. Decomposition is rapid and year-round, and the annual turnover rate of leaf material is high; up to 60–80%. This turnover is aided by the rich diversity of large herbivores found in savannas, where up to 60% of the biomass can be consumed in a given year. Dung beetles are important components of the nutrient cycle due to their role in breaking down animal droppings. The high herbivore diversity and production is mirrored by the great variety of predators and scavengers found in savannas.

Desert Biomes

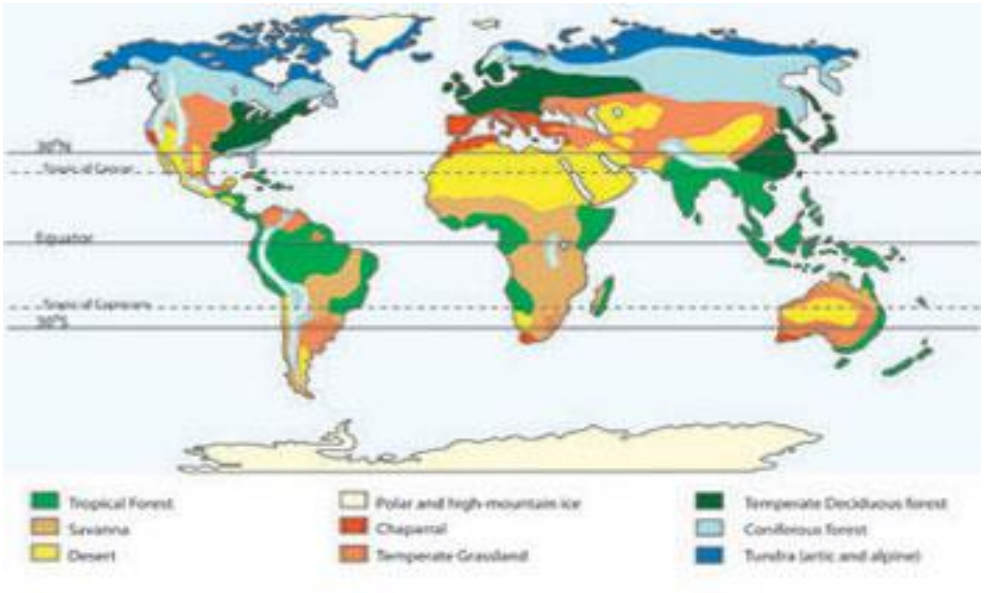


Figure 4: Biomes of the world
Biomes are regions of similar climate and dominant plant types.

Deserts generally occur in a band around the world between 15–30° N and S latitude (Figure 4). They cover between 26–35% of the land surface of the Earth. The climate of deserts is dominated by low precipitation, generally below 250 mm yr^{-1} (Figure 7). However, there is a lot of variability in desert types, with hot deserts, cold deserts, high elevation deserts, and rain shadow deserts. Consequently, there is a great deal of variation in the biodiversity, productivity and organisms found in different types of desert.

The dominant plant biomass in most deserts is composed of perennial shrubs with extensive roots and small, gray or white leaves. However, in warm deserts, therophytes (annual plants) can make up most of the species diversity (Figure 2). Desert annuals can survive unpredictable dry periods as seeds. Seeds may remain viable in the soil for several years, until the appropriate rainfall and temperature conditions occur, after which they will germinate. These annuals grow rapidly, completing their life cycle in a few weeks, then flowering and setting seed before soil water reserves are depleted. Winter desert annuals in North American deserts can generate over 1 kg m^{-2} of biomass in a wet year.
With the exception of large blooms of annuals, net primary productivity in most deserts is low and extremely variable. There is a positive relationship between productivity and precipitation, and values can range from near 0 to 120 g m^{-2} yr^{-1}. Just as with savannas, productivity will vary with soil depth and local drainage patterns (e.g., washes).

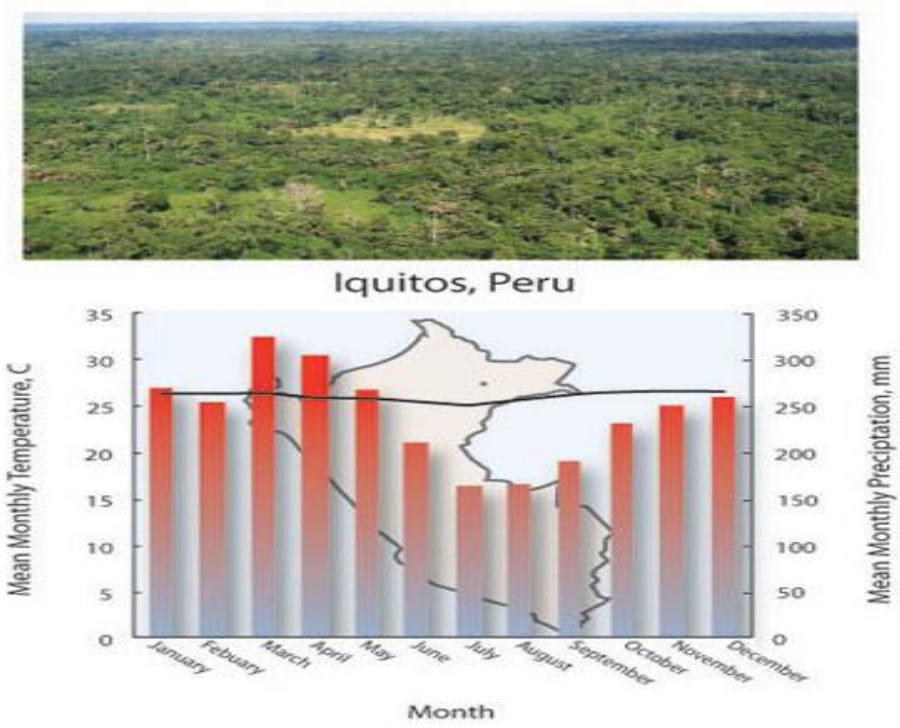


Figure 5: Tropical forest biome climate diagram
Climate in these areas show little seasonal variation with high yearly rainfall and relatively constant, warm temperatures.

Grassland Biomes

Grassland biomes occur primarily in the interiors of continents (Figure 4) and are characterized by large seasonal temperature variations, with hot summers and cold winters (Figure 8). Precipitation varies, with a strong summer peak. The type of grassland community that develops, and the productivity of grasslands, depends strongly upon precipitation. Higher precipitation leads to tall grass prairie with a high biodiversity of grasses and forbs. Lower precipitation leads to short grass prairies and arid grasslands.

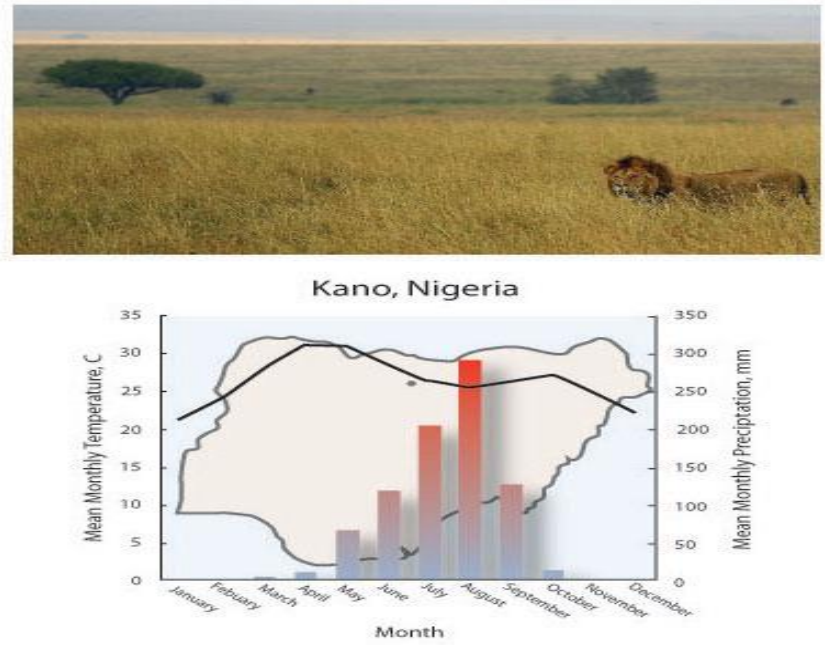
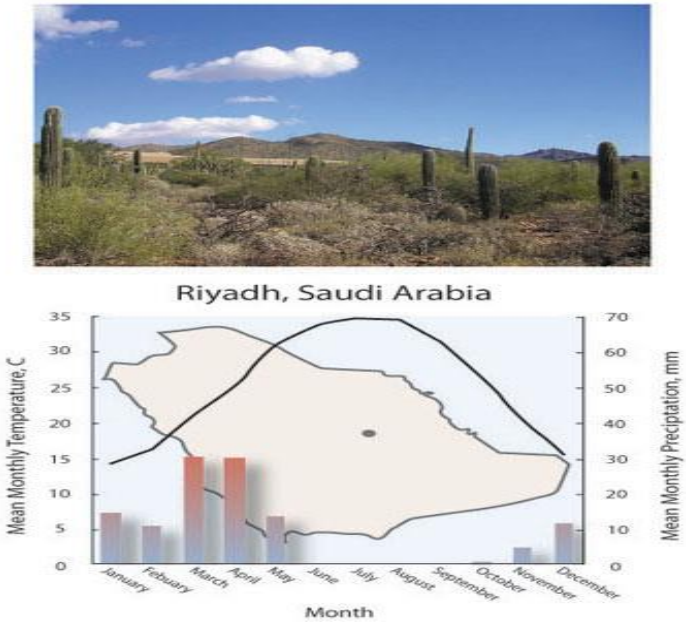


Figure 6: Savanna biome climate diagram
Savannas are located north and south of tropical forest biomes and are characterized by lower yearly rainfall and longer dry seasons.

Net primary productivity in dry grasslands may be 400 g m^{-2} yr^{-1}, while higher precipitation may support up to 1 kg m^{-2} yr^{-1}. Grasslands grade into deciduous forest biomes on their wetter margins, and deserts on their drier margins. The borders between grasslands and other biomes are dynamic and shift according to precipitation, disturbance, fire and drought. Fire and drought will favor grassland over forest communities.



There is a greater variability in desert types, with hot deserts, cold deserts, high elevation deserts, and rain shadow deserts.

Three major selective forces dominate the evolution of plant traits in grasslands, recurring fire, periodic drought, and grazing. These factors have led to the dominance of hemicryptophytes in grasslands with perennating organs located at or below the soil surface. Many grasses have below ground rhizomes connecting above ground shoots or tillers. Grass blades grow from the bottom up, with actively dividing meristems at the base of the leaf. Thus when grazers eat the grass blade, the meristem continues to divide and the blade can continue to grow. Grasses are often decay-resistant, and recurring cool, fast moving surface fires started by lightning at the end of summer aid in nutrient recycling. Fires stimulate productivity and the germination of fire resistant seeds.

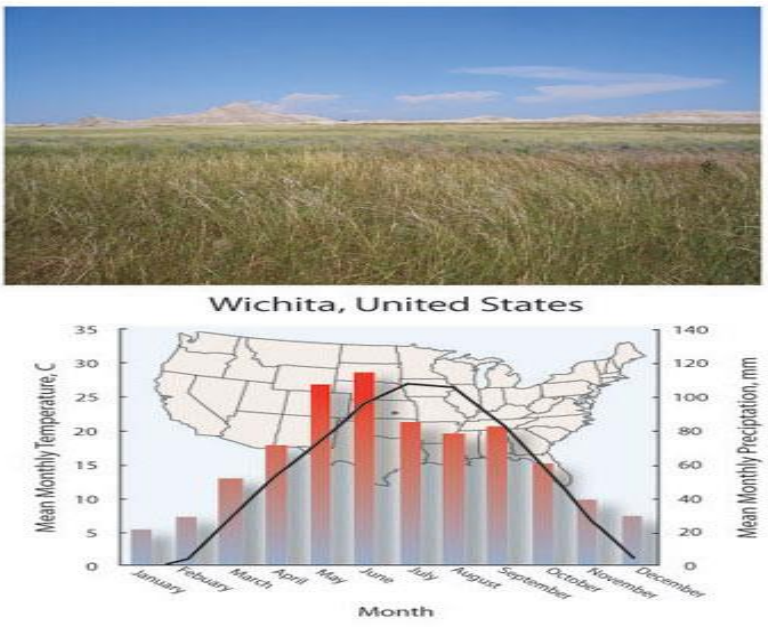


Figure 8: Grassland biome climate diagram

Grassland biomes occur primarily in the interiors of continents and are characterized by large seasonal temperature variations, with hot summers and cold winters.

Many of the world's largest terrestrial animals are found in grasslands. Animals such as gray kangaroos (*Macropus giganteus*) in Australia, Bison (*Bison bonasus*) and horses (*Equus* spp.) in Eurasia and North America were part of species rich assemblages of grazing animals, their predators, and scavengers. Remnant herds in North America suggest that disturbances due to grazers increased local biodiversity by creating openings that rare species could colonize. Large grazers also accelerated plant decomposition through their droppings, creating nutrient hotspots that altered species composition.

Temperate Deciduous Forest Biome

Temperature deciduous forests occur in mid-latitudes (Figure 4) where cool winters, warm summers, and high year round precipitation occurs (Figure 9). Net primary productivity ranges from 600–1500 g m^{-2} yr^{-1} with high litter production. Litter serves as a major pathway for nutrient recycling. This biome is named for the dominant trees that drop their leaves during the winter months. These forests may have an overstory of 20–30 m tall trees, an understory of 5–10 m trees and shrubs, a shrub layer around 1–2 m in height, and a ground layer of herbaceous plants. Biodiversity is relatively high in this biome due to the niche partitioning allowed by the multiple forest layers. More complex forests are associated with a greater number of animal species; for example, bird species diversity shows a positive correlation with forest height and number of layers.

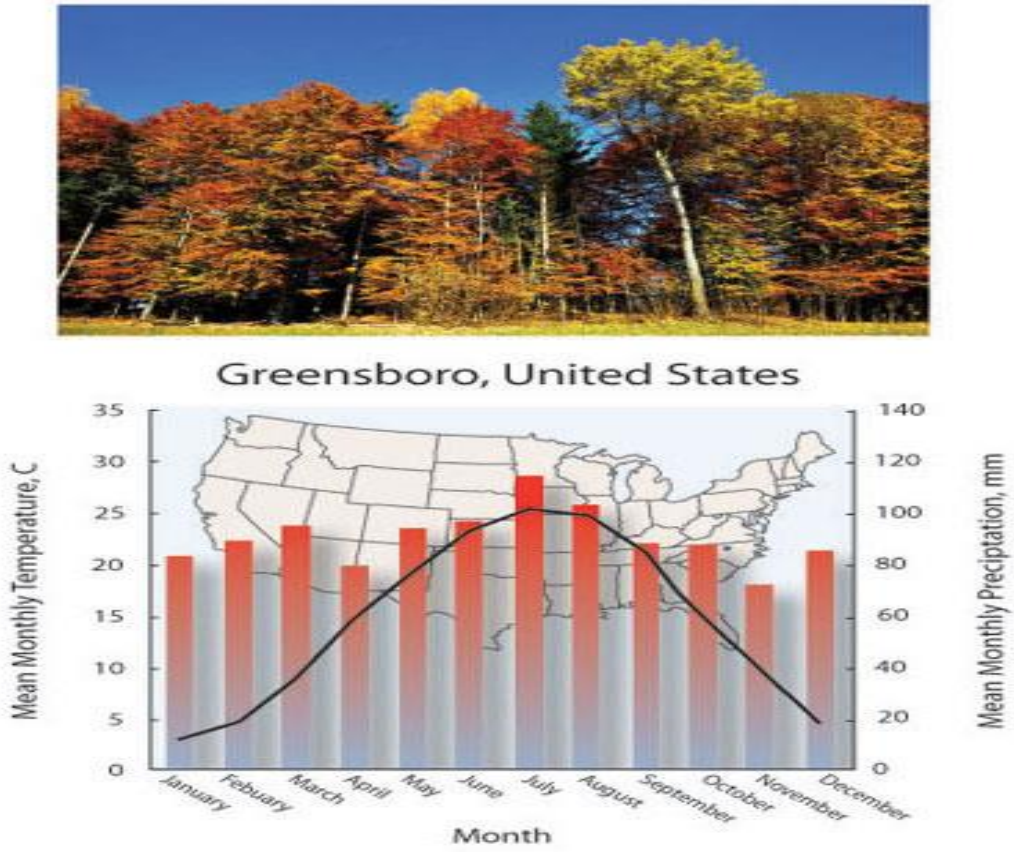


Figure 9: Temperate deciduous forest climate diagram
Temperature deciduous forests occur in mid-latitudes and are characterized by cool winters, warm summers, and high year round precipitation occurs.

Mediterranean Climate Biomes

This small biome (about 1.8 million square km) is separated into five separate regions between 30–40 degrees N and S latitude (Figure 4) with hot, dry summers, and cool, moist winters (Figure 10). Unrelated evergreen, sclerophyllous shrubs and trees have evolved independently in each of these areas, representing a striking example of convergent evolution. Net primary productivity varies from 300–600 g m^{-2} yr^{-1}, dependent upon water availability, soil depth, and age of the stand. Stand productivity decreases after 10–20 years as litter and woody biomass accumulates. Recurring fires aid in nutrient cycling and many plants show fire-induced or fire-promoted flowering. Some species are able to resprout from buds protected by the soil, while others germinate from decay-resistant seeds that lie dormant in the soil until a fire promotes their germination. Therophytes make up a large component of the flora, and their appearance is associated with openings created by fires.

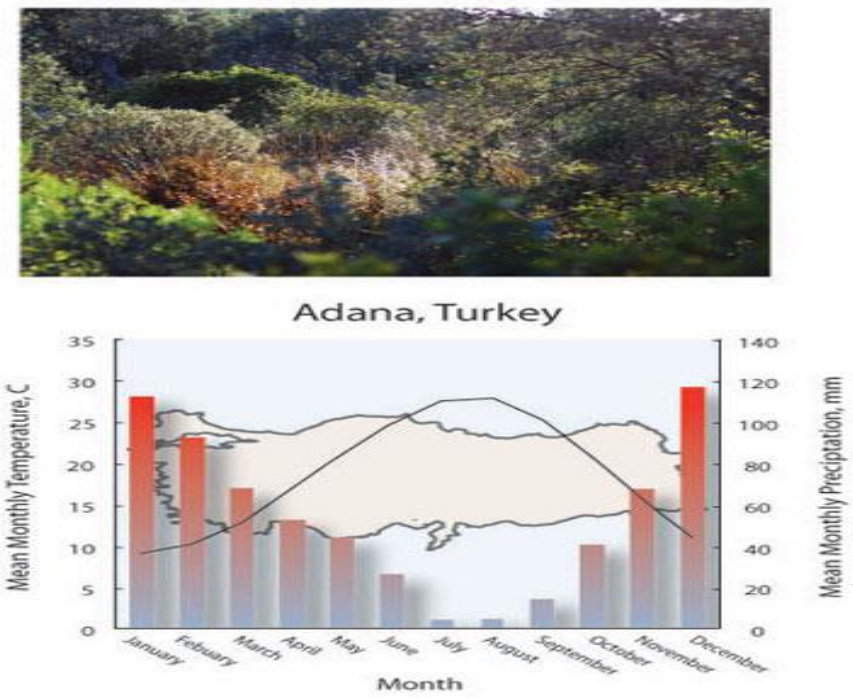


Figure 10: Mediterranean biome climate diagram
There are five separate regions between 30-40 degrees N and S latitude with hot, dry summers, and cool, moist winters.

Northern Coniferous Forest Biome

Located at higher latitudes is a biome dominated by needle-leaved, drought tolerant, evergreen trees (Figure 4), and a climate consisting of long, cold winters and short, cool summers (Figure 11). Biodiversity is low in this two-layered forest made up of an overstory of trees and a ground layer of herbs or mosses. The overstory in much of the boreal forest is made up of only one or two species. The low biodiversity is mirrored by low net primary productivity of 200–600 g m^{-2} yr^{-1}. Productivity varies with precipitation, the length of the frost-free period, and local soil drainage. In flooded areas, sphagnum bogs may develop. The acidic tissue of sphagnum, and the anoxic, flooded conditions, slows decomposition, resulting in the production of peat bogs.

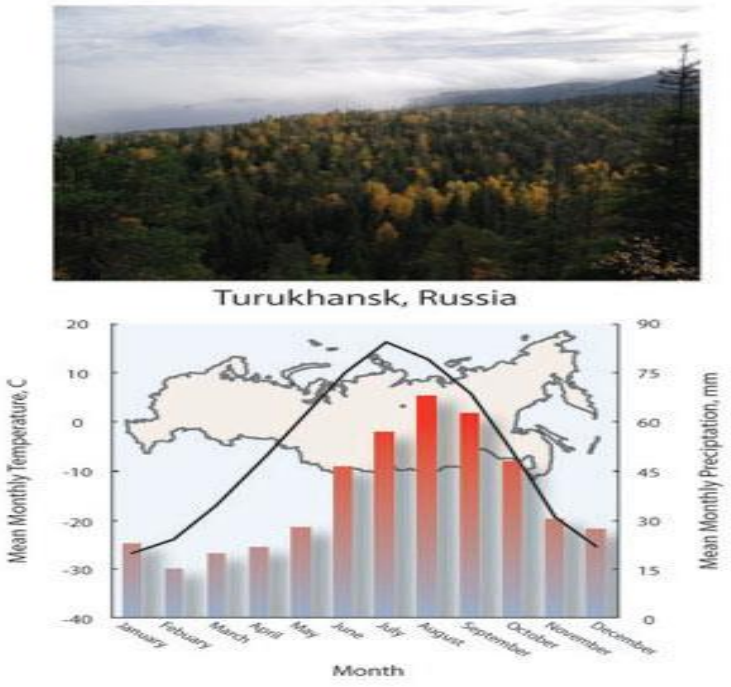


Figure 11: Boreal forest biome climate diagram

Boreal forests are characterized by needle-leaved, drought tolerant, evergreen trees, and a climate consisting of long, cold winters and short, cool summers.

Biomass in tree trunks and long-lived evergreen leaves results in nutrients being stored in the plants. Low temperatures lead to slow decomposition and high litter accumulation. Up to 60% of the biomass may be tied up in litter and humus. Soils are heavily leached, and permafrost underlies much of the soil. Consequently, trees have shallow root systems and rely on extensive mycorrhizal associations for nutrient uptake.

Tundra Biome

At latitudes beyond the boreal forest tree line lies a marshy area (Figure 4) where growing seasons are very short and temperatures are below zero degrees Celsius for much of the year (Figure 12). Because of these low temperatures and short growing seasons, net primary productivity is very low in the tundra, between 100–200 g m^{-2} yr^{-1}. Productivity varies with snowfall depth and local drainage. Rocky fields and dry meadows will have lower productivity than moist, low-lying areas and wet meadows.

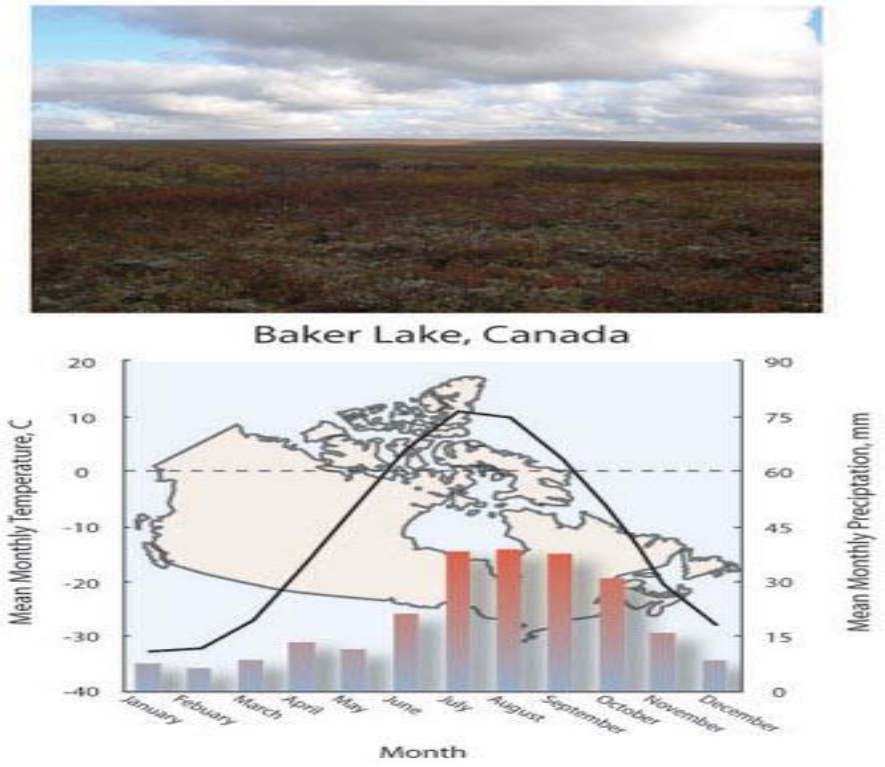


Figure 12: Tundra biome climate diagram

Very short growing seasons and temperatures that are below zero degrees Celsius for much of the year characterize tundras.

Biodiversity in the tundra is low and dominated by mosses, lichens, and low-growing perennial shrubs. The tundra biome contains only about 3% of the world's flora. Up to 60% of the flora can be made up of long-lived hemicryptophytes. Windy conditions and low temperatures select for low growing shrubs, often with tightly-packed, rounded canopies with closely spaced leaves and branches. Wind and ice damage help form this shape by pruning branches. The canopy morphology reduces wind speeds and absorbs solar radiation, resulting in canopy temperatures on sunny days more than 10° C above air temperature.

Soils are low in nutrients due to slow decomposition rates and plants retain nutrients in long-lived evergreen tissues. Nitrogen fixation by lichens with cyanobacterial components is a major source of soil nitrogen. Animals have extended hibernation periods or migrate seasonally.

5. Basic Ecological Concepts and Processes- Energy in Ecosystem, Food Chain and Food Web.

Energy has been defined as the capacity to do work. Energy exists in two forms potential and kinetic.
Potential energy is the energy at rest {i.e., stored energy) capable of performing work. Kinetic energy is the energy of motion (free energy).
It results in work performance at the expense of potential energy. Conversion of potential energy into kinetic energy involves the imparting of motion.
The source of energy required by all living organisms is the chemical energy of their food. The chemical energy is obtained by the conversion of the radiant energy of sun.
The radiant energy is in the form of electromagnetic waves which are released from the sun during the transmutation of hydrogen to helium. The chemical energy stored in the food of living organisms is converted into potential energy by the arrangement of the constituent atoms of food in a particular manner. In any ecosystem there should be unidirectional flow of energy.

This energy flow is based on two important Laws of Thermodynamics which are as follows:

(1) The first law of Thermodynamics:

It states that the amount of energy in the universe is constant. It may change from one form to another, but it can neither be created nor destroyed. Light energy can be neither created nor destroyed as it passes through the atmosphere. It may, however, be transformed into another type of energy, such as chemical energy or heat energy. These forms of energy cannot be transformed into electromagnetic radiation.

(2) The second law of Thermodynamics:

It states that non-random energy (mechanical, chemical, radiant energy) cannot be changed without some degradation into heat energy. The change of energy from one form to another takes place in such a way that a part of energy assumes waste form (heat energy). In this way, after transformation the capacity of energy to perform work is decreased. Thus, energy flows from higher to lower level.
Main source of energy is sun. Approximately 57% of sun energy is absorbed in the atmosphere and scattered in the space. Some 35% is spent to heat water and land areas and to evaporate water. Of the approximately 8% of light energy striking plant surface, 10% to 15% is reflected, 5% is transmitted and 80 to 85% is absorbed; and an average of only 2% (0.5 to 3.5%) of the total light energy striking on a leaf is used in photosynthesis and rest is transformed into heat energy.

Energy flow in Ecosystems:

Living organisms can use energy in two forms radiant and fixed energy. Radiant energy is in the form of electromagnetic waves, such as light. Fixed energy is potential chemical energy bound in various organic substances which can be broken down in order to release their energy content.

Organisms that can fix radiant energy utilizing inorganic substances to produce organic molecules are called autotrophs. Organisms that cannot obtain energy from abiotic source but depend on energy-rich organic molecules synthesized by autotrophs are called heterotrophs. Those which obtain energy from living organisms are called consumers and those which

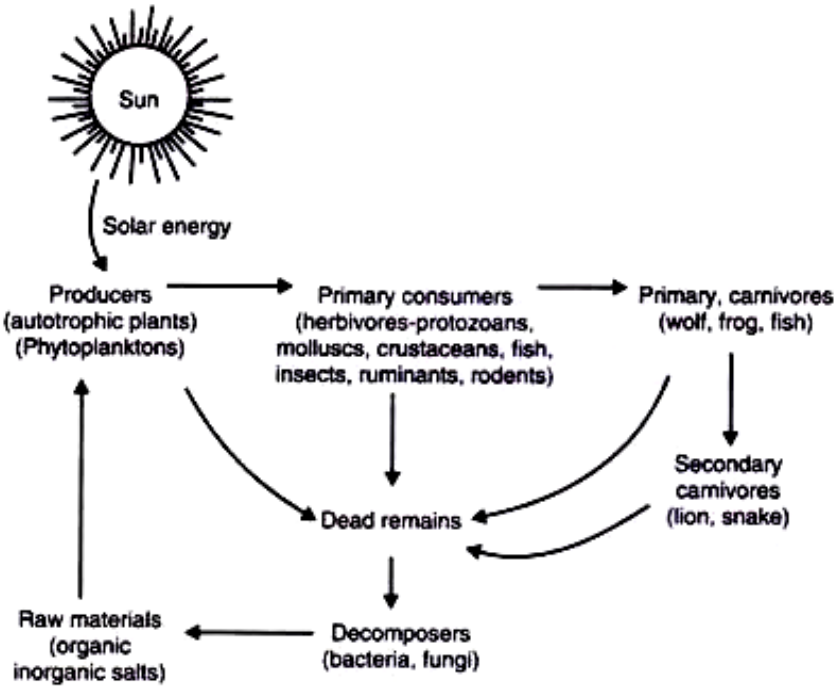


Fig. 3.7. Flow of energy at different levels of ecosystem.

obtain energy from dead organisms are called decomposers (Fig. 3.7).

When the light energy falls on the green surfaces of plants, a part of it is transformed into chemical energy which is stored in various organic products in the plants. When the herbivores consume plants as food and convert chemical energy accumulated in plant products into kinetic energy, degradation of energy will occur through its conversion into heat. When herbivores are consumed by carnivores of the first order (secondary consumers) further degradation will occur. Similarly, when primary carnivores are consumed by top carnivores, again energy will be degraded.

Trophic level:
The producers and consumers in ecosystem can be arranged into several feeding groups, each known as trophic level (feeding level). In any ecosystem, producers represent the first trophic level, herbivores present the second trophic level, primary carnivores represent the third trophic level and top carnivores represent the last level.

Food Chain:
In the ecosystem, green plants alone are able to trap in solar energy and convert it into chemical energy. The chemical energy is locked up in the various organic compounds, such as carbohydrates, fats and proteins, present in the green plants. Since virtually all other living organisms depend upon green plants for their energy, the efficiency of plants in any given area in capturing solar energy sets the upper limit to long-term energy flow and biological activity in the community.

The food manufactured by the green plants is utilized by themselves and also by herbivores. Animals feed repeatedly. Herbivores fall prey to some carnivorous animals. In this way one form of life supports the other form. Thus, food from one trophic level reaches to the other trophic level and in this way a chain is established. This is known as food chain.

A food chain may be defined as the transfer of energy and nutrients through a succession of organisms through repeated process of eating and being eaten. In food chain initial link is a green plant or producer which produces chemical energy available to consumers. For example, marsh grass is consumed by grasshopper, the grasshopper is consumed by a bird and that bird is consumed by hawk.

Thus, a food chain is formed which can be written as follows:
Marsh grass → grasshopper → bird → hawk

Food chain in any ecosystem runs directly in which green plants are eaten by herbivores, herbivores are eaten by carnivores and carnivores are eaten by top carnivores. Man forms the terrestrial links of many food chains.

Food chains are of three types:
1. Grazing food chain

2. Parasitic food chain

3. Saprophytic or detritus food chain

1. Grazing food chain:
The grazing food chain starts from green plants and from autotrophs it goes to herbivores (primary consumers) to primary carnivores (secondary consumers) and then to secondary carnivores (tertiary consumers) and so on. The gross production of a green plant in an ecosystem may meet three fates—it may be oxidized in respiration, it may be eaten by herbivorous animals and after the death and decay of producers it may be utilized by decomposers and converters and finally released into the environment. In herbivores the assimilated food can be stored as carbohydrates, proteins and fats, and transformed into much more complex organic molecules.

The energy for these transformations is supplied through respiration. As in autotrophs, the energy in herbivores also meets three routes respiration, decay of organic matter by microbes and consumption by the carnivores. Likewise, when the secondary carnivores or tertiary consumers eat primary carnivores, the total energy

assimilated by primary carnivores or gross tertiary production follows the same course and its disposition into respiration, decay and further consumption by other carnivores is entirely similar to that of herbivores.

Thus, it is obvious that much of the energy flow in the grazing food chain can be described in terms of trophic levels as outlined below:

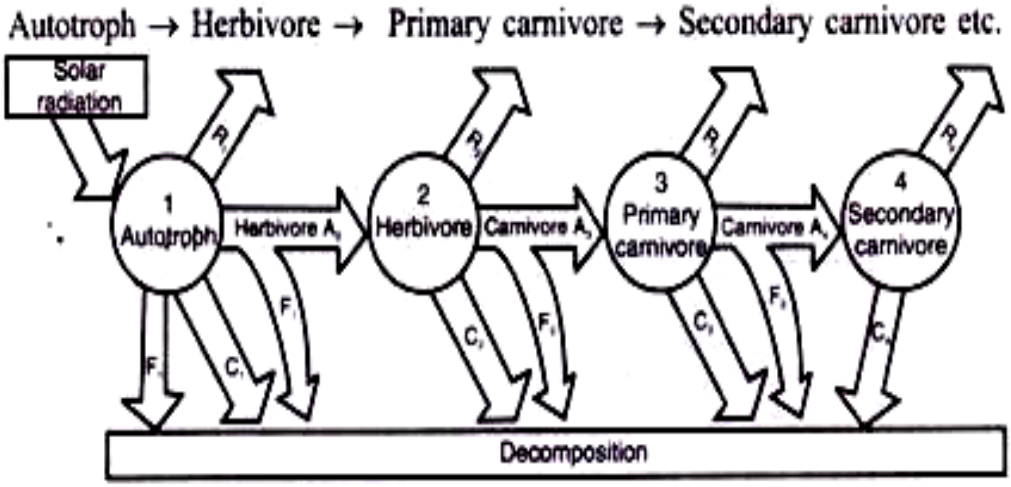


Fig. 3.8. Diagrammatic representation of a grazing food chain showing input and losses of energy at each trophic level. Trophic levels are numbered and used as subscripts to letters indicating energy transfer. A—assimilation of food by the organisms at the trophic level; F—energy lost in the form of faeces and other excretory products; C—energy lost through decay; and R—energy lost to respiration.

A schematic representation of grazing food chain showing input and losses of energy has been presented.

2. Parasitic food chain:
It goes from large organisms to smaller ones without outright killing as in the case of predator.

3. Detritus food chain:
The dead organic remains including metabolic wastes and exudates derived from grazing food chain are generally termed detritus. The energy contained in detritus is not lost in ecosystem as a whole, rather it serves as a source of energy for a group of organisms called detritivores that are separate from the grazing food chain. The food chain so formed is called detritus food chain (Fig. 3.9).

In some ecosystems more energy flows through the detritus food chain than through grazing food chain. In detritus food chain the energy flow remains as a continuous passage rather than as a stepwise flow between discrete entities. The organisms in the detritus food chain are many and include algae, fungi, bacteria, slime moulds, actinomycetes, protozoa, etc. Detritus organisms ingest pieces of partially decomposed organic matter, digest them partially and after extracting some of the chemical energy in the food to run their metabolism, excrete the remainder in the form of simpler organic molecules.

The waste from one organism can be immediately utilized by a second one which repeats the process. Gradually, the complex organic molecules present in the organic wastes or dead tissues are broken down to much simpler compounds, sometimes to carbon dioxide and water and all that are left are humus. In a normal environment the humus is quite stable and forms an essential part of the soil. Schematic representation of detritus food chain is given in Fig. 3.9.

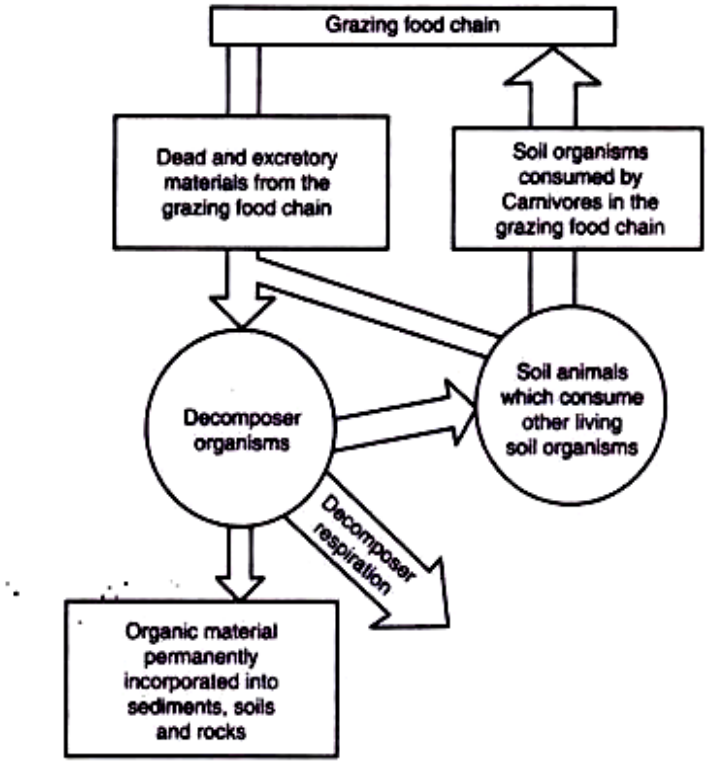


Fig. 3.9. Diagrammatic representation of the detritus food chain showing energy transfers between it and the grazing food chain, as well as energy losses to the detritus food chain.

Food web:

Many food chains exist in an ecosystem, but as a matter of fact these food chains are not independent. In ecosystem, one organism does not depend wholly on another. The resources are shared specially at the beginning of the chain. The marsh plants are eaten by variety of insects, birds, mammals and fishes and some of the animals are eaten by several predators.

Similarly, in the food chain grass → mouse → snakes → owls, sometimes mice are not eaten by snakes but directly by owls. This type of interrelationship interlinks the individuals of the whole community. In this way, food chains become interlinked. A complex of interrelated food chains makes up a food web. Food web maintains the stability of the ecosystem. The greater the number of alternative pathways the more stable is the community of living things. Fig. 3.10. illustrates a food web in ecosystem.

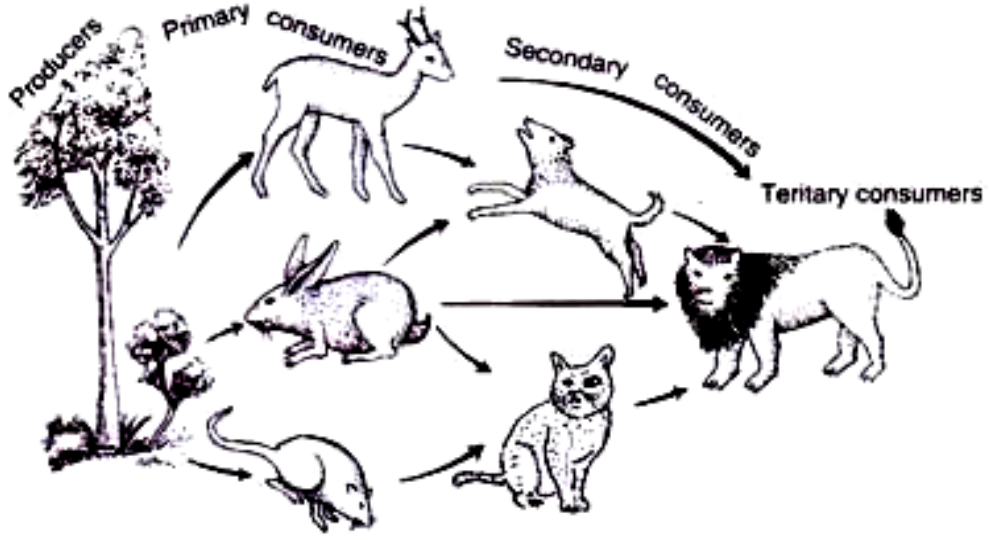


Fig. 3.10. Food web in an ecosystem.

Ecological pyramid:
The trophic structure of an ecosystem can be indicated by means of ecological pyramid. At each step in the food chain a considerable fraction of the potential energy is lost as heat. As a result, organisms in each trophic level pass on lesser energy to the next trophic level than they actually receive. This limits the number of steps in any food chain to 4 or 5. Longer the food chain the lesser energy is available for final members. Because of this tapering off of available energy in the food chain a pyramid is formed that is known as ecological pyramid. The higher the steps in the ecological pyramid the lower will be the number of individuals and the larger their size.

The idea of ecological pyramids was advanced by C.E. Elton (1927). There are different types of ecological pyramids. In each ecological pyramid, producer level forms the base and successive levels make up the apex. Three types of pyramidal relations may be found among the organisms at different levels in the ecosystem.

These are as follows:
1. Pyramid of numbers,

2. Pyramid of biomass (biomass is the weight of living organisms), and

3. Pyramid of energy.

1. Pyramid of numbers:
It depicts the numbers of individuals in producers and in different orders of consumers in an ecosystem. The base of pyramid is represented by producers which are the most abundant. In the successive levels of consumers, the number of organisms goes on decreasing rapidly until there are a few carnivores.

The pyramid of numbers of an ecosystem indicates that the producers are ingested in large numbers by smaller numbers of primary consumers. These primary consumers are eaten by relatively smaller number of secondary consumers and these secondary consumers, in turn, are consumed by only a few tertiary consumers (Fig. 3.11, 3.12a).

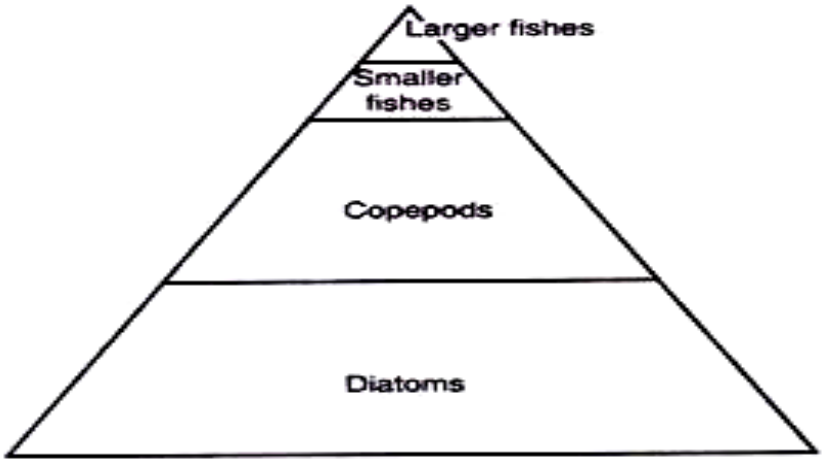


Fig. 3.11. A pyramid of numbers of a lake ecosystem.

This type of pyramid is best presented by taking an example of Lake Ecosystem. In this type of pyramid the base trophic level is occupied by producer elements—algae, diatoms and other hydrophytes which are most abundant. At the second trophic level come the herbivores or zooplanktons which are lesser in number than producers.

The third trophic level is occupied by carnivores which are still smaller in number than the herbivores and the top is occupied by a few top carnivores. Thus, in the ecological pyramid of numbers there is a relative reduction in number of organisms and an increase in the size of body from base to apex of the pyramid. In parasitic food chain starting from tree, the pyramid of numbers will be inverted (Fig. 3.12).

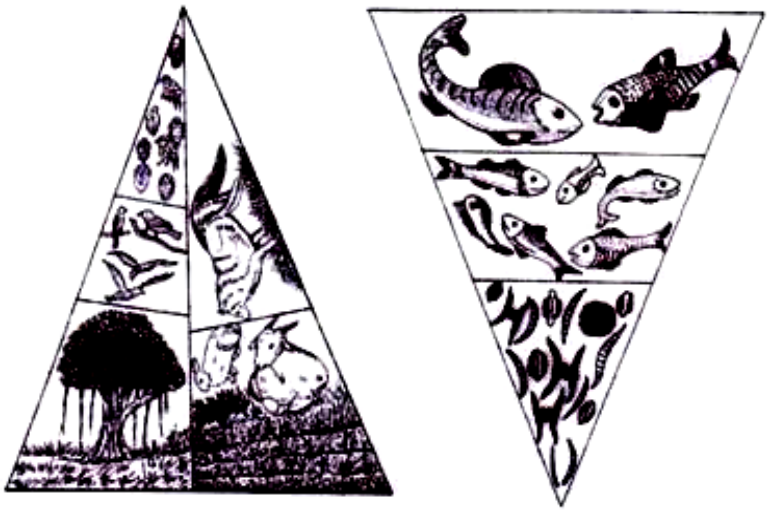


Fig. 3.12 (*a* & *b*). Pyramids of number
(*a*) Fig. 3.12 a & b. Up-right Pyramids of numbers in a grassland and cultivated field
(*b*) Pyramid of numbers (inverted) of diseased tree (Parasitic ecosystem)

2. Pyramid of biomass of organisms:
The living weights or biomass of the members of the food chain present at any one time form the pyramid of biomass of organisms. This indicates, by weight or other means of measuring materials, the total bulk of organisms or fixed energy present at one time. Pyramid of biomass indicates the decrease of biomass in each tropic level from base to apex, e.g., total biomass of producers is more than the total biomass of the herbivores.

Likewise, the total biomass of secondary consumers will be lesser than that of herbivores and so on (Fig. 3.13, 3.14 a, b). Since some energy and material are lost in each successive link, the total mass supported at each level is limited by the rate at which the energy is being stored below. This usually gives sloping pyramid for most of the communities in terrestrial and shallow water ecosystems. The pyramid of biomass in a pond ecosystem will be inverted as shown in Fig. 3.13 b.

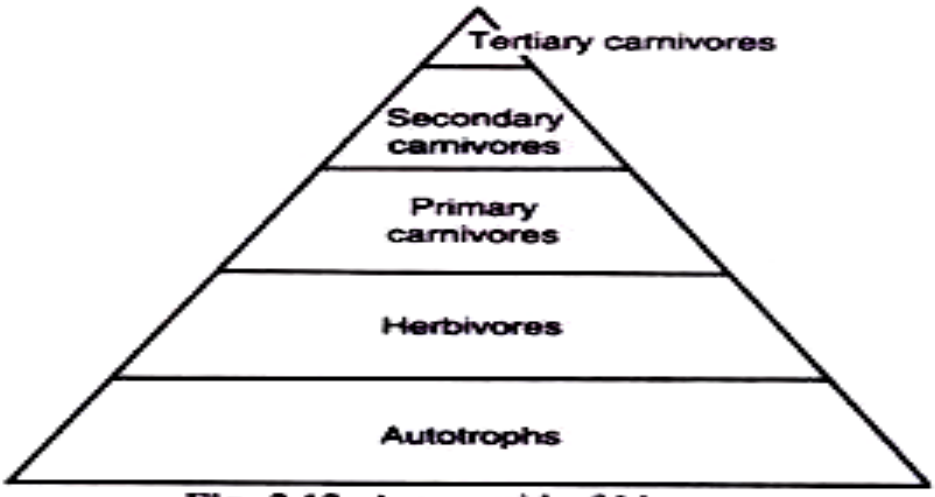


Fig. 3.13. A pyramid of biomass

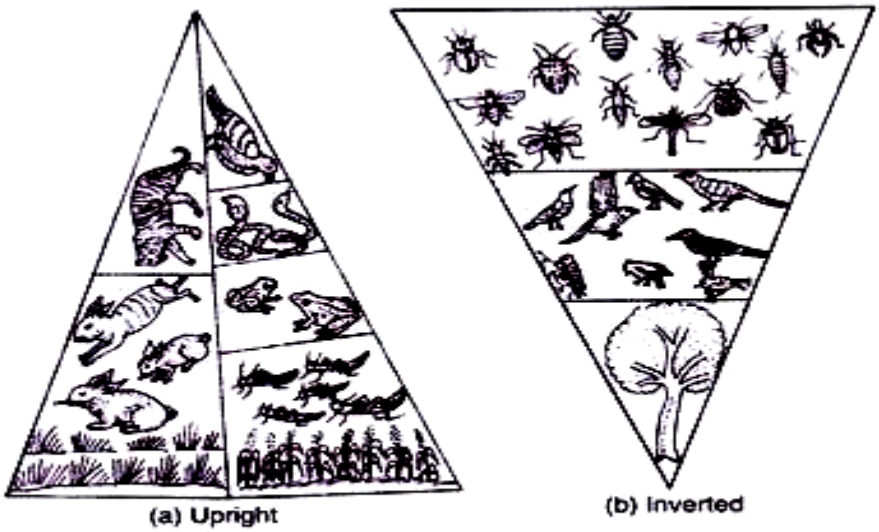


Fig. 3.14. (*a* & *b*). Pyramids of biomass
(*a*) A grassland ecosystems showing upright-triangular
(*b*) Inverted pyramid of biomass of an aquatic ecosystem.

3. Pyramid of energy:

This depicts not only the amount of total energy utilized by the organisms at each trophic level of food chain but more important, the actual role of various organisms in transfer of energy. At the producer level the total energy will be much greater than the energy at the successive higher trophic level.

Some producer organisms may have small biomass but the total energy they assimilate and pass on to consumers may be greater than that of organisms with much larger biomass. Higher trophic levels are more efficient in energy utilization but much heat is lost in energy transfer. Energy loss by respiration also progressively increases from lower to higher trophic states (Fig. 3.15).

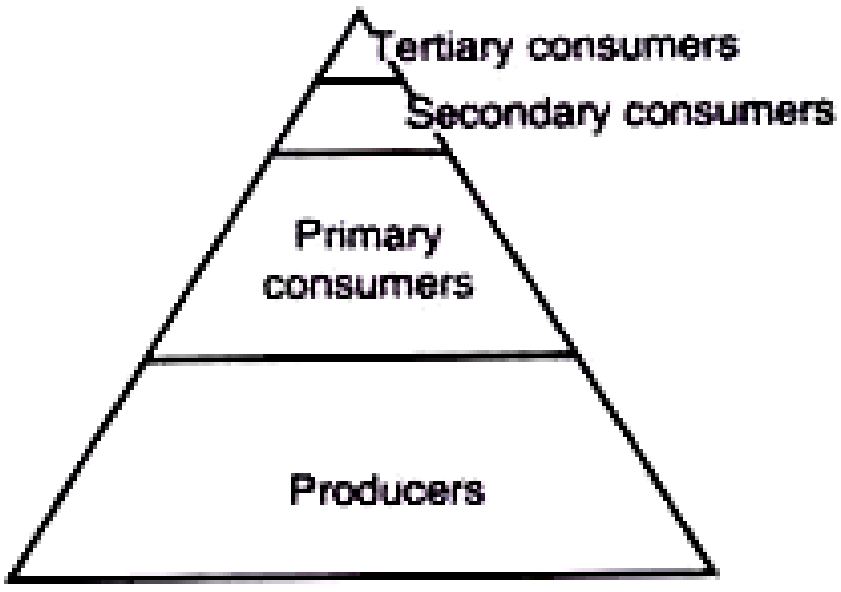


Fig. 3.15. A pyramid of energy

In the energy flow process, two things become obvious. Firstly there is one way along which energy moves i.e. unidirectional flow of energy. Energy comes in the ecosystem from outside source i.e. sun. The energy captured by autotrophs does not go back to the sun, the energy that passes from autotrophs to herbivores does not revert back and as it moves progressively through the various trophic levels, it is no longer available to the previous levels.

Thus due to unidirectional flow of energy, the system would collapse if the supply from primary source, the sun is cut off. Secondly, there occurs a progressive decrease in energy level at each trophic level which is accounted largely by the energy dissipated as heat in metabolic activities.

Productivity:
The relationship between the amount of energy accumulated and the amount of energy utilized within one trophic level of food chain has an important bearing on how much energy from one trophic level passes on to the next trophic level in the food chain. The ratio of output of energy to input of energy is referred to as ecological efficiency.

Different kinds of efficiencies can be measured by the following parameters:
(i) Ingestion which indicates the quantity of food or energy taken by trophic level. This is also called exploitation efficiency.
(ii) Assimilation indicates the amount of food absorbed and fixed into energy rich organic substances which are stored or combined with other molecules to build complex molecules such as proteins, fats etc.
(iii) Respiration which indicates the energy lost in metabolism.
Primary Productivity:
The fraction of fixed energy a trophic level passes on to the next trophic level is called production. Green plants fix solar energy and accumulate it in organic forms as chemical energy. Since it is the first and basic form of energy storage, the rate at which the energy accumulates in the green plants or producers is known as primary productivity.

Primary productivity is the rate at which energy is bound or organic material is created by photosynthesis per unit area of earth's surface per unit time. It is most often expressed as energy in calories / cm^2 / yr or dry organic matter in g / m^2 / yr (g/m^2 x 8.92 = lb / acre). The amount of organic matter present at a given time per unit area is called standing crop or biomass and as such productivity, which is a rate, is quite different from biomass or standing crop.
The standing crop is usually expressed as dry weight in g/m^2 or kg/m^2 or t/ha (metric tons) or 10^6g/hectare. Primary productivity is the result of photosynthesis by green plants including algae of different colours. Bacterial photosynthesis or chemosynthesis, although of small significance may also contribute to primary productivity. The total solar energy trapped in the food material by photosynthesis is referred to as gross primary productivity (G.P.P.).
A good fraction of gross primary production is utilized in respiration of green plants. The amount of energy bound in organic matter per unit area and time that is left after respiration in plants is net primary production (N.P.P.) or plant growth. Only the net primary productivity is available for harvest by man and other animals. Net productivity of energy = gross productivity—energy lost in respiration.

Secondary Productivity:
The rates at which the heterotrophic organisms resynthesize the energy-yielding substances is termed as secondary productivity. Secondary productivities are the productivities of animals and saprobes in communities. The amount of energy stored in the tissues of consumers or heterotrophs is termed as net secondary production and the total plant material ingested by herbivores is grass secondary production. Total plant material ingested by herbivores minus the materials lost as faeces is equal to Ingested Secondary Production.

Environmental factors affecting the production processes in an ecosystem are as follows:
1. Solar radiation and Temperature

2. Moisture. Leaf water potential, soil moisture and precipitation fluctuation and transpiration.

3. Mineral nutrition. Uptake of minerals from the soil, rhizosphere effects, fire effects, salinity, heavy metals, nitrogen metabolism.

4. Biotic activities. Grazing, above ground herbivores, below ground herbivores, predators and parasites, diseases of primary producers.

5. Impact of human population. Pollutions of different sorts, ionizing radiations like atomic explosions, etc.

There are three fundamental concepts of productivity:
1. Standing crop

2. Materials removed

3. Production rate.

1. Standing crop:
It is the abundance of the organisms existing in the area at any one time. It may be expressed in terms of number of individuals, as biomass of organisms, as energy content or in some other suitable terms. Measurement of standing crop reveals the concentration of individuals in the various populations of ecosystem.

2. The materials removed:
The second concept of productivity is the materials removed from the area per unit time. It includes the yield to man, organisms removed from the ecosystem by migration, and the material withdrawn as organic deposit.

3. The production rate:
The third concept of productivity is the production rate. It is the rate at which the growth processes are going forward within the area. The amount of material formed by each link in the food chain per unit of time per unit area or volume is the production rate.

All the three major groups of organisms—producers, consumers and reducers are the functional kingdoms of natural communities. The three represent major directions of evolution and are characterised by different modes of nutrition. Plants feed primarily by photosynthesis, animals feed primarily by ingesting food that is digested and absorbed in the alimentary canal and the saprobes feed by absorption and have need for an extensive surface of absorption. The principal kinds of organisms among saprobes are the unicellular bacteria, yeasts, chytrids or lower fungi and higher fungi with mycelial bodies.

In terrestrial communities as much as 90% of net primary production remains un-harvested and are utilized as dead tissue by saprobes and soil animals. The saprobes have a larger and more essential role than animals in degrading dead organic matter to inorganic forms and in such ecosystems, secondary production by reducers (decomposers) should exceed that by consumers, though the former is even more difficult to measure than the latter.

Biomass of decomposers with their microscopic cells and filaments embedded in food sources is also difficult to measure and that is small in relation to their productivity and significance for the ecosystem. Small masses of reducers degrade and transform larger masses of organic matter to inorganic remnants. In so doing decomposers disperse back to the environment the energy of photosynthesis accumulated in the organic compounds that are decomposed.

Thus they have a major role in the energy flow of ecosystems. A community or ecosystem, like an organism, is an open energy system. The continuous intake of energy in photosynthesis replaces the energy dissipated to environment by respiration and biological activity and the system does not run-down through the loss of free energy to maximum entropy.

If the amount of energy entrapped is greater than the energy dissipated, the pool of biologically useful energy of organic bonds increases. This results in increase of community biomass and consequently the community grows; such is the case in succession. If energy intake is lesser than energy dissipation, the community biomass will decrease and it must, in some sense, retrogress. If energy intake and loss are in balance, the pool of organic energy is in steady state; such is the case in climax communities.

Three aspects of this steady state may be recognized:
(i) The steady state of population of climax communities in which equal birth and death rates in population

keep the number of individuals relatively constant,

(ii) The steady state of energy flow,

(iii) The steady state of the matter of community, where addition of material by photosynthesis and organic synthesis is balanced by loss of material through respiration and decomposition.

Methods of Measuring Primary Production:
There are several parameters for measuring primary production and the methods of measuring primary production are based on those parameters.

The methods are discussed here as under:
1. Harvest method:
It involves removal of vegetation periodically and weighing the material. For measuring above ground production, the above ground plant parts are clipped at ground level, dried to constant weight at 80°C and weighed. The dry weight in g/m^2 /year gives the ground production. Below ground production is estimated by using frequent core sampling technique of Dahlman and Kucera (1965). It is expressed in terms of weight in gm per unit area per year. In terms of energy one gm dry weight of plant material contains 4 to 5 kcal.
The limitations of harvest method are as follows:
(i) The amount of plant material consumed by herbivores and the food oxidized during respiration process of the plants is not accounted.

(ii) Root biomass is neglected.

(iii) Photosynthetic trans located to underground parts of plants are not known.

In spite of these limitations the method is used all over for measuring net assimilation rate (NAR) and relative growth rate (RGR).

2. Carbon dioxide assimilation method:
Utilization of CO_2 in photosynthesis or its liberation during respiration is measured by infrared gas analysis or by passing the gas through Baryta water $Ba(OH)_2$ and titrating the same. The CO_2 removed from incoming gas chamber is taken to be synthesized into organic matter by the green plants. Performing the experiment in light and dark chambers the net and gross production can be measured.
In the lighted chamber photosynthesis and respiration take place simultaneously and the CO_2 coming out from the chamber is the unused gas of the atmosphere plus gas from the respiration of plant parts. In the dark chamber all CO_2 is due to respiration.
Net production = Gross production—Respiration

3. Oxygen production method:
In the aquatic vegetation CO_2 gas analysis method is not used but oxygen evolution method is generally used. The light and dark bottle technique is employed for measuring primary production of aquatic plant. In this method two bottles, one transparent and the other opaque are filled with water at a given depth of lake, closed, maintained at that depth for some time and then brought to laboratory for determination of oxygen content in the water. The decrease of oxygen in dark bottle is due to respiratory activity while increase of O_2 in light bottle is due to photosynthesis. The total increase of O_2 in light bottle plus the amount of O_2 decreased in dark bottle express gross productivity (O_2 value multiplied by 0.375 gives an equivalent of carbon assimilation). Recently, oxygen electrodes have been used for estimating oxygen content in water.
4. Chlorophyll method:
Gesner (1949) pointed out that the amount of chlorophyll/m^2 is almost limited to a narrow range of 0.1 to 3.0 gm regardless of the age of individuals or the species present therein. There is direct correlation between the amount of chlorophyll and dry matter production in different types of communities with varying light conditions.
The relation of total amount of chlorophyll to the photosynthetic rate is referred to as assimilation ratio or rate of production/gm chlorophyll. Total chlorophyll per unit area is greater in land plants as compared to that in aquatic plants. In marine ecosystem the rate of carbon assimilation is 3.7 g/ hr/g of chlorophyll. The

relationship between area based chlorophyll and dry matter production in terrestrial ecosystems has been worked out by Japanese ecologists Argua and Monsi (1963).

5. Other methods:
Pandeya (1971), Sharma (1972) and several other ecologists have evolved correlation coefficients for evaluating biomass and productivity in forest trees by measuring their diameter at breast height (DBH), height, canopy cover, etc.

Methods of establishing regression are as below:
(i) Diameter of trees in sample quadrats is measured at breast height and the height repeated is determined for each tree.

(ii) Different diameter and height classes are determined for each species.

(iii) A set of sample trees are cut and subjected to a detailed analysis for dry weight of stems, twigs, leaves and roots.

(iv) Regression values are computed for the sets of trees belonging to each girth class, relating the biomass of each fraction to the diameter at breast height.

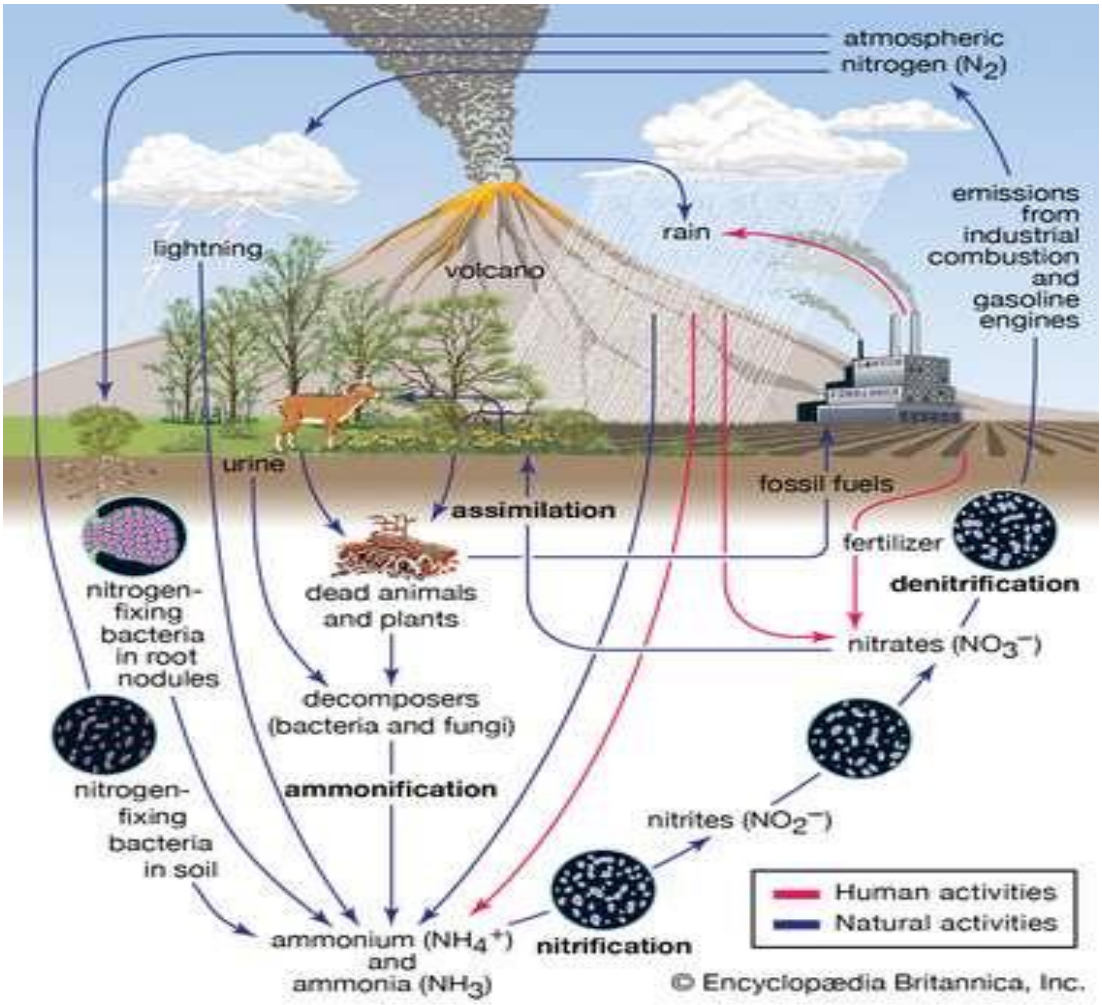
(v) The regression values are used to compute the probable biomass and Production each tree in the sample area. These values for each species when pooled give biomass and production rate of trees per unit area in the forest. Age of the trees markedly influences the annual net production.

6. Biogeochemical Cycles- Concept, Gaseous and Sedimentary Cycle, Nitrogen, Sulphur and Phosphorous cycles.

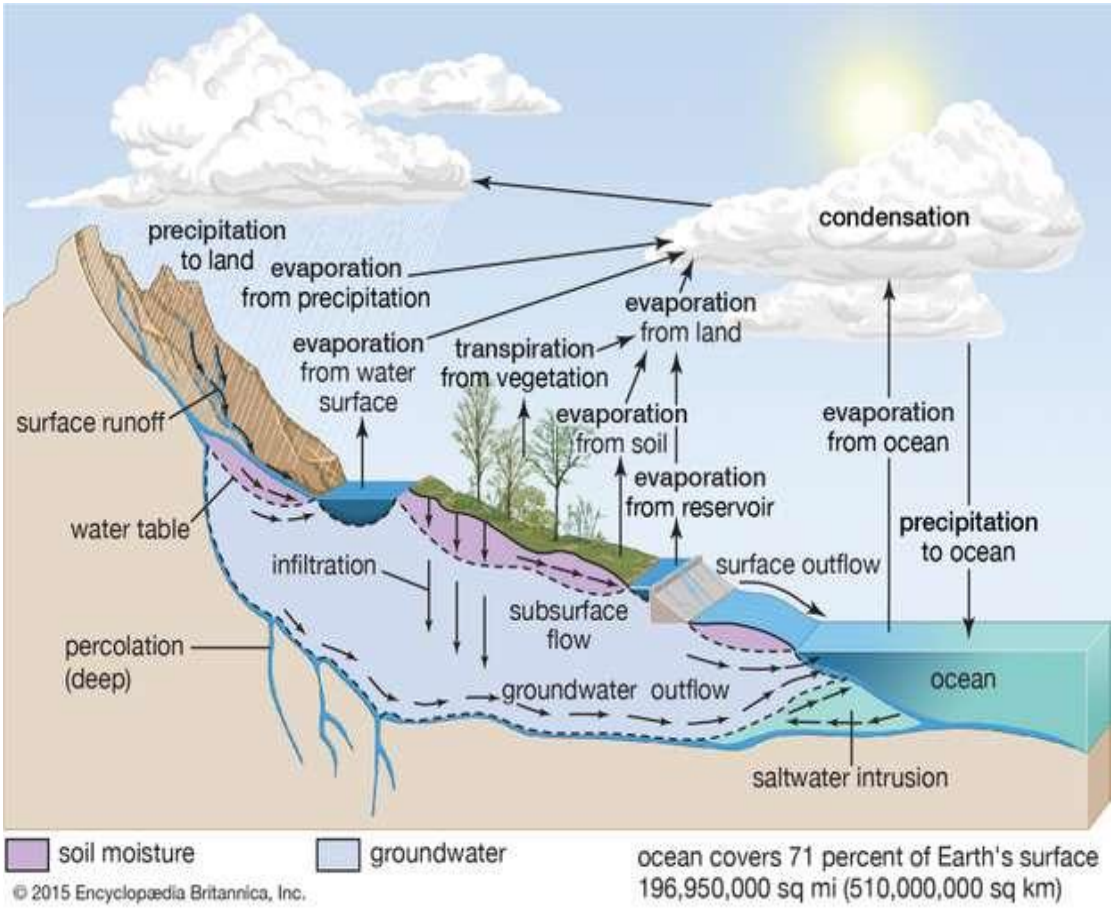
In ecology and Earth science, a **biogeochemical cycle** or **substance turnover** or **cycling of substances** is a pathway by which a chemical substance moves through biotic (biosphere) and abiotic (lithosphere, atmosphere, and hydrosphere) compartments of Earth. There are biogeochemical cyclesfor the chemical elements calcium , carbon, hydrogen, mercury, nitrogen, oxygen, phosphorus, selenium, and sulfur; molecular cycles for water and silica; macroscopic cycles such as the rock cycle; as well as human-induced cycles for synthetic compounds such as polychlorinated biphenyl (PCB). In some cycles there are *reservoirs* where a substance remains for a long period of time (such as an ocean or lake for water).

Elements within biogeochemical cycles flow in various forms from the nonliving (abiotic) components of the biosphere to the living (biotic) components and back. In order for the living components of a major ecosystem (e.g., a lake or a forest) to survive, all the chemical elements that make up living cells must be recycled continuously. Each biogeochemical cycle can be considered as having a reservoir (nutrient) pool—a larger, slow-moving, usually abiotic portion—and an exchange (cycling) pool—a smaller but more-active portion concerned with the rapid exchange between the biotic and abiotic aspects of an ecosystem.

Biogeochemical cycles can be classed as gaseous, in which the reservoir is the air or the oceans (via evaporation), and sedimentary, in which the reservoir is Earth's crust. Gaseous cycles include those of nitrogen, oxygen, carbon, and water; sedimentary cycles include those of iron, calcium, phosphorus, sulfur, and other more-earthbound elements. Gaseous cycles tend to move more rapidly than do sedimentary ones and to adjust more readily to changes in the biosphere because of the large atmospheric reservoir. Local accumulations of carbon dioxide (CO_2), for example, are soon dissipated by winds or taken up by plants. Extraordinary disturbances (such as global warming) and more-frequent local disturbances (such as wildfires and storm-driven events) can, however, seriously affect the capacity for self-adjustment.



GASEOUS AND SEDIMENTARY CYCLE:



Sedimentary cycles vary from one element to another, but each cycle consists fundamentally of a solution (or water-related) phase and a rock (or sediment) phase. In the solution phase, weathering releases minerals from Earth's crust in the form of salts, some of which dissolve in water, pass through a series of organisms, and ultimately reach the deep seas, where they settle out of circulation indefinitely. In the rock phase, other salts deposit out as sediment and rock in shallow seas, eventually to be weathered and recycled. Report Advertisement

Sedimentary cycle A cycle which comprises the weathering of an existing rock, followed by the erosion of minerals, their transport and deposition, then burial. First-cycle sediments are characterized by the presence of less resistant minerals and rock fragments. If this material is reworked through a second cycle, the less resistant minerals will be eliminated, or altered to more stable products. The more sedimentary cycles that a sediment has passed through, the more mature it will become and it will be dominated by well-rounded, resistant minerals

Plants and some animals obtain their nutrient needs from solutions in the environment. Other animals acquire the bulk of their needs from the plants and animals that they consume. After the death of an organism, the elements fixed in its body are returned to the environment through the action of decomposers (decay organisms such as bacteria, insects, and fungi) and become available to other living organisms again.

PHOSPHORUS CYCLE:

Phosphorus is an important element for all forms of life. As phosphate (PO4), it makes up an important part of the structural framework that holds DNA and RNA together. Phosphates are also a critical component of ATP—the cellular energy carrier—as they serve as an energy release' for organisms to use in building proteins or contacting muscles. Like calcium, phosphorus is important to vertebrates; in the human body, 80% of phosphorous is found in teeth and bones.

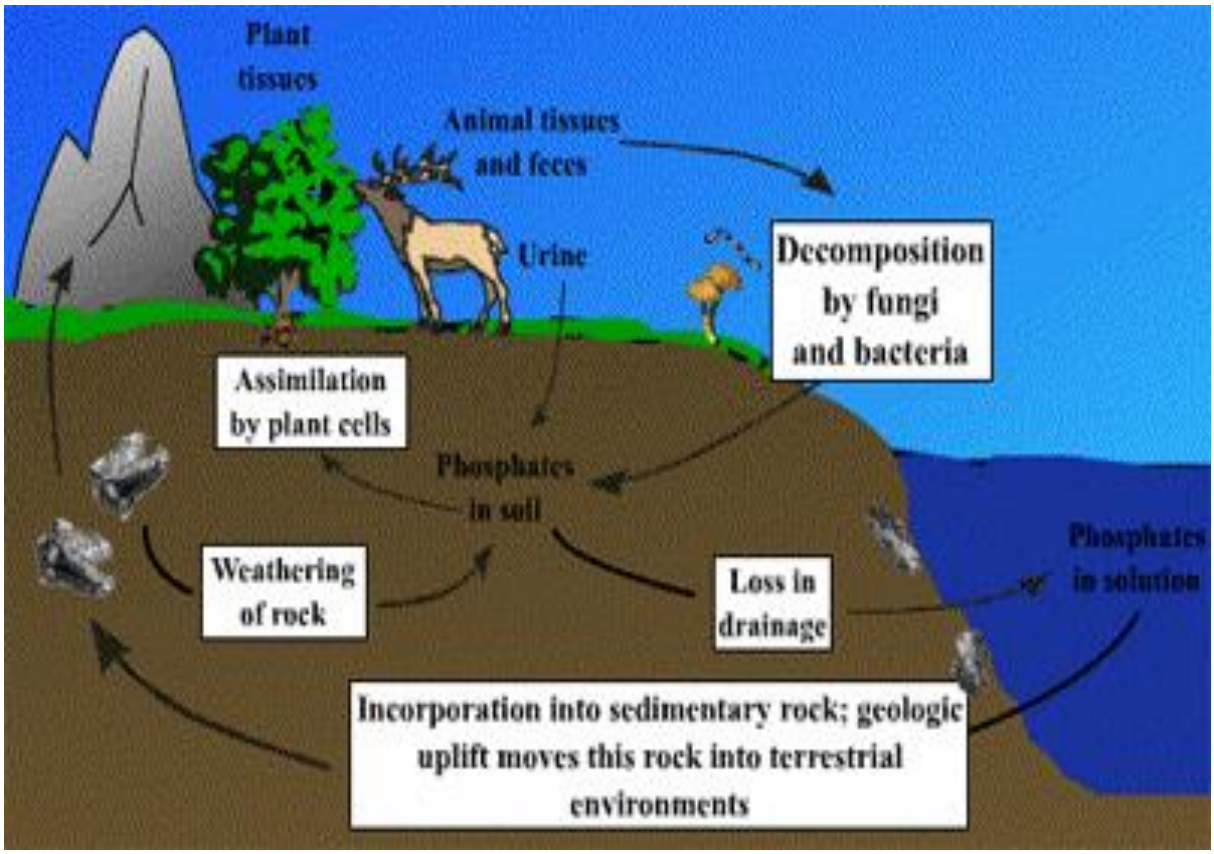
The phosphorus cycle differs from the other major biogeochemical cycles in that it does not include a gas phase; although small amounts of phosphoric acid (H_3PO_4) may make their way into the atmosphere, contributing—in some cases—to acid rain. The water, carbon, nitrogen and sulfur cycles all include at least one phase in which the element is in its gaseous state. Very little phosphorus circulates in the atmosphere because at Earth's normal temperatures and pressures, phosphorus and its various compounds are not gases. The largest reservoir of phosphorus is in sedimentary rock.

It is in these rocks where the phosphorus cycle begins. When it rains, phosphates are removed from the rocks (via weathering) and are distributed throughout both soils and water. Plants take up the phosphate ions from the soil. The phosphates then moves from plants to animals when herbivores eat plants and carnivores eat plants or herbivores. The phosphates absorbed by animal tissue through consumption eventually returns to the soil through the excretion of urine and feces, as well as from the final decomposition of plants and animals after death.

The same process occurs within the aquatic ecosystem. Phosphorus is not highly soluble, binding tightly to molecules in soil, therefore it mostly reaches waters by traveling with runoff soil particles. Phosphates also enter waterways through fertilizer runoff, sewage seepage, natural mineral deposits, and wastes from other industrial processes. These phosphates tend to settle on ocean floors and lake bottoms. As sediments are stirred up, phosphates may reenter the phosphorus cycle, but they are more commonly made available to aquatic organisms by being exposed through erosion. Water plants take up the waterborne phosphate which then travels up through successive stages of the aquatic food chain.

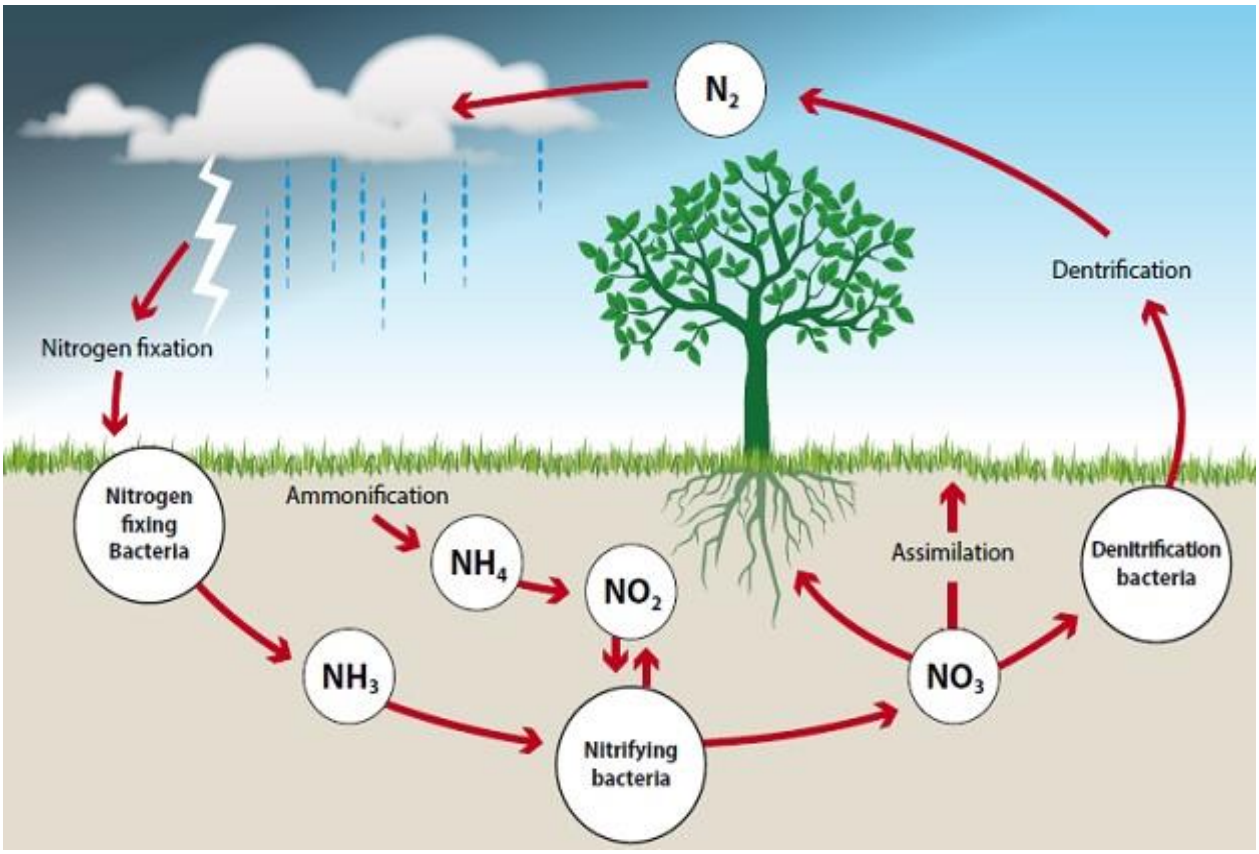
While obviously beneficial for many biological processes, in surface waters an excessive concentration of phosphorus is considered a pollutant. Phosphate stimulates the growth of plankton and plants, favoring weedy species over others. Excess growth of these plants tend to consume large amounts of dissolved oxygen, potentially suffocating fish and other marine animals, while also blocking available sunlight to bottom dwelling species. This is known as eutrophication.

Humans can alter the phosphorus cycle in many ways, including in the cutting of tropical rain forests and through the use of agricultural fertilizers. Rainforest ecosystems are supported primarily through the recycling of nutrients, with little or no nutrient reserves in their soils. As the forest is cut and/or burned, nutrients originally stored in plants and rocks are quickly washed away by heavy rains, causing the land to become unproductive. Agricultural runoff provides much of the phosphate found in waterways. Crops often cannot absorb all of the fertilizer in the soils, causing excess fertilizer runoff and increasing phosphate levels in rivers and other bodies of water. At one time the use of laundry detergents contributed to significant concentrations of phosphates in rivers, lakes, and streams, but most detergents no longer include phosphorus as an ingredient.



NITROGEN CYCLE: circulation of nitrogen in various forms through nature. Nitrogen, a component of proteins and nucleic acids, is essential to life on Earth. Although 78 percent by volume of the atmosphere is nitrogen gas, this abundant reservoir exists in a form unusable by most organisms. Through a series of microbial transformations, however, nitrogen is made available to plants, which in turn ultimately sustain all animal life. The steps, which are not altogether sequential, fall into the following classifications: nitrogen fixation, nitrogen assimilation, ammonification, nitrification, and denitrification. Nitrogen fixation, in which nitrogen gas is converted into inorganic nitrogen compounds, is mostly (90 percent) accomplished by certain bacteria and blue-green algae. A much smaller amount of free nitrogen is fixed by abiotic means (e.g., lightning, ultraviolet radiation, electrical equipment) and by conversion to ammonia through the Haber-Bosch process. Nitrates and ammonia resulting from nitrogen fixation are assimilated into the specific tissue compounds of algae and higher plants. Animals then ingest these algae and plants, converting them into their own body compounds. The remains of all living things—and their waste products—are decomposed by microorganisms in the process of ammonification, which yields ammonia (NH_3) and ammonium (NH_4^+). (Under anaerobic, or oxygen-free, conditions, foul-smelling putrefactive products may appear, but they too are converted to ammonia in time.) Ammonia can leave the soil or be converted into other nitrogen compounds, depending in part on soil conditions.

Nitrification, a process carried out by nitrifying bacteria, transforms soil ammonia into nitrates (NO_3^-), which plants can incorporate into their own tissues.
Nitrates also are metabolized by denitrifying bacteria, which are especially active in water-logged anaerobic soils. The action of these bacteria tends to deplete soil nitrates, forming free atmospheric nitrogen.



SULPHUR CYCLE:

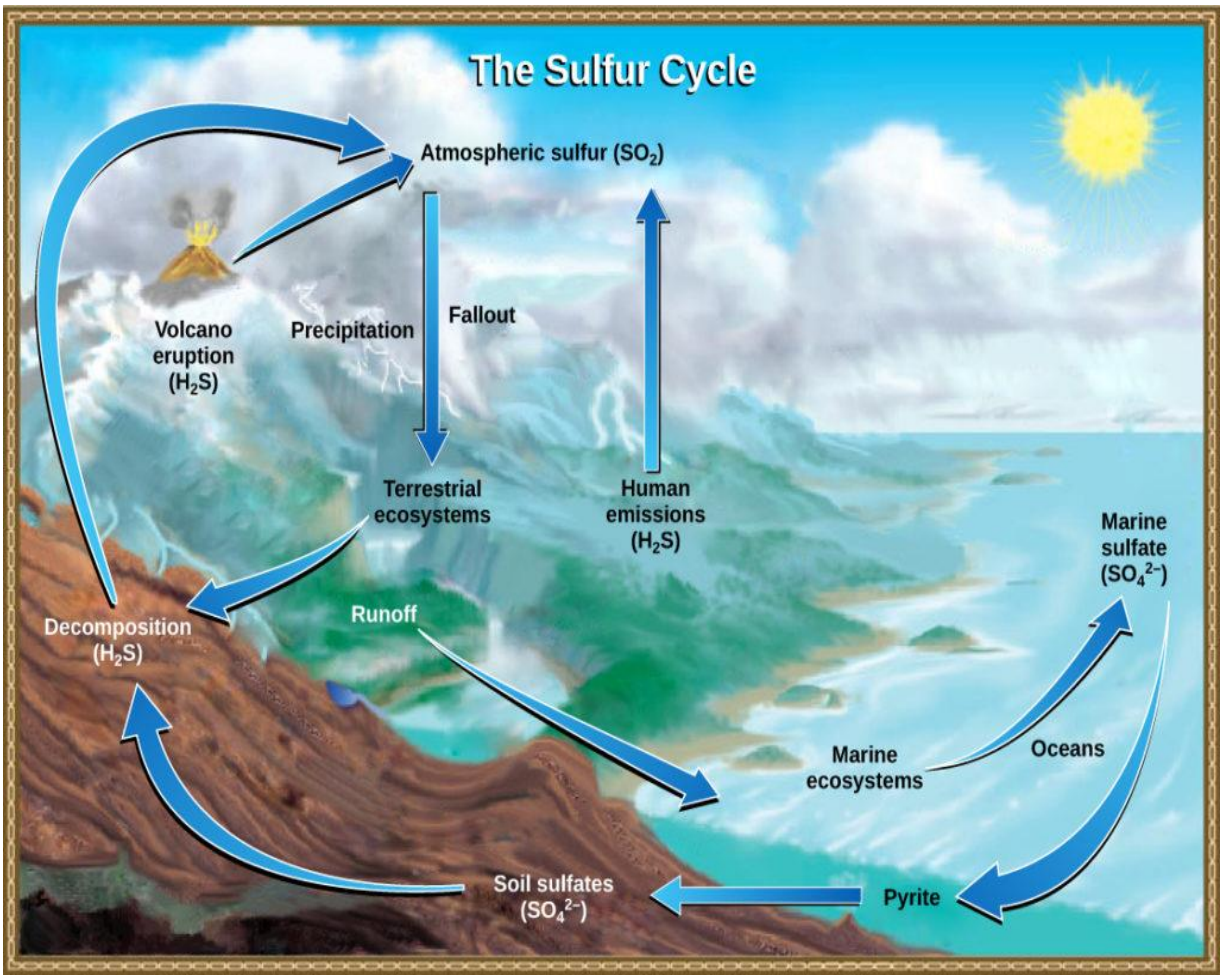
Sulfur, an essential element for the macromolecules of living things, is released into the atmosphere by the burning of fossil fuels, such as coal. As a part of the amino acid cysteine, it is involved in the formation of disulfide bonds within proteins, which help to determine their 3-D folding patterns, and hence their functions. As shown in Figure 1, sulfur cycles between the oceans, land, and atmosphere. Atmospheric sulfur is found in the form of sulfur dioxide (SO_2) and enters the atmosphere in three ways: from the decomposition of organic molecules, from volcanic activity and geothermal vents, and from the burning of fossil fuels by humans.

On land, sulfur is deposited in four major ways: precipitation, direct fallout from the atmosphere, rock weathering, and geothermal vents (Figure 2). Atmospheric sulfur is found in the form of sulfur dioxide (SO_2), and as rain falls through the atmosphere, sulfur is dissolved in the form of weak sulfuric acid (H_2SO_4). Sulfur can also fall directly from the atmosphere in a process called **fallout**. Also, the weathering of sulfur-containing rocks releases sulfur into the soil. These rocks originate from ocean sediments that are moved to land by the geologic uplifting of ocean sediments. Terrestrial ecosystems can then make use of these soil sulfates (SO_4^-), and upon the death and decomposition of these organisms, release the sulfur back into the atmosphere as hydrogen sulfide (H_2S) gas.

Sulfur enters the ocean via runoff from land, from atmospheric fallout, and from underwater geothermal vents. Some ecosystems rely on chemoautotrophs using sulfur as a biological energy source. This sulfur then supports marine ecosystems in the form of sulfates.

Human activities have played a major role in altering the balance of the global sulfur cycle. The burning of large quantities of fossil fuels, especially from coal, releases larger amounts of hydrogen sulfide gas into the atmosphere. As rain falls through this gas, it creates the phenomenon known as acid rain. **Acid rain** is corrosive rain caused by rainwater falling to the ground through sulfur dioxide gas, turning it into weak sulfuric acid, which causes damage to aquatic ecosystems. Acid rain damages the natural environment by lowering the pH of lakes, which kills many of the resident fauna; it also affects the man-made environment through the chemical degradation of buildings. For example, many marble monuments, such as the Lincoln Memorial in Washington,

DC, have suffered significant damage from acid rain over the years. These examples show the wide-ranging effects of human activities on our environment and the challenges that remain for our future.



7. Environmental pollution- Pollution and pollutant- Concept, definition and characteristics.

a. Air pollution- Source and types of air pollutant and their chemistry, photochemical reactions, green house and global warming, O_3 depletion, acid rain, air pollutant in India

b. Water pollution- Source and type of water pollution, effect of water pollution on ecosystem, heavy metals and their effect on biota, nuclear pollution and thermal pollution

c. Electronic waste (e waste), sources and types, constituents of e waste, recycling of e waste, impact of e waste on environment and its management

d. Soil pollution- Sources and classes of soil pollutants and their environmental effects, solid waste-pollution and disposal problems, waste- effect disposal and management

e. Pesticides, classification, chemical pollution, effect on living organisms.

Air pollution

Air pollution occurs when harmful or excessive quantities of substances including gases, particles, and biological molecules are introduced into Earth's atmosphere. It may cause diseases, allergies and even death to humans; it may also cause harm to other living organisms such as animals and food crops, and may

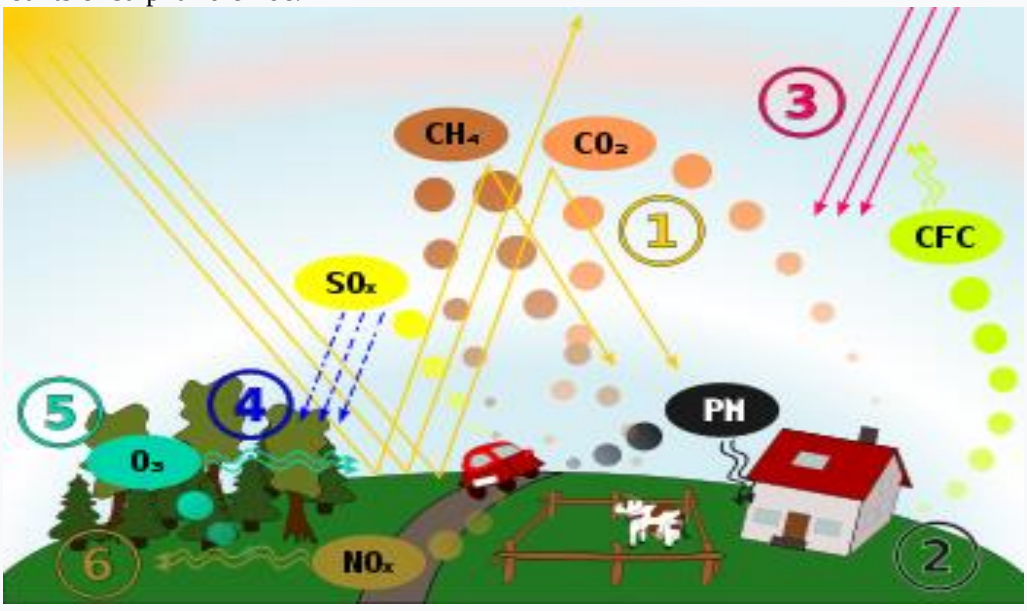
damage the natural or built environment. Both human activity and natural processes can generate air pollution.

Indoor air pollution and poor urban air quality are listed as two of the world's worst toxic pollution problems in the 2008 Blacksmith Institute World's Worst Polluted Places report. Outdoor air pollution alone causes 2.1 to 4.21 million premature deaths annually. According to the 2014 World Health Organization report, air pollution in 2012 caused the deaths of around 7 million people worldwide, an estimate roughly echoed by the International Energy Agency.

Pollutants:

An air pollutant is a material in the air that can have adverse effects on humans and the ecosystem. The substance can be solid particles, liquid droplets, or gases. A pollutant can be of natural origin or man-made. Pollutants are classified as primary or secondary. Primary pollutants are usually produced by processes such as ash from a volcanic eruption. Other examples include carbon monoxide gas from motor vehicle exhausts or Sulphur dioxide released from factories. Secondary pollutants are not emitted directly. Rather, they form in the air when primary pollutants react or interact. Ground level ozone is a prominent example of secondary pollutants. Some pollutants may be both primary and secondary: they are both emitted directly and formed from other primary pollutants.

Before flue-gas desulphurization was installed, the emissions from this power plant in New Mexico contained excessive amounts of sulphur dioxide.



Schematic drawing, causes and effects of air pollution: (1) greenhouse effect, (2) particulate contamination, (3) increased UV radiation, (4) acid rain, (5) increased ground-level ozone concentration, (6) increased levels of nitrogen oxides.

Thermal oxidizers are air pollution abatement options for hazardous air pollutants (HAPs), volatile organic compounds (VOCs), and odorous emissions

Pollutants emitted into the atmosphere by human activity include:

- Carbon dioxide– Because of its role as a greenhouse gas it has been described as "the leading pollutant" and "the worst climate pollution". Carbon dioxide is a natural component of the atmosphere, essential for plant life and given off by the human respiratory system. This question of terminology has practical effects, for example as determining whether the U.S. Clean Air Act is deemed to regulate CO_2 emissions.CO_2 currently forms about 410 parts per million (ppm) of earth's atmosphere, compared to about 280 ppm in pre-industrial times, and billions of metric tons of CO_2 are emitted annually by burning of fossil fuels. CO2 increase in earth's atmosphere has been accelerating.
- Sulfur oxides (SO_x) – particularly Sulphur dioxide, a chemical compound with the formula SO_2. SO2 is produced by volcanoes and in various industrial processes. Coal and petroleum often contain Sulphur compounds, and their combustion generates Sulphur dioxide. Further oxidation of SO_2, usually in the presence of a catalyst such as NO_2, forms H_2SO_4, and thus acid rain. This is one of the causes for concern over the environmental impact of the use of these fuels as power sources.
- Nitrogen oxides (NO_x) – Nitrogen oxides, particularly nitrogen dioxide, are expelled from high temperature combustion, and are also produced during thunderstorms by electric discharge. They can be seen as a brown haze dome above or a plume downwind of cities. Nitrogen dioxide is a chemical compound with the formula NO_2. It is one of several nitrogen oxides. One of the most prominent air pollutants, this reddish-brown toxic gas has a characteristic sharp, biting odor.
- Carbon monoxide (CO) – CO is a colorless, odorless, toxic yet non-irritating gas. It is a product of combustion of fuel such as natural gas, coal or wood. Vehicular exhaust contributes to the majority of carbon monoxide let into our atmosphere. It creates a smog type formation in the air that has been linked to many lung diseases and disruptions to the natural environment and animals. In 2013, more than half of the carbon monoxide emitted into our atmosphere was from vehicle traffic and burning one gallon of gas will often emit over 20 pounds of carbon monoxide into the air.
- Volatile organic compounds (VOC) – VOCs are a well-known outdoor air pollutant. They are categorized as either methane (CH_4) or non-methane (NMVOCs). Methane is an extremely efficient greenhouse gas which contributes to enhance global warming. Other hydrocarbon VOCs are also significant greenhouse gases because of their role in creating ozone and prolonging the life of methane in the atmosphere. This effect varies depending on local air quality. The aromatic NMVOCs benzene, toluene and xylene are

suspected carcinogens and may lead to leukemia with prolonged exposure. 1, 3-butadiene is another dangerous compound often associated with industrial use.

- Particulate matter / particles, alternatively referred to as particulate matter (PM), atmospheric particulate matter, or fine particles, are tiny particles of solid or liquid suspended in a gas. In contrast, aerosol refers to combined particles and gas. Some particulates occur naturally, originating from volcanoes, dust storms, forest and grassland fires, living vegetation, and sea spray. Human activities, such as the burning of fossil fuels in vehicles, power plants and various industrial processes also generate significant amounts of aerosols. Averaged worldwide, anthropogenic aerosols—those made by human activities—currently account for approximately 10 percent of our atmosphere. Increased levels of fine particles in the air are linked to health hazards such as heart disease, altered lung function and lung cancer. Particulates are related to respiratory infections and can be particularly harmful to those already suffering from conditions like asthma.
- Persistent free radicals connected to airborne fine particles are linked to cardiopulmonary disease.
- Toxic metals, such as lead and mercury, especially their compounds.
- Chlorofluorocarbons (CFCs) – harmful to the ozone layer; emitted from products are currently banned from use. These are gases which are released from air conditioners, refrigerators, aerosol sprays, etc. On release into the air, CFCs rise to the stratosphere. Here they come in contact with other gases and damage the ozone layer. This allows harmful ultraviolet rays to reach the earth's surface. This can lead to skin cancer, eye disease and can even cause damage to plants.
- Ammonia – emitted mainly by agricultural waste. Ammonia is a compound with the formula NH_3. It is normally encountered as a gas with a characteristic pungent odor. Ammonia contributes significantly to the nutritional needs of terrestrial organisms by serving as a precursor to foodstuffs and fertilizers. Ammonia, either directly or indirectly, is also a building block for the synthesis of many pharmaceuticals. Although in wide use, ammonia is both caustic and hazardous. In the atmosphere, ammonia reacts with oxides of nitrogen and Sulphur to form secondary particles.
- Odors — such as from garbage, sewage, and industrial processes
- Radioactive pollutants – produced by nuclear explosions, nuclear events, war explosives, and natural processes such as the radioactive decay of radon.

Secondary pollutants include:

- P**articulate**s created from gaseous primary pollutants and compounds in photochemical smog. Smog is a kind of air pollution. Classic smog results from large amounts of coal burning in an area caused by a mixture of smoke and Sulphur dioxide. Modern smog does not usually come from coal but from vehicular and industrial emissions that are acted on in the atmosphere by ultraviolet light from the sun to form secondary pollutants that also combine with the primary emissions to form photochemical smog.
- **Ground level ozone** (O_3) formed from NO_x and VOCs. Ozone (O_3) is a key constituent of the troposphere. It is also an important constituent of certain regions of the stratosphere commonly known as the Ozone layer. Photochemical and chemical reactions involving it drive many of the chemical processes that occur in the atmosphere by day and by night. At abnormally high concentrations brought about by human activities (largely the combustion of fossil fuel), it is a pollutant and a constituent of smog.
- **Peroxyacetyl nitrate** ($C_2H_3NO_5$) – similarly formed from NO_x and VOCs.

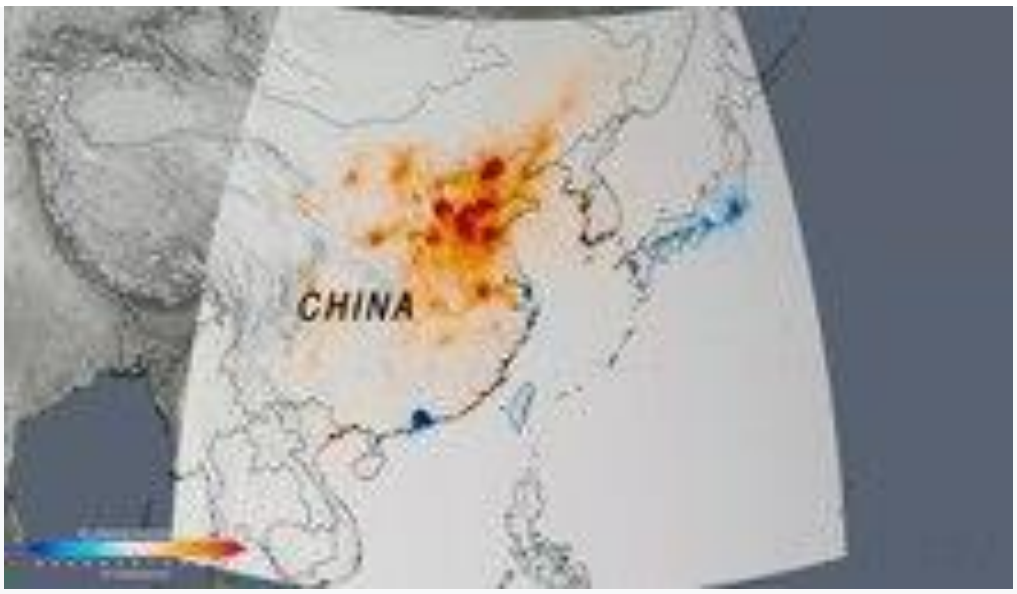
Minor air pollutants include:

- A large number of minor hazardous air pollutants. Some of these are regulated in USA under the Clean Air Act and in Europe under the Air Framework Directive

A variety of persistent organic pollutants, which can attach to particulates

This video provides an overview of a NASA study on the human fingerprint on global air quality.

Persistent organic pollutants (POPs) are organic compounds that are resistant to environmental degradation through chemical, biological, and photolytic processes. Because of this, they have been observed to persist in the environment, to be capable of long-range transport, bio-accumulate in human and animal tissue, bio-magnify in food chains, and to have potentially significant impacts on human health and the environment.



Source:

Mean acidifying emissions (air pollution) of different foods per 100g of protein	
Food Types	**Acidifying Emissions (g SO_2eq per 100g protein)**
Beef	343.6
Cheese	165.5
Pork	142.7
Lamb and Mutton	139.0
Farmed Crustaceans	133.1
Poultry	102.4
Farmed Fish	65.9
Eggs	53.7
Groundnuts	22.6
Peas	8.5
Tofu	6.7

There are various locations, activities or factors which are responsible for releasing pollutants into the atmosphere. These sources can be classified into two major categories.

Anthropogenic (man-made) sources:

Controlled burning of a field outside of Statesboro, Georgia in preparation for spring planting.

Smoking of fish over an open fire in Ghana, 2018

These are mostly related to the burning of multiple types of fuel.

- Stationary sources include smoke stacks of fossil fuel power stations (see for example environmental impact of the coal industry), manufacturing facilities (factories) and waste incinerators, as well as furnaces and other types of fuel-burning heating devices. In developing and poor countries, traditional biomass burning is the major source of air pollutants; traditional biomass includes wood, crop waste and dung.
- Mobile sources include motor vehicles, marine vessels, and aircraft.

- Controlled burn practices in agriculture and forest management. Controlled or prescribed burning is a technique sometimes used in forest management, farming, prairie restoration or greenhouse gas abatement. Fire is a natural part of both forest and grassland ecology and controlled fire can be a tool for foresters. Controlled burning stimulates the germination of some desirable forest trees, thus renewing the forest.
- Fumes from paint, hair spray, varnish, aerosol sprays and other solvents. These can be substantial; emissions from these sources was estimated to account for almost half of pollution from volatile organic compounds in the Los Angeles basin in the 2010s.
- Waste deposition in landfills, which generate methane. Methane is highly flammable and may form explosive mixtures with air. Methane is also an asphyxiant and may displace oxygen in an enclosed space. Asphyxia or suffocation may result if the oxygen concentration is reduced to below 19.5% by displacement.
- Military resources, such as nuclear weapons, toxic gases, germ warfare and rocketry.
- Fertilized farmland may be a major source of nitrogen oxides.

Natural sources

Dust storm approaching Stratford, Texas.

- Dust from natural sources, usually large areas of land with little or no vegetation
- Methane, emitted by the digestion of food by animals, for example cattle
- Radon gas from radioactive decay within the Earth's crust. Radon is a colorless, odorless, naturally occurring, radioactive noble gas that is formed from the decay of radium. It is considered to be a health hazard. Radon gas from natural sources can accumulate in buildings, especially in confined areas such as the basement and it is the second most frequent cause of lung cancer, after cigarette smoking.
- Smoke and carbon monoxide from wildfires. During periods of actives wildfires, smoke from uncontrolled biomass combustion can make up almost 75% of all air pollution by concentration.[27]
- Vegetation, in some regions, emits environmentally significant amounts of Volatile organic compounds (VOCs) on warmer days. These VOCs react with primary anthropogenic pollutants—specifically, NO_x, SO_2, and anthropogenic organic carbon compounds — to produce a seasonal haze of secondary pollutants. Black gum, poplar, oak and willow are some examples of vegetation that can produce abundant VOCs. The VOC production from these species result in ozone levels up to eight times higher than the low-impact tree species.
- Volcanic activity, which produces sulphur, chlorine, and ash particulates

Emission factors:

Beijing air on a 2005-day after rain (left) and a smoggy day (right)

Air pollutant emission factors are reported representative values that attempt to relate the quantity of a pollutant released to the ambient air with an activity associated with the release of that pollutant. These factors are usually expressed as the weight of pollutant divided by a unit weight, volume, distance, or duration of the activity emitting the pollutant (e.g., kilograms of particulate emitted per tonne of coal burned). Such factors facilitate estimation of emissions from various sources of air pollution. In most cases, these factors are simply averages of all available data of acceptable quality, and are generally assumed to be representative of long-term averages.

There are 12 compounds in the list of persistent organic pollutants. Dioxins and furans are two of them and intentionally created by combustion of organics, like open burning of plastics. These compounds are also endocrine disruptors and can mutate the human genes.

The United States Environmental Protection Agency has published a compilation of air pollutant emission factors for a wide range of industrial sources. The United Kingdom, Australia, Canada and many other countries have published similar compilations, as well as the European Environment Agency.

Exposure

Up to 30 % of Europeans living in cities are exposed to air pollutant levels exceeding EU air quality standards.

And around 98 % of Europeans living in cities are exposed to levels of air pollutants deemed damaging to health by the World Health Organization's more stringent guidelines.

Air pollution risk is a function of the hazard of the pollutant and the exposure to that pollutant. Air pollution exposure can be expressed for an individual, for certain groups (e.g. neighborhoods or children living in a country), or for entire populations. For example, one may want to calculate the exposure to a hazardous air pollutant for a geographic area, which includes the various microenvironments and age groups. This can be calculate as an inhalation exposure. This would account for daily exposure in various settings (e.g. different indoor micro-environments and outdoor locations). The exposure needs to include different age and other demographic groups, especially infants, children, pregnant women and other sensitive subpopulations. The exposure to an air pollutant must integrate the concentrations of the air pollutant with respect to the time spent in each setting and the respective inhalation rates for each subgroup for each specific time that the subgroup is in the setting and engaged in particular activities (playing, cooking, reading, working, spending time in traffic, etc.). For example, a small child's inhalation rate will be less than that of an adult. A child engaged in vigorous exercise will have a higher respiration rate than the same child in a sedentary activity. The daily exposure, then, needs to reflect the time spent in each micro-environmental setting and the type of activities in these settings. The air pollutant concentration in each micro activity/micro-environmental setting is summed to indicate the exposure. For some pollutants such as black carbon, traffic related exposures may dominate total exposure despite short exposure times since high concentrations coincide with proximity to major roads or participation to (motorized) traffic. A large portion of total daily exposure occurs as short peaks of high concentrations, but it remains unclear how to define peaks and determine their frequency and health impact.

Indoor air quality:

Air quality monitoring, New Delhi, India.

A lack of ventilation indoors concentrates air pollution where people often spend the majority of their time. Radon (Rn) gas, a carcinogen, is exuded from the Earth in certain locations and trapped inside houses. Building materials including carpeting and plywood emit formaldehyde (H_2CO) gas. Paint and solvents give off volatile organic compounds (VOCs) as they dry. Lead paint can degenerate into dust and be inhaled. Intentional air pollution is introduced with the use of air fresheners, incense, and other scented items. Controlled wood fires in stoves and fireplaces can add significant amounts of smoke particulates into the air, inside and out. Indoor pollution fatalities may be caused by using pesticides and other chemical sprays indoors without proper ventilation.

Carbon monoxide poisoning and fatalities are often caused by faulty vents and chimneys, or by the burning of charcoal indoors or in a confined space, such as a tent. Chronic carbon monoxide poisoning can result even from poorly-adjusted pilot lights. Traps are built into all domestic plumbing to keep sewer gas and hydrogen sulfide, out of interiors. Clothing emits tetrachloroethylene, or other dry cleaning fluids, for days after dry cleaning.

Though its use has now been banned in many countries, the extensive use of asbestos in industrial and domestic environments in the past has left a potentially very dangerous material in many localities. Asbestosis is a

chronic inflammatory medical condition affecting the tissue of the lungs. It occurs after long-term, heavy exposure to asbestos from asbestos-containing materials in structures. Sufferers have severe dyspnea (shortness of breath) and are at an increased risk regarding several different types of lung cancer. As clear explanations are not always stressed in non-technical literature, care should be taken to distinguish between several forms of relevant diseases. According to the World Health Organization (WHO), these may defined as; asbestosis, lung cancer, and Peritoneal Mesothelioma (generally a very rare form of cancer, when more widespread it is almost always associated with prolonged exposure to asbestos).

Biological sources of air pollution are also found indoors, as gases and airborne particulates. Pets produce dander, people produce dust from minute skin flakes and decomposed hair, dust mites in bedding, carpeting and furniture produce enzymes and micrometer-sized fecal droppings, inhabitants emit methane, mold forms on walls and generates mycotoxins and spores, air conditioning systems can incubate Legionnaires' disease and mold, and houseplants, soil and surrounding gardens can produce pollen, dust, and mold. Indoors, the lack of air circulation allows these airborne pollutants to accumulate more than they would otherwise occur in nature.

Health effects:

In 2012, air pollution caused premature deaths on average of 1 year in Europe, and was a significant risk factor for a number of pollution-related diseases, including respiratory infections, heart disease, COPD, stroke and cancer. The health effects caused by air pollution may include difficulty in breathing, wheezing, coughing, asthma and worsening of existing respiratory and cardiac conditions. These effects can result in increased medication use, increased doctor or emergency department visits, more hospital admissions and premature death. The human health effects of poor air quality are far reaching, but principally affect the body's respiratory system and the cardiovascular system. Individual reactions to air pollutants depend on the type of pollutant a person is exposed to, the degree of exposure, and the individual's health status and genetics the most common sources of air pollution include particulates, ozone, nitrogen dioxide, and Sulphur dioxide. Children aged less than five years that live in developing countries are the most vulnerable population in terms of total deaths attributable to indoor and outdoor air pollution.

Mortality

The World Health Organization estimated in 2014 that every year air pollution causes the premature death of some 7 million people worldwide. Studies published in March 2019 indicated that the number may be around 8.8 million.

India has the highest death rate due to air pollution India also has more deaths from asthma than any other nation according to the World Health Organization. In December 2013 air pollution was estimated to kill 500,000 people in China each year. There is a positive correlation between pneumonia-related deaths and air pollution from motor vehicle emissions.

Annual premature European deaths caused by air pollution are estimated at 430,000-800,000 an important cause of these deaths is nitrogen dioxide and other nitrogen oxides (NOx) emitted by road vehicles. In a 2015 consultation document the UK government disclosed that nitrogen dioxide is responsible for 23,500 premature UK deaths per annum. Across the European Union, air pollution is estimated to reduce life expectancy by almost nine months. Causes of deaths include strokes, heart disease, COPD, lung cancer, and lung infections.

Urban outdoor air pollution is estimated to cause 1.3 million deaths worldwide per year. Children are particularly at risk due to the immaturity of their respiratory organ systems.

The US EPA estimated in 2004 that a proposed set of changes in diesel engine technology (Tier 2) could result in 12,000 fewer premature mortalities, 15,000 fewer heart attacks, 6,000 fewer emergency department visits by children with asthma, and 8,900 fewer respiratory-related hospital admissions each year in the United States.

The US EPA has estimated that limiting ground-level ozone concentration to 65 parts per billion, would avert 1,700 to 5,100 premature deaths nationwide in 2020 compared with the 75-ppb standard. The agency projected the more protective standard would also prevent an additional 26,000 cases of aggravated asthma, and more than a million cases of missed work or school. Following this assessment, the EPA acted to protect public health by lowering the National Ambient Air Quality Standards (NAAQS) for ground-level ozone to 70 parts per billion (ppb).

A new economic study of the health impacts and associated costs of air pollution in the Los Angeles Basin and San Joaquin Valley of Southern California shows that more than 3,800 people die prematurely (approximately 14 years earlier than normal) each year because air pollution levels violate federal standards.

The number of annual premature deaths is considerably higher than the fatalities related to auto collisions in the same area, which average fewer than 2,000 per year.

Diesel exhaust (DE) is a major contributor to combustion-derived particulate matter air pollution. In several human experimental studies, using a well-validated exposure chamber setup, DE has been linked to acute vascular dysfunction and increased thrombus formation.

The mechanisms linking air pollution to increased cardiovascular mortality are uncertain, but probably include pulmonary and systemic inflammation.

Cardiovascular disease

A 2007 review of evidence found ambient air pollution exposure is a risk factor correlating with increased total mortality from cardiovascular events (range: 12% to 14% per 10 microg/m^3 increase).

Air pollution is also emerging as a risk factor for stroke, particularly in developing countries where pollutant levels are highest. A 2007 study found that in women, air pollution is not associated with hemorrhagic but with ischemic stroke. Air pollution was also found to be associated with increased incidence and mortality from coronary stroke in a cohort study in 2011. Associations are believed to be causal and effects may be mediated by vasoconstriction, low-grade inflammation and atherosclerosis other mechanisms such as autonomic nervous system imbalance have also been suggested.

Lung disease

Research has demonstrated increased risk of developing asthma and COPD from increased exposure to traffic-related air pollution. Additionally, air pollution has been associated with increased hospitalization and mortality from asthma and COPD. Chronic obstructive pulmonary disease (COPD) includes diseases such as chronic bronchitis and emphysema.

A study conducted in 1960–1961 in the wake of the Great Smog of 1952 compared 293 London residents with 477 residents of Gloucester, Peterborough, and Norwich, three towns with low reported death rates from chronic bronchitis. All subjects were male postal truck drivers aged 40 to 59. Compared to the subjects from the outlying towns, the London subjects exhibited more severe respiratory symptoms (including cough, phlegm, and dyspnea), reduced lung function (FEV_1 and peak flow rate), and increased sputum production and purulence. The differences were more pronounced for subjects aged 50 to 59. The study controlled for age and smoking habits, so concluded that air pollution was the most likely cause of the observed differences. More recent studies have shown that air pollution exposure from traffic reduces lung function development in children and lung function may be compromised by air pollution even at low concentrations. Air pollution exposure also cause lung cancer in nonsmokers.

It is believed that much like cystic fibrosis, by living in a more urban environment serious health hazards become more apparent. Studies have shown that in urban areas patients suffer mucus hyper secretion, lower levels of lung function, and more self-diagnosis of chronic bronchitis and emphysema.

Cancer (lung cancer)

Cancer is mainly the result of environmental factors.

A review of evidence regarding whether ambient air pollution exposure is a risk factor for cancer in 2007 found solid data to conclude that long-term exposure to PM2.5 (fine particulates) increases the overall risk of non-accidental mortality by 6% per a 10 microg/m^3 increase. Exposure to PM2.5 was also associated with an increased risk of mortality from lung cancer (range: 15% to 21% per 10 microg/m^3 increase) and total cardiovascular mortality (range: 12% to 14% per a 10 microg/m^3 increase). The review further noted that living close to busy traffic appears to be associated with elevated risks of these three outcomes – increase in lung cancer deaths, cardiovascular deaths, and overall non-accidental deaths. The reviewers also found

suggestive evidence that exposure to PM2.5 is positively associated with mortality from coronary heart diseases and exposure to SO_2 increases mortality from lung cancer, but the data was insufficient to provide solid conclusions. Another investigation showed that higher activity level increases deposition fraction of aerosol particles in human lung and recommended avoiding heavy activities like running in outdoor space at polluted areas.

In 2011, a large Danish epidemiological study found an increased risk of lung cancer for patients who lived in areas with high nitrogen oxide concentrations. In this study, the association was higher for non-smokers than smokers. An additional Danish study, also in 2011, likewise noted evidence of possible associations between air pollution and other forms of cancer, including cervical cancer and brain cancer.

In December 2015, medical scientists reported that cancer is overwhelmingly a result of environmental factors, and not largely down to bad luck. Maintaining a healthy weight, eating a healthy diet, minimizing alcohol and eliminating smoking reduces the risk of developing the disease, according to the researchers.

Children

In the United States, despite the passage of the Clean Air Act in 1970, in 2002 at least 146 million Americans were living in non-attainment areas—regions in which the concentration of certain air pollutants exceeded federal standards. These dangerous pollutants are known as the criteria pollutants, and include ozone, particulate matter, Sulphur dioxide, nitrogen dioxide, carbon monoxide, and lead. Protective measures to ensure children's health are being taken in cities such as New Delhi, India where buses now use compressed natural gas to help eliminate the "pea-soup" smog. A recent study in Europe has found that exposure to ultrafine particles can increase blood pressure in children. According to a WHO report-2018, polluted air is a main cause poisoning millions of children under the age of 15 years and ruining their lives which resulting to death of some six hundred thousand children annually.

Infants:

Ambient levels of air pollution have been associated with preterm birth and low birth weight. A 2014 WHO worldwide survey on maternal and perinatal health found a statistically significant association between low birth weights (LBW) and increased levels of exposure to PM2.5. Women in regions with greater than average PM2.5 levels had statistically significant higher odds of pregnancy resulting in a low-birth weight infant even when adjusted for country-related variables. The effect is thought to be from stimulating inflammation and increasing oxidative stress.

A study by the University of York found that in 2010 exposure to PM2.5 was strongly associated with 18% of preterm births globally, which was approximately 2.7 million premature births. The countries with the highest air pollution associated preterm births were in South and East Asia, the Middle East, North Africa, and West sub-Saharan Africa.

A study performed by Wang, et al. between the years of 1988 and 1991 has found a correlation between sulphur Dioxide (SO2) and total suspended particulates (TSP) and preterm births and low birth weights in Beijing. A group of 74,671 pregnant women, in four separate regions of Beijing, were monitored from early pregnancy to delivery along with daily air pollution levels of sulphur Dioxide and TSP (along with other particulates). The estimated reduction in birth weight was 7.3 g for every 100 $\mu g/m^3$ increase in SO2 and 6.9g for each 100 $\mu g/m^3$ increase in TSP. These associations were statistically significant in both summer and winter, although, summer was greater. The proportion of low birth weight attributable to air pollution, was 13%. This is the largest attributable risk ever reported for the known risk factors of low birth weight. Coal stoves, which are in 97% of homes, are a major source of air pollution in this area.

Brauer et al. studied the relationship between air pollution and proximity to a highway with pregnancy outcomes in a Vancouver cohort of pregnant woman using addresses to estimate exposure during pregnancy. Exposure to NO, NO2, CO PM10 and PM2.5 were associated with infants born small for gestational age (SGA). Women living <50meters away from an expressway or highway were 26% more likely to give birth to a SGA infant.

"Clean" areas

Even in the areas with relatively low levels of air pollution, public health effects can be significant and costly, since a large number of people breathe in such pollutants. A study published in 2017 found that even in areas of the U.S. where ozone and PM2.5 meet federal standards, Medicare recipients who are exposed to more air pollution have higher mortality rates. A 2005 scientific study for the British Columbia Lung Association

showed that a small improvement in air quality (1% reduction of ambient PM2.5 and ozone concentrations) would produce $29 million in annual savings in the Metro Vancouver region in 2010. This finding is based on health valuation of lethal (death) and sub-lethal (illness) affects.

Central nervous system

Data is accumulating that air pollution exposure also affects the central nervous system.

In a June 2014 study conducted by researchers at the University of Rochester Medical Center, published in the journal Environmental Health Perspectives, it was discovered that early exposure to air pollution causes the same damaging changes in the brain as autism and schizophrenia. The study also shows that air pollution also affected short-term memory, learning ability, and impulsivity. Lead researcher Professor Deborah Cory-Slechta said that "When we looked closely at the ventricles, we could see that the white matter that normally surrounds them hadn't fully developed. It appears that inflammation had damaged those brain cells and prevented that region of the brain from developing, and the ventricles simply expanded to fill the space. Our findings add to the growing body of evidence that air pollution may play a role in autism, as well as in other neuro developmental disorders." Air pollution has a more significant negative effect on males than on females.

In 2015, experimental studies reported the detection of significant episodic (situational) cognitive impairment from impurities in indoor air breathed by test subjects who were not informed about changes in the air quality. Researchers at the Harvard University and SUNY Upstate Medical University and Syracuse University measured the cognitive performance of 24 participants in three different controlled laboratory atmospheres that simulated those found in "conventional" and "green" buildings, as well as green buildings with enhanced ventilation. Performance was evaluated objectively using the widely used Strategic Management Simulation software simulation tool, which is a well-validated assessment test for executive decision-making in an unconstrained situation allowing initiative and improvisation. Significant deficits were observed in the performance scores achieved in increasing concentrations of either volatile organic compounds (VOCs) or carbon dioxide, while keeping other factors constant. The highest impurity levels reached are not uncommon in some classroom or office environments. Air pollution increases the risk of dementia in people over 50 years old.

Agricultural effects:

In India in 2014, it was reported that air pollution by black carbon and ground level ozone had reduced crop yields in the most affected areas by almost half in 2011 when compared to 1980 levels.

Economic effects:

Air pollution costs the world economy $5 trillion per year as a result of productivity losses and degraded quality of life, according to a joint study by the World Bank and the Institute for Health Metrics and Evaluation (IHME) at the University of Washington. These productivity losses are caused by deaths due to diseases caused by air pollution. One out of ten deaths in 2013 was caused by diseases associated with air pollution and the problem is getting worse. The problem is even more acute in the developing world. "Children under age 5 in lower-income countries are more than 60 times as likely to die from exposure to air pollution as children in high-income countries." The report states that additional economic losses caused by air pollution, including health costs and the adverse effect on agricultural and other productivity were not calculated in the report, and thus the actual costs to the world economy are far higher than $5 trillion.

Historical disasters:

The world's worst short-term civilian pollution crisis was the 1984 Bhopal Disaster in India. Leaked industrial vapors from the Union Carbide factory, belonging to Union Carbide, Inc., U.S.A. (later bought by Dow Chemical Company), killed at least 3787 people and injured from 150,000 to 600,000. The United Kingdom suffered its worst air pollution event when the December 4 Great Smog of 1952 formed over London. In six days more than 4,000 died and more recent estimates put the figure at nearer 12,000. An accidental leak of anthrax spores from a biological warfare laboratory in the former USSR in 1979 near Sverdlovsk is believed to have caused at least 64 deaths. The worst single incident of air pollution to occur in the US occurred in Donora, Pennsylvania in late October, 1948, when 20 people died and over 7,000 were injured.

Alternatives to pollution

There are now practical alternatives to the principal causes of air pollution:

- Areas downwind (over 20 miles) of major airports more than double total particulate emissions in air, even when factoring in areas with frequent ship calls, and heavy freeway and city traffic like Los Angeles. Aviation biofuel mixed in with jet fuel at a 50/50 ratio can reduce jet derived cruise altitude particulate emissions by 50-70%, according to a NASA led 2017 study (however, this should imply ground level benefits to urban air pollution as well).
- Ship propulsion and idling can be switched too much cleaner fuels like natural gas. (Ideally a renewable source but not practical yet)
- Combustion of fossil fuels for space heating can be replaced by using ground source heat pumps and seasonal thermal energy storage.
- Electric power generation from burning fossil fuels can be replaced by power generation from nuclear and renewables. For poor nations, heating and home stoves that contribute much to regional air pollution can be replaced by a much cleaner fossil fuel like natural gas, or ideally, renewables.
- Motor vehicles driven by fossil fuels, a key factor in urban air pollution, can be replaced by electric vehicles. Though lithium supply and cost is a limitation, there are alternatives. Herding more people into clean public transit such as electric trains can also help. Nevertheless, even in emission-free electric vehicles, rubber tires produce significant amounts of air pollution themselves, ranking as 13th worst pollutant in Los Angeles.
- Reducing travel in vehicles can curb pollution. After Stockholm reduced vehicle traffic in the central city with a congestion tax, nitrogen dioxide and PM10 pollution declined, as did acute pediatric asthma attacks.
- Bio digesters can be utilized in poor nations where slash and burn is prevalent, turning a useless commodity into a source of income. The plants can be gathered and sold to a central authority that will break it down in a large modern bio digester, producing much needed energy to use.
- Induced humidity and ventilation both can greatly dampen air pollution in enclosed spaces, which was found to be relatively high inside subway lines due to braking and friction and relatively less ironically inside transit buses than lower sitting passenger automobiles or subways.

Reduction efforts:

Various air pollution control technologies and strategies are available to reduce air pollution. At its most basic level, land-use planning is likely to involve zoning and transport infrastructure planning. In most developed countries, land-use planning is an important part of social policy, ensuring that land is used efficiently for the benefit of the wider economy and population, as well as to protect the environment.

Because a large share of air pollution is caused by combustion of fossil fuels such as coal and oil, the reduction of these fuels can reduce air pollution drastically. Most effective is the switch to clean power sources such as wind power, solar power, hydro power which don't cause air pollution. Efforts to reduce pollution from mobile sources includes primary regulation (many developing countries have permissive regulations), expanding regulation to new sources (such as cruise and transport ships, farm equipment, and small gas-powered equipment such as string trimmers, chainsaws, and snowmobiles), increased fuel efficiency (such as through the use of hybrid vehicles), conversion to cleaner fuels or conversion to vehicles. Titanium has been researched for its ability to reduce air pollution. Ultraviolet light will release free electrons from material, thereby creating free radicals, which break up VOCs and NOx gases. One form is super hydrophilic

In 2014, Prof. Tony Ryan and Prof. Simon Armitage of University of Sheffield prepared a 10 meter by 20 meter-sized poster coated with microscopic, pollution-eating nanoparticles of titanium dioxide. Placed on a building, this giant poster can absorb the toxic emission from around 20 cars each day.

A very effective means to reduce air pollution is the transition to renewable energy. According to a study published in Energy and Environmental Science in 2015 the switch to 100% renewable energy in the United States would eliminate about 62,000 premature mortalities per year and about 42,000 in 2050, if no biomass were used. This would save about $600 billion in health costs a year due to reduced air pollution in 2050, or about 3.6% of the 2014 U.S. gross domestic product.

Control devices

The following items are commonly used as pollution control devices in industry and transportation. They can either destroy contaminants or remove them from an exhaust stream before it is emitted into the atmosphere.

- **Particulate control**
  - Mechanical collectors (dust cyclones, multicyclones)

- Electrostatic precipitators an electrostatic precipitator (ESP), or electrostatic air cleaner is a particulate collection device that removes particles from a flowing gas (such as air), using the force of an induced electrostatic charge. Electrostatic precipitators are highly efficient filtration devices that minimally impede the flow of gases through the device, and can easily remove fine particulates such as dust and smoke from the air stream.
- Bag houses Designed to handle heavy dust loads, a dust collector consists of a blower, dust filter, a filter-cleaning system, and a dust receptacle or dust removal system (distinguished from air cleaners which utilize disposable filters to remove the dust).
- Particulate scrubbers Wet scrubber is a form of pollution control technology. The term describes a variety of devices that use pollutants from a furnace flue gas or from other gas streams. In a wet scrubber, the polluted gas stream is brought into contact with the scrubbing liquid, by spraying it with the liquid, by forcing it through a pool of liquid, or by some other contact method, so as to remove the pollutants.

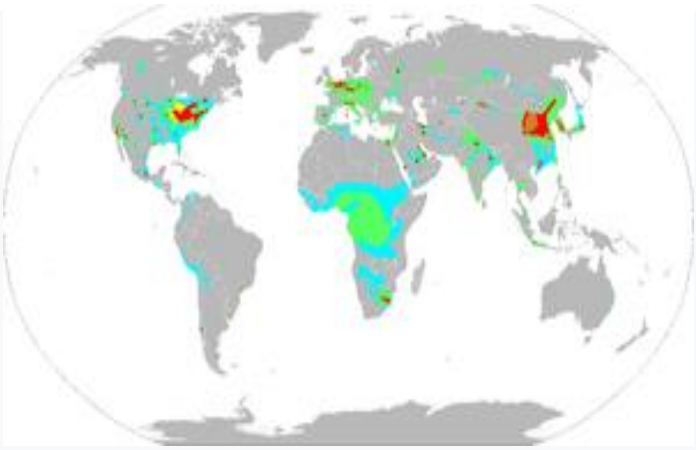
Hotspots:

Air pollution hotspots are areas where air pollution emissions expose individuals to increased negative health effects. They are particularly common in highly populated, urban areas, where there may be a combination of stationary sources (e.g. industrial facilities) and mobile sources (e.g. cars and trucks) of pollution. Emissions from these sources can cause respiratory disease, childhood asthma, cancer, and other health problems. Fine particulate matter such as diesel soot, which contributes to more than 3.2 million premature deaths around the world each year, is a significant problem. It is very small and can lodge itself within the lungs and enter the bloodstream. Diesel soot is concentrated in densely populated areas, and one in six people in the U.S. live near a diesel pollution hot spot.

While air pollution hotspots affect a variety of populations, some groups are more likely to be located in hotspots. Previous studies have shown disparities in exposure to pollution by race and/or income. Hazardous land uses (toxic storage and disposal facilities, manufacturing facilities, major roadways) tend to be located where property values and income levels are low. Low socioeconomic status can be a proxy for other kinds of social vulnerability, including race, a lack of ability to influence regulation and a lack of ability to move to neighborhoods with less environmental pollution. These communities bear a disproportionate burden of environmental pollution and are more likely to face health risks such as cancer or asthma.

Studies show that patterns in race and income disparities not only indicate a higher exposure to pollution but also higher risk of adverse health outcomes. Communities characterized by low socioeconomic status and racial minorities can be more vulnerable to cumulative adverse health impacts resulting from elevated exposure to pollutants than more privileged communities. Blacks and Latinos generally face more pollution than whites and Asians, and low-income communities bear a higher burden of risk than affluent ones. Racial discrepancies are particularly distinct in suburban areas of the Southern United States and metropolitan areas of the Midwestern and Western United States. Residents in public housing, who are generally low-income and cannot move to healthier neighborhoods, are highly affected by nearby refineries and chemical plants.

Cities



Nitrogen dioxide concentrations as measured from satellite 2002–2004

Deaths from air pollution in 2004

Air pollution is usually concentrated in densely populated metropolitan areas, especially in developing countries where environmental regulations are relatively lax or nonexistent. However, even populated areas in developed countries attain unhealthy levels of pollution, with Los Angeles and Rome being two examples. Between 2002 and 2011 the incidence of lung cancer in Beijing near doubled. While smoking remains the leading cause of lung cancer in China, the number of smokers is falling while lung cancer rates are rising.

Most polluted cities by PM	
Particulate matter, µg/m^3 (2004)	**City**
168	Cairo, Egypt
150	Delhi, India
128	Kolkata, India (Calcutta)
125	Tianjin, China
123	Chongqing, China
109	Kanpur, India
109	Lucknow, India
104	Jakarta, Indonesia
101	Shenyang, China

Governing urban air pollution:

In Europe, Council Directive 96/62/EC on ambient air quality assessment and management provides a common strategy against which member states can "set objectives for ambient air quality in order to avoid, prevent or reduce harmful effects on human health and the environment ... and improve air quality where it is unsatisfactory".

On 25 July 2008 in the case Dieter Janecek v Freistaat Bayern CURIA, the European Court of Justice ruled that under this directive citizens have the right to require national authorities to implement a short term action plan that aims to maintain or achieve compliance to air quality limit values.

This important case law appears to confirm the role of the EC as centralised regulator to European nation-states as regards air pollution control. It places a supranational legal obligation on the UK to protect its citizens from dangerous levels of air pollution, furthermore superseding national interests with those of the citizen.

In 2010, the European Commission (EC) threatened the UK with legal action against the successive breaching of PM10 limit values. The UK government has identified that if fines are imposed, they could cost the nation upwards of £300 million per year.

In March 2011, the Greater London Built-up Area remains the only UK region in breach of the EC's limit values, and has been given 3 months to implement an emergency action plan aimed at meeting the EU Air Quality Directive. The City of London has dangerous levels of PM10 concentrations, estimated to cause 3000 deaths per year within the city. As well as the threat of EU fines, in 2010 it was threatened with legal action for scrapping the western congestion charge zone, which is claimed to have led to an increase in air pollution levels.

In response to these charges, Boris Johnson, Mayor of London, has criticised the current need for European cities to communicate with Europe through their nation state's central government, arguing that in future "A great city like London" should be permitted to bypass its government and deal directly with the European Commission regarding its air quality action plan.

This can be interpreted as recognition that cities can transcend the traditional national government organizational hierarchy and develop solutions to air pollution using global governance networks, for example through transnational relations. Transnational relations include but are not exclusive to national governments and intergovernmental organizations, allowing sub-national actors including cities and regions to partake in air pollution control as independent actors.

Particularly promising at present are global city partnerships. These can be built into networks, for example the C40 Cities Climate Leadership Group, of which London is a member. The C40 is a public 'non-state' network of the world's leading cities that aims to curb their greenhouse emissions. The C40 has been identified as 'governance from the middle' and is an alternative to intergovernmental policy. It has the potential to improve urban air quality as participating cities "exchange information, learn from best practices and consequently mitigate carbon dioxide emissions independently from national government decisions".

Green house and global warming

The continuous rise in temperature of the planet is really upsetting. The root cause for this is global warming. Global warming begins when sunlight reaches the Earth. The clouds, atmospheric particles, reflective ground surfaces and surface of oceans then sends back about 30 % of sunlight back into the space, whilst the remaining is absorbed by oceans, air and land. This consequently heats up the surface of the planet and atmosphere, making life feasible. As the Earth warms up, this solar energy is radiated by thermal radiation and infrared rays, propagating directly out to space thereby cooling the Earth. However, some of the outgoing radiation is re-absorbed by carbon dioxide, water vapors, ozone, methane and other gases in the atmosphere and is radiated back to the surface of Earth. These gases are commonly known as greenhouse gases due to their heat-trapping capacity. It must be noted that this re-absorption process is actually good as the Earth’s average surface temperature would be very cold if there was no existence of greenhouse gases. The dilemma began when the concentration of greenhouse gases in the atmosphere was artificially increased by humankind at an alarming rate since the past two centuries. As of 2004, over 8 billion tons of carbon dioxide was pumped thermal radiation is further hindered by increased levels of greenhouse gases resulting in a phenomenon known as human enhanced global warming effect. Recent observations regarding global warming have substantiated the theory that it is indeed a human enhanced greenhouse effect that is causing the planet to heat up. The planet has experienced the largest increase in surface temperature over the last 100 years. Between 1906 and 2006, the Earth’s average surface temperature augmented between 0.6 to 0.9 degrees Celsius, however out per year.

Millions of pounds of methane gas are generated in landfills and agricultural decomposition of biomass and animal manure. Nitrous oxide is released into the atmosphere by various nitrogen-based fertilizers including urea and di-ammonium phosphate and other soil management utilizations. Once released, these greenhouse gases stay in the atmosphere for decades or even longer. According to Intergovernmental Panel on Climate Change (IPCC), carbon dioxide and methane levels have increased by 35 % and 148 % since the industrial revolution of 1750.

Greenhouse Effect

While other planets in the solar system of the Earth are either roasting hot or bitterly cold, Earth's surface has relatively mild, steady temperatures. Earth enjoys these temperatures because of its atmosphere, which is the thin layer of gases that cover and protect the planet. However, 97 % of climate scientists and researchers agree that humans have changed the Earth's atmosphere in dramatic ways over the past two centuries, resulting in global warming. To understand global warming, it is first necessary to become familiar with the greenhouse effect. As Fig.1 depicts, the natural greenhouse effect normally traps some portion of heat in such a way that our planet is safe from reaching freezing temperatures while human enhanced greenhouse effect leads to global warming. This is due to burning of fossil fuels which increase the amount of greenhouse gases (carbon dioxide, methane and oxides of nitrogen) present in the atmosphere.

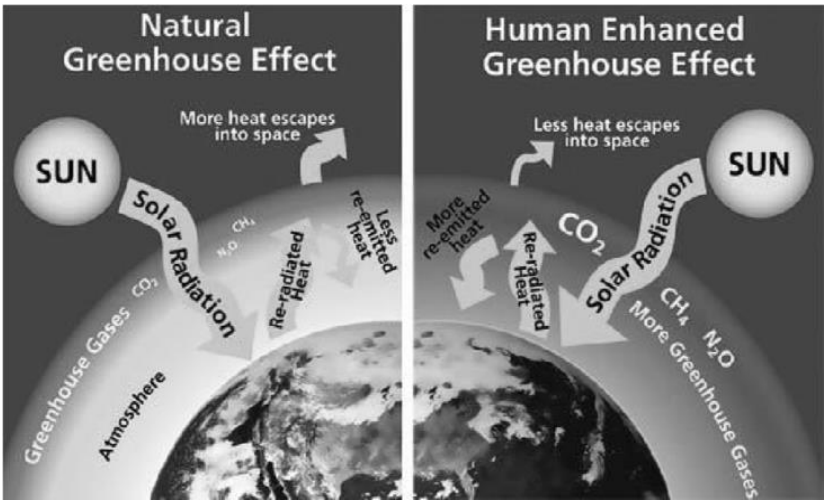


Fig.1 Types of greenhouse effects

The trade of incoming and outgoing radiation that heats up the Earth is often referred to as the greenhouse effect because a greenhouse works in a similar way (Fig.2). Incoming ultraviolet radiation easily passes through the glass walls of a greenhouse and is absorbed by the plants and hard surfaces inside. Weaker infrared radiation, however, has difficulty passing through the glass walls and is trapped inside, therefore, warming the greenhouse. This effect lets tropical plants prosper inside a greenhouse, even during a cold season

Fig. 2 Plants embodied in a greenhouse

A similar phenomenon takes place in a car which is parked outside on a cold sunny day. Incoming solar radiation warms the interior of the car but outgoing thermal radiation is trapped inside the closed windows of the cars. This entrapment basically warms up the car. This trapping occurs in such a way that the hot air does not rise and does not lose energy though convention. This phenomenon is depicted in Fig. 3.

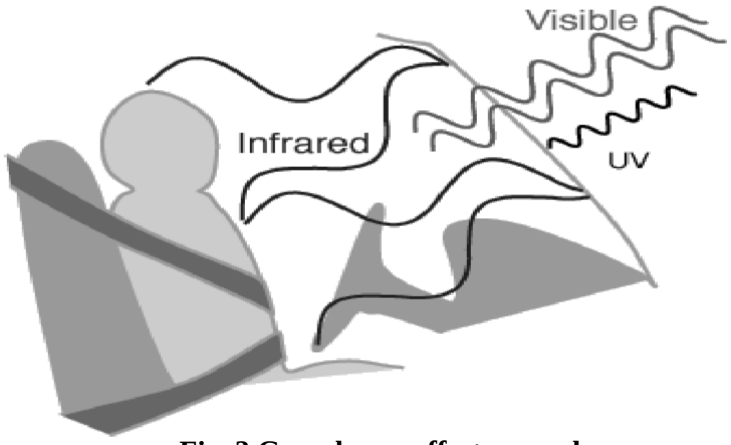


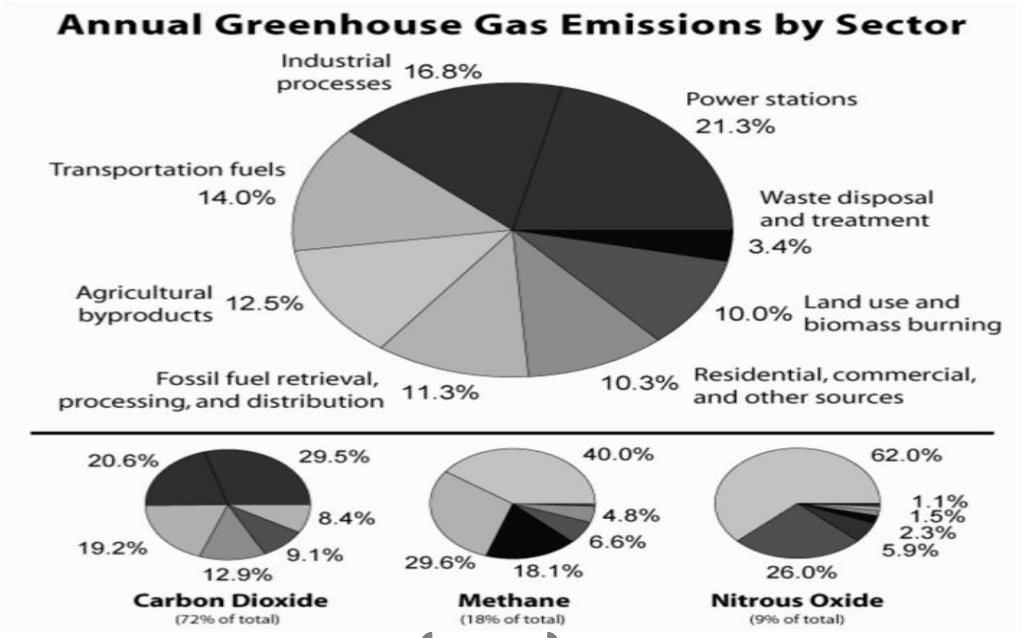
Fig. 3 Greenhouse effect example

In the words of Michael Daley, an Associate Professor of Environmental Science at Lasell College: "Gas molecules that absorb thermal infrared radiation, and are in significant enough quantity, can force the climate system. These types of gas molecules are called greenhouse gases". Carbon dioxide and other greenhouse gases act like a mantle, absorbing infrared radiation and preventing it from escaping into the outer space. The net effect is the regular heating of the Earth's atmosphere and surface.

The greenhouse effect, combined with increasing levels of greenhouse gases and the resulting global warming, is expected to have philosophical implications. If global warming continues unrestrained and nothing effective is done to limit this evil, it will cause significant climate change, a rise in sea levels, extreme weather events and other ruthless natural, environmental and social impacts.

Greenhouse Gases: A Hazard

There are many greenhouse gases which are mainly emitted by human activity. The first and foremost in the list is carbon dioxide. Excessive burning of fossil fuels like coal and oil is the major factor for producing this gas. Moreover, deforestation i.e. removal of trees for acquiring lands also causes large amount of carbon dioxide in the atmosphere. Cement manufacture also contributes carbon dioxide to atmosphere when calcium carbonate is heated generating lime and carbon dioxide. The second culprit gas is methane, commonly known as natural gas. It is produced as a result of agricultural activities such as livestock digestion, paddy rice farming and use of manure. Methane is also produced due to improper management of waste. Nitrous oxides are generated mainly by fertilizers. Moreover, fluorinated gases such as chlorofluorocarbons (CFCs) are chiefly a result of various industrial processes and refrigeration. Fig.4 shows pictorially the distribution of greenhouse gases. These gases are playing their negative part in increasing the havoc of global warming. They are continuously causing an increase in the earth's temperature.



Causes of Global warming:

The major cause of global warming is the greenhouse gases. They include carbon dioxide, methane, nitrous oxides and in some cases chlorine and bromine containing compounds. The build-up of these gases in the atmosphere changes the radiative equilibrium in the atmosphere. Their overall effect is to warm the Earth's surface and the lower atmosphere because greenhouse gases absorb some of the outgoing radiation of Earth and re-radiate it back towards the surface. The net warming from 1850 to the end of the 20th century was equivalent to nearly 2.5 W/m^2 with carbon dioxide contribution about 60 % to this figure, methane about 25 per cent, with nitrous oxides and halocarbons providing the remainder. In 1985, Joe Farman, of the British Antarctic Survey, published an article showing the decrease in ozone levels over Antarctica during the early 1980s. The response was striking: large scale international scientific programmes were mounted to prove that CFCs (used as aerosol propellants in industrial cleaning fluids and in refrigeration tools) were the cause of the problem. Even more important was abrupt international action to curb the emissions of CFCs. The second major cause of global warming is the depletion of ozone layer. This happens mainly due to the presence of chlorine containing source gases. When ultraviolet light is present, these gases dissociate releasing chlorine atoms which then catalyses ozone destruction. Aerosols present in the atmosphere are also causing global warming by changing the climate in two different ways. Firstly, they scatter and absorb solar and infrared radiation and secondly, they may alter the microphysical and chemical properties of clouds and perhaps affect their lifetime and extent. The scattering of solar radiation acts to cool the planet, while absorption of solar radiation by aerosols warms the air directly instead of permitting sunlight to be absorbed by the surface of the Earth. The human contribution to the amount of aerosols in the atmosphere is of various forms. For instance, dust is a by-product of agriculture. Biomass burning generates a mixture of organic droplets and soot particles. Many industrial processes produce a wide diversity of aerosols depending on what is being burned or generated in the manufacturing process. Moreover, exhaust emissions from various sorts of transport produce a rich mixture of pollutants that are either aerosols from the outset or are transformed by chemical reactions in the atmosphere to form aerosols.

Global Warming: The Effects

Predicting the consequences of global warming is one of the most difficult tasks faced by the climate researchers. This is due to the fact that natural processes that cause rain, snowfall, hailstorms, rise in sea levels is reliant on many diverse factors. Moreover, it is very hard to predict the size of emissions of greenhouse gases in the future years as this is determined majorly through technological advancements and political decisions. Global warming produces many negative effects some of which are described here. Firstly, extra water vapour which is present in the atmosphere falls again as rain which leads to floods in various regions of the world. When the weather turns warmer, evaporation process from both land and sea rises. This leads to drought in the regions where increased evaporation process is not compensated by increased precipitation. In some areas of the world, this will result in crop failure and famine particularly in areas where the temperatures are already high. The extra water vapour content in the atmosphere will fall again as extra rain hence causing flood. Towns and villages which are dependent on the melting water from snowy mountains may suffer drought and scarcity of water supply. It is because the glaciers all over the world are shrinking at a very rapid rate and melting of ice appears to be faster than previously projected. According to Intergovernmental Panel on Climate Change (IPCC), about one-sixth of the total population of the world lives in the regions which shall be affected by a decrease in melting water. The warmer climate will likely cause more heat waves, more violent rainfall and also amplification in the severity of hailstorms and thunderstorms. Rising of sea levels is the most deadly effect of global warming, the rise in temperature is causing the ice and glaciers to melt rapidly. This will lead to rise of water levels in oceans, rivers and lakes that can pilot devastation in the form of floods.

As evident from Fig. 5, temperature anomalies are projected to increase in coming years. Before, the 20^{th} century, the situation was well under control but the beginning of the current century, the situation started to worsen .This was all due to increase in global warming majorly due to the fact that new industries and power houses started operation and emitted harmful gases which cause the planet to heat up. This data is based on the research carried out by different climate and environmental research agencies.

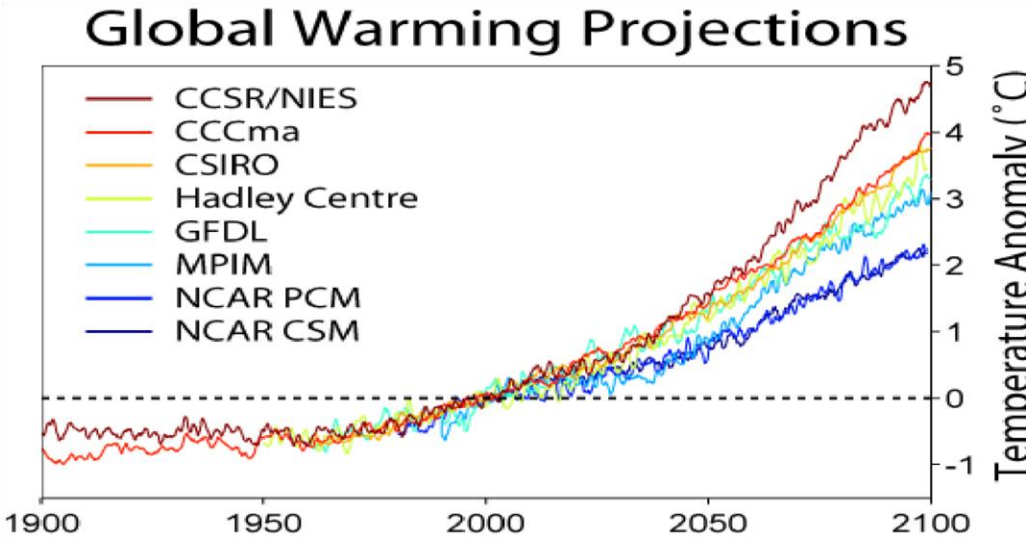


Fig. 5 Global warming projections by various

Science and Engineering research agencies

Similarly, Fig.6 elaborates the risks and impacts of global warming in years to come. As can be inferred from figure, we are currently experiencing severity of extreme climate events in the form of thunderstorms, floods and earthquakes. This destruction will take a sharp hike if nothing is done to stop this menace. Fig. 7 depicts global mean temperature in the recent years according to National Aeronautics and Space Administration (NASA). The trend clearly puts up a serious question for us. How will we survive on earth given the rise in temperature to prevail?

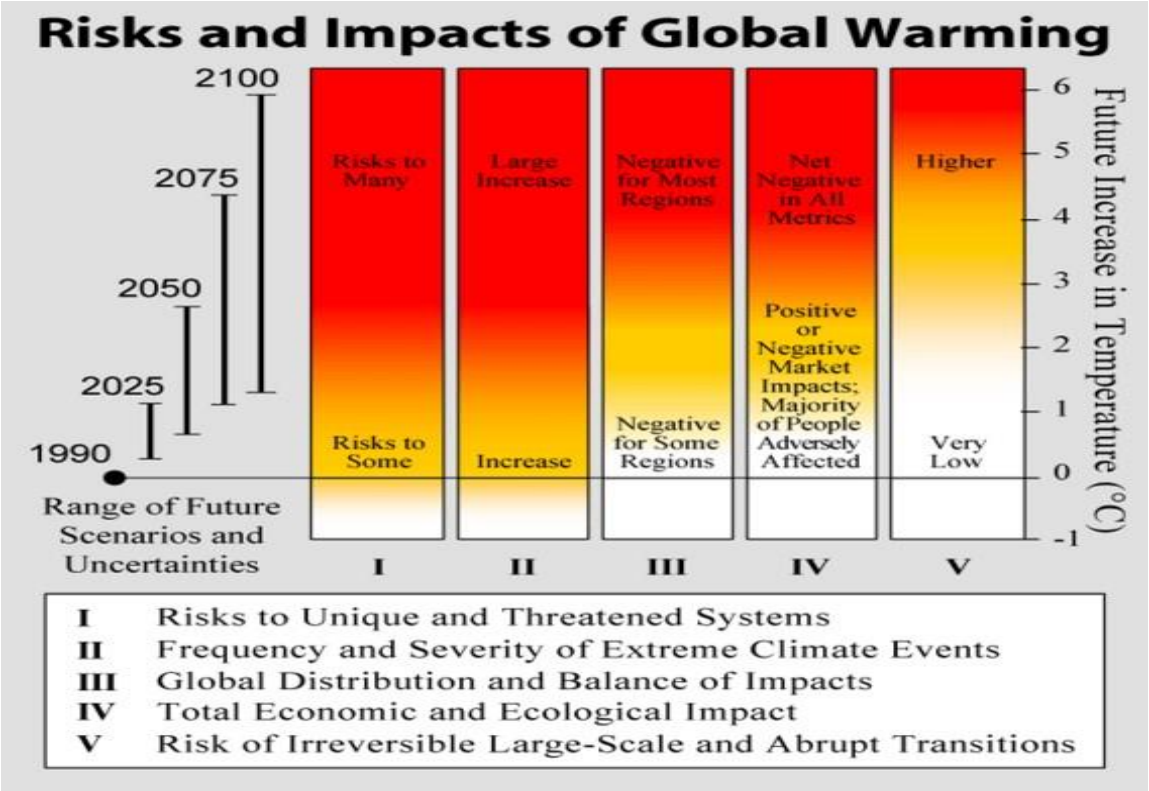


Fig. 6 An assessment of the relative impact and risks connected with global warming. The bars are color-coded to show level of impact/concern for each factor as a function of temperature increase

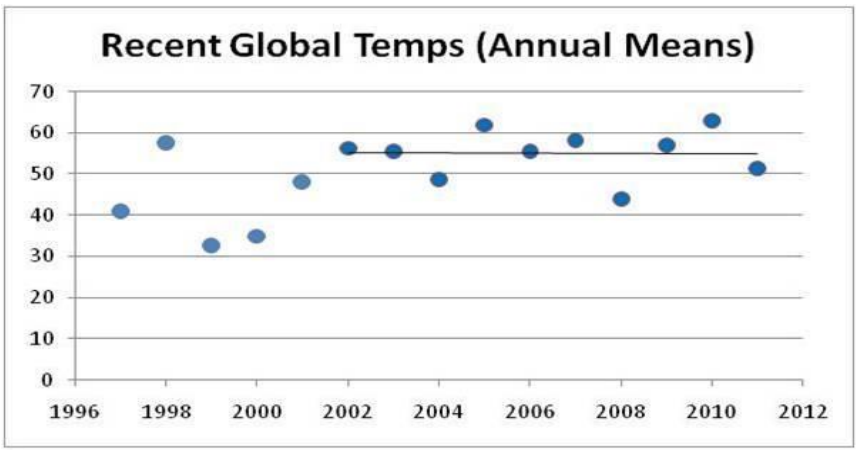


Fig. 7 Recent global mean temperatures according to NASA

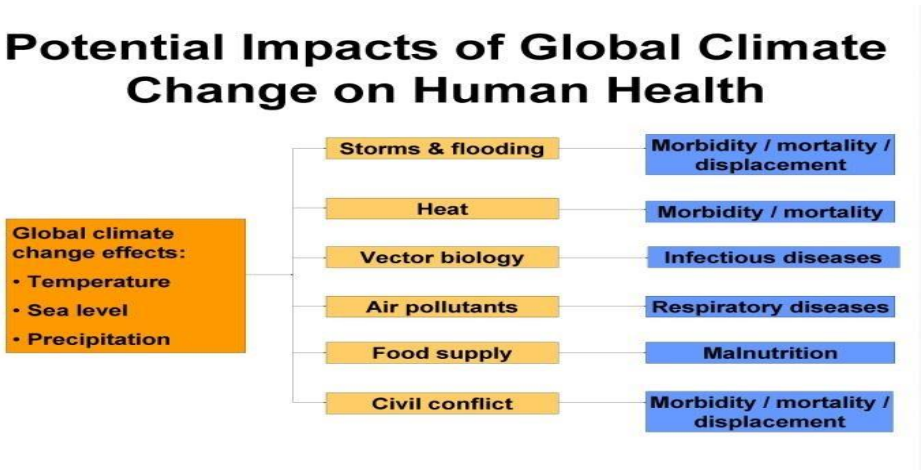
Effects on Living Beings

Global warming can severely affect the health of living beings. Excess heat can cause stress which may lead to blood pressure and heart diseases. Crop failures and famines, which are a direct consequence of heating up of earth, can cause a decline in human body resistance to viruses and infections. Global warming may also transfer various diseases to other regions as people will shift from regions of higher temperatures to regions of comparatively lower temperatures. Warmer oceans and other surface waters may lead to severe cholera outbreaks and harmful infections in some types of sea food.

Moreover, it is an established fact that warmer temperatures lead to dehydration which is a major cause of kidney stones. A medical team from

The Children's Hospital of Philadelphia examined the health proceedings of more than 60,000 Americans alongside weather records. They discovered that individuals were most likely to be hospitalized with kidney stones three days after a temperature rise. Since 1994, kidney stone incidence has risen from about one in 20 people to one in 11. This trend is likely to increase as the globe gets hotter. According to Luis Ostrosky, M.D. of the Division of Infectious Diseases at The University of Texas Health Science Centre at Houston Medical School and medical director for epidemiology at Memorial Hermann-Texas Medical Centre: “One infection that is definitely making a weird pattern is valley fever”. In his words, “This is a fungal infection we used to see only in California, Arizona, New Mexico and a little in Texas, but last year we found it for the first time in Washington State. “This potentially deadly condition caused apprehension in California when the number of cases increased drastically during 2010 and 2011. Valley fever infections have been on the rise, probably because of warming climates and drought causing dust storms. Dry soil and wind can carry spores that spread the virus. Hotter and drier climates are projected to increase the amount of dusting carrying this disease. Researchers have already noticed a rise in mosquito-borne disease like dengue fever and malaria due to warmer and longer summers. Perhaps the most prominent mosquito-borne disease, West Nile Virus, has already experienced a sharp increase in annual cases. According to the U.S. Centres for Disease Control and Prevention, the summer of 2012 was the nastiest West Nile season on record, the likely reason was that summer's scorching heat and drought. Lyme disease is another dangerous disease which is transmitted mainly through bites from certain tick species.

Fig. 8 describes in the form of a block diagram that how alterations in global climate can affect human health. The bitterest fact is that it can cause various diseases and deprive human beings of the food.



Global warming is also affecting animals. They need to move to cooler places in order to survive. This process has been observed in various places, for instance, in the Alps, in mountainous Queensland in Australia, and in the misty forests of Costa Rica. Fish in the North Sea have been reported to move northwards too .The impacts on species are becoming noteworthy to such an extent that their movements can be used as a sign of a warming world. They are the silent witnesses of the swift changes being inflicted on the Earth. Scientists and researchers predict that global warming is gradually damaging the ecosystems of various species and is playing a very unconstructive role in making them extinct. For instance Asia's only ape – the orangutan – is in bottomless trouble. Its last remaining strongholds in the rainforests of Indonesia are being endangered by a range of pressures, including climate change, putting the animal at the menace of extinction within a few decades. With global warming continually increasing the duration and frequency of droughts, bushfires are occurring more often in these heavily logged forests, further fragmenting the orang-utan's living domain. Similarly, in Africa, elephants face a series of threats including shrinking living space, which brings them more regularly into divergence with people. With this reduced living space, elephants will be unable to escape any changes to their natural habitat caused by global warming, including more common and longer dry periods, placing further pressure on their survival.

Alternative Energy Sources

The hazards caused by global warming are tremendous. Excessive use of fossil fuels such as coal, natural gas and oil play a part in it too. The usage of fossil fuels should be discontinued immediately. The most significant solution to put an end to this disaster is the use of alternative energy sources. They include wind, solar, bio mass, geothermal and hydro. The most noteworthy point in using these sources is their clean nature. They do not produce any sort of pollution or toxic gases that can lead to global warming. They are environmentally friendly and pose no threat to ecological balance. However, their high installation and setup costs may drive energy companies away from them at first but in the long run they are surely beneficial for everyone. Most importantly, fossil fuels will deplete one day and sooner or later, we have to turn to renewable energy sources for energy production. Thus, the eventual solution to end global warming is to use alternative energy sources. Fig. 9 depicts in a pictorial way that earth can be saved from the hazards of global warming if we utilize renewable energy sources.

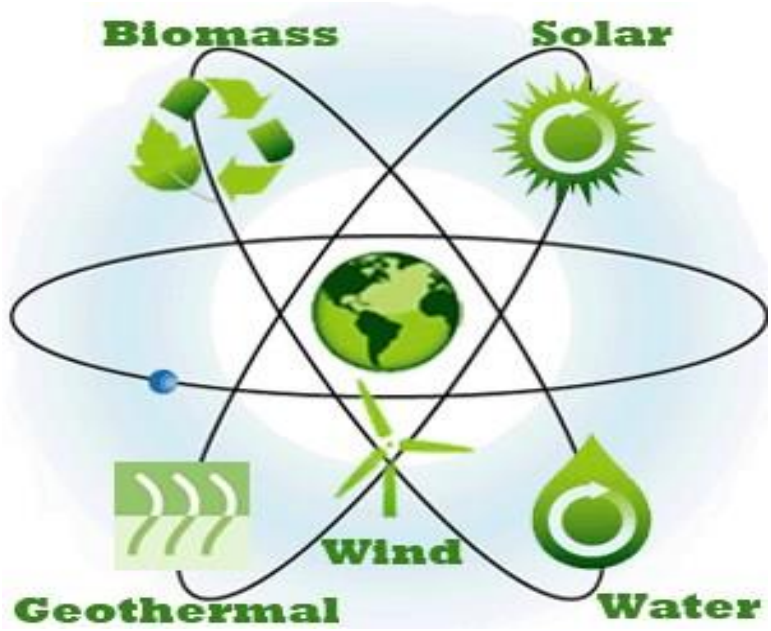


Fig. 9 save earth from global warming by using renewable energy sources

To counteract the medical hazards of global warming, it is essential to turn to renewable energy sources. Public, in general, should be responsible about their decisions on energy conservation methods. This will ensure a healthy atmosphere and stable climate for our future generations. Governments should devise and pass policies which encourage the energy companies and people, in general, to use renewable energy instead of conventional energy, Nongovernmental organizations (NGOs) should distribute pamphlets to people motivating them to use alternative sources of energy and discourage them from using fossil fuels. They should also explain to them the hazards which the usage of fossil fuels will cause. Many developed countries are already generating huge amounts of power using renewables. These countries should extend their helping hand to developing countries to combat the evil of global warming collectively. Using

renewable energy is the most effective way to curtain the emission of gases which play a major role in global warming.

Fig. 10 and Fig. 11 show that the use of renewables is gradually increasing. The figure should be much more than present so that we can tackle the problem of global warming timely and effectively

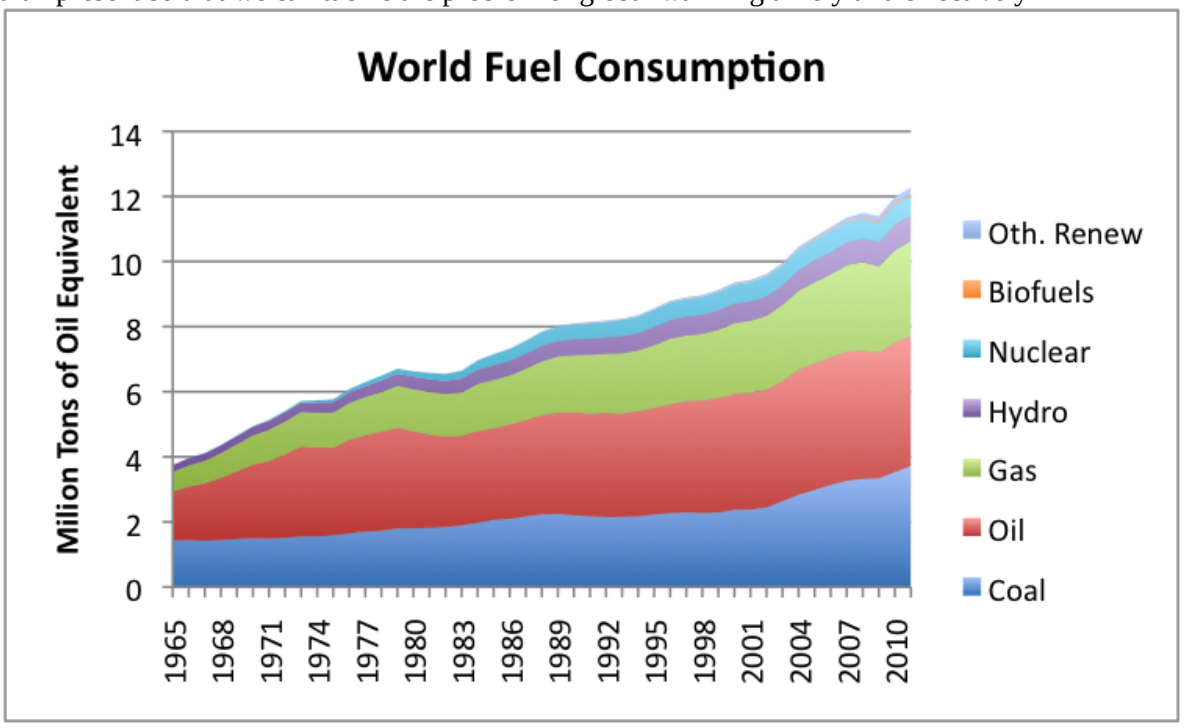


Fig. 10 World fuel consumption in recent years

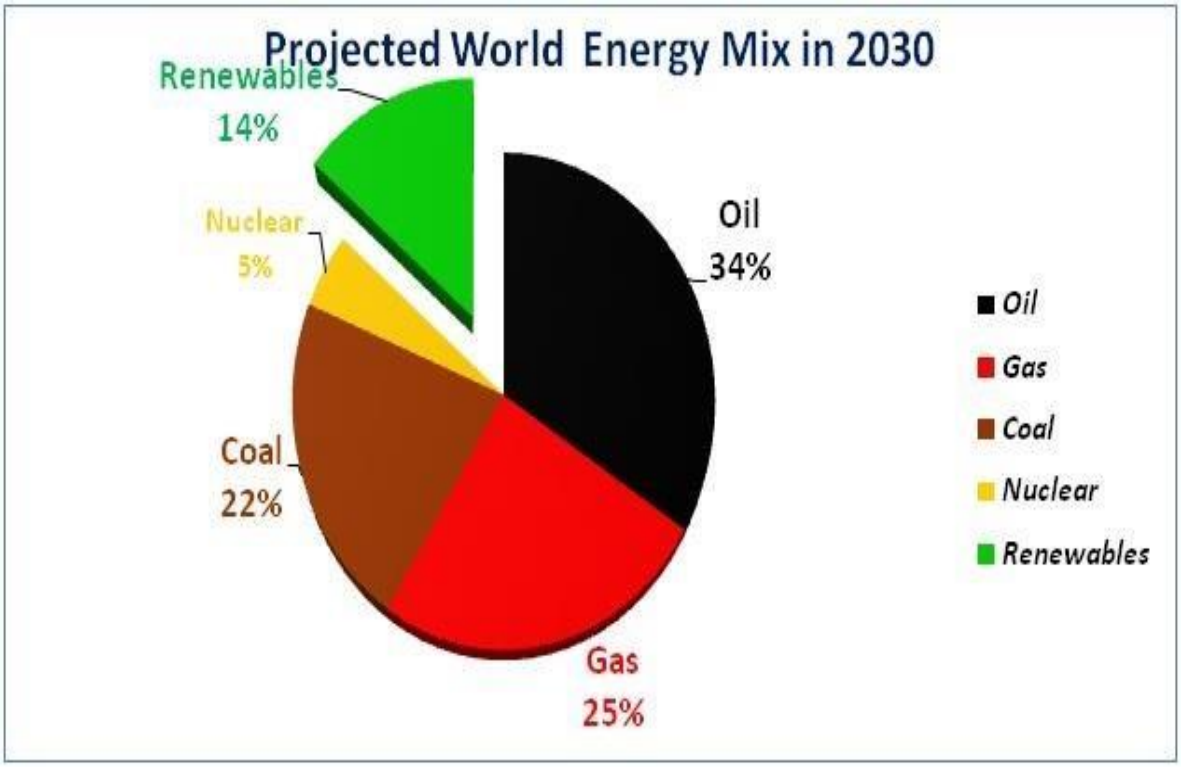


Fig .11 Projected world energy mix in 2030

Other Solutions

As elaborated earlier, toxic emissions are a major cause of global warming, a likely solution to reduce harmful emissions is to cut the usage of vehicles which produce them. This has not been met with much success as many people refuse to cut down their practice of using cars. No doubt, some people have started to use bicycles and public transport, whereas some other prefer to walk but these numbers are relatively small. It should be noted that fuel economy and emission rates are chief factors to consider regarding the

car choice. Hybrid cars have higher efficiency and lower emission rates. Keeping the tires inflated will help improve mileage and air filters should be frequently replaced to cut down harmful emissions. People should share the ride with friends or co-workers to reduce the total number of vehicles on the road. Print and social media can play an effective role in curbing the problem. It should use the philosophy of automobile advertisements to encourage drivers to conserve energy and reduce pollution. Awareness campaigns can be started using placards, posters and logos similar to shown in Figures 12-14. They are a very useful way to demonstrate that global warming is not good for the planet. Recycling is also a good way to reduce global warming. People should use rechargeable batteries instead of disposable ones. Quality products should be bought that have a long life. Shopping should be done from local markets which reduce transportation. Even small individual efforts like lowering the thermostats in winter and using compact fluorescent lamps instead of incandescent lamps can aid to address the issue of global warming. Reforestation schemes must be started to grow a large number of trees. Forest degradation and deforestation must be discouraged at government level. Nuclear power is also a possible solution as this power results in fewer emissions but this method should be used with care as it can lead to severe accidents therefore, the major hurdle is to overcome the security, propagation, waste disposal and high costs of nuclear power if this method has to be made practical.

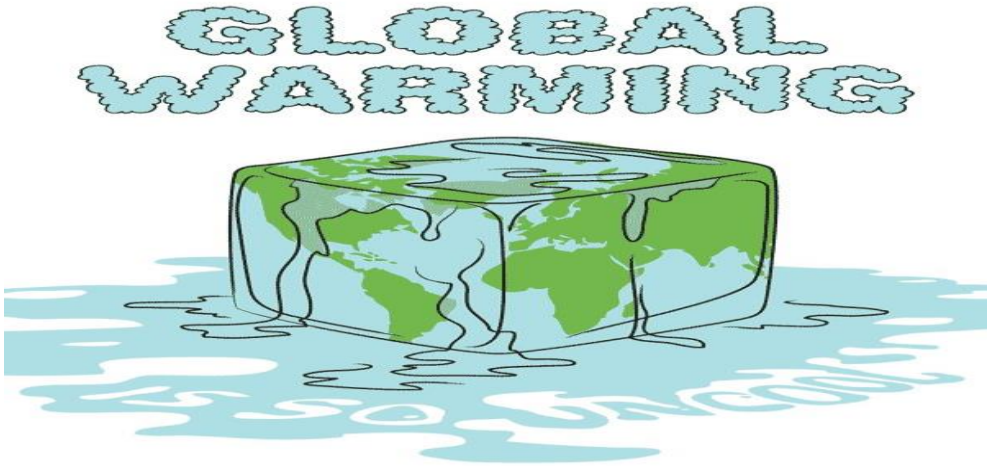


Fig. 12 shows symbolically how global warming is causing the earth to melt

Fig. 13 showing a symbolic representation to stop global warming

Fig. 14 depicting that how human beings are destroying the earth for their own benefits

The scientific and environmental community is on the same page regarding the bitter reality of global warming and the involvement of human factor in it. The paper discussed here has only dented the surface of what is a very intricate line of scientific and engineering exploration. Global warming is a big hazard and appropriate measures must be taken to tackle this serious problem. This problem is not only causing trouble to the human beings but also to animals and plants. Melting of polar ice caps will lead to floods which can cause mayhem everywhere. Rise of sea levels will devastate agricultural and fishing activities. To embark upon these problems, some remedial steps must be timely taken which include but are not limited to the use of renewable sources of energy and stopping deforestation. Innovative solutions must be brought forward to end this hazard once and forever.

Ozone Layer Depletion and Its Effects

The ozone layer is a layer in Earth's atmosphere which contains relatively high concentrations of ozone (O_3). This layer absorbs 93-99% of the sun's high frequency ultraviolet light, which is potentially damaging to life on earth [1]. Over 91% of the ozone in Earth's atmosphere is present here. It is mainly located in the lower portion of the stratosphere from approximately 10 km to 50 km above Earth, though the thickness varies seasonally and geographically. The ozone layer was discovered in 1913 by the French physicists Charles Fabry and Henri Buisson. Its properties were explored in detail by the British meteorologist G. M. B. Dobson, who developed a simple spectrophotometer (the Dobson meter) that could be used to measure stratospheric ozone from the ground. Between 1928 and 1958 Dobson established a worldwide network of ozone monitoring stations which continues to operate today. The "Dobson unit", a convenient measure of the total amount of ozone in a column overhead, is named in his honor.

Ozone

Without ozone, life on Earth would not have evolved in the way it has. The first stage of single cell organism development requires an oxygen-free environment. This type of environment existed on earth over 3000 million years ago. As the primitive forms of plant life multiplied and evolved, they began to release minute amounts of oxygen through the photosynthesis reaction (which converts carbon dioxide into oxygen)

The buildup of oxygen in the atmosphere led to the formation of the ozone layer in the upper atmosphere or stratosphere. This layer filters out incoming radiation in the "cell-damaging" ultraviolet (UV) part of the spectrum. Thus with the development of the ozone layer came the formation of more advanced life forms. Ozone is a form of oxygen. The oxygen we breathe is in the form of oxygen molecules (O_2) - two atoms of oxygen bound together. Normal oxygen which we breathe is colourless and odourless. Ozone, on the other hand, consists of three atoms of oxygen bound together (O_3). Most of the atmosphere's ozone occurs in the region called the stratosphere. Ozone is colourless and has a very harsh odour. Ozone is much less common than normal oxygen. Out of 10 million air molecules, about 2 million are normal oxygen, but only 3 are ozone. Most ozone is produced naturally in the upper atmosphere or stratosphere. While ozone can be found through the entire atmosphere, the greatest concentration occurs at altitudes between 19 and 30 km above the Earth's surface. This band of ozone-rich air is known as the "ozone layer". Ozone also occurs in very small amounts in the lowest few kilometres of the atmosphere, a region known as the troposphere. It is produced at ground level through a reaction between sunlight and volatile organic compounds (VOCs) and nitrogen oxides (NO_x), some of which are produced by human activities such as driving cars. Ground-level ozone is a component of urban

smog and can be harmful to human health. Even though both types of ozone contain the same molecules, their presence in different parts of the atmosphere has very different consequences. Stratospheric ozone blocks harmful solar radiation - all life on Earth has adapted to this filtered solar radiation. Ground-level ozone, in contrast, is simply a pollutant. It will absorb some incoming solar radiation, but it cannot make up for ozone losses in the stratosphere.

Ozone Hole

In some of the popular news media, as well as in many books, the term "ozone hole" has and often still is used far too loosely. Frequently, the term is employed to describe any episode of ozone depletion, no matter how minor. Unfortunately, this sloppy language trivializes the problem and blurs the important scientific distinction between the massive ozone losses in Polar Regions and the much smaller, but nonetheless significant, ozone losses in other parts of the world. Technically, the term "ozone hole" should be applied to regions where stratospheric ozone depletion is so severe that levels fall below 200 Dobson Units (D.U.), the traditional measure of stratospheric ozone. Normal ozone concentration is about 300 to 350 D.U. Such ozone loss now occurs every springtime above Antarctica, and to a lesser extent the Arctic where special meteorological conditions and very low air temperatures accelerate and enhance the destruction of ozone loss by man-made ozone depleting chemicals (ODCs).

Ozone Layer

The ozone layer is not really a layer at all, but has become known as such because most ozone particles are scattered between 19 and 30 kilometers (12 to 30 miles) up in the Earth's atmosphere, in a region called the stratosphere. The concentration of ozone in the ozone layer is usually under 10 parts ozone per million. Without the ozone layer, a lot of ultraviolet (UV) radiation from the Sun would not be stopped reaching the Earth's surface, causing untold damage to most living species. In the 1970s, scientists discovered that chlorofluorocarbons (CFCs) could destroy ozone in the stratosphere. Ozone is created in the stratosphere when UV radiation from the Sun strikes molecules of oxygen (O2) and causes the two oxygen atoms to split apart. If a freed atom bumps into another O2, it joins up, forming ozone (O3). This process is known as photolysis. Ozone is also naturally broken down in the stratosphere by sunlight and by a chemical reaction with various compounds containing nitrogen, hydrogen and chlorine. These chemicals all occur naturally in the atmosphere in very small amounts. In an unpolluted atmosphere there is a balance between the amount of ozone being produced and the amount of ozone being destroyed. As a result, the total concentration of ozone in the stratosphere remains relatively constant. At different temperatures and pressures (i.e. varying altitudes within the stratosphere), there are different formation and destruction rates. Thus, the amount of ozone within the stratosphere varies according to altitude. Ozone concentrations are highest between 19 and 23 km. Most of the ozone in the stratosphere is formed over the equator where the level of sunshine striking the Earth is greatest. It is transported by winds towards higher latitudes. Consequently, the amount of stratospheric ozone above a location on the Earth varies naturally with latitude, season, and from day-to-day. Under normal circumstances highest ozone values are found over the Canadian Arctic and Siberia, whilst the lowest values are found around the equator. The ozone layer over Canada is normally thicker in winter and early spring, varying naturally by about 25% between January and July. Weather conditions can also cause considerable daily variations.

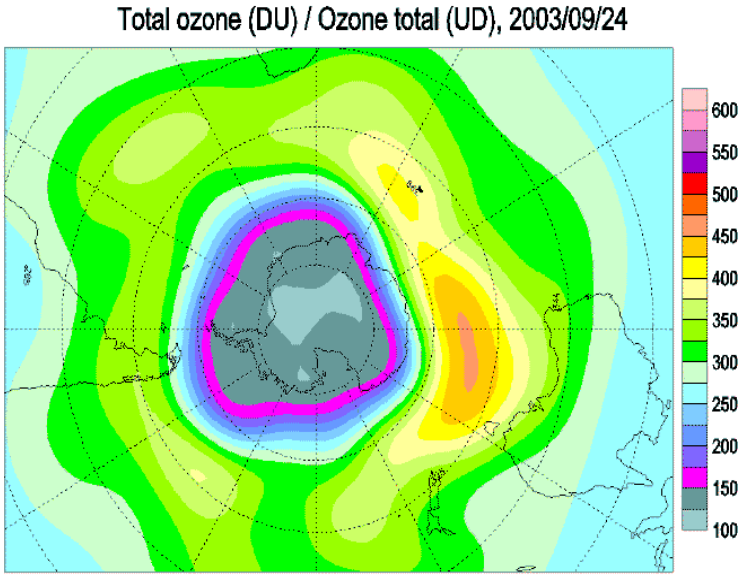


Fig.1 ozone layer depletion over Antarctica Ozone depletion over India

With so much worry about the rapid ozone depletion taking place in various parts of the earth, Indian scientists are closely monitoring the ozone layer over India for possible depletion trends. Opinions are many and varied. According to S K
Srivastava, head of the National Ozone Centre in New Delhi, there is no trend to show total ozone depletion over India. V.Thaphyal and S M Kulshresta of the Indian Meteorological Department also point out that for the period 1956 to 1986 "ozone measurements exhibit year to year variability, but do not show any increasing or decreasing trend over India."
However, former director of the National Ozone Centre, K Chatterji, now with Development Alternatives, warns that there is no case for complacency. He asserts that his calculations exhibit an ozone depletion trend in the upper, layers of the stratosphere over New Delhi and Pune from 1980 to 1983 in the month of October when the Antarctic ozone hole is at its maximum. Since India already receives high doses of ultraviolet (UV-B) radiation, and is at the threshold go to speak, effects of ozone layer depletion could he far more disastrous in India. A P Mitra, former director general of the Council of Scientific and Industrial Research, clarifies that while there is no trend in the total ozone value, there is some evidence of ozone depletion at higher altitudes - at about 30 to 40 km - even over the tropics. He argues, however, that there is insufficient data and that the depletion may be due to solar cycles and other natural phenomena. However, the effects of CFCs and belong cannot be ruled out. Total column ozone data has been recorded over India for a long time. A network of stations using Dobson spectrophotometers to mea- sure total ozone, some six times a day, covers Srinagar, New Delhi, Varanasi, Ahmedabad, Pone and Kodaikanal. Ozone profiles are also regularly recorded using balloons. Ozone levels are the lowest during November and December and the highest in summer. Across the country, variations do exist. In Kodaikanal, the total ozone is 240 to 280 Dobson units (DU), in New Delhi 270 to 320 DU and in Srinagar 290 to 360 DU. One Dobson unit is the equivalent of 0.01 mm of compressed gas at a pressure of 760 rare mercury and 0°C.B N Srivastava of the National Physical Laboratory, who been working on incident UV radiation levels, says that during summer, at noon, the UV-B radiation with a wavelength of 290 nanometer (nm) is equivalent to levels attained in the Antarctica during the ozone hole period. He warns that even a slight depletion of the ozone layer over India may lead to large percentage changes in UV-B radiation over the country. **Measuring Ozone Depletion**
The most common stratospheric ozone measurement unit is the Dobson Unit (DU). The Dobson Unit is named after the atmospheric ozone pioneer G.M.B. Dobson who carried out the earliest studies on ozone in the atmosphere from the 1920s to the 1970s. A Dobson Unit measures the total amount of ozone in an overhead column of the atmosphere. Dobson Units are measured by how thick the layer of ozone would be if it were compressed into one layer at 0 degrees Celsius and with a pressure of one atmosphere above it. Every 0.01 millimeter thickness of the layer is equal to one Dobson Unit. The average amount of ozone in the stratosphere across the globe is about 300 DU (or a thickness of only 3mm at 0°C and 1 atmospheric pressure!). Highest levels of ozone are usually found in the mid to high latitudes, in Canada and Siberia (360DU). When stratospheric ozone falls below 200 DU this is considered low enough to represent the beginnings of an ozone hole. Ozone holes of course commonly form during springtime above Antarctica, and to a lesser extent the Arctic.
The Ozone Hole 2009- Situation at 2009
November the 2009 ozone hole is now waning, with much of the continent experiencing a stratospheric spring warming. The residual vortex is over the Weddell Sea and Antarctic Peninsula and here minimum values are around 160 DU and depletion exceeds 50%. Ozone values outside the polar vortex have dropped to near 400 DU, and inside the vortex ozone values are increasing as the atmosphere warms. The temperature of the ozone layer over Antarctica is now rising, though a small area is still cold enough for polar stratospheric clouds (PSCs) to exist. During the early winter, the polar vortex was often rather more elliptical than it was in 2008, and this lead to some early depletion in circumpolar regions as stratospheric clouds became exposed to sunlight. It reverted to a more circular circulation as winter progressed and this led to another relatively slow start to the growth of the ozone hole (as measured by NASA/SBUV2), with the "hole" not beginning until mid-August. The vortex became more elliptical again in late August, with South Georgia being affected by the fringes of the ozone hole between September 2 and 6. The hole grew to reach an area of around 24 million square kilometers by mid-September, but had declined to 12 million square kilometres by mid-November. It is now a little larger than the average for the past decade. The tip of South America and South Georgia were affected by the fringes of the ozone hole from September 24 to September 30 and again from October 3 to October 7.

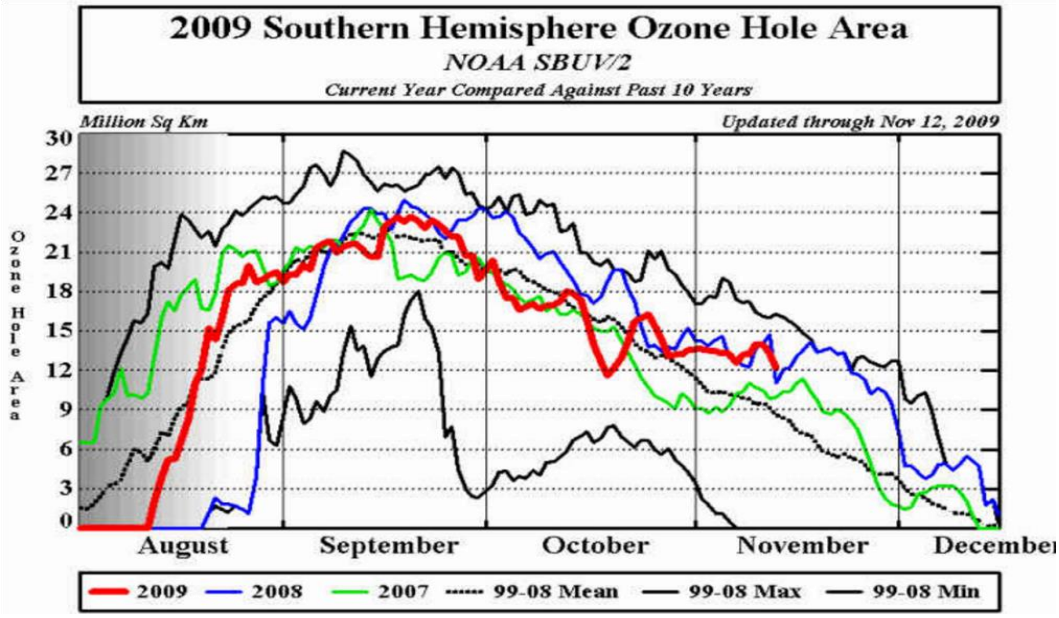
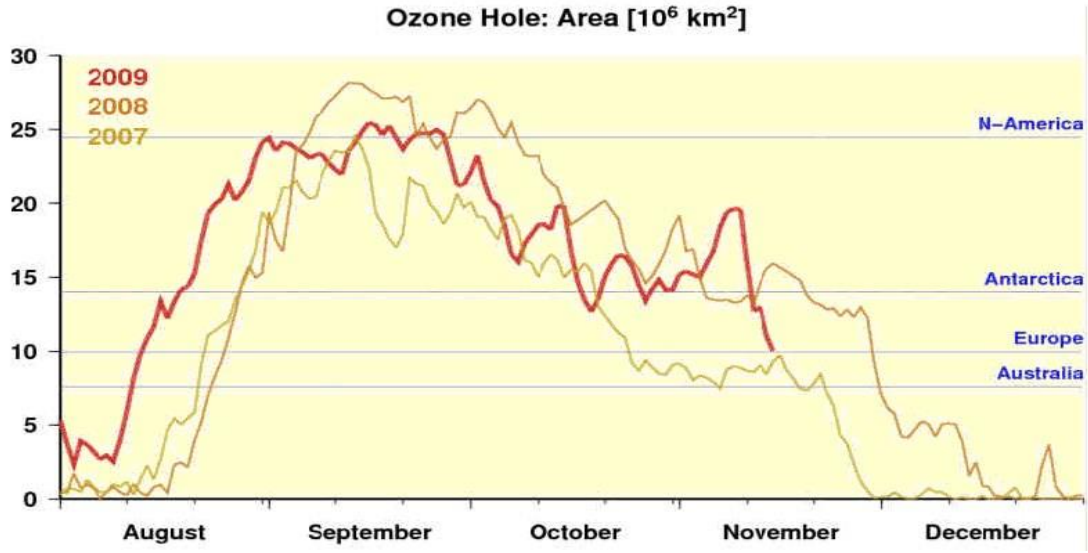


Fig.2 ozone hole area variation



Ozone Layer Recovery

The ozone depletion caused by human-produced chlorine and bromine compounds is expected to gradually disappear by about the middle of the 21st century as these compounds are slowly removed from the stratosphere by natural processes. This environmental achievement is due to the landmark international agreement to control the production and use of ozone-depleting substances. Full compliance would be required to achieve this expected recovery. Without the Montreal Protocol and its Amendments, continuing use of chlorofluorocarbons (CFCs) and other ozone-depleting substances would have increased the stratospheric abundances of chlorine and bromine tenfold by the mid-2050s compared with the 1980 amounts. Such high chlorine and bromine abundances would have caused very large ozone losses, which would have been far larger than the depletion observed at present. In contrast, under the current international agreements that are now reducing the human-caused emissions of ozone-depleting gases, the net troposphere concentrations of chlorine- and bromine-containing compounds started to decrease in 1995. Because 3 to 6 years are required for the mixing from the troposphere to the stratosphere, the stratospheric abundances of chlorine are starting to reach a constant level and will slowly decline thereafter. With full compliance, the international agreements will eventually eliminate most of the emissions of the major ozone-depleting gases. All other things being constant, the ozone layer would be expected to return to a normal state during the middle of the next century. This slow recovery, as compared with the relatively rapid onset of the ozone depletion due to CFC and bromine-containing halons emissions, is related primarily to the time required for natural processes to eliminate the CFCs and halons from the atmosphere. Most of the CFCs and halons have atmospheric residence times of about 50 to several hundred years.

Ozone depletion occurs when the natural balance between the production and destruction of stratospheric ozone is tipped in favour of destruction. Although natural phenomena can cause temporary ozone loss, chlorine and bromine released from man-made compounds such as CFCs are now accepted as the main cause of this depletion. It was first suggested by Drs. M. Molina and S. Rowland in 1974 that a man-made group of compounds known as the chlorofluorocarbons (CFCs) were likely to be the main source of ozone depletion. However, this idea was not taken seriously until the discovery of the ozone hole over Antarctica in 1985 by the Survey. Chlorofluorocarbons are not "washed" back to Earth by rain or destroyed in reactions with other chemicals. They simply do not break down in the lower atmosphere and they can remain in the atmosphere from 20 to 120 years or more. As a consequence of their relative stability, CFCs are instead transported into the stratosphere where they are eventually broken down by ultraviolet (UV) rays from the Sun, releasing free chlorine. The chlorine becomes actively involved in the process of destruction of ozone. The net result is that two molecules of ozone are replaced by three of molecular oxygen, leaving the chlorine free to repeat the process:

$Cl + O_3 = ClO + O_2$

$ClO + O = Cl + O_2$

Ozone is converted to oxygen, leaving the chlorine atom free to repeat the process up to 100,000 times, resulting in a reduced level of ozone. Bromine compounds, or halons, can also destroy stratospheric ozone. Compounds containing chlorine and bromine from man-made compounds are known as industrial halocarbons. Emissions of CFCs have accounted for roughly 80% of total stratospheric ozone depletion. Thankfully, the developed world has phased out the use of CFCs in response to international agreements to protect the ozone layer. However, because CFCs remain in the atmosphere so long, the ozone layer will not fully repair itself until at least the middle of the 21st century. Naturally occurring chlorine has the same effect on the ozone layer, but has a shorter life span in the atmosphere.

Chlorofluorocarbons

Chlorofluorocarbons or CFCs (also known as Freon) are non-toxic, non-flammable and non-carcinogenic. They contain fluorine atoms, carbon atoms and chlorine atoms. The 5 main CFCs include CFC-11 (trichlorofluoromethane - $CFCl_3$), CFC-12 (dichloro-difluoromethane - CF_2Cl_2), CFC-113 (trichloro-trifluoroethane - $C_2F_3Cl_3$), CFC-114 (dichloro-tetrfluoroethane - $C_2F_4Cl_2$), and CFC-115 (chloropentafluoroethane - C_2F_5Cl).CFCs are widely used as coolants in refrigeration and air conditioners, as solvents in cleaners, particularly for electronic circuit boards, as a blowing agents in the production of foam (for example fire extinguishers), and as propellants in aerosols. Indeed, much of the modern lifestyle of the second half of the 20th century had been made possible by the use of CFCs. Man-made CFCs however, are the main cause of stratospheric ozone depletion. CFCs have a lifetime in the atmosphere of about 20 to 100 years, and consequently one free chlorine atom from a CFC molecule can do a lot of damage, destroying ozone molecules for a long time. Although emissions of CFCs around the developed world have largely ceased due to international control agreements, the damage to the stratospheric ozone layer will continue well into the 21st century.

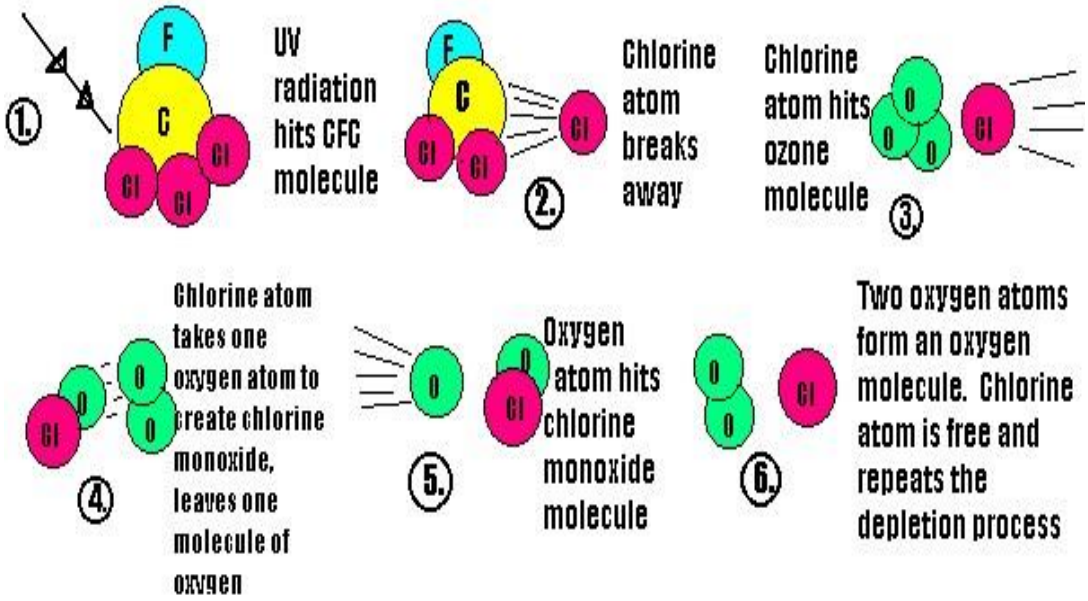


Fig. 4 ozone depletion reaction

Rocket Launches:

The global market for rocket launches may require more stringent regulation in order to prevent significant damage to Earth's stratospheric ozone layer in the decades to come, according to a new study by researchers in California and Colorado. Future ozone losses from unregulated rocket launches will eventually exceed ozone losses due to chlorofluorocarbons, or CFCs, which stimulated the 1987 Montreal Protocol banning ozone-depleting chemicals, said Martin Ross, chief study author from The Aerospace Corporation in Los Angeles. The study, which includes the University of Colorado at Boulder and Embry-Riddle Aeronautical University, provides a market analysis for estimating future ozone layer depletion based on the expected growth of the space industry and known impacts of rocket launches." As the rocket launch market grows, so will ozone-destroying rocket emissions," said Professor Darin Toohey of CU-Boulder's atmospheric and oceanic sciences department. "If left unregulated, rocket launches by the year 2050 could result in more ozone destruction than was ever realized by CFCs."Since some proposed space efforts would require frequent launches of large rockets over extended periods, the new study was designed to bring attention to the issue in hopes of sparking additional research, said Ross. "In the policy world uncertainty often leads to unnecessary regulation," he said. "We are suggesting this could be avoided with a more robust understanding of how rockets affect the ozone layer." Current global rocket launches deplete the ozone layer by no more than a few hundredths of 1 percent annually, said Toohey. But as the space industry grows and other ozone-depleting chemicals decline in the Earth's stratosphere, the issue of ozone depletion from rocket launches is expected to move to the forefront. Highly reactive trace-gas molecules known as radicals dominate stratospheric ozone destruction, and a single radical in the stratosphere can destroy up to 10,000 ozone molecules before being deactivated and removed from the stratosphere.

Ozone depletion

In 1974, after millions of tons of CFCs had been manufactured and sold; chemists F. Sherwood Rowland and Mario Molina of the University of California began to wonder where all these CFCs ended up. Rowland and Molina theorized that ultraviolet (UV) rays from the Sun would break up CFCs in the stratosphere, and that the free chlorine atoms would then enter into a chain reaction, destroying ozone. Many people, however, remained unconvinced of the danger until the mid-1980s, when a severe springtime depletion of ozone was first monitored by the British Antarctic Survey above Antarctica. The depletion above the South Pole was so severe that the British geophysicist, Joe Farman, who first measured it, assumed his spectrophotometer must be broken and sent the device back to England to be repaired. Once the depletion was verified, it came to be known throughout the world through a series of NASA satellite photos as the Antarctic Ozone Hole. Laboratory studies backed by satellite and ground-based measurements, show that free chlorine reacts very rapidly with ozone. They also show that the chlorine oxide formed in that reaction undergoes further processes that regenerate the original chlorine, allowing the sequence to be repeated up to 100,000 times. This process is known as a "chain reaction". Similar reactions also take place between bromine and ozone. Observations of the Antarctic ozone hole have given a convincing and unmistakable demonstration of these processes. Scientists have repeatedly observed a large number of chemical species over Antarctica since 1986. Among the chemicals measured were ozone and chlorine monoxide, which is the reactive chemical identified in the laboratory as one of the participants in the ozone-destroying chain reactions. The satellite maps shown in the figure below relate the accumulation of chlorine monoxide observed over Antarctica and the subsequent ozone depletion that occurs rapidly in a few days over very similar areas.

EFFECT OF OZONE LAYER DEPLETION

i) Effect on human and animal health-

Increased penetration of solar UV-B radiation is likely to have profound impact on human health with potential risks of eye diseases, skin cancer and infectious diseases. UV radiation is known to damage the cornea and lens of the eye. Chronic exposure to UV-B could lead to cataract of the cortical and posterior subcapsular forms. UV-B radiation can adversely affect the immune system causing a number of infectious diseases. In light skinned human populations, it is likely to develop nonmelanoma skin cancer (NMSC). Experiments on animals show that UV exposure decreases the immune response to skin cancers, infectious agents and other antigens.

ii) Effect on terrestrial plant-

Antarctic springtime ozone hole will shrink by five to 10 per cent between 2000 and 2020. In sharp contrast, the cosmic It is a known fact that the physiological and developmental processes of plants are affected by UV-B radiation. Scientists believe that an increase in UV-B levels would necessitate using more UV-B tolerant

cultivar and breeding new tolerant ones in agriculture. In forests and grasslands increased UV-B radiation is likely to result in changes in species composition (mutation) thus altering the bio-diversity in different ecosystems. UV-B could also affect the plant community indirectly resulting in changes in plant form, secondary metabolism, etc. These changes can have important implications for plant competitive balance, plant pathogens and bio-geochemical cycles.

iii) Effects on Aquatic Ecosystems-

While more than 30 percent of the world's animal protein for human consumption comes from the sea alone, it is feared that increased levels of UV exposure can have adverse impacts on the productivity of aquatic systems. High levels of exposure in tropics and subtropics may affect the distribution of phytoplanktons which form the foundation of aquatic food webs. Reportedly a recent study has indicated 6-12 percent reduction in phytoplankton production in the marginal ice zone due to increases in UV-B. UV-B can also cause damage to early development stages of fish, shrimp, crab, amphibians and other animals, the most severe effects being decreased reproductive capacity and impaired larval development.

iv) Effects on Bio-geo-chemical Cycles-

Increased solar UV radiation could affect terrestrial and aquatic bio-geo-chemical cycles thus altering both sources and sinks of greenhouse and important trace gases, e.g. carbon dioxide (CO2), carbon monoxide (CO), carbonyl sulphide (COS), etc. These changes would contribute to biosphere-atmosphere feedbacks responsible for the atmosphere build-up of these gases.

v) Effects on air quality-

Reduction of stratospheric ozone and increased penetration of UV-B radiation result in higher photo dissociation rates of key trace gases that control the chemical reactivity of the troposphere. This can increase both production and destruction of ozone and related oxidants such as hydrogen peroxide which are known to have adverse effects on human health, terrestrial plants and outdoor materials. Changes in the atmospheric concentrations of the hydroxyl radical (OH) may change the atmospheric lifetimes of important gases such as methane and substitutes of chlorofluoro carbons (CFCs).

vi) Effects on Materials-

An increased level of solar UV radiation is known to have adverse effects on synthetic polymers, naturally occurring biopolymers and some other materials of commercial interest. UV-B radiation accelerates the photo degradation rates of these materials thus limiting their lifetimes. Typical damages range from discoloration to loss of mechanical integrity. Such a situation would eventually demand substitution of the affected materials by more photo stable plastics and other materials in future. In 1974, two United States (US) scientists Mario Molina and F. Sherwood Rowland at the University of California were struck by the observation of Lovelock that the CFCs were present in the atmosphere all over the world more or less evenly distributed by appreciable concentrations. They suggested that these stable CFC molecules could drift slowly up to the stratosphere where they may breakdown into chlorine atoms by energetic UV-B and UB-C rays of the sun. The chlorine radicals thus produced can undergo complex chemical reaction producing chlorine monoxide which can attack an ozone molecule converting it into oxygen and in the process regenerating the chlorine atom again. Thus the ozone destroying effect is catalytic and a small amount of CFC would be destroying large number of ozone molecules. Their basic theory was then put to test by the National Aeronautic Space Authority (NASA) scientists and found to be valid, ringing alarm bells in many countries and laying the foundation for international action.

vii) Effects on Climate Change-

Ozone depletion and climate change are linked in a number of ways, but ozone depletion is not a major cause of climate change. Atmospheric ozone has two effects on the temperature balance of the Earth. It absorbs solar ultraviolet radiation, which heats the stratosphere. It also absorbs infrared radiation emitted by the Earth's surface, effectively trapping heat in the troposphere. Therefore, the climate impact of changes in ozone concentrations varies with the altitude at which these ozone changes occur. The major ozone losses that have been observed in the lower stratosphere due to the human-produced chlorine- and bromine-containing gases have a cooling effect on the Earth's surface. On the other hand, the ozone increases that are estimated to have occurred in the troposphere because of surface-pollution gases have a warming effect on the Earth's surface, thereby contributing to the "greenhouse" effect. In comparison to the effects of changes in other atmospheric gases, the effects of both of these ozone changes are difficult to calculate accurately. In the figure below, the upper ranges of possible effects for the ozone changes are indicated by the open bars, and the lower ranges are indicated by the solid bars.

viii) Effects on Ultraviolet Radiation-

The depletion of the ozone layer leads, on the average, to an increase in ground-level ultraviolet radiation, because ozone is an effective absorber of ultra-violet radiation. The Sun emits radiation over a wide range of energies, with about 2% in the form of high-energy, ultraviolet (UV) radiation. Some of this UV radiation (UV-B) is especially effective in causing damage to living beings, the largest decreases in ozone during the past 15 years have been observed over Antarctica, especially during each September and October when the ozone hole forms. During the last several years, simultaneous measurements of UV radiation and total ozone have been made at several Antarctic stations. In the late spring, the biologically damaging ultraviolet radiation in parts of the Antarctic continent can exceed that in San Diego, California, where the Sun is much higher above the horizon. In areas where smaller ozone depletion has been observed, UV-B increases are more difficult to detect. In particular, detection of trends in UV-B radiation associated with ozone decreases can be further complicated by changes in cloudiness, by local pollution, and by difficulties in keeping the detection instrument in precisely the same condition over many years. Prior to the late 1980s, instruments with the necessary accuracy and stability for measurement of small long-term trends in ground-level UV-B were not available. Therefore, the data from urban locations with older, less-specialized instruments provide much less reliable information, especially since simultaneous measurements of changes in cloudiness or local pollution are not available. When high-quality measurements have been made in other areas far from major cities and their associated air pollution, decreases in ozone have regularly been accompanied by increases in UV-B. This is shown in the figure below, where clear-sky measurements performed at six different stations demonstrate that ozone decreases lead to increased UV-B radiation at the surface in amounts that are in good agreement with that expected from calculations (the "model" curve).

INTERNATIONAL ACTIONS:

The first international action to focus attention on the dangers of ozone depletion in the stratosphere and its dangerous consequences in the long run on life on earth was focused in 1977 when in a meeting of 32 countries in Washington D.C. a World plan on action on Ozone layer with UNEP as the coordinator was adopted. As experts began their investigation, data piled up and in 1985 in an article published in the prestigious science journal, “Nature” by Dr. Farman pointed out that although there is overall depletion of the ozone layer all over the world, the most severe depletion had taken place over the Antarctica. This is what is famously called as "the Antarctica Ozone hole". His findings were confirmed by Satellite observations and offered the first proof of severe ozone depletion and stirred the scientific community to take urgent remedial actions in an international convention held in Vienna on March 22, 1985. This resulted in an international agreement in 1987 on specific measures to be taken in the form of an international treaty known as the Montreal Protocol on Substances That Deplete the Ozone Layer. Under this Protocol the first concrete step to save the Ozone layer was taken by immediately agreeing to completely phase out chlorofluorocarbons (CFC), Halons, Carbon tetrachloride (CTC) and Methyl chloroform (MCF) as per a given schedule

A. Montreal Protocol :

In 1985 the Vienna Convention established mechanisms for international co-operation in research into the ozone layer and the effects of ozone depleting chemicals (ODCs). 1985 also marked the first discovery of the Antarctic ozone hole. On the basis of the Vienna Convention, the Montreal Protocol on Substances that Deplete the Ozone Layer was negotiated and signed by 24 countries and by the European Economic Community in September 1987. The Protocol called for the Parties to phase down the use of CFCs, halons and other man-made ODCs. The Montreal Protocol represented a landmark in the international environmentalist movement. For the first time whole countries were legally bound to reducing and eventually phasing out altogether the use of CFCs and other ODCs. Failure to comply was accompanied by stiff penalties. The original Protocol aimed to decrease the use of chemical compounds destructive to ozone in the stratosphere by 50% by the year 1999. The Protocol was supplemented by agreements made in London in 1990 and in Copenhagen in 1992, where the same countries promised to stop using CFCs and most of the other chemical compounds destructive to ozone by the end of 1995. Fortunately, it has been fairly easy to develop and introduce compounds and methods to replace CFC compounds. In order to deal with the special difficulties experienced by developing countries it was agreed that they would be given an extended period of grace, so long as their use of CFCs did not grow significantly. China and India, for example, are strongly increasing the use of air conditioning and cooling devices

B. Australian Chlorofluorocarbon Management Strategy

It provides a framework for the responsible management and use of CFCs in Australia. The strategy recognizes some continuing need for these chemicals in pharmaceutical and laboratory uses, but commits to their gradual

phasing out.

C. Environmental Protection (Ozone Protection) Policy2000

This WA policy aims to minimize the discharge of ozone-depleting substances into the environment, and has been extended to cover use of alternative refrigerants (where relevant). This has been done to prevent current stocks of ozone-depleting substances from being released to the atmosphere by trade's people that are not accredited, or with inadequate training and/or equipment working on systems that contain these substances.

D. United Nations Environment Programme:

Has published several assessments of the environmental effects of ozone depletion (United Nations Environment Programme, 1998; World Meteorological Organization, 2002).

E. Ozone Protection and Synthetic Greenhouse Gas Management Act 1989 **(and associated regulations and amendments)**

Was implemented by the Commonwealth Government to meet its commitments under the Montreal Protocol.

F. Ultraviolet index forecast

The Bureau of Meteorology has developed a model to predict the amount of ultraviolet exposure and the times of day at which it will occur for 45 WA locations. It is designed to help people minimize their exposure to dangerous levels of ultraviolet radiation.

Under the auspices of United Nations Environment Programme (UNEP), Governments of the world, including the United States have cooperatively taken action to stop ozone depletion with the "The Montreal Protocol on Substances that Deplete the Ozone Layer", signed in 1987. Scientists are concerned that continued global warming will accelerate ozone destruction and increase stratospheric ozone depletion. Ozone depletion gets worse when the stratosphere (where the ozone layer is), becomes colder. Because global warming traps heat in the troposphere, less heat reaches the stratosphere which will make it colder. Greenhouse gases act like a blanket for the troposphere and make the stratosphere colder. In other words, global warming can make ozone depletion much worse right when it is supposed to begin its recovery during the next century. Maintain programs to ensure that ozone-depleting substances are not released and ongoing vigilance is required to this effect. In fact, global warming, acid rain, ozone layer depletion, and ground-level ozone pollution all pose a serious threat to the quality of life on Earth. They are separate problems, but, as has been seen, there are links between each. The use of CFCs not only destroys the ozone layer but also leads to global warming./

Acid rain: Causes, Effects and Solutions:

Acid rain, or acid deposition, is a broad term that includes any form of precipitation that contains acidic components, such as sulfuric acid or nitric acid, according to the Environmental Protection Agency (EPA).
The precipitation is not necessarily wet or liquid; the definition includes dust, gasses, rain, snow, fog and hail. The type of acid rain that contains water is called wet deposition. Acid rain formed with dust or gasses is called dry deposition

Dead trees line a riverbank near Norilsk, Russia, formerly a major industrial center in Siberia, and one of the most polluted cities in the world, according to National Geographic

Causes-

The term acid rain was coined in 1852 by Scottish chemist Robert Angus Smith, according to the Royal Society of Chemistry, which calls him the "father of acid rain." Smith decided on the term while examining rainwater chemistry near industrial cities in England and Scotland. He wrote about his findings in 1872 in the book "Air and Rain: The Beginnings of a Chemical Climatology."
In the 1950s, scientists in the United States started studying the phenomenon, and in the 1960s and early 1970s, acid rain became recognized as a regional environmental issue that affected Western Europe and eastern North America.

Though manmade pollutants are currently affecting most acidic precipitation, natural disasters can be a factor as well. For example, volcanoes can cause acid rain by blasting pollutants into the air. These pollutants can be carried around the world in jet streams and turned into acid rain far from the volcano.

After an asteroid supposedly wiped out the dinosaurs 65.5 million years ago, sulfur trioxide was blasted into the air. When it hit the air, it turned into sulfuric acid, generating a downpour of acid rain, according to a paper published in 2014 in the journal Nature Geoscience.
Even before that, over 4 billion years ago, it is suspected that the air may have had 10,000 times as much carbon dioxide as today. Geologists from the University of Wisconsin-Madison backed up this theory be studying rocks and publishing the results in a 2008 issue of the journal Earth and Planetary Science Letters. "At [those levels of carbon dioxide], you would have had vicious acid rain and intense greenhouse [effects]. That is a condition that will dissolve rocks," said study team member John Valley. [Early Earth Marred by Acid Rain]
Sulfur dioxide (SO_2) and nitrogen oxides (NOx) released into the air by fossil-fuel power plants, vehicles and oil refineries are the biggest cause of acid rain today, according to the EPA. Two thirds of sulfur dioxide and one fourth of nitrogen oxide found in the atmosphere come from electric power generators.
A chemical reaction happens when sulfur dioxide and nitrogen oxides mix with water, oxygen and other chemicals in the air. They then become sulfuric and nitric acids that mix with precipitation and fall to the ground. Precipitation is considered acidic when its pH level is about 5.2 or below, according to Encyclopedia Britannica. The normal pH of rain is around 5.6.

Effects-

Acid rain affects nearly everything. Plants, soil, trees, buildings and even statues can be transformed by the precipitation.

Acid rain has been found to be very hard on trees. It weakens them by washing away the protective film on leaves, and it stunts growth. A paper released in the online version of the journal of Environmental Science and Technology in 2005 showed evidence of acid rain stunting tree growth.
"By providing the only preserved soil in the world collected before the acid rain era, the Russians helped our international team track tree growth for the first time with changes in soil from acid rain," said Greg Lawrence, a U.S. Geological Survey scientist who headed the effort. "We've known that acid rain acidifies surface waters, but this is the first time we've been able to compare and track tree growth in forests that include soil changes due to acid rain."

Acid rain can also change the composition of soil and bodies of water, making them uninhabitable for local animals and plants. For example, healthy lakes have a pH of 6.5 or higher. As acid rain raises the level of acidity, fish tend to die off. Most fish species can't survive a water pH of below 5. When the pH becomes a 4, the lake is considered dead, according to National Atmospheric Deposition Program.
It can additionally deteriorate limestone and marble buildings and monuments, like gravestones.

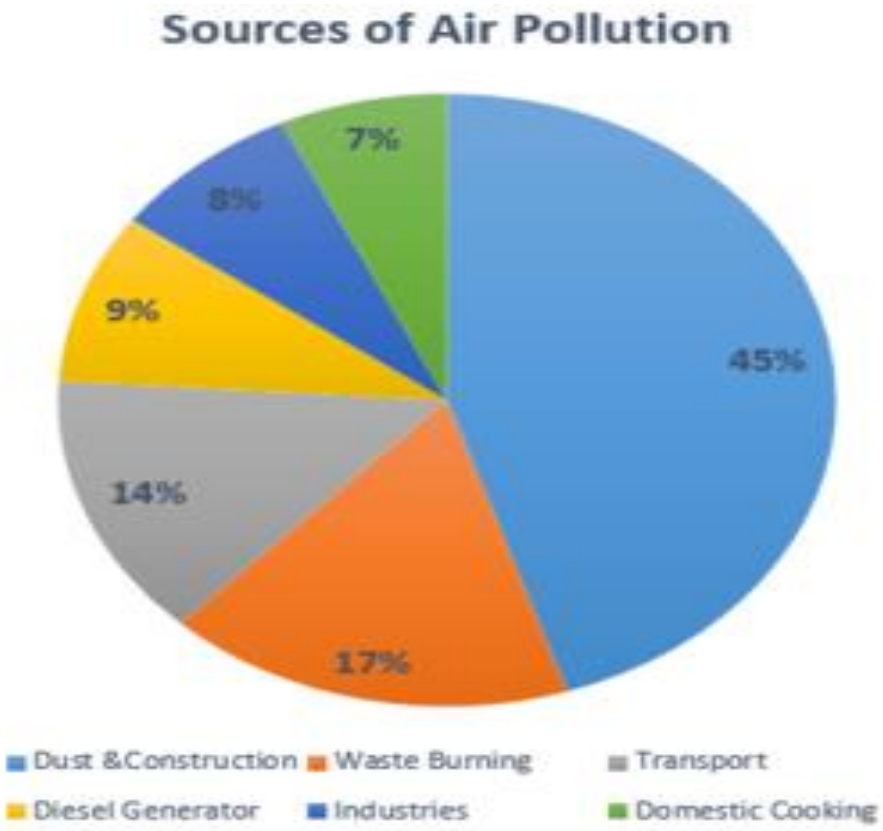
Solutions-

There are several solutions to stopping manmade acid rain. Regulating the emissions coming from vehicles and buildings is an important step, according to the EPA. This can be done by restricting the use of fossil fuels and focusing on more sustainable energy sources such as solar and wind power.

Also, each person can do their part by reducing their vehicle use. Using public transportation, walking, riding a bike or carpooling is a good start, according to the EPA. People can also reduce their use of electricity, which is

widely created with fossil fuels, or switch to a solar plan. Many electricity companies offer solar packages to their customers that require no installation and low costs.

Air pollution in India:



Dust & Construction contribute about 45% to the air pollution in India, which is followed by Waste Burning. Dust & Construction activities are mostly in the urban areas while Waste Burning is in the rural areas (agriculture).Air pollution occurs when harmful or excessive quantities of substances including gases, particles, and biological molecules are introduced into the Earth's atmosphere. Air pollution in India is a serious issue, ranking higher than smoking, high blood pressure, child and maternal malnutrition, and risk factors for diabetes. At least 140 million people breathe air 10 times or more over the WHO safe limit and 13 of the world's 20 cities with the highest annual levels of air pollution are in India. Air pollution contributes to the premature deaths of 2 million Indians every year. In urban areas, most emissions come from vehicles and industry, whereas in rural areas, much of the pollution stems from biomass burning for cooking and keeping warm. In autumn and winter months, large scale crop residue burning in agriculture fields – a low cost alternative to mechanical tilling – is a major source of smoke, smog and particulate pollution India has a low per capita emissions of greenhouse gases but the country as a whole is the third largest after China and the United States. A 2013 study on non-smokers has found that Indians have 30% lower lung function compared to Europeans.

The Air (Prevention and Control of Pollution) Act was passed in 1981 to regulate air pollution and there have been some measurable improvements. However, the 2016 Environmental Performance Index ranked India 141 out of 180 countries.

In 2015, Government of India, together with IIT Kanpur launched the National Air Quality Index. In 2019, India launched 'The National Clean Air Programme's with tentative national target of 20%-30% reduction in PM2.5 and PM10 concentrations by 2024, considering 2017 as the base year for comparison. It will be rolled out in 102 cities that are considered to have air quality worse than the National Ambient Air Quality Standards.

Causes:

Cooking fuel in rural India is prepared from a wet mix of dried grass, fuel wood pieces, hay, leaves and mostly cow/livestock dung. This mix is patted down into disc-shaped cakes, dried, and then used as fuel in stoves. When it burns, it produces smoke and numerous indoor air pollutants at concentrations 5 times higher than coal.

Fuel and biomass burning

A rural aburo stove using biomass cakes, fuel wood and trash as cooking fuel. Surveys suggest over 100 million households in India use such stoves (chullahs) every day, 2–3 times a day. Clean burning fuels and electricity are unavailable in rural parts and small towns of India because of poor rural highways and limited energy generation infrastructure.Fuel wood and biomass burning is the primary reason for near-permanent haze and smoke observed above rural and urban India, and in satellite pictures of the country. Fuel wood and biomass cakes are used for cooking and general heating needs. These are burnt in cook stoves known as chullah or chulha piece in some parts of India. These cook stoves are present in over 100 million Indian households, and are used two to three times a day, daily. Some reports, including one by the World Health Organization, claim 300,000 to 400,000 people die of indoor air pollution and carbon monoxide poisoning in India because of biomass burning and use of chullahs the air pollution is also the main cause of the Asian brown cloud which is delaying the start of the monsoon. Burning of biomass and firewood will not stop unless electricity or clean burning fuel and combustion technologies become reliably available and widely adopted in rural and urban India.

India is the world's largest consumer of fuel wood, agricultural waste and biomass for energy purposes. From the most recent available nationwide study, India used 148.7 million tonnes coal replacement worth of fuel-wood and biomass annually for domestic energy use. India's national average annual per capita consumption of fuel wood, agricultural waste and biomass cakes was 206 kilogram coal equivalent. The overall contribution of

fuel wood, including sawdust and wood waste, was about 46% of the total, the rest being agri waste and biomass dung cakes. Traditional fuel (fuel wood, crop residue and dung cake) dominates domestic energy use in rural India and accounts for about 90% of the total. In urban areas, this traditional fuel constitutes about 24% of the total. India burns tenfold more fuel wood every year than the United States; the fuel wood quality in India is different from the dry firewood of the United States; and, the Indian stoves in use are less efficient, thereby producing more smoke and air pollutants per kilogram equivalent.

Fuel adulteration

Some Indian taxis and auto-rickshaws run on adulterated fuel blends. Adulteration of gasoline and diesel with lower-priced fuels is common in South Asia, including India. Some adulterants increase emissions of harmful pollutants from vehicles, worsening urban air pollution. Financial incentives arising from differential taxes are generally the primary cause of fuel adulteration. In India and other developing countries, gasoline carries a much higher tax than diesel, which in turn is taxed more than kerosene meant as a cooking fuel, while some solvents and lubricants carry little or no tax.

As fuel prices rise, the public transport driver cuts costs by blending the cheaper hydrocarbon into highly taxed hydrocarbon. The blending may be as much as 20–30 percent. For a low wage driver, the adulteration can yield short term savings that are significant over the month. The consequences to long term air pollution, quality of life and effect on health are simply ignored. Also ignored are the reduced life of vehicle engine and higher maintenance costs, particularly if the taxi, auto-rickshaw or truck is being rented for a daily fee.

Adulterated fuel increases tailpipe emissions of hydrocarbons (HC), carbon monoxide (CO), oxides of nitrogen (NO_x) and particulate matter (PM). Air toxin emissions - which fall into the category of unregulated emissions - of primary concern are benzene and polyaromatic hydrocarbons (PAHs), both well known carcinogens. Kerosene is more difficult to burn than gasoline, its addition results in higher levels of HC, CO and PM emissions even from catalyst-equipped cars. The higher sulfur level of kerosene is another issue. Fuel adulteration is essentially an unintended consequence of tax policies and the attempt to control fuel prices, in the name of fairness. Air pollution is the ultimate result. This problem is not unique to India, but prevalent in many developing countries including those outside of south Asia. This problem is largely absent in economies that do not regulate the ability of fuel producers to innovate or price based on market demand.

Traffic congestion

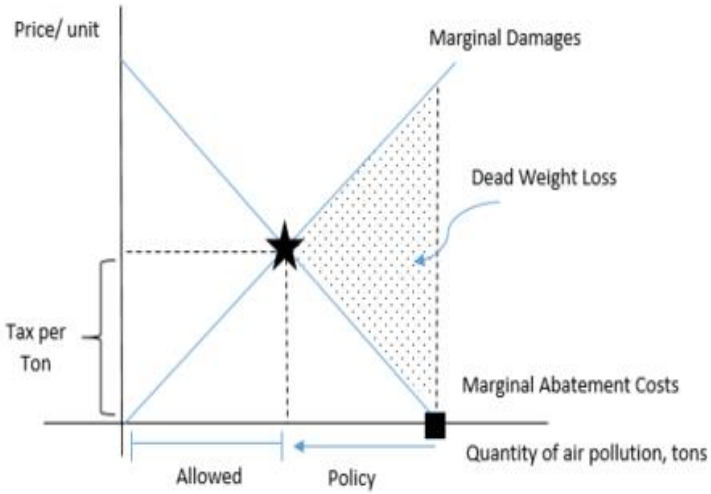
Traffic congestion is severe in India's cities and towns. Traffic congestion is caused for several reasons, some of which are: increase in number of vehicles per kilometer of available road, a lack of intra-city divided-lane highways and intra-city expressways networks, lack of inter-city expressways, traffic accidents and chaos due to poor enforcement of traffic laws.

Traffic congestion reduces average traffic speed. At low speeds, scientific studies reveal, vehicles burn fuel inefficiently and pollute more per trip. For example, a study in the United States found that for the same trip, cars consumed more fuel and polluted more if the traffic was congested, than when traffic flowed freely. At average trip speeds between 20 and 40 kilometers per hour, the cars pollutant emission was twice as much as when the average speed was 55 to 75 kilometers per hour. At average trip speeds between 5 and 20 kilometers per hour, the cars pollutant emissions were 4 to 8 times as much as when the average speed was 55 to 70 kilometers per hour. Fuel efficiencies similarly were much worse with traffic congestion.

Traffic gridlock in Delhi and other Indian cities is extreme. The average trip speed on many Indian city roads is less than 20 kilometers per hour; a 10 kilometer trip can take 30 minutes, or more. At such speeds, vehicles in India emit air pollutants 4 to 8 times more than they would with less traffic congestion; Indian vehicles also consume a lot more carbon footprint fuel per trip, than they would if the traffic congestion was less. Emissions of particles and heavy metals increase over time because the growth of the fleet and mileage outpaces the efforts to curb emissions.

In cities like Bangalore, around 50% of children suffer from asthma

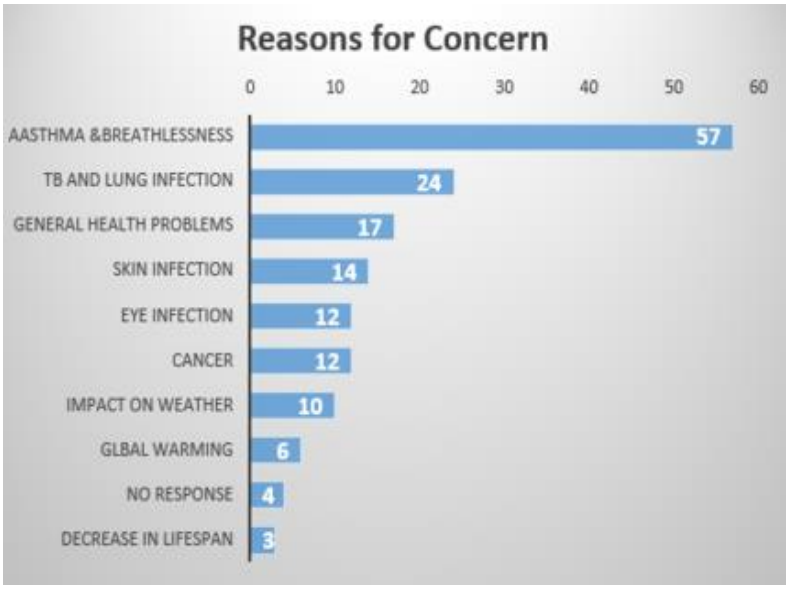
Greenhouse gas emissions



Market for Air Pollution: The box represents the current status quo and the star represents the ideal, socially optimal point to be at. The shaded area represents the dead weight loss. The MAC curve denotes the additional cost of achieving one more unit decrease in level of emissions. MD denotes the additional damage caused by an additional unit of emission.

India was the third largest emitter of carbon dioxide in 2017 at 6.82% share of CO2 emissions, after China (27.21%) and the United States (14.58%). According to a report by the Global Carbon Project, "“after low growth during 2014 to 2016, fossil CO2 emissions have now risen two years in a row, with a 1.6 per cent rise in 2017 and a projected 2.7 per cent (range 1.8 per cent to 3.7 per cent) rise expected in 2018, reaching a record high of 37.1 (plus or minus 2) billion tonnes of CO2. The peak in global CO2 emissions is not yet in sight.” About 65 percent of India's carbon dioxide emissions in 2009 was from heating, domestic uses and power sector. About 9 percent of India's emissions were from transportation (cars, trains, two wheelers, aeroplanes, others). India's coal-fired, oil-fired and natural gas-fired thermal power plants are inefficient and offer significant potential for CO_2 emission reduction through better technology. Compared to the average emissions from coal-fired, oil-fired and natural gas-fired thermal power plants in European Union (EU-27) countries, India's thermal power plants emit 50 to 120 percent more CO_2 per kWh produced.This is in significant part to inefficient thermal power plants installed in India prior to its economic liberalisation in the 1990s

Effects



Health costs of air pollution

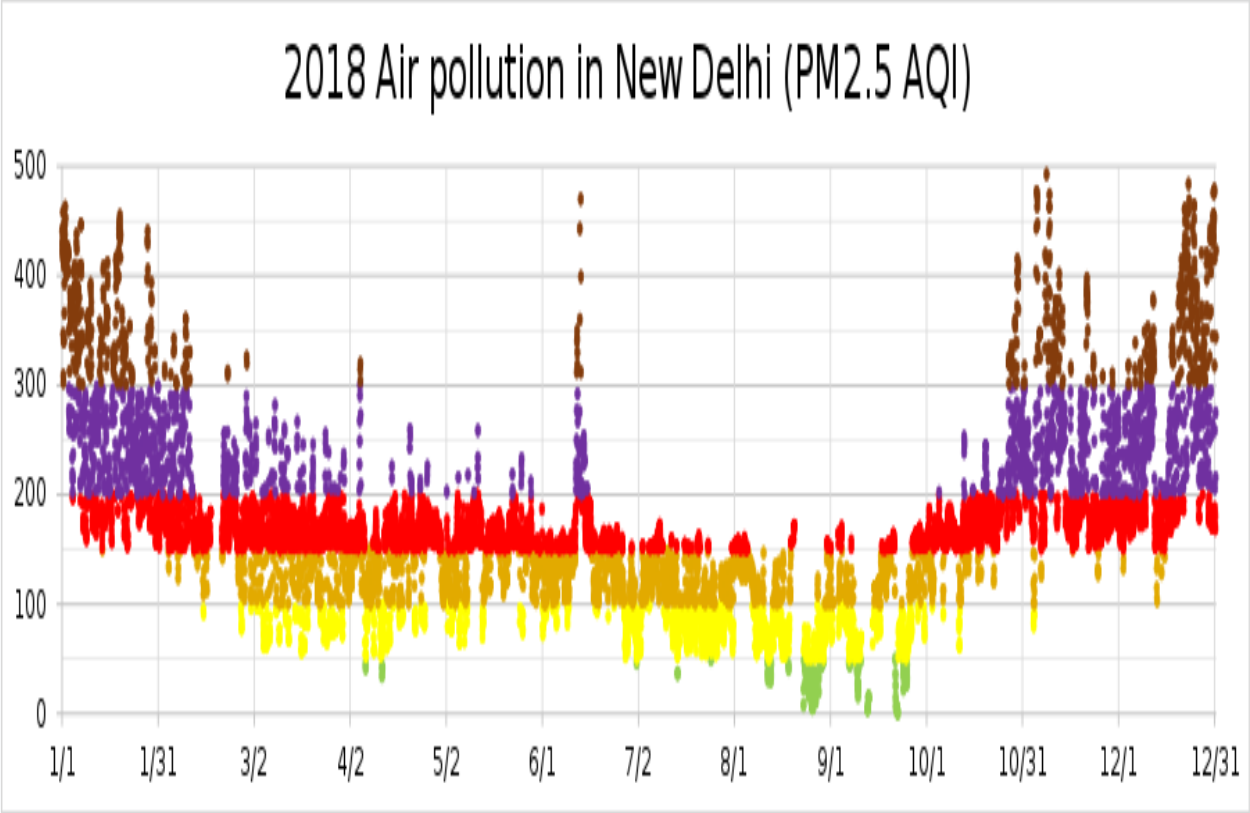
Asthma is the leading health problem faced by Indians. Not surprisingly, it accounts for more than 50% of the health problems caused by air pollution.One of the most important reasons for concern for the growing air pollution in the country is its effects on the health of individuals. Exposure to particulate matter for a long time can lead to respiratory and cardiovascular diseases such as asthma, bronchitis, lung cancer and heart attacks. The Global Burden of Disease Study for 2010, published in 2013, had found that outdoor air pollution was the fifth-largest killer in India and around 620,000 early deaths occurred from air pollution-related diseases in 2010.According to a WHO study, 13 of the 20 most-polluted cities in the world are in India; however, the accuracy and methodology of the WHO study was questioned by the Government of India.

Over a million Indians die prematurely every year due to air pollution, according to the non-profit Health Effects Institute. Over two million children -- half the children in Delhi -- have abnormalities in their lung function, according to the Delhi Heart and Lung Institute. Over the past decade air pollution has increased in India significant. Asthma is the most common health problem faced by Indians and it accounts for more than half of the health issues caused by air pollution.

State-Wise Trends:

According to the WHO, India has14 out of the 15 most polluted cities in the world in terms of PM 2.5 concentrations. Other Indian cities that registered very high levels of PM2.5 pollutants are Delhi, Patna, Agra, Muzzaffarpur, Srinagar, Gurgaon, Jaipur, Patiala and Jodhpur, followed by Ali Subah Al-Salem in Kuwait and a few cities in China and Mongolia.

Air Quality Index (AQI) is a number used to communicate the level of pollution in the air and it essentially tells you the level of pollution in the air in a given city on a given day. The AQI of Delhi was placed under the "severe-plus category" when it touched 574, by the System of Air Quality and Weather Forecasting And Research. In May 2014 the World Health Organisation announced New Delhi as the most polluted city in the world. In November 2016, the Great smog of Delhi was an environmental event which saw New Delhi and adjoining areas in a dense blanket of smog, which was the worst in 17 years.



2018 Air Pollution in NewDelhi (PM2.5 AQI). A surge on June 14 was caused by dust storms brought on by a combination of extreme heat and powerful downdraft winds.[26]

Hazardous Very Unhealthy Unhealthy Unhealthy for Sensitive Groups Moderate Good

Top 20 Cities in India with the highest level of PM 2.5	
Cities	PM2.5 Levels
Delhi	153
Patna	149
Gwalior	144
Raipur	134
Ahmedabad	100
Lukhnow	96
Firozabad	96
Kanpur	93
Amritsar	92
Ludhiana	91
Allahbad	88
Agra	88
Khanna	88

India's Central Pollution Control Board now routinely monitors four air pollutants namely sulphur dioxide (SO2), oxides of nitrogen (NOx), suspended particulate matter (SPM) and respirable particulate matter (PM10). These are target air pollutants for regular monitoring at 308 operating stations in 115 cities/towns in 25 states and 4 Union Territories of India. The monitoring of meteorological parameters such as wind speed and direction, relative humidity and temperature has also been integrated with the monitoring of air quality. The monitoring of these pollutants is carried out for 24 hours (4-hourly sampling for gaseous pollutants and 8-hourly sampling for particulate matter) with a frequency of twice a week, to yield 104 observations in a year.

The key findings of India's central pollution control board are:

- Most Indian cities continue to violate India's and world air quality PM10 targets. Respirable particulate matter pollution remains a key challenge for India. Despite the general non-attainment, some cities showed far more improvement than others. A decreasing trend has been observed in PM10 levels in cities like Solapur and Ahmedabad over the last few years. This improvement may be due to local measures taken to reduce sulphur in diesel and stringent enforcement by the government.
- A decreasing trend has been observed in sulphur dioxide levels in residential areas of many cities such as Delhi, Mumbai, Lucknow, Bhopal during last few years. The decreasing trend in sulphur dioxide levels may be due to recently introduced clean fuel standards, and the increasing use of LPG as domestic fuel instead of coal or fuelwood, and the use of CNG instead of diesel in certain vehicles.
- A decreasing trend has been observed in nitrogen dioxide levels in residential areas of some cities such as Bhopal and Solapur during last few years. The decreasing trend in sulphur dioxide levels may be due to recently introduced vehicle emission standards, and the increasing use of LPG as domestic fuel instead of coal or fuelwood.
- Most Indian cities greatly exceed acceptable levels of suspended particulate matter. This may be because of refuse and biomass burning, vehicles, power plant emissions, industrial sources.
- The Indian air quality monitoring stations reported lower levels of PM10 and suspended particulate matter during monsoon months possibly due to wet deposition and air scrubbing by rainfall. Higher levels of particulates were observed during winter months possibly due to lower mixing heights and more calm conditions. In other words, India's air quality worsens in winter months, and improves with the onset of monsoon season.
- The average annual SOx and NOx emissions level and periodic violations in industrial areas of India were significantly and surprisingly lower than the emission and violations in residential areas of India
- Of the four major Indian cities, air pollution was consistently worse in Delhi, every year over 5-year period (2004–2018). Kolkata was a close second, followed by Mumbai. Chennai air pollution was least of the four.

Steps Taken/ Policy Recommendations

- The government in Delhi launched an Odd-Even Rule in November, 2017 which is based on the Odd-Even rationing method: This meant that cars running with number plates ending in Odd digits could only be driven on certain days of the week, while the even digit cars could be driven on the remaining days of the week.
- Local governments of various states also implemented measures such as tighter vehicle emissions' norms, higher penalties for burning rubbish and better control of road dust
- The Indian government has committed to a 50% reduction in households using solid fuel for cooking
- Some goals set for future are:
    - Clean up the transportation sector by introducing 1,000 electric public transport buses to its 5,50-string feet
    - Meet a goal of 25% of private vehicles to be electricity powered by 2023
    - Provide farmers with a machine called a Happy Seeder which converts agricultural residue to fertilizer
    - Analyze health data and study the efficiency of different room filtration systems in areas where indoor air pollution is highest
    - Identify effective ways to inform the public about air pollution data
    - Launch new citizen science programs to better document exposures

- Reduce Carbon Emissions: "According to Inter-governmental Panel on Climate Change, to limit warming well below 2 degree Celsius, CO2 emissions should decline by about 20 per cent by 2030 and reach net zero around 2075; to limit warming below 1.5 degree Celsius, CO2 emissions should decline by 50 per cent by 2030 and reach net zero by around 2050.

Water Pollution

British poet W. H. Auden once noted, "Thousands have lived without love, not one without water." Yet while we all know water is crucial for life, we trash it anyway. Some 80 percent of the world's wastewater is dumped—largely untreated—back into the environment, polluting rivers, lakes, and oceans.

This widespread problem of water pollution is jeopardizing our health. Unsafe water kills more people each year than war and all other forms of violence combined. Meanwhile, our drinkable water sources are finite: Less than 1 percent of the earth's freshwater is actually accessible to us. Without action, the challenges will only increase by 2050, when global demand for freshwater is expected to be one-third greater than it is now.

But while most Americans have access to safe drinking water, potentially harmful contaminants—from arsenic to copper to lead—have been found in the tap water of every single state in the nation.

Still, we're not hopeless against the threat to clean water. To better understand the problem and what we can do about it, here's an overview of what water pollution is, what causes it, and how we can protect ourselves.

What Is Water Pollution?

Water pollution occurs when harmful substances—often chemicals or microorganisms—contaminate a stream, river, lake, ocean, aquifer, or other body of water, degrading water quality and rendering it toxic to humans or the environment.

Water pollution is the contamination of water bodies, usually as a result of human activities. Water bodies include for example lakes, rivers, oceans, aquifers and groundwater. Water pollution results when contaminants are introduced into the natural environment.

What Are the Causes of Water Pollution?

Water is uniquely vulnerable to pollution. Known as a "universal solvent," water is able to dissolve more substances than any other liquid on earth. It's the reason we have Kool-Aid and brilliant blue waterfalls. It's also why water is so easily polluted. Toxic substances from farms, towns, and factories readily dissolve into and mix with it, causing water pollution.

Categories of Water Pollution

Groundwater-

When rain falls and seeps deep into the earth, filling the cracks, crevices, and porous spaces of an aquifer (basically an underground storehouse of water), it becomes groundwater—one of our least visible but most important natural resources. Nearly 40 percent of Americans rely on groundwater, pumped to the earth's surface, for drinking water. For some folks in rural areas, it's their only freshwater source. Groundwater gets polluted when contaminants—from pesticides and fertilizers to waste leached from landfills and septic systems—make their way into an aquifer, rendering it unsafe for human use. Ridding groundwater of contaminants can be difficult to impossible, as well as costly. Once polluted, an aquifer may be unusable for decades, or even thousands of years. Groundwater can also spread contamination far from the original polluting source as it seeps into streams, lakes, oceans.

Surface water-

Covering about 70 percent of the earth, surface water is what fills our oceans, lakes, rivers, and all those other blue bits on the world map. Surface water from freshwater sources (that is, from sources other than the ocean) accounts for more than 60 percent of the water delivered to American homes. But a significant pool of that water is in peril. According to the most recent surveys on national water quality from the U.S. Environmental Protection Agency, nearly half of our rivers and streams and more than one-third of our lakes are polluted and unfit for swimming, fishing, and drinking. Nutrient pollution, which includes nitrates and phosphates, is the leading type of contamination in these freshwater sources. While plants and animals need these nutrients to grow, they have become a major pollutant due to farm waste and fertilizer runoff. Municipal and industrial waste discharges contribute their fair share of toxins as well. There's also all the random junk that industry and individuals dump directly into waterways

Ocean water

Eighty percent of ocean pollution (also called marine pollution) originates on land—whether along the coast or far inland. Contaminants such as chemicals, nutrients, and heavy metals are carried from farms, factories, and cities by streams and rivers into our bays and estuaries; from there they travel out to sea. Meanwhile, marine debris—particularly plastic—is blown in by the wind or washed in via storm drains and sewers. Our seas are

also sometimes spoiled by oil spills and leaks—big and small—and are consistently soaking up carbon pollution from the air. The ocean absorbs as much as a quarter of man-made carbon emissions.

Point source-

When contamination originates from a single source, it's called point source pollution. Examples include wastewater (also called effluent) discharged legally or illegally by a manufacturer, oil refinery, or wastewater treatment facility, as well as contamination from leaking septic systems, chemical and oil spills, and illegal dumping. The EPA regulates point source pollution by establishing limits on what can be discharged by a facility directly into a body of water. While point source pollution originates from a specific place, it can affect miles of waterways and ocean.

Nonpoint source-

Nonpoint source pollution is contamination derived from diffuse sources. These may include agricultural or stormwater runoff or debris blown into waterways from land. Nonpoint source pollution is the leading cause of water pollution in U.S. waters, but it's difficult to regulate, since there's no single, identifiable culprit.

Transboundary-

It goes without saying that water pollution can't be contained by a line on a map. Transboundary pollution is the result of contaminated water from one country spilling into the waters of another. Contamination can result from a disaster—like an oil spill—or the slow, downriver creep of industrial, agricultural, or municipal discharge.

The Most Common Types of Water Contamination

Agricultural

Toxic green algae in Copco Reservoir, northern California

Not only is the agricultural sector the biggest consumer of global freshwater resources, with farming and livestock production using about 70 percent of the earth's surface water supplies, but it's also a serious water polluter. Around the world, agriculture is the leading cause of water degradation. In the United States, agricultural pollution is the top source of contamination in rivers and streams, the second-biggest source in wetlands, and the third main source in lakes. It's also a major contributor of contamination to estuaries and groundwater. Every time it rains, fertilizers, pesticides, and animal waste from farms and livestock operations wash nutrients and pathogens—such bacteria and viruses—into our waterways. Nutrient pollution, caused by excess nitrogen and phosphorus in water or air, is the number-one threat to water quality worldwide and can cause algal blooms, a toxic soup of blue-green algae that can be harmful to people and wildlife.

Sewage and wastewater

Used water is wastewater. It comes from our sinks, showers, and toilets (think sewage) and from commercial, industrial, and agricultural activities (think metals, solvents, and toxic sludge). The term also includes storm water runoff, which occurs when rainfall carries road salts, oil, grease, chemicals, and debris from impermeable surfaces into our waterways.

More than 80 percent of the world's wastewater flows back into the environment without being treated or reused, according to the United Nations; in some least-developed countries, the figure tops 95 percent. In the United States, wastewater treatment facilities process about 34 billion gallons of wastewater per day. These

facilities reduce the amount of pollutants such as pathogens, phosphorus, and nitrogen in sewage, as well as heavy metals and toxic chemicals in industrial waste, before discharging the treated waters back into waterways. That's when all goes well. But according to EPA estimates, our nation's aging and easily overwhelmed sewage treatment systems also release more than 850 billion oil pollution

Big spills may dominate headlines, but consumers account for the vast majority of oil pollution in our seas, including oil and gasoline that drips from millions of cars and trucks every day. Moreover, nearly half of the estimated 1 million tons of oil that makes its way into marine environments each year comes not from tanker spills but from land-based sources such as factories, farms, and cities. At sea, tanker spills account for about 10 percent of the oil in waters around the world, while regular operations of the shipping industry—through both legal and illegal discharges—contribute about one-third. Oil is also naturally released from under the ocean floor through fractures known as seeps.

Radioactive substances

Radioactive waste is any pollution that emits radiation beyond what is naturally released by the environment. It's generated by uranium mining, nuclear power plants, and the production and testing of military weapons, as well as by universities and hospitals that use radioactive materials for research and medicine. Radioactive waste can persist in the environment for thousands of years, making disposal a major challenge. Consider the decommissioned Hanford nuclear weapons production site in Washington, where the cleanup of 56 million gallons of radioactive waste is expected to cost more than $100 billion and last through 2060. Accidentally released or improperly disposed of contaminants threaten groundwater, surface water, and marine resources.

What Are the Effects of Water Pollution?

On human health

To put it bluntly: Water pollution kills. In fact, it caused 1.8 million deaths in 2015, according to a study published in The Lancet. Contaminated water can also make you ill. Every year, unsafe water sickens about 1 billion people. And low-income communities are disproportionately at risk because their homes are often closest to the most polluting industries.

Waterborne pathogens, in the form of disease-causing bacteria and viruses from human and animal waste, are a major cause of illness from contaminated drinking water. Diseases spread by unsafe water include cholera, giardia, and typhoid. Even in wealthy nations, accidental or illegal releases from sewage treatment facilities, as well as runoff from farms and urban areas, contribute harmful pathogens to waterways.

Thousands of people across the United States are sickened every year by Legionnaires' disease (a severe form of pneumonia contracted from water sources like cooling towers and piped water), with cases cropping up from California's Disneyland to Manhattan's Upper East Side.

A woman using bottled water to wash her three-week-old son at their home in Flint, MichiganTodd McInturf/The Detroit News/AP

Meanwhile, the plight of residents in Flint, Michigan—where cost-cutting measures and aging water infrastructure created the recent lead contamination crisis—offers a stark look at how dangerous chemical and other industrial pollutants in our water can be. The problem goes far beyond Flint and involves much more than lead, as a wide range of chemical pollutants—from heavy metals such as arsenic and mercury to pesticides and nitrate fertilizers—are getting into our water supplies. Once they're ingested, these toxins can cause a host of health issues, from cancer to hormone disruption to altered brain function. Children and pregnant women are particularly at risk.

Even swimming can pose a risk. Every year, 3.5 million Americans contract health issues such as skin rashes, pinkeye, respiratory infections, and hepatitis from sewage-laden coastal waters, according to EPA estimates.

On the environment

In order to thrive, healthy ecosystems rely on a complex web of animals, plants, bacteria, and fungi—all of which interact, directly or indirectly, with each other. Harm to any of these organisms can create a chain effect, imperiling entire aquatic environments.

When water pollution causes an algal bloom in a lake or marine environment, the proliferation of newly introduced nutrients stimulates plant and algae growth, which in turn reduces oxygen levels in the water. This dearth of oxygen, known as eutrophication, suffocates plants and animals and can create "dead zones," where waters are essentially devoid of life. In certain cases, these harmful algal blooms can also produce neurotoxins that affect wildlife, from whales to sea turtles.

Chemicals and heavy metals from industrial and municipal wastewater contaminate waterways as well. These contaminants are toxic to aquatic life—most often reducing an organism's life span and ability to reproduce—and make their way up the food chain as predator eats prey. That's how tuna and other big fish accumulate high quantities of toxins, such as mercury. Marine ecosystems are also threatened by marine debris, which can strangle, suffocate, and starve animals. Much of this solid debris, such as plastic bags and soda cans, gets swept into sewers and storm drains and eventually out to sea, turning our oceans into trash soup and sometimes consolidating to form floating garbage patches. Discarded fishing gear and other types of debris are responsible for harming more than 200 different species of marine life.

Meanwhile, ocean acidification is making it tougher for shellfish and coral to survive. Though they absorb about a quarter of the carbon pollution created each year by burning fossil fuels, oceans are becoming more acidic. This process makes it harder for shellfish and other species to build shells and may impact the nervous systems of sharks, clownfish, and other marine life

What Can You Do to Prevent Water Pollution?

With your actions

It's easy to tsk-tsk the oil company with a leaking tanker, but we're all accountable to some degree for today's water pollution problem. Fortunately, there are some simple ways you can prevent water contamination or at least limit your contribution to it: Reduce your plastic consumption and reuse or recycle plastic when you can.

- Properly dispose of chemical cleaners, oils, and non-biodegradable items to keep them from ending up down the drain.
- Maintain your car so it doesn't leak oil, antifreeze, or coolant.
- If you have a yard, consider landscaping that reduces runoff and avoid applying pesticides and herbicides.
- If you have a pup, be sure to pick up its poop.

Electronic waste

E-waste or electronic waste is created when an electronic product is discarded after the end of its useful life. The rapid expansion of technology means that a very large amount of e-waste is created every minute.

Electronic waste or e-waste may be defined as discarded computers, office electronic equipment, entertainment device electronics, mobile phones, television sets, and refrigerators. This includes used electronics which are destined for reuse, resale, salvage, recycling, or disposal as well as re-usables (working and repairable electronics) and secondary scraps (copper, steel, plastic, etc.). The term "waste" is reserved for residue or material which is dumped by the buyer rather than recycled, including residue from reuse and recycling operations, because loads of surplus electronics are frequently commingled (good, recyclable, and non-recyclable). Several public policy advocates apply the term "e-waste" and "e-scrap" broadly to all surplus electronics. Cathode ray tubes (CRTs) are considered one of the hardest types to

recycle.
CRTs have relatively high concentration of lead and phosphors (not to be confused with phosphorus), both of which are necessary for the display. The United States Environmental Protection Agency (EPA) includes discarded CRT monitors in its category of "hazardous household waste" but considers CRTs that have been set aside for testing to be commodities if they are not discarded, speculatively accumulated, or left unprotected from weather and other damage. These CRT devices are often confused between the DLP Rear Projection TV, both of which have a different recycling process due to the materials they are composed of.

Environmental impact

The processes of dismantling and disposing of electronic waste in developing countries led to a number of environmental impacts as illustrated in the graphic. Liquid and atmospheric releases end up in bodies of water, groundwater, soil, and air and therefore in land and sea animals – both domesticated and wild, in crops eaten by both animals and human, and in drinking water.

One study of environmental effects in Guiyu, China found the following:

- Airborne dioxins – one type found at 100 times levels previously measured
- Levels of carcinogens in duck ponds and rice paddies exceeded international standards for agricultural areas and cadmium, copper, nickel, and lead levels in rice paddies were above international standards
- Heavy metals found in road dust – lead over 300 times that of a control village's road dust and copper over 100 times

The environmental impact of the processing of different electronic waste components

E-Waste Component	Process Used	Potential Environmental Hazard
Cathode ray tubes (used in TVs, computer monitors, ATM, video cameras, and more)	Breaking and removal of yoke, then dumping	Lead, barium and other heavy metals leaching into the ground water and release of toxic phosphorus
Printed circuit board (image behind table – a thin plate on which chips and other electronic components are placed)	De-soldering and removal of computer chips; open burning and acid baths to remove metals after chips are removed.	Air emissions and discharge into rivers of glass dust, tin, lead, brominated dioxin, beryllium cadmium, and mercury
Chips and other gold plated components	Chemical stripping using nitric and hydrochloric acid and burning of chips	PAHs, heavy metals, brominated flame retardants discharged directly into rivers acidifying fish and flora. Tin and lead contamination of surface and groundwater. Air emissions of brominated dioxins, heavy metals, and PAHs
Plastics from printers, keyboards, monitors, etc.	Shredding and low temp melting to be reused	Emissions of brominated dioxins, heavy metals, and hydrocarbons
Computer wires	Open burning and stripping to remove copper	PAHs released into air, water, and soil.

Information security

E-waste presents a potential security threat to individuals and exporting countries. Hard drives that are not

properly erased before the computer is disposed of can be reopened, exposing sensitive information. Credit card numbers, private financial data, account information, and records of online transactions can be accessed by most willing individuals. Organized criminals in Ghana commonly search the drives for information to use in local scams. Electronic files about government contracts have been discovered on hard drives found in Agbogbloshie. Multimillion-dollar agreements from United States security institutions such as the Defense Intelligence Agency (DIA), the Transportation Security Administration, and Homeland Security have all resurfaced in Agbogbloshie.

E-waste management
Recycling

Computer monitors are typically packed into low stacks on wooden pallets for recycling and then shrink-wrapped.

E-waste can be managed by properly disposing and managing your electronic waste .We should give our waste to the e waste dealers and should follow the precautions as they contain many heavy metals.

Recycling

One of the major challenges is recycling the printed circuit boards from the electronic wastes. The circuit boards contain such precious metals as gold, silver, platinum, etc. and such base metals as copper, iron, aluminum, etc. One way e-waste is processed is by melting circuit boards, burning cable sheathing to recover copper wire and open- pit acid leaching for separating metals of value.

Consumer awareness efforts

A campaign to promote e-waste recycling in Ghana.

The U.S. Environmental Protection Agency encourages electronic recyclers to become certified by demonstrating to an accredited, independent third party auditor that they meet specific standards to safely recycle and manage electronics. This should work so as to ensure the highest environmental standards are being maintained. Two certifications for electronic recyclers currently exist and are endorsed by the EPA. Customers are encouraged to choose certified electronics recyclers. Responsible electronics recycling reduces environmental and human health impacts, increases the use of reusable and refurbished equipment and reduces

Energy use while conserving limited resources. The two EPA-endorsed certification programs are Responsible Recyclers Practices (R2) and E-Stewards. Certified companies ensure they are meeting strict environmental standards which maximize reuse and recycling, minimize exposure to human health or the environment, ensure safe management of materials and require destruction of all data used on electronics.[60] Certified electronics recyclers have demonstrated through audits and other means that they continually meet specific high environmental standards and safely manage used electronics. Once certified, the recycler is held to the particular standard by continual oversight by the independent accredited certifying body. A certification board accredits and oversees certifying bodies to ensure that they meet specific responsibilities and are competent to audit and provide certification.

Benefits of recycling

Recycling raw materials from end-of-life electronics is the most effective solution to the growing e-waste problem. Most electronic devices contain a variety of materials, including metals that can be recovered for future uses. By dismantling and providing reuse possibilities, intact natural resources are conserved and air and water pollution caused by hazardous disposal is avoided. Additionally, recycling reduces the amount of greenhouse gas emissions caused by the manufacturing of new products. Another benefit of recycling e-waste is that many of the materials can be recycled and re-used again. Materials that can be recycled include "ferrous (iron-based) and non-ferrous metals, glass, and various types of plastic." “Non-ferrous metals, mainly aluminum and copper can all be re-smelted and re- manufactured. Ferrous metals such as steel and iron can be also be re-used." Due to the recent surge in popularity in 3D printing, certain 3D printers have been designed (FDM variety) to produce waste that can be easily recycled which decreases the amount of harmful pollutants in the atmosphere. The excess plastic from these printers that comes out as a byproduct can also be reused to create new 3D printed creations.

Soil Pollution

Soil contamination Soil contamination or soil pollution as part of land degradation is caused by the presence of xenobiotics (human-made) chemicals or other alteration in the natural soil environment. It is typically caused by industrial activity, agricultural chemicals or improper disposal of waste. The most common chemicals involved are petroleum hydrocarbons, polynuclear aromatic hydrocarbons (such as naphthalene and benzo(a)pyrene), solvents, pesticides, lead, and other heavy metals. Contamination is correlated with the degree of industrialization and intensity of chemical substance. The concern over soil contamination stems primarily from health risks, from direct contact with the contaminated soil, vapours from the contaminants, and from secondary contamination of water supplies within and underlying the soil. Mapping of contaminated soil sites and the resulting cleanups are time consuming and expensive tasks, requiring extensive amounts of geology, hydrology, chemistry, computer modeling skills, and GIS in Environmental Contamination, as well as an appreciation of the history of industrial chemistry. In North America and Western Europe the extent of contaminated land is best known, with many of countries in these areas having a legal framework to identify and deal with this environmental problem. Developing countries tend to be less tightly regulated despite some of them having undergone significant industrialization. Causes Pesticides and herbicides Agents of war Health effects Ecosystem effects Cleanup options By country People's Republic of China European Union United Kingdom Canada India See also References External links Soil pollution can be caused by the following (non-exhaustive list) Excavation showing soil contamination at a disused gasworks in England. Contents Causes Micro plastics Oil spills Mining and activities by other heavy industries Accidental spills may happen during activities, etc. Corrosion of underground storage tanks (including piping used to transmit the contents) Acid rain Intensive farming Agrochemicals, such as pesticides, herbicides and fertilizers Petrochemicals Industrial accidents Road debris Drainage of contaminated surface water into the soil Ammunitions, chemical agents, and other agents of war Waste disposal Oil and fuel dumping Nuclear wastes Direct discharge of industrial wastes to the soil Discharge of sewage Landfill and illegal dumping Coal ash Electronic waste The most common chemicals involved are petroleum hydrocarbons, solvents, pesticides, lead, and other heavy metals. Any activity that leads to other forms of soil degradation (erosion, compaction, etc.) may indirectly worsen the

contamination effects in that soil remediation becomes more tedious. Historical deposition of coal ash used for residential, commercial, and industrial heating, as well as for industrial processes such as ore smelting, were a common source of contamination in areas that were industrialized before about 1960. Coal naturally concentrates lead and zinc during its formation, as well as other heavy metals to a lesser degree. When the coal is burned, most of these metals become concentrated in the ash (the principal exception being mercury). Coal ash and slag may contain sufficient lead to qualify as a "characteristic hazardous waste", defined in the USA as containing more than 5 mg/l of extractable lead using the TCLP procedure. In addition to lead, coal ash typically contains variable but significant concentrations of polynuclear aromatic hydrocarbons (PAHs; e.g., benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(cd)pyrene, phenanthrene, anthracene, and others). These PAHs are known human carcinogens and the acceptable concentrations of them in soil are typically around 1 mg/kg. Coal ash and slag can be recognised by the presence of off-white grains in soil, gray heterogeneous soil, or (coal slag) bubbly, vesicular pebble-sized grains. Treated sewage sludge, known in the industry as biosolids, has become controversial as a "fertilizer". As it is the byproduct of sewage treatment, it generally contains more contaminants such as organisms, pesticides, and heavy metals than other soil. In the European Union, the Urban Waste Water Treatment Directive allows sewage sludge to be sprayed onto land. The volume is expected to double to 185,000 tons of dry solids in 2005. This has good agricultural properties due to the high nitrogen and phosphate content. In 1990/1991, 13% wet weight was sprayed onto 0.13% of the land; however, this is expected to rise 15 fold by 2005. Advocates say there is a need to control this so that pathogenic microorganisms do not get into water courses and to ensure that there is no accumulation of heavy metals in the top soil. Pesticides and herbicides A pesticide is a substance used to kill a pest. A pesticide may be a chemical substance, biological agent (such as a virus or bacteria), antimicrobial, disinfectant or device used against any pest. Pests include insects, plant pathogens, weeds, mollusks, birds, mammals, fish, nematodes (roundworms) and microbes that compete with humans for food, destroy property, spread or are a vector for disease or cause a nuisance. Although there are benefits to the use of pesticides, there are also drawbacks, such as potential toxicity to humans and other organisms. Herbicides are used to kill weeds, especially on pavements and railways. They are similar to auxins and most are biodegradable by soil bacteria. However, one group derived from trinitrotoluene (2:4 D and 2:4:5 T) have the impurity dioxin, which is very toxic and causes fatality even in low concentrations. Another herbicide is Paraquat. It is highly toxic but it rapidly degrades in soil due to the action of bacteria and does not kill soil fauna. Insecticides are used to rid farms of pests which damage crops. The insects damage not only standing crops but also stored ones and in the tropics it is reckoned that one third of the total production is lost during food storage. As with fungicides, the first insecticides used in the nineteenth century were inorganic e.g. Paris Green and other compounds of arsenic. Nicotine has also been used since the late eighteenth century. There are now two main groups of synthetic insecticides – **1. Organochlorines** include DDT, Aldrin, Dieldrin and BHC. They are cheap to produce, potent and persistent. DDT was used on a massive scale from the 1930s, with a peak of 72,000 tonnes used 1970. Then usage fell as the harmful environmental effects were realized. It was found worldwide in fish and birds and was even discovered in the snow in the Antarctic. It is only slightly soluble in water but is very soluble in the bloodstream. It affects the nervous and endocrine systems and causes the eggshells of birds to lack calcium causing them to be easily breakable. It is thought to be responsible for the decline of the numbers of birds of prey like ospreys and peregrine falcons in the 1950s – they are now recovering. As well as increased concentration via the food chain, it is known to enter via permeable membranes, so fish get it through their gills. As it has low water solubility, it tends to stay at the water surface, so organisms that live there are most affected. DDT found in fish that formed part of the human food chain caused concern, but the levels found in the liver, kidney and brain tissues was less than 1 ppm and in fat was 10 ppm, which was below the level likely to cause harm. However, DDT was banned in the UK and the United States to stop the further buildup of it in the food chain. U.S. manufacturers continued to sell DDT to developing countries, who could not afford the expensive replacement chemicals and who did not have such stringent regulations governing the use of pesticides.

2. Organophosphates e.g. parathion, methyl parathion and about 40 other insecticides are available nationally. Parathion is highly toxic, methyl-parathion is less so and Malathion is generally considered safe as it has low toxicity and is rapidly broken down in the mammalian liver. This group works by preventing normal nerve transmission as cholinesterase is prevented from breaking down the transmitter substance acetylcholine, resulting in uncontrolled muscle movements. The disposal of munitions, and a lack of care in manufacture of munitions caused by the urgency of production, can contaminate soil for extended periods. There is little published evidence on this type of contamination largely because of restrictions placed by governments of many countries on the publication of material related to war effort. However, mustard gas stored during World War II has contaminated some sites for up to 50 years and the testing of Anthrax as a potential biological

weapon contaminated the whole island of Gruinard. Contaminated or polluted soil directly affects human health through direct contact with soil or via inhalation of soil contaminants which have vaporized; potentially greater threats are posed by the infiltration of soil contamination into groundwater aquifers used for human consumption, sometimes in areas apparently far removed from any apparent source of above ground Agents of war Health effects contamination. This tends to result in the development of pollution-related diseases. Health consequences from exposure to soil contamination vary greatly depending on pollutant type, pathway of attack and vulnerability of the exposed population. Chronic exposure to chromium, lead and other metals, petroleum, solvents, and many pesticide and herbicide formulations can be carcinogenic, can cause congenital disorders, or can cause other chronic health conditions. Industrial or man-made concentrations of naturally occurring substances, such as nitrate and ammonia associated with livestock manure from agricultural operations, have also been identified as health hazards in soil and groundwater. Chronic exposure to benzene at sufficient concentrations is known to be associated with higher incidence of leukemia. Mercury and cyclodienes are known to induce higher incidences of kidney damage and some irreversible diseases. PCBs and cyclodienes are linked to liver toxicity. Organophosphates and carbonates can induce a chain of responses leading to neuromuscular blockage. Many chlorinated solvents induce liver changes, kidney changes and depression of the central nervous system. There is an entire spectrum of further health effects such as headache, nausea, fatigue, eye irritation and skin rash for the above cited and other chemicals. At sufficient dosages a large number of soil contaminants can cause death by exposure via direct contact, inhalation or ingestion of contaminants in groundwater contaminated through soil. The Scottish Government has commissioned the Institute of Occupational Medicine to undertake a review of methods to assess risk to human health from contaminated land. The overall aim of the project is to work up guidance that should be useful to Scottish Local Authorities in assessing whether sites represent a significant possibility of significant harm (SPOSH) to human health. It is envisaged that the output of the project will be a short document providing high level guidance on health risk assessment with reference to existing published guidance and methodologies that have been identified as being particularly relevant and helpful. The project will examine how policy guidelines have been developed for determining the acceptability of risks to human health and propose an approach for assessing what constitutes unacceptable risk in line with the criteria for SPOSH as defined in the legislation and the Scottish Statutory Guidance. Not unexpectedly, soil contaminants can have significant deleterious consequences for ecosystems. There are radical soil chemistry changes which can arise from the presence of many hazardous chemicals even at low concentration of the contaminant species. These changes can manifest in the alteration of metabolism of endemic microorganisms and arthropods resident in a given soil environment. The result can be virtual eradication of some of the primary food chain, which in turn could have major consequences for predator or consumer species. Even if the chemical effect on lower life forms is small, the lower pyramid levels of the food chain may ingest alien chemicals, which normally become more concentrated for each consuming rung of the food chain. Many of these effects are now well known, such as the concentration of persistent DDT materials for avian consumers, leading to weakening of egg shells, increased chick mortality and potential extinction of species. Effects occur to agricultural lands which have certain types of soil contamination. Contaminants typically alter plant metabolism, often causing a reduction in crop yields. This has a secondary effect upon soil conservation, since the languishing crops cannot shield the Earth's soil from erosion. Some of these chemical contaminants have long half-lives and in other cases derivative chemicals are formed from decay of primary soil contaminants. Cleanup or environmental remediation is analyzed by environmental scientists who utilize field measurement of soil chemicals and also apply computer models (GIS in Environmental Contamination) for analyzing transport and fate of soil chemicals. Various technologies have been developed for remediation of oil-contaminated soil and sediments. There are several principal strategies for remediation: Excavate soil and take it to a disposal site away from ready pathways for human or sensitive ecosystem contact. This technique also applies to dredging of bay muds containing toxins. Ecosystem effects Cleanup options Aeration of soils at the contaminated site (with attendant risk of creating air pollution) Thermal remediation by introduction of heat to raise subsurface temperatures sufficiently high to volatize chemical contaminants out of the soil for vapor extraction. Technologies include ISTD, electrical resistance heating (ERH), and ET-DSP. Bioremediation, involving microbial digestion of certain organic chemicals. Techniques used in bioremediation include landfarming, biostimulation and bioaugmentating soil biota with commercially available microflora. Extraction of groundwater or soil vapor with an active electromechanical system, with subsequent stripping of the contaminants from the extract. Containment of the soil contaminants (such as by capping or paving over in place). Phytoremediation, or using plants (such as willow) to extract heavy metals. Myco-remediation, or using fungus to metabolize contaminants and accumulate heavy metals. Remediation of oil contaminated sediments with self-collapsing air microbubbles. Surfactant leaching Various national standards for concentrations of particular contaminants include the United States EPA Region 9

Preliminary Remediation Goals (U.S. PRGs), the U.S. EPA Region 3 Risk Based Concentrations (U.S. EPA RBCs) and National Environment Protection Council of Australia Guideline on Investigation Levels in Soil and Groundwater. The immense and sustained growth of the People's Republic of China since the 1970s has exacted a price from the land in increased soil pollution. The State Environmental Protection Administration believes it to be a threat to the environment, to food safety and to sustainable agriculture. According to a scientific sampling, 150 million mu (100,000 square kilometres) of China's cultivated land have been polluted, with contaminated water being used to irrigate a further 32.5 million mu (21,670 square kilometres) and another 2 million mu (1,300 square kilometres) covered or destroyed by solid waste. In total, the area accounts for one-tenth of China's cultivatable land, and is mostly in economically developed areas. An estimated 12 million tonnes of grain are contaminated by heavy metals every year, causing direct losses of 20 billion yuan ($2.57 billion USD). According to the received data from Member states, in the European Union the number of estimated potential contaminated sites is more than 2.5 million and the identified contaminated sites around 342 thousand. Municipal and industrial wastes contribute most to soil contamination (38%), followed by the industrial/commercial sector (34%). Mineral oil and heavy metals are the main contaminants contributing around 60% to soil contamination. In terms of budget, the management of contaminated sites is estimated to cost around 6 billion Euros (€) annually. Generic guidance commonly used in the United Kingdom are the Soil Guideline Values published by the Department for Environment, Food and Rural Affairs (DEFRA) and the Environment Agency. These are screening values that demonstrate the minimal acceptable level of a substance. Above this there can be no assurances in terms of significant risk of harm to human health. These have been derived using the Contaminated Land Exposure Assessment Model (CLEA UK). Certain input parameters such as Health Criteria Values, age and land use are fed into CLEA UK to obtain a probabilistic output. Guidance by the Inter Departmental Committee for the Redevelopment of Contaminated Land (ICRCL) has been formally withdrawn by DEFRA, for use as a prescriptive document to determine the potential need for remediation or further assessment. By country People's Republic of China European Union United Kingdom the CLEA model published by DEFRA and the Environment Agency (EA) in March 2002 sets a framework for the appropriate assessment of risks to human health from contaminated land, as required by Part IIA of the Environmental Protection Act 1990. As part of this framework, generic Soil Guideline Values (SGVs) have currently been derived for ten contaminants to be used as "intervention values". These values should not be considered as remedial targets but values above which further detailed assessment should be considered; see Dutch standards. Three sets of CLEA SGVs have been produced for three different land uses, namely residential (with and without plant uptake) allotments commercial/industrial It is intended that the SGVs replace the former ICRCL values. The CLEA SGVs relate to assessing chronic (long term) risks to human health and do not apply to the protection of ground workers during construction, or other potential receptors such as groundwater, buildings, plants or other ecosystems. The CLEA SGVs are not directly applicable to a site completely covered in hard standing, as there is no direct exposure route to contaminated soils. To date, the first ten of fifty-five contaminant SGVs have been published, for the following: arsenic, cadmium, chromium, lead, inorganic mercury, nickel, selenium ethyl benzene, phenol and toluene. Draft SGVs for benzene, naphthalene and xylene have been produced but their publication is on hold. Toxicological data (Tox) has been published for each of these contaminants as well as for benzo[a]pyrene, benzene, dioxins, furans and dioxin-like PCBs, naphthalene, vinyl chloride, 1,1,2,2 tetrachloroethane and 1,1,1,2 tetrachloroethane, 1,1,1 trichloroethane, tetrachloroethene, carbon tetrachloride, 1,2-dichloroethane, trichloroethene and xylene. The SGVs for ethyl benzene, phenol and toluene are dependent on the soil organic matter (SOM) content (which can be calculated from the total organic carbon (TOC) content). As an initial screen the SGVs for 1% SOM are considered to be appropriate. In March 2009, the issue of Uranium poisoning in Punjab attracted press coverage. It was alleged to be caused by fly ash ponds of thermal power stations, which reportedly lead to severe birth defects in children in the Faridkot and Bhatinda districts of Punjab. The news reports claimed the uranium levels were more than 60 times the maximum safe limit. In 2012, the Government of India confirmed that the ground water in Malwa belt of Punjab has uranium metal that is 50% above the trace limits set by the United Nations' World Health Organization (WHO). Scientific studies, based on over 1000 samples from various sampling points, could not trace the source to fly ash and any sources from thermal power plants or industry as originally alleged. The study also revealed that the uranium concentration in ground water of Malwa district is not 60 times the WHO limits, but only 50% above the WHO limit in 3 locations. This highest concentration found in samples was less than those found naturally in ground waters currently used for human purposes elsewhere, such as Finland. Research is underway to identify natural or other sources for the uranium.

Soil Pollution has gradually become a major challenge that we need to overcome for establishing a healthy environment. Weathering of earth's crusts by different processes leads to the formation of soil that accumulates

over the centuries. The soil is the home for a large part of bacterial biodiversity and other microscopic and macroscopic living organisms.

Example of Soil Pollution

However, let us consider our very own country India. Indian economy is largely dependent on agriculture. Thus, we Indians give very high priority to the development of agriculture, fisheries, and livestock. Therefore, for surplus production, it is very important to protect crops from any type of damage that occurs due to insects, weeds, rodents and other crop diseases.

So, how do we protect crops? The very obvious answer is pesticides and herbicides. However, do you know these pesticides and herbicides is a leading cause of soil pollution? Therefore, it is very important to judiciously use pesticides because it contains lots of different harmful chemicals. Therefore, to improve soil and prevent soil pollution it is important to limit the use of pesticides and herbicides.

Causes of Soil Erosion

Definition of Soil Pollution

Soil pollution refers to anything that causes contamination of soil and degrades the soil quality. It occurs when the pollutants causing the pollution reduce the quality of the soil and convert the soil inhabitable for microorganisms and macro organisms living in the soil.

Soil contamination or soil pollution can occur either because of human activities or because of natural processes. However, mostly it is due to human activities. The soil contamination can occur due to the presence of chemicals such as pesticides, herbicides, ammonia, petroleum hydrocarbons, lead, nitrate, mercury, naphthalene, etc in an excess amount.

The primary cause of soil pollution is a lack of awareness in general people. Thus, due to many different human activities such as overuse of pesticides the soil will lose its fertility. Moreover, the presence of excess chemicals will increase the alkalinity or acidity of soil thus degrading the soil quality. This will in turn cause soil erosion. This soil erosion refers to soil pollution.

Causes of Soil Pollution

Soil pollution can be natural or due to human activity. However, it mostly boils down to the activities of the human that causes the majority of soil pollution such as heavy industries, or pesticides in agriculture.

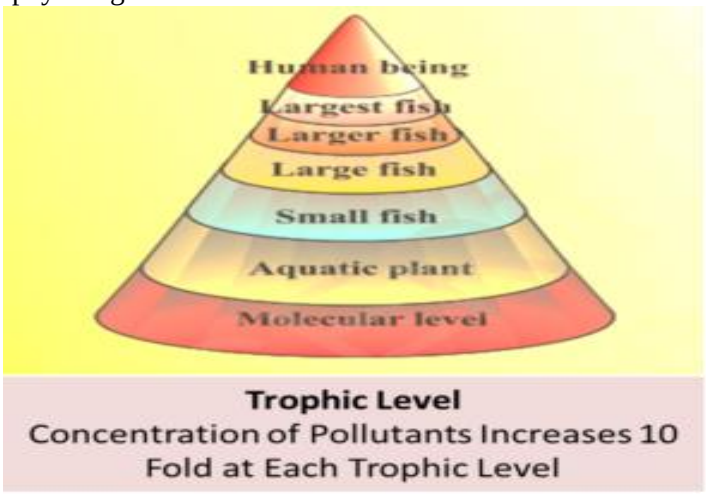
Pesticides

Before World War II, the chemical nicotine chemical present in the tobacco plants was used as the pest controlling substance in agricultural practices. However, DDT was found to be extremely useful for malaria control and as pest control of many insects during World War II. Therefore, it was used for controlling many diseases.

Hence, post-war, people started using it as pest control in agriculture for killing rodents, weeds, insects, etc and avoiding the damages due to these pests. However, everyone gradually the adverse effects of this chemical which led to the ban of this chemical in many parts of the world including India.

Moreover, pests became resistance to DDT due to the chemicals regular use. Hence this led to the introduction of other harmful chemicals such as Aldrin and Dieldrin. Pesticides are synthetic toxic chemicals that definitely kill

different types of pests and insects causing damage to agriculture but it has many ecological repercussions.
They are generally insoluble in water and non-biodegradable. Therefore, these chemicals will not gradually decompose and keep on accumulating in the soil. Therefore, the concentration of these chemicals will increase when the transfer of these chemicals take place from lower to higher trophic level via the food chain. Hence, it will cause many metabolic and physiological disorders in humans.



Chlorinated Organic toxins
The harmful effect of DDT and other chemicals led to the introduction of less persistent organic and more-biodegradable substance such as carbamates and organophosphates. However, these chemicals act as harmful toxins for nerves, hence they are more dangerous to humans. It led to pesticides related to the death of field workers in some agricultural fields.

Causes and Effects of Water Pollution

Herbicides
Slowly, the industries began production of herbicides like sodium arsenite (Na3AsO3), sodium chlorate (NaClO3), etc. Herbicides can decompose in a span of few months. However, even they affect the environment and are not environmental friendly. Even though they are not as harmful as organo-chlorides but most of the herbicides are toxic. They are known to cause birth defects.
Furthermore, research suggests that spraying herbicides causes more insect attack and diseases of plants in comparison to manual weeding. One thing to note here is all the above factors occupy just a small portion of the causes. Majority of the causes is related to manufacturing activities in chemical and industrial processes that are released in nature or environment.

Inorganic Fertilizers
Excessive use of inorganic nitrogen fertilizers leads to acidification of soil and contaminate the agricultural soil. Also known as agrochemical pollution.

Industrial Pollution
The incorrect way of chemical waste disposal from different types of industries can cause contamination of soil. Human activities like this have led to acidification of soil and contamination due to the disposal of industrial waste, heavy metals, toxic chemicals, dumping oil and fuel, etc.

Inferior Irrigation Practices
Poor irrigation methods increase the soil salinity. Moreover, excess watering, improper maintenance of canals and irrigation channels, lack of crop rotation and intensive farming gradually decreases the quality of soil over time and cause degradation of land.

Solid Waste
Disposal of plastics, cans, and other solid waste falls into the category of soil pollution. Disposal of electrical goods such as batteries causes an adverse effect on the soil due to the presence of harmful chemicals. For instance, lithium present in batteries can cause leaching of soil.

Urban Activities
Lack of proper waste disposal, regular constructions can cause excessive damage to the soil due to lack of proper drainage and surface run-off. These waste disposed of by humans contain chemical waste from residential areas. Moreover leaking of sewerage system can also affect soil quality and cause soil pollution by changing the chemical composition of the soil.

After-Effects of Soil Pollution
Soil pollution is not only the problem in India but it is a global problem. It causes harmful effect on the soil and the

environment at large. Contamination of soil will decrease the agricultural output of a land. Major soil pollution after effects are:

Inferior Crop Quality

It can decrease the quality of the crop. Regular use of chemical fertilizers, inorganic fertilizers, pesticides will decrease the fertility of the soil at a rapid rate and alter the structure of the soil. This will lead to decrease in soil quality and poor quality of crops. Over the time the soil will become less productive due to the accumulation of toxic chemicals in large quantity.

Harmful Effect on Human Health

It will increase the exposure to toxic and harmful chemicals thus increasing health threats to people living nearby and on the degraded land. Living, working or playing in the contaminated soil can lead to respiratory diseases, skin diseases, and other diseases. Moreover, it can cause other health problems.

Water Sources Contamination

The surface run-off after raining will carry the polluted soil and enter into different water resource. Thus, it can cause underground water contamination thereby causing water pollution. This water after contamination is not fit for human as well as animal use due to the presence of toxic chemicals.

Negative Impact on Ecosystem and Biodiversity

Soil pollution can cause an imbalance of the ecosystem of the soil. The soil is an important habitat and is the house of different type of microorganisms, animals, reptiles, mammals, birds, and insects. Thus, soil pollution can negatively impact the lives of the living organisms and can result in the gradual death of many organisms. It can cause health threats to animals grazing in the contaminated soil or microorganisms residing in the soil.

Therefore, human activities are responsible for the majority of the soil pollution. We as humans buy things that are harmful and not necessary, use agricultural chemicals (fertilizers, pesticides, herbicides, etc.), drop waste here and there. Without being aware we harm our own environment.

Therefore, it is very important to educate people around you the importance of environment if they are not aware. Prevention of soil erosion will help to cease soil pollution. Thus, it is our small steps and activities that can help us to achieve a healthier planet for us. Therefore, it is essential for industries, individuals and businesses to understand the importance of soil and prevent soil pollution and stop the devastation caused to plant and animal life.

Control of Soil Pollution:

With the rapid pace of industrialization and increasing population density, numerous pollutants have posed a serious threat to living organisms. Extensive solid wastes and the use of biocides etc. have put the interest of agriculture and aqua-culture at cross purposes.

The various approaches to control soil pollution are as follows:

1. Production of Natural Fertilizers. Organic wastes contained in animal dung can be used for preparing compost manure and biogas rather than throwing them wastefully polluting the soil. The use of bio-pesticides should be encouraged in place of toxic pesticides.
2. Ban on Toxic Chemicals. Ban should be imposed on chemicals, biocides, pesticides which are fatal to plants and animals. Thus banning the use of highly toxic and resistant pesticides or regulating their use only for special purposes under thorough monitoring can control soil pollution.
3. Using biological weapons to control soil pollution.
4. Plantation and recycling of trees to prevent soil erosion. Other control measures are:
5. Launching of extensive afforestation and community forestry programmes.
6. Implementing deterrent measures against deforestation. Conservation of soil to prevent the loss of precious top soil by erosion and to maintain it in a fertile state for agricultural purposes.
7. Sponsoring more intensive R and D efforts on bio-fertilizers, microbial degradation of wastes, utilization of wastes by recovery, reusing and .recycling solid wastes, safer treatment and disposal of hazardous wastes.
8. Security land-fills have to be constructed for permanent disposal of hazardous and recalcitrant industrial wastes.
9. **Transforming intensive agriculture into a sustainable system by measures such as**
    (i) Maintaining a healthy soil community in order to regenerate soil fertility by providing organic manures, increasing fallow periods, avoiding excessive use of chemical fertilizers.
    (ii) Infusing bio-diversity in agriculture by sowing mixed crops and adopting crop rotation etc.
10. Effective treatment of domestic sewage by suitable biological and chemical methods and adopting modern techniques of sludge disposal.
11. Municipal wastes have to be properly collected by segregation, treated and disposed scientifically. Recycling of glass, paper, and plastics should be done carefully.
12. Industrial wastes have to be properly treated at source, by segregation of wastes or adopting integrated waste treatment methods. Basic slag, the major byproduct of steel industries, can be used for making nitrophosphatic fertilizer.

13. Enforcing environment audit for industries and promoting eco labelled products.
14. Implementing stringent and pro-active population control programmes.
15. Formulation of stringent pollution control legislation and effective implementation with powerful administrative machinery.
16. Imparting informal and formal public awareness programmes to educate people at large regarding health hazards and undesirable effects due to environmental pollution. Mass media, educational institutions and voluntary agencies should be involved to achieve these objectives.
17. Extending market support for recoverable products through fiscal concessions.

Pesticides:

The following points highlight the three main types of bio-pesticides. The types are: 1. Bio-Insecticides 2. Bio-Nematicides 3. Bio-Herbicides.

Bio-Pesticide: Type # 1. Bio-Insecticides:

Microorganisms like bacteria, fungi and viruses show greatest commercial importance as bio- control agents than the commercial synthetic pesticides. With the more progress of scientific achievement the bio-insecticide becomes popular in different countries.

i) Bacterial:

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. The bacterium has been shown to be successful against a wide range of more than 1 50 insects.

It shows insecticidal activity against larvae of Lepidoptera. B. thuringiensis var. israelensis (BTI) is highly active against larvae of mosquito vectors of malaria. Unlike DDT, the pathogen is environmentally safe in use and mosquito does not show any resistance against the bacterium. It also shows excellent result to control black fly — the carrier of widespread river blindness in Africa.

Four different toxins are produced by the B. thuringiensis and about 16 formulations have been prepared based on the above toxins. These are used in different countries like USA, Russia, France, Germany etc. Some of the registered products like Sporcine, Condor, Cutlass, Thuricide, Foil etc., are commonly used.

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 sugarcane. In USA, different registered formulations prepared from B. thuringiensis are used to control pests of different crops like Alfalfa caterpillar (Alfalfa), Bollworm (Cotton), Cabbage worm (Cabbage and Cauliflower). Orange dog (Orange), Crape leaf folder (Grapes) etc.

With the help of recombinant DNA technology, 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. Later, similar success has been achieved in different crop plants like tobacco, cotton etc., by using the similar technique. Now-a-days, Bt cotton is very popular among the farmers.

ii) Fungal:

Different entomogenous fungi have been used to protect different crops from insect damage. Species of the different genera like Aschersonia, Beauveria, Coelomomyces, Entomophthora, Hirsutella etc., are in common use. Some registered products are also produced and used by different countries like Australia, Brazil, China, France, Japan, USA, UK etc.

List of some of the products, producing fungi in parenthesis and insects on which it acts are:

i. Aseronija (Aschersonia aleyrodis) — Whitefly of many crops.

ii. Boverin and Boverol (Beauveria brassiana) — Pine caterpillar, Green leaf hipper, Colorado potato beetle etc.

iii. Mycotal and Vertalec (Verticillium lecanii) — Whitefly and Aphids of glasshouse crop.

iii) Viral:

Viruses are also very much effectively used as bioinsecticide. There are three major groups of viruses that can infect different insects.

These are:

(a) Nuclear polyhedrosis viruses (NPV),

(b) Granulosis viruses (GV) of Baculoviridae, and

(c) Cytoplasmic polyhedrosis viruses (CPV) of Reoviridae.

The NPVs are effective against moths and butterflies, while CPVs are effective mainly on caterpillars. These are used in different countries like USA, UK, Canada, lapan, Germany etc.

List if some viruses, registered trade names in parenthesis and target insects are given below:

i. NPV (ELCAR) — Tobacco budworm and Cotton bollworm.
ii. NPV (GYPCHEK) — Gypsy moth.
iii. NPV (VfROX) — European sawfly.
iv. CPV (MATSUKEMIN) — Pine caterpillar.
v. GV (MATEX) — Insects of different food crops like Codling moths.

Bio-Pesticide: Type # 2. 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 adhesive branch which may stick with the body of nematode on accidental contact.

iv) Formation of hyphal mesh:

The mycelium 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.

Bio-Pesticide: Type # 3. 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.

Possible Harmful Effects:

Increased, indiscriminate and uncontrolled use of pesticides and other agricultural chemicals for increased crop production has polluted soil, air and water. Acharjyo (1996) quoted an assessment wherein 25% of the estimated 34,000 tonnes of pesticides annually used in India find their way to sea.

DDT for example, enters into food chain through meat food animals, fish, predators of crop eating pests (rats, frogs, reptiles, insects etc.), birds and other omnivorous or scavenging animals. A general sketch of translocation of these chemicals through plant and animal tissues to human beings is presented.

Acharjyo (1996) himself attended to fatal poisoning in free living elephants who had consumed paddy crop sprayed with insecticide. The harmful effects on human physiology are varied in nature depending upon chemical and degree of exposure.

These are:

1. Carcinogenic
2. Mutagenic
3. Teratogenic
4. Allergy
5. Acute Toxicity or Poisoning.

1. Carcinogenic Effect:

A substance that produces cancer is called Carcinogen and its cancer producing activity is called as Carcinogenic effect. The toxic dose of a Carcinogen is quite higher than the Carcinogenic dose. In food producing animals treated with or fed Carcinogenic compound, no residues are permitted in the edible tissue at the time of slaughter (e.g. di-ethyl- stilbestrtol).

The carcinogenic molecule interact with various intra-cellar components like protein, DNA, RNA, glycogen, phospholipids and glutathione and form a chemical receptor complex called adduct. The reaction is irreversible and therefore, the dissociation of the adduct to the free chemical and free receptor does not occur.

This leads to damage of the cellular component such as DNA which is permanent in nature. A cell has a natural capability to remove a carcinogen bound to DNA at the rate of approximately 1 x 105carcinogen molecule/cell/12 hours.

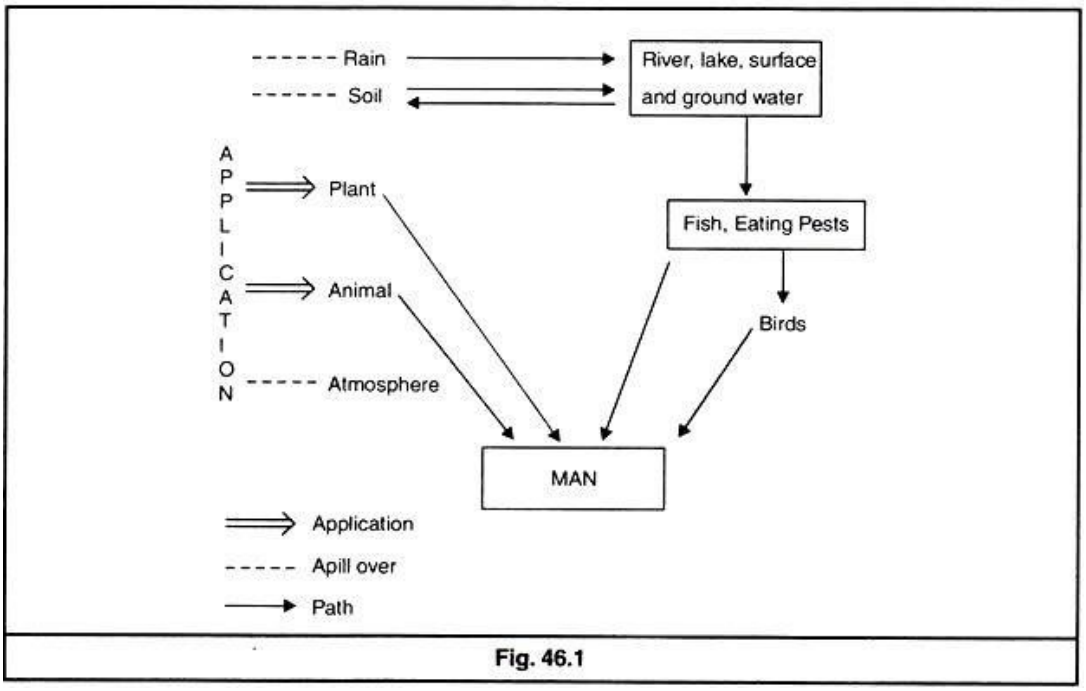


Fig. 46.1

It has been observed that the radiolabeled 2-acetyl-amino-fluorine and azo dyes remained in hepatic DNA and glycogen for months. Therefore, the persistent covalently bound drug residue in food products would constitute a health hazard. For veterinary drugs the drug residue in meat, milk and eggs should not be more than 1 ppb or 1ppt.

2. Mutagenic Effect:

The chemical agents which damage the genetic component of a cell is called as mutagen and its activity is mutagenic effects. The mutagenic affect shown by a chemical substance is either by gene mutation or by chromosomal aberration.

A mutagen can cause three types of genetic injury of germinal or somatic cells:

1. Point mutation
2. Gene elimination
3. Chromosomal breakage.

The genetic mutagenesis is more important because it is hazardous to future generation while the somatic mutagenesis may lead to aging which is due to accumulation of damage DNA. The polycyclic hydrocarbon, natural or synthetic, have mutagenic and carcinogenic properties.

3. Teratogenic Effect:

The drug or chemical agents which produce a toxic effect on the embryo or fetus during gestation is called as teratogen. This lead to congenital malformation. The drug thalidomide is a well-known teratogen and causes phocomelia or 'seal limb' in children.

The feed containing methallibure an anterior pituitary inhibitor to control oestrus cycle in swine, results into congenital deformity in new born piglets. A number of compound derived from plant have teratogenic effect in animals.

4. Drug Allergy or Hypersensitivity:

Allergic reactions to drugs or chemicals are quite similar to the allergic responses to protein, carbohydrate and lipid and may include anaphylaxis, serum sickness, cutaneous reactions and delayed hypersensitive response. In humans, allergic or hypersensitive response to drugs appears to be more commonly associated with antibiotics, especially penicillin. However, its allergic response to animal is not well known.

5. Acute Toxicity:

A. Organophosphate:

According to a study in California, 75% of systemic poisoning with agricultural chemicals in human beings is due to phosphate esters.

For all organophosphate esters the approximate residual range is 0.003 ppm.

Symptoms in Animals:

The symptoms in animals after intoxication with organophosphate esters is due to inhibition of cholinesterases (these esterases hydrolyze acetylcholine, butyrylcholine, benzoyl choline, acetyl-B-methyl choline etc. depending on the species). This reflects stimulation of the autonomic and central nervous systems and subsequent accumulation of acetylcholine.

The prolongation and intensification of acetylcholine action results in two degree of response. The initial action in on smooth muscles, cardiac muscle and exocrine glands and is quite comparable to stimulation of the post ganglionic parasympathetic nerve.
This early signs of toxicity resembles with those of muscarine and hence this phase is also known as muscarinic action of acetylcholine and most common signs are intestinal cramp,
tightness in the chest, blurred vision, headache, diarrhoea, decrease in blood pressure and salivation. This action can be counteracted by atropin.
The second phase results from stimulation of the peripheral motor system and all autonomic ganglia. This phase resembles with the classical action of nicotine, therefore, it is also known as nicotinic action of acetylcholine.
The complexity of toxic action during the second stage includes neuromuscular and ganglionic blockage. The ultimate effect is stimulation and/or paralysis of the somatic, autonomic and central nervous system. Sings of toxicity depends upon the toxicant, vehicle, route of administration dosage etc.
Gross Pathology in Animals:
This includes demyelination of peripheral nerves, Anterior horn cell degeneration, and fatty degeneration of spinal cord white matter. This results in a syndrome resembling “Jake leg” or “ginger jake” paralysis.
Prognosis:
It depends upon the exposure, type of compound and adequacy of treatment.

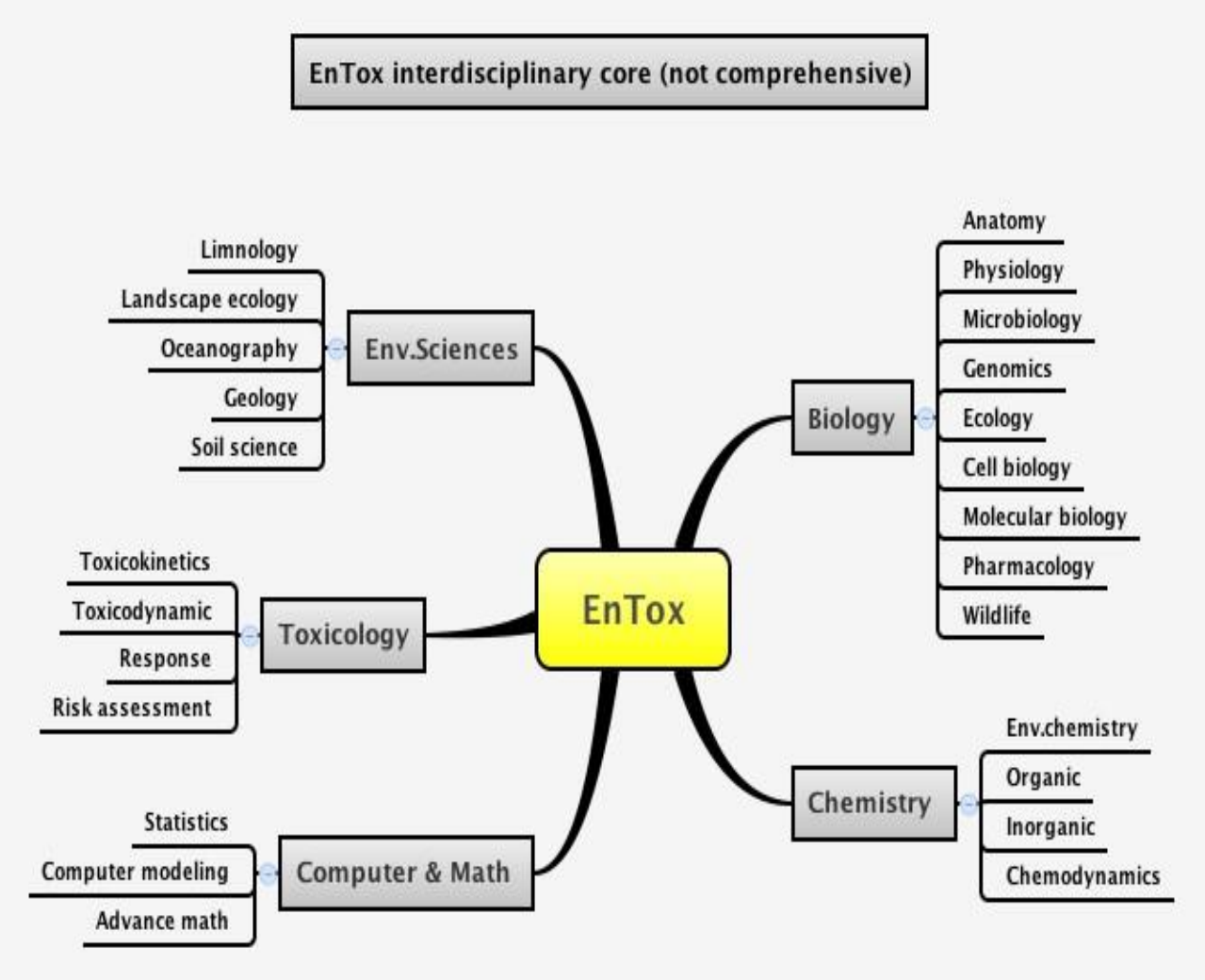
8. Environmental toxicology - Principles and Mechanisms

Environmental toxicology
Environmental toxicology is a multidisciplinary field of science concerned with the study of the harmful effects of various chemical, biological and physical agents on living organisms. Ecotoxicology is a subdiscipline of environmental toxicology concerned with studying the harmful effects of toxicants at the population and ecosystem levels.

Rachel Carson is considered the mother of environmental toxicology, as she made it a distinct field within toxicology in 1962 with the publication of her book Silent Spring, which covered the effects of uncontrolled pesticide use. Carson's book was based extensively on a series of reports by Lucille Farrier Stickel on the ecological effects of the pesticide DDT.

Organisms can be exposed to various kinds of toxicants at any life cycle stage, some of which are more sensitive than others. Toxicity can also vary with the organism's placement within its food web. Bioaccumulation occurs when an organism stores toxicants in fatty tissues, which may eventually establish a trophic cascade and the biomagnification of specific toxicants. Biodegradation releases carbon dioxide and water as by-products into the environment. This process is typically limited in areas affected by environmental toxicants.

Harmful effects of such chemical and biological agents as toxicants from pollutants, insecticides, pesticides, and fertilizers can affect an organism and its community by reducing its species diversity and abundance. Such changes in population dynamics affect the ecosystem by reducing its productivity and stability.
Although legislation implemented since the early 1970s had intended to minimize harmful effects of environmental toxicants upon all species, McCarty (2013) has warned that "longstanding limitations in the implementation of the simple conceptual model that is the basis of current aquatic toxicity testing protocols" may lead to an impending environmental toxicology "dark age"



Overview of the interdisciplinarity of environmental toxicology

9. Environmental Laws and Policies.

Environmental laws around the world International law

Global and regional environmental issues are increasingly the subject of international law. Debates over environmental concerns implicate core principles of international law and have been the subject of numerous international agreements and declarations.

Customary international law is an important source of international environmental law. These are the norms and rules that countries follow as a matter of custom and they are so prevalent that they bind all states in the world. When a principle becomes customary law is not clear cut and many arguments are put forward by states not wishing to be bound. Examples of customary international law relevant to the environment include the duty to warn other states promptly about icons of an environmental nature and environmental damages to which another state or states may be exposed, and Principle 21 of the Stockholm Declaration ('good neighbourliness' or sicutere).

Numerous legally binding international agreements encompass a wide variety of issue-areas, from terrestrial, marine and atmospheric pollution through to wildlife and biodiversity protection. International environmental agreements are generally multilateral (or sometimes bilateral) treaties (a.k.a. convention, agreement, protocol,

etc.). Protocols are subsidiary agreements built from a primary treaty. They exist in many areas of international law but are especially useful in the environmental field, where they may be used to regularly incorporate recent scientific knowledge. They also permit countries to reach agreement on a framework that would be contentious if every detail were to be agreed upon in advance. The most widely known protocol in international environmental law is the Kyoto Protocol, which followed from the United Nations Framework Convention on Climate Change.

While the bodies that proposed, argued, agreed upon and ultimately adopted existing international agreements vary according to each agreement, certain conferences, including 1972's United Nations Conference on the Human Environment, 1983's World Commission on Environment and Development, 1992's United Nations Conference on Environment and Development and 2002's World Summit on Sustainable Development have been particularly important. Multilateral environmental agreements sometimes create an International Organization, Institution or Body responsible for implementing the agreement. Major examples are the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and the International Union for Conservation of Nature (IUCN).

International environmental law also includes the opinions of international courts and tribunals. While there are few and they have limited authority, the decisions carry much weight with legal commentators and are quite influential on the development of international environmental law. One of the biggest challenges in international decisions is to determine an adequate compensation for environmental damages. The courts include the International Court of Justice (ICJ), the international Tribunal for the Law of the Sea (ITLOS), the European Court of Justice, European Court of Human Rights and other regional treaty tribunals.

Asia

The Asian Environmental Compliance and Enforcement Network (AECEN) is an agreement between 16 Asian countries dedicated to improving cooperation with environmental laws in Asia. These countries include Cambodia, China, Indonesia, India, Maldives, Japan, Korea,

Malaysia, Nepal, Philippines, Pakistan, Singapore, Sri Lanka, Thailand, Vietnam, and Lao PDR.

European Union

The European Union issues secondary legislation on environmental issues that are valid throughout the EU (so called regulations) and many directives that must be implemented into national legislation from the 28 member states (national states). Examples are the Regulation (EC) No. 338/97 on the implementation of CITES; or the Natura 2000 network the centerpiece for nature & biodiversity policy, encompassing the bird Directive (79/409/EEC/ changed to 2009/147/EC)and the habitats directive (92/43/EEC). Which are made up of multiple SACs (Special Areas of Conservation, linked to the habitats directive) & SPAs (Special Protected Areas, linked to the bird directive), throughout Europe.

EU legislation is ruled in Article 249 Treaty for the Functioning of the European Union (TFEU). Topics for common EU legislation are:

- Climate change
- Air pollution
- Water protection and management
- Waste management
- Soil protection
- Protection of nature, species and biodiversity
- Noise pollution
- Cooperation for the environment with third countries (other than EU member states)
- Civil protection

Middle East

The U.S. Environmental Protection Agency is working with countries in the Middle East to improve "environmental governance, water pollution and water security, clean fuels and vehicles, public participation, and pollution prevention."

Australia

The Environment Protection and Biodiversity Conservation Act 1999 is the center piece of environmental legislation in the Australian Government. It sets up the "legal framework to protect and manage nationally and

internationally important flora, fauna, ecological communities and heritage places". It also focuses on protecting world heritage properties, national heritage properties, wetlands of international importance, nationally threatened species and ecological communities, migratory species, Commonwealth marine areas, Great Barrier Reef Marine Park, and the environment surrounding nuclear activities. Commonwealth v Tasmania (1983), also known as the "Tasmanian Dam Case", is the most influential case for Australian environmental law.

Brazil

The Brazilian government created the Ministry of Environment in 1992 in order to develop better strategies of protecting the environment, use natural resources sustainably, and enforce public environmental policies. The Ministry of Environment has authority over policies involving environment, water resources, preservation, and environmental programs involving the Amazon.

China

Ministry of Environmental Protection of the People's Republic of China

According to the U.S. Environmental Protection Agency, "China has been working with great determination in recent years to develop, implement, and enforce a solid environmental law framework. Chinese officials face critical challenges in effectively implementing the laws, clarifying the roles of their national and provincial governments, and strengthening the operation of their legal system." Explosive economic and industrial growth in China has led to significant environmental degradation, and China is currently in the process of developing more stringent legal controls. The harmonization of Chinese society and the natural environment is billed as a rising policy priority.

India:

Indian environmental law

In India, Environmental law is governed by the Environment Protection Act, 1986. This act is enforced by the Central Pollution Control Board and the numerous State Pollution Control Boards. Apart from this, there are also individual legislations specifically enacted for the protection of Water, Air, Wildlife, etc. Such legislations include :-

- The Water (Prevention and Control of Pollution) Act, 1974
- The Water (Prevention and Control of Pollution) Cess Act, 1977
- The Forest (Conservation) Act, 1980
- The Air (Prevention and Control of Pollution) Act, 1981
- Air (Prevention and Control of Pollution) (Union Territories) Rules, 1983
- The Biological Diversity Act, 2002 and the Wild Life Protection Act, 1972
- Batteries (Management and Handling) Rules, 2001
- Recycled Plastics, Plastics Manufacture and Usage Rules, 1999
- The National Green Tribunal established under the National Green Tribunal Act of 2010 has jurisdiction over all environmental cases dealing with a substantial environmental question and acts covered under the Water (Prevention and Control of Pollution) Act, 1974.
- Water (Prevention and Control of Pollution) Cess Rules, 1978
- Ganga Action Plan, 1986
- The Forest (Conservation) Act, 1980
- The Public Liability Insurance Act, 1991 and the Biological Diversity Act, 2002. The acts covered under Indian Wild Life Protection Act 1972 do not fall within the jurisdiction of the National Green Tribunal. Appeals can be filed in the Hon'ble Supreme Court of India.
- Basel Convention on Control of Transboundary Movements on Hazardous Wastes and Their Disposal, 1989 and Its Protocols
- Hazardous Wastes (Management and Handling) Amendment Rules, 2003

Japan

The Basic Environmental Law is the basic structure of Japan's environmental policies replacing the Basic Law for Environmental Pollution Control and the Nature Conservation Law. The updated law aims to address "global environmental problems, urban pollution by everyday life, loss of accessible natural environment in urban areas and degrading environmental protection capacity in forests and farmlands."

Russia

The Ministry of Natural Resources and Environment of the Russian Federation makes regulation regarding "conservation of natural resources, including the subsoil, water bodies, forests located in designated conservation areas, fauna and their habitat, in the field of hunting, hydrometeorology and related areas,

environmental monitoring and pollution control, including radiation monitoring and control, and functions of public environmental policy making and implementation and statutory regulation."

10. Suggested Readings

1. Jones Allan M. Environmental Biology (1997) by Routledge New Fetter Lane, London EC4P 4EE2.
2. Shukla, R.S. & Chandel, P.S. Plant Ecology, Latest Ed., S. Chandel and Co.
3. Dhaliwal, G. S., Sngha, G. S. and Ralhan, P. K. 1998. Fundamentals of Environmental Science. Kalyani Publishers.
4. Kaushik, A. and Kaushik, C. P. 2014. Perspective in environmental studies. New Age International Ltd. Publishers, New Delhi.
5. https://www.wikipedia.org/
6. http://www.biologydiscussion.com/
7. Ecology Global insights and Investigation by Peter Stiling

11. Assignment

1. Define ecosystem and ecology.
2. What are food chain and food web?
3. Describe carbon cycle with diagram.
4. Describe nitrogen cycle with diagram.
5. Discuss different laws for the protection of environment.
6. Define water pollution. State its cause and effects.
7. Define air pollution. State its cause and effects
8. Discuss the laws and policies in environmental pollution
9. Montreal protocol
10. Xenobiotics

All the materials are self written and collected from ebook, journals and websites.

POST GRADUATE DEGREE PROGRAMME (CBCS)
IN
BOTANY

SEMESTER - III

Course: BOSCT 3.2

(Forensic Botany)

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING
UNIVERSITY OF KALYANI
KALYANI – 741 235,
WEST BENGAL

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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 UGC Regulations, 2017 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.) Sankar Kumar Ghosh, 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.

Prof Manas Mohan Adhikary
Director
Directorate of Open and Distance Learning
University of Kalyani

SYLLABUS
COURSE – BOSCT 3.2
Forensic Botany
(Full Marks – 50)

Course	Group	Details Contents Structure		Study hour
BOSCT3.2	Forensic Botany	**Unit 1. Introduction of forensic botany**	1. Introduction: Introduction to forensic botany	1
		Unit 2. Collection and preservation of botanical evidences	2. Collection and preservation of botanical evidences: Botanical samples, outdoor crime scene consideration	1
		Unit 3. . Analysis of samples I	3 Analysis of samples I: Plant anatomy; pollen analysis; DNA analysis; plant DNA typing	1
		Unit 4. . Analysis of samples II	4. Analysis of samples II: DNA analysis; plant DNA typing	1
		Unit 5. Classic forensic botany cases I	5. Classic forensic botany cases I: Case histories by using Plant anatomy and systematic, Palynology, Plant ecology	1
		Unit 6. Classic forensic botany cases II	6. Classic forensic botany cases II: Case histories by using Limnology, Plant Molecular Biology and DNA, Drug enforcement and DNA.	1

Content

COURSE – BOSCT 3.2

(Forensic Botany)

Soft Core Theory Paper **Credit = 2**

Content Structure

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1. Introduction
2. Course Objectives
3. Introduction: Introduction to forensic botany
4. Collection and preservation of botanical evidences: Botanical samples, outdoor crime scene consideration
5. Analysis of samples: Plant anatomy; pollen analysis; DNA analysis; plant DNA typing.
6. Classic forensic botany cases: Case histories by using Plant anatomy and systematic, Palynology, Plant ecology, Limnology, Plant Molecular Biology and DNA, Drug enforcement and DNA.
7. Let's sum up
8. Suggested Reading
9. Assignment

1. Introduction

Forensic science is the application of sciences such as physics, chemistry, biology, computer science and engineering to matters of law. Forensic science plays a vital role in the criminal justice system by providing scientifically based information through the analysis of physical evidence. During an investigation, evidence is collected at a crime scene or from a person, analyzed in a crime laboratory and then the results presented in court. Each crime scene is unique, and each case presents its own challenges. Over the course of a Forensic Science degree you'll study a wide range of aspects of the discipline.

2. Course Objectives

You should gather knowledge after studying the course:

- Understand, from a legal standpoint, the importance of properly securing a crime scene
- Classify the various types of biological evidence that may be obtained at a crime scene Use technology to perform descriptive and inferential data analysis for one or two variables.
- Gather information about outdoor crime scence.
- Analyze the biological samples in legal proceedings
- Describe DNA typing and Drug enforcement.
- Understand different case histories by using Plant anatomy and systematics, Palynology, Plant ecology, Limnology, Molecular biology and DNA.

3. Introduction: Introduction of forensic botany

"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 to matters 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.

- Application of botany in law enforcement i.e. scientific use of plant materials to solve crimes
- 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.
- 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
- These forms of physical evidences can be sometimes traced to an individual suspect
- 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).
- 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.

"Matters of Law" include:

- **Crimes**
  - Homicide, sexual assault, burglary, etc.
- **Disputes among individuals**
  - Wrongful death, patents, etc.
- **Establishing rights**
  - Parentage; immigration, land disputes
- **Investigation of disasters**
  - Natural and man-made

CIVIL LAW	CRIMINAL LAW

• **case filed by a private party**
  ○ **a corporation**
  ○ **an individual**
• **Penalty:**
a guilty defendant pays the plaintiff *(a person who brings a case against another in a court of law)* for losses caused by their actions.
  ○ no incarceration
	• **case filed by the government**
	• **Penalty:**
	a guilty defendant is punished by
	  ○ incarceration (in jail/prison)
	  ○ fine paid to the gov't
	  ○ execution (death penalty)

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 hairs on 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 abundant types 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, or trunk
- 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.

4. Collection and preservation of botanical evidences: Botanical samples, outdoor crime scene consideration

General Biological Samples

Today, the primary tool for individualizing biological evidence is DNA analysis. However, one of the more effective challenges to DNA involves attacking the integrity of the sample before it arrives at the forensic laboratory. Indeed, the ability to introduce DNA findings in court has been adversely affected by challenging evidence collection and preservation procedures. Detailed evidence collection protocols have been well described in a variety of textbooks. The specific collection method employed will depend on the state and condition of the biological evidence. In general, an investigator should collect a significant quantity of material to ensure recovery of sufficient DNA for testing purposes. However, it is important to limit collecting additional dirt, grease, fluids, etc. from the surrounding area since many substances are known to adversely affect forensic analysis methods such as the DNA typing process. Carefully collect each item to minimize contamination using disposable gloves, forceps, sterile swabs and scalpels, particle masks, and so forth as appropriate for the sample. Package each item of evidence separately according to established forensic practices to minimize cross-contamination. The evidentiary value may be seriously compromised if several items are packaged together. In addition, biological samples such as wet bloodstains should be packaged in sealed paper bags, not in airtight containers, which retain moisture and promote bacterial growth. It is useful to place any moistened swabs in a specially designed cardboard swab collection box that facilitates sample drying during transport. Once the samples have been collected and packaged, they should be promptly delivered to the forensic laboratory. To minimize specimen deterioration, items should be stored in a cool, dry environment until they are submitted for testing.

Botanical Samples

The collection of plant fragments, seeds, flowers, and fruits should all be performed by hand. Whole plants and any fragments that may potentially be useful for a physical match should be collected as well as any pieces associated with a body. Botanical fragments in and on motor vehicles should be collected; in particular, the wheel wells, in and under floor mats, the undercarriage, pedals, windshield wipers, vents, trunk, and engine compartments should be fully examined. Care should be taken to avoid vacuuming a vehicle for microscopic trace evidence until all plant fragments have been collected by hand. Botanical evidence should be collected in paper, not plastic, when possible. Plant matter in stomach contents would most likely be collected during autopsy by the medical examiner. Fecal matter deserves a special note on collection methods since it differs from most aspects of botanical collection. For fecal matter and later possible plant identification, the sample should be kept moist. If a fresh deposit, then the sample should be stored in glass or plastic. Dried feces or soiled clothing can be placed in paper bags and sealed. For microscopic analysis for plant cell identification, a 10% formalin solution as a preservative is acceptable. However, if plant DNA analysis may be performed, storing the sample in a freezer is optimal.

Outdoor Crime Scene Considerations

Outdoor crime scenes also warrant a special note. Investigator and technicians are often working under greater time pressure because, given certain weather conditions, some of the physical evidence can be altered or destroyed. Animals and insects can change the condition of the body, and the rate of sample decomposition can be great. The size or scope of the scene may be largeor somewhat undefined and hence challenging to secure. The media and the general public can interfere or impede the collection process.

A complete record of all crime scene personnel is essential. Elimination fingerprints, shoeprints, and DNA samples may need to be collected from scene technicians and other individuals to understand the significance of the physical evidence. For example, a cigarette butt recovered near the location of the crime could be used for DNA analysis. If the evidentiary DNA profile excludes the suspect, it could point to a different perpetrator. However, if a neighbor or a police officer was the source of a cigarette butt or shoeprint at the scene, a different conclusion could be more valid. Hence, it is vital that access to the scene be restricted to persons associated with the investigation.

Examination of a Body

The body of the victim/decedent must be thoroughly examined and documented prior to sample collection. Describe and sketch/photograph the location of any possible injury, trace evidence, or stains on the victim. Note potential bite marks, bruises, abrasions, and so forth. All trace evidence, including blood and body fluids, prints, soil, hairs and fibers, pollen, plant fragments, and stains, should be photographed in place prior to collection and packaging. Body orifices should be examined for semen and other body fluids, hairs and fibers, and other trace materials. It is important to collect the evidence when possible prior to moving the body. Physical evidence has been lost or altered when the body is placed in a body bag and the evidence is then washed away at the autopsy room. In addition, to minimize the recovery of skin cells from the victim, collect any blood/body fluid stains from the putative suspect as gently as possible. This can be accomplished by lightly swabbing the stained area. Pubic combings are regularly collected to identify evidence that could have been transferred from the perpetrator. Fingernail scrapings or clippings are often collected in homicides and sexual assaults. Here, too, it is important to avoid applying excessive force in this process to minimize collecting the victim's blood/skin. In general, collecting too much biological material from the victim may mask or impede the detection of foreign biological evidence, that is, from the perpetrator. It is

also important to document (sketch and photograph) any pattern evidence on the victim's clothing, such as blood spatter, smears, and swipes. Carefully collect the victim's clothing to minimize the loss of important trace evidence and preserve any pattern evidence. It is often useful to have the victim disrobe over a large piece of clean paper. If clothing must be cut from the victim, avoid cutting or damaging any possible bullet/knife holes.

5. Analysis of samples: Plant anatomy; pollen analysis; DNA analysis; plant DNA typing

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 Plant 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.

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

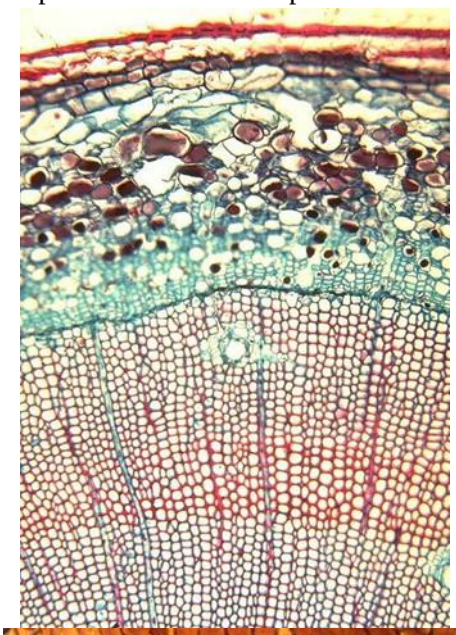
Since 1930s: became increasingly more common in forensic applications (Lane et al., 1990). Cell wall: particularly important for two reasons:

◊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)

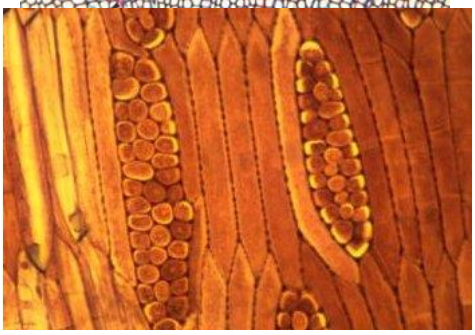
The Vascular Cambium

- 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 elongated shapes

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.

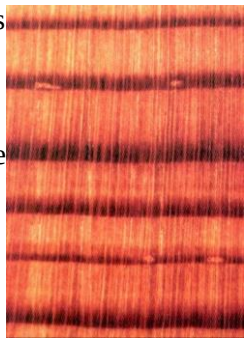
Xylem: principle strengthening and water conducting tissue of the stem, roots, and branches.

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.

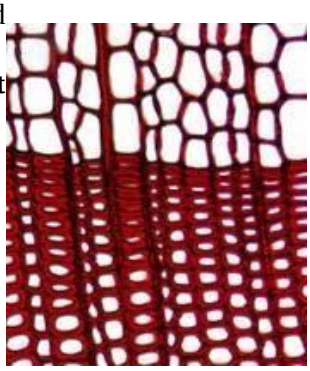
Vessel: the composite, tube-like structure found in hardwoods from the fusion of cells in a longitudinal column.
Fibre: an elongated cell with pointed ends and a thick or infrequently thin wall.

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

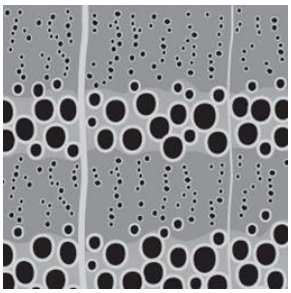
- Growth interrupted by environment (e.g. defoliation)
- Slow growth may cause formation of latewood type cells

Discontinuous rings

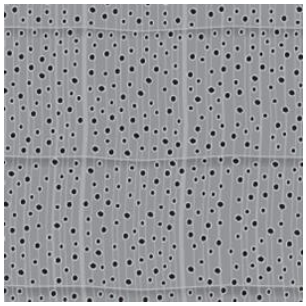
- Cambium was dormant in one region
- One-sided crowns, suppressed, or over mature trees

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.
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.



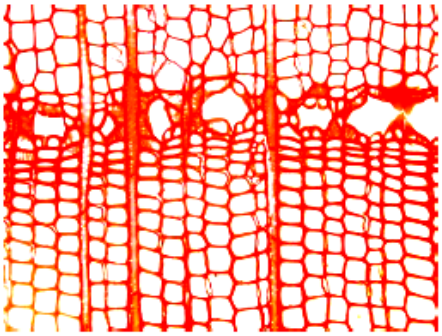
Non porous- Softwoods (gymnospermous wood) don't have vessel cells. However, different softwoods have different growth-ring characteristics. In white pine, the rings are non-distinct, and stain uptake is fairly even, as in diffuse porous woods. In yellow pine, where the rings are clearly visible, stain uptake in early wood is more pronounced than in latewood, as in ring-porous woods.

Criteria for identification

- Structure of tracheid walls and xylem rays

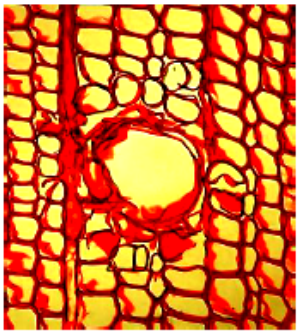
- Resin canals
- Pitting in rays
- Crystals

Resin canals:

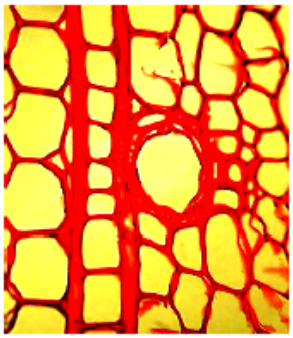


Traumatic resin canals

(produced by injury)



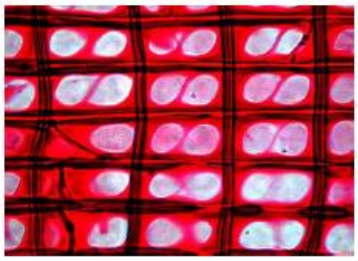
Resin canals with thin-walled epithelial cells



Resin canals with thick-walled epithelial cells

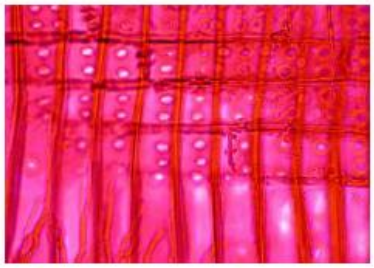
The wood formed immediately after wounding will contain traumatic resin canals. Wounding may be caused by freezing, fires, or mechanical damage. Traumatic resin canals do not have as regular a shape as the normal resin canals, and are formed by some species that normally do not have resin canals.

Pits:



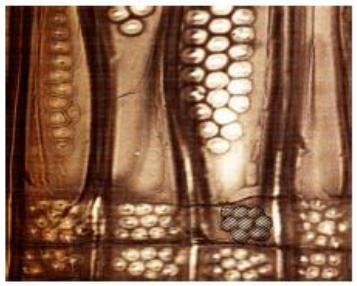
Large window-like pits

(large pinoid pits-occupying practically



Cupressoid pits

(The pit aperture is approx. the same size as the pit border)



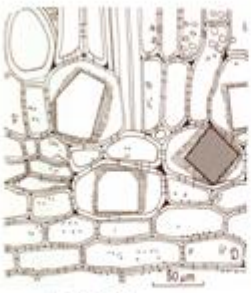
Araucaroid (pits

2 or more rows alternating pits side by side)

Crystals:

- ❖ In ordinary cells
- In chambered cells
- In idioblasts
- Raphides/ druses
- Oil/ mucilage cells

In chambered cells
(*Citrus maxima*)



In idioblasts
(*Zelkova acuminata*)

Raphides
(*Morinda lucida*)

Oil cells
(*Litsea populifolia*)

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 location where it was found.

Three ways in which the roots can be used to date the remains or otherwise characterize the burial site:

(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 to be 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 her last 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).

Wood features in determining Art Fraud:

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, from which 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 objects and 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 spruce from 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 were constructed.

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.

Analyzing Pollen and Spore Samples:

- 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.
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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?

Case study:

Srebrenica massacre or Srebrenica genocide (part of the Bosnian War)

Some of the more than 6,100 gravestones at the Srebrenica-Potočari Memorial and Cemetery for the Victims of the 1995 massacre	
Location	**Srebrenica, Bosnia and Herzegovina**
Date	**11–22 July 1995**
Target	**Bosniak men and boys**
Attack type	**Military assault, mass murder, ethnic cleansing, genocide**
Deaths	**8,373**
Perpetrators	**Army of Republika Srpska Scorpions 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

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 had a 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.

“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 pollen grain 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.

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 that the clothing had been in mountains in the vicinity of Mount Holdsworth or a similar mountain scene where *Nothofagus menziesii* was growing.

Example 4:

Part I: Two male intruders entered a house in which the sole female occupant slept having left the back door unlocked for the return of her husband.
She awoke and saw strangers in her bedroom.
The intruders ran off, one leaving a jacket behind on the kitchen floor.

One of the intruders subsequently returned to recover his jacket, but in his rush to leave the house he brushed against a flowering *Hypericum* bush growing just outside the back door.
A suspect was arrested later that day and charged with indecent assault on a female and burglary, but denied any involvement and refused to name any associate.
Part II: A day following the offence the suspect's clothes were taken for forensic examination.
Pollen analysis of selected parts of his clothing showed that his track pants contained 14% *Hypericum* pollen, denim jacket 24%, and polo shirt 27.5%.
Traces of *Hypericum* pollen occurred on other items. Most of these pollen grains still had their cell contents preserved and were on the clothing in clumps consistent with having recently been collected by the clothing and not having been aerially dispersed.
The pollen from the *Hypericum* bush was identical in colour, shape, development, and size range to the pollen from the clothing.
The clothes had so much *Hypericum* pollen on them that they had to have been in direct and intimate contact with a flowering bush.
Part III: The suspect may have been in contact with *Hypericum* elsewhere, but detailed investigations indicated that this was unlikely.
This is but one way in which forensic palynology can assist law enforcement agencies to determine the history behind a criminal action, and demonstrates that forensic palynology should be considered as an integral part of any criminal investigation.
Pollen evidence is by its nature circumstantial and often cannot be used on its own to convict, or more strictly to determine the truth.

DNA analysis:
DNA is composed of four chemical constituents (labelled A, T, C and G), known as bases, attached to a sugar backbone which can form a strand millions of bases long. There are two such strands in DNA, which run in opposite directions. The bases pair up to form a twisted ladder. Each base pair exclusively with one other base on the opposite strand: A to T and G to C.
Forensic DNA analysis focuses on examining specific sections of DNA that are known to be particularly variable between individuals in order to create a DNA profile. The part of the DNA that is examined is called a locus (plural loci), which is a unique site along DNA of a chromosome characterised by a specific sequence of bases. Currently, an individual's entire genome is not analysed to create his or her DNA profile. This means that part or all of the same DNA profile could be shared by more than one person. The statistical analysis of forensic DNA data therefore focuses on establishing the weight of evidence that should be attached to the similarity etween the DNA profile of a person of interest and DNA taken from a crime scene.
DNA profiling/ typing/ fingerprinting:
DNA profiling (also called DNA fingerprinting, DNA tying) is the process of determining an individual's DNA characteristics, which are as unique as fingerprints. DNA analysis intended to identify a species, rather than an individual, is called DNA barcoding.
DNA profiling is a forensic technique in criminal investigations, comparing criminal suspects' profiles to DNA evidence so as to assess the likelihood of their involvement in the crime. It is also used in parentage testing, to establish immigration eligibility, and in genealogical and medical research. DNA profiling has also been used in the study of animal and plant populations in the fields of zoology, botany, and agriculture.
Who invented it?
The process of DNA fingerprinting was invented by Alec Jeffries at the University of Leicester in England in 1984.

Methods of DNA typing:
Sample collection:
A sample of DNA is taken from

- Blood
- Hair follicles
- Saliva

- ❖ Semen
- ❖ Body tissue cells, such as cheek epithelial cells

Cells are then broken down to release their DNA

"Touch" DNA

Humans shed tens of thousands of skin cells each day, and these cells may be transferred to surfaces we touch. Touch DNA has been successfully sampled (by swabbing) items such as

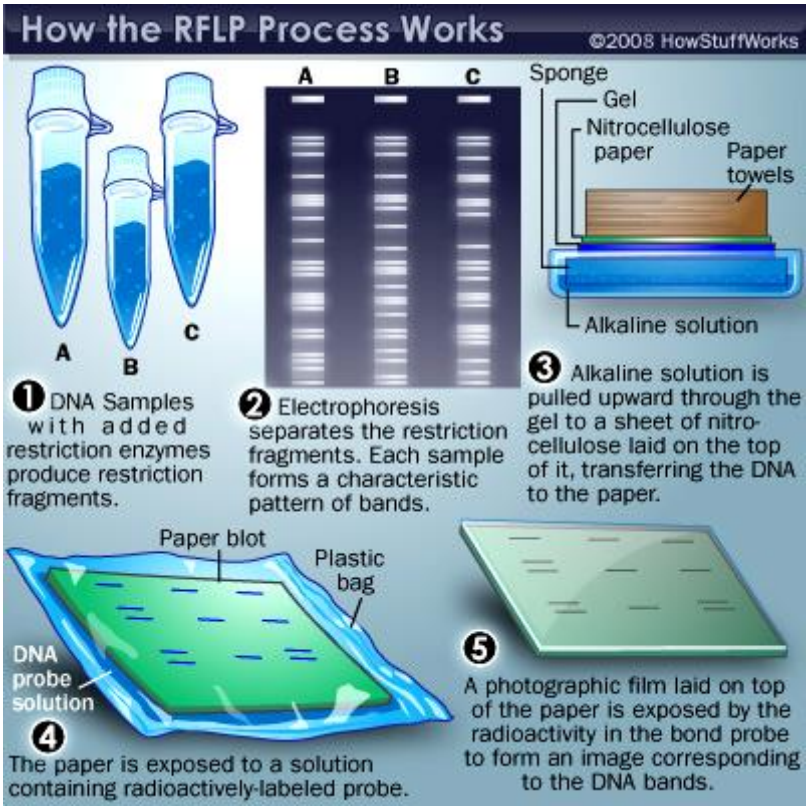
• Door knobs
• Steering wheels
• Gun grips
• Eating utensils

DNA extraction

When a sample such as blood or saliva is obtained, the DNA is only a small part of what is present in the sample. Before the DNA can be analyzed, it must be extracted from the cells and purified. There are many ways this can be accomplished, but all methods follow the same basic procedure. The cell and nuclear membranes need to be broken up to allow the DNA to be free in solution. Once the DNA is free, it can be separated from all other cellular components. After the DNA has been separated in solution, the remaining cellular debris can then be removed from the solution and discarded, leaving only DNA. The most common methods of DNA extraction include organic extraction (also called phenol chloroform extraction), Chelex extraction, and solid phase extraction. Differential extraction is a modified version of extraction in which DNA from two different types of cells can be separated from each other before being purified from the solution. Each method of extraction works well in the laboratory, but analysts typically selects their preferred method based on factors such as the cost, the time involved, the quantity of DNA yielded, and the quality of DNA yielded. After the DNA is extracted from the sample, it can be analyzed, whether it is by RFLP analysis or quantification and PCR analysis.

RFLP analysis

The first methods for finding out genetics used for DNA profiling involved RFLP analysis. DNA is collected from cells and cut into small pieces using a restriction enzyme (a restriction digest). This generates DNA fragments of differing sizes as a consequence of variations between DNA sequences of different individuals. The fragments are then separated on the basis of size using gel electrophoresis.

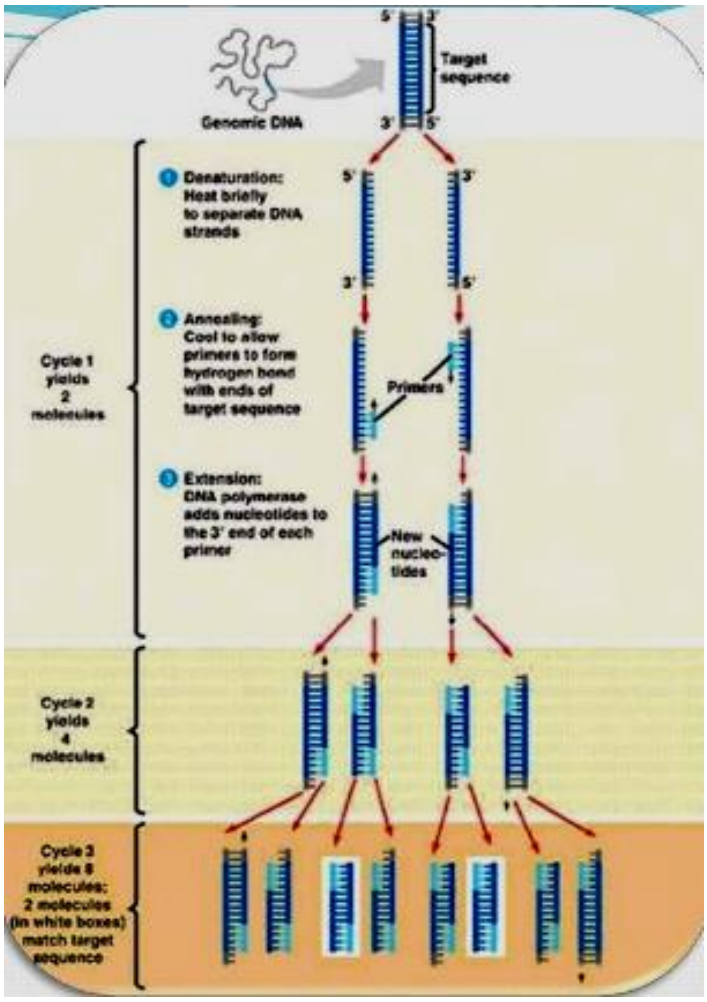


The separated fragments are then transferred to a nitrocellulose or nylon filter; this procedure is called a Southern blot. The DNA fragments within the blot are permanently fixed to the filter, and the DNA strands are denatured. Radiolabeled probe molecules are then added that are complementary to sequences in the genome that contain repeat sequences. These repeat sequences tend to vary in length among different individuals and are called variable number tandem repeat sequences or VNTRs. The probe molecules hybridize to DNA fragments containing the repeat sequences and excess probe molecules are washed away. The blot is then exposed to an X-ray film. Fragments of DNA that have

bound to the probe molecules appear as fluoresent bands on the film.
The Southern blot technique requires large amounts of non-degraded sample DNA. Also, Karl Brown's original technique looked at many minisatellite loci at the same time, increasing the observed variability, but making it hard to discern individual alleles (and thereby precluding paternity testing). These early techniques have been supplanted by PCR-based assays.

Polymerase chain reaction (PCR) analysis

Developed by Kary Mullis in 1983, a process was reported by which specific portions of the sample DNA can be amplified almost indefinitely (Saiki et al. 1985, 1985) The process, polymerase chain reaction (PCR), mimics the biological process of DNA replication, but confines it to specific DNA sequences of interest. With the invention of the PCR technique, DNA profiling took huge strides forward in both discriminating power and the ability to recover information from very small (or degraded) starting samples.



PCR greatly amplifies the amounts of a specific region of DNA. In the PCR process, the DNA sample is denatured into the separate individual polynucleotide strands through heating. Two oligonucleotide DNA primers are used to hybridize to two corresponding nearby sites on opposite DNA strands in such a fashion that the normal enzymatic extension of the active terminal of each primer (that is, the 3' end) leads toward the other primer. PCR uses replication enzymes that are tolerant of high temperatures, such as the thermostable Taq polymerase. In this fashion, two new copies of the sequence of interest are generated. Repeated denaturation, hybridization, and extension in this fashion produce an exponentially growing number of copies of the DNA of interest. Instruments that perform thermal cycling are readily available from commercial sources. This process can produce a million-fold or greater amplification of the desired region in 2 hours or less.

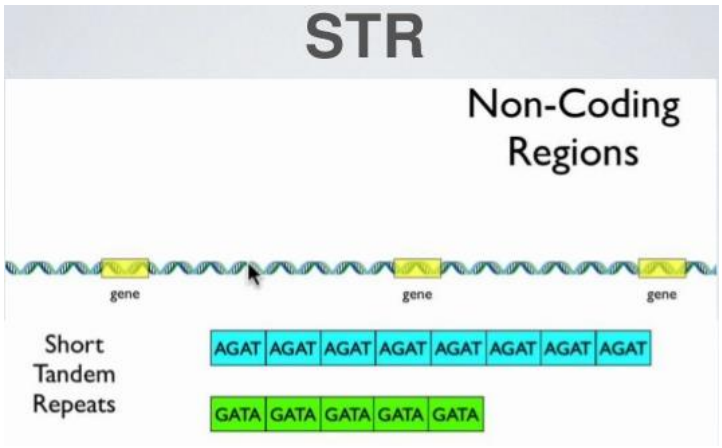
Early assays such as the HLA-DQ alpha reverse dot blot strips grew to be very popular due to their ease of use, and the speed with which a result could be obtained. However, they were not as discriminating as RFLP analysis. It was also difficult to determine a DNA profile for mixed samples, such as a vaginal swab from a sexual assault victim.

However, the PCR method was readily adaptable for analyzing VNTR, in particular STR loci. In recent years, research in human DNA quantitation has focused on new "real-time" quantitative PCR (qPCR) techniques. Quantitative PCR methods enable automated, precise, and high-throughput measurements. Inter-laboratory studies have demonstrated the importance of human DNA quantitation on achieving reliable interpretation of STR typing and obtaining consistent results across laboratories.

STR analysis

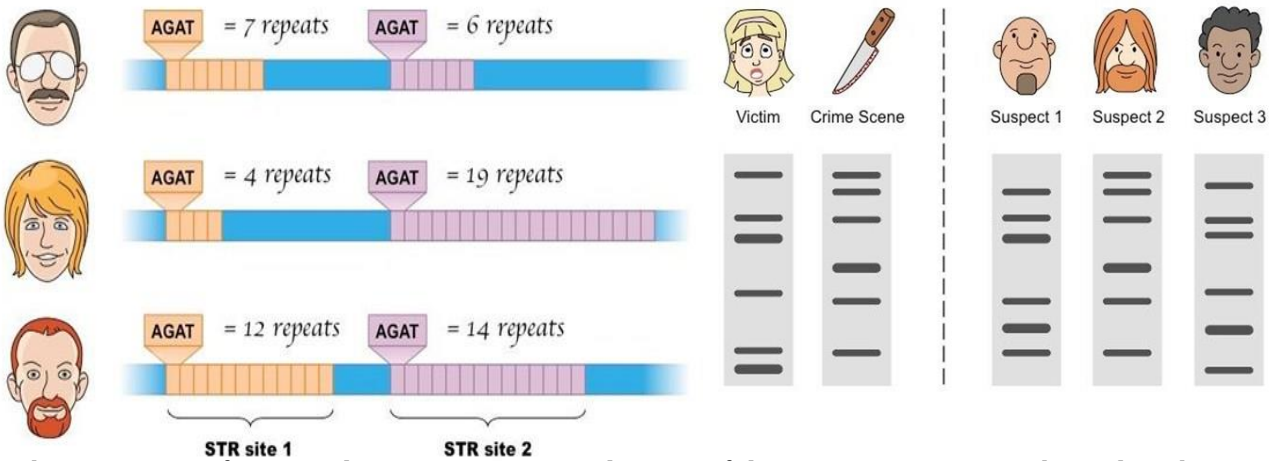
The system of DNA profiling used today is based on polymerase chain reaction (PCR) and uses simple sequences or short tandem repeats (STR). This method uses highly polymorphic regions that

have short repeated sequences of DNA (the most common is 4 bases repeated, but there are other lengths in use, including 3 and 5 bases). Because unrelated people almost certainly have different numbers of repeat units, STRs can be used to discriminate between unrelated individuals. These STR loci (locations on a chromosome) are targeted with sequence-specific primers and amplified using PCR. The DNA fragments that result are then separated and detected using electrophoresis. There are two common methods of separation and detection, capillary electrophoresis (CE) and gel electrophoresis.



Each STR is polymorphic, but the number of alleles is very small. Typically each STR allele will be shared by around 5 - 20% of individuals. The power of STR analysis comes from looking at multiple STR loci simultaneously. The pattern of alleles can identify an individual quite accurately. Thus STR analysis provides an excellent identification tool. The more STR regions that are tested in an individual the more discriminating the test becomes.

From country to country, different STR-based DNA-profiling systems are in use. In North America, systems that amplify the CODIS 20 core loci are almost universal, whereas in the United Kingdom the DNA-17 17 loci system (which is compatible with The National DNA Database) is in use, and Australia uses 18 core markers. Whichever system is used, many of the STR regions used are the same. These DNA-profiling systems are based on multiplex reactions, whereby many STR regions will be tested at the same time.



The true power of STR analysis is in its statistical power of discrimination. Because the 20 loci that are currently used for discrimination in CODIS are independently assorted (having a certain number of repeats at one locus does not change the likelihood of having any number of repeats at any other locus), the product rule for probabilities can be applied. This means that, if someone has the DNA type of ABC, where the three loci were independent, we can say that the probability of having that DNA type is the probability of having type A times the probability of having type B times the probability of having type C. This has resulted in the ability to generate match probabilities of 1 in a

quintillion (1x1018) or more. However, DNA database searches showed much more frequent than expected false DNA profile matches. Moreover, since there are about 12 million monozygotic twins on Earth, the theoretical probability is not accurate.
In practice, the risk of contaminated-matching is much greater than matching a distant relative, such as contamination of a sample from nearby objects, or from left-over cells transferred from a prior test. The risk is greater for matching the most common person in the samples: Everything collected from, or in contact with, a victim is a major source of contamination for any other samples brought into a lab. For that reason, multiple control-samples are typically tested in order to ensure that they stayed clean, when prepared during the same period as the actual test samples. Unexpected matches (or variations) in several control-samples indicates a high probability of contamination for the actual test samples. In a relationship test, the full DNA profiles should differ (except for twins), to prove that a person was not actually matched as being related to their own DNA in another sample.

AFLP

Another technique, AFLP, or amplified fragment length polymorphism was also put into practice during the early 1990s. This technique was also faster than RFLP analysis and used PCR to amplify DNA samples. It relied on variable number tandem repeat (VNTR) polymorphisms to distinguish various alleles, which were separated on a polyacrylamide gel using an allelic ladder (as opposed to a molecular weight ladder). Bands could be visualized by silver staining the gel. One popular focus for fingerprinting was the D1S80 locus. As with all PCR based methods, highly degraded DNA or very small amounts of DNA may cause allelic dropout (causing a mistake in thinking a heterozygote is a homozygote) or other stochastic effects. In addition, because the analysis is done on a gel, very high number repeats may bunch together at the top of the gel, making it difficult to resolve. AmpFLP analysis can be highly automated, and allows for easy creation of phylogenetic trees based on comparing individual samples of DNA. Due to its relatively low cost and ease of set-up and operation, AmpFLP remains popular in lower income countries.

Y-chromosome analysis:

Recent innovations have included the creation of primers targeting polymorphic regions on the Y-chromosome (Y-STR), which allows resolution of a mixed DNA sample from a male and female or cases in which a differential extraction is not possible. Y-chromosomes are paternally inherited, so Y-STR analysis can help in the identification of paternally related males. Y-STR analysis was performed in the Sally Hemings controversy to determine if Thomas Jefferson had sired a son with one of his slaves. The analysis of the Y-chromosome yields weaker results than autosomal chromosome analysis. The Y male sex-determining chromosome, as it is inherited only by males from their fathers, is almost identical along the patrilineal line. This leads to a less precise analysis than if autosomal chromosomes were testing, because of the random matching that occurs between pairs of chromosomes as zygotes are being made.

Mitochondrial analysis

For highly degraded samples, it is sometimes impossible to get a complete profile of the 13 CODIS STRs. In these situations, mitochondrial DNA (mtDNA) is sometimes typed due to there being many copies of mtDNA in a cell, while there may only be 1-2 copies of the nuclear DNA. Forensic scientists amplify the HV1 and HV2 regions of the mtDNA, and then sequence each region and compare single-nucleotide differences to a reference. Because mtDNA is maternally inherited, directly linked maternal relatives can be used as match references, such as one's maternal grandmother's daughter's son. In general, a difference of two or more nucleotides is considered to be an exclusion. Heteroplasmy and poly-C differences may throw off straight sequence comparisons, so some expertise on the part of the analyst is required. mtDNA is useful in determining clear identities, such as those of missing people when a maternally linked relative can be found. mtDNA testing was used in determining that Anna Anderson was not the Russian princess she had claimed to be, Anastasia Romanov. mtDNA can be obtained from such material as hair shafts and old bones/teeth. Control mechanism based on interaction point with data. This can be determined by tooled placement in sample.

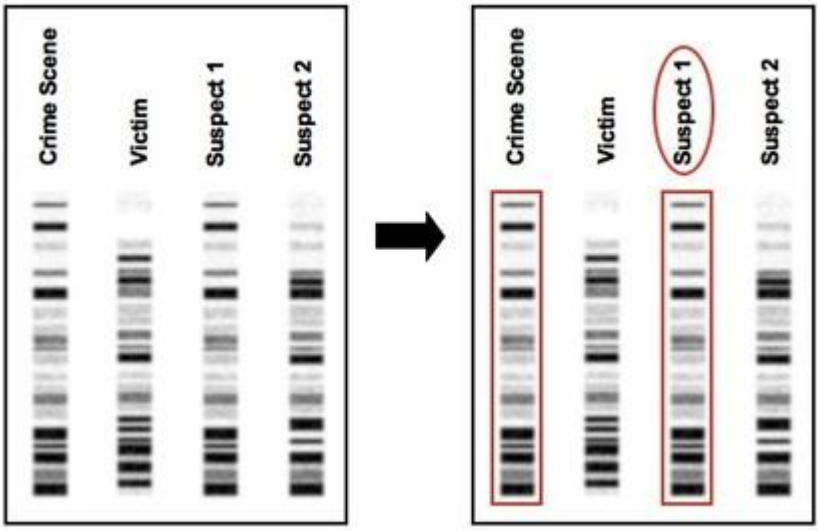
Applications of DNA Fingerprinting:

Since its discovery, DNA fingerprints have been used as evidences in the identification of criminals in murder case, rapists in rape case, parents in case of doubt-ful parentage etc. This method has also been used in a wide range of other applications, including immigration cases, disputes invol-ving purebred dogs, and in animal conserva-tion studies.

In Forensic Analysis:

If a DNA profile from tissue found at a crime scene matches with that of a suspect, it does not prove that the tissue belongs to the suspect; instead, it excludes all those who have a different DNA profile. Therefore, to generate five to more DNA profiles from the same sample, diffe-rent probes have to be used.

The more pro-files that match, between the sample and the suspect, the more unlikely it is that the sample at the crime scene came from someone other than the suspect. Sometimes DNA fin-gerprinting in criminal case may not involve the suspect's own DNA, but rather the DNA of plants or animals that are closely present at the crime scene.



In Paternity case:

If a mother of a new-born accuses a particular man of being the father of her child, and the man denies it, the DNA typing or fingerprinting can at least reach conclusive decisions. In this case, DNA samples are taken from blood samples of mother, baby and the sus-pected father.

DNAs are cut with restriction enzyme for the marker to be analysed and the resulting fragments are separated by elec-trophoresis, transferred to a membrane filter by Southern blotting and probed with a labeled mono-locus STR or VNTR probe.

After autoradiography or chemiluminescence detection, the DNA banding pattern – the DNA fingerprint or DNA profile is analyzed to compare the samples. The data shown can be interpreted as follows. In second lane two DNA fragments are shown from mother's sample.

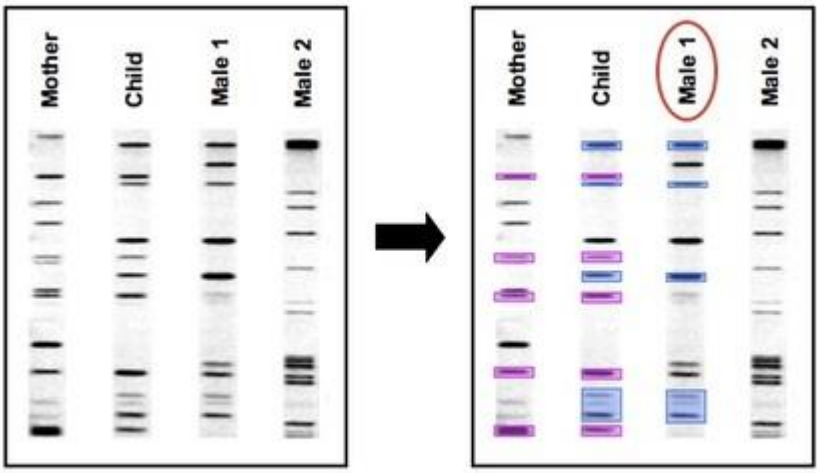
Therefore, the mother is heterozygous for one particular pair of alleles at the STR or VNTR locus under study. Similarly, two DNA fragments are also detected in the third lane for the baby.

It indicates that the baby is also hetero-zygous. It is also shown that one fragment for the baby matches the larger fragment of the mother's DNA. The other fragment of the baby's DNA is much larger, indicating presence of more repeats in that allele. In the autoradiogram it is also shown that the lar-ger fragment of the baby's DNA matches with one DNA fragment of the alleged father.

We know that the baby receives one allele from its mother and one from its father. The present data indicate that the man shares an allele with the baby, but he denied being its father. If the man had no alleles in common with the baby, then the data would have proved that he is not the father. This is called exclusion result. The inclusion result that indicates positive identity is difficult to establish through DNA fingerprinting.

It depends on frequencies of STR or VNTR alle-les identified by the probe in the ethnic popu-lation from which the accused person comes. However, for better' confirmation, it is suggested to probe the DNA fragment with different mono-locus STRs or VNTRs and if the data for each probe indicated

that the same person contributed a particular allele to the child, then the conclusion regarding the paternity will be more confirmed.



Other Applications of DNA Fingerprinting:

(i) Detection of genetically modified organisms (GMOs):

Genetically modified crops typically contain certain genes that were introduced for the development of new crops. In general, these genes express a parti-cular promoter and a particular transcription terminator, enabling PCR primers to be designed on the basis of these sequences. Such primers can be used to test their pre-sence.

A positive result will indicate that the plant is genetically modified or that the food contains one or many GMOs. On the other hand a negative result does not rule out the presence of a GMO, because the plant may have been genetically modified using genes with a different promoter or terminator.

(ii) Phylogenetic relationship:

Analysis and comparative study of DNA extracted from very ancient organisms, such as 40 mil-lion year old fossil leaf or 20 million year old insect in amber, or 40,000 year old mammoth with present day related organisms may be helpful to determine the actual phylogenetic relationship among present-day descendant.

(iii) Determination of variability in popu-lation or ethnic groups may be done by DNA typing.

(iv) In horses, dogs etc. pedigree status can be determined for certain breeds to pre-pare their breed registration.

(v) Conservation biology may also use DNA typing to determine genetic variability among endangered species.

(vi) Presence of pathogenic strains of bacteria like E. coli in food can be tested by DNA typing.

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.

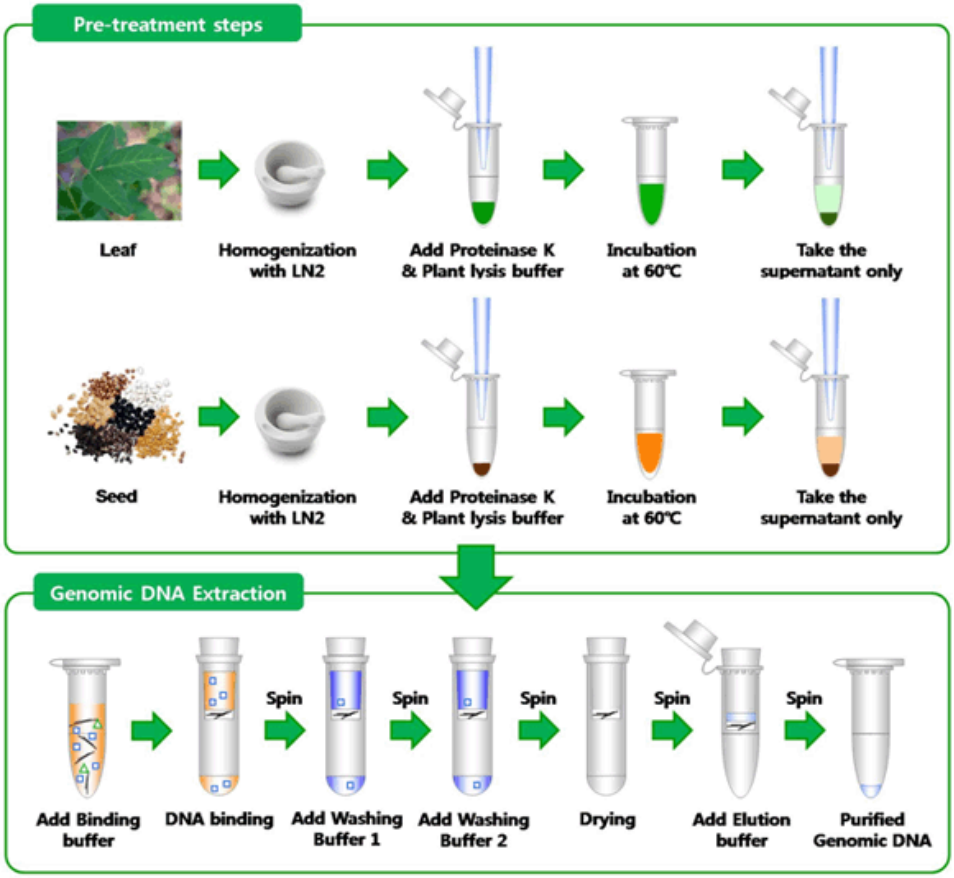
Preparation of Genomic DNA from Plant Tissue

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:

Cold, sterile water Liquid nitrogen
Extraction buffer (100 mM Tris-Cl, pH 8; 100 mM EDTA; 250 mM NaCl; 100 µg/mL proteinase K)
10% (wt/vol) N-lauroylsarcosine Isopropanol
TE buffer (10 mM Tris-Cl, pH 8; 1 mM EDTA) Cesium chloride (CsCl)
10 mg/mL ethidium bromide CsCL-saturated isopropanol Ethanol
3M sodium acetate, pH 5.2
Beckman JA-14, JA-20, JA-21, and Vti80 rotors

- 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.

Applications of DNA fingerprinting in forensic botany:

In theory, DNA fingerprints obtained from plant fragments should be able to provide important evidence in crime investigations but success has been limited so far, probably due to problems with isolating DNA of sufficient quality from poorly preserved plant material. SSR markers are often chosen for forensic work since they work comparably well also with heavily degraded DNA. One famous early case, however, involved RAPD analysis of seed pods of the Palo Verde tree, *Cercidium* sp., recovered both from the crime site and from the pick-up truck of a suspect, while another case made use of SSR and RAPD analysis to compare fragments from clonally reproducing bryophytes (mosses) collected both on the crime site and on the suspect himself. In subsequent experiments, a high likelihood of picking up fragments of bryophytes by walking outdoors wearing rubber boots was shown, as well as the ability to isolate DNA of sufficient quality after several months of storing bryophyte material under adverse conditions. These facts together with the high level of clonality in many bryophyte species make them an ideal target for forensic analysis. In yet another criminal case, seedlings of the inbreeding herbaceous knotweed Polygonum aviculare obtained from germinating seeds found in the wheelhouse of a suspect's car tire, and from a large number of soil samples taken at the crime site and various reference localities, were analyzed with AFLP.

Detection of adulterations of food, drink and medicinal products is another area for forensic botany. Licensing arrangements sometimes require that a specified clone, cultivar or landrace is utilized in the manufacturing of food and beverages. Thus, well-defined grapevine clones must be used to receive "appellation d'origine controllée" labelling in France. In one study, musts (that is, freshly pressed grape juice destined for wine-making) from two different grape cultivars could be identified using two SSR markers. In another study, musts containing different proportions of two grape cultivars were analyzed with densitometry measurements of the SSR amplification products after separation and staining on polyacrylamide gels. In Greece, Nemea wines are marketed with protected denomination of origin (PDO). Instead of using only the prescribed cultivar 'Agiorgitiko' , the more productive 'Cabernet Sauvignon' is sometimes added. DNA samples from fresh and fermented products, containing various mixtures of these two cultivars, were therefore subjected to a CAPS assay. Presence of the adulterant could be detected down to 10% throughout the fermentation process.

Olive oil is also often marketed with PDO labelling. RAPD, ISSR and SSR analysis of Portuguese olive oils allowed the determination of geographic origin of the cultivars on which they had been based . Similarly all 10 olive cultivars involved in samples of Italian oil samples could be identified with only one AFLP primer pair. For rice, the adulteration of the expensive Basmati rice is an important issue, not only for European and US customs but also for consumers. Basmati cultivars have often been mixed with crossbred Basmati varieties and long-grain non-Basmati varieties. Several DNA-based markers have been proposed, and some were commercialized for adulteration tests, such as the multiplexed SSR markers developed by Archak and colleagues. DNA analyses of various plant-based food products have similarly been used for authentication. The presence of the apple 'Annurca' could thus be verified by SSR analysis in highly processed nectar and purée products. Using relatively short SSR target sequences (below 160 bp), it was also possible to amplify genomic DNA from canned pear fruit and fruit juice while markers with longer target sequences failed.

Medicinal drugs constitute another important product area where adulterants cause major problems. Based on nine SNP sites, all populations except two could be distinguished in DNA isolated from the dried stems of the orchid Dendrobium officinale, which is a valuable source of 'Fengdou' drugs used in traditional Chinese medicine. The latter two populations could instead be distinguished using a more complex procedure known as suppression subtraction hybridization which involves PCR amplification, differential DNA fragment cloning and sequencing. Using these protocols, origination of the plant material could be determined for 50 drug samples obtained at a commercial market. For more information on DNA marker use in medicinal plants, see the reviews by Nybom and Weising and Sarwat and colleagues.

A variety of DNA marker methods have been used to demonstrate infringement of Plant Breeder's Rights, either in court or, in our experience much more common, leading to a settlement outside of court. A related field concerns the identification of plants, the possession of which is considered illegal. Thus several studies have been published on the identification of Cannabis sativa specimens as part of drug enforcement. In one approach, 15 SSR loci were combined into a single multiplex to enable fast and user-friendly discrimination between Cannabis genotypes. One of the detected genotypes, however, proved to be very common in police seizure-derived evidence material, suggesting that many illicit growers had access to the same clone. This clonal propagation of course makes it difficult to determine the origination of a particular batch. A related DNA marker application concerns violation of trade restrictions. A special situation is encountered when products from protected trees are involved since woody tissue usually yields heavily degraded DNA. Nevertheless, a set of SNP markers derived from cpDNA intergenic spacers have proven useful for identification of tropical tree species using wood-derived DNA samples.

6. Classic forensic botany cases: Case histories by using Plant anatomy and systematic, Palynology, Plant ecology, Limnology, Plant Molecular Biology and DNA, Drug enforcement and 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 used to 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 pattern of 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 provide a 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 was 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 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 already elapsed.

- 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's home, 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 creating a physical barrier.

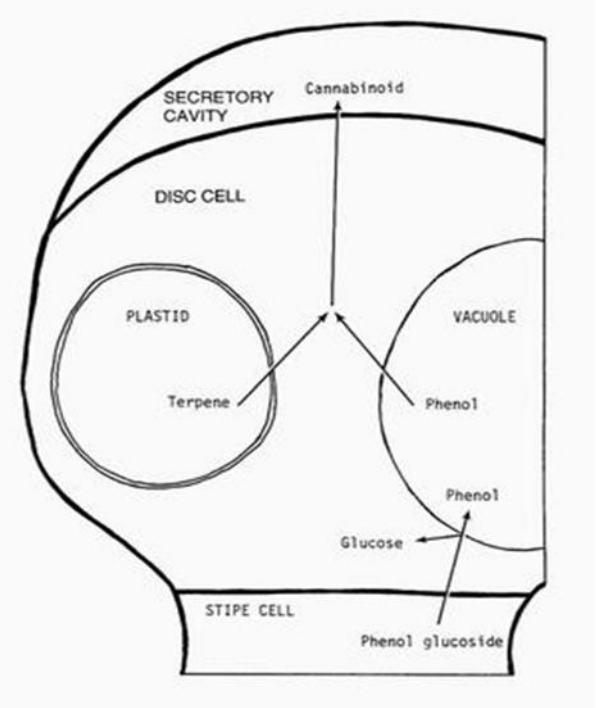
The Cannabinoids and Terpenoids in Resin:

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 THC group, 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 link a 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.

7. Let's sum up

- Forensic botany is a marriage of many disciplines and results ultimately in their application to matters 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 for species identification.
- 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.
- Plant evidence is long lasting, which means that plant parts to remain identifiable for 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.
- The collection of plant fragments, seeds, flowers, and fruits should all be performed by hand. Whole plants and any fragments that may potentially be useful for a physical match should be collected as well as any pieces associated with a body. Botanical fragments in and on motor vehicles should be collected; in particular, the wheel wells, in and under floor mats, the undercarriage, pedals, windshield wipers, vents, trunk, and engine compartments should be fully examined.

- Outdoor crime scenes also warrant a special note. Investigator and technicians are often working under greater time pressure because, given certain weather conditions, some of the physical evidence can be altered or destroyed.
- All trace evidence, including blood and body fluids, prints, soil, hairs and fibers, pollen, plant fragments, and stains, should be photographed in place prior to collection and packaging. Body orifices should be examined for semen and other body fluids, hairs and fibers, and other trace materials. It is important to collect the evidence when possible prior to moving the body.
- 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.
- 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 location where it was found.
- Characteristic cell types from food plants can be used to identify a victim's last meal. Knowledge about which can be useful in determining the victim's whereabouts or actions prior to death. Some of these cell types include: sclereids, starch grains, raphide crystals, ruse crystals and silica bodies.
- 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-number of pollen grains, type of pollen grains, found in a certain area and at a particular time.
- 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 5 years with the implementation of mitochondrial DNA sequencing, and plant and animal DNA testing in the forensic arena.
- 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.
- 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.

8. Suggested readings

- Coyle. H. M. Forensic_Botany-Principles and Applications to Criminal Casework CRC PRESS Boca Raton London New York Washington, D.C.
- https://en.wikipedia.org/
- http://www.notesonzoology.com/dna/dna-fingerprinting/

9. Assignment

1. What is ‘touch’ DNA?
2. What do you meant by pollen fingerprinting?
3. Describe the application anatomical samples in foresic botany
4. Write a short note on outdoor crime scene
5. Differentiate between civil and criminal law.
6. Write one famous case study solved through use of plant anatomical evidences.
7. How Drug enforcement help to solve forensic cases
8. What is typing? Give an overview of DNA typing.
9. Mention the role of pollen in case study. What are the advantages?

10. How palynological evidences helps to solve criminal cases.
11. What is Limnology?
12. Name two DNA markers and mention its role in forensic botany.
13. How Diatoms help to solve forensic cases.
14. Distinguish ring porous wood and diffuse porous wood.
15. Briefly describe different steps of DNA fingerprinting with suitable diagram
16. What botanical evidence can do? How botanical samples are collected?

All the materials are self writing and collected from ebook, journals and websites.

POST GRADUATE DEGREE PROGRAMME (CBCS)
IN
BOTANY

SEMESTER - III

Course: BOSCT 3.3

(Medical Mycology)

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING
UNIVERSITY OF KALYANI
KALYANI – 741 235,
WEST BENGAL

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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 UGC Regulations, 2017 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.) Sankar Kumar Ghosh, 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.

Prof Manas Mohan Adhikary
Director
Directorate of Open and Distance Learning
University of Kalyani

SYLLABUS
COURSE – BOSCT3.3
Medical Mycology
(Full Marks –50)

Course	Group	Details Contents Structure		Study hour
BOSCT3.3	**Medical Mycology**	**Unit 1. Fungi as human pathogens**	Fungi as human pathogens, host-pathogen interaction, pathogenecity factors	1
		Unit 2. Mycoses	Mycoses: types, symptoms and treatment	1
		Unit 3. Aflatoxin and other fungal toxins	Diseases caused by aflatoxin and other fungal toxins and their impact on human health	1
		Unit 4. Mycetismus and mushroom poisoning	Mycetismus and mushroom poisoning	1
		Unit 5. Fungi as allergens	Fungi as allergens	1
		Unit 6. Fungi and its therapeutic uses	Fungi and its products used for therapeutic purposes	1

Content

COURSE – BOSCT3.3

Medical mycology

Soft Core Theory Paper **Credit: 2**

Content Structure

1. Introduction

Medical Mycology is the study of fungi that produce disease in humans and other animals, and of the diseases they produce, their ecology, and their epidemiology.
Certain mushrooms, especially polypores like reishi were thought to be able to benefit a wide variety of health ailments. Medicinal mushroom research in the United States is currently active, with studies taking place at City of Hope National Medical Center, as well as the Memorial Sloan–Kettering Cancer Center.
Fungi play an important role in addressing major global challenges. Use of fungal processes and products can lead to increased sustainability through more efficient use of natural resources. Applications range from upgrading bio-waste for value added products to use of renewable plant biomass as a substitute for oil-based products such as biochemicals, plastics, fertilizer, and fuel.

Fungal inoculum, introduced into soil together with seed, can promote more robust plant growth through increasing plant uptake of nutrients and water, a robustness of importance for maintaining crop yields under climate change condition. Fungal enzymes can lead to production of food ingredients with prebiotic effects for a healthier human gut biota and hence greater resilience towards life-style diseases. Similarly, use of fungi can be a short cut to healthier animal feed and less use of antibiotics in, for example, meat production, one of the current prime sources of multiple drug resistant bacteria. Fungi are one of nature´s most promising hotspots for finding new drug candidates and antimicrobials. Last but not least, fungi have interesting potential as the new way of manufacturing biological medicines and a wide spectrum of new value added bio-based products. All such uses of fungi, fungal products and fungal processes reflect the efforts of mycologists over generations.

Similar efforts lie behind work to cure and prevent life threatening human mycoses, to control mycotoxin contaminations, and to counteract fungal spoilage of materials and ‘sick building’ syndrome. As a scientific

discipline mycology has built comprehensive understanding of the fungal kingdom: fungal biodiversity, physiology, genetics, ecology, pathogenesis, nutrition. Mycology includes understanding at the system, organismal and molecular level. This knowledge and insight constitute the platform that has given rise to uses of fungi in industry, agriculture, food and feed, medicine and health. Mycology must grow fast beyond where it is today. The potential of fungi for a more sustainable world must be released to address global challenges of climate change, higher demands on natural resources, and the increased burden of lifestyle diseases. Genome sequencing was developed first for the human genome after which bacterial genomes were quickly sequenced. But mycology is catching up. Interestingly, up to now, industry makes use only of a minute portion of the fungal kingdom. Fungal biodiversity is a resource pool for the future. However, fungal diversity is endangered by loss of habitat, causing loss of species and loss of biodiversity in general.

We need to stimulate mycology globally and work more efficiently together to take good care of this diversity and unlock the full potential of the fungal kingdom for future use all over the world. The discipline of mycology needs to be developed to a stage where it can recruit talent for the new generation of mycological researchers and for building the skills needed for the world to change towards the new and more sustainable bioeconomy.

In summary what is needed is: Increased understanding of the fungal kingdom, phylogeny and phylogenomics as a basis for understanding the fungal life-form generally, and for expanding the exploitation of fungal biodiversity for more value added uses; the mycological platform, building mycological know how and skills in all parts of the world. There is a global need for the bioeconomy, for increased resource efficiency and upgrading of biowaste to healthier food and feed ingredients, materials and fuel; a stronger global mycology network, including globally distributed databases (embracing genotypic and phenotypic data), improved opportunities for networking activities, talent recruitment, research education, and for broadening the IMA activities to real global inclusiveness and perspective; Open access mycology, a new concept where cultures and information go together to support knowledge dissemination, enabling distributed uses of fungi for upgrading bio-waste resources to higher value; a concerted effort to build a stronger focus on the role of fungi in nature, how they interact with substrate and other organisms, including global research efforts on the fungal secretome, as a basis for increased collaboration between academia and industry within the field of mycology; revisiting the traditional use of microbial fungi for food, the advanced mycological heritage, revitalized through molecular studies, giving insight in use of microbial consortia for food processing to inspire also the next generation of biobased products; and not the least: excellence in teaching of mycology, to be developed in a global scale to provide a better platform for recruitment of talent, for stimulating a fascination of fungal life, and for building broader skills for the bioeconomy to unfold.

Last, but most importantly, further studies are urgently needed to prevent, control and cure serious human mycoses caused by *Coccidioides* species. The number of fatalities caused by this fungal disease, especially in Africa, approaches that caused by tuberculosis and malaria, but far less effort has been invested in preventing and curing human mycoses. Perhaps basic studies of the secretome
(including substances bound to the outer wall structures), and new molecular insights, instruments and technologies could bring us a step further in this very difficult significantly under prioritized area of health and pharmaceutical investment.

2. Objectives

After completion of the module students will be benifited about this subject matters are :

- About the current conceptual models for the pathogenesis of medically important fungi, with a focus on the most frequently encountered pathogens – Candida, Cryptococcus, and Aspergillus
- The molecular manipulation of Candida, Cryptococcus, and Aspergillus
- The experience with mammalian, invertebrate, and cell culture models to assess virulence and analyze different types of host-pathogen interactions

●To present a broad perspective on experimental issues pertinent to pathogenic fungi, such as the definition and determination of virulence, the determination of host responses relevant to infection, and the quantification of antifungal susceptibility
●The techniques relevant to the analysis of the function of fungal gene products such as determination of essentiality, microscopic analysis of morphology and fluorescent protein fusions, comparison of RNA expression profiles of wild-type and mutant strains, assessment of chromosome content
● Insight into the clinical aspects of fungal diseases from the perspective of the host and the pathogen
●An introduction to tools for comparative genome and transcriptional analysis
●About the discuss research ethics, professional development (academic, industrial, or government careers), and issues specific to the medical mycology field.
●Apply the knowledge and skills acquired to identify various fungi species.

3. Fungi as human pathogens, host-pathogen interaction, pathogenecity factors

A) Introduction:

In 1860, Louis Pasteur is solicited by Napoleon III to cure a "disease" affecting wines exported to England. His work will eventually result in the identification of yeast (*Mycoderma vini*) as the agent responsible for the transformation of the sugar of the grape in alcohol and of a bacterium (Mycoderma aceti) as the agent responsible for the acidification and thus the wine "disease".

He then proposed to heat the wine to "cure" the disease setting the bases of the pasteurization principle. Some years later, he demonstrated the implication of the microsporidia *Nosema bombycis* in the Silkworm disease that was then devastating the silk industry in France and he found a way to limit contagion. These founding discoveries lifted for the first time the veil on microscopic fungi and resulted some years later in the spectacular extension of the fungal kingdom way beyond the well-known mushrooms.

Nowadays, about 120,000 species of fungi have been described in varying degrees of details using classical botanical description but recent advances in sequencing technologies and computer sciences associated with the analysis of a large set of very diverse biotopes spectacularly changed our vision on fungal diversity. A recent estimate of nearly 4 million predicted fungal species makes this kingdom the most diverse of the domain Eukarya Pathogenic fungi also affect human beings.

Whereas superficial fungal infections are usually benign, invasive infections are much harder to treat and they have an astonishing impact on human health, being responsible for major mortality rate. Although human fungal diseases have been neglected, the most recent studies estimate that they kill more than 1.6 million people every year.

The impact of fungi on human health is an ever-increasing issue. Invasive or chronic fungal infections affect 4.9 million persons every year in the word. The financial impact is proportionally high. For instance, fungal diseases have been reported to have cost more than $7.2 billion dollars in the United States in 2017. The epidemiology of fungal infections is highly dependent on the type of patients affected and on the ecology of the pathogenic fungi. As such, for opportunistic fungal infections, the prevalence of fungal diseases can depend on the underlying associated diseases. Thus, Pneumocystis and Cryptococcus infections, which are associated with the AIDS outbreak, are prevalent in poor countries that do not have a general access to antiretroviral therapy. In contrast, invasive candidiasis and allergic bronchopulmonary or invasive aspergillosis, which are associated with cancer and surgery, are more often diagnosed in rich countries. The epidemiology of primary fungal pathogens is mainly dependant on the natural prevalence of the fungi in the environment. For instance, Paracoccidioidomycosis is one of the most prevalent systemic mycoses in Latin America and its epidemiology is mainly restricted by the geographical distribution of different Paracoccidioides species in these regions. For similar reasons, the burden of histoplasmosis is reaching that of tuberculosis in Latin America.

Fungal infections are in general very difficult to cure and the mortality remains very high even when validated antifungal treatments can be used. The first reason is that no efficient vaccine is yet available and the arsenal of antifungal molecules available is limited and not available in most countries. Actually, very few classes of antifungal molecules are used to treat fungal infections and there is concern about their toxicity. Notably,

echinochandins are the only class of antifungal molecules that has been developed over the last 15 years. The underlying disease (AIDS, cancer, …) weakening the host immunity and type of patients (neonates, elderlies..) with altered immune responses and mostly affected by opportunistic fungal pathogens also explain therapeutic failures.

Drug resistance is an emerging issue. **First,** species naturally resistant to some antifungal drugs are emerging or show increased incidence in recent years due to large use of antifungal prophylaxis and use of more acute fungal identification tools. **Second**, prolonged antifungal treatments in clinics or intensive usage of antifungal drugs of the same classes in agriculture are associated with the emergence of resistance isolates. The last reason is economical. As said above a large part of fungal infections affect poor people with limited access to antifungal drugs. For instance, the current guidelines for treatment of cryptococcocal meningitis published by the World Health Organization in 2018, are short-course induction regimen with amphotericin B deoxycholate and flucytosine, followed by long term fluconazole treatment. However, the price and the absence of license strongly limit the access of these drugs in the countries the most impacted by this disease. Amphotericin B is thus not available in more than 25% of the countries and flucytosine cannot be obtained in more than 75% of them.

Lastly, the high mortality and morbidity associated with fungal diseases is due to the difficulties we have to perform early diagnostic. The failure exists in part because sensitive molecular tools to diagnose early and specifically this type of diseases are often lacking. In that sense, the fact that some commensal fungi of the human body such as *Candida albicans, Malassezia* sp. and very common environmental fungi such as *Aspergillus fumigatus* can also be responsible for invasive life-threatening fungal infections renders these diagnostics challenging.

In order to fight fungal diseases, new diagnostic tools and antifungal drugs with improved efficiency are needed. In this context, we need a greater understanding of the virulence factors used by fungal pathogens and of the biology of pathogenic fungi during the infection, including their responses to variations in their environment.

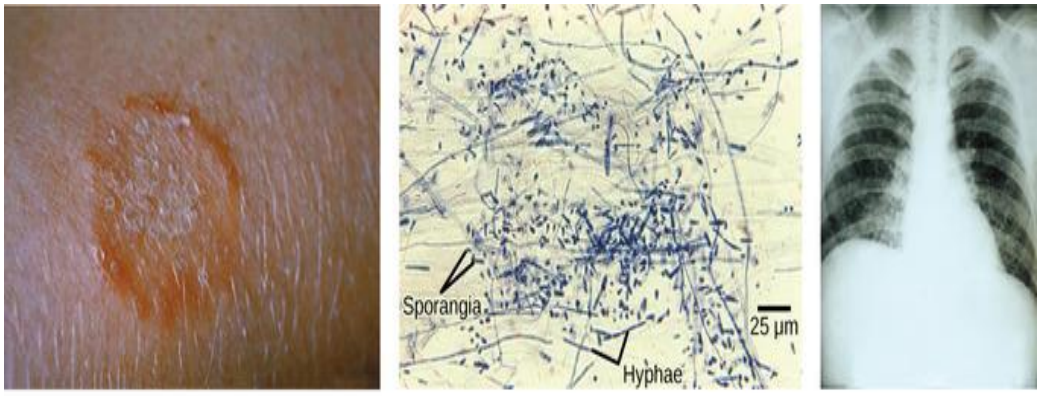
Pathogenic fungi cause disease in humans and in other organisms, which is called as fungal pathogenesis. Fungal pathogens can be divided into two general classes' primary pathogens and opportunistic pathogens. Currently, there has been a dramatic increase in fungal infections of this type, in particular candidiasis, cryptococcosis, aspergillosis, and zygomycosis. More recently described mycoses of this category include hyalohyphomycosis and phaeohyphomycosis.

The superficial mycoses -these are superficial cosmetic fungal infections of the skin or hair shaft.

Dermatophytosis - ringworm or tinea - ringworm of scalp, glabrous skin, and nails caused by a closely related group of fungi known as dermatophytes.

The subcutaneous mycoses - these are chronic, localized infections of the skin and subcutaneous tissue following the traumatic implantation of the aetiologic agent.

Infectious disease mycology - these are fungal infections of the body caused by dimorphic fungal pathogens.



Ringworm presents as a red ring on skin; (b) *Trichophyton violaceum,* shown in this bright field light micrograph, causes superficial mycoses on the scalp; (c) *Histoplasma capsulatum* is an ascomycete that infects airways and causes symptoms similar to influenza.

B) Host-pathogen interactions:

Fungi present intra- and/or extracellular host–parasite interfaces, the parasitism phenomenon being dependent on complementary surface molecules. The entry of the pathogen into the host cell is initiated by the fungus adhering to the cell surface, which generates an uptake signal that may induce its cytoplasmatic internalization. Furthermore, microbial pathogens use a variety of their surface molecules to bind to host extracellular matrix (ECM) components to establish an effective infection. On the other hand, integrins mediate the tight adhesion of cells to the ECM at sites referred to as focal adhesions and also play a role in cell signaling. The phosphorylation process is an important mechanism of cell signaling and regulation; it has been implicated recently in defense strategies against a variety of pathogens that alter host-signaling pathways in order to facilitate their invasion and survival within host cells. The study of signal transduction pathways in virulent fungi is especially important in view of their putative role in the regulation of pathogenicity.
Not every possible invasive speciesis pathogenic in humans – how pathogenic a particular organismis depends strongly on the host it is trying to invade. The defences that must be evaded and the conditions that must be adapted to vary widely in their details from host to host, and will often require a unique set of machinery. Therefore, most pathogens evolve to target a particular host almost exclusively. It is important to recognise that interactions pathogens may have with non-human hosts are hints rather than concrete answers as to their interactions with humans.

Table 1 Definitions of common terms associated with host-pathogen interactions, emphasising the dual sources of pathogenicity – pathogen and host

Term	*Definition (as per Casadevall and Pirofski, 1999)*
Pathogen	A microbe capable of causing host damage; host damage can result from either direct microbial action or the host immune response
Pathogenicity	The capacity of a microbe to cause damage in a host
Virulence	The relative capacity of a microbe to cause damage in a host
Virulence factor	A component of a pathogen that damages the host; this can include components essential for viability

Casadevall, A., Pirofski, L.A., 1999. Host-pathogen interactions: Redefining the basic concepts of virulence and pathogenicity. Infection and Immunity 67(8), 3703–3713.

Primary Pathogens
We have chosen to consider primary pathogens as those fungi that cause disease in noncompromised patients. This distinction is necessarily a gray one, because ***C. neoformans***, a model opportunist, sometimes causes disease in healthy individuals, and the primary pathogens, such as *C. immitis*, are much more virulent in immuno compromised patients. Furthermore, infection by a primary pathogen
often leads to subclinical disease. However, the distinction is worth making in the effort to understand general mechanisms of pathogenesis.
Opportunistic Pathogens
Opportunistic pathogens incite disease in hosts whose local or systemic immune attributes have been impaired, damaged, or are innately dys functional. The pathogenesis of opportunistic infections involves production of virulence factors that allow individual organisms to be commensals during times when humans have normal immune systems. Then, as the immune system wanes, the organism takes its opportunity of being in the right place at the right time to continue growth. This unregulated growth then leads to invasive infection.
Adherence:
For most fungal infections, the ability of the host to resist the physical clearing of the infectious agent is important. For example, the lungs have effective means of clearing foreign particles, but *C. immitis*, *Aspergillus* species, *H. capsulatum*, and *C. neoformans* all infect via the bronchial route and must avoid clearance.
C. albicans also must adhere to various host surfaces both as a commensal to avoid being washed out of its various niches and as a pathogen during the onset of hematogenous infections.

A large number of studies have demonstrated the importance of adherence in various pathogens. A 120-kDa cell wall adhesin, WI-1, which contains 34 copies of a 25–amino acid tandem repeat, has been isolated from the surface of all *Blastomyces dermatitidis* examined. This adhesin mediates attachment to human monocyte-derived macrophages mainly through binding complement type 3 receptors.

This protein is both released into the growth medium and found on the cell wall; free WI-1 seems to be recaptured by the fungus and binds with the cell wall via covalent and noncovalent interactions. The disruption of the gene prevents binding to and infection of macrophages, diminishes adhesion, and attenuates the virulence of the fungus. Hence, in blastomycosis a single molecule affects adhesion both at the site of entry and during later stages of infection.

Adhesion in C. albicans has been the focus of much investigation, and several gene products have been implicated. Three of the most intensely studied adhesion mechanisms involve the HWP1 gene product, the ALS gene family, and the INT1 gene product.

A genomic screen, using insertion mutagenesis, of *Candida glabrata* yielded a mutant that was unable to adhere to cultured laryngeal cells. The mutant was not affected in virulence, suggesting that there are multiple adhesins involved in pathogenesis in this yeast

Penetration

The first step in fungal infection is introduction of the agent to the host. Infections may be limited to portal of entry or they may become systemic, disseminating either via hematogenous or contiguous routes.

Movement from the infecting surface into the bloodstream requires tissue damage. This damage can be preexisting or can occur either by mechanical penetration or newtissue necrosis. Hence, the ability of fungi to penetrate host cells is crucial for progression of infection in the setting of intact skin or gut barriers. For Candida, it is the ability of hyphae to grow through host cell walls that is proposed to account for the importance of polymorphism in virulence. A. fumigatus and other true molds are able to penetrate blood vessels and grow along the vessel lumen as they invade tissue and can use the same or different virulence factors in that process to cross layers of tissue, unlike bacteria whose infections conform to tissue planes.

Hyphae respond thigmotropically (movement toward or away from a touch stimulus) and morphologically to cues such as the presence of a surface, pores, grooves, and ridges. Growth on some firm surfaces elicits a helical growth response. Hyphae follow grooves and ridges of inert substrates and penetrate pores of filtration membranes. Thus, thigmotropism may enhance the ability of a hypha to invade epithelia of a host at sites of weakened integrity or to follow vasculature.

Fungi may also spread from the site of infection throughout the host by such mechanisms as host phagocytosis. *C. albicans* invades endothelial cells through being phagocytosed. *H. capsulatum* is phagocytosed by macrophages but

Table 2
Principal signaling events in fungus and during interaction with host cells

Fungus	Cell type	Signaling pathway		References
		Fungus	Cell/fungus interaction	
Candida albicans	Macrophages endothelial Y → M	cAMP/PKA: virulence, filamentation MAPK: virulence, filamentation Ras 1: morphogenesis/Ras 2: pseudohyphae Ras 1/Ras 2: viability Ca^{+2}/Calmodulin: Y → M	PKC: survival, virulence MAPK/ERK 1/2/ p90RSK: phagocytosis	101,102,105,111,113, 115,126
Fonsecaea pedrosoi	Macrophages epithelial	PKC: invasion	PKC: attachment, invasion	104
Saccharomyces cerevisiae	Macrophages	cAMP/PKA: nutrient MAPK: nutrient GPA1/STE: mating	MAPK/ERK 1/2/ p90RSK: phagocytosis	111,115
Aspergillus fumigatus	Endothelial	cAMP: virulence	cAMP/PKA: gene regulation Ras: virulence	107
Cryptococcus neoformans	Phagocytes	GPA, Rac1: mating GPA: nutrient Ras 1: virulence, filamentation	PI-3K/Ras/MEK/ERK: phagocytosis	111,117
Paracoccidioides brasiliensis	Y → M	PKA G protein, Ras cAMP, GPA: putative Rho1, SEP1, FLB1: signal, polarity, shape PKC1: putative Ca^{+2}/Calmodulin: morphogenetic differentiation Y → M		112,123,124
Histoplasma capsulatum	Y → M	Ca^{+2}/Calmodulin: differentiation Y → M		125

PKC – Protein Kinase C; cAMP – cyclic adenosine 5′ monophosphate; MAPK – mitogen activated protein kinase; PKA: cyclic AMP-dependent protein kinase; Y: yeast; M: mycelium.

does not seem to be killed and multiplies within the phagosome, a characteristic shared by the closely related fungus Blastomyces dermatitidis.

Signaling:

The study of signal transduction pathways in virulent fungi is especially important in view of their putative role in the regulation of pathogenicity. Table 2 shows the principal signaling events in fungus and during interaction with host cells.

C) Pathogenecity factors:

1) Nutritional and Metabolic Factors **-** In order to flourish in the host, fungi need to be able to carry out biosynthetic reactions while concentrating relatively scarce nutrients like Ca^{2+} and $Fe^{2+.}$ Experiments with auxotrophic mutants of *C. albicans* (auxotrophs require a nutrient that the parent organism, the prototroph, does not require) have shown that the inability to synthesize purines, pyrimidines, or heme de novo significantly diminishes virulence. The ability to synthesize fatty acids has also been shown to be essential for *C. albicans* both in a systemic mouse model and in a rat model of oropharyngeal disease. Deletion of the FAS2 gene led to an avirulent Candida strain that was an auxotroph for several fatty acids. The heterozygote was also diminished in its virulence.

Glycolytic enzymes constitute a group of *C. albicans* proteins that are immunogenic during oral and esophageal infections. Changes in glycolytic gene expression accompany the dimorphic transition in *C. albicans* and reflect the underlying physiological status of the cells during morphogenesis. Also, there is a broad spectrum of variability in the expression of genes controlling the utilization of alternative carbon and nitrogen sources.

Urease production has been shown to be a virulence factor in *C. neoformans* in mouse intravenous and inhalation models but not in rabbit meningitis models. The authors suggest that this enzyme may not be needed for maintenance in the cerebrospinal fluid but may be essential for establishment or maintenance of a disseminated model of infection.

2. Necrotic Factors **-** Necrotic factors are vehicles of virulence because they allow the fungus to overcome structural barriers that the human host uses to prevent invasive infection.

Most necrotic factors are enzymes. Because the majority of fungal pathogens are opportunists, these enzymes may have evolved for saprophytic purposes and might be considered nutritional factors, but it seems more likely that their major role in infection is degradation of host tissue.

Cellular and tissue damage at the site of the organism are characteristic of many fungal infections. Among the factors that are thought to contribute to this damage are extracellular degradative enzymes, such as proteinases, phosphatases, and DNAses. The evidence adduced for the role of these enzymes is usually diminished virulence in a mutant lacking the activity.

Although such findings are persuasive, it is important to bear in mind that a failure to demonstrate involvement of a particular activity is not conclusive because many of these genes are members of families, and several of the products may be involved in pathogenesis.

The earliest identification of a potential necrotic factor was the extracellular proteinase of *C. albicans*, first identified by Staib. Further work, largely by Ruchel and coworkers, showed that this enzyme was usually found at infection sites. Because this was the first identifiable potential virulence factor, it has been the subject of much investigation and has been a dominant influence on thinking about fungal pathogenesis.

Several different names were originally given to the gene for this enzyme, such as Opa1 and EPR, but all correspond to members of the secreted aspartyl proteinase (SAP) gene family. A mutant lacking all nine SAP genes has not been isolated, but the role of several genes has been deduced using individual gene mutants. Six of the SAP genes have been disrupted. Single disruptions of SAP1, SAP2, or SAP3 each caused diminished virulence,

A proteinase associated with *C. immitis* may function in the compartmentalization of the spherules into endospores, and the endospores have proteinase activity associated with them. This activity may function to break down lung tissue, the primary site of initial infection, and allow dissemination of the fungus. Cole et al and Yu et al have isolated the genes for a proteinase and a urease, both of which may be important in pathogenesis.

C. albicans is known to secrete phospholipases and to possess a phospholipase gene family. Extracellular phospholipases have a role in the pathogenicity of *C. albicans*, as blood isolates produce significantly more extracellular phospholipase activity than do commensal strains.

3. Morphology

Morphological versatility - Almost all pathogenic fungi can grow in more than one form. Aspergillus species, which are classical filamentous molds, form conidia that are the infectious agent. The major exception is *C. neoformans*, which apparently exists only in the yeast form in vivo. In vitro it also grows mostly as a yeast; however, it does form filaments during the mating process, and the small basidiospores have been proposed to be the agents of infection. Furthermore, there is a strong correlation between MAT® (mating-type alpha) and virulence in Cryptococcus, and MAT® cells, in contrast to MATa cells, can undergo haploid fruiting, which involves formation of filaments and basidia, in the absence of a sexual partner.

Specific parasitic cell forms - *H. capsulatum*, Blastomyces dermatitidis, and several (but not all) species of Candida can grow both as yeasts and as hyphae. In *C. albicans*, both the yeast and hyphal forms are found at the site of infection, whereas in Histoplasma and Blastomyces, the yeast form seems to be the major, if not the exclusive, parasitic form. The transition to the parasitic form in *H. capsulatum* leads to a specific pattern of gene expression; this pattern facilitates many of the steps important in infection, including blocking acidification of the phagolysosome and synthesis of the calcium-binding protein, Cbp1p. In contrast to *C. albicans*, the *H. capsulatum* hypha seems to play no role in vivo.

Multiple parasitic cell forms - Dimorphic fungi regulate their cellular morphology in response to environmental conditions. For example, ellipsoidal single cells of C. albicans (blastospores) predominate in rich media, whereas filaments composed of elongated cells attached end-to-end form in response to starvation, serum, and other conditions. A variety of environmental changes, including a shift from an aerobic to a fermentative metabolism or growth on particular compounds such as N-acetyl glucosamine, cause C. albicans to switch from yeast to filamentous growth. This change is accompanied by changes in carbohydrate metabolism and an interruption of electron transfer within the cell.

Both temperature (a shift to 37±C) and pH can regulate C. albicans dimorphism. The complex regulation of these pathways is reviewed in Brown & Gow.

Phenotypic switching - The capacity of fungi to undergo an epigenetic change (regulation of expression of gene activity without alteration of genetic structure) in colony morphology has come to be called phenotypic switching. This phenomenon was often observed in C. albicans, but its significance was not understood until Soll's lab rediscovered it and set out to analyze its role in pathogenesis. Phenotypic switching is characterized by a reversible change, usually occurring between per cell division, in some property or properties of the cell. Slutsky et al found that colonies arising from cells plated on Lee's medium showed a standard (smooth) phenotype in 99.99% of the cases, but the remaining 0.01% had a wrinkled surface. Careful observation showed that there were a number of potential colony phenotypes and that the cells could pass from any one of these phenotypes to any other.

4. Adaptations of Specific Organisms

Growth at different - pHs C. albicans can grow both at acid and at basic pHs, a reflection of its ability to colonize several niches, ranging from the acid vagina to the neutral oropharyngeal tract. This ability to tolerate a wide pH range is important in several models of virulence. *C. albicans* has two pH-regulated genes, PHR2 and PHR1, the former expressed at acid pH and the latter at neutral and basic pH. Deletion of one or the other leads to an inability to grow at the pH at which the gene is expressed. DeBernardis and coworkers showed that virulence of the disruptants was related to the niche: The PHR2 deletion was avirulent in the vagina, whereas the PHR1 deletion showed reduced virulence in a systemic model.

Toxin production - A. fumigatus produces gliotoxin, but it is not known whether clinically significant amounts of gliotoxin are produced in human disease. Gliotoxin inhibits macrophage phagocytosis via DNA fragmentation and apoptosis, targets the neutrophil respiratory burst, and inhibits T-cell activation and proliferation. Filamentous fungi also produce several ribotoxins, alpha-sarcin, restrictocin, and mitogillin, but the biological function of these potent toxins is unknown.

Melanin - Melanins are scavengers of reactive oxygen intermediaries, making organisms relatively resistant to leukocyte attack. Melanin synthesis is catalysed by a membrane-bound phenoloxidase with a substrate

specificity for phenolic compounds that contain hydroxyl or amino groups, such as L-DOPA and dopamine. Melanin is deposited in the cell wall of *C. neoformans*.
The brown color of this pigment can be revealed by growth on birdseed agar or by the Masson-Fontana stain. Although much attention has been focused on the role of melanin in cryptococcosis, this substance is constitutively produced in the dematiaceous fungi, such as Cladosporium and Wangiella dermatitidis. The role of melanin in *W. dermatitidis* has been extensively examined. Melanin seems to be important in initiating an infection, because melanin-deficient strains are much
less infective on a colony-forming–unit basis but can cause the same neurological symptoms once infection is established.
Iron and calcium - Iron is an essential element for the growth and metabolism of fungi. C. neoformans capsular polysaccharide synthesis is increased by limitation of ferric iron. Most pathogenic microbes elaborate siderophores (molecules that can bind iron) to mobilize iron from ferric ligands. One such siderophore, deferoxamine, which is used as an iron chelator in medical practice, has a known association with the development of zygomycete infections because zygomycetes use the iron in deferoxamine efficiently, unlike *C. albicans* or *A. fumigatus*.
Surface properties - The best known of the fungal immunoevasion systems is the capsule of C. neoformans. A viscous polysaccharide capsule composed of glucuronoxyomannan and other components, the capsule is believed to present a surface not recognized by phagocytes, downregulate cytokine secretion, inhibit leukocyte accumulation, induce suppressive T-cells, inhibit antigen presentation, and inhibit lymphoproliferation. Hence, it serves as a barrier to host defenses in a variety of ways. Its importance as a virulence factor is demonstrated by a comprehensive series of analyses of acapsular mutants.
Mating type - Virulence in *C. neoformans* is associated with theMAT® genotype. Most clinical isolates are serotype A, and this serotype seems to exist exclusively in the MAT® configuration. In pathogenic isolates of the other three serotypes, B through D, MAT® seems to predominate as well. In contrast to *S. cerevisiae*, the MAT locus in C. neoformans contains the gene for the transcription factor STE12. Its orthologue in S. cerevisiae is required for the hormonal response leading to mating as well as for pseudohyphal growth. In contrast, Chang and coworkers have shown that this transcription factor controls the expression of several virulence factors, including melanin and capsule formation in serotype D cells. *C. neoformans* serotype D cells deleted for STE12alpha are therefore avirulent, and replacement of the gene restores virulence. Such cells are still able to mate.

4. Mycoses: types, symptoms and treatment

Mycosis is a fungal infection of animals, including humans. Mycoses are common and a variety of environmental and physiological conditions can contribute to the development of fungal diseases. Inhalation of fungal spores or localized colonization of the skin may initiate persistent infections; therefore, mycoses often start in the lungs or on the skin.
Fungal infections of the skin was the 4th most common disease in 2010 affecting 984 million people. An estimated 1.6 million people die each year of fungal infections.

General Concepts

Classification of Mycoses

The clinical nomenclatures used for the mycoses are based on the **(1)** site of the infection **(2)** route of acquisition of the pathogen and **(3)** type of virulence exhibited by the fungus.

Classification Based on Site

Mycoses are classified as superficial, cutaneous, subcutaneous, or systemic (deep) infections depending on the type and degree of tissue involvement and the host response to the pathogen.

Classification Based on Route of Acquisition

Infecting fungi may be either exogenous or endogenous. Routes of entry for exogenous fungi include airborne, cutaneous or percutaneous. Endogenous infection involves colonization by a member of the normal flora or reactivation of a previous infection.

Classification Based on Virulence

Primary pathogens can establish infections in normal hosts. Opportunistic pathogens cause disease in individuals with compromised host defense mechanisms.

Epidemiology

The primary pathogens have relatively well-defined geographic ranges; the opportunistic fungi are ubiquitous.

Introduction

Current magnitude and problems of mycoses:

Fungal infections or mycoses cause a wide range of diseases in humans. Mycoses range in extent from superficial infections involving the outer layer of the stratum corneum of the skin to disseminated infection involving the brain, heart, lungs, liver, spleen, and kidneys. The range of patients at risk for invasive fungal infections continues to expand beyond the normal host to encompass patients with the acquired immuno deficiency syndrome; those immunosuppressed due to therapy for cancer and organ transplantation, and those undergoing major surgical procedures. Each of these patient populations has a high risk of developing invasive fungal infections. As the population at risk continues to expand so also does the spectrum of opportunistic fungal pathogens infecting these patients also continue to increase? Many of the deeply invasive mycoses are difficult to diagnose early and often difficult to treat effectively. The development of new approaches to diagnosis and treatment of invasive fungal infections is the subject of intensive research.

Concepts of classification

Fungal infections may be classified according to the site of infection, route of acquisition, and type of virulence. When classified according to the site of infection, fungal infections are designated as superficial, cutaneous, subcutaneous, and deep (Fig. 75-1).

Superficial mycoses are limited to the stratum corneum and essentially elicit no inflammation. Cutaneous infections involve the integument and its appendages, including hair and nails. Infection may involve the stratum corneum or deeper layers of the epidermis. Inflammation of the skin is elicited by the organism or its products. Subcutaneous mycoses include a range of different infections characterized by infection of the subcutaneous tissues usually at the point of traumatic inoculation. An inflammatory response develops in the subcutaneous tissue frequently with extension into the epidermis.

Deep mycoses involve the lungs, abdominal viscera, bones and or central nervous system. The most common portals of entry are the respiratory tract, gastrointestinal tract, and blood vessels (Fig. 75-2).

When classified according to the route of acquisition, a fungal infection may be designated as exogenous or endogenous in origin. If classified as exogenous, an infecting organism may be transmitted by airborne, cutaneous, or percutaneous routes. An endogenously-acquired fungal infection may be acquired from colonization or reactivation of a fungus from a latent infection.

Fungi may be classified also according to virulence, as primary pathogens or as opportunistic pathogens. A primary pathogen may establish infection in an immunologically normal host; whereas, an opportunistic pathogen requires some compromise of host defenses in order for infection to become established.

Superficial and Cutaneous Mycoses

Superficial Mycoses include the following fungal infections and their etiological agent: black piedra (*Piedraia hortae*), white piedra (*Trichosporon beigelii*), pityriasis versicolor (*Malassezia furfur*), and tinea nigra (*Phaeoannellomyces werneckii*).

Pityriasis versicolor is a common superficial mycosis, which is characterized by hypopigmentation or hyperpigmentation of skin of the neck, shoulders, chest, and back. Pityriasis versicolor is due to *Malassezia furfur* which involves only the superficial keratin layer. Black piedra is a superficial mycosis due to *Piedraia hortae* which is manifested by a small firm black nodule involving the hair shaft. By comparison, white piedra due to *T. beigelii* is characterized by a soft, friable, beige nodule of the distal ends of hair shafts. Tinea nigra most typically presents as a brown to black silver nitrate-like stain on the palm of the hand or sole of the foot.

Cutaneous Mycoses may be classified as dermatophytoses or dermatomycoses.

Dermatophytoses are caused by the agents of the genera *Epidermophyton, Microsporum,* and *Trichophyton*. Dermatomycoses are cutaneous infections due to other fungi, the most common of which are *Candida* spp. The dermatophytoses are characterized by an anatomic site-specificity according to genera.

For example, *Epidermophyton floccosum* infects only skin and nails, but does not infect hair shafts and follicles.

Whereas, *Microsporum* spp. infect hair and skin, but do not involve nails. *Trichophyton* spp. may infect hair, skin, and nails.

Subcutaneous Mycoses

There are three general types of subcutaneous mycoses: chromoblastomycosis, mycetoma, and sporotrichosis. All appear to be caused by traumatic inoculation of the etiological fungi into the subcutaneous tissue. Chromoblastomycosis is a subcutaneous mycosis characterized by verrucoid lesions of the skin (usually of the lower extremities); histological examination reveals muriform cells (with perpendicular septations) or so-called "copper pennies" that are characteristic of this infection.

Chromoblastomycosis is generally limited to the subcutaneous tissue with no involvement of bone, tendon, or muscle. By comparison, mycetoma is a suppurative and granulomatous subcutaneous mycosis, which is destructive of contiguous bone, tendon, and skeletal muscle. Mycetoma is characterized by the presence of draining sinus tracts from which small but grossly visible pigmented grains or granules are extruded. These grains are microcolonies of fungi causing the infection.

Chromoblastomycosis and mycetoma are caused by only certain fungi. The most common causes of chromoblastomycosis are Fonsecaea pedrosoi, Fonsecaea compacta, Cladosporium carionii, and Phialophora verrucosa. The causes of mycetoma are more diverse but can be classified as eumycotic and actinomycotic mycetoma. Within the United States, the most common agent of eumycotic mycetoma is Pseudallescheria boydii and the most common cause of actinomycotic mycetoma is Nocardia*brasiliensis*. Many of the fungi causing mycetoma are pigmented brown to black.

These organisms are known as dematiaceous (melanized) fungi. The melanin pigment is deposited in the cell walls of these organisms. These fungi may produce a range of infections from superficial to subcutaneous to deep (visceral) infection characterized by the presence of dematiaceous hyphal and/or yeast-like cells in tissue. Such deep infections due to dematiaceous fungi are termed phaeohyphomycosis.

Sporotrichosis is the third general class of subcutaneous mycoses. This infection is due to *Sporothrix schenckii* and involves the subcutaneous tissue at the point of traumatic inoculation. The infection usually spreads along cutaneous lymphatic channels of the extremity involved.

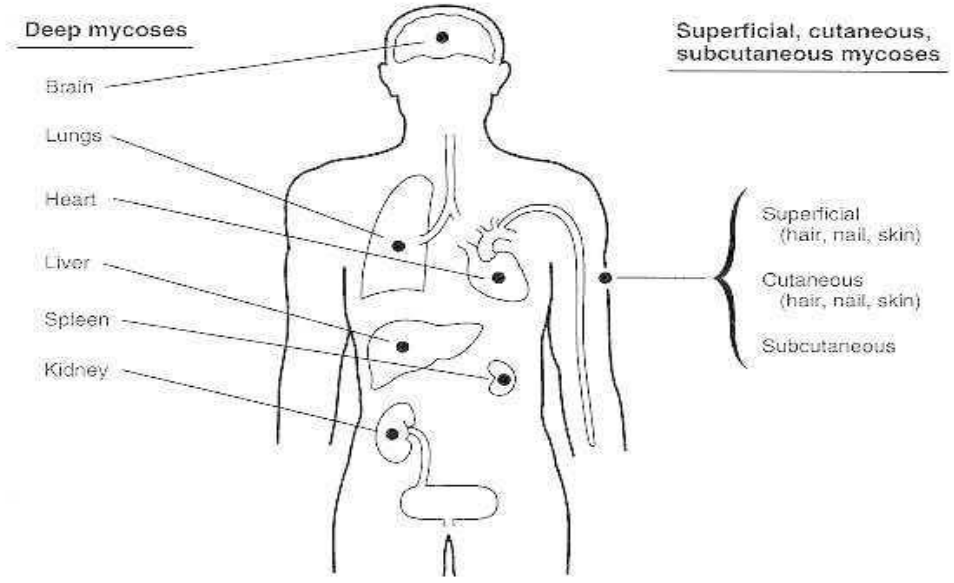


Figure 75.1

Deep Mycoses

General Concepts

Primary versus opportunistic mycoses

Deep mycoses are caused by primary pathogenic and opportunistic fungal pathogens. The primary pathogenic fungi are able to establish infection in a normal host; whereas, opportunistic pathogens require a compromised host in order to establish infection (e.g., cancer, organ transplantation, surgery, and AIDS). The primary deep

pathogens usually gain access to the host via the respiratory tract. Opportunistic fungi causing deep mycosis invade via the respiratory tract, alimentary tract, or intravascular devices.

The primary systemic fungal pathogens include *Coccidioides immitis, Histoplasma capsulatum, Blastomyces dermatitidis,* and *Paracoccidioides brasiliensis*.

The opportunistic fungal pathogens include *Cryptococcus neoformans, Candida* spp., *Aspergillus* spp., *Penicillium marneffei*, Zygomycetes, *Trichosporon beigelii*, and *Fusarium* spp.

Dimorphism in the Pathogenic Fungi

Fungal dimorphism is the morphological and physiological conversion of certain fungi from one phenotype to another when such fungi change from one environment to another. Dimorphic fungi include *C. immitis, H. capsulatum, B. dermatitidis, P. brasiliensis, P. marneffei*, and *S. schenckii*, and certain opportunistic fungi such as *Candida albicans* and *Penicillium marneffei*.

Various environmental host factors control fungal dimorphism. These factors include amino acids, temperature, carbohydrates, and trace elements (e.g. zinc). Among the primary pathogens and *S schenckii*, the morphological transformation is from a hyphal form to a yeast-like form (or spherule in the case of *C immitis*) in tissue (Fig. 75-3). However, the dimorphism of *Candida albicans* is somewhat different in that the organism transforms from a budding yeast-like structures (blastoconidia) to filamentous structures known as germ tubes. Other filamentous structures may later develop as pseudohyphae and hyphae. *Penicillium marneffei* is unique in being the only *Penicillium* species pathogenic to humans. It undergoes dimorphic conversion *in vivo* to transversely dividing sausage-shaped cells.

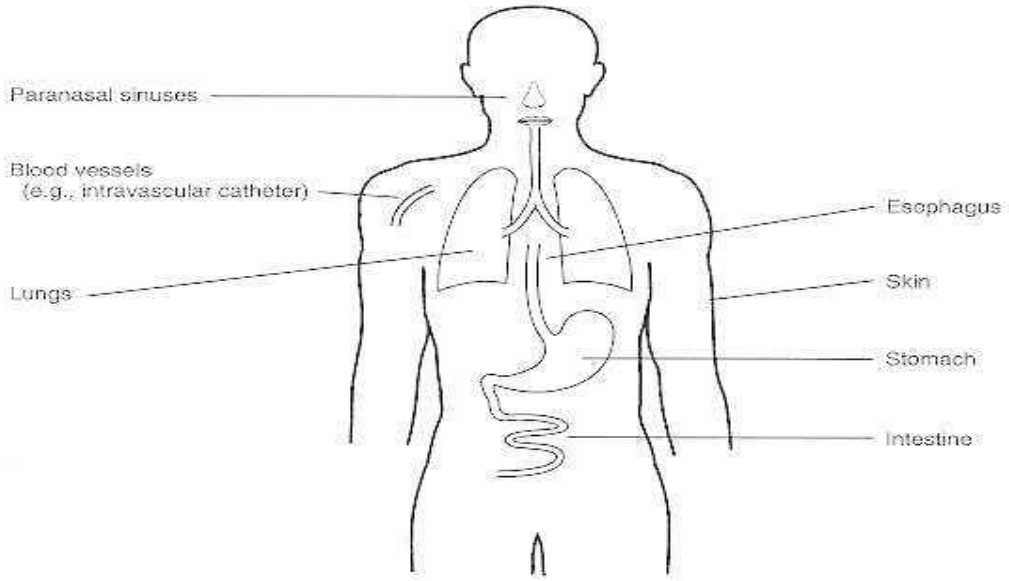


Figure 75.2

Primary Mycoses

Most cases of primary deep mycoses are asymptomatic or clinically mild infections occurring in normal patients living or traveling in endemic areas. However, patients exposed to a high inoculum of organisms or those with altered host defenses may suffer life-threatening progression or reactivation of latent foci of infection.

The arthroconidia of *C. immitis* are inhaled and convert in the lung to spherules. Most cases of coccidioidomycosis are clinically occult or mild infections in patients who inhale infective arthroconidia. However, some patients have progressive pulmonary infection and also may suffer dissemination to the brain, bone, and other sites. *Coccidioides* meningitis is a life-threatening infection requiring lifelong treatment.

Histoplasmosis is a primary pulmonary infection resulting from inhalation of conidia of *Histoplasma capsulatum* which convert in vivo into the blastoconidial (budding yeast) form. Dissemination to the hilar and mediastinal lymph nodes, spleen, liver, bone marrow, and brain may be life-threatening in infants and other immunocompromised patients. Histoplasmosis (like tuberculosis) is characterized by intracellular growth of the pathogen in macrophages and a granulomatous reaction in tissue. These

Granulomatous foci may reactivate and cause dissemination of fungi to other tissues. These patterns of primary infection and reactivation are similar to those of *Mycobacterium tuberculosis*. Histoplasmosis also may be associated with a chronic inflammatory process known as fibrosing mediastinitis, where scar tissue (formed in response to *H capsulatum*) encroaches on vital structures in the mediastinum.
Blastomycosis, similar to histoplasmosis, is a primary pulmonary infection resulting from inhalation of conidia from the mycelial phase of *Blastomyces dermatitidis* which convert in vivo to the parasitic yeast phase. Blastomycosis (due to *B. dermatitidis*) in the blastoconidial phase also causes a primary pulmonary infection. The organism elicits a granulomatousreaction often associated with a marked fibrotic reaction. The clinical pattern of pulmonary blastomycosis is one of chronic pneumonia. Dissemination occurs most commonly to the skin, bone, and, in males, prostate.

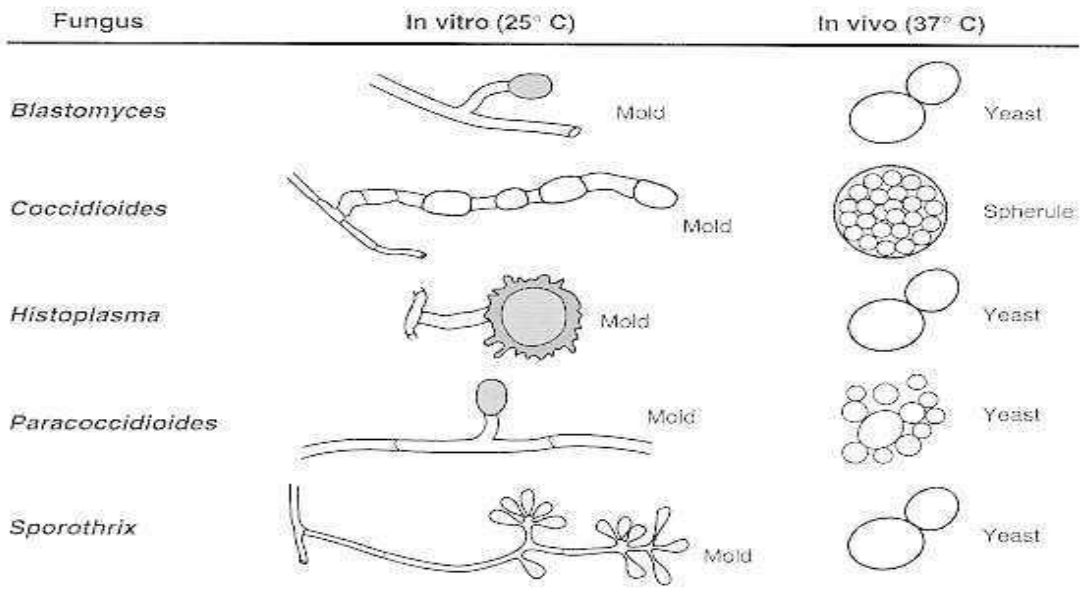


Figure 75.3

Opportunistic Mycoses
Candidiasis
Candidiasis (due to *C albicans* and other *Candida* spp.) is the most common opportunistic fungal infection. *Candida albicans* is the most common cause of candidiasis. Candidiasis may be classified as superficial or deep. Superficial candidiasis may involve the epidermal and mucosal surfaces, including those of the oral cavity, pharynx, esophagus, intestines, urinary bladder, and vagina. The alimentary tract and intravascular catheters are the major portals of entry for deep (or visceral) candidiasis.
The kidneys, liver, spleen, brain, eyes, heart, and other tissues are the major organ sites involved in deep or visceral candidiasis. The principal risk factors predisposing to deeply invasive candidiasis are protracted courses of broad spectrum antibiotics, cytotoxic chemotherapy, corticosteroids, and vascular catheters.
Aspergillosis
Invasive aspergillosis most frequently involves the lungs and paranasal sinuses. This fungus may disseminate from the lungs to involve the brain, kidneys, liver, heart, and bones. The main portal of entry for aspergillosis is the respiratory tract, however, injuries to the skin may also introduce the organism into susceptible hosts.
Quantitative and functional defects in circulating neutrophils are key risk factors for development of invasive aspergillosis. For example, neutropenia due to cytotoxic chemotherapy and systemic corticosteroids are common predisposing factors for invasive aspergillosis.

Zygomycosis

Zygomycosis due to *Rhizopus, Rhizomucor, Absidia, Mucor* species, or other members of the class of Zygomycetes, also causes invasive sinopulmonary infections. An especially life-threatening form of zygomycosis (also known as mucormycosis), is known as the rhinocerebral syndrome, which occurs in diabetics with ketoacidosis. In addition to diabetic ketoacidosis, neutropenia and corticosteroids are other major risk factors for zygomycosis. *Aspergillus* spp and the Zygomycetes have a strong propensity for invading blood vessels.

Cryptococcosis

Cryptococcosis is most typically an opportunistic fungal infection that most frequently causes pneumonia and/or meningitis. Defective cellular immunity, especially that associated with the acquired immune deficiency syndrome, is the most common risk factor for developing cryptococcosis.

Phaeohyphomycosis

Phaeohyphomycosis is an infection by brown to black pigmented fungi of the cutaneous, superficial, and deep tissues, especially brain. These infections are uncommon, life-threatening, and occur in various immunocompromised states.

Hyalohyphomycosis

Hyalohyphomycosis is an opportunistic fungal infection caused by any of a variety of normally saprophytic fungi with hyaline hyphal elements.

For example, *Fusarium* spp. infects neutropenic patients to cause pneumonia, fungemia, and disseminated infection with cutaneous lesions.

Basic Concepts of Environmental Epidemiology

The epidemiology of dimorphic primary pathogens may be contrasted with that of the opportunistic fungal pathogens. The primary pathogens have a relatively well-defined geographic range of endemic infection in immunocompromised hosts. By comparison, the opportunistic fungi (e.g. *Aspergillus* spp.) are ubiquitously distributed with the frequency of infection being dependent upon a population of immunocompromised hosts. *Penicillium marneffei*, an opportunistic pathogen, appears to be geographically restricted to the East Asia, particularly Thailand and China.

Control and Treatment

Hospital-acquired fungal infections may be reduced by maintaining the lowest possible concentration of fungal spores in the ambient air of the institution. Ideally, a "spore-free" environment should be sought. Antifungal therapy, which is reviewed in depth elsewhere, is an area of intense investigation. New antifungal compounds will hopefully improve the efficacy and reduce toxicity of treatment of invasive fungal infections.

5. Diseases caused by aflatoxin and other fungal toxins and their impact on human health

One of the more important effects of postharvest decays of fruits and vegetables, especially of seeds, is the induction of diseases of animals and humans caused by the consumption of feeds and foods invaded by certain common fungi. These fungi produce toxic substances called **mycotoxins**. The diseases they cause are called **mycotoxicoses**.

Ergotism (St. Anthony's fire) of humans and animals, caused by eating ergotcontaining wheat and rye bread and feeds, and poisoning of humans from eating poisonous mushrooms, are classic examples of mycotoxicoses and have been known for a long time. The magnitude of the mycotoxin problem began to be appreciated during World War II, when it was noted that the consumption of moldy grain led to necroses of the skin, hemorrhage, liver and kidney failure, and death in numerous humans and animals.

Similar symptoms also appeared in horses fed moldy hay. In 1960, a large number of young turkeys died in England after they were fed contaminated peanut feed. That led to intensive research on mycotoxins, which established that they are a global problem. Mycotoxins pose an ever-present threat to the health of humans and animals. When they are present in relatively high concentrations they cause acute disease symptoms. Perhaps even more serious are the chronic effects on health and productivity caused by the constant presence of subacute dosages of mycotoxins in the food and feed consumed throughout the world, particularly in developing countries.

Most mycotoxicoses are caused by the common and widespread fungi Aspergillus, Penicillium, and Fusarium. Some may result in severe illness and death. Aspergillus and Penicillium produce their toxins mostly in stored

seeds and hay, but also on commercially processed foods and feeds, including meats, cheeses, and spices. Infection of seeds usually takes place in the field. Fusarium produces its toxins primarily on corn and other grains infected in the field or after they are stored.

Many other common fungi that infect agricultural commodities or contaminate food produce several mycotoxins.

Mycotoxins differ in their chemical formula, in the products in, and conditions under which they are produced, in their effects on various animals and humans, and in their degree of toxicity. Several different fungi, however, produce some of the same or closely related toxins.

ASPERGILLUS TOXINS — AFLATOXINS:

Aflatoxins are produced by *Aspergillus flavus* and several other species of *Aspergillus*. Aflatoxins are produced in infected cereal seeds and most legumes, but they often reach a rather low and probably nontoxic concentration (about 50 ppb). During some years, a rather high percentage (30% or more) of the corn harvest over large areas contains more than 100 ppb aflatoxin, which is five times that allowed in food for humans and in feed for sensitive animals such as chickens.

However, in peanuts, cottonseed, fishmeal, Brazil nuts, and probably other seeds or nuts grown in warm and humid regions, aflatoxin is produced at high concentrations (up to 1000 ppb or more) and causes mostly chronic or occasionally acute mycotoxicoses in humans and domestic animals. Aflatoxins exist in a variety of derivatives with varying effects. Some of these toxins, when ingested with the feed by dairy cattle, are excreted in the milk in still toxic form.

The symptoms of mycotoxicoses caused by aflatoxin in animals, and presumably humans, vary widely with the particular toxin and animal species, dosage, age of the animal, and so on. Young ducklings and turkeys fed high dosages of aflatoxin become severely ill and die.

Pregnant cows, calves, fattening pigs, mature cattle, and sheep fed low dosages of aflatoxin over long periods develop weakening, intestinal bleeding, debilitation, reduced growth, nausea, refusal of feed, predisposition to other infectious diseases, and may abort. Moreover, most of the ingested aflatoxin is taken up by the liver, and, in some experiments, animals given feed containing even less than the permissible amount of aflatoxin (20 ppb) almost invariably developed liver cancer.

FUSARIUM TOXINS

Three groups of toxins, zearalenones, trichothecenes, and fumonisins, are produced by several species of *Fusarium*, primarily in moldy corn.

Deoxynivalenol, also known as **vomitoxin** or **DON** is produced by the fungus *Gibberella zeae* (anamorph *Fusarium graminearum*), the cause of Gibberella ear rot of corn and of head blight (scab) of wheat. The mycotoxin at first causes reduced feeding by the animals and, thereby, slower gain or loss of weight. At higher concentrations of the mycotoxin, the animals are induced to vomit and totally refuse to eat.

Zearalenones seem to be most toxic to swine, in which they cause abnormalities and degeneration of the reproductive system, the so-called estrogenic syndrome. Female swine fed zearalenone-containing feed develop swollen vulvas bearing bleeding lesions and atrophying, nonfunctioning ovaries. They are susceptible to abor tion, and piglets that are born are small and weak. Male swine show signs of feminization, namely atrophy of the testes and enlargement of the mammary glands.

Fumonisins are produced by ***Fusarium moniliforme***, which causes Fusarium ear rot of corn that affects as much as 90% of the corn fields. Fumonisins are the cause of blind staggers (equine leukoencephalomalacia) in horses, donkeys and mules, pulmonary edema in swine, and, possibly, cancer in humans.

Trichothecins (or **trichothecenes**), of which there are more than 100, are produced by species of *Fusarium* and by several other fungi. They are most toxic when fed to swine, in which they cause, among other symptoms, listlessness or inactivity, degeneration of the cells of the bone marrow, lymph nodes, and intestines, diarrhea, bleeding, and death. Other animals, however, such as cows, chicks, and lambs, are also affected.

Other Aspergillus Toxins and Penicillium Toxins

In addition to aflatoxins, species of *Aspergillus* also produce other toxins in infected grains. The same or similar toxins are also produced in grains infected by species of *Penicillium*. The most important such toxins are **ochratoxins**, which cause degeneration and necrosis of the liver and kidney, along with several other symptoms, in domestic animals. Some ochratoxins can persist in the meat of animals fed contaminated feed and can be transmitted to humans through the food chain.

Yellowed-rice toxins, primarily citreoviridin, citrinin, and luteoskyrin, are all produced by species of *Penicillium* growing in stored rice, barley, corn, and dried fish. They cause toxicoses associated with various diseases, nervous and circulatory disorders, and degeneration of the kidneys and liver.
Tremorgenic toxins cause marked body tremors and excessive discharge of urine, followed by convulsive seizures that often end in death. They are produced by species of both *Aspergillus* and *Penicillium* infecting foodstuffs in storage and also in refrigerated foods, grains, and cereal products. **Patulin** is produced by *Penicillium* and *Aspergillus*. It causes edema and bleeding in lungs and brain, damage to kidneys, and paralysis of motor nerves and it also induces cancer in higher organisms.
It is commonly found to occur naturally in foodstuffs such as fruit or juices made with fruit partly infected with *Penicillium*, in naturally molded bread and bakery products, and in most commercial apple products. Thus, patulin may constitute a serious health hazard for humans as well as for animals.
Ergotism is the oldest known mycotoxicosis. It is caused by several toxic substances contained in the sclerotia (ergots) of the ergot fungus (*Claviceps*) when they contaminate grain crops, such as rye, barley, sorghum, millet, wheat, and wild grasses, and are ingested by humans and animals. Ergotism is expressed as convulsions and limb swellings, followed by gangrene of body extremities and of burning sensations (St. Anthony's fire). Ground-up ergots have been used in the past to stop heavy bleeding, as happens, e.g., during labor or accidents.
Fescue toxicosis affects cattle and horses feeding on plants of the perennial grass tall fescue infected systemically with the fungus *Acremonium*. The fungus is an endophyte growing internally through the plant without invading its cells. The fungus actually seems to make the infected plants more resistant to stress, particularly drought. Horses eating tall fescue plants infected with the fungus show only reproductive disorders. Cattle feeding on such plants, in addition to reduced calving and lower milk production, show reduced weight gains, elevated body temperature, and rough hair coat; moreover, as in ergotism, feet or other body extremities may develop gangrene and drop off ("fescue foot").

6. Mycetismus and mushroom poisoning

Mushroom poisoning (also known as mycetismus) refers to harmful effects from ingestion of toxic substances present in a mushroom. It is common for the local population to consume wild mushrooms that grow in forests. Deadly toxins are amatoxin produced by *Amanita* spp. causes liver damage, gyrometrine by *Gyremitra* spp. causes neurotoxicity gastrointestinal upset; non- lethal toxins are psilocybin & psilocin produced by *Psilocybe* spp. causes CNS arousal and hallucinations, coprine by *Coprinus* spp. causes illness.
Mushroom poisoning (also known as **mycetism** or **mycetismus**) refers to harmful effects from ingestion of toxic substances present in a mushroom. These symptoms can vary from slight gastrointestinal discomfort to death. The toxins present are secondary metabolites produced by the fungus. Mushroom poisoning is usually the result of ingestion of wild mushrooms after misidentification of a toxic mushroom as an edible species. The most common reason for this misidentification is close resemblance in terms of colour and general morphology of the toxic mushrooms species with edible species.
To prevent mushroom poisoning, mushroom gatherers familiarize themselves with the mushrooms they intend to collect as well as with any similar-looking toxic species. In addition, edibility of mushrooms may depend on methods of preparation for cooking. The edibility or toxicity of some species varies with geographic location.
Folk traditions:
Many folk traditions concern the defining features of poisonous mushrooms. However, there are no general identifiers for poisonous mushrooms, so such traditions are unreliable. Guidelines to identify particular mushrooms exist, and will serve if one knows which mushrooms are toxic.
Examples of **erroneous** folklore "rules" include:

- "Poisonous mushrooms are brightly colored." – Indeed, fly agaric, usually bright-red to orange or yellow, is narcotic and hallucinogenic, although no human deaths have been reported. The deadly destroying angel, in contrast, is an unremarkable white. The deadly *Galerinas* are brown. Some choice edible species (chanterelles, *Amanita caesarea*, *Laetiporus sulphureus*, etc.) are brightly colored, whereas most poisonous species are brown or white.

- "Insects/animals will avoid toxic mushrooms." – Fungi that are harmless to invertebrates can still be toxic to humans; the death cap, for instance, is often infested by insect larvae.
- "Poisonous mushrooms blacken silver." – None of the known mushroom toxins have a reaction with silver.
- "Poisonous mushrooms taste bad." – People who have eaten the deadly *Amanitas* have reported that the mushrooms tasted quite good.
- "All mushrooms are safe if cooked/parboiled/dried/pickled/etc." – While it is true that some otherwise-inedible species can be rendered safe by special preparation, many toxic species cannot be made toxin-free. Many fungal toxins are not particularly sensitive to heatand so are not broken down during cooking; in particular, α-amanitin, the poison produced by the death cap (*Amanita phalloides*) and others of the genus, is not denatured by heat.
- "Poisonous mushrooms will turn rice red when boiled." – A number of Laotian refugees were hospitalized after eating mushrooms (probably toxic *Russula* species) deemed safe by this folklore rule and this misconception cost at least one person her life.
- "Poisonous mushrooms have a pointed cap. Edible ones have a flat, rounded cap." – The shape of the mushroom cap does not correlate with presence or absence of mushroom toxins, so this is not a reliable method to distinguish between edible and poisonous species. Death cap, for instance, has a rounded cap when mature.
- "Boletes are, in general, safe to eat." – It is true that, unlike a number of *Amanita* species in particular, in most parts of the world, there are no known deadly varieties of the genus *Boletus*, which reduces the risks associated with misidentification. However, mushrooms like the Devil's bolete are poisonous both raw and cooked and can lead to strong gastrointestinal symptoms, and other species like the lurid bolete require thorough cooking to break down toxins. As with other mushroom *genera*, proper caution is, therefore, advised in determining the correct species.

Causes:

New species of fungi are continuing to be discovered, with an estimated number of 800 new species registered annually. This, added to the fact that many investigations have recently reclassified some species of mushrooms from edible to poisonous has made older classifications insufficient at describing what now is known about the different species of fungi that are harmful to humans. Thus, contrary to what older registers state, it is now thought that of the approximately 100,000 known fungi species found worldwide, about 100 of them are poisonous to humans. However, by far the majority of mushroom poisonings are not fatal, and the majority of fatal poisonings are attributable to the *Amanita phalloides* mushroom. A majority of these cases are due to mistaken identity. This is a common occurrence with *A. phalloides* in particular, due to its resemblance to the Asian paddy-straw mushroom, *Volvariella volvacea*. Both are light-colored and covered with a universal veil when young.

*Amanita*s can be mistaken for other species, as well, in particular when immature. On at least one occasion they have been mistaken for *Coprinus comatus*. In this case, the victim had some limited experience in identifying mushrooms, but did not take the time to correctly identify these particular mushrooms until after he began to experience symptoms of mushroom poisoning.

The author of *Mushrooms Demystified*, David Arora cautions puffball-hunters to beware of *Amanita* "eggs", which are *Amanita*s still entirely encased in their universal veil. *Amanita*s at this stage are difficult to distinguish from puffballs. Foragers are encouraged to always cut the fruiting bodies of suspected puffballs in half, as this will reveal the outline of a developing *Amanita* should it be present within the structure.

A majority of mushroom poisonings in general are the result of small children, especially toddlers in the "grazing" stage, ingesting mushrooms found in the lawn. While this can happen with any mushroom, *Chlorophyllum molybdites* is often implicated due to its preference for growing in lawns. *C. molybdites* causes severe gastrointestinal upset but is not considered deadly poisonous.

A few poisonings are the result of misidentification while attempting to collect hallucinogenic mushrooms for recreational use. In 1981, one fatality and two hospitalizations occurred following consumption of *Galerina autumnalis*, mistaken for a *Psilocybe* species. *Galerina* and *Psilocybe* species are small, brown, and sticky, and can be found growing together. However, *Galerina*

contains amatoxins, the same poison found in the deadly *Amanita* species. Another case reports kidney failure following ingestion of *Cortinarius orellanus*, a mushroom containing orellanine.

It is natural that accidental ingestion of hallucinogenic species also occurs, but is rarely harmful when ingested in small quantities. Cases of serious toxicity have been reported in small children.[14] *Amanita pantherina*, while containing the same hallucinogens as *Amanita muscaria* (e.g., ibotenic acid and muscimol), has been more commonly associated with severe gastrointestinal upset than its better-known counterpart.

Although usually not fatal, *Omphalotus* spp., "Jack-o-lantern mushrooms," are another cause of sometimes significant toxicity. They are sometimes mistaken for chanterelles. Both are bright- orange and fruit at the same time of year, although *Omphalotus* grows on wood and has true gills rather than the veins of a *Cantharellus*. They contain toxins known as illudins, which causes gastrointestinal symptoms.

Bioluminescent species are generally inedible and often mildly toxic.

Clitocybe dealbata, which is occasionally mistaken for an oyster mushroom or other edible species contains muscarine.

Toxicities can also occur with collection of morels. Even true morels, if eaten raw, will cause gastrointestinal upset. Typically, morels are thoroughly cooked before eating. *Verpa bohemica*, although referred to as "thimble morels" or "early morels" by some, have caused toxic effects in some individuals. *Gyromitra* spp., "false morels", are deadly poisonous if eaten raw. They contain a toxin called gyromitrin, which can cause neurotoxicity, gastrointestinal toxicity, and destruction of the blood cells. The Finns consume *Gyromitra esculenta* after parboiling, but this may not render the mushroom entirely safe, resulting in its being called the "fugu of the Finnish cuisine".

A more unusual toxin is coprine, a disulfiram-like compound that is harmless unless ingested within a few days of ingesting alcohol. It inhibits aldehyde dehydrogenase, an enzyme required for breaking down alcohol. Thus, the symptoms of toxicity are similar to being hung over— flushing, headache, nausea, palpitations, and, in severe cases, trouble breathing. *Coprinus* species, including *Coprinopsis atramentaria*, contain coprine. *Coprinus comatus* does not, but it is best to avoid mixing alcohol with other members of this genus.

Recently, poisonings have also been associated with *Amanita smithiana*. These poisonings may be due to orellanine, but the onset of symptoms occurs in 4 to 11 hours, which is much quicker than the 3 to 20 days normally associated with orellanine.

Paxillus involutus is also inedible when raw, but is eaten in Europe after pickling or parboiling. However, after the death of the German mycologist Dr Julius Schäffer, it was discovered that the mushroom contains a toxin that can stimulate the immune system to attack its own red blood cells. This reaction is rare, but can occur even after safely eating the mushroom for many years. Similarly, *Tricholoma equestre* was widely considered edible and good, until it was connected with rare cases of rhabdomyolysis.

In the fall of 2004, thirteen deaths were associated with consumption of *Pleurocybella porrigens* or "angel's wings". In general, these mushrooms are considered edible. All the victims died of an acute brain disorder, and all had pre-existing kidney disease. The exact cause of the toxicity was not known at this time and the deaths cannot be definitively attributed to mushroom consumption.

However, mushroom poisoning is not always due to mistaken identity. For example, the highly toxic ergot *Claviceps purpurea*, which grows on rye, is sometimes ground up with rye, unnoticed, and later consumed. This can cause devastating, even fatal effects, which is called ergotism.

Cases of idiosyncratic or unusual reactions to fungi can also occur. Some are probably due to allergy, others to some other kind of sensitivity. It is not uncommon for an individual person to experience gastrointestinal upset associated with one particular mushroom species or genus.

Poisonous mushrooms contain a variety of different toxins that can differ markedly in toxicity. Symptoms of mushroom poisoning may vary from gastric upset to life-threatening organ failure resulting in death. Serious symptoms do not always occur immediately after eating, often not until the toxin attacks the kidney or liver, sometimes days or weeks later.

The most common consequence of mushroom poisoning is simply gastrointestinal upset. Most "poisonous" mushrooms contain gastrointestinal irritants that cause vomiting and diarrhea (sometimes requiring hospitalization), but usually no long-term damage. However, there are a number of recognized mushroom toxins with specific, and sometimes deadly, effects:

Toxin	**Toxicity**	**Effects**
Alpha-amanitin	Deadly	Causes often fatal liver damage 1–3 days after ingestion. Principal toxin in the death cap.
Phallotoxin	Non-lethal	Causes extreme gastrointestinal upset. Found in various mushrooms.
Orellanine	Deadly	Redox cycler similar to paraquat. Causes kidney failure within 3 weeks after ingestion. Principal toxin in genus *Cortinarius*.
Muscarine	Potentially deadly	Causes SLUDGE syndrome. Found in various mushrooms. Antidote is atropine

Monomethylhydrazine(MMH)	Deadly	Causes brain damage, seizures, gastrointestinal upset, and hemolysis. Metabolic poison. Principal toxin in genus *Gyromitra*. Antidote is large doses of intravenous pyridoxinehydrochloride[22]
Coprine	Non-lethal	Causes illness when consumed with alcohol. Principal toxin in genus *Coprinus*.
Ibotenic acid	Potentially deadly	Excitotoxin. Principal toxin in *Amanita muscaria*, *A. pantherina*, and *A. gemmata*.
Muscimol	Psychoactive	Causes CNS depression and hallucinations. Principal toxin in *Amanita muscaria*, *A. pantherina*, and *A. gemmata*.
Psilocybin and psilocin	Psychoactive	Causes CNS arousal and hallucinations. Principal Effects in psilocybin mushrooms, many of which belonging to the genus *Psilocybe* (often used recreationally).
Arabitol	Non-lethal	Causes diarrhea in some people.
Bolesatine	Non-lethal	Causes gastrointestinal irritation, vomiting, nausea.
Ergotamine	Deadly	Affects the vascular system and can lead to loss of limbs and/or cardiac arrest. Found in genus *Claviceps*.

The period of time between ingestion and the onset of symptoms varies dramatically between toxins, some taking days to show symptoms identifiable as mushroom poisoning.

- **Alpha-amanitin**: For 6–12 hours, there are no symptoms. This is followed by a period of gastrointestinal upset (vomiting and profuse, watery diarrhea). This stage is caused primarily by the phallotoxins and typically lasts 24 hours. At the end of this second stage is when severe liver damage begins. The damage may continue for another 2–3 days. Kidney damage can also occur. Some patients will require a liver transplant. Amatoxins are found in some mushrooms in the genus *Amanita*, but are also found in some species of *Galerina* and *Lepiota*. Overall, mortality is between 10 and 15 percent. Recently, *Silybum marianum* or blessed milk thistlehas been shown to protect the liver from amanita toxins and promote regrowth of damaged cells.
- **Orellanine**: This toxin causes no symptoms for 3–20 days after ingestion. Typically around day 11, the process of kidney failure begins, and is usually symptomatic by day 20. These symptoms can include pain in the area of the kidneys, thirst, vomiting, headache, and fatigue. A few species in the very large genus *Cortinarius* contain this toxin. People having eaten mushrooms containing orellanine may
- experience early symptoms as well, because the mushrooms often contain other toxins in addition to

orellanine. A related toxin that causes similar symptoms but within 3–6 days has been isolated from *Amanita smithiana* and some other related toxic *Amanita*s.

- **Muscarine**: Muscarine stimulates the muscarinic receptors of the nerves and muscles. Symptoms include sweating, salivation, tears, blurred vision, palpitations, and, in high doses, respiratory failure. Muscarine is found in mushrooms of the genus *Omphalotus*, notably the Jack o' Lantern mushrooms. It is also found in *A. muscaria*, although it is now known that the main effect of this mushroom is caused by ibotenic acid. Muscarine can also be found in some *Inocybe* species and *Clitocybe* species, in particular *Clitocybe dealbata*, and some red-pored *Boletes*.
- **Gyromitrin**: Stomach acids convert gyromitrin to monomethylhydrazine (MMH), a compound employed in rocket fuel. It affects multiple body systems. It blocks the important neurotransmitter GABA, leading to stupor, delirium, muscle cramps, loss of coordination,[23] tremors, and/or seizures. It causes severe gastrointestinal irritation, leading to vomiting and diarrhea. In some cases, liver failure has been reported. It can also cause red blood cells to break down, leading to jaundice, kidney failure, and signs of anemia. It is found in mushrooms of the genus *Gyromitra*. A gyromitrin-like compound has also been identified in mushrooms of the genus *Verpa*.
- **Coprine**: Coprine is metabolized to a chemical that resembles disulfiram. It inhibits aldehyde dehydrogenase (ALDH), which, in general, causes no harm, unless the person has alcohol in their bloodstream while ALDH is inhibited. This can happen if alcohol is ingested shortly before or up to a few days after eating the mushrooms. In that case the alcohol cannot be completely metabolized, and the person will experience flushed skin, vomiting, headache, dizziness, weakness, apprehension, confusion, palpitations, and sometimes trouble breathing. Coprine is found mainly in mushrooms of the genus *Coprinus*, although similar effects have been noted after ingestion of *Clitocybe clavipes*.
- **Ibotenic acid**: Decarboxylates into muscimol upon ingestion. The effects of muscimol vary, but nausea and vomiting are common. Confusion, euphoria, or sleepiness are possible. Loss of muscular coordination, sweating, and chills are likely. Some people experience visual distortions, a feeling of strength, or delusions. Symptoms normally appear after 30 minutes to 2 hours and last for several hours. *A. muscaria*, the "Alice in Wonderland" mushroom, is known for the hallucinatory experiences caused by muscimol, but *A. pantherina* and *A. gemmata* also contain the same compound. While normally self-limiting, fatalities have been associated with *A. pantherina*, and consumption of a large number of any of these mushrooms is likely to be dangerous.
- **Psilocybin**: Dephosphorylates into the psychoactive psilocin upon ingestion, which acts as a psychedelic drug. Symptoms begin shortly after ingestion. The effects can include euphoria, visual and religious hallucinations, and heightened perception. However, some persons experience fear, agitation, confusion, and schizophrenia-like symptoms. All symptoms generally pass after several hours. Some (though not all) members of the genus *Psilocybe* contain psilocybin, as do some *Panaeolus*, *Copelandia*, *Conocybe*, *Gymnopilus*, and others. Some of these mushrooms also contain baeocystin, which has effects similar to psilocin.
- **Arabitol**: A sugar alcohol, similar to mannitol, which causes no harm in most people but causes gastrointestinal irritation in some. It is found in small amounts in oyster mushrooms, and considerable amounts in *Suillus* species and *Hygrophoropsis aurantiaca* (the "false chanterelle").

Some mushrooms contain less toxic compounds and, therefore, are not severely poisonous. Poisonings by these mushrooms may respond well to treatment. However, certain types of mushrooms, such as the Amanitas, contain very potent toxins and are very poisonous; so even if symptoms are treated promptly mortality is high. With some toxins, death can occur in a week or a few days.

7. Fungi as allergen

Introduction:

Fungi have interminably affected humans in various ways, including plant and animal diseases, and superficial and systemic mycoses in humans. Fungi are also involved in many hypersensitivity diseases and toxin-induced

health hazards in humans. Diseases associated with inhalation of fungal spores include toxic pneumonia, hypersensitivity pneumonitis, tremors, chronic fatigue syndrome, and kidney failure.

Allergy is one of the most common ailments, affecting more than 20% of the population of the industrialized world. Allergy develops upon sensitization with extraneous proteins from various sources. The allergens constitute foreign proteins from animals and plant sources. Exposure to allergens results in the induction of IgE antibody, which is thought to be the central player in the induction of allergy. The exposure to allergens is by inhalation, contact, ingestion, or injection. The major allergic manifestations induced by fungi are asthma, rhinitis, allergic bronchopulmonary mycoses, and hypersensitivity pneumonitis.

Clinically, the presenting symptoms are sneezing, nasal discharge, coughing, wheezing, and shortness of breath, with evidence of reversible airway obstruction, urticaria,
angioedema, and anaphylaxis.

Mold allergy constitutes 25% to 30% of all allergic asthma cases. Patients sensitized to the allergens demonstrate immunologic and inflammatory responses. The immunologic responses include both humoral and cellmediated responses. Lung inflammation resulting from antigen exposure leads to airway remodeling and hyperre activity.

The mechanism involved in this disease is complex and is frequently dependent on the nature of allergens, underlying diseases, concentration of allergens, duration of exposure, and genetic predisposition of the host.

Fungi and Their Allergens:

Fungi are eukaryotic, nonchlorophyllous, unicellular to multicellular organisms that are present in all types of environments. Although most fungi are saprophytic, a few of them are parasitic in humans and animals. The concentration of fungal spores in the environment depends on many factors, including climate and vegetation. The types of fungi present indoors and their prevalence depends on moisture, ventilation, presence or absence of carpets, pets, and houseplants. The fungal spore count indoors and outdoors varies considerably depending on these factors.

The indoor fungi constitute transient spores from outdoor air and those growing indoors. *Alternaria* and *Drechslera* are major outdoor molds, but have been reported in some exclusive indoor environments. Similarly, *Aspergillus* and *Penicillium* are found indoors and outdoors, albeit less commonly reported outdoors. Other fungi, such as *Cladosporium*, *Epicoccum*, and *Fusarium*, have been reported in both indoor and outdoor environments. Aerobiologic studies of indoor and outdoor fungal flora are necessary for evaluating the allergic status of patients. The role of fungi in allergy has been established based on clinical histories of patients and in vivo and in vitro diagnostic tests using specific fungal antigens.

A number of allergenic fungi have been identified. Those most frequently encountered are listed in Table 1. The most important among these are *Alternaria*, *Aspergillus*, *Penicillium*, *Cladosporium*,
and *Fusarium*. The extracts from these fungi have been used in skin testing patients and for demonstrating IgE antibody by in vitro assays.

Fungal allergens are usually proteins, polysaccharides, or glycoproteins. These molecules, on interaction with the immune system, produce IgE antibody in atopic individuals.

More than 300 allergens from plants, animals, and fungi have been reported, and some show cross-reactivity resulting from the chemical nature and sequence similarities they share. Based on the physicochemical characteristics, the allergens associated with disease have been classified as:

1) hydrolytic enzymes, such as proteases; **2)** nonhydrolytic enzymes, such as enolases; **3)** inhibitors,
such as trypsin and amylase inhibitors; **4)** transport proteins, such as lipocalins; and **5)** regulatory proteins, such as heat-shock proteins. In addition, allergens with diverse chemical nature and unknown biochemical function and activities constitute the majority of the allergens.

Recombinant Allergens

The crude extracts from mycelia, spores, and culture filtrates of fungi contain considerable amounts of nonantigenic components, including potent toxins. For effective diagnosis of fungal allergy, well-characterized, pure, and standardized antigens are essential. The complexity and the variability of the
fungal allergens have complicated the process of obtaining purified and standardized antigens. Considerable research has been directed to purify relevant antigens from fungi.

Table 1. Fungi associated with IgE-mediated allergy

Zygomycetes	Ascomycetes	Basidiomycetes	Deuteromycetes (fungi imperfecti)		Yeasts
Mucor	*Chaetomium*	*Agaricus*	*Acremonium*	*Helminthosporium*	*Saccharomyces*
Rhizopus	*Claviceps*	*Boletus*	*Alternaria*	*Neurospora*	*Candida*
	Daldinia	*Calvatia*	*Aspergillus*	*Nigrospora*	*Rhodotorula*
	Didymella	*Coprinus*	*Aureobasidium*	*Paecilomyces*	
	Erysiphe	*Ganoderma*	*Botrytis*	*Penicillium*	
	Eurotium	*Lentinus*	*Cephalosporium*	*Phoma*	
	Microsphaera	*Merulius*	*Chrysosporium*	*Scopulariopsis*	
		Pleurotus	*Cladosporium*	*Stachybotrys*	
		Psilocybe	*Coniosporium*	*Stemphylium*	
		Puccinia	*Curvularia*	*Torula*	
		Tilletia	*Cylindrocarpon*	*Trichoderma*	
		Urocystis	*Drechslera*	*Trichophyton*	
		Ustilago	*Epicoccum*	*Trichothecium*	
			Fusarium	*Ulocladium*	
			Gliocladium	*Wallemia*	

These include fractionation methods, including size exclusion chromatography, affinity chromatography using monoclonal antibodies, partition chromatography, electrophoresis, isoelectric focusing, and chromatofocusing. None of these methods has yielded relevant allergens useful in the diagnosis of mold allergy. However, recently, using molecular biology to clone and express relevant standardizable antigens from mold has gained significant progress.

Currently, many allergens have been cloned, and the proteins expressed. Such allergens are currently being evaluated for standardization. Many techniques have been used to clone complementary DNA (cDNA), encoding allergens and antigens.

Approximately 70 such allergens have been reported from various fungi and approved by the International Allergen Nomenclatural Committee. Most of them have been expressed by cloned genes from the various fungi, and their amino acid sequences have been ascertained. The T- and Bcell epitopes of some of these allergens have been investigated and mapped. Currently, cloned allergens are available for immunologic assays from *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, yeast, and a few *Basidiomycetes*.

The cloning of allergens consists of the isolation of messenger RNA (mRNA) from 72 to 96-hour-old actively growing cultures. The total RNA from the mycelium can be extracted with guanidium isothiocyanate or any of the new commercial preparations available.

The mRNA can be purified using oligo (dT) cellulose chromatography. The purified mRNA was then reverse transcribed to obtain cDNA. Of the several methods available to clone the gene from the cDNA library, the amplification of the desired DNA fragments by polymerase chain reaction (PCR) is the most efficient and frequently used method.

Although this method is faster than other cloning methods, it has the inherent limitation that the sequence of the allergen must be known. Hence, the strategy is to construct an expression library that can be screened using sera-containing, allergen-specific IgE antibody from patients. Libraries constructed on bacteriophage lambda vectors must be used to immobilize the particles to a solid phase before testing the reactivity with the sera. The allergens encoding genes will be processed using lambda coding, and the protein will be expressed. Recently, phage-display libraries were also constructed to aid in the understanding of the immune responses of the allergens expressed.

Among the various vectors used for expression of fungal allergens, the pET (pET expression protocol; Novagen, Madison, WI) has been the most favored prokaryotic expression system. This system utilizes the T7 polymerase-based expression of the proteins in *Escherichia coli*. The expression of the target protein is induced by a T7 RNA polymerase in the bacterial host, *E. coli*. The allergen expressed can be purified using a histidine tag at the N- or C-terminal end of the protein. The expressed allergen can be purified using single step Ni-agarose affinity chromatography. Most of the allergens from *Aspergillus*, *Penicillium*, *Alternaria*, and *Cladosporium* have been cloned in high-expression prokaryotic systems to obtain recombinant allergens for in vitro immunoassays and skin tests. Most of these allergens showed similar skin-test reactivity and in vitro IgE binding with native allergens.

The IgE binding of these allergens is frequently directed to the protein parts of the molecules, and, hence, posttranslational modification of the molecules is not a significant factor in the IgE binding.
For antigens with posttranslational modification, it is possible to express the clones in eukaryotic systems. One of the expression systems that has been used for high-level expression of recombinant proteins is the yeast *Picchia pastoris*. The target gene in *Picchia* is under the control of an alcohol oxidase promoter. Many *Aspergillus fumigatus* proteins and glycoproteins have been expressed using this system. Recently, a phage display library was developed in which the gene products were displayed on the surface of filamentous phages. Compared with screening of lambda libraries, the screening of cDNA libraries displayed on phage surfaces has several advantages. The phages can multiply in a suitable host, and, hence, can be used to infect *E. coli*, and thereby amplify the phages with affinity to the ligand. Phages with protein having specific binding to the desired ligand can be enriched by successive rounds of phage growth and selection. Such concentrated phages can be used in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot for analysis and evaluation.
The pJuFo phagemid is a useful cloning vector for phage display library. Numerous *Aspergillus*, *Alternaria*, and *Cladosporium* allergens displayed on phages have been constructed, and their usefulness in IgE recognition has been ascertained.

Allergens from Fungi

Although there are many allergenic molds capable of causing **Type 1 hypersensitivity**, the most frequently involved fungi are *Aspergillus*, *Penicillium*, *Cladosporium*, and *Alternaria*.
Significant allergens from these and other fungi have been cloned and the protein expressed. Because these four genera represent the most common fungal allergens, they are considered here in detail.

***Aspergillus* species**

Aspergillus species are saprophytic fungi widely distributed in nature and are associated with a number of human diseases. Several recombinant allergens from *A. fumigatus* have been identified and purified from cDNA and phage display libraries of *A. fumigatus*. Most of these proteins showed specific binding to IgE from asthmatic and allergic bronchopulmonary aspergillosis (ABPA) patients. The molecular structures cover a wide range of functional proteins, including toxins, enzymes, heat shock proteins, and several unique proteins lacking homology to any of the known proteins. Asp
f1, a ribotoxin that inhibits protein translation, was found to be toxic to Epstein-Barr virus (EBV)–transformed and phytohemagglutinin (PHA)–stimulated peripheral blood mononuclear cells (PBMCs). This allergen showed positive skin test reactivity in 80% of ABPA patients and 50% of
asthmatic patients. However, because of the high toxicity and reactivity with skin test positive asthmatics and some normals, the usefulness of this allergen in diagnosis is questioned. This allergen demonstrated 13 linear epitopes binding to IgE. Asp f 1 also showed Th1- and Th2-specific epitopes when studied in a murine model of allergic aspergillosis.
Aspergillus fumigatus major allergen Asp f2 is a 310 amino acid protein that has a fibrinogen-binding property. The gene encoding Asp f2 has two introns of 83 and 52 nucleotides and four potential glycosylation sites. There was strong sequence homology shown with Asp nd 1, a fibrinogen binding
protein from *Aspergillus nidulans*. Asp f2 also showed homology with pH-regulatory proteins from *Candida albicans*. The native protein purified using monoclonal antibody measured 37 kDa, whereas the *E. coli* expressed proteins measuring only 32 to 35 kDa. However, when the protein was expressed in the eukaryotic host, *P. pastoris,* it measured 70 to 75 kDa. Asp f2 reacted strongly with IgE antibody from ABPA patients. Approximately 87% of ABPA patients from the United States and Switzerland carried IgE antibody to Asp f2 in their sera, whereas only very few skin test positive asthmatics and normal controls carried the IgE antibody. The *Picchia*-expressed proteins showed
more reactivity with IgG antibody in the sera of patients with ABPA and asthma. Deletion mutants of Asp f2 varied in their binding to IgE antibody from ABPA and non-ABPA controls. IgE-binding epitope analysis demonstrated nine linear IgE-binding regions spanning the entire sequence. By selective deletion of amino acids, a few conformational IgE-binding epitopes also have been identified.
Recent studies confirm that one of these epitopes also functions as a T-cell epitope. Of the eight cysteine residues in the molecule, the two cysteines at 204 and 267 positions were found to be essential for IgE binding.

Asp f3, a 18.5-kDa protein with homology to *Candida boidinii* peroxisomal protein showed skin test reactivity and in vitro reactivity with serum IgE of ABPA patients. Recently
it was found that this allergen is valuable in the serodiagnosis of ABPA. More than 80% of ABPA patients carried IgE to Asp f3, whereas only a few asthmatics carried IgE.
The epitope and structure analysis indicate that the 10 Nterminal amino acids and 143 to 150 C-terminal amino acids of Asp f3 are important in binding IgE in the sera of ABPA patients. Epitope analysis demonstrated seven IgE-binding regions spanning the entire sequence of Asp f3. Asp f4, an intracellular protein allergen with unknown biochemical function, showed strong reactivity with IgE from sera of cystic fibrosis (CF) patients with asthma or ABPA. Asp f5, a metalloproteinase, demonstrated skin test reactivity in more than 70% of allergic asthmatics without ABPA, whereas Asp f 5 showed reactivity in more than 90% of ABPA patients. Although its diagnostic significance
is not clear, Asp f5 might be used as an indicator for sensitization to *Aspergillus*. Asp f6, manganese superoxide dismutase (MNSOD), has a strong homology to MNSODs of human, bovine, fruit fly, gum tree, yeast, and *E. coli*. Asp f6 reacted positively in skin tests and bound to IgE in the sera of ABPA patients. Recently its usefulness as a significant allergen for detecting IgE antibody in the sera of ABPA patients has been reported.
Asp f8 has homology with acidic P2 ribosomal proteins isolated from *Alternaria* and *Cladosporium*. This allergen reacted with IgE antibody in the sera of patients and showed skin test reactivity in ABPA patients. Asp f10, an aspartic protease, showed only low prevalence of IgE binding in ABPA patients. Asp f11, a cyclophilin that has homology with similar proteins from *Malassezia furfur*, reacted with IgE from 50% of ABPA patients. The heat shock protein Asp f12 has homologous counterparts in *C. albicans, Saccharomyces, Trypanasoma*, housefly, mouse, and humans because of the extremely conserved *HSP* gene.
Asp f16, which has no known biologic function, showed strong binding to IgG from ABPA patients. This antigen showed sequence homology with Asp f9 and a membrane protein from *Saccharomyces*. Other minor allergens isolated from *A. fumigatus* and related *Aspergillus* spp. also bound to the IgE antibody from ABPA and allergic asthma patients. Several of these *Aspergillus fumigatus* allergens also exhibited high sequence homologies with known, functional proteins and enzymes. Alkaline serine proteinases with allergenic properties such as Asp f13, Asp fl13, and Aspo13 from *A. fumigatus*, *Aspergillus flavus*, and *Aspergillus oryzae*, respectively, have been reported. Similar serine proteinases, Pen b 13, Pen c 13, and Pen ch 13, with sequence homology to *Aspergillus* proteinases, have also been identified from various species of *Penicillium*.
Recently, another group of homologous vacuolar serine proteinases from *Aspergillus* and *Penicillium*, Aspf 18, Aspn 18, Pench 18, and Peno 18, with conserved sequence, have been reported. *A. flavus* extracts demonstrated IgE-antibody binding in 44% of asthmatic patients studied by immuno blotting. Additionally, a 34-kDa alkaline serine proteinase, Asp fl13, which demonstrates significant IgE antibody binding, was purified, and its enzyme activity was ascertained.

***Penicillium* species**

Species belonging to the genus *Penicillium* are prevalent indoor fungi. Inhalation of *Penicillium* spores in quantities comparable with those encountered by natural exposure can induce both immediate and late asthma in sensitive persons. Among more than 100 different *Penicillium* species, *Penicillium citrinum*, together with *Penicillium chrysogenum* (*Penicillium notatum*), *Penicillium oxalicum*, *Penicillium brevicompactum*, and *Penicillium spinulosum*, were the five most frequently recovered species of *Penicillium* in the United States, whereas *P. citrinum* was the most prevalent
Penicillium species reported from Taiwan.
Approximately 12 antigens from *P. citrinum* and 11 antigens from *P. chrysogenum* have been shown to react with IgE from patients' sera by immunoblotting. Recently, several *Penicillium* allergens have also been characterized at the molecular level. Among the *Penicillium* allergens, the 32 to 34 kDa alkaline and/or vacuolar serine proteases were identified as the major allergens of *P. citrinum*, *P. brevicompactum*, *P. chrysogenum*, and *P. oxalicum*. Immunoblotting data showed that IgE antibodies against components of these prevalent *Penicillium* species could be detected in the sera of approximately 16% to 26% of the asthmatic patients.
The alkaline serine protease allergens with strong IgE binding properties have been isolated from *P. citrinum* (Pen c 13) and *P. chrysogenum* (Pench 13), and the vacuolar serine proteases from *P. citrinum* (Pen c 18), *P.*

oxalicum (Pen o 18), and *P. chrysogenum* (Pen ch 18). The mature Pen ch 13 allergen is formed by the removal of the preprosequence of the precursor molecule. Besides N-terminal cleavage, the mature Pen c 18 and Pen o 18 also undergo C-terminal processing. The IgE cross-reactivity among the allergens in *Penicillium* and *Aspergillus* spp. has been detected. In addition to the reactivity with IgE antibody, serine proteases (Pench 13) also demonstrated histamine releasing activity from peripheral blood leukocytes of asthmatic patients.

Besides the serine protease allergens, a 68-kDa allergen *N*acetylglucosaminidase and an allergenic heat shock protein belonging to the hsp 70 family have also been identified from *P. chrysogenum* and *P. citrinum*, respectively. They have been designated by the Allergen Nomenclature Subcommittee as Pen ch 20 and Pen c 19, respectively. An 18 kDa peroxisomal membrane protein (Pen c 3), similar to Asp f3, and enolase (Pen c 22), similar to Asp f22, were also identified from *P. citrinum*. Cross-reacting IgE antibodies have been reported against these allergens.

Alternaria alternata

Alternaria alternata, a member of the imperfect fungi, is one of the most important among all allergenic fungi. The spores produced by imperfect fungi vary in shape, size, texture, color, number of cells, and thickness of the cell wall.

Alternaria has dark "dictyospores," with both horizontal and vertical septa. Airborne fungal spores of *Alternaria* and *Cladosporium* are found throughout the world. The airborne spores of these two fungi are generally considered to be important causes of both allergic rhinitis and allergic asthma.

Several allergens from *A. alternata* have been purified and characterized either by conventional fractionation or by employing molecular biology techniques. Alt a 1, a major allergen of *Alternaria*, binds to the IgE of more than 80% of asthmatic patients with allergy to this fungus.

Several variants and isoforms of Alt a 1 have been reported. A reduced form of Alt a 1 produces a doublet pattern on SDS-PAGE with molecular weight of 14.5 and 16.0 kDa. In immunoblots with human atopic serum, this doublet demonstrated reactivity with IgE of patients sensitized to *Alternaria*. Achatz *et al*. described three additional cDNA clones encoding functionally active *A. alternata* allergens. Alt a 7, the 22 kDa allergen, demonstrated more than 70% sequence homology with the YCP4 protein of *Saccharomyces cerevisiae*, whereas Alt a 6, the 11 kDa protein, demonstrated homology with ribosomal P2 protein. However, these allergens reacted diversely with IgE from sera of allergic patients.

Cladosporium herbarum

Cladosporium herbarum is distributed widely in our environment and is a major source of inhalant allergens. *A. alternate* is the predominant fungus demonstrated in warm and humid climates, whereas *Cladosporium* is the leading allergenic mold observed in cooler climates. Approximately 60 antigens from *Cladosporium herbarum* have been identified by crossed immunoelectrophoresis, and approximately 36 of them have been shown to react with IgE from patients' sera. Three major *C. herbarum* allergens have been purified and characterized. Cla h 1 is a small, 13-kDa acidic allergen composed of five isoallergens (isoelectric point [pI] 3.4–4.4), and Cla h 2, a slightly larger molecule

(23 kDa), is a glycoprotein (pI 5.0) with more than 80% carbohydrates. The protein part retained the IgE-binding property even after removing the carbohydrate from the allergen. Cla h 4, the ribosomal P2 protein, is a low-molecular weight (11-kDa) acidic allergen (pI 3.94) with high alanine and serine content. It shares 60% sequence homology with ribosomal P2 protein. Achatz *et al*. reported that purified recombinant *C. herbarum* (Cla h 6; 48 kDa) bound strongly to IgE antibodies in 20% of *Alternaria* allergic patients, as measured by immunoblots.

Enolase has been found to be a highly conserved major allergen in most fungi, and might contribute to allergen cross-reactivity in mold allergy. Approximately 20% of the serum IgE from patients sensitized to *Alternaria* and *Cladosporium* showed binding to enolase. An allergenic HSP 70 has also been isolated from this organism.

Other Fungi There are several other fungi also involved in allergy and asthma (Table 1). These fungi might be seasonal or might be present throughout the year. Some of these fungi are predominantly saprophytic, whereas others are parasites of humans, animals, or birds. In addition, the prevalence of several of these molds is seasonal or dependent on the vegetation.

However, because of their lesser prevalence, not much attention has been paid to isolate and purify the relevant allergens. The pathogenic fungi such as *Trichophyton* and *Candida* also can elicit allergic responses in humans.

Antigens from most of these fungi are species specific, but they also can have cross-reactive epitopes.

Many of the *Basidiomycetes* spp. have been implicated in seasonal allergy and are listed in Table 1. Although *Basidiomycetes* spores are predominantly outdoors, indoor air, especially in damp conditions, show the presence of *Basidiomycetes* antigen incorporated with dust and are a major cause of respiratory allergy. Studies conducted in Europe and the United States demonstrate that 25% to 30% of patients with respiratory allergy have been sensitized to *Basidiomycetes*. The predominant species involved in allergy are *Boletus, Coprinus,* and *Pleurotus*. Other mushrooms such as *Ganoderma, Clavatis, Psilocybe, Schizophyllum,* and *Lentinus* have been reported as causing allergy.

Epitopes

Epitopes are sites or regions of an allergen molecule that interact with T and/or B cells of the immune system. The allergens that interact with IgE are the B-cell epitope, whereas those presented to T cells after processing by antigen presenting cells (APC) as peptide fragments linked to major histocompatibility (MHC) molecules are the T-cell epitopes.

T-cell epitopes

T cells recognize small peptide fragments derived by processing the antigen by APC and presented as MHC complexes on the surface to T-cell receptors (TCR). The TCRs rearrange themselves on the surface, resulting in only one receptor on each T cell. The binding of the MHC antigen complex to the TCR triggers the activation of the T cell. T-cell epitope presentation to the TCR involves MHC class II molecules and is presented frequently by single human leukocyte antigen (HLA) molecules such as DR, DQ, or DP. The minimum peptide length that can bind and initiate a T-cell response is between 7 and 10 residues, although peptides with more than 10 residues are also presented efficiently. Based on the physicochemical studies of peptides and their functional activities, several T-cell epitopes have been identified.

The only fungal allergen studied extensively so far with regard to cell-mediated immune response and T-cell epitope mapping is Asp f1 from *A. fumigatus*. The Asp f1–specific T-cell lines established from ABPA have been characterized as $CD4^+$ Th2 cells based on their cytokine secretion pattern. Most of the Asp f1–specific T-cell clones isolated from PBMCs of ABPA responded to two peptide fragments of Asp f1 representing amino acid (aa) residues aa 46 to 65 and aa 106 to 125 and restricted by HLA-DR2 and HLADR5 alleles. In a mouse model of ABPA, epitopes of Asp f 1 demonstrated differential induction of $CD4^+$ Th2 and $CD4^+$ Th1 cells as indicated by the cytokine patterns. Tcell clones were generated from ABPA patients against Asp f 2. Stimulation of these T-cell clones and PBMCs from ABPA patients using synthetic peptides from Asp f2 sequence identified aa 54 to 74 as a major T-cell epitope. This epitope was further defined, and it was found that T-cell clones stimulated with aa 60–74 secreted interleukin (IL)-5, but not interferon (IFN)-γ.

B-cell epitopes

B-cell epitopes of the allergens might be sequential (linear) and topographic (conformational). Linear epitopes are short sequences of amino acids with up to eight residues located and exposed on protein surfaces where the adjacent hydrophilic amino acids contribute to the interaction with antibody.

The identification of sequential epitopes is readily achieved by the systematic synthesis of overlapping short peptides spanning the sequence of the protein allergen. The second type of epitope is discontinuous, in which the amino acid residues of the antigenic determinants are not sequentially contiguous, but brought close together in space by folding of the polypeptide chain.

Both sequential and conformational epitopes have been identified in Asp f 2, from *A. fumigatus*. The epitope mapping of linear peptides synthesized on derivative cellulose membranes demonstrated distinct IgE-binding epitopes of three to seven amino acids long and were distributed throughout the whole molecule. However, IgE antibody binding of recombinant polypeptide fragments representing various identified epitopes suggest the possible involvement of other structural constraints in the proper folding and threedimensional structure of Asp f2. B-cell epitope mapping of Asp f2 revealed conserved B-cell epitope sequences present in other *Aspergillus* species as well as in the proteins from *Candida*.

Two immunodominant epitopes of Asp f 1, from the Cterminal end of the protein (aa 115–149) with strong T-cell proliferative responses as well as IgE antibody binding with sera from ABPA patients, have been reported.

Structure Function Properties of Allergens and Allergic Responses

The molecular structure of allergens and their interaction with the immune system of the host is not fully understood. Recently, because of the availability of purified recombinant allergens, limited attempts have been made in this direction.

The sequence comparison and homology search indicates the distribution of unique and shared epitopes in some pollen allergens. However, only very few structure function studies have been conducted with mold allergens. Structure function responses of one of the major allergens of *Aspergillus*, Asp f2, were studied after engineering the allergen using molecular techniques. Deletion of N, C, and N´ and C´ terminals showed diverse binding reactivity with IgE antibody. Nor C-terminal end is essential for the conformation of the molecule as both N and C deleted mutants failed to react with IgE. Postglycosylation of the protein demonstrated less IgE binding, but more IgG binding compared with nonglycosylated Asp f2. The role of cysteine residues in Asp f2 was studied, and the results indicate that cysteines at positions C264 and C204 are essential for the IgE binding.

Very little is known about the three-dimensional structure of the mold allergens. The three-dimensional structure is suggested for restrictocin, a closely related ribotoxin belonging to Asp f1 family. It has three alpha helical turns and five stranded antiparallel beta sheets. Recently, Asp f 6 (MNSOD) from *A. fumigatus* was studied, and the 3-D structure has been elucidated. It has a homotetrameric structure with four conserved manganese ions and exposed amino acids with potential cross-reactivity with other MNSODs. Therefore, 3-D structure of allergens will be valuable in determining the cross-reactivity and specificity of the allergen.

Correlations between Airborne Prevalence and Clinical Relevance

Ascomycetes, basidiomycetes, and zygomycetes are the major fungal groups that contain genera known to produce allergens and elicit allergic reactions. Collectively, these groups contribute most of the spores found in air. Early sampling methods for airborne fungal particles had several biases. These methods were nonvolumetric, which tends to favor large spores or spores that disperse in clumps. Recovered fungi were usually detected by their growth on culture medium, which selects for fungi able to grow on the medium used. Analysis of the sample usually consisted of identifying the cultures on the basis of sporulation characters, which excludes species that do not reproduce in culture. Many anamorphic fungi are easily recovered by these methods, however, and most early research on fungal allergens centered on allergenic species of these.

As a result of more recent studies, in which nonviable, volumetric air sampling procedures have been used with direct microscopic analyses, it has been determined that conidia represent only 30 to 60% of airborne spores. The bulk of the remaining spores are ascospores and basidiospores. Recent evidence also indicates that the prevalence of hypersensitivity (skin test reactivity) to basidiospore and that to conidial allergens are also comparable. Thus, the intensity of exposure may indeed determine clinical relevance. The largest gap in our knowledge of aeroallergens pertains to ascospores, the sexual spores of ascomycetes. They constitute much of the balance of the airspora that is neither conidia nor basidiospores.

Very little is known about ascospore allergens, although a certain degree of similarity to the allergens of conidial fungi might be inferred since the majority of the conidial fungi are anamorphs of ascomycete fungi.

Prevalence of Fungal Allergy

Allergic inhalant diseases such as asthma or rhinitis afflict 20% of the population in the United States and other industrialized countries, of which 10% have significant or severe allergic disease. Skin test results further suggest that at least 3 to 10% of adults and children worldwide are affected by fungal allergy. The exact prevalence has not been established since reports of skin test reactivity to fungi range from 3 to 91%, depending upon the population studied, extracts used, and species tested. Prevalence of reactivity is dependent upon the source and batch of allergen as well as the selection criteria for the test subjects. For example, reactions to commercial *Cladosporium herbarum* extracts varied from 12 to 65% in the same population. Likewise, an experimental *E. nigrum* mycelium extract detected sensitivity in 70% of another population, whereas only 6% of the same group reacted

to a commercial extract. Clearly, the true prevalence of fungal allergy will be unknown until standardized extracts are used to test well-defined populations.

Allergen and Allergen-Specific IgE Detection

Skin testing is the simplest method to detect IgE directed against a specific allergen. Two methods commonly used to diagnose allergies to inhalants, such as pollens, mites, or animal danders, are prick and intradermal skin testing. Skin prick testing correlates better with clinical history, radioallergosorbent test (RAST), and provocative inhalation challenge than the more sensitive intradermal test does. Intradermal tests also give false-positive results more frequently than prick testing does.

The RAST is an in vitro test designed to measure the circulating allergen-specific IgE antibodies of a patient. The RAST is generally considered less sensitive than skin testing, although a RAST conducted with partially purified fungal allergens can be comparable to skin tests in both sensitivity and specificity. However, the use of exquisitely specific skin test reagents may indicate a lower prevalence of reactivity since less-specific reagents will cross-react with more species of fungi. Thus, cross-reactive antigens could simultaneously detect allergies to several different species with only a single skin test and provide a more robust screening reagent for fungal allergy.

Crossed immunoelectrophoresis (CIE) was used extensively for antigen analysis in the 1970s and 1980s, especially for complex mixtures such as allergen extracts. Crossed-line immunoelectrophoresis (CLIE) can identify common antigenic components in different sources and was used for early cross-reactivity studies. Allergen identification is possible with crossed radioimmunoelectrophoresis (CRIE), a method in which a CIE agarose gel is overlaid with patient serum and any bound IgE is detected with radiolabeled anti-IgE. Although still useful to demonstrate antigenic purity, these methods have been replaced largely by immunoprinting (Western blotting [immunoblotting]) procedures. The basophil histamine release test is another valuable in vitro assay, but it has limited clinical usefulness since it requires fresh leukocytes. False-negative results can occur, so the clinical symptoms of the patient must be considered when interpreting test results. Other methods of in vitro allergy testing, based on enzyme or chemiluminescent detection, are becoming commercially available and are generating much interest. None is as yet widely accepted.

Diagnosis of Fungal Allergy

Diagnosis of any allergic disease is based on the clinical symptoms of the patient, determination of exposure to the allergen, results of skin and in vitro tests (particularly the RAST), and sometimes, if necessary, provocative inhalation challenge testing. Most subjects sensitive to fungi are also sensitive to other inhalant allergens, thus increasing the difficulty of diagnosis.

Allergic symptoms may occur in definite patterns that can provide clues to the diagnostician. Fungal allergic symptoms are not usually considered seasonal, however, as are pollenrelated ailments. Moreover, many fungal spores derived from different species are similar morphologically; this can limit the completeness of spore identification in air sampling surveys.

Outdoor, indoor, and occupational exposures can all induce fungal sensitivity. Consequently, the variety of possibly allergenic fungi is far greater than can feasibly be included in any panel of skin test reagents. This emphasizes the value of environmental sampling, since the range of fungi to be considered can be narrowed effectively if an aerobiological identification is available. However, this would require sampling and analysis by certified personnel.

On the basis of aerobiologic surveys conducted in different locations of the world, skin test studies, and fungal allergen characterization, the consensus is that skin test panels should include, at the minimum, *Alternaria alternata*, *Aspergillus fumigatus*, *Cladosporium herbarum*, *E. nigrum*, *Fusarium roseum*, and *Penicillium chrysogenum*. An optimal screening panel of fungal allergens is not currently available. It is also important to note that this list does not include basidiomycetes, primarily because suitable extracts are not commercially available.

Provocative challenge with specific fungal allergens can provide a definitive diagnosis of fungus-induced allergic disease. False-positive results can occur, however, so experienced investigators must perform and interpret these tests, which include nasal, conjunctival, or bronchial provocation.

Inhalation challenge can be an important tool in cases where conflicting or questionable data have been gathered, and it can confirm the clinical usefulness of allergen preparations in research settings as well. Conjunctival testing is less commonly used because of its poor reproducibility.

Despite the introduction of provocative challenge and the development of sophisticated in vitro tests, making the diagnosis of mold allergy remains difficult. Standardized extracts are not available, the average person is constantly exposed to a wide variety of propagules, and many subjects are also allergic to nonfungal inhalant

allergens. Additionally, the symptoms may be due to a fungus either whose spores are not restricted to a given season or whose particular spore type is not tallied independently by a local pollen or spore station, if one exists. These factors would obscure any correlation between spore count and symptoms that might indicate the offending species.

Development of standardized fungal extracts and more extensive and detailed reporting of pollen or spore counts would contribute significantly to the reliability of diagnoses of fungal allergies.

Specific Immunotherapy

The use of specific immunotherapy for fungal allergy is debatable. Specific immunotherapy for type I allergy should be based on reducing allergic reactions to those allergens that are recognized by the patients' IgE antibodies. The establishment of patients' IgE reactivity profile (allergogram) with recombinant allergens will be valuable in selecting the components against which a substantial IgE response is mounted. Selection of allergens according to the patients' allergogram might, therefore, improve both diagnostic and therapeutic specificity of the allergens. Allergograms might also help in monitoring the effectiveness of immunotherapy. With the availability of recombinant allergens, structure function studies might be carried out, resulting in valuable information in our understanding of the immunopathogenesis of mold allergy. This information might contribute to more focused and refined immunomodulatory therapy for controlling the disease.

TABLE 3. Purified fungal allergens

Fungal species	Nomenclature		Molecular mass (kDa)	Isoelectric point (pI)	Reference
	Standard	Provisional/alternate			
Alternaria alternata		Alt-1	31	4.3	221
	Alt a 1[a,b]		28		125
		Alt a 29K[a,b]	29	4.2–4.6	38
	Alt a 1_{1563}[b]		31	4.0–4.5	143
		Alt a Bd29K[b]	29	4.2	42
		gp 70[c]	70	3.5	153
		Not yet designated[c]	60–66		29
		Basic peptide	31	9.5–9.8	25
Aspergillus fumigatus	Asp f 1	Ag 3	18		103
		Ag 20 kDa	20	5–6	165
Cladosporium herbarum	Cla h 1	Ag 32	13	3.4–4.4	13
	Cla h 2	Ag 54	23	5.0	13
Trichophyton tonsurans	Tri t 1		30		43
Calvatia cyathiformis		Cal c Bd9.3	16	9.3	74
Psilocybe cubensis		Psi c Bd48kD	48		158
	Psi c 2	Cyclophilin	16		81
Saccharomyces cerevisiae		Enolase	51		17
Candida albicans		Enolase	48		52
		Alcohol dehydrogenase	40		179

8. Fungi and its products used for therapeutic purposes

Medicinal fungi are those fungi which produce medically significant metabolites or can be induced to produce such metabolites using biotechnology. The range of medically active compounds that have been identified include antibiotics, anti-cancer drugs, cholesterolinhibitors, psychotropic drugs, immunosuppressants and even fungicides. Although initial discoveries centred on simple moulds of the type that cause spoilage of food, later work identified useful compounds across a wide range of fungi. Although fungi products have been used in traditional and folk medicines, probably since pre-history, the ability to identify beneficial properties and then extract the active ingredient started with the discovery of penicillin by Alexander Fleming in 1928. Since that time, many additional antibiotics have been discovered and the potential for fungi to synthesize biologically active molecules, useful in a wide range of clinical therapies, has been extensively exploited.

Pharmacological research has now isolated antifungal, antiviral, and antiprotozoan, isolates from fungi.The fungus with probably the longest record of medicinal use, *Ganoderma lucidum*, is known in Chinese as líng zhī ("spirit plant"), and in Japanese as mannentake ("10,000-year mushroom"). In ancient Japan, *Grifola*

frondosa was worth its weight in silver, although no significant therapeutic benefits have been demonstrated in humans.

Application:

Cancer :

Table 1: Diversity of nutraceutical and therapeutic metabolite producing mushrooms found in order Agaricales (nomenclature source: Mycobank).

Family	Important genera	Therapeutic/nutritional value	References
Hygrophoraceae	*Hygrophorus*	Fatty acids with bactericidal and fungicidal activity prepared from *Hygrophorus eburneus*. Hygrophamides are important constituents of cell membranes and assumed to play important roles as antigens and their receptors.	[9, 14]
Tricholomataceae	*Clitocybe, Tricholoma*	*Tricholoma magnivelare*, the pine mushroom, or the white matsutake, is another edible mushroom known to enhance fertility and virility, strengthen the immune system, have anticancer activity, and even work as an aphrodisiac.	[9]
Amanitaceae	*Amanita*	*A. phalloides*, also known as "death cup," is a well-known poisonous mushroom of this genus and is also reported to be used in tumor therapy.	[9, 15]
Agaricaceae	*Agaricus*	*A. campestris* and *A. rodmani* are edible. *A. brunnescens* (*bisporus*) is grown commercially.	[9]
Strophariaceae	*Stropharia, Naematoloma, Psilocybe*	The well-known edible members of the genus *Stropharia* include *Stropharia rugosoannulata* and *Stropharia aeruginosa*. These mushrooms are a low calorie and fat-free food choice, known to assist in weight loss. *Psilocybe cubensis*'s principal active compounds are psilocybin and psilocin which have mind-altering effects like euphoria and visual and mental hallucinations.	[9, 11]
Coprinaceae	*Coprinus Panaeolus*	*Coprinus atramentarius* is edible unless consumed with alcohol. *Coprinus comatus* has antioxidant properties.	[9]
Pluteaceae	*Pluteus Volvariella*	*P. cervinus, P. atromarginatus*, and *V. volvacea* all are edible.	[9]
Cortinariaceae	*Cortinarius Galerina*	*Cortinarius* sp. is inedible and some are poisonous too.	[16]
Psathyrellaceae	*Psathyrella*	*Psathyrella atroumbonata* Pegler is one of the most valuable edible mushrooms in Nigeria as it is very rich in protein and fibres.	[17]
Inocybaceae	*Inocybe*	*Inocybe aeruginascens* contains the formerly known alkaloids psilocybin, psilocin, baeocystin, and aeruginascin.	[16]
Strophariaceae	*Pholiota*	Polysaccharide isolated from *Pholiota nameko* (PNPS-1) led to significant decreases in very low-density lipoprotein/low-density lipoprotein cholesterol and an increase in high-density lipoprotein cholesterol. A novel lectin from *Pholiota adiposa* showed antiproliferative activity.	[18, 19]
Marasmiaceae	*Marasmius, Omphalotus*	*Marasmius oreades*, the fairy ring mushroom, is a good edible species containing Cu, Fe, Zn, folic acid, protein, and all the essential amino acids required by man.	[9]
Omphalotaceae	*Lentinula*	*Lentinula edodes* is low in sodium and glucose, rich source of fibre, and ideal for diabetics. In Japan, it is used as a natural treatment of cancer because of its complex carbohydrate, lentinan. It is also a source of selenium, an antioxidant that is said to prevent cancer.	[9]
Lyophyllaceae	*Termitomyces*	Methanolic extract of *Termitomyces albuminosus* mycelia showed high antioxidant properties. Polysaccharide-rich fraction of *Termitomyces eurhizus* accelerates healing of indomethacin induced gastric ulcer. Analgesic and anti-inflammatory effects of the dry matter of culture broth of *Termitomyces albuminosus* and its extracts.	[20–22]
Pleurotaceae	*Pleurotus*	Ribonucleases (RNases: mol. wt. 10.7 kDa) have been isolated and characterized from *P. ostreatus* that has the potentiality to neutralize HIV through degradation of viral genetic material.	[23]
Hydnangiaceae	*Laccaria*	*Laccaria edulis, Laccaria laccata, and Laccaria proxima* are all edible species.	[24]
Physalacriaceae	*Flammulina Armillaria*	*Flammulina velutipes* is rich in peroxidase, superoxide dismutase, and others and can prevent some severe diseases like cancer and coronary heart disease.	[25]

Paclitaxel is synthesised using *Penicillium raistrickii* and plant cell fermentation. Fungi can synthesize other mitotic inhibitors including vinblastine, vincristine, podophyllotoxin, griseofulvin, aurantiamine, oxaline , and neoxaline. 11,11'-Dideoxyverticillin A, an isolate of marine *Penicillium*, was used to create dozens of semi-synthetic anticancer compounds. 11,11'-Dideoxyverticillin A, andrastin A, barceloneic acid A, and barceloneic acid B, are farnesyl transferase inhibitors that can be made by *Penicillium*. 3-O-Methylfunicone, anicequol, duclauxin, and rubratoxin B, are anticancer/cytotoxic metabolites of *Penicillium*.

TABLE 2: Diversity of nutraceutical and therapeutic metabolite producing mushrooms found in order Aphyllophorales.

Family	Important genera	Therapeutic properties	Bioactive components	Mode of action	References
Hymenochaetaceae	*Inonotus Phellinus*	*I. obliquus* (chaga mushroom) is used for cancer, gastritis, ulcer and tuberculosis of bones, treatment of breast, liver, uterine, and gastric cancer, hypertension, and diabetes.	Phenolic compounds, melanin and lanostane-type terpenoids, and betulinic acid (pentacyclic triterpenoids)	Stimulates body to produce natural killer cells to battle infection and tumor growth	M. Blackwell, "unpublished data,", 1994
		I. dryadeus possess antibacterial activity.	Nonlinear, complex (1–3) and (1–6) β-D-glucans, a type of polysaccharide		
Ganodermataceae	*Ganoderma*	*Ganoderma lucidum* (also known as Reishi) shows antitumor, immune-modulating activities for treatment of hypoglycemosis, hepatoprotection, and the effect on blood vessel system. This mushroom has been used in traditional Chinese and Japanese medicines. Reishi is commonly prescribed for a host of conditions such as anxiety, high blood pressure, bronchitis, insomnia, and asthma but is particularly renowned for its use in hepatitis and other diseases of liver. It also prevents "fatty-liver" and cirrhosis due to alcohol abuse.	Mucopolysaccharides and polysaccharide-protein complexes	Activates the immune response of the host and leads to the induction of cell differentiation and metabolizing enzymes, the inhibition of angiogenesis, and the expression of the urokinase-type plasminogen activator (uPA) and the uPA receptor in cancer cells	[26]
		Ganoderma applanatum have been reported to show immunostimulating properties, fight cancer, stop pain, eliminate indigestion, and reduce phlegm, antibiotic and antiviral.	β-D-glucans and their protein complexes, for example, xyloglucans and acidic β-D-glucan containing uronic acid, dietary fibres, lectins, and terpenoids		
Cantharellaceae	*Cantharellus*	The edible species *C. cibarius*, commonly known as chanterelles, have antimicrobial activity against some Gram positive and Gram negative bacteria, yeasts, filamentous fungi, and actinomycetes. They are also known for their antitumor, antioxidant activity and antigenotoxic potential.	Ethyl alcohol, chloroform, and ethanol		[27, 28]

Table 2: Continued.

Family	Important genera	Therapeutic properties	Bioactive components	Mode of action	References
Schizophyllaceae	*Schizophyllum*	Pharmacologically, *S. commune* is very important because it produces polysaccharide which shows anticancer activity in xenography and clinical practice.	Schizophyllan (β-D-glucan)	Immunomodulation	[29]
Sparassidaceae	*Sparassis*	*Sparassis crispa* (cauliflower fungus), its fruiting bodies have antitumor properties and also contain chemicals which may stimulate immune system and inhibit growth of superbug MRSA (methicillin-resistant *Staphylococcus aureus*).	Bioactive β-D-glucan, phenyl derivatives, chalcones, and sesquiterpenoids	Exhibits various biological activities, including enhancement of the hematopoietic response and induction of cytokine *production*	[30, 31]
Polyporaceae	*Polyporus*	*Polyporus umbellatus* have anticancer and diuretic properties. From *Polyporus alveolaris*, a polypeptide with antifungal properties has been isolated from the fresh fruit bodies of this species named alveolarin.	Nucleotides, nucleosides, proteins, amino acids, vitamins, polysaccharides, and triterpenoids	Their diuretic property has been used to get rid of the toxins that are responsible for causing infections in the urinary tract and is also found to be very effective against urethral blockages	[32]

Table 3: Diversity of nutraceutical and therapeutic metabolite producing mushrooms found in order Auriculariales.

Family	Important genera	Therapeutic/nutritional value	Bioactive component	References

Table 4: Diversity of nutraceutical and therapeutic metabolite producing mushrooms found in class Gasteromycetes.

Order	Family	Important genera	Therapeutic/nutritional value	References
Lycoperdales	Lycoperdaceae Geastraceae	*Calvatia* *Lycoperdon* *Radiigera* *Geastrum*	*Calvatia gigantea* and *Calvatia caelata* are used in burnt cases due to their anesthetic nature. *Calvatia* spp. are also used to stop bleeding from wounds. Calvacin isolated from *Calvatia gigantea* having antitumor activity. *Lycoperdon pusillum* is used to cure sores, abrasion or bruises, deep cut, hemorrhages, and urinary infections.	[35–37]
Tulostomatales	Calostomataceae Tulostomataceae	*Calostoma* *Tulostoma* *Battarrea*	*Calostoma cinnabarina* has been recorded to be an edible mushroom. Antibacterial activity has been reported in *Tulostoma obesum*.	[38, 39]
Sclerodermatales		*Scleroderma* *Astraeus* *Pisolithus*	Polysaccharide from *Astraeus hygrometricus* has been reported for macrophage stimulation.	[40]
Phallales	Phallaceae Clathraceae	*Mutinus* *Pseudocolus* *Linderia*	—	[9]
Nidulariales	Nidulariaceae Sphaerobolaceae	*Nidularia* *Sphaerobolus*	—	[9]

Table 11: Source, type, and bioactivity of some mushroom polysaccharides.

Mushroom name	Polysaccharide source	Type of polysaccharide	Bioactivity	References
Pleurotus tuber-regium	Sclerotium, mycelium	β-D-glucan	Hepatoprotective, antibreast cancer	[64]
Ganoderma lucidum	Fruiting body, culture broth	Heteroglycan, mannoglucan, glycopeptide	Hyperglycemia, immunomodulating, antitumor, antioxidative, antidecrepitude	[64]
Auricularia auricular	Fruiting body	Glucan	Hyperglycemia, immunomodulating, antitumor, anti-inflammatory, antiradiative	[64]
Schizophyllum commune	Mycelium	Glucan, schizophyllan	Antitumor	[64]
Hericium erinaceus	Fruiting body, mycelium	Heteroglycan, heteroglycan peptide	Hyperglycemia, immunomodulating, antitumor	[64]
Lentinus edodes	Culture broth, fruiting body	Mannoglucan, polysaccharide-protein complex, glucan, lentinan	Immunomodulating, antitumor, antiviral	[64]
Sclerotinia sclerotiorum	Sclerotium	Glucan, scleroglucan (SSG)	Antitumor	[64]
Polystictus versicolor	Fruiting body, culture broth, mycelium	Heteroglycan, glycopeptide, Krestin (PSK)	Immunomodulating, antitumor, antiradiative, hyperglycemia, anti-inflammatory	[64]
Grifola frondosa	Fruiting body	Proteoglycan, glucan, galatomannan, heteroglycan, and grifolan	Immunomodulating, antitumor, antiviral, hepatoprotective	[64]
Inonotus obliquus	Fruiting body, mycelium	Glucan	Antitumor, immunomodulating	[64]
Agaricus blazei	Fruiting body, mycelium	Glucan, heteroglycan, glucan protein, Glucomannan-protein complex	Antitumor	[64]
Flammulina velutipes	Fruiting body, mycelium	Glucan-protein complex, glycoprotein	Antitumor, anti-inflammatory, antiviral, immunomodulating	[64]
Ganoderma applanatum	Fruiting body	Glucan	Antitumor	[64]
Polyporus umbellatus	Mycelium	Glucan	Antitumor, immunomodulating	[64]
Clitopilus caespitosus	Fruiting body	Glucan	Antitumor	[64]
Pleurotus citrinopileatus	Fruiting body	Galactomannan	Antitumor	[64]
Trametes robiniophila	Mycelium	Proteoglycan	Immunomodulating, hepatoprotective, anticancer	[64]
Tremella fuciformis	Fruiting body, mycelium, culture broth	Heteroglycan	Hyperlipidemia, hyperglycemia, immunomodulating, antitumor, antidecrepitude, antithrombus	[64]
Tremella aurantialba	Fruiting body, mycelium	Heteroglycan	Immunomodulating, hyperglycemia	[64]
Pleurotus ostreatus	Fruiting body	Glycoprotein	Antitumor, hyperglycemia, antioxidant	[64]
Morchella esculenta	Fruiting body	Heteroglycan	Hyperglycemia, antitumor	[64]
Omphalia lapidescens	Fruiting body	Glucan	Anti-inflammatory, immunomodulating	[64]
Phellinus linteus	Fruiting body	Glucan	Antitumor	[64]
Armillariella tabescens	Mycelium	Heteroglycan	Antitumor	[64]
Dictyophora indusiata	Fruiting body	Heteroglycan, mannan, glucan	Antitumor, hyperlipidemia	[64]
Tricholoma mongolium	Fruiting body	Glucan	Antitumor	[64]

Table 11: Continued.

Mushroom name	Polysaccharide source	Type of polysaccharide	Bioactivity	References
Cordyceps sp.	Fruiting body, mycelium, culture broth	Glucan, heteroglycan	Antitumor, immunomodulating, antitumor, hyperglycemia	[64]
Coriolus versicolor	Fruiting body, mycelium	Polysaccharides PSK and PSP	Antiviral effect on HIV and cytomegalovirus *in vitro* and anticancer	[65]
Marasmius androsaceus	Fruiting body, mycelium	Polysaccharide	Analgesic/sedative effect	[66]
Volvariella sp.	Fruiting body, mycelium	Polysaccharide	Cardiac tonic	[66]
Coprinellus disseminatus	Fruiting body, mycelium	Polysaccharide	Antitumor	[67]
Trametes coriolus	Fruiting body, mycelium	Polysaccharide PSP-a glycopeptides	Antitumor and immunostimulant	[67]
Tricholomopsis rutilans	Fruiting body, mycelium	Polysaccharides extract	Anticarcinogenic activity Antioxidative and anti-inflammatory	[65]
Tremella fuciformis	Fruiting body, mycelium	Polysaccharide	Hypocholesteric and hypolipidemic	[67]
Schizophyllum commune	Fruiting body, mycelium	Polysaccharide schizophyllan	Antitumor effect	[67]

Penicillium is a potential source of the leukemia medicine asparaginase.

Some countries have approved Beta-glucan fungal extracts lentinan, polysaccharide-K, and polysaccharide peptide as immunologic adjuvants. Evidence suggests this use as effective in prolonging and improving the quality of life for patients with certain cancers, although the Memorial Sloan-Kettering Cancer Center observes that "well designed, large scale studies are needed to establish the role of lentinan as a useful adjunct to cancer treatment". According to Cancer Research UK, "there is currently no evidence that any type of mushroom or mushroom extract can prevent or cure cancer". Fungal metabolites such as ergosterol, clavilactones, and triterpenoids are efficient Cdk inhibitors that lead to G1/S or G2/M arrest of cancer cells. Other metabolites, such as panepoxydone, are inhibitors of NF-κB. Fucose and mannose fragments of fungal cell wall are antagonists of VEGF-receptors antagonists of VEGF-receptors.

Antibacterial agents (antibiotics)

Alexander Fleming led the way to the beta-lactam antibiotics with the *Penicillium* mold and penicillin. Subsequent discoveries included alamethicin, aphidicolin, brefeldin A, Cephalosporin, cerulenin, citromycin, eupenifeldin, fumagillin, fusafungine, fusidic acid, itaconic acid, MT81, nigrosporin B, usnic acid, verrucarin A, vermiculine and many others.

Antibiotics retapamulin, tiamulin, and valnemulin are derivatives of the fungal metabolite pleuromutilin. Plectasin, austrocortilutein, austrocortirubin, coprinol, oudemansin

A, strobilurin, illudin, pterulone, and sparassol are antibiotics isolated from basidiomycete species.

Cholesterol biosynthesis inhibitors

Statins are an important class of cholesterol-lowering drugs; the first generation of statins were derived from fungi.The Pravastatin precursor mevastatin can be extracted from *Penicillium*. Lovastatin, the first commercial statin, was extracted from a fermentation broth of *Aspergillus terreus*. Industrial production is now capable of producing 70 mg lovastatin per kilogram of substrate. The red yeast rice fungus, *Monascus purpureus*, can synthesize lovastatin, mevastatin, and the simvastatin precursor monacolin J. Zaragozic acids were isolated from ascomycota. Nicotinamide riboside, a cholesterol biosynthesis inhibitor, is made by *Saccharomyces cerevisiae*. Other compounds include Simvastatin, a semi-synthetic derivative of Lovastatin.

Table 12: Miscellaneous therapeutic metabolites of mushrooms and their bioactivity.

Mushroom name	Active principle	Activity	Reference
Ganoderma lucidum Common name: Reishi	Triterpenes, Ganoderiol F Ganoderic acid B Ganodermanontriol. Ganodermadiol Polysaccharide (ganopoly) Triterpenes Polysaccharides Ganoderon A, Ganoderon B, Ganoderon C β-D-glucan Glycoprotein	Active against HIV-1 Cytotoxic to hepatoma cells Active against herpes simplex virus-1 Causing palliative effect on cancer related symptoms and hepatitis B treatment Protection against atherosclerosis Hypoglycemic effect during treatment of type 2 diabetics Immunostimulative antitumor activity Antitumor activity	[65, 67]
Ganoderma applanatum	Steroids Applanoxidic acid G Applanoxidic acid G	Antagonist to both Gram positive and Gram negative bacteria Active against influenza virus type A Active against mouse skin tumor promoters Useful in treatments of pain	[65]
Lentinula edodes Common name: shiitake	Oxalic acid present on caps Polysaccharide lentinan Emitanin Soluble lignin Eritadenine (a nucleotide derivative)	Antibacterial activity Inhibits Sarcoma 180 and HIV induced cytopathic effect Used in tumor/chemo/radiotherapy/surgery Anti-HIV activities Antilipidemic activity and reduces risk of atherosclerosis	[65]
Flammulina velutipes	Sesquiterpenes, proteins, and polysaccharides Enokipodins Velutin protein Alcoholic and hot water extracts of its sporophores	Antitumor Antifungal and antibacterial Inactivating ribosome and inhibiting HIV-1 reverse transcriptase Cytostatic effects against tumor cells Antiallergic and anticomplementary effects	[65]
Auricularia- auricula judae	Acidic heteroglycans	Antitumor	[65]
Auricularia polytricha	Antiplatelet compound Adenosine	Arhterosclerotic activity	[65]
Pleurotus ostreatus	Lovastatin	Arhterosclerotic activity	[65]
Grifola frondosa	Polysaccharide β-D-glucan Maitake D-fraction Proteins with β-D-glucan (Grifolan) Xyloglucan, fucomannoglucan Heteroglucans proteins Mannogalactofucan Heteroxylan Galactomannoglycan	Antiviral activity and immunostimulatory effect HIV-antitumor activity Antitumor activity	[67]
Laetiporus sulphureus	Dehydrotrametenolicacid	Hypoglycemic activity Insulin sensitizer and reducing hyperglycemia	[67]
Lenzites betulina	Betulinam A	Radical scavenger	[67]
Cordyceps militaris	Polysaccharides (Cordycepic acid and β-D-glucans) Triterpenoids Nucleosides (cordycepin, 3-deoxyadenosine) Proteins, amino acids Vitamins (B-complex and ascorbic acid) Minerals (trace elements)	Cancer Fatigue Respiratory and pulmonary diseases Liver and renal disorders Male and female sexual dysfunctions Cardiovascular diseases	[65]
Cordyceps sinensis	Adenosine, cordycepin, and ergosterol	Antioxidative property	[68]

Table 12: Continued.

Mushroom name	Active principle	Activity	Reference
Morchella esculenta	Polysaccharides, proteins, enzymes, vitamins, minerals, and amino acids No cholesterol	Cures pneumonia, fever, cough, cold, and stomachache Cures diseases for pregnant and lactating minerals For respiratory ailments	[65]
Pleurotus sajor-caju	Proteins having polysaccharide xyloglucan, xyloproteins	Antitumor	[67]
Agaricus campestris	Glycoproteins	Antitumor	[67]
Boletus edulis		Antitumor	[67]
Calvatia gigantea	Calvacin	Antitumor	[67]
Cyathus stercoreus	Polyketide Cyathusals A, B, and C Pulvinatal antioxidant effect	Antitumor	[67]
Tricholomopsis rutilans	Polysaccharides Phenol fosmicin B Sterols	Antitumor, anti-inflammatory, and antioxidant	[67]
Oudemansiella radicata	E-b methoxy acrylate	Antibiotic effect	[66]
Collybia velutipes	Eritadenine	Decreasing cholesterol	[66]

Antifungals

Some antifungals are derived or extracted from other fungal species. Griseofulvin is derived from a number of *Penicillium* species, caspofungin is derived from Glarea lozoyensis. Strobilurin, azoxystrobin, micafungin, and echinocandins, are all extracted from fungi. Anidulafungin is a derivative of an *Aspergillus* metabolite.

Immunosuppressants

Ciclosporin, was discovered in *Tolypocladium inflatum*. Bredinin was discovered in *Eupenicillium brefeldianum*. Mycophenolic acid was discovered in *Penicillium stoloniferum*. Thermophilic fungi were the source of the fingolimod precursor myriocin. *Aspergillus* synthesizes immuno suppressants gliotoxin and endocrocin. Subglutinols are immuno suppressants isolated from *Fusarium subglutinans*. Other compounds include mizoribine.

Malaria

Codinaeopsin, efrapeptins, zervamicins, and antiamoebin, are made by fungi.

Diabetes

Many fungal isolates act as DPP-4 inhibitors, alpha-glucosidase inhibitors, and alpha amylase inhibitors *in vitro*. Ternatin is a fungal isolate that suppresses hyperglycemia. Aspergillusol A is an alpha-glucosidase inhibitor made by *Aspergillus*. Sclerotiorin is an aldose reductase inhibitor made by *Penicillium*.

Psychotropic effects

Main article: Psilocybin mushroom

A number of fungi have well documented psychotropic effects, some of them severe and associated with sometimes acute and life-threatening side-effects. Well known amongst these is *Amanita muscaria*, the fly agaric. More widely used informally are a range of fungi collectively known as "magic mushrooms", which contain psilocybin and psilocin.

The history of bread-making is also peppered with references to deadly ergotism caused by ergot, most commonly *Claviceps purpurea*, a parasite of cereal crops. A number of therapeutically useful drugs have subsequently been extracted from ergot including ergotamine, pergolide and cabergoline.

Psychotropic compounds created from ergot alkaloids also include dihydroergotamine, methysergide, methylergometrine, hydergine, nicergoline, lisuride, b romocriptine, cabergoline, pergolide. *Polyozellus multiplex* synthesizes prolyl

endopeptidase inhibitors polyozellin, thelephoric acid, kynapcins. Neurotrophic fungal isolates include L-theanine, tricholomalides, scabronines, termitomycesphins. Many fungi synthesize the partial, non-selective, serotonin receptor agonist/analog psilocin.

A number of other fungal species, including species of *Aspergillus* and *Penicillium*, have been induced to produce ergot alkaloids.

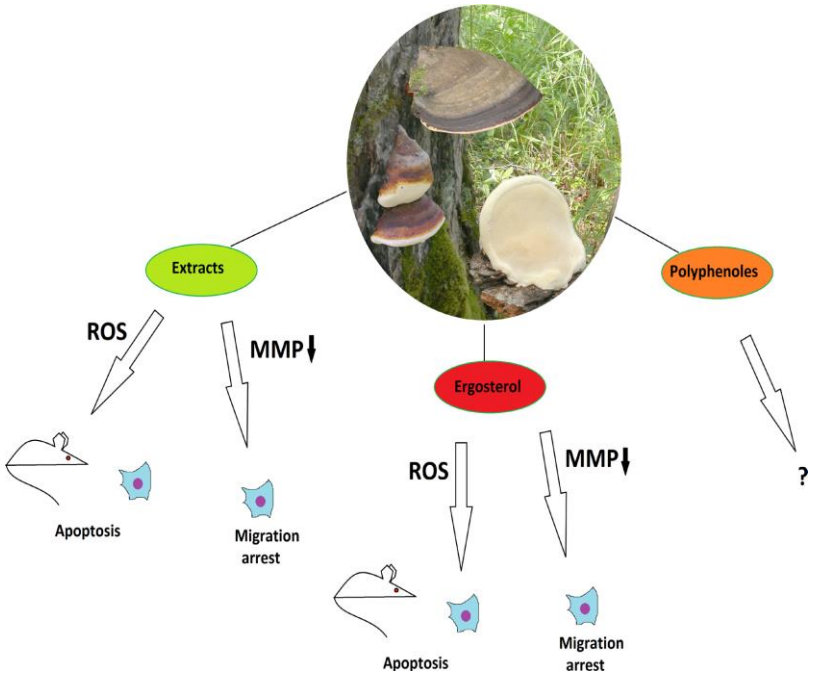


Figure 1: The anticancer properties of *Fomitopsis pinicola*. Effects of different mushroom derivatives and their mechanisms of actions on various models are depicted. Mouse and cell icons indicate results obtained on animal and cell models, respectively. ROS – reactive oxygen species, MMP↓ – downregulation of matrix metalloproteinases.

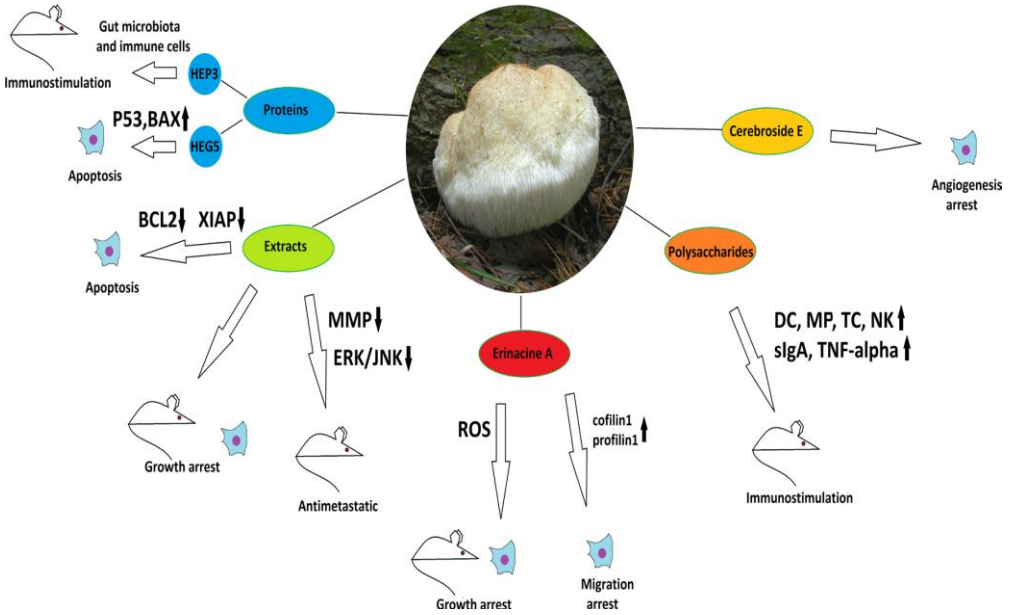


Figure 2: The anticancer properties of *Hericium erinaceus*. Effects of different mushroom derivatives and their mechanisms of actions in various models are depicted. Mouse and cell icons indicate results obtained on

animal and cell models, respectively. Arrows up and down reflect up- or down-regulation of respective proteins or pathways. ROS – reactive oxygen species, MMP - matrix metalloproteinases, DC – dendritic cells, MP – macrophages, TC – T-cells, NK – natural killers. Other proteins/pathways are mentioned under their standard names.

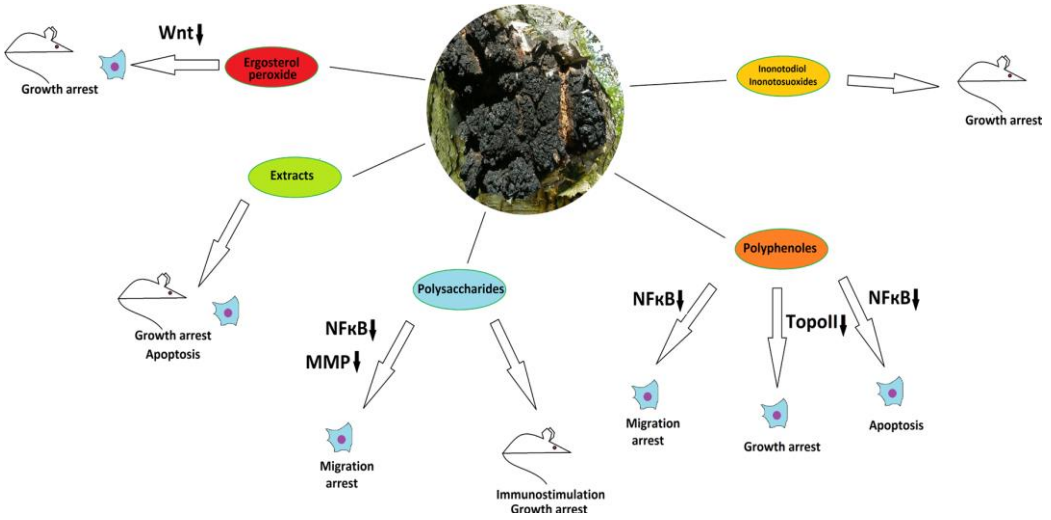


Figure 3: The anticancer properties of *Inonotus obliquus*. Effects of different mushroom derivatives and their mechanisms of actions in various models are depicted. Mouse and cell icons indicate results obtained on animal and cell models, respectively. Arrows up and down reflect up- or down-regulation of respective proteins or pathways. ROS – reactive oxygen species, MMP - matrix metalloproteinases, TopoII – topoisomerase II. Other proteins/pathways are mentioned under their standard names.

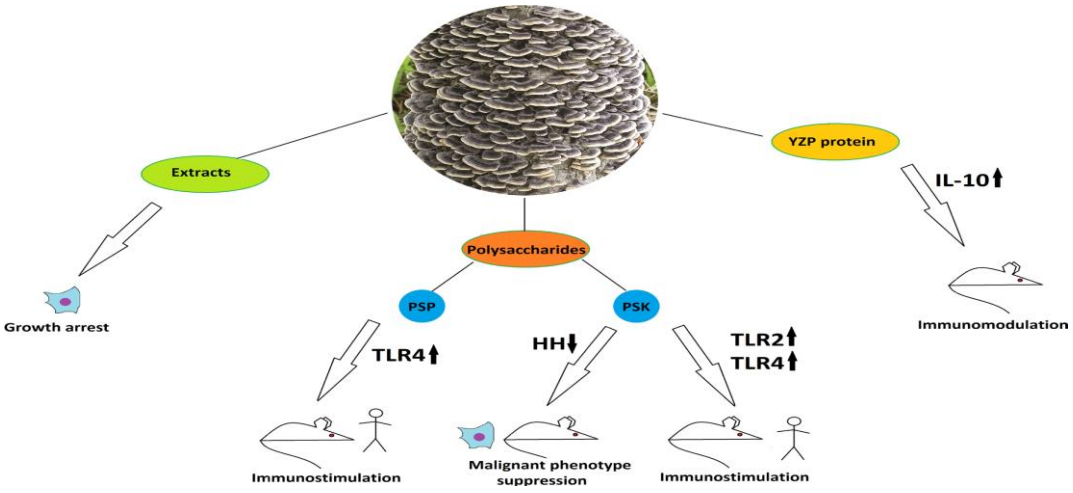
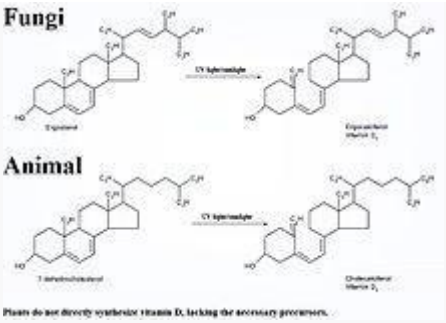


Figure 4: The anticancer properties of *Trametes versicolor*. Effects of different mushroom derivatives and their mechanisms of actions in various models are depicted. Human, mouse and cell icons indicate results obtained in human patients, animal and cell models, respectively. Arrows up and down reflect up- or down-regulation of respective proteins or pathways. PSP – polysaccharopeptide, PSK – polysaccharide Krestin, HH – Hedgehog pathway, TLR2, TLR4 – Toll-like receptors 2 and 4. IL-10 – Interleukin 10.

Vitamins

Fungi are a source of ergosterol which can be converted to vitamin D upon exposure to ultravioletlight to synthesize vitamins D_2 (ergocalciferol), D_4 (22-dihydroergocalciferol), and D_1(Lumisterol+D_2).



The photochemistry of vitamin D biosynthesis

Phytase

Aspergillus niger is used to produce recombinant phytase, an enzyme added to animal feeds to improve absorption of phosphorus.

Edible species which contain drugs (biologically active constituents) include:

- *Agaricus subrufescens* (*Agaricus blazei*/*brasiliensis*, almond mushroom) is a fungus associated with Brazil and Japan. Blazein, a bioactive steroid, was isolated from *A. subrufescens*.
- The adenosine analog cordycepin was originally isolated from *Cordyceps*. Other *Cordyceps* isolates include, cordymin, cordycepsidone, and cordyheptapeptide. CS-4 is commercially sold as *C. sinensis*, but Cs-4 has recently been confirmed to be a different species from the Cordyceps species used in traditional Chinese medicine. CS-4 is properly known as *Paecilomyces hepiali*. *Hirsutella sinensis* is the accepted asexual form of *C. sinensis*.
- *Ganoderma lucidum* (Ling zhi, mannentake, reishi) contains p-hydroxybenzoic acid, cinnamic acid, and lanostane-type triterpenoidssuch as ganoderic acids.
- *Hydnellum peckii* has yielded atromentin, a compound isolated from the mycorrhiza, and subsequently its biosynthesis has been characterized.
- *Lentinula edodes* (Shiitake) has been used as a source of Lentinan, AHCC, and eritadenine.
- *Schizophyllum commune* (Split gill) has yielded schizophyllan (SPG, sizofiran, sonifilan). Hydrophobins were originally isolated from *S. commune*. A chemically analogous polysaccharide, scleroglucan, is an isolate of *Sclerotium rolfsii*.
- *Tolypocladium inflatum Gams* yields the immunosuppressant ciclosporin.
- *Trametes versicolor* (*Coriolus versicolor*, yun zhi, kawaratake, turkey tail) have produced protein-bound polysaccharides PSK and PSP (polysaccharopeptide) from different mycelia strains.
- *Ustilago maydis* (Mexican truffle, huitlacoche, corn fungus) synthesises ustilagine and ustilagic acid.

9. Suggested Readings

1. "California Poison Action Line: Mushrooms". Retrieved 2008-02-18.
2. Ian Robert Hall (2003). Edible and Poisonous Mushrooms of the World. Timber Press. p. 103. ISBN 0-88192-586-1.
3. Centers for Disease Control (CDC) (June 4, 1982). "Mushroom Poisoning among Laotian Refugees – 1981". MMWR. USA: CDC. **31** (21): 287–8. PMID 6808348. Retrieved 2008-08-04.
4. "Woman died of mushroom poisoning". BBC News. 2010-03-18.
5. Pitel, Laura (2010-03-19). "Amphon Tuckey died after eating death cap mushrooms picked at botanic gardens". The Times. London.
6. Graeme, Kimberlie A. "Mycetism: A review of the recent literature." Journal of medical toxicology (2014): 1–17.

10. Assignment

1. What is host parasite interaction?
2. Describe different Pathogenecity factors
3. Chemical structure of aflatoxin and its effect
4. Disuss different Mushroom poisioning effect on human
5. Mentions diferents Therapeutic use of fungi and its significance
6. What is Allergens? Disuss it prevalence, diagnostic actor and immuno therapy
7. Spectrum of mycosis and types of classification
8. What is host-pathgoen interaction and disuss it signalling factor related in this phenomenon
9. What is hypersentivity ? discuss its differents types in relation to fungal allergens
10. Name different metablites of fungal which is utlised in cancer

All the materials are self written and collected from ebook, journals and websites.

POST GRADUATE DEGREE PROGRAMME (CBCS)
IN
BOTANY

SEMESTER - III

Course: BOSCT 3.4

(Stress Physiology)

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING
UNIVERSITY OF KALYANI
KALYANI – 741 235,
WEST BENGAL

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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 UGC Regulations, 2017 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.) Sankar Kumar Ghosh, 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.

Prof Manas Mohan Adhikary
Director
Directorate of Open and Distance Learning
University of Kalyani

SYLLABUS
COURSE – BOSCT 3.4
Stress Physiology
(Full Marks – 50)

Course	Group	Details Contents Structure		Study hour
BOHCT3.4	**Stress Physiology**	**Unit 1. Water stress**	1. Definition and categories of abiotic stress: water stress and their impact on plant's life with regards to productivity.	1
		Unit 2. Salinity stress	2. Salinity stress and gene expression.	1
		Unit 3. Oxidative stress and antioxidation strategies	3. Oxidative stress and antioxidation strategies in plants and their cellular regulation.	1
		Unit 4. Transcriptome analysis and analysis of DNA markers	4. Transcriptome analysis in stress mediated responses to plants. 5. Analysis of DNA markers in assisted breeding for stress tolerance.	1
		Unit 5. Temperature stress	6 Temperature stress and HSPs structure and functions in plants.	1
		Unit 6. Development of transgenic and Crop designing	7. Development of transgenic for stress tolerance. 8. Crop designing for stress tolerance and climate resilient plants.	1

Content

COURSE – BOSCT 3.4

(Stress Physiology)

Soft Core Theory Paper **Credit = 2**

Content Structure

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1. Introduction
2. Course Objectives
3. Definition and categories of abiotic stress: water stress and their impact on plant's life with regards to productivity.
4. Salinity stress and gene expression.
5. Oxidative stress and antioxidation strategies in plants and their cellular regulation.
6. Transcriptome analysis in stress mediated responses to plants.
7. Analysis of DNA markers in assisted breeding for stress tolerance.
8. Temperature stress and HSPs structure and functions in plants.
9. Development of transgenic for stress tolerance.
10. Crop designing for stress tolerance and climate resilient plants.
11. Let's sum up
12. Suggested Reading
13. Assignment

1. Introduction

Any change in the surrounding environment may disrupt homeostasis. Environmental modulation of homeostasis may be defined as biological stress. Thus, it follows that plant stress implies some adverse effect on the physiology of a plant induced upon a sudden transition from some optimal environmental condition where homeostasis is maintained to some suboptimal condition which disrupts this initial homeostatic state. Thus, plant stress is a relative term since the experimental design to assess the impact of a stress always involves the measurement of a physiological phenomenon in a plant species under a suboptimal, stress condition compared to the measurement of the same physiological phenomenon in the same plant species under optimal conditions.

2. Course Objectives

You should gather knowledge after studying the course:

- To know impact of water stress in plants life..
- Explain categories of abiotic stress.
- An understanding on oxidative stress and antioxidation strategies
- transcriptome analysis in stress mediated response to plant.
- Define HSPs , ROS and Salt gland
- To design crop for stress tolerance.

3. Definition and categories of abiotic stress: water stress and their impact on plant's life with regards to productivity

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.

Abiotic stress:

Abiotic stress is defined as the negative impact of non-living factors on the living organisms in a specific environment. The non-living variable must influence the environment beyond its normal range of variation to adversely affect the population performance or individual physiology of the organism in a significant way.

Whereas a biotic stress would include living disturbances such as fungi or harmful insects, abiotic stress factors, or stressors, are naturally occurring, often intangible and inanimate factors such as intense sunlight, temperature or wind that may cause harm to the plants and animals in the area affected. Abiotic stress is essentially unavoidable. Abiotic stress affects animals, but plants are especially dependent, if not solely dependent, on environmental factors, so it is particularly constraining. Abiotic stress is the most harmful factor concerning the growth and productivity of crops worldwide. Research has also shown that abiotic stressors are at their most harmful when they occur together, in combinations of abiotic stress factors**.**

Categories of abiotic stress

There are different types of abiotic stress. Those are

- Water stress,
- Heat stress,
- Cold stress,
- Osmotic stress,
- Salinity stress etc.

Water stress and their impacts on plant's life:

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

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.

Cell expansion is a turgor-driven process and is extremely sensitive to water deficit. Cell expansion is described by the relationship

$$GR = m(Yp - Y)$$

Where, GR is growth rate, Yp is turgor, Y is the yield threshold (the pressure below which the cell wall resists plastic, or nonreversible, deformation), and m is the wall extensibility (the responsiveness of the wall to pressure).

This equation shows that a decrease in turgor causes a decrease in growth rate. Note also that besides showing that growth slows down when stress reduces Yp, The ewquation shows that Yp need decrease only to the value of Y, not to zero, to eliminate expansion. In normal conditions, Y is usually only 0.1 to 0.2 MPa less than Yp, so small decreases in water content and turgor can slow down or fully stop growth. Water stress not only decreases turgor, but also decreases m and increases Y. Water-deficient plants tend to become rehydrated at night, and as a result substantial leaf growth occurs at that time. Nonetheless, because of changes in m and Y, the growth rate is still lower than that of unstressed plants having the same turgor.

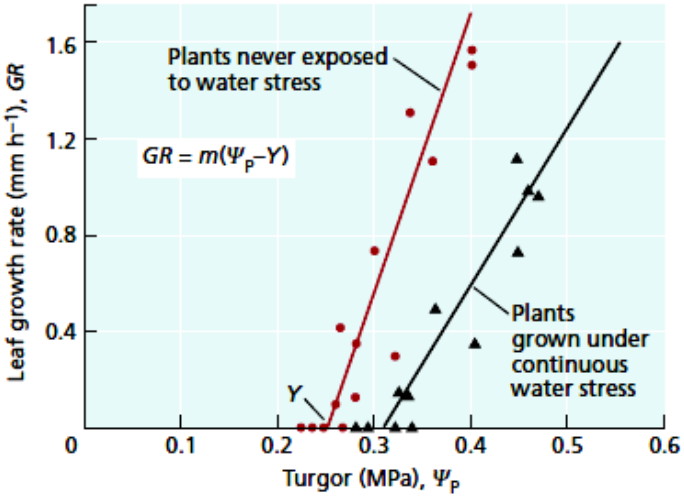


FIGURE 25.1 Dependence of leaf expansion on leaf turgor. Sunflower (*Helianthus annuus*) plants were grown either with ample water or with limited soil water to produce mild water stress. After rewatering, plants of both treatment groups were stressed by the withholding of water, and leaf growth rates (*GR*) and turgor (Ψ_p) were periodically measured. Both decreased extensibility (*m*) and increased threshold turgor for growth (*Y*) limit the leaf's capacity to grow after exposure to stress. (After Matthews et al. 1984.)

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.

FIGURE 25.2 The leaves of young cotton (*Gossypium hirsutum*) plants abscise in response to water stress. The plants at left were watered throughout the experiment; those in the middle and at right were subjected to moderate stress and severe stress, respectively, before being watered again. Only a tuft of leaves at the top of the stem is left on the severely stressed plants. (Courtesy of B. L. McMichael.)

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.

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. When the mesophyll becomes mildly dehydrated, two things happen:

1. Some of the ABA stored in the chloroplasts is released to the apoplast (the cell wall space) of the mesophyll cell (Hartung et al. 1998). The redistribution of ABA depends on pH gradients within the leaf, on the weak-acid properties of the ABA molecule, and on the permeability properties of cell membranes. The redistribution of ABA makes it possible for the transpiration stream to carry some of the ABA to the guard cells.

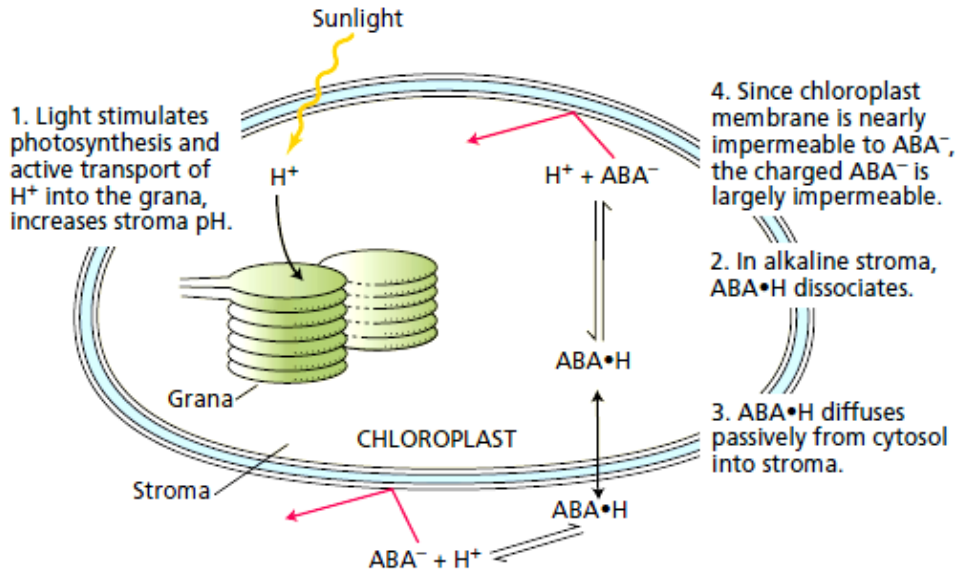


FIGURE 25.3 Accumulation of ABA by chloroplasts in the light. Light stimulates proton uptake into the grana, making the stroma more alkaline. The increased alkalinity causes the weak acid ABA•H to dissociate into H^+ and the ABA^- anion. The concentration of ABA•H in the stroma is lowered below the concentration in the cytosol, and the concentration difference drives the passive diffusion of ABA•H across the chloroplast membrane. At the same time, the concentration of ABA^- in the stroma increases, but the chloroplast membrane is almost impermeable to the anion (red arrows), which thus remains trapped. This process continues until the ABA•H concentrations in the stroma and the cytosol are equal. But as long as the stroma remains more alkaline, the total ABA concentration (ABA•H + ABA^-) in the stroma greatly exceeds the concentration in the cytosol.

2. ABA is synthesized at a higher rate, and more ABA accumulates in the leaf apoplast. The higher ABA concentrations resulting from the higher rates of ABA synthesis appear to enhance or prolong the initial closing effect of the stored ABA.

Besides ABA (Sauter et al. 2001), other signals, such as pH and inorganic ion redistribution, appear to play a role in long-distance signaling between the roots and the shoots.

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.

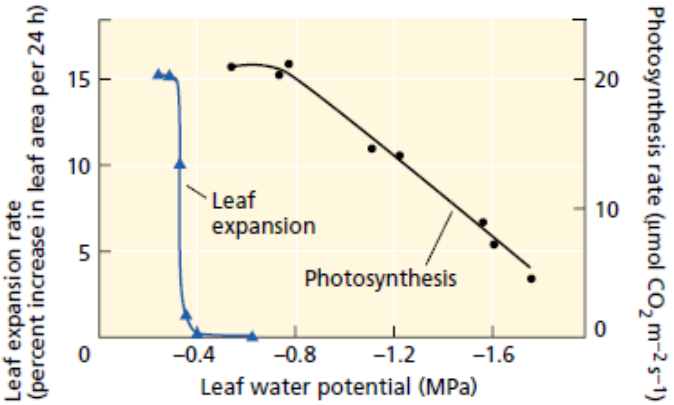


FIGURE 25.4 Effects of water stress on photosynthesis and leaf expansion of sunflower (*Helianthus annuus*). This species is typical of many plants in which leaf expansion is very sensitive to water stress, and it is completely inhibited under mild stress levels that hardly affect photosynthetic rates. (After Boyer 1970.)

Osmotic Adjustment of Cells Helps Maintain Plant Water Balance:

As the soil dries, its matric potential becomes more negative. Plants can continue to absorb water only as long as their water potential (Yw) is lower (more negative) than that of the soil water. Osmotic adjustment, or accumulation of solutes by cells, is a process by which water potential can be decreased without an accompanying decrease in turgor or decrease in cell volume.

Yw = Ys + Yp.

The change in cell water potential results simply from changes in solute potential (Ys), the osmotic component of Yw. Osmotic adjustment is a net increase in solute content per cell that is independent of the volume changes that result from loss of water. The decrease in Ys is typically limited to about 0.2 to 0.8 MPa, except in plants adapted to extremely dry conditions. Most of the adjustment can usually be accounted for by increases in concentration of a variety of common solutes, including sugars, organic acids, amino acids, and inorganic ions (especially K+).

Cytosolic enzymes of plant cells can be severely inhibited by high concentrations of ions. The accumulation of ions during osmotic adjustment appears to be restricted to the vacuoles, where the ions are kept out of contact with enzymes in the cytosol or subcellular organelles. Because of this compartmentation of ions, other solutes must accumulate in the cytoplasm to maintain water potential equilibriumwithin the cell.

These other solutes, called **compatible solutes (or compatible osmolytes)**, are organic compounds that do not interfere with enzyme functions. Commonly accumulated compatible solutes include the amino acid proline, sugar alcohols (e.g., sorbitol and mannitol), and a quaternary amine called glycine betaine. Synthesis of compatible solutes helps plants adjust to increased salinity in the rooting zone.

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.

Water Deficit Increases Wax Deposition on the Leaf Surface:

A common developmental response to water stress is the production of a thicker cuticle that reduces water loss from the epidermis (cuticular transpiration). Although waxes are deposited in response to water deficit both on the surface and within the cuticle inner layer, the inner layer may be more important in controlling the rate of water loss in ways that are more complex than by just increasing the amount of wax present.

A thicker cuticle also decreases CO_2 permeability, but leaf photosynthesis remains unaffected because the epidermal cells underneath the cuticle are nonphotosynthetic.

Water Deficit Alters Energy Dissipation from Leaves:

In warm, dry climates, an experienced farmer can decide whether plants need water simply by touching the leaves because a rapidly transpiring leaf is distinctly cool to the touch. When water stress limits transpiration, the leaf heats up unless another process offsets the lack of cooling. Because of these effects of transpiration on leaf temperature, water stress and heat stress are closely interrelated.

Maintaining a leaf temperature that is much lower than the air temperature requires evaporation of vast quantities of water. This is why adaptations that cool leaves by means other than evaporation (e.g., changes in leaf size and leaf orientation) are very effective in conserving water. When transpiration decreases and leaf temperature becomes warmer than the air temperature, some of the extra energy in the leaf is dissipated as sensible heat loss.

Osmotic Stress Induces Crassulacean Acid Metabolism in Some Plants:

Crassulacean acid metabolism (CAM) is a plant adaptation in which stomata open at night and close during the day. The leaf-to-air vapor pressure difference that drives transpiration is much reduced at night, when both leaf and air are cool. As a result, the water-use efficiencies of CAM plants are among the highest measured.

A CAM plant may gain 1 g of dry matter for only 125 g of water used—a ratio that is three to five times greater than the ratio for a typical C3 plant. CAM is very prevalent in succulent plants such as cacti. Some succulent species display facultative CAM, switching to CAM when subjected to water deficits or saline conditions. This switch in metabolism is a remarkable adaptation to stress, involving accumulation of the enzymes phosphoenolpyruvate (PEP) carboxylase, pyruvate–orthophosphate dikinase, and NADP malic enzyme, among others.

Osmotic Stress Changes Gene Expression:

As noted earlier, the accumulation of compatible solutes in response to osmotic stress requires the activation of the metabolic pathways that biosynthesize these solutes. Several genes coding for enzymes associated with osmotic adjustment are turned on (up-regulated) by osmotic stress and/or salinity, and cold stress. These genes encode enzymes such as the following (Buchanan et al. 2000):

- Δ′1-Pyrroline-5-carboxylate synthase, a key enzyme in the proline biosynthetic pathway
- Betaine aldehyde dehydrogenase, an enzyme involved in glycine betaine accumulation
- myo-Inositol 6-O-methyltransferase, a rate-limiting enzyme in the accumulation of the cyclic sugar alcohol called pinitol

Several other genes that encode well-known enzymes are induced by osmotic stress. The expression of glyceraldehyde- 3-phosphate dehydrogenase increases during osmotic stress, perhaps to allow an increase of carbon flow into organic solutes for osmotic adjustment. Enzymes involved in lignin biosynthesis are also controlled by osmotic stress.

Reduction in the activities of key enzymes also takes place. The accumulation of the sugar alcohol mannitol in response to osmotic stress appears not to be brought about by the up-regulation of genes producing enzymes involved in mannitol biosynthesis, but rather by the down-regulation of genes associated with sucrose production and mannitol degradation.

A large group of genes that are regulated by osmotic stress was discovered by examination of naturally desiccating embryos during seed maturation. These genes code for so-called **LEA proteins** (named for late embryogenesis abundant), and they are suspected to play a role in cellular membrane protection. Although the function of LEA proteins is not well understood, they accumulate in vegetative tissues during episodes of osmotic stress. The proteins encoded by these genes are typically

hydrophilic and strongly bind water. Their protective role might be associated with an ability to retain water and to prevent crystallization of important cellular proteins and other molecules during desiccation. They might also contribute to membrane stabilization.

Table 25.2
The five groups of late embryogenesis abundant (LEA) proteins found in plants

Group (family name)[a]	Protein(s) in the group	Structural characteristics and motifs	Functional information/ proposed function
Group 1 (D-19 family)	Cotton D-19 Wheat Em (early methionine-labeled protein) Sunflower Ha ds10 Barley B19	Conformation is predominantly random coil with some predicted short α helices Charged amino acids and glycine are abundant	Contains more water of hydration than typical globular proteins Overexpression confers water deficit tolerance on yeast cells
Group 2 (D-11 family) (also referred to as dehydrins)	Maize DHN1, M3, RAB17 Cotton D-11 *Arabidopsis* pRABAT1, ERD10, ERD14 *Craterostigma* pcC 27-04, pcC 6-19 Tomato pLE4, TAS14 Barley B8, B9, B17 Rice pRAB16A Carrot pcEP40	Variable structure includes α helix–forming lysine-rich regions The consensus sequence for group 2 dehydrins is EKKGIMDKIKELPG The number of times this consensus repeats per protein varies Often contains a poly(serine) region Often contains regions of variable length rich in polar residues and either Gly or Ala., and Pro	Often localized to the cytoplasm or nucleus More acidic members of the family are associated with the plasma membrane May act to stabilize macromolecules at low water potential
Group 3 (D-7 family)	Barley HVA1 (ABA-induced) Cotton D-7 Wheat pMA2005, pMA1949 *Craterostigma* pcC3-06	Eleven amino-acid consensus sequence motif TAQAAKEKAXE is repeated in the protein Contains apparent amphipathic α helices Dimeric protein	Transgenic plants expressing HVA1 demonstrate enhanced water deficit stress tolerance D-7 is an abundant protein in cotton embryos (estimated concentration 0.25 m*M*) Each putative dimer of D-7 may bind as many as ten inorganic phosphates and their counterions
Group 4 (D-95 family)	Soybean D-95 *Craterostigma* pcC27-45	Slightly hydrophobic N-terminal region is predicted to form amphipathic α helices	In tomato, a gene encoding a similar protein is expressed in response to nematode feeding
Group 5 (D-113 family)	Tomato LE25 Sunflower Hads11 Cotton D-113	Family members share sequence homology at the conserved N terminus N-terminal region is predicted to form α helices C-terminal domain is predicted to be a random coil of variable length and sequence Ala, Gly, and Thr are abundant in the sequence	Binds to membranes and/or proteins to maintain structure during stress Possibly functions in ion sequestration to protect cytosolic metabolism When LE25 is expressed in yeast, it confers salt and freezing tolerance D-113 is abundant in cottonseeds (up to 0.3 m*M*)

[a]The protein family names are derived from the cotton seed proteins that are most similar to the family.
Source: After Bray et al. 2000.

Stress-Responsive Genes Are Regulated by ABADependent and ABA-Independent Processes:
Studies of the promoters of several stress-induced genes have led to the identification of specific regulatory sequences for genes involved in different stresses. For example, the RD29 gene contains DNA sequences that can be activated by osmotic stress, by cold, and by ABA (Yamaguchi-Shinozaki and Shinozaki 1994; Stockinger et al. 1997).
The promoters of ABA-regulated genes contain a sixnucleotide sequence element referred to as the **ABA response element (ABRE),** which probably binds transcriptional factors involved in ABA-regulated gene activation. The promoters of these genes, which are regulated by osmotic stress in an

ABA-dependent manner, contain an alternative nine-nucleotide regulatory sequence element, the **dehydration response element (DRE)** which is recognized by an alternative set of proteins regulating transcription. Thus the genes that are regulated by osmotic stresses appear to be regulated either by signal transduction pathways mediated by the action of ABA (ABA-dependent genes), or by an ABA-independent, osmotic stress–responsive signal transduction pathway.
At least two signaling pathways have been implicated in the regulation of gene expression in an ABA-independent manner. Transacting transcription factors (called DREB1 and DREB2) that bind to the DRE elements in the promoters of osmotic stress–responsive genes are apparently activated by an ABA-independent signalling cascade. Other ABA-independent, osmotic stress–responsive genes appear to be directly controlled by the so-called MAP kinase signaling cascade of protein kinases. Other changes in gene expression appear to be mediated via other mechanisms not involving DREBs.

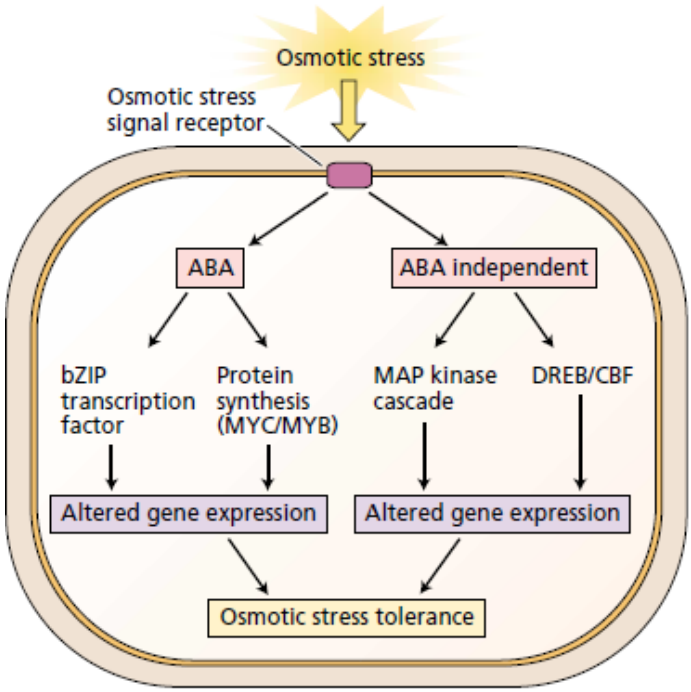


FIGURE 25.9 Signal transduction pathways for osmotic stress in plant cells. Osmotic stress is perceived by an as yet unknown receptor in the plasma membrane activating ABA-independent and an ABA-dependent signal transduction pathways. Protein synthesis participates in one of the ABA-dependent pathways involving MYC/MYB. The bZIP ABA-dependent pathway involves recognition of ABA-responsive elements in gene promoters. Two ABA-independent pathways, one involving the MAP kinase signaling cascade and the other involving DREBP/CBF-related transcription factors have also been demonstrated. (After Shinozaki and Yamaguchi-Shinozaki, 2000.)

4. Salinity stress and gene expression

Salinity stress and gene expression:
Under natural conditions, terrestrial higher plants encounter high concentrations of salts close to the seashore and in estuaries where seawater and freshwater mix or replace each other with the tides. Far inland, natural salt seepage from geologic marine deposits can wash into adjoining areas, rendering them unusable for agriculture. However, a much more extensive problem in agriculture is the accumulation of salts from irrigation water. Evaporation and transpiration remove pure water (as vapor) from the soil, and this water loss concentrate solutes in the soil. When irrigation water contains a high concentration of solutes and when there is no opportunity to flush out accumulated salts to a

drainage system, salts can quickly reach levels that are injurious to salt-sensitive species. It is estimated that about one-third of the irrigated land on Earth is affected by salt. In this section we discuss how plant function is affected by water and soil salinity, and we examine the processes that assist plants in avoiding salinity stress.

According to an estimate, about one third of irrigated land on Earth is affected by salt stress. Na^+, Cl^-, HCO_3^- , K^+, Ca^{2+}, Mg^{2+} and SO_4^{2+} ions usually contribute to the soil salinity. Higher concentrations of sodium, chloride and carbonate ions are potentially toxic to salt sensitive plants. Presence of high salt concen-trations in the soil is a common and important stress factor in deserts.

Salt Accumulation in Soils Impairs Plant Function and Soil Structure:

In discussing the effects of salts in the soil, we distinguish between high concentrations of Na^+, referred to as **sodicity**, and high concentrations of total salts, referred to as **salinity**. The two concepts are often related, but in some areas Ca^{2+}, Mg^{2+} and SO_4^{2+}, as well as NaCl, can contribute substantially to salinity. The high Na^+ concentration of a sodic soil can not only injure plants directly but also degrade the soil structure, decreasing porosity and water permeability. Asodic clay soil known as caliche is so hard and impermeable that dynamite is sometimes required to dig through it!

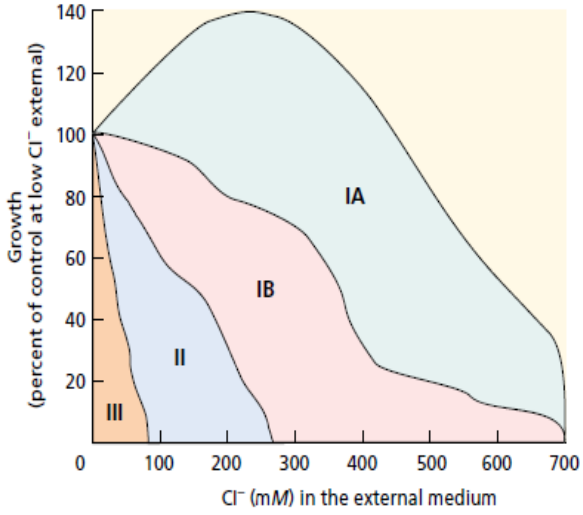
In the field, the salinity of soil water or irrigation water is measured in terms of its electrical conductivity or in terms of osmotic potential. Pure water is a very poor conductor of electric current; the conductivity of a water sample is due to the ions dissolved in it. The higher the salt concentration in water, the greater its electrical conductivity and the lower its osmotic potential (higher osmotic pressure).

The quality of irrigation water in semiarid and arid regions is often poor. In the United States the salt content of the headwaters of the Colorado River is only 50 mg L–1, but about 2000 km downstream, in southern California, the salt content of the same river reaches about 900 mg L–1, enough to preclude growth of some salt-sensitive crops, such as maize.

Salinity Depresses Growth and Photosynthesis in Sensitive Species:

Plants can be divided into two broad groups on the basis of their response to high concentrations of salts. **Halophytes** are native to saline soils and complete their life cycles in that environment. **Glycophytes** (literally "sweet plants"), or nonhalophytes, are not able to resist salts to the same degree as halophytes. Usually there is a threshold concentration of salt above which glycophytes begin to show signs of growth inhibition, leaf discoloration, and loss of dry weight.

Among crops, maize, onion, citrus, pecan, lettuce, and bean are highly sensitive to salt; cotton and barley are moderately tolerant; and sugar beet and date palms are highly tolerant (Greenway and Munns 1980). Some species that are highly tolerant of salt, such as *Suaeda maritima* (a salt marsh plant) and *Atriplex nummularia* (a saltbush), show growth stimulation at Cl^- concentrations many times greater than the lethal level for sensitive species.



Group IA (halophytes) includes sea blite (*Suaeda maritima*) and salt bush (*Atriplex nummularia*). These species show growth stimulation with Cl^- levels below 400 n*M*.

Group IB (halophytes) includes Townsend's cordgrass (*Spartina x townsendii*) and sugar beet (*Beta vulgaris*).These plants tolerate salt, but their growth is retarded.

Group II (halophytes and nonhalophytes) includes salt-tolerant halophytic grasses that lack salt glands, such as *Festuca rubra* subsp. red fescue (*littoralis*) and *Puccinellia peisonis*, and nonhalophytes, such as cotton (*Gossypium* spp.) and barley (*Hordeum vulgare*). All are inhibited by high salt concentrations. Within this group, tomato (*Lycopersicon esculentum*) is intermediate, and common bean (*Phaseolus vulgaris*) and soybean (*Glycine max*) are sensitive.

The species in **Group III (very salt-sensitive nonhalophytes)** are severely inhibited or killed by low salt concentrations. Included are many fruit trees, such as citrus, avocado, and stone fruit.

FIGURE 25.14 The growth of different species subjected to salinity relative to that of unsalinized controls. The curves dividing the regions are based on data for different species. Plants were grown for 1 to 6 months. (From Greenway and Munns 1980.)

Salt Injury Involves Both Osmotic Effects and Specific Ion Effects:

Dissolved solutes in the rooting zone generate a low (more negative) osmotic potential that lowers the soil water potential. The general water balance of plants is thus affected because leaves need to develop even lower water potential to maintain a "downhill" gradient of water potential between the soil and the leaves.

This effect of dissolved solutes is similar to that of a soil water deficit, and most plants respond to excessive levels of soil salinity in the same way as described earlier for water deficit. A major difference between the low-water-potential environments caused by salinity versus soil desiccation is the total amount of water available. During soil desiccation a finite amount of water can be obtained from the soil profile by the plant, causing ever decreasing water potentials. In most saline environments a large (essentially unlimited) amount of water at a constant, low water potential is available.

Of particular importance here is the fact that most plants can adjust osmotically when growing in saline soils. Such adjustment prevents loss of turgor (which would slow cell growth) while generating a lower water potential, but these plants often continue to grow more slowly after this adjustment for an unknown reason that curiously,is not related to insufficient turgor (Bressan et al. 1990)

In addition to the plant responses to low water potential, specific **ion toxicity effects** also occur when injurious concentrations of ions—particularly Na^+, Cl^-, or SO_4^{2-} accumulate in cells. Under nonsaline conditions, the cytosol of higher-plant cells contains 100 to 200 mM K^+ and 1 to 10 mM Na+, an ionic environment in which many enzymes function optimally. An abnormally high ratio of Na^+ to K^+ and high concentrations of total salts inactivate enzymes and inhibit protein synthesis. At a high concentration, Na^+ can displace Ca^{2+} from the plasma membrane of cotton root hairs, resulting in a change in plasma membrane permeability that can be detected as leakage of K^+ from the cells (Cramer et al. 1985).

Photosynthesis is inhibited when high concentrations of Na^+ and/or Cl^- accumulate in chloroplasts. Since photosynthetic electron transport appears relatively insensitive to salts, either carbon metabolism or photophosphorylation may be affected. Enzymes extracted from salt-tolerant species are just as sensitive to the presence of NaCl as enzymes from salt-sensitive glycophytes are. Hence the resistance of halophytes to salts is not a consequence of salt-resistant metabolism. Instead, other mechanisms come into play to avoid salt injury, as discussed in the following section.

Plants Use Different Strategies to Avoid Salt Injury:

Plants minimize salt injury by excluding salt from meristems, particularly in the shoot, and from leaves that are actively expanding and photosynthesizing. In plants that are salt sensitive, resistance to moderate levels of salinity in the soil depends in part on the ability of the roots to prevent potentially harmful ions from reaching the shoots. The Casparian strip imposes a restriction to the movements of ions into the xylem. To bypass the Casparian strips, ions need to move from the apoplast to the symplastic pathway across cell membranes.

This transition offers salt-resistant plants a mechanism to partially exclude harmful ions. Sodium ions enter roots passively (by moving down an electrochemical-potential gradien), so root cells must use energy to extrude Na^+ actively back to the outside solution. By contrast, Cl^- is excluded by negative electric potential across the cell membrane, and the low permeability of root plasma membranes to this ion. Movement of Na^+ into leaves is further minimized by absorption of Na^+ from the transpiration stream (xylem sap) during its movement from roots to shoots and leaves.

Some salt-resistant plants, such as salt cedar (*Tamarix* sp.) and salt bush (*Atriplex* sp.), do not exclude ions at the root, but instead have salt glands at the surface of the leaves. The ions are transported to these glands, where the salt crystallizes and is no longer harmful. In general, halophytes have a greater capacity than glycophytes for ion accumulation in shoot cells.

Although some plants, such as mangroves, grow in saline environments with abundant water supplies, the ability to acquire that water requires that they make osmotic adjustments to obtain water from the low-water-potential external environment. As discussed earlier in relation to water deficit, plant cells can adjust their water potential (Yw) in response to osmotic stress by lowering their solute potential (Ys). Two intracellular processes contribute to the decrease in Ys: the accumulation of ions in the vacuole and the synthesis of compatible solutes in the cytosol.

As mentioned earlier in the chapter, compatible solutes include glycine betaine, proline, sorbitol, mannitol, pinitol, and sucrose. Specific plant families tend to use one or two of these compounds in preference to others. The amount of carbon used for the synthesis of these organic solutes can be rather large (about 10% of the plant weight). In natural vegetation this diversion of carbon to adjust water potential does not affect survival, but in agricultural crops it can reduce growth and therefore total biomass and harvestable yields.

Many halophytes exhibit a growth optimum at moderate levels of salinity, and this optimum is correlated with the capacity to accumulate ions in the vacuole, where they can contribute to the cell osmotic potential without damaging the salt-sensitive enzymes. To a lesser extent, this process also occurs in more salt-sensitive glycophytes, but the adjustment may be slower.

Besides making adjustments in water potential, plants adjusting to salinity stress undergo the other osmotic stress–related acclimations described earlier for water deficit. For example, plants subjected to salt stress can reduce leaf area and or drop leaves via leaf abscission just as during episodes of osmotic stress. In addition, changes in gene expression associated with osmotic stress are similarly associated with salinity stress. Keep in mind, however, that in addition to acclimation to a low-water-potential environment, plants experiencing salinity stress must cope with the toxicity of high ion concentrations associated with salinity stress.

Ion Exclusion Is Critical for Acclimation and Adaptation to Salinity Stress:

In terms of metabolic energy, use of ions to balance tissue water potential in a saline environment clearly has a lower energy cost for the plant than use of carbohydrates or amino acids, the production of which has a significantly higher energy cost. On the other hand, high ion concentrations are toxic to many cytosolic enzymes, so ions must be accumulated in the vacuole to minimize toxic concentrations in the cytosol.

Because NaCl is the most abundant salt encountered by plants under salinity stress, transport systems that facilitate compartmentation of Na^+ into the vacuole are critical (Binzel et al. 1988). Both Ca^{2+} and K^+ affect intracellular Na^+ concentrations (Zhong and Läuchli 1994). At high concentrations of Na^+, K^+ uptake through a high-affinity K^+–Na^+ transporter, HKT1, is inhibited, and the transporter operates as an Na+ uptake system. Calcium, on the other hand, enhances K^+/Na^+ selectivity and in so doing increases salt tolerance (Liu and Zhu 1997).

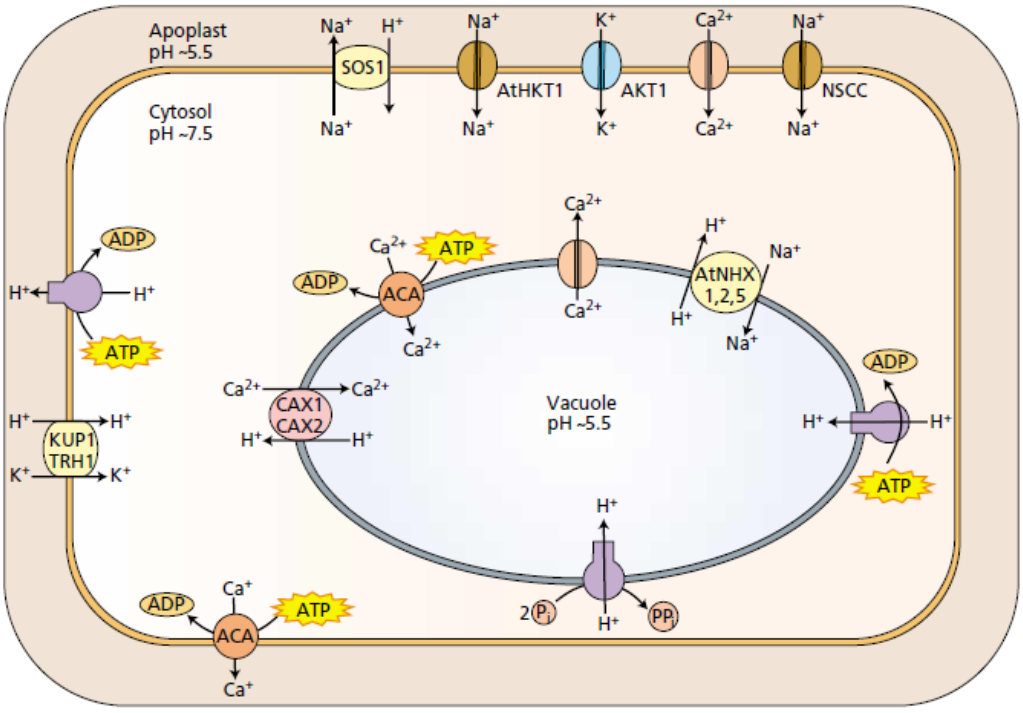


FIGURE 25.15 Membrane transport proteins mediating sodium, potassium, and calcium transport during salinity stress. SOS1, plasma membrane Na^+/H^+ antiporter; ACA, plasma/tonoplast membrane Ca^{2+}-ATPase; KUP1/TRH1, high-affinity K^+-H^+ co-transporter; atHKT1, sodium influx transporter; AKT1, K^+_{in} channel; NSCC, non selective cation channel; CAX1 or 2, Ca^{2+}/H^+ antiporter; atNHX1, 2 or 5, endomembrane Na^+/H^+ antiporter. Also indicated in the figure are proteins that have been implicated in ion homeostasis, but whose molecular identity is either not presently known or cofirmed in plants. These include plasma membrane and tonoplast calcium channel proteins, and vacuolar proton-pumping ATPases and pyrophosphatases. The membrane potential difference across the plasma membrane is typically 120 to 200 mV, negative inside; across the tonoplast 0 to 20 mV; positive inside.

Sodium Is Transported across the Plasma Membrane and the Tonoplast:

H^+ pumps in the plasma membrane and tonoplast provide the driving force (H^+ electro-chemical potential) for secondary transport of ions. An ATPase is primarily responsible for the large ΔpH and membrane potential gradient found across the plasma membrane. Avacuolar H^+-ATPase generates a ΔpH and membrane potential across the tonoplast (Hasegawa et al. 2000).

Activity of these pumps is required for the secondary transport of excess ions associated with plant responses to salinity stress. This is indicated by findings showing that the activity of these H+ pumps is increased by salinity, andminduced gene expression may account for some of this upregulation.

Energy-dependent transport (efflux) of Na^+ from the cytosol of plant cells across the plasma membrane is mediated by the gene product of the SOS1 (salt overly sensitive1) gene that function as a Na^+–H^+ antiporter.

The SOS1 antiporter is regulated by the gene products of at least two other genes, referred to as SOS2 and SOS3 (Shi et al. 2000). SOS2 is a serine/threonine kinase that is apparently activated by calcium through the function of SOS3, a calcium-regulated protein phosphatase. Vacuolar compartmentation of Na^+ results in part from the activity of a family of Na^+–H^+ antiporters such as Arabidopsis AtNHX1. Transgenic Arabidopsis and tomato plants overexpressing the gene that encodes AtNHX1 exhibit enhanced salt tolerance (Apse et al. 1999; Quintero et al. 2000).

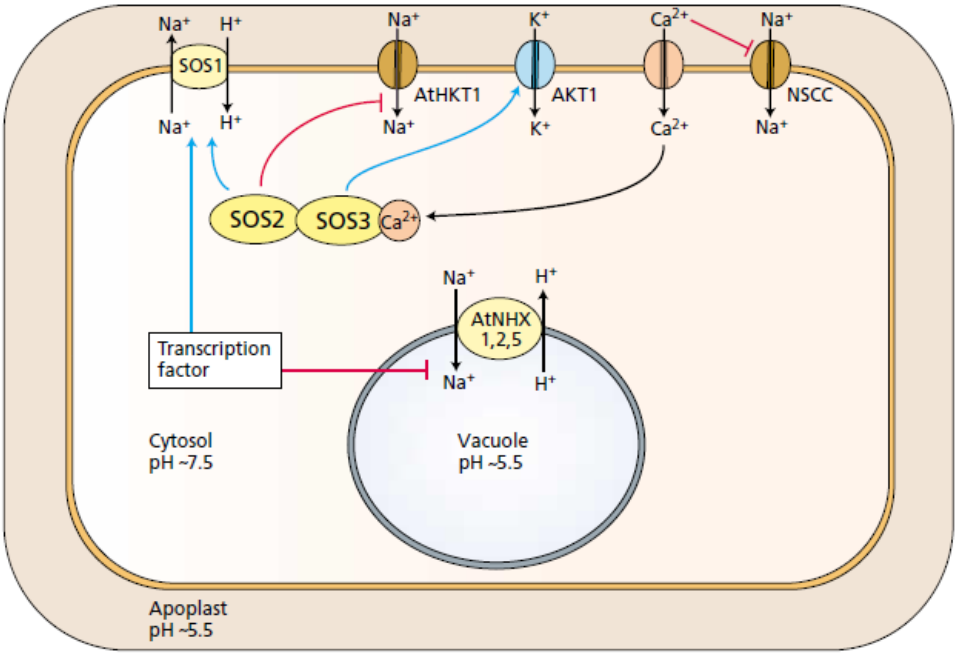


FIGURE 25.16 The regulation of ion homeostasis by the SOS signal transduction pathway, salinity stress, and calcium levels. Red arrows indicate positive regulation of the effected transport protein while blue arrows indicate negative regulation. Proteins shown in yellow are activated by salinity stress. SOS1, plasma membrane Na^+/H^+ antiporter; SOS2, serine/threonine kinase; SOS3, Ca^{2+} binding protein; HKT1, sodium influx transporter; AKT1, K^+_{in} channel; NSCC, non selective cation channel; NHX1, 2 or 5, endomembrane Na^+/H^+ antiporter; shown in orange is an undertermined calcium channel protein. Salinity stress activates a calcium channel leading to an increase in cytosolic calcium that activates the SOS cascade through SOS3. The SOS cascade must negatively regulate HKT1 which in turn secondarily regulates AKT1. At the same time, the SOS cascade increases the activity of SOS1 and AKT1. Working through an as yet undefined transcription factor the SOS cascade increases transcription of SOS1 while decreasing transcription of NHX gene(s). At low calcium NSCC can also function as an alternative sodium influx system, but this transporter is inhibited at high calcium levels. The membrane potential difference across the plasma membrane is typically 120 to 200 mV, negative inside, that of the tonoplast is 0 to 20 mV, positive inside.

5. Oxidative stress and antioxidation strategies in plants and their cellular regulation.

Oxidative stress:

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting

damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Oxidative stress from oxidative metabolism causes base damage, as well as strand breaks in DNA. Base damage is mostly indirect and caused by reactive oxygen species (ROS) generated, e.g. O^{2-} (superoxide radical), OH° (hydroxyl radical) and H_2O_2 (hydrogen peroxide). Further, some reactive oxidative species act as cellular messengers in redox signaling. Thus, oxidative stress can cause disruptions in normal mechanisms of cellular signaling.

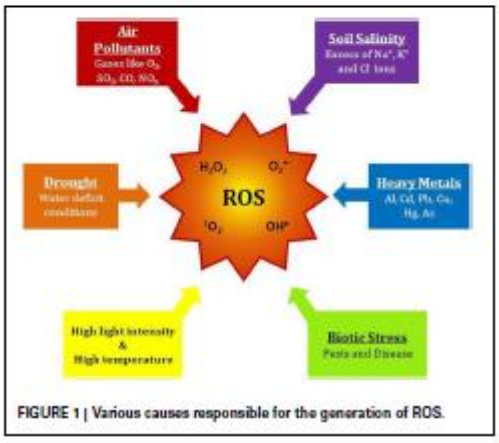


FIGURE 1 | Various causes responsible for the generation of ROS.

Types of ROS:

Phototrophs convert light energy from the sun into biochemical energy and therefore are crucial for sustaining life on Earth.The rice they have to pay for this is to face the risk of oxidative damages, because of the different types of ROS, namely, $^1O_2$ (singlet oxygen), H_2O_2 (hydrogen peroxide),$O\bullet^{-2}$ (superoxide radical), and OH• (hydroxyl radical),generated as unwanted by products. These are generated from only 1–2% of total O_2 consumed by plants (Bhattacharjee,2005). The reactions generating the different ROS members are shown in figure.

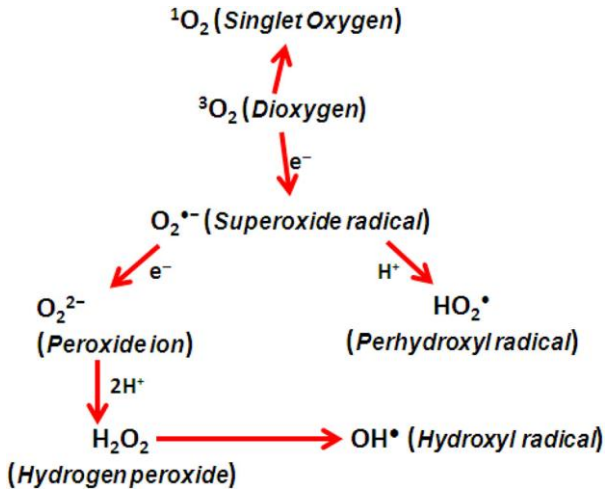


Table 1 | Different members of the ROS family and their attributes.

ROS	$t_{1/2}$	Migration distance	Sources	Mode of action	Reaction with DNA	Reaction with protein	Reaction with DNA	Scavenging systems
Superoxide ($O_2^{\bullet-}$)	1–4 µs	30 nm	Membranes, Chloroplasts, Mitochondria	Reacts with double bond containing compounds such as (Fe-S) proteins	No	Via the Fe-center	Extremely low	SOD
Hydroxyl radical (OH•)	1 µs	1 nm	Membranes, Chloroplasts, Mitochondria	Extremely reactive with all biomolecules	Rapidly reacting	Rapidly reacting	Rapidly reacting	Flavonoids and Proline
Hydrogen Peroxide (H_2O_2)	1 ms	1 µm	Membranes, Chloroplasts, Mitochondria, Peroxisomes	Oxidizes proteins and forms OH• via $O_2^{\bullet-}$	No	Attacks the Cys residue	Extremely low	CAT, POXs and Flavonoids
Singlet Oxygen ($^1O_2$)	1–4 µs	30 nm	Membranes, Chloroplasts, Mitochondria	Oxidizes proteins, PUFAs and DNA	Reacts with G residue	Attacks Trp, His, Tyr, Met and Cys residues	PUFA	Carotenoids and α-Tocopherol

Superoxide radical ($O\bullet^{-2}$)

The ROS is being constantly generated in the chloroplasts due to partial reduction of O_2 or as a result of transfer of energy to O_2. The superoxide radical ($O\bullet^{-2}$) is formed mainly in the thylakoid localized PSI during non-cyclic electron transport chain (ETC), as well as other cellular compartments. Normally, H_2O is generated when cytochrome c oxidase interacts with O_2. Occasionally, O_2 reacts with the different ETC components to give rise to the $O\bullet^{-2}$. It is usually the first ROS to be formed. Superoxide radical ($O\bullet^{-2}$) can also undergo further reactions tgenerate other members of the ROS family.

$$O_2^{\bullet-} + Fe^{3+} \rightarrow {}^1O_2 + Fe^{2+}$$
$$2O_2^{\bullet-} + 2H^+ \rightarrow O_2 + H_2O_2Fe^{3+}$$
$$Fe^{2+} + H_2O_2 + Fe^{3+} \rightarrow Fe^{3+} + OH^- + OH^\bullet$$

Singlet oxygen ($_1O^2$)

Singlet Oxygen is an a typical ROS which is generated not by electron transfer to O_2, but rather by the reaction of chlorophyll (Chl) triplet state in the antenna system with O_2.

$$Chl \rightarrow 3Chl$$
$$3Chl + 3O_2 \rightarrow Chl + {}_1O^2$$

Environmental stresses like salinity, drought and heavy metals cause stomatal closure, leading to insufficient intra cellular CO_2 concentration.This favors the formation of $_1O^2$. Singlet oxygen can cause severe damages to both the photosystems, PSI and PSII, and puts the entire photosynthetic machinery into jeopardy. Even though $_1O^2$ has a short half-life of about 3 μs (Hatz et al.,2007), it can manage to diffuse some 100 nano meters and causes damage to wide range of targets. These include molecules like proteins, pigments, nucleicacids and lipids (Wagner et al., 2004; Krieger-Liszkay et al.,2008), and is the major ROS responsible for light-induced loss of PSII activity,eliciting cellular death. Plants have managed to efficiently scavenge $_1O^2$ with the help of β-carotene, tocopherol, plastoquinone and canals or eact with the D1 protein of PSII.

Hydrogen peroxide (H_2O_2):

Hydrogen peroxide, a moderately reactive ROS is formed when $O\bullet^{-2}$ undergoes both univalent reduction as well as protonation. It can occur both non-enzymatically by being dismutated to H_2O_2 under low pH conditions or mostly by a reaction catalysed by SOD.

$$2O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2$$
$$2O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2$$

H_2O_2 is produced in plant cells not only under normal conditions, but also by oxidative stress, caused by factors like drought, chilling, intense light, UV radiation, wounding and pathogen infection (Sharmaetal.,2012). Due to stomatal closure and its limited fixation, Ribulose1,5-bisphosphate (RuBP) oxygenation is favoured and thus photorespiration is enhanced. This accounts for more than 70% of the H_2O_2 produced as a result of drought stress (Noctor et al., 2002). The major sources of H_2O_2 production in plant cells include the ETC in the chloroplast, mitochondria, ER, cell membrane, β-oxidation of fatty acid and photorespiration. Additional sources comprise of different reactions involving photooxidation by NADPH oxidase and xanthine oxidase (XOD).

Hydroxyl radical (OH•):

Among its family members, hydroxyl radical (OH•) is the most reactive and the most toxic ROS known. It is generated at neutral pH by the Fenton reaction between H_2O_2 and $O\bullet^{-2}$ catalyzed by transition metals like Fe (Fe^{2+}, Fe^{3+}).

$$H_2O_2 + O_2^{\bullet-} \rightarrow OH^- + O_2 + OH^\bullet$$

It has the capability to damage different cellular components by lipid peroxidation (LPO), protein damage and membrane destruction. Since there is no existing enzymatic system to scavenge this toxic radical, excess accumulation of OH• causes the cellular death (Pinto et al., 2003).

Sites of ROS Production in Plant Cells

The ROS is being produced under both normal and stressful conditions at various locations in the chloroplasts, mitochondria, peroxisomes, plasma membranes, ER and the cell wall. In presence of light, chloroplasts and peroxisomes are the major sources of ROS production, while the mitochondrion is the leading producer of ROS under dark conditions (Choudhury et al., 2013).

Chloroplast

The chloroplast comprises of an extremely ordered system of thylakoid membranes which houses the light capturing photosynthetic machinery as well as anatomical requirements for efficient light harvesting (Pfannschmidt, 2003). The photosystems, PSI and PSII which form the core of the light harvesting system in the thylakoids are the major sources of ROS production. Abiotic stress factors like drought, salinity, temperature extremes, all of which cause water stress and limit CO_2 concentrations, coupled with excess light, leads to the formation of $O\bullet^{-2}$ at the PS, via the Mehler reaction.

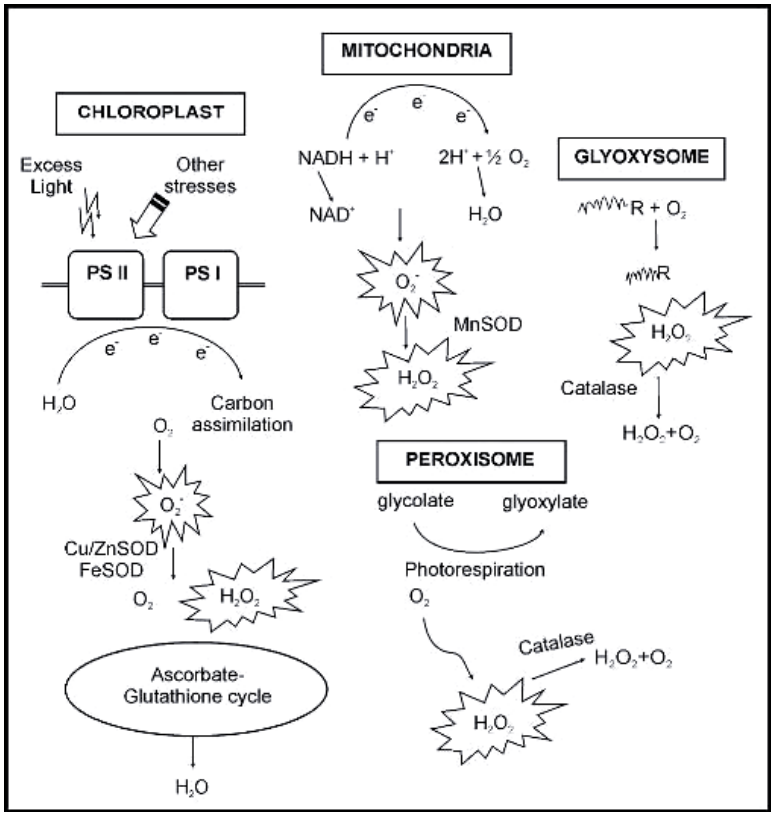
Subsequently, a membrane-bound Cu/Zn SOD at the PSI converts $O\bullet^{-2}$ into H_2O_2 (Miller et al., 2010). The other accomplices of leaking electrons from the ETC of PSI are the 2Fe-2S and the 4Fe-4S clusters. In the PSII, seepage of electrons occurs, via the QA and QB electron acceptors and is responsible for the generation of $O\bullet^{-2}$. The superoxide radical then goes onto converting itself into more toxic ROS like OH• via H_2O_2 intermediate by the Fenton reaction at the Fe-S centers. The PSII is also responsible for the generation of $^1O_2$ and this occurs in two ways. Firstly, when environmental stress upsets the delicate balance between light harvesting and energy utilization, it leads to the formation of triplet Chl (3Chl*) which on reacting with dioxygen ($^3O_2$) liberates singlet oxygen ($^1O_2$) (Karuppanapandian et al., 2011). Secondly, when the ETC is over reduced, the light harvesting complex (LHC) at the PSII generates $^1O_2$ (Asada, 2006). The $^1O_2$ accumulating in the chloroplast causes peroxidation of membrane lipids, and especially Polyunsaturated Fatty Acids (PUFA) and damages membrane proteins which put the P680 reaction center of PSII at risk. It could also directly lead to cell death (Møller etal., 2007; Triantaphylidès et al., 2008). The involvement of the chloroplast in oxidative stress-induced programmed cell death was revealed when animal anti-apoptotic Bcl-2 was expressed in transgenic tobacco (Chen and Dickman, 2004). The $^1O_2$ can also initiate a genetic program, via the EXECUTOR1 and EXECUTOR2 pathways and lead to growth inhibition in plants (Lee et al., 2007). Thus, the chloroplast is a major source of ROS production in plants. To ensure the continual survival of plants under stress, controlling and scavenging the ROS in the chloroplast is very essential, as shown in transgenic plants, as well in stress-tolerant cultivars (Tseng et al., 2007).

Mitochondria

Mitochondria are also the site of generation of harmful ROS, like H_2O_2 and $O\bullet^{-2}$ (Navrot et al., 2007), though in a smaller scale. Plant mitochondria differ from animal counterparts in having O_2 and carbohydrate-rich environment (Rhoads et al., 2006) and also being involved in photorespiration. The mitochondrial ETC (mtETC) is the major culprit as it houses sufficiently energized electrons to reduce O_2 to form the ROS. The two major components of the mtETC responsible for producing ROS are Complex I and Complex III (Møller etal., 2007; Noctor et al., 2007). The NADH Dehydrogenase or Complex I directly reduces O_2 to $O\bullet^{-2}$ in its flavoprotein region. The ROS production at Complex I is further enhanced when there is reverse electron flow from Complex III to Complex I due to lack of NAD^+-linked substrates. This reverse flow of electrons is controlled by ATP hydrolysis (Turrens, 2003). In Complex III, ubiquinone in its fully reduced form donates an electron to Cytochrome c1 leaving behind an unstable ubisemiquinone semi-radical which favors leakage of electrons to O_2, thereby generating $O\bullet^{-2}$ (Murphy, 2009). Other sources of ROS production in the mitochondria are the various enzymes present in the mitochondrial matrix. This include enzymes like aconitase which directly produces ROS and others like 1-Galactono-γ-lactone dehydrogenase (GAL) which indirectly produce ROS by feeding electrons to the ETC (Rasmusson et al., 2008). Even though $O\bullet^{-2}$ is the leading ROS in the mitochondria, it is converted to H2O2 by the Mn-SOD and the APX (Sharma et al., 2012). Estimates show that 1–5% of the total O2 consumption by the mitochondria is diverted toward production of H_2O_2. Mitochondrion generally produces ROS during normal conditions, but is greatly boosted at times of abiotic stress conditions (Pastore et al., 2007). Such stressful conditions affect the tight coupling of ETC and ATP synthesis, leading to over reduction of electron carriers like ubiquinone (UQ) pool, thereby generating ROS (Rhoads et al., 2006; Blokhina and Fagerstedt, 2010).

Since respiratory rate increases during drought, the mitochondrial ATP synthesis increases to compensate for the lower rate of chloroplast ATP synthesis, enhancing the mitochondrial ROS production (Atkin and Macherel, 2009). To counteract this oxidative stress in the mitochondria, two enzymes, Mitochondrial Alternative Oxidase (AOX) and Mitochondrial SOD (Mn-SOD) are very crucial. The AOX maintains the reduced state of the UQ pool and cuts down the ROS production. Its importance is evident from the fact that Arabidopsis lacking a functional AOX is sensitive to drought stress and has altered transcription profiles of different components of the antioxidant machinery (Ho et al., 2008). On the other hand, the higher activity of Mn-SOD clearly made the difference between a salt-tolerant cultivar and a salt-sensitive cultivar of tomato under salinity stress (Mittova et al., 2003).

Peroxisomes



Peroxisomes are single-membrane-bound spherical microbodies and are the major sites of intracellular H_2O_2 production due to their integral oxidative metabolism (Luis et al., 2006; Palma et al., 2009). They also produce $O\bullet^{-2}$, like chloroplasts and mitochondria during the course of various metabolic process. The $O\bullet^{-2}$ is generated at two different locations. The Xanthine oxidase (E.C.1.17.3.2), located in the peroxisomal matrix, metabolizes both xanthine and hypoxanthine into uric acid and generate $O\bullet^{-2}$ as a by-product. Second is the NADPH-dependent small ETC, composed of NADH and Cyt b localized in the peroxisomal membrane which utilizes O2 as the electron acceptor and releases $O\bullet^{-2}$ into the cytosol. Additionally, Peroxisomal Membrane Polypeptides (PMPs) of molecular masses 18, 29, and 132 kDa are the three integral membrane proteins responsible for $O\bullet^{-2}$ production. The NADH acts as the electron donor of the 18 and 32 kDa PMPs, whereas the 29 kDa PMP uses the NADPH as the electron donor to reduce Cytochrome c. During stressful conditions, when the availability of water is low and stomata remains closed, the ratio of CO_2 to O_2 reduces considerably which causes increased photorespiration leading to glycolate formation. This glycolate is oxidized by the glycolate oxidase in peroxisome to release H_2O_2, making it the leading producer of H_2O_2 during photorespiration (Noctor et al., 2002). Besides, there are other supplemental metabolic processes like β-oxidation of fatty acids, flavin oxidase pathway and the disproportionation of $O\bullet^{-2}$ radicals for peroxisomal ROS production.

Apoplast

Apoplast, the diffusible space around the plant cell membrane is responsible for converting the incoming CO_2 into a soluble, diffusible form which enters the cytosol to undergo photosynthesis. At times of adverse environmental conditions, stress signals combined with abscisic acid (ABA) make the apoplast a prominent site for H_2O_2 production (Hu et al., 2006). The NADPH oxidases expressed by the AtRbohD and AtRbohF in the guard cells and the mesophyll cells of *Arabidopsis*, account for generating the apoplastic ROS which is required for ABA-induced stomatal closure (Kwak et al., 2003). Besides these enzymes, there are additional ROS-generating enzymes which comprise of pH dependent peroxidases (POXs), cell wall-linked oxidases, germin-like oxalate oxidases and polyamine oxidases, all of which mainly produce H_2O_2.

Plasma Membranes

Plasma membrane which surrounds the entire plant cell plays an important role in interacting with the ever changing environmental conditions and provides information necessary for the continual survival of the cell. The NADPH-dependent-oxidases which are localized in the plasma membrane are in the spotlight due to their gene expression and presence of different homologs during different stress conditions (Apel and Hirt, 2004). The NADPH oxidase produces $O\bullet^{-2}$ by transferring electrons from cytosolic NADPH to O_2, which either spontaneously dismutates to H_2O_2 or is catalyzed by SOD. The fact that NADPH oxidase plays an important role in plant defense against pathogenic infection and abiotic stress conditions (Kwak et al., 2003) is well supported.

Cell Walls

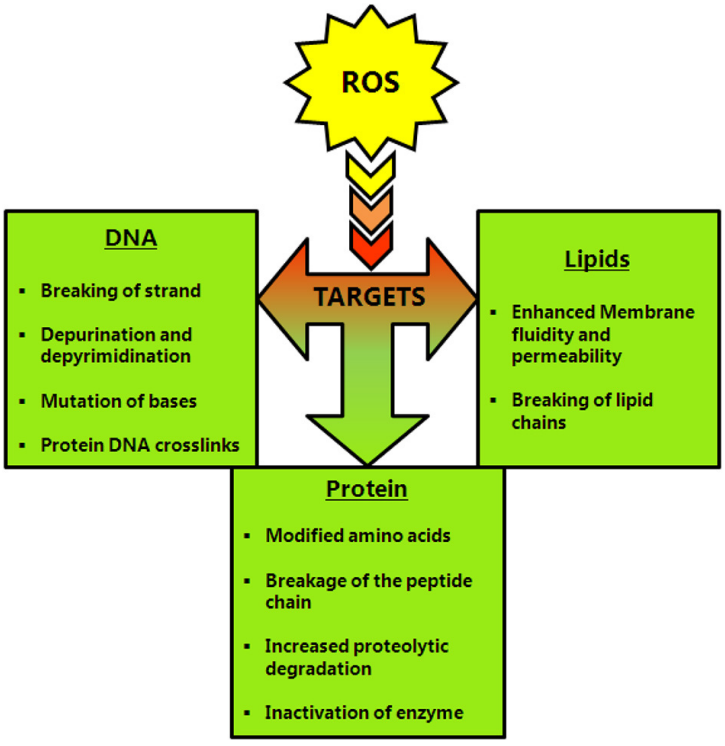
During stress, the cell wall-localized lipoxygenase (LOX) causes hydroperoxidation of polyunsaturated fatty acids (PUFA) making it active source of ROS like $OH\bullet$, $O\bullet^{-2}$, H_2O_2, and $^1O_2$. The cell wall-localized diamine oxidases utilize diamines or polyamines to generate ROS in the cell wall. During pathogen attack, lignin precursors undergo extensive cross-linking, via H_2O_2-mediated pathways to reinforce the cell wall with lignin (Higuchi, 2006).

Endoplasmic Reticulum (ER)

The NADPH-mediated electron transport involving $CytP_{450}$, localized in the ER generates $O^{\bullet-2}$ (Mittler, 2002). Organic substrate, RH interacts with the $CytP_{450}$ followed by reduction by a flavoprotein to give rise to a free radical intermediate (Cyt P_{450} R−). This intermediate promptly reacts with triplet oxygen ($^3O_2$) to form an oxygenated complex (Cyt P_{450}-ROO−). The complex may occasionally decompose to Cyt P_{450}-Rh by generating $O^{\bullet-2}$ as byproduct**.**

Targets of ROS:

ROS is known to cause damages to biomolecules such as lipids, proteins and DNA.



Lipids:

Lipids form a major portion of the plasma membrane which envelopes the cell and helps it to adapt to the changing environment. However, under stressful conditions, when the level of ROS rise above the threshold value, LPO becomes so damaging that it is often considered as the single parameter to gauge lipid destruction. LPO starts a chain reaction and further exacerbates oxidative stress by creating lipid radicals which damages proteins and DNA. The two main targets of the ROS in membrane phospholipids are the double bond between C-atoms and the ester linkage between glycerol and fatty acids. The PUFA which are important components of the plasma membrane are the

hotspots for ROS damage. PUFAs like linoleic and linolenic acid are specifically prone to attack by ROS like $_1O^2$ and OH•. The hydroxyl radical (OH•) is the most damaging member as it has the ability to trigger a cyclic chain reaction and cause further peroxidation of other PUFAs.
The entire process of LPO can be divided into three distinct phases, Initiation, Progression, and Termination. Initiation involves energizing the O_2 (a rate limiting step) to give rise to radicals like $O^{\bullet -2}$ and OH•. These ROS react with the methylene groups of the PUFA, yielding conjugated dienes, lipid peroxyl radicals and hydroperoxides (Smirnoff, 2000).

$$\text{PUFA-H} + \text{OH}^{\bullet} \rightarrow \text{PUFA}^{\bullet}\text{(PUFA alkyl radical)} + \text{H}_2\text{O}$$

$$\text{PUFA}^{\bullet} + \text{O}_2 \rightarrow \text{PUFA-OO}^{\bullet}\text{(Peroxyl radical)}$$

The PUFA peroxyl radical once formed possesses the ability to further propagate the LPO by extracting one H-atom from adjoining PUFA side chains.

$$\text{PUFA-OO}^{\bullet} + \text{PUFA} - \text{H} \rightarrow \text{PUFA} - \text{OOH} + \text{PUFA}^{\bullet}$$

The lipid hydroperoxide (PUFA-OOH) undergoes cleavage by reacting with reduced metals such as $Fe^{2+.}$

$$\text{PUFA-OOH} + \text{Fe}^{2+} \rightarrow \text{PUFA-}\,\text{O}^{\bullet} + \text{Fe}^{3+}$$

The lipid hydroperoxides can also undergo decomposition to form different reactive species such as lipid alkoxyl radicals, aldehydes, alkanes, lipid epoxides, and alcohols. LPO terminates with the formation of different lipid dimers caused by different lipid derived radicals.

$$\text{PUFA}^{\bullet} + \text{PUFA}^{\bullet} \rightarrow \text{PUFA} + \text{PUFA}$$

(Fatty Acid Dimer)

$$\text{PUFA}^{\bullet} + \text{PUFA-OO}^{\bullet} \rightarrow \text{PUFA-OO-PUFA}$$

(Peroxide bridged Dimer)

$$\text{PUFA-OO}^{\bullet} + \text{PUFA-OO}^{\bullet} \rightarrow \text{PUFA-OO-PUFA} + \text{O}_2$$

(Peroxide bridged Dimer)

Overall, the LPO increases membrane fluidity causing the membrane to be leaky to substances which otherwise enter the cell through special channels, damage the membrane proteins, deactivate the membrane receptors, membrane-localized enzymes and ion-channels.

Proteins:

The ROS produced during stress conditions causes the oxidation of proteins. The protein undergoes different types of modifications which may either be direct or indirect. During direct modifications, the activity of the protein becomes varied as a result of different chemical modifications such as nitrosylation, carboxylation, disulfide bond formation, and glutathionylation. Protein carbonylation is often used as a marker for evaluating protein oxidation (Møller etal., 2007). Indirect modification of proteins can occur as a result of interaction with the products of LPO. The ROS concentration, on crossing its threshold value, leads to the site-specific modification of amino acids like Arg, Lys, Pro, Thr, and Trp, and increased susceptibility to proteolytic degradation (Møller etal., 2007). The amino acids differ in their susceptibility to ROS attack. Amino acids containing thiol groups and sulfur are the most vulnerable. The Cys and Met are both prone to damage by the reactive $_1O^2$ and OH•. The enzymes containing iron-sulfur centers are irreversibly inactivated on getting oxidized by $O^{\bullet -2}$. The oxidized proteins thus become better targets for proteolytic digestion by getting primed for ubiquitination-mediated proteosomal degradation.

DNA:

Since the plant nuclear DNA is well protected by histones and associated proteins, both mitochondrial and chloroplastic DNA bears the brunt of the ROS attack due to lack of protective histones as well as the close proximity to ROS production machinery. Oxidative damage of DNA as a result of ROS

occurs at multiple levels which include oxidation of the deoxyribose sugar residue, modification of the nucleotide base, abstraction of a nucleotide, breaks in either DNA strand, and cross-linking of the DNA and protein. The hydroxyl radical not only damages the deoxyribose sugar backbone by extracting H-atom, but also reacts with double bonds of the purine and pyrimidine bases (Halliwell, 2006). The ROS abstracts the C-4 H-atom of the deoxyribose sugar and forms a deoxyribose radical which reacts further to cause single strand breaks in the DNA (Evans et al., 2004). The damaged products as a result of base oxidation include the most common 8-hydroxyquinine and other less common ones like hydroxyl methyl urea, dehydro-2′-deoxyguanosine, thymine glycol, and thymine and adenine ring opened. The OH• is also notorious for creating DNA-protein cross-links when it reacts with either DNA or associated proteins. These cross-links are not easily reparable and may be lethal to the plant cell, if not repaired in time before commencement of critical cellular processes like replication or transcription.

Antioxidation strategies:

The ROS defense mechanism consists of the antioxidant machinery which helps to mitigate the above mentioned oxidative stress-induced damages.The antioxidant machinery has two arms with the enzymatic components and non-enzymatic antioxidants.

Enzymatic antioxidants:

The enzymes localized in the different subcellular compartments and comprising the antioxidant machinery include Superoxide Dismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (APX), Monodehydro ascorbate reductase (MDHAR), Dehydroascorbate reductase (DHAR), Glutathione Reductase (GR) and Guaiacol Peroxidase (GPX).

Superoxide Dismutase (SOD):

SOD (E.C.1.15.1.1) belongs to the family of metalloenzymes omnipresent in all aerobic organisms. Under environmental stresses, SOD forms the first line of defense against ROS-induced damages. The SOD catalyzes the removal of $O\bullet^{-2}$ by dismutating it into O_2 and H_2O_2. This removes the possibility of OH• formation by the Haber-Weiss reaction. SODs are classified into three isozymes based on the metal ion it binds, Mn-SOD (localized in mitochondria), Fe-SOD (localized in chloroplasts) and Cu/Zn-SOD (localized in cytosol, peroxisomes and chloroplasts) (Mittler,2002). SOD has been found to be upregulated by abiotic stress conditions (Boguszewskaetal.,2010).

$$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \rightarrow 2H_2O_2 + O_2$$

Catalase (CAT):

CAT (E.C.1.11.1.6) is a tetrameric heme-containing enzyme responsible for catalysing the dismutation of H_2O_2 into H_2O and O_2. It has high affinity for H_2O_2, but lesser specificity for organic peroxides (R-O-O-R). It has a very high turn over rate (6 × 106 molecules of H_2O_2 to H_2O andO_2 min−1) and is unique amongst antioxidant enzymes in not requiring a reducing equivalent. Peroxisomes are the hotspots of H_2O_2 production due to β-oxidation of fatty acids, photorespiration, purine catabolism and oxidative stress (Mittler,2002). However, recent reports suggest that CAT is also found in other subcellular compartments such as the cytosol, chloroplast and the mitochondria, though significant CAT activity is yet to be seen (Mhamdi etal.,2010).

Angiosperms have been reported to have three CAT genes. CAT1 is expressed in pollens and seeds (localized in peroxisomes and cytosol), CAT2 predominantly expressed in photosynthetic tissues but also in roots and seeds (localized in peroxisomes and cytosol) and finally CAT3 is found to be expressed in leaves and vascular tissues (localized in the mitochondria).Stressful conditions demand greater energy generation and expenditure of the cell.

This is fulfilled by increased catabolism which generates H_2O_2. CAT removes the H_2O_2 in an energy efficient way.

$$H_2O_2 \rightarrow H_2O + (1/2)O_2$$

Ascorbate peroxidase (APX):

APX (E.C.1.1.11.1) is an integral component of the Ascorbate-Glutathione (ASC-GSH) cycle. While CAT predominantly scavenges H_2O_2 in the peroxisomes, APX performs the same function in the

cytosol and the chloroplast. The APX reduces H_2O_2 to H_2O and DHA, using Ascorbic acid (AA) as a reducing agent.

$$H_2O_2 + AA \rightarrow 2H_2O + DHA$$

The APX family comprises of five isoforms based on different amino acids and locations, viz., cytosolic, mitochondrial, peroxisomal and chloroplastid (stromal and thylakoidal) (Sharma and Dubey, 2004). Since APX is widely distributed and has a better affinity for H_2O_2 than CAT, it is a more efficient scavenger of H_2O_2 at times of stress.

Monodehydroascorbate reductase (MDHAR):

MDHAR (E.C.1.6.5.4) is responsible for regenerating AA from the short-lived MDHA, using NADPH as a reducing agent, ultimately replenishing the cellular AA pool. Since it regenerates AA, it is co-localized with the APX in the peroxisomes and mitochondria, where APX scavenges H_2O_2 and oxidizes AA in the process (Mittler,2002). MDHAR has several isozymes which are confined in chloroplast, mitochondria, peroxisomes, cytosol and glyoxysomes.

$$MDHA + NADPH \rightarrow AA + NADP^+$$

Dehydroascorbate reductase (DHAR)

DHAR (M.C.1.8.5.1) reduces dehydroascorbate (DHA) to AA using Reduced Glutathione (GSH) as an electron donor (Eltayeb et al., 2007). This makes it another agent, apart from MDHAR, which regenerates the cellular AA pool. It is critical in regulating the AA pool size in both symplast and apoplast, thus maintaining the redox state of the plant cell (Chen andGallie,2006). DHAR is found abundantly in seeds, roots and both green and etiolated shoots.

$$DHA + 2GSH \rightarrow AA + GSSG$$

Glutathione Reductase (GR)

GR (E.C.1.6.4.2) is a flavoprotein oxidoreductase which uses NADPH as a reductant to reduce GSSG to GSH. Reduced glutathione (GSH) is used upto regenerate AA from MDHA and DHA, and as a result is converted to its oxidized form (GSSG).

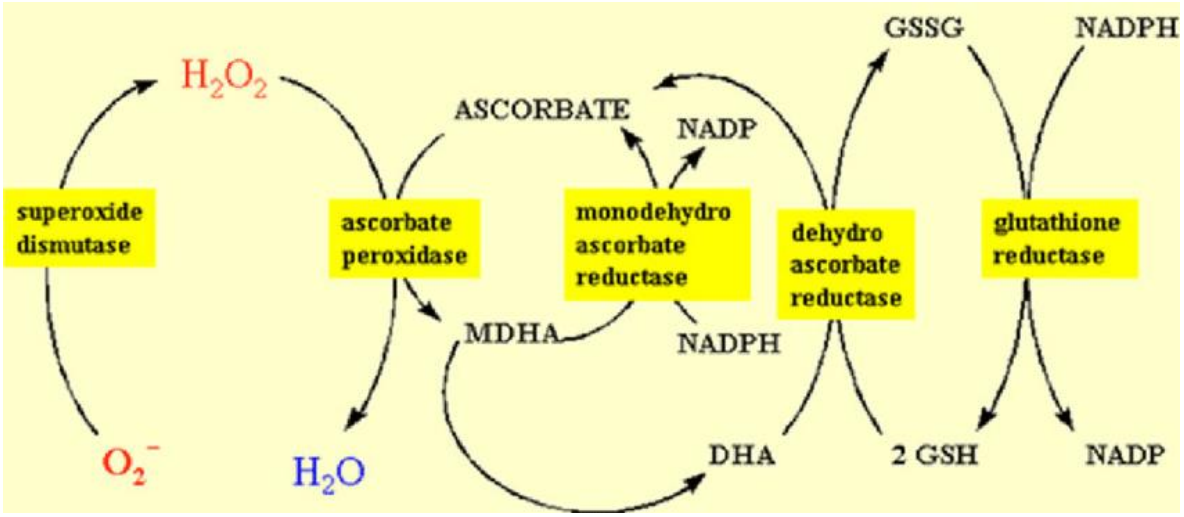
GR, a crucial enzyme of ASC-GSH cycle catalyzes the formation of a disulphide bond in glutathione disulphide to maintain a high cellular GSH/GSSG ratio.It is predominantly found in chloroplasts with small amounts occurring in the mitochondria and cytosol. GSH is a low molecular weight compound which plays the role of a reductant to prevent thiol groups from getting oxidized and react with detrimental ROS members like $_1O^2$ and OH•.

$$GSSG + NADPH \rightarrow 2GSH + NADP^+$$

Guaiacol peroxidase (GPX)

GPX (E.C.1.11.1.7) is a heme-containing enzyme composed of 40–50 kDa monomers, which eliminates excess H_2O_2 both during normal metabolism as well as during stress. It plays a vital role in the biosynthesis of lignin as well as defends against biotic stress by degrading indole acetic acid (IAA) and utilizing H_2O_2 in the process.GPX prefers aromatic compounds like guaiacol and pyragallol(Asada,1999) as electron donors.Since GPX is active intracellularly (cytosol, vacuole), in the cell wall and extracellularly, it is considered as the key enzyme in the removal of H_2O_2.

$$H_2O_2 + GSH \rightarrow H_2O + GSSG$$



The glutathione-ascorbate cycle

Non-enzymatic antioxidants:

The non-enzymatic antioxidants form the other half of the antioxidant machinery, comprising of AA, GSH, α-tocopherol, carotenoids, phenolics, flavonoids, and amin oacid cumosmolyte proline.They not only protect different components of the cell from damage, but also play a vital role in plant growth and development by tweaking cellular process like mitosis, cell elongation, senescence and cell death (de Pinto and DeGara,2004).

Ascorbic Acid (AA)

AA is the most abundant and the most extensively studied antioxidant compound. It is considered powerful as it can donate electrons to a wide range of enzymatic and non-enzymatic reactions. Majority of AA in plant cells is the result of Smirnoff-Wheeler pathway, catalysed by L-galactano-γ-lactone dehydrogenase in the plant mitochondria, with the remaining being generated from D-galacturonic acid. 90% of the AA pool is concentrated not only in the cytosol, but also substantially in apoplast, thus making it the first line of defense against ROS attack(Barnesetal., 2002). AA is oxidized in two successive steps, starting with oxidation into MDHA, which if not reduced immediately to ascorbate, disproportionates to AA and DHA.It reacts with H_2O_2, OH•,$O^{\bullet -2}$, and regenerates α-tocopherol from to copheroxyl radical, there by protecting the membranes from oxidative damage(Shao et al.,2005). It also protects and preserves the activities of metal binding enzymes. AA in its reduced state acts as the cofactor of violaxanthinede-epoxidase and maintains the dissipation of the excess excitation energy (Smirnoff,2000).

Reduced glutathione (GSH)

Glutathione is a low molecular weight thiol tripeptide (γ-glutamyl-cysteinyl-glycine) abundantly found in almost all cellular compartments like cytosol, ER, mitochondria, chloroplasts, vacuoles, peroxisomes and even the apoplast. It is involved in a wide range of processes like cell differentiation, cell growth/division, cell death and senescence, regulation of sulphate transport, detoxification of xenobiotics, conjugation of metabolites, regulation of enzymatic activity,synthesis of proteins and nucleotides, synthesis of phytochelatins and finally expression of stress responsive genes (MullineauxandRausch, 2005). This versatility of GSH is all due to its high reductive potential. A central cysteine residue with nucleophilic character is the source of its reducing power. GSH scavenges H_2O_2, $_1O^2$, OH•, and$O^{\bullet -2}$ and protects the different biomolecules by forming adducts (glutathiolated) or by reducing the min presence of ROS or organic free radicals and generating GSSG as a by-product. GSH also plays a vital role in regenerating AA to yield GSSG.The GSSG thus generated is converted back to GSH, either by denovo synthesis or enzymatically by GR. This ultimately replenishes the cellular GSH pool. GSH also helps in the formation of phytochelatins via phytochelatins ynthase (Roychoudhuryetal., 2012a), which helps to chelate heavy metal ions and thus scavenges another potential source of ROS formation in plants (RoyChoudhuryetal.,2012b).

α-Tocopherol

The α-tocopherol belongs to a family of lipophilic antioxidants which are efficient scavengers of ROS and lipid radicals, making them indispensable protectors and essential components of biological membranes (Holländer-Czytkoetal.,2005;Kiffinetal., 2006). The α-tocopherol has the highest antioxidant capability among the four isomers (α-, β-, γ-, δ-). Tocopherols are known for their ability to protect lipids and other membrane constituents of the chloroplasts by reacting with O_2 and quenching its excess energy, thus protecting the PSII, both structurally and functionally. Tocopherol also serves as an effective free radical trap by halting the chain propagation step of the LPO cycle. It reacts with the lipid radicals RO•, ROO • and RO∗ at the membrane-water interface,where α-tocopherol reduces them and itself gets converted into TOH•. The TOH• radical undergoes recycling to its reduced form by interacting with GSH and AA (Igamberdievetal., 2004).

Carotenoids

Carotenoids belong to family of lipophilic antioxidants which are localized in the plastids of both photosynthetic and non-photosynthetic plant tissues. They belong to a group of antennae molecules which absorbs light in the 450–570 nm and transfers the energy to the chlorophyll molecule. Carotenoids exhibit their antioxidative activity by protecting the photosynthetic machinery in four ways, (a) reacting with LPO products to end the chain reactions, (b) scavenging $_1O^2$ and generating heatas a by-product, (c) preventing the formation of $_1O^2$ by reacting with 3Chl∗ and excited chlorophyll (Chl∗) and (d) dissipating the excess excitation energy, via the xanthophyll cycle.

Flavonoids

Flavonoids are widely found in the plant kingdom occurring commonly in the leaves, floral organs and pollen grains. Flavonoids can be classified into four classes on the basis of their structure, flavonols, flavones, isoflavones and anthocyanins.They have diverse roles in providing pigmentation in flowers, fruits and seeds involved in plant fertility and germination of pollen and defense against plant pathogens.Flavonoids have been considered as a secondary ROS scavenging system in plants experiencing damage to the photosynthetic apparatus, due to the excess excitation energy (Fini etal., 2011). They also have a role in scavenging $_1O^2$ and all eviate the damages caused to the outer envelope of the chloroplastic membrane (Agatietal.,2012).

Proline

Proline, an osmolyte is also regarded as a powerful antioxidant. It is widely used across the different kingdoms as a nonenzymatic antioxidant to counter act the damaging effects of different ROS members. Proline is synthesized using glutamic acid as a substrate; via a pyrroline5-carboxylate (P5C) intermediate.This pathway in plants is catalysed by two enzymes, ð1-pyrroline-5-carboxylate synthetase (P5CS) and Pyrroline-5-carboxylate reductase (P5CR). It is an efficient scavenger of OH• and $_1O^2$ and can inhibit the damages due to LPO.

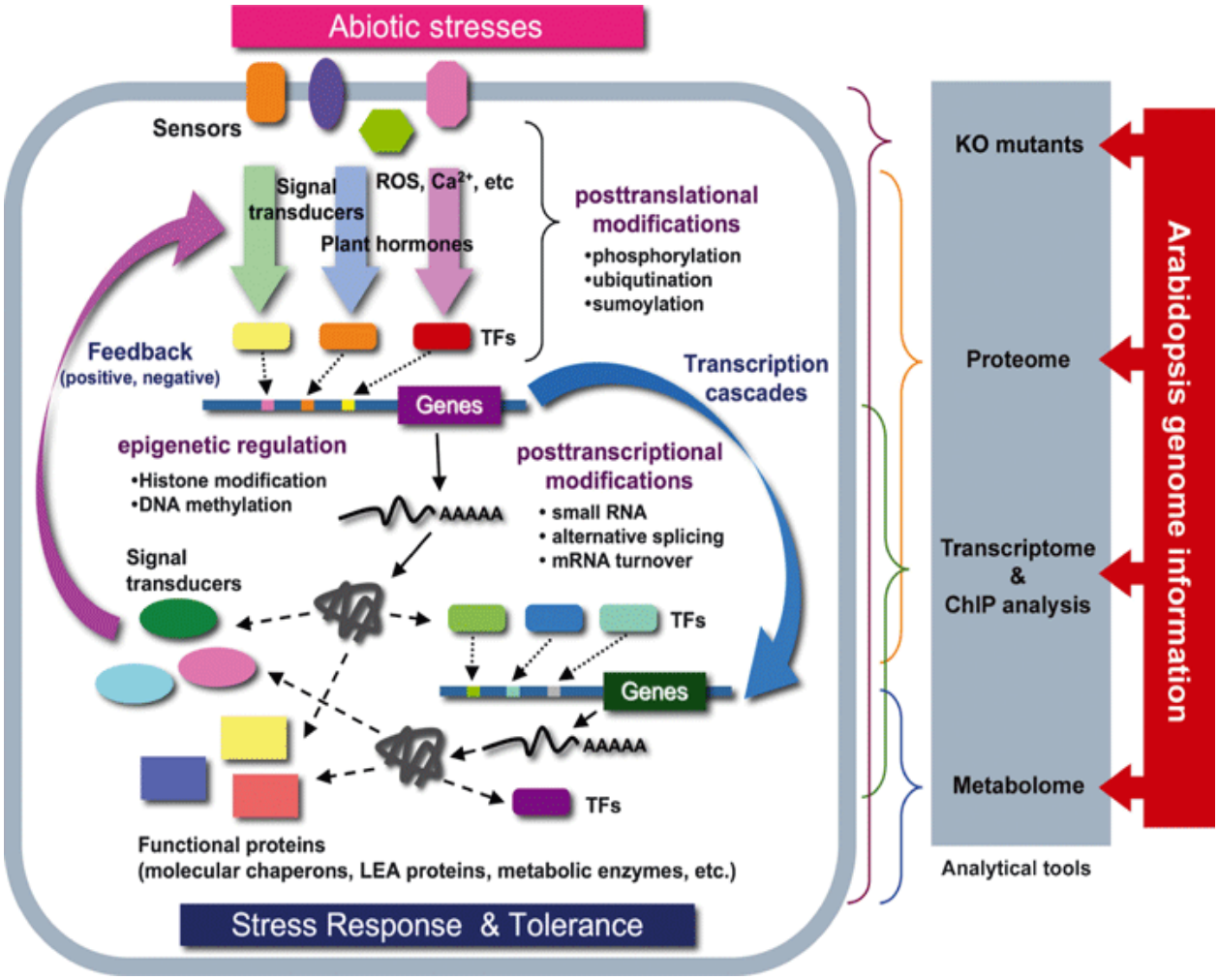
6. Transcriptome analysis in stress mediated responses to plants.

Transcriptomics:

Transcriptomics is often considered as a step next to genomics in the study of biological systems. The transcriptome is the set of all mRNA molecules, or "transcripts", produced in one organism or cell type under a given set of conditions. It is more complex than genomics, mostly because an organism's genome is rather constant (with the exception of mutations), while a transcriptome differs from cell to cell and constantly changes with changing external environmental conditions. Thus, the transcriptome reflects the genes that are being actively expressed at any given time, with the exception of mRNA degradation phenomena such as transcriptional attenuation.

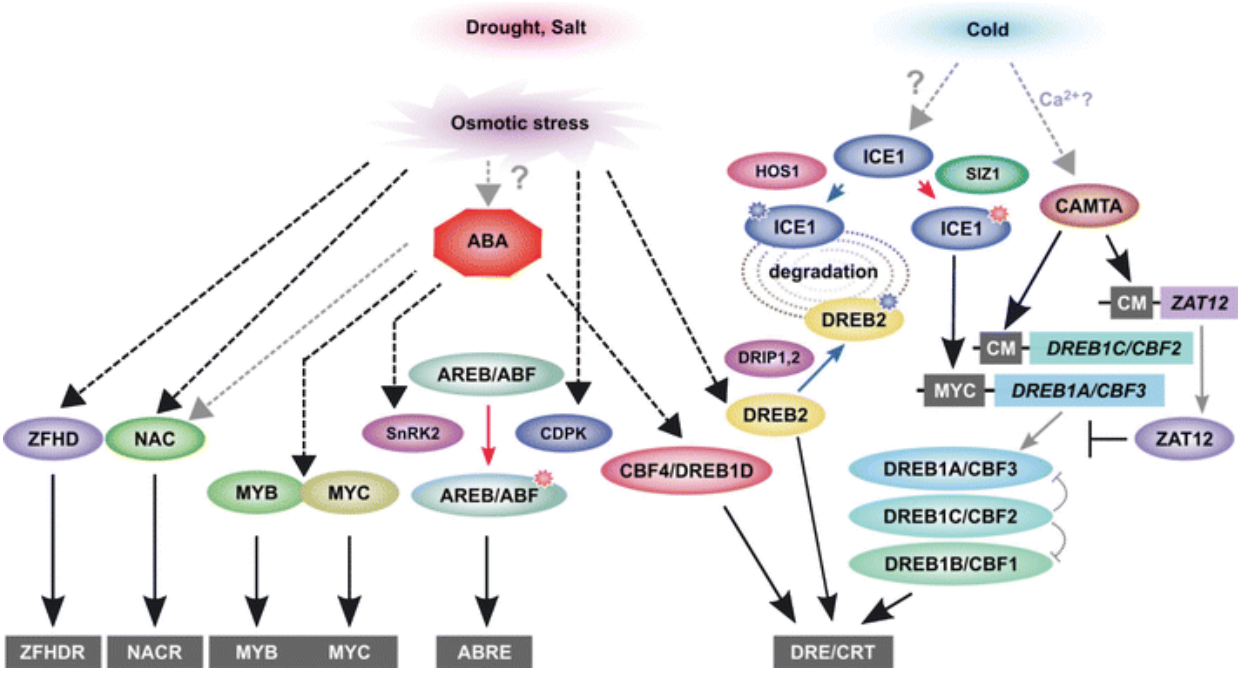
Abiotic stresses have been shown to cause accumulation of many intracellular substances, including nucleic acids, proteins, carbohydrates and amino acids. After the introduction of molecular biological techniques into plant biology, a great deal of effort went into the identification of stress-inducible genes, such as RD29A, using differential screening or differential display techniques for various plant species, including Arabidopsis. These studies succeeded in isolating genes that are presumed to function in stress responses and tolerance. Over-expression of some of these genes in planta confers some abiotic stress tolerance (Bartels and Sunkar, 2005; Umezawa et al., 2006b). More importantly, using the expression of such inducible genes as markers, an overall scheme of transcriptional regulation was developed. In the emerging picture, transcriptional activation occurs at distinct time points in response to stress stimuli. The various induction phases for stress-inducible genes are due to their varying dependency on de novo synthesis of proteins or signaling molecules, such as abscisic acid (ABA) (Yamaguchi-Shinozaki and Shinozaki, 2006). These findings suggest that abiotic stress responses are never simple, and that each induction phase may be controlled by a different signaling mechanism and different transcription factors. Identification of the relevant factors in each pathway has been addressed using responsive genes as markers. For example, an abiotic stress-responsive cis-element, dehydration responsive element (CRE)/C-repeat (CRT) (A/GCCGAC), was identified, which in turn has triggered important studies to identify the transcriptional regulating factors, DRE-binding protein (DREB)/C-repeat binding factor (CBF), and their post-translational regulatory mechanisms (Thomashow, 2001; Shinozaki and Yamaguchi-Shinozaki, 2007). In addition, genetic screens for mutations that affect the expression of stress-inducible genes have allowed the identification of novel components in the regulatory system (Chinnusamy et al., 2002). These results have provided a basic picture of gene regulatory networks in abiotic stress responses in plants. However, the stress-inducible genes isolated in this way were largely restricted to those with higher expression levels, and thus our knowledge on gene expression profiles was limited.

Complete determination of the genomes of Arabidopsis, Oryza sativa spp. japonica cv. Nipponbare, and other plants has changed the situation dramatically. The complete genome sequence has enabled genome-wide gene expression profiling in response to various abiotic stresses (e.g. using AtGenExpress; Kilian et al., 2007). Using microarray technology, genes responding to abiotic stresses have now been identified more comprehensively than ever before. Comprehensive transcriptome analysis revealed the relationships among stress-regulated transcripts, and enabled the prediction of their cis-regulatory elements (Kilian et al., 2007; Weston et al., 2008). Ma and Bohnert (2007) showed a clear correlation between expression profiles and the 5′ regulatory motifs of stress-regulated genes. These analyses indicated that stress-regulated genes are controlled by a complicated regulatory network. This type of network has been proposed based on transcriptome data using various theoretical approaches (Chen and Zhu, 2004; Ma et al., 2007; Long et al., 2008).
Determination of the function of stress-inducible genes has been addressed by the reverse genetic approach, aided by the use of insertional mutation lines. The products of stress-inducible genes identified in vast microarray experiments can be classified into two groups (Shinozaki et al., 2003): one containing mainly proteins functioning in direct abiotic tolerance [e.g. late embryogenesis abundant (LEA) proteins], and the other consisting of regulators for intracellular signaling and stress-inducible gene expression (e.g. protein kinases such as MAP kinases, phosphatases, phospholipid metabolic enzymes, and various types of transcription factors). The identification of stress-inducible signal transducers gave rise to the idea that plants have developed flexible cellular response mechanisms to efficiently respond to various abiotic stresses. Figure 1 shows the current understanding of abiotic stress responses in conjunction with the contribution of *Arabidopsis* genome information.



Regulation of Transcription in Abiotic Stress Responses

As described above, many transcription factors involved in stress responses have been identified. As an example, we describe here the transcriptional regulatory system for cold and drought (or osmotic) stresses (Figure 2). The DREB1/CBF family comprises APETALA2 (AP2) type transcription factors that recognize DRE/CRT and function in cold stress responses. Expression of DREB1A/CBF3 or DREB1C/CBF2 is regulated at the transcriptional level by INDUCER OF CBF EXPRESSION 1 (ICE1) (Chinnusamy et al., 2003) or calmodulin binding transcription activator (CAMTA) (Doherty et al., 2009), respectively. ICE1 is a MYC-type transcription factor that has also been shown to regulate stomata formation (Kanaoka et al., 2008). CAMTA transcription factors recognize Conserved Motif (CM) sequences providing a link with the Ca2+ signaling that is activated in the abiotic stress response, as CAMTA proteins have a calmodulin-binding domain. ZAT12, a zinc finger protein, is also implicated in the regulation of DREB/CBF expression (Vogel et al., 2005). DREB2, another AP2-type transcription factor that recognizes DRE/CRT, is involved in drought or salinity stress responses. Osmotic stress activates several other transcription factors, including zinc finger homeodomain (ZFHD) proteins and NAM ATAF CUC2 (NAC). ZFHD1 binds the CACTAAATTGTCAC motif, named ZFHDR, in the promoter region of EARLY RESPONSE TO DEHYDRATION 1 (ERD1). NAC proteins recognize a MYC-like target sequence and activate ERD1 (Tran et al., 2004, 2006). Osmotic stress increases the ABA level, which in turn activates sets of genes. ABA-responsive transcription factors (AREB/ABF), with a bZIP type DNA-binding domain that binds the ABA-responsive element (T/CACGTGGC), have a pivotal role in ABA-dependent gene activation (Choi et al., 2000; Uno et al., 2000). MYB and MYC transcription factors are synthesized de novo under osmotic stress conditions, and cooperatively activate stress-inducible genes such as RD22 (Abe et al., 2003).



Transcriptional regulatory network functioning in drought, salinity and cold stress

Some stress-associated transcription factors are themselves regulated at the transcriptional level, constituting a transcriptional cascade. Other transcription factors are regulated by post-translational modifications. Such regulation of transcription factors may be required for rapid and fine-tuned regulation under abiotic stress conditions. There is much evidence showing that ubiquitination, which usually induces degradation of the target protein, plays a pivotal role in abiotic stress responses (Vierstra, 2009). DREB2 was shown to be regulated by DREB-INTERACTING PROTEIN 1 and 2 (DRIP1 and DRIP2), which are RING finger E3 ligases, through ubiquitination (Qin et al., 2008).

ICE1 was demonstrated to be under the control of HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENE 1 (HOS1), another RING finger protein (Dong et al., 2006). Sumoylation is also involved in the regulation of transcription factors in abiotic stress responses. In contrast to ubiquitination, sumoylation regulates the activity of target proteins. A recent study showed that a SUMO E3 ligase, SIZ1, sumoylated ICE1 and enhanced its activation of DREB1A/CBF3 (Miura et al., 2007).

As in other organisms, transcription factors in plant systems are regulated by phosphorylation. The ABA-responsive transcription factors ABI5 and AREB/ABF of Arabidopsis and TRAB1 of rice are regulated by the phosphorylation of multiple Ser/Thr residues. SNF1-related kinase 2 (SnRK2)-type protein kinases (Kobayashi et al., 2005; Furihata et al., 2006) and Ca2+-dependent protein kinase (CDPK) are good candidates for regulators of AREB/ABFs (Choi et al., 2005; Kaplan et al., 2006; Zhu et al., 2007). SnRK2s are activated by osmotic stress or ABA (Boudsocq et al., 2004), while CDPKs are activated by increased intracellular Ca2+ levels induced by various stimuli (Harper et al., 2004) (see below). Presumably, such transcription factors function as a hub component that integrates multiple signal inputs under abiotic stress conditions.

Post-Transcriptional Regulation of Abiotic Stress-Inducible Transcripts

Recent studies have indicated that post-transcriptional regulation contributes to stress responses more than was previously thought. Transcribed RNA goes through various modifications: addition of a 5′ cap structure, splicing, and 3′ polyA addition. After these processes, mRNA is exported actively from the nucleus to the cytoplasm, where translation occurs and unnecessary or abnormal mRNA is promptly degraded. Each step is regulated coordinately (Houseley and Tollervey, 2009). Genetic studies on Arabidopsis mutants exhibiting an abnormal response to abiotic stress or ABA revealed that mRNA processing and metabolism have a close link with stress responses (Fedoroff, 2002a; Kuhn and Schroeder, 2003; Hirayama and Shinozaki, 2007). For example, RNA helicases are implicated in abiotic stress responses in various organisms including plants (Owttrim, 2006). In addition, Iida et al. (2004) showed that alternative splicing, which enables production of diverse polypeptides from one gene, is regulated by various abiotic stresses, such as cold stress. Moreover, cold stress changed the alternative splicing profiles of splicing factors, suggesting that complex multi-step regulation controls the splicing profiles in abiotic stress responses (Iida et al., 2004; Reddy, 2007). Alternative splicing events are considerably conserved between Arabidopsis and rice, indicating their importance (Wang and Brendel, 2006).

Small RNAs control stress responses

A very wellknown example is the natural cis-antisense transcript (NAT) siRNA (nat-siRNA) derived from the overlapping region of the sense–antisense pair P5CDH and SRO5. P5CDH encodes D1 - pyrroline-5-carboxylate dehydrogenase, which functions in proline catabolism, while the function of SRO5 is unknown. Borsani et al. showed that under salt stress, accumulation of SRO5 transcripts and 24-nt nat-siRNAs is upregulated, whereas that of P5CDH is downregulated. In this model, the 24-nt natsiRNAs guide the initial cleavage of the P5CDH transcript, which generates secondary 21-nt nat-siRNAs by phasing cleavage. The secondary nat-siRNAs also suppress P5CDH transcripts and, after accumulation of the osmoprotectant proline, the plant acquires tolerance to salt stress. A bioinformatics study by Henz et al. showed that small RNAs registered in public databases are not enriched in cis-NATs when compared with non-overlapping neighboring gene pairs, and then suggested that siRNA-mediated silencing does not play a major role in global regulation of cis-NAT expression. Therefore, production of nat-siRNAs from overlapping gene pairs may rarely occur, at least in Arabidopsis. In addition, a homology search showed that P5CDH homologs exist in the genomes of rice, soybean, poplar, grape, Lotus japonicus, and Physcomitrella patens, but SRO5 homologs are not present on the antisense strand of the P5CDH genes in these six plant species. It is still unknown whether this nat-siRNA functions only in tolerance to salt stress in Arabidopsis.

Cytoplasmic RNA-containing granules involved in stress responses

Transcriptome analyses show mRNA accumulation, but this does not necessarily mean that mRNAs of expressed genes are actively translated. In addition to the transcriptome profile, it is important to examine which mRNAs are translated, degraded, or temporarily stored during stress treatments. Transcribed mRNAs form messenger ribonucleo protein complexes (mRNPs), depending on developmental stages or environmental conditions. Polysome-associated mRNAs are usually

translated. On the contrary, non-translated mRNA is localized in two kinds of cytoplasmic mRNP granules; an mRNA processing body (P-Body, PB) or a stress granule (SG). In yeast and mammals, the PB contains components of mRNA decay machinery such as the DCP1/DCP2 decapping complex and 50 –30 exoribonuclease XRN1, and executes 50 –30 destruction of unnecessary mRNAs. On the contrary, the SG contains translation initiation factors such as eIF4E, eIF4G, and eIF4A, the 40S ribosomal subunit, the poly(A)-binding protein and some RNAbinding proteins, and stores non-translated mRNAs that have been stalled during initiation of translation. In yeast and mammals, cells subjected to environmental stresses show increases in the number of SGs and their assembly. Because stress responses often involve a transient inhibition of the initiation of translation, SGs accumulate during a wide range of stress responses. SGs often dock to P bodies, which suggests that mRNAs move between these two compartments. Recent reports have revealed that PBs and SGs also exist in plants. Weber et al. observed dynamic changes in the assembly of these granules during heat stress, using DCP1, DCP2, and XRN4 as markers of PBs, or eIF4E, RBP47, and UBP1 as markers of SGs. UBP1 and RBP47 are RRM-type RNA-binding proteins and plant orthologs of the TIA-1 protein, a component of SGs in mammals. Some mRNAs are destined to be translated (polysome), degraded (P-body, PB), or temporarily stored (stress granule, SG) during stress treatment. Some are processed by gene activation such as a histone modification, and some are regulated at a post-transcriptional level (e.g., siRNA, miRNA, or antisense RNA). www.sciencedirect.com Current Opinion in Plant Biology 2010, 13:132–138 30 -UTR. Weber et al. also revealed that UBP1 and RBP47 predominantly localize in the nucleus under control conditions, while they re-localize in several cytoplasmic granules when cells are exposed to heat-stress conditions. Cycloheximide treatment, which blocks translation elongation, prevents the formation of SGs. Therefore, plant SGs are regulated in a dynamic exchange with polysomes. Yeast two-hybrid analyses and in vitro binding assays identified UBA1 and UBA2, RRM-type RNA-binding proteins, as UBP1-interacting partners. UBA2a localizes throughout the nucleus under control conditions. However, ABA treatment results in relocalization of UBA2a into nuclear speckle structures that are storage sites of spliceosomal components. The levels of splice variants of UBA2a and UBA2c transcripts are upregulated by wounding. It is possible that some RRM-type RNA-binding proteins play important roles in regulation of mRNA metabolism and cell homeostasis under stress conditions. Microarray analyses showed that some stress-responsive transcripts such as RD20, COR15A, AtGolS2, and PP2C are upregulated in the mutant of DCP2 that encodes a component of the decapping complex. Taken together, the results of studies on SGs and PBs suggest that they are key cytoplasmic structures for controlling gene expression during plant stress responses.

Histone modification under abiotic stress conditions

Changes of transcriptional states in response to environmental stresses are coupled with chromatin remodeling. In general, these changes are accompanied by posttranslational modification of histone N-tails such as acetylation, methylation, and phosphorylation. Various modifications of histones occur during stress responses in plants. The chromatin immunoprecipitation (ChIP) assay is the best method to detect the state of histone modification under stress conditions. Tri-methylation of histone H3 Lys27 (H3K27me3) is generally a negative marker of transcription. Using ChIP assays, Kwon et al. demonstrated that H3K27me3 modification gradually decreases at the loci of two cold-responsive genes, COR15A and AtGolS3, during exposure to cold temperatures. When coldexposed plants are returned to normal conditions, transcriptions of these genes are repressed to their initial levels, but the cold-triggered decrease in H3K27me3 is still maintained. This decrease does not enhance the induction of transcription when plants are returned to cold temperatures. Using ChIP assays, Kim et al. monitored histone H3 modifications at the coding regions of four dehydrationstress-responsive genes, RD29A, RD29B, RD20, and an AP2 transcription factor (At2g20880), during dehydration stress. At all four gene loci, there was an enrichment of H3K4me3 (tri-methylation of H3 Lys4) and H3K9ac (acetylation of H3 Lys9), a positive marker of gene activation, in response to dehydration stress. Dehydration stress resulted in enrichment of H3K23ac and H3K27ac at the coding regions of RD29B, RD20, and At2g20880, but not at the coding region of RD29A. H3K4me3 enrichment occurred on the coding regions of RD29A and At2g20880 after accumulation of Pol II. However, these reports describe only isolated examples of chromatin modification during stress responses.

Large-scale analyses using ChIP-seq or ChIP-chip methods are necessary to increase our understanding of the roles of histone modification in abiotic stress responses.

7. Analysis of DNA markers in assisted breeding for stress tolerance

Molecular markers:

In the early part of the 20th century, scientist discovered that, Mendelian factor controlling inheritances (genes) are organized in linear order on cytogenetically defined structure called chromosomes. It was shown that, combination of genes can be inherited in a group (i.e. they are linked together because they are close to each other on the same chromosomes. The individual genes flanking within a defined close interval are known as molecular DNA markers. Molecular markers are identifiable DNA sequence, found at specific locations of the genome and associated with the inheritance of a trait or linked gene (FAO, 2004). Thottappilly et al (2000), refer to molecular markers as naturally occurring polymorphism which include proteins and nucleic acids that are detectably different. Rapid advances are genome research and molecular biology has led to the use of DNA markers in plant breeding. Target genes in a segregating population can be identified with the assistance of DNA makers so as to accelerate traditional breeding programs (Thottappilly et al, 2000). Markers must be polymorphic (i.e. they must exist in different forms so that the chromosome carrying the mutant gene can be distinguished from the chromosome with normal gene by form of the marker it carries. Polymorhism can be detected at three levels: morphological, biochemical or molecular. Recently, the term DNA fingerprinting /profiling is used to describe the combined use of several single locus detection systems and are being used as versatile tools for investigating various aspects of plant genomes. These include characterization of genetic variability, genome fingerprinting, genome mapping, gene localization, analysis of genome evolution, population genetics, taxonomy, plant breeding and diagnostics (Joshi et al, 2011) The development of DNA (or molecular markers) has irreversibly change the disciplines of plant genetics and breeding (Collard and Mackill, 2006), According to Joshi et al (2011),an ideal DNA makers should however poses the following properties.(i) Highly polymorphism, which is the simultaneous occurrence of a trait at the same population of two or more discontinues variants or genotypes.

(i) Co dominant inheritance- different form of marker should be detected in a diploid organism to allow discrimination of homozygote and heterozygote.

(ii) Frequent occurrence in genome

(iii) Selective neutral behaviour (the DNA sequences of any organism are neutral to environmental conditions or management practices)

(iv) Easy access (availability)

(v) Easy and fast assay

(vi) Reproducible – highly reproducibility and

(vii) Easy exchange of data between laboratories.

It is extremely difficult for a single genetic marker to possess all properties above. Depending on the type of study to be undertaken a marker system can be identified that would fulfill at least a few of the above characteristics.

a) Types and description of DNA markers

i. Non-PCR based genetic markers (Restriction fragment length polymorphism): The first and forest molecular markers system called the Restriction Fragment length Polymorphism (RFLP), was developed in early 1980 (Farooq and Azam, 2002). The RFLPs are simply inherited naturally occurring Mendelian characters. Genetic information is stored in the DNA sequence on a chromosome and variation in this sequence is the basis for the genetic diversity within species. Plants are able to replicate their DNA with high accuracy and rapidity, but many mechanisms causing changes (mutation) in the DNA are operative (Joshi et al, 2011).This leads to simple or large-base pair changes as a result of inversion, translocation, transpositions or deletion which may occur, resulting in a loss or gain of a recognition sites and in turn lead to restriction fragment of different lengths. This marker was first reported by Botstein et al,

(1980); in the detection of DNA polymorphism (Agarwal et al, 2008). Genomic restriction fragment of different length between genotypes can be detected on southern blots and by a suitable probe. In this method, DNA is digested with restriction enzyme like EcoR1, which cut the DNA at specific sequences, electrophoresed, blotted on a membrane and probed with a labelled clone. RFLP marker provides a way to directly follow chromosome segments during recombination as they follow Mendelian rules and greatly aid in the construction of genetic maps. When an F1 plants undergoes meiosis to produce gametes, its chromosomes will undergo recombination by crossing over and this recombination is the basis of conventional genetic mapping and when use, RFLP markers, require hybridization of probe DNA with sampled plant DNA.

Polymerase chain reaction Based markers

A decade after the emergence of AFLP, there was another breakthrough which involves the use of PCR in 1990 (Farooq and Azam, 2002). PCR is an in vitro method of nucleic acid synthesis by which a particular segment of DNA can be specifically replicated (Mullis and Faloona, 1987). The process involves two oligonucleotide primers that flank the DNA fragment of interest and amplification is achieved by a series of repeated cycles of heat denaturation of the DNA, annealing of the primer to their complementary sequences, and extension of the annealed primers with a thermophilic DNA polymerase.

Since the extension products themselves are also complementary to primers, successive cycles of amplification essentially double the amount of the target DNA synthesized in the previous cycle and the result is an exponential accumulation of the specific target fragment. Genomic DNA from two different individual often produces different amplification and a particular fragment generated from one individual but not for other represent DNA polymorphism and can be used as genetic markers. The pattern of amplified bands so could be use for genomic fingerprint (Welsh and McClelland 1990).

a) Randomly - amplified polymorphic DNA marker

The randomly-amplified polymorphic DNA marker (RAPD), detects nucleotide sequence polymorphism in DNA by using a single primer of arbitrary nucleotide sequence (Oligonucleotide primer, mostly ten bases long) (William et al, 1991). In this reaction, a single species of primer anneals to thegenomic DNA at two different sites on complementary strands of DNA template.

b) Amplified Fragment Length Polymorphism (AFLP)

AFLPS are fragments of DNA that have been amplified using directed primers from restriction of genomic DNA (Metthes et al, 1998). In this approach the sample DNA is enzymatically cut up into small fragments (as with RFLP analysis), but only a fraction of fragments are studied following selective PCR amplification (Liu et al, 1994). It is a combination of RFLP and RADP methods. AFLP technique shares some characteristic withboth RFLP and RAPD analysis (Farooq and Azam, 2002) and combines the specifically of restriction analyses with PCR amplification.

Other markers systems.

c) Simple sequence repeat or short tandem repeats (SSRs) or micro satellites

These are ideal genetic markers for detecting differences between and within species of genes of all eukaryotes (Farooq and Azam, 2002). It consist of tandemly repeated 2-7 base pair units arranged in repeats of mono-, di-, tri-, tetra and penta-nucleotides (A,T, AT, GA, AGG, AAAG etc) with different lengths of repeat motifs. These repeats are widely distributed throughout the plants and animal genomes that display high level of genetic variation based on differences in the number of tandemly repeating units of a locus. The variation in the number of tandemly repeated units results in highly polymorphic banding pattern (Farooq and Azam, 2002) which are detected by PCR, using locus specific flanking region primers where they are known.

Some other microsatellites based on the same principle include the following:

(i) Randomly Amplified Microsatellite Polymorphism (RAMP): This is a micro satellite – based marker which shows a high degree of allellic polymorphism, but they are labor-intensive (Agarwal and Shrivastava, 2008). On the other hand RAPD markers are inexpensive but exhibit a low degree of polymorphism. To compensate for the weaknesses of these approaches, a technique termed as RAMP was developed (Wu et al, 1994). The technique involves a radiolabeled primer consisting of a 51 anchor and 31 repeats which is used to amplify genomic DNA in the presence or absence of RAPD primers. (Agarwal and Shrivastava, 2008).

(ii) The Sequence Characterized Amplified Region (SCAR): The SCARS are PCR-based markers that represent genomic DNA fragments at genetically defined loci that are identified by PCR amplification using sequence specific oligonucleotide primer (McDermoth et al, 1994).
(iii) Simple Primer Amplification Reaction (SPAR): SPAR uses the single SSR oligonucleotide principles.
(iv) Sequence – Related Amplified Polymorphism (SRAP): The aim of SRAP technique (Li and Quiros, 2001) is the amplification of open reading frames (ORFs). It is base on two-primer amplification using the AT- or GC- rich cores to amplify intragenic fragment for polymorphism detection (Agarwal and Shirvastava, 2008).
(v) Target region amplification polymorphism (TRAP): The TRAP technique (Hu and Vick, 2003) is a rapid and efficient PCR-based technique, which utilizes bioinformatics tools and expressed sequence tag (EST) database information to generate polymorphic markers, around targeted candidate gene sequences.

Molecular Markers as Tools for Dissecting Quantitative Traits
Plant adaptation to variable environments is reflected by an interrelated set of complex physiological and morphological traits, each with an intricate regulatory system. By integrating physiological and genetic strategies, we can obtain a deeper understanding of the underlying molecular mechanisms, which opens the way towards a more targeted breeding approach for higher stress tolerance in crop plants. The breakthrough that has made this approach possible was the introduction of easy-to-use DNA markers that brought QTL mapping into the mainstream, making it possible to efficiently map the genetic loci controlling complex traits. This was made possible through genetic linkage analysis, allowing the construction of linkage maps, and the identification of QTLs controlling particular traits based on statistical methods that help establish the association between molecular markers and phenotypic data.
A Dissecting Complex Traits Using QTL Mapping
Although the theoretical underpinnings of modern QTL mapping were introduced earlier in the twentieth century, the method was limited in application due to the dependence on morphological markers to tag genes (Sax 1923; Thoday 1961). It was not until the introduction of molecular markers, starting with isozymes, that QTL mapping could provide comprehensive coverage of the genome in scanning the loci that control complex traits (Tanksely 1993). Once DNA markers such as RFLPs and SSRs became widely available for most plant species, QTL mapping was quickly adopted. To date, there are over 10,000 mapped QTLs reported for rice and maize in the Gramene database (www.gramene.org). One of the key advantages of QTL mapping is the ability to map genes underlying many different traits and trait components using the same mapping population and the same genetic linkage map. For abiotic stress tolerance, it becomes possible to test different physiological components and compare the QTL locations for these with the QTLs for tolerance or yield under stress to identify the causal factors. Furthermore, use of permanent mapping populations, such as recombinant inbred lines (RILs) or chromosomal segment substitution lines (CSSLs) enables testing stress tolerant traits in replicated experiments across different environments, which can help differentiate the QTLs based on their effectiveness at different stress levels. Once important QTL targets are identified, i.e., large-effect QTLs from the donor that provide increased stress tolerance, these can be captured as single introgressions in a set of nearisogenic lines (NILs), which can help unravel the complexity of different traits by limiting the variation between lines to focus only on the locus of interest. The NILs then provide the foundation for further physiological characterization, finemapping, and ultimately cloning of the QTL to identify the causal gene.
B Gene Discovery: Genomics and Positional Cloning
Not long after QTL mapping became commonplace, another breakthrough arrived that brought genetic mapping to the DNA sequence level, i.e., the first high-quality complete sequencing of a plant, *Arabidopsis* (*Arabidopsis* Genome Initiative 2000), which was followed soon after by the complete sequencing of rice (International Rice Genome Sequencing Project 2005). Having the Havingthe complete DNA sequence was an instant boon to genetic mapping as it presented an opportunity to make a universal consensus map that can bring together genetic mapping data from disparate sources into a single physical map based on the DNA sequence. This eliminated problems

with ambiguous marker orders and variable map distances, and allowed previously mapped genes, QTLs, and markers to be integrated regardless of the original mapping population. In addition, it provided many new markers across the genome, which is essential for fine-mapping. For example, the rice microsatellite map grew from 500 simple sequence repeat (SSR) markers using conventional techniques (Temnykh et al. 2001); to 2,740 SSRs using limited sequence data (McCouch et al. 2002); to 18,828 SSRs using the complete rice genome sequence (International Rice Genome Sequencing Project 2005). In the future, single nucleotide polymorphism (SNP) markers will further increase the number of available markers and will enable more cost-effective high-throughput genotyping techniques

These advances have improved the efficiency of fine-mapping and have made cloning a QTL, to isolate the casual gene, much easier in Arabidopsis (Lukowitz et al. 2000; Jander et al. 2002) and in rice (Ashikari and Matsuoka 2006), in spite of the challenges still existing (Salvi and Tuberosa 2005). Most of the QTLs cloned to date were associated with morphological attributes such as plant height, fruit characteristics or flowering time (Paran and Zamir 2003), and similar approaches can be used successfully to identify genes controlling key steps for other physiological traits. Several major QTLs associated with tolerance of abiotic stresses have been identified and fine-mapped in rice, with the first examples of map-based cloning being the salinity tolerance QTL SKC1 (Ren et al. 2005) and the submergence tolerance QTL SUB1 (Xu et al. 2006). The fine-mapping and cloning of QTLs has also given more confidence in the results of the primary QTL studies, which have proven to be highly accurate upon retrospect (Price 2006). Once a QTL is cloned, knowledge of the underlying sequence allows further probing into allelic variation at the causal gene level, which can help identify the functional nucleotide polymorphism (FNP) that controls the change in phenotype. The FNP can then be used to develop a functional or perfect marker that directly assesses the desired phenotype at the molecular level. For example, the cloning of the fragrance gene led to a perfect marker for aroma in rice at an 8 bp deletion (Bradbury et al. 2005), and the cloning of the gene for red pericarp in rice led to the identification of a 14 bp deletion that has been developed into a marker for red rice (Sweeney et al. 2006). These functional markers can have several advantages in marker-assisted breeding, especially as they will always co-segregate with the desired phenotype, eliminating the danger of recombination between the linked markers and the target gene. This also allows for rapid diagnosis of the allele state at that gene across diverse germplasm accessions (Andersen and Lubberstedt 2003; Mackill and McNally 2005). In a number of cases, however, the FNP may be elusive, especially if multiple sequence changes can result in the same phenotype. In this case, the FNP marker may work well with a particular source of the gene, but may not work across all germplasm accessions that have the trait.

C Strategies for Marker-Assisted Selection

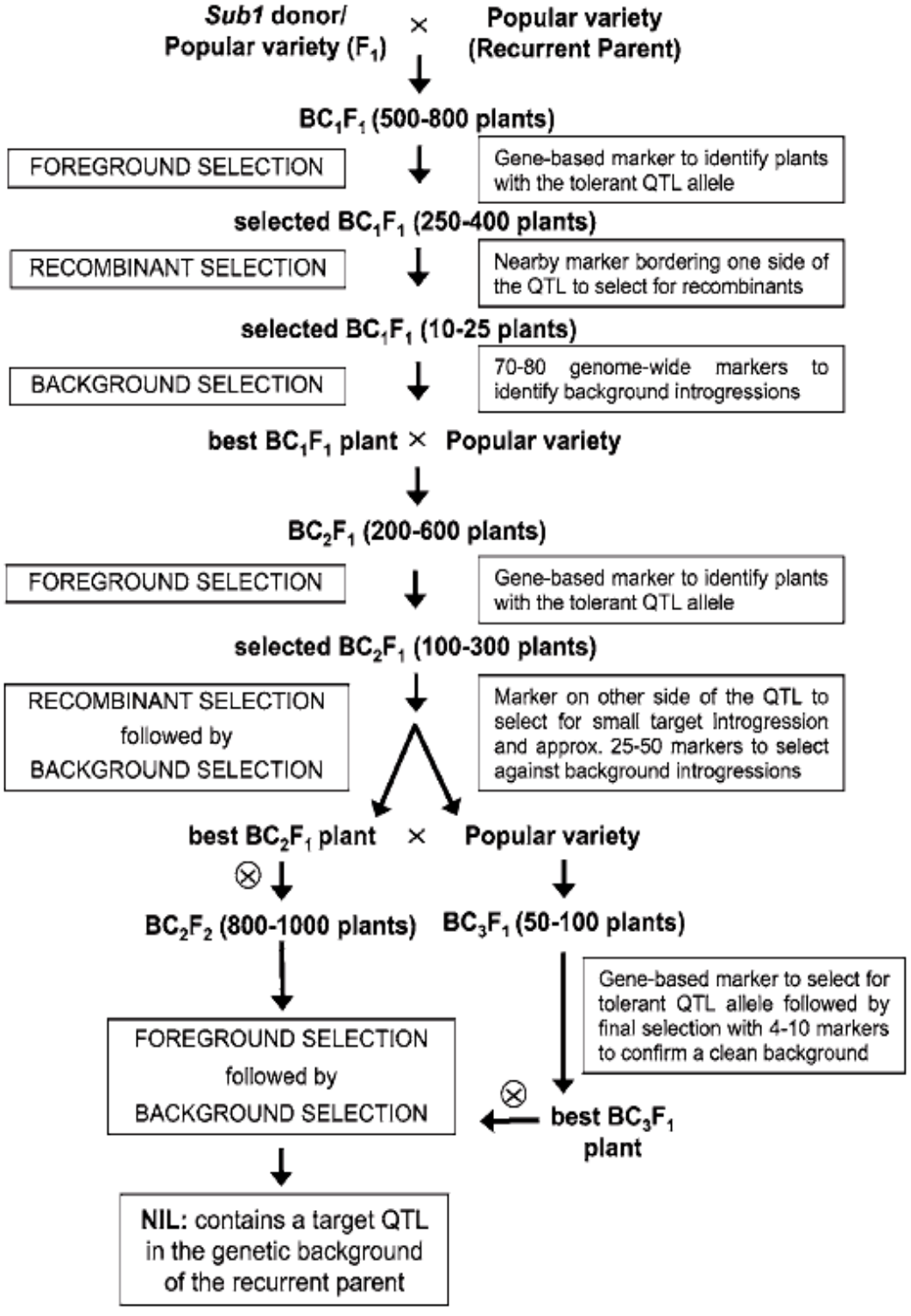
The recent advances in genomics have paved the way for clear and reliable methods for MAS in plants: from QTL identification, NIL development and fine-mapping to transferring the QTL into popular varieties using a precise markerassisted backcrossing (MABC) strategy (Mackill 2006; Collard et al. 2005; Collard and Mackill 2008; Collard et al. 2008). MABC involves the manipulation of genomic regions involved in the expression of particular traits of interest through DNA markers, and combines the power of a conventional backcrossing program with the ability to differentiate parental chromosomal segments. The efficiency of a MABC program depends on a number of factors, including the size and reliability of the target QTL effect, the precision of the target gene/QTL fine-map, the rate of polymorphism when identifying background markers, as well as the cost, speed, and failure rate of the markers employed in each customized MABC system. For each set of parents and for each target QTL, a customized MABC package needs to be developed with optimized foreground markers to select the QTL target, recombinant markers flanking the locus of interest to reduce linkage drag and an evenly-spaced set of polymorphic background markers across the genome to select the recurrent parent background (Neeraja et al. 2007; Collard and Mackill 2008). QTL mapping has been Fig. 1. Example of the marker-assisted backcrossing scheme used to transfer the SUB1 QTL for submergence tolerance into six mega-varieties, showing recommended numbers of plants and markers for each step to develop a Sub1-converted near-isogenic line (NIL), at either the BC2 F2 or BC3 F2 generation, depending on the number of background introgressions remaining at the BC2 F1 generation and the size of the target introgression desired (Neeraja et al. 2007; E. Septiningsih,

unpublished). 456 20 Marker Assisted Breeding progressing at an accelerating pace over the past decade, but few products using this technology have been released to farmers. Constraints to the use of marker-assisted selection for quantitative traits include:

• Poor resolution of QTLs on the genetic map
• Small effects of many QTLs
• Interaction of QTLs with environment or genetic background
• Poor selection of appropriate parents for mapping populations
• The expense of genotyping, limiting the number of samples that can be processed

If these constraints are carefully addressed, breeders would be much more likely to use MABC to develop stress-tolerant varieties.

The proper selection of QTL targets, combined with the development of an optimized MABC package consisting of tested markers and appropriate donors, is essential for the successful implementation of MABC for any breeding targets. The selection of the QTL target and appraisal for its usefulness in a MABC program needs to weigh the benefits of a markerassisted program versus conventional selection. For example, often trait components can be used directly for selection in plant breeding depending on their degree of association with plant adaptability or yield under specific environments, the cost and precision of their assessment and their interaction with the environment. The genetic control of these traits can be affected by factors such as the number of genes involved, extent of association with undesirable pleiotropic effects, or adverse genetic linkage. Traits such as yield, nutrient acquisition and tolerance of abiotic stresses consist of several underlying components that need to be combined to achieve higher performance. At present, inadequate progress has been made in using these physiological criteria for the largescale breeding needed to combine multiple traits of importance, and their use has been essentially limited to the identification of parental lines. The value of using markers as a surrogate for direct selection of stress tolerance components or yield under stress will often depend on how difficult are the phenotyping techniques and the amount of replicated trials required versus how reliable are the linked markers in predicting the phenotype after its transfer to a recipient variety. Practically, it is advisable to introgress QTLs of important agronomic or adaptive values into varieties that are well known to farmers and are covering large areas. This will ensure that the new varieties will be used immediately by farmers, millers and marketing channels, who are usually cautious when taking on new varieties (Mackill 2006). Provided that popular varieties normally have limited lifespan, MABC also needs to be integrated with conventional breeding to incorporate useful QTLs into elite breeding lines. So far, the greatest success in MABC for improving tolerance of biotic and abiotic stresses has been achieved with QTLs proven to provide high levels of tolerance in many different genetic backgrounds and environments (Collard and Mackill 2008; Collard et al. 2008). A good example in cereals is the introgression of SUB1, the major QTL for submergence tolerance, into several popular rice varieties (Xu et al. 2006; Neeraja et al. 2007). Future breeding objectives, however, may require more complex situations, such as the pyramiding of multiple QTLs having more subtle effects that are effective during different developmental stages, or the combining of QTLs for different abiotic stress tolerance into the same genetic background. Up to now, MABC has been successful in transferring traits whose expression is controlled by a single gene or by a gene that controls most of the phenotypic variance of the trait. However, the effective use of MABC in combining several genomic regions which control a single trait or a few independent traits required for a desired phenotype still awaits further development of more efficient technologies and innovative strategies. An example of a successful approach used in the transfer of a single QTL into several popular rice varieties is presented in Fig.



Example of the marker-assisted backcrossing scheme used to transfer the SUB1 QTL for submergence tolerance into six mega-varieties

MAS for Abiotic Stress Tolerance in Plants:

Rice

Drought stress is one of the major constraints in rice production and that too especially in rainfed conditions. Therefore, identification and introgression of consistent QTLs for drought resistance can prove to be an effective strategy to tackle the rice production in drought-prone areas. Although a number of QTLS have been identified in rice for drought resistance, the progress on marker-assisted backcrossing (MAB)- based introgression of the identified QTLS is limited (Table 1.1). Prince et al. (2015) conducted SSR-based QTL mapping on RIL population derived from a cross of IR20 and Nootripathu for physiological and yield traits under drought. Three QTLs mapped on chromosome 1 (RM8085), chromosome 4 (I12S), and chromosome 6 (RM6836) for physiological and yield traits can be effectively utilized for introgression into elite rice lines for stable yield production under drought stress-prone ecologies. In an another study, RILs and association mapping population were utilized for identification of QTLs for deep rooting as it is an important trait for imparting drought tolerance in rice and usually represented by ratio of deep rooting (RDR). SNP-based genotyping resulted into mapping of six QTLS for RDR on chromosomes 1, 2, 4, 7, and 10 (Lou et al. 2015). Sangodele et al. (2014) identified ten SSR genotyping-based QTLs for physiological, productivity, and related traits under drought using backcross inbred lines (BILs) derived from the cross of Swarna and WAB 450 and grown in a poly house in a randomized block design. The drought resistance imparted due to the positive alleles from WAB 450 can help in providing stability in grain yield under drought stress. Lang et al. (2013) worked on BC2F2 population derived from a cross of OM1490 and WAB880–1-38-18-20-P1- HB and identified four QTLS related to root length and root dry weight. The phenotypic variation explained by the QTLS for dry root weight lies in the range of 20.7% to 30.8%.

In the era of climate change, heat stress threatens the rice production globally. In this study, two different populations (biparental F2 population and three-way F2 population) derived from cross of heat-tolerant variety Giza178 × IR64 and IR64 × Milyang23 × Giza178, respectively. Results – four QTLs, namely, qHTSF1.2, qHTSF2.1, qHTSF3.1, and qHTSF4.1 and two QTLs, qHTSF6.1 and qHTSF11.2 – were identified in the biparental F2 and three-way F2 population, respectively, through linked SNP markers. The SNP markers can be further fine mapped to develop SNP chips for marker-assisted crop improvement (Ye et al. 2015). In a population of chromosome segment substitution lines derived from a cross of Sasanishiki (Japonica ssp. heat susceptible) and Habataki (Indica spp. heat tolerant), 11 QTLs were mapped through SSR markers on chromosomes 1, 2, 3, 4, 5, 7, 8, 10, and 11 for spikelet fertility, daily flowering time, and pollen shedding under heat stress. Three novel QTLs, namely, PSLht4.1, qPSLht7, and qPSLht10.2, were identified for the first time, and among them qPSLht4.1 was found to impart heat tolerance at varying temperature regimes and therefore can be successfully utilized for rice lines for better pollen shedding as well as pollen growth on stigma under heat stress (Zhao et al. 2016).

Submergence is a problem of serious concern in rice-growing ecologies particularly in South and Southeast Asia. Although *SUB1* gene has been utilized in rice to survive under complete submergence for a period of fortnight, then also novel QTLs are required to be identified for longer-term submergence. A cross between IR72 and Madabaru was made to develop F2:3 population, and using SNP markers, four QTLs were identified on chromosomes 1, 2, 9, and 12. A major QTL found on chromosome 1 explained the phenotypic variance of 52.3%. Gene pyramiding can be utilized for combining this novel QTL with *SUB1* and thereby further improving the submergence tolerance in rice growing in flood-prone areas (Septiningsih et al. 2012). Manangkil et al. (2013) identified 32 QTLS for seedling vigor under submergence in backcross inbred lines derived from a cross of Nipponbare × Kasalath and Nipponbare. RFLP markers mapped 7, 11, and 14 QTLs for shoot length, root length, and shoot fresh weight on chromosomes 1, 3, 4, 6, and 7. Recombinant inbred lines (RILs) derived from a cross of IR42 and FR13A led to detection of five QTLs on chromosomes 1, 4, 8, 9, and 10. The novel QTLs have a tremendous potential to augment *SUB1* for better rice production under submergence conditions (Gonzaga et al. 2016).

Salinity is another important stress affecting the production of rice globally. Hossain et al. (2015) conducted QTL mapping in F2 population derived from a cross of salinity-tolerant Cheriviruppu with sensitive cultivar Pusa Basmati 1 (PB1) using 131 SSR markers and mapped 16 QTLs for different traits such as pollen fertility, Na+ concentration, and Na/K ratio on chromosomes 1, 7, 8, and 10.

Similarly, another experiment resulted into identification of 16 major QTLs for various traits related to salinity tolerance such as shoot potassium concentration, sodium-potassium ratio, salt injury score, plant height, and shoot dry weight in RILs developed from a cross of Bengal with Pokkali. A total of 85 additive QTLs were mapped through 9303 SNPs on chromosomes 1, 2, 3, 4, 6, 7, 8, 10, 11, and 12 (Leon et al. 2016). These QTLs for salinity tolerance can be effectively utilized for marker-assisted breeding-based rice improvement for salinity tolerance.

Lowland rice is hampered by the problem of iron (Fe) toxicity owing to excess ferrous iron (Fe^{2+}) formation in reduced soils. African rice (*Oryza glaberrima*) is a potential source of genes for resistance to iron toxicity because of its adaptability to adverse soil conditions and higher rusticity. Therefore, SSR-based QTL mapping carried out in BC3DH lines derived from the backcross of *O. sativa* (Caiapo)/*O. glaberrima* (MG12)/*O. sativa* (Caiapo) under Fe^{2+} condition in hydroponics resulted in identification of 28 QTLs for 11 morphological and physiological traits on chromosome 5 and 10 (Dufey et al. 2015). Similarly, Wu et al. (2014) identified seven QTLs for leaf bronzing score on chromosome 1, 2, 4, 7, and 12 in RIL population derived from a cross between IR29 and Pokkali.

Crop	QTLs/Loci	Mapping Population	Cross(s)	Genotyping Markers	Stress
Rice	3 QTLS (physiological and yield traits)	RILs	IR20 × Nootripathu	SSRs	Drought
Rice	6 QTLS (ratio of deep rooting)	RILS	Zhenshan97B × IRAT109	SNP	Drought
Rice	4 QTLS (root length and root dry weight)	BC_2F_2	OM1490 × WAB880–1–38-18-20-P1-HB	SSRs	Drought
Rice	15 QTLS (1000 grain weight, leaf temperature, relative water content, grain weight per plant, relative water content, productive tillers, grain number per plant, panicle weight, productive tillers, and spikelet fertility)	BIL	Swarna × WAB 450	SSR	Drought
Rice	11 QTLs (spikelet fertility, daily flowering time, and pollen shedding level)	CSSLs	Sasanishiki × Habataki	SSR	Heat
Rice	8 QTLS (spikelet fertility)	Three-way cross	(IR64 × Milyang23) × Giza178	SNP	Heat
Rice	5 QTLS (submergence tolerance beyond SUB1)	RILs	IR42 × FR13A	SSR	Water logging/ submergence
Rice	32 QTLS (shoot length, root length, and shoot fresh weight)	BILs	(Nipponbare×Kasalath) × Nipponbare	RFLPs	Water logging/ submergence
Rice	4 QTLS (submergence)	F_{23}	IR72 × Madabaru	SNP	Water logging/ submergence
Rice	85 QTLs (shoot potassium concentration, sodium-potassium ratio, salt injury score, plant height, and shoot dry weight.)	RILs	Bengal × Pokkali	SNP	Salinity
Rice	16 QTLS (pollen fertility, Na^+ concentration, and Na/K ratio in the flag leaf)	F_2	Cherivirippu × Pusa Basmati1	SSR	Salinity

Wheat

Moisture stress is a limiting factor in wheat production globally which can be addressed effectively through introgression of drought-tolerant QTLs in wheat cultivars. Merchuk-Ovnat et al. (2016) identified three QTLs for yield and biomass in RILs derived from a cross of wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) and *durum* (*T. turgidum* ssp. durum) and bread wheat (*T. aestivum*) on chromosomes 1BL, 2BS, and 7AS where wild emmer wheat acts as source of drought resistance. In another study on identification of QTLs for drought resistance in wheat, Barakat et al. (2015) identified 13 QTLs for abscisic acid content in F4 population derived from a cross of drought-sensitive (Yecora Rojo) and drought-tolerant (Pavon 76) using different markers (SRAP, TRAP, and SSR). The QTLs mapped on chromosomes 3B, 4A, and 5A through linked markers (Barc164, Wmc96, and Trap9) can be used for breeding drought-tolerant cultivars. Similarly, QTL mapping

conducted in F2 population derived from cross of tolerant cultivar, Chakwal-86, with sensitive cultivar, 6544-6, using SSR markers mapped four QTLs for photosynthesis, cell membrane stability, and relative water content on chromosome 2A (Malik et al. 2015). Zhang et al. (2013a) identified 22 QTLs on chromosomes 1B, 2A, 2B, 3B, 4A, 5D, 6A, 6D, 7B, and 7D for different traits like coleoptile length, seedling height, longest root length, root number, seedling fresh weight, stem and leaf fresh weight, root fresh weight, seedling dry weight, stem and leaf dry weight, root dry weight, root-to-shoot fresh weight ratio, and root-to-shoot dry weight ratio in two RIL populations derived from Weimai 8 × Luohan 2 and Weimai 8 × Yannong 19, respectively. Six QTLs found to be major or main effect QTLs can be utilized for drought improvement in wheat breeding program. Root architectural traits can play an important role in imparting resistance to drought in wheat. Four QTLs and two QTLs for seminal root angle and seminal root number, respectively, were mapped through DArT and SSR markers in a doubled haploid population derived from a cross of SeriM82 and Hartog. Four QTLs for seminal root angle were located on chromosomes 2A, 3D, 6A, and 6B, while for two QTLs for seminal root number on 4A and 6A (Christopher et al. 2013).

Wheat-growing ecologies are severely affected due to heat (high temperature) stress during grain filling period. In present scenario terminal heat stress is a major concern for wheat production globally. Parameters of chlorophyll fluorescence kinetics (PCFKs) can be utilized for identification and understanding of heat stresstolerant cultivars. Therefore, QTL mapping was done in a DH population derived from a cross of Chinese cultivars, Hanxuan 10 and Lumai 14, using SSR and AFLP markers under controlled conditions. In the study, a total of seven QTLs were mapped on chromosomes 1A, 1B, 2B, 4A, and 7D for traits related to PCFKs such as initial fluorescence, maximum fluorescence, variable fluorescence, and maximum quantum efficiency of photosystem II. These identified QTLs can be effectively utilized for further deep understanding of genetic basis of chlorophyll fluorescence kinetics (Azam et al. 2015). Similarly, Talukder et al. (2014) targeted mapping of QTLs for thylakoid membrane damage (TMD), plasma membrane damage (PMD), and SPAD chlorophyll content (SCC) in RIL population developed from a cross of Ventnor and Karl 92. This is the first study on the development of genotype by sequencing SNP (GBS-SNP) marker-based linkage map in wheat. There are a total of five QTLs for TMD, PMD, and SCC on chromosomes 1B, 1D, 1B, 2B, 6A, and 7A with a maximum phenotypic variance of 33.5% for PMD. Tiwari et al. (2013) conducted SSR-based QTL mapping in DH population derived from a cross of Berkutwith and Krichauff and identified a total of seven stable QTLs on chromosomes 1D, 6B, 2D, and 7A. Three, two, and one QTLs were identified for grain filling duration, thousand grain weight, grain yield, and canopy temperature.

Salinity is a major concern for wheat production globally. Improved wheat cultivars with salinity tolerance can be developed through identification and introgression of QTLs imparting tolerance to salinity. Proportions of dead leaves (%DL) are an important parameter for identification of tolerant cultivars at early vegetative stage. SSR-based association studies carried out in 119 wheat varieties led to identification of a single QTL for %DL on chromosome 4B (Turki et al. 2015). Xu et al. (2013) identified a total of 18 additive and 16 epistatic QTLs in a RIL population comprising of 131 individuals, derived from a cross of Chuan 35,050 and Shannong 483. QTLs for shoot K^+/Na^+ concentration and for total dry weight mapped on chromosome 5A and chromosome 4B, respectively.

Globally, irrigated and high rainfall-prone wheat ecologies are severely affected by water logging. There have been very limited studies on identification of QTLs for waterlogging tolerance in wheat. Two independent RIL populations, International Triticeae Mapping Initiative (ITMI) population "W7984/Opata85" and "SHWL1 × Chuanmai 32" (SC) population, were mapped for root dry weight index, shoot dry weight index, and total dry weight index. DArT and SSR-based mapping led to identification of 36 and 10 QTLs in and SC population, respectively. These QTLs can be effectively utilized for development of waterlogging-tolerant varieties in wheat breeding program (Yu and Chen 2013).

Acidic soils tend to limit the wheat production globally due to aluminum (Al) toxicity, and development of Al-tolerant cultivars can effectively address the problem of wheat production in acidic soils. A RIL mapping population developed using Al-tolerant Chinese landrace FSW and Al-sensitive US spring wheat cultivar Wheaton was validated for previously identified QTL FSW. Out of three

previously identified QTL for net root growth during Al stress, two QTLs were validated through earlier reported SSRs to be present on chromosome 4DL and 3BL. The linked SSR markers can be effectively utilized for development of AL-tolerant cultivars through MAS (Dai et al. 2013).

Maize

Drought is also a major abiotic constraint in maize that causes drastic yield reduction and economic losses in all over the world. Several researchers identified a large number of QTLs using different mapping population with different molecular markers, and these QTLs can address the drought problem effectively. Almeida et al. (2013) evaluated three tropical biparental populations under water stress (WS) and well-watered (WW) regimes to identify genomic regions responsible for grain yield (GY) and anthesis-silking interval (ASI) and identified a total of 83 and 62 QTL through individual environment analyses for GY and ASI, respectively. Six constitutively expressed meta-QTLs mapped on chromosomes 1, 4, 5, and 10 for GY. One mQTL on chromosome 7 for GY and one on chromosome 3 for ASI were found to be "adaptive" to WS conditions. In another study, Almeida et al. (2014) identified a total of 203 QTLs for different drought-related morphophysiological traits using biparental population, and a clusters of QTL were observed on chromosomes 1, 3, 4, 5, 7, and 10. Li et al. (2016) evaluated 5000 inbred lines from 30 joint linkage association mapping population using 365 SNPs for genome-wide association, and these SNPs associated with drought-related traits, located in 354 candidate genes. Fifty-two of these genes showed significant differential expression in the inbred line B73 under the well-watered and water-stressed conditions (Li et al. 2016).

Waterlogging is an important abiotic stress that leads to significant yield losses in high rainfall-prone maize-growing ecologies. The most sustainable and viable approach to tackle the waterlogging problem is through MAS-based incorporation of QTLs for waterlogging tolerance in cultivars. Zaidi et al. (2015) assessed the genetic variation in a population of recombinant inbred lines (RILs) derived from crossing a waterlogging-tolerant line (CAWL-46-3-1) to an elite but sensitive line (CML311-2-1-3) and observed significant range of variation for grain yield under waterlogging stress along with a number of other secondary traits such as brace roots (BR), chlorophyll content (SPAD), % stem, and root lodging (SandRL) among the RILs. Genotyping with 331 polymorphic single SNP markers using KASP (Kompetitive Allele Specific PCR) platform revealed a total of 18 QTLs on chromosomes 1, 2, 3, 4, 5, 7, 8, and 10.

Low temperature or cold is another type of abiotic stress that hampers the growth and yield of maize. Allam et al. (2016) developed two independent RIL populations from the crosses of B73 × P39 and B73 × IL14h and identified a total of 27 QTLs for germination and early growth under field condition. SNP genotyping mapped the QTLs on chromosomes 1, 2, 3, 4, 5, 6, 7, 8, and 9. Revilla et al. (2016) conducted a genome-wide association analysis in temperate maize inbred lines for pyramiding of cold tolerance genes and recorded traits for days from sowing to emergence and relative chlorophyll content using two panels of 306 dent and 292 European flint maize inbred lines. A total of 47 flint inbreds and 4 dent inbreds harbored the favorable alleles for 6 significant QTLs and 3 main effect QTLs, respectively, under cold conditions. These findings for cold tolerance will open new avenues for the genetic improvement of maize genotypes through marker-assisted selection or genome-wide selection.

Salinity also affects the maize production in the ecologies with higher proportion of saline soil. Different traits related to salt tolerance such as shoot length, root length, ratio of root length, and shoot length shoot fresh weight, root fresh weight, plant fresh weight, plant dry weight, shoot dry weight, root dry weight, ratio root dry weight, and shoot dry weight were targeted for mapping the QTLs in F2:3 population developed from a cross between B73 and CZ-7 under greenhouse. SSR genotyping mapped 15 QTLs for target traits on chromosomes 1, 2, 4, 5, 6, 7, 8, 9, and 10 (Hoque et al. 2015).

Chickpea

Chickpea contributes a greater proportion to total pulse production, but it is vulnerable to low moisture in drought-prone areas. SSR-based QTL mapping conducted in a RIL population comprising of 181 individuals, derived from a cross of ILC588 S. and ILC3279, identified 93 QTLs for drought resistance score, plant height, days to flowering, and days to maturity along with seven yield-related traits such as grain yield, biological yield, harvest index, the number of pods/3 plants, percentage of empty pods, 100-seed weight, and seed number/3 plants. The QTLs mapped on LG3 and LG4 can

play an immense role in chickpea molecular breeding programs for drought (Hamwieh et al. 2013). In addition to drought, salinity is also an important abiotic stress hampering the chickpea production globally. Pushpavalli et al. (2015) identified 48 QTLs for days to 50% flowering and maturity and days after sowing in RILs derived from cross of ICCV 2 and JG 11. Twenty-eight SSRs and SNPs mapped the QTLs for salinity tolerance-related traits on linkage group 5 and 7.

Common Bean

Drought stress is a serious concern for common bean (*Phaseolus vulgaris* L.) production globally. A total of 14 QTLs were detected for traits such as number of days to flower, maturity, and seed fill; harvest index and pod harvest index; and yield and yield components including number of pods per plant, seeds per pod, 100-seed weight, and seed yield per day in RIL population derived from a cross of drought tolerant line SEA5 and CAL96 cultivar under. QTLs mapped under drought stress with the help of SNP markers on chromosomes 1, 3, 4, 7, 8, and 9 can be used for development of drought-tolerant cultivars in common bean molecular breeding program (Mukeshimana et al. 2014).

Soybean Al Tolerance

Soybean [*Glycine max* (L.) Merr.] serving as an important source of protein and oil is highly sensitive to aluminum (Al) toxicity existing in acidic soils globally. Therefore, breeding for Al-tolerant cultivars can provide a sustainable solution for Al toxicity in soybean. Root extension is an important trait that imparts tolerance to AL-toxicity under acidic soils. Two QTLs for root extension mapped on linkage group 8 and 16 in RILs derived from a cross of young and PI 416937 and evaluated under Al stress conditions (Abdel-Haleem et al. 2014). Salt tolerance is another abiotic stress that hampers the soybean production in a significant manner. Ha et al. (2013) identified a single QTL for salt tolerance in RIL population derived from a cross of PI 483463 and Hutcheson. SSR- and SNP-based genotyping mapped the QTL for leaf scorching between SSR03_1335 and SSR03_ 1359 on chromosome 3. The linked markers can be effectively utilized for identification of salt-tolerant cultivars possessing desirable QTL and thereby enhancing the pace of soybean molecular breeding program for salt tolerance.

Pea

Winter pea crop production is hampered by the problem of frost in temperate regions. Therefore, there is a requirement of identification of genomic regions that can contribute to frost-tolerant cultivar development in winter pea. A total of 161 QTLs have been identified in a RIL population derived from cross of JI1491 and Came or for different traits such as height at harvest, number of basal branches per plant, pod number per plant, seed number per plant, seed number per pod, seed weight per plant, 1000-seed weight, straw dry weight per plant, biomass dry weight per plant, seed protein content, and harvest index under six environments. The mapping carried out with the help of SSR and SNP markers located the QTLs on seven linkage groups (Klein et al. 2014). Pea is an important legume crop whose production is affected by low moisture in drought-prone ecologies. Iglesias-García et al. (2015) identified ten QTLs (relative water content in soil and leaves) in RILs derived from a cross of P665 and cv. Messire using SSR markers. The QTLS located on linkage groups 1, 3, and 4 were found to be linked to markers such as A6, AA175, AC74, AD57, AB141, AB64, Psblox2, PsAAP2_SNP4, and DipeptIV_SNP1, and thereby these markers can aid for selection of drought-tolerant genotypes possessing desired QTLs.

8. Temperature stress and HSPs structure and functions in plants.

Heat stress and heat shock:

Most tissues of higher plants are unable to survive extended exposure to temperatures above 45°C. Nongrowing 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 **thermotolerance**. 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.

High Leaf Temperature and Water Deficit Lead to Heat Stress:

Many CAM, succulent higher plants, such as *Opuntia* and *Sempervivum*, are adapted to high temperatures and can tolerate tissue temperatures of 60 to 65°C under conditions of intense solar radiation in summer. Because CAM plants keep their stomata closed during the day, they cannot cool by transpiration. Instead, they dissipate the heat from incident solar radiation by re-emission of longwave (infrared) radiation and loss of heat by conduction and convection.

On the other hand, typical, nonirrigated C_3 and C_4 plants rely on transpirational cooling to lower leaf temperature. In these plants, leaf temperature can readily rise 4 to 5°C above ambient air temperature in bright sunlight near midday, when soil water deficit causes partial stomatal closure or when high relative humidity reduces the potential for evaporative cooling.

Increases in leaf temperature during the day can be pronounced in plants from arid and semiarid regions experiencing drought and high irradiance from sunshine. Heat stress is also a potential danger in greenhouses, where low air speed and high humidity decrease the rate of leaf cooling. A moderate degree of heat stress slows growth of the whole plant. Some irrigated crops, such as cotton, use transpirational cooling to dissipate heat. In irrigated cotton, enhanced transpirational cooling is associated with higher agronomic yields.

At High Temperatures, Photosynthesis Is Inhibited before Respiration:

Both photosynthesis and respiration are inhibited at high temperatures, but as temperature increases, photosynthetic rates drop before respiratory rates. The temperature at which the amount of CO_2 fixed by photosynthesis, equals the amount of CO_2 released by respiration, in a given time interval is called the **temperature compensation point**.

At temperatures above the temperature compensation point, photosynthesis cannot replace the carbon used as a substrate for respiration. As a result, carbohydrate reserves decline, and fruits and vegetables lose sweetness. This imbalance between photosynthesis and respiration is one of the main reasons for the deleterious effects of high temperatures.

In the same plant the temperature compensation point is usually lower for shade leaves than for sun leaves that are exposed to light (and heat). Enhanced respiration rates relative to photosynthesis at high temperatures are more detrimental in C_3 plants than in C_4 or CAM plants because the rates of both dark respiration and photorespiration are increased in C_3 plants at higher temperatures.

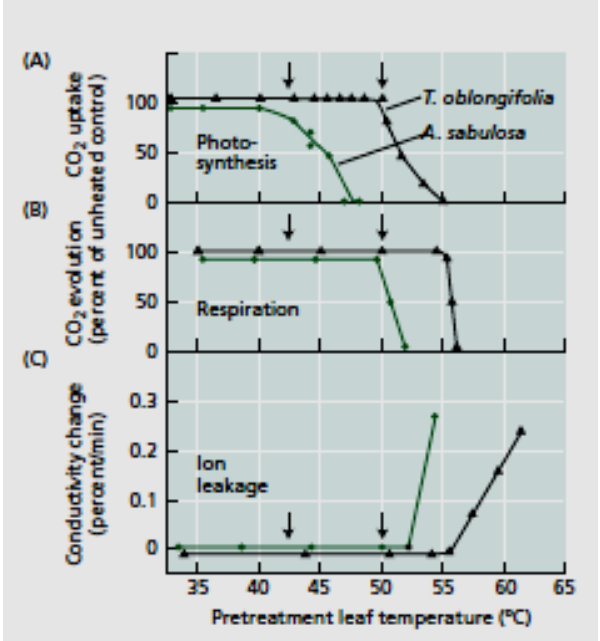


FIGURE 25.10 Response of frosted orache (*Atriplex sabulosa*) and Arizona honeysweet (*Tidestromia oblongifolia*) to heat stress. Photosynthesis (A) and respiration (B) were measured on attached leaves, and ion leakage (C) was measured in leaf slices submerged in water. At the beginning of the experiment, control rates were measured at a noninjurious 30°C. Attached leaves

Plants Adapted to Cool Temperatures Acclimate Poorly to High Temperatures:
The extent to which plants that are genetically adapted to a given temperature range can acclimate to a contrasting temperature range is illustrated by a comparison of the responses of two C_4 species: *Atriplex sabulosa* (frosted orache, family Chenopodiaceae) and *Tidestromia oblongifolia* (Arizona honeysweet, family Amaranthaceae).
A. sabulosa is native to the cool climate of coastal northern California, and *T. oblongifolia* is native to the very hot climate of Death Valley, California, where it grows in a temperature regime that is lethal for most plant species. When these species were grown in a controlled environment and their growth rates were recorded as a function of temperature, *T. oblongifolia* barely grew at 16°C, while *A. sabulosa* was at 75% of its maximum growth rate. By contrast, the growth rate of *A. sabulosa* began to decline between 25 and 30°C, and growth ceased at 45°C, the temperature at which T. *oblongifolia* growth showed a maximum.
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.
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.
Heat shock protein (HSPs):
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.

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.

A Transcription Factor Mediates HSP Accumulation in Response to Heat Shock

All cells seem to contain molecular chaperones that are constitutively expressed and function like HSPs. These chaperones are called **heat shock cognate proteins**. However, when cells are subjected to a stressful, but nonlethal heat episode, the synthesis of HSPs dramatically increases while the continuing translation of other proteins is dramatically lowered or ceases. This heat shock response appears to be mediated by a specific transcription factor (HSF) that acts on the transcription of HSP mRNAs.

In the absence of heat stress, HSF exists as monomers that are incapable of binding to DNA and directing transcription. Stress causes HSF monomers to associate into trimers that are then able to bind to specific sequence elements in DNA referred to as heat shock elements (HSEs). Once bound to the HSE, the trimeric HSF is phosphorylated and promotes the transcription of HSP mRNAs. HSP70 subsequently binds to HSF, leading to the dissociation of the HSF/HSE complex, and the HSF is subsequently recycled to the monomeric HSF form. Thus, by the action of HSF, HSPs accumulate until they become abundant enough to bind to HSF, leading to the cessation of HSP mRNAproduction.

HSPs Mediate Thermotolerance

Conditions that induce thermal tolerance in plants closely match those that induce the accumulation of HSPs, but that correlation alone does not prove that HSPs play an essential role in acclimation to heat stress. More conclusive experiments show that expression of an activated HSF induces constitutive synthesis of HSPs and increases the thermotolerance of *Arabidopsis*. Studies with Arabidopsis plants containing an antisense DNA sequence that reduces HSP70 synthesis showed that the high-temperature extreme at which the plants could survive was reduced by 2°C compared with controls, although the mutant plants grew normally at optimum temperatures (Lee and Schoeffl 1996).

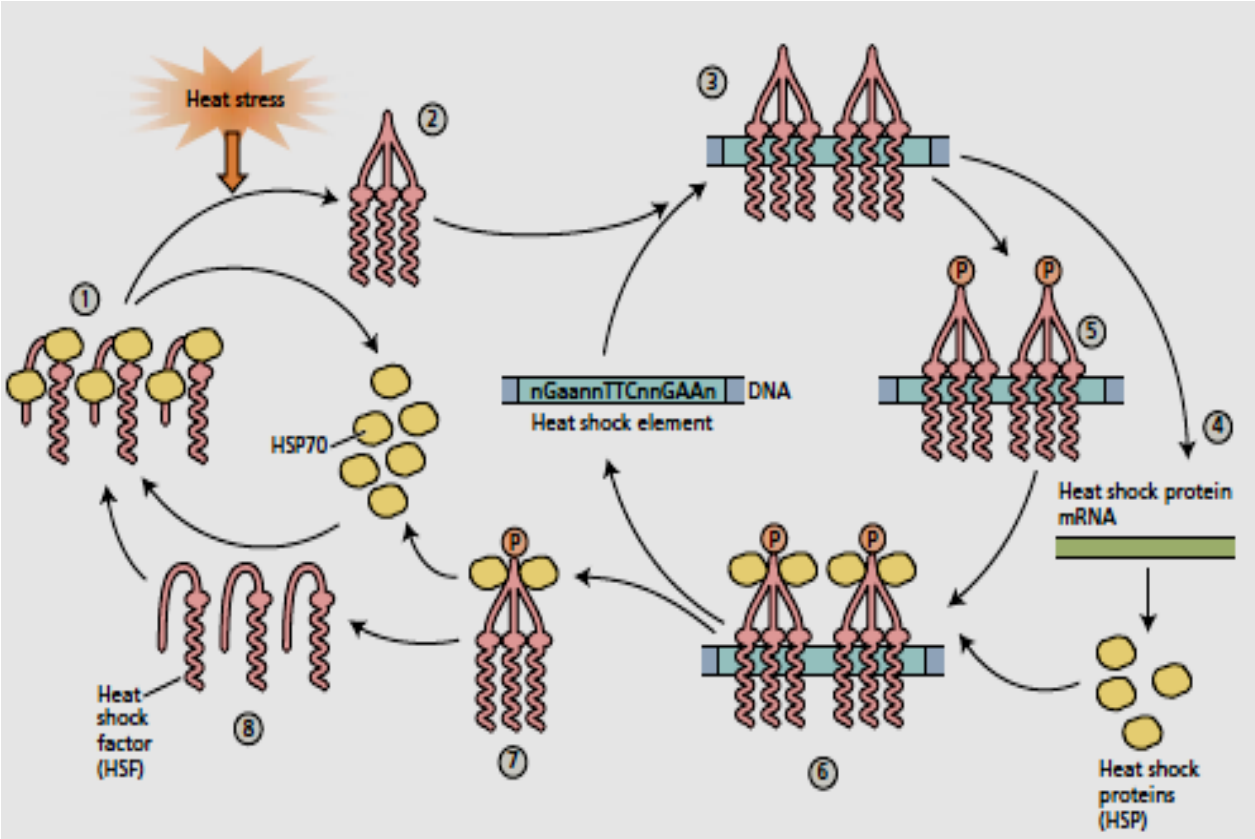


FIGURE 25.11 The heat shock factor (HSF) cycle activates the synthesis of heat shock protein mRNAs. In nonstressed cells, HSF normally exists in a monomeric state (1) associated with HSP70 proteins. Upon the onset of an episode of heat stress, HSP70 dissociates from HSF which subsequently trimerizes (2). Active trimers bind to heat shock elements (HSE) in the promoter of heat shock protein (HSP) genes (3), and activate the transcription of HSP mRNAs leading to the translation of HSPs among which are HSP70 (4). The HSF trimers associated with the HSE are phosphorylated (5) facilitating the binding of HSP70 to the phosphorylated trimers (6). The HSP70 trimer complex (7) dissociates from the HSE and disassembles and dephosphorylates into HSF monomers (8), which subsequently bind HSP reforming the resting HSP70/HSF complex. (After Bray et al. 2000.)

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. CAX_1 and CAX_2 are transport proteins, ACA: Ca^{2+} ATPase.

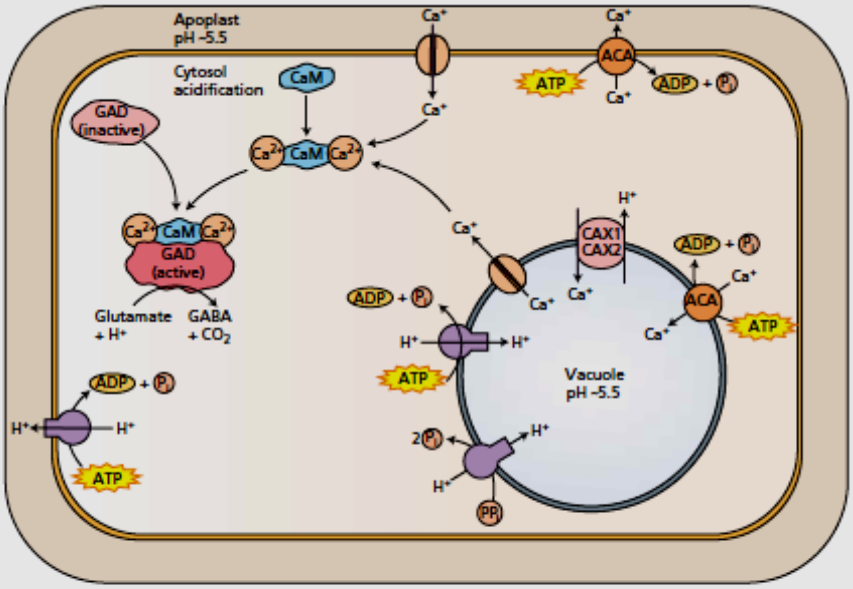


FIGURE 25.12 Heat stress causes a reduction in cytosolic pH from the normal slightly alkaline value, probably by inhibiting proton-pumping ATPases and pyrophosphatases that pump protons across the plasma membrane or into the vacuole. Additionally, heat stress effects a change in calcium homeostasis inside 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

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.

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).

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 because 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.

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.

9. Development of transgenic for stress tolerance

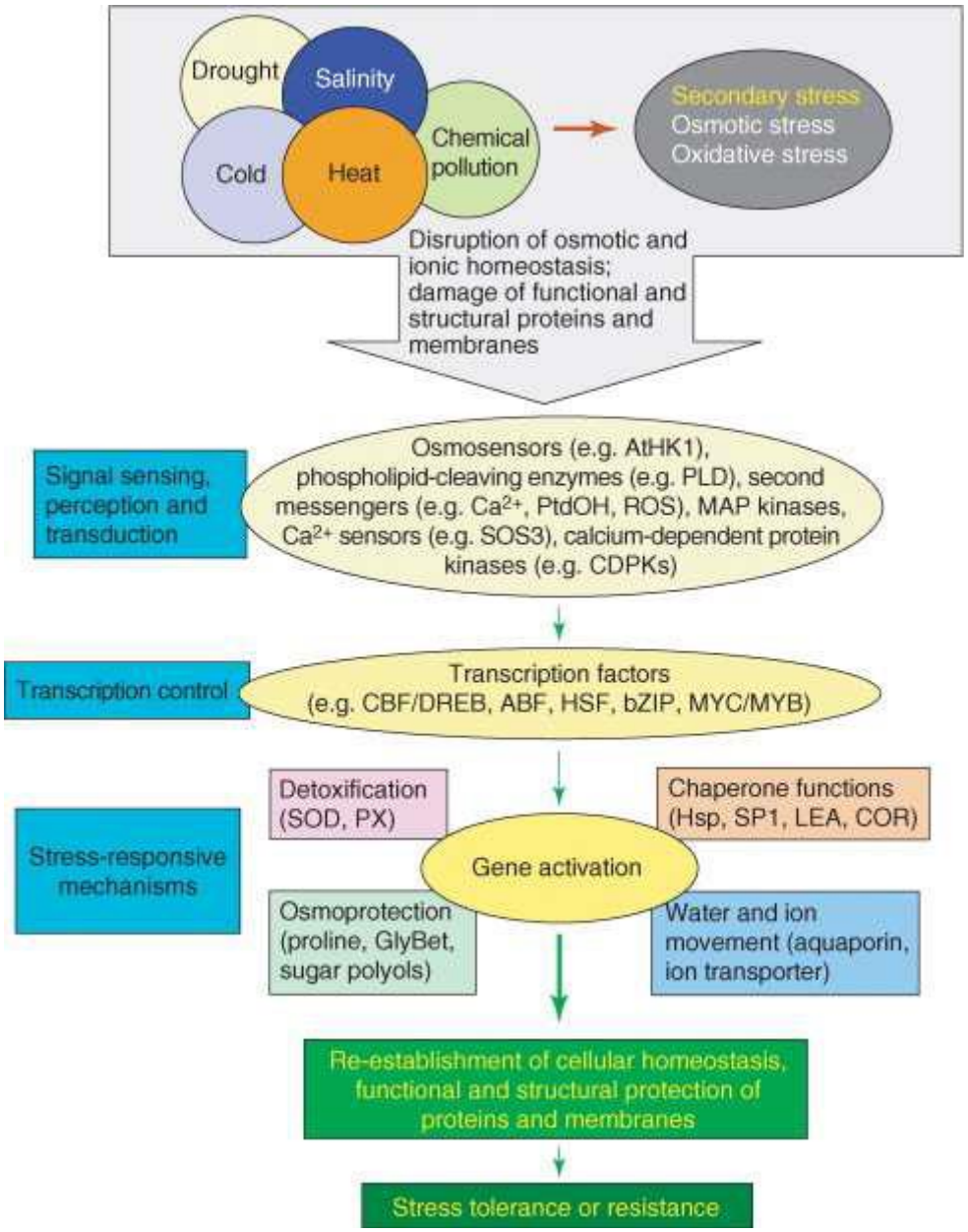
Introduction:

As we all know that Agriculture is totally dependent on climate. So a variety of Abiotic Stresses causing a serious crop loss of about >50 % on an average thus limiting the agricultural productivity world wide. By 2025, 30% of crop production will be at risk due to the declining water availability. World Bank projects that the climate change will depress crop yields by 20% or more by the year 2050. (Narendra Tuteja, 2012) Efforts have been made by Plant breeder in developing abiotic stress resistant crop plants but are not sufficient enough. Thus the role of Transgenic Approach in crop improvement has become of great importance in assuring worlds future food security.

Transgenic techniques are being used to introduce biotic and abiotic stress related genes in crop plants (Ashraf et al., 2008). Plant responses under abiotic stresses are complex involving number of genes with additive effects, hence prospects of improve stress tolerance are not very bright. In spite of it,

researchers have exerted efforts during last decade to generate transgenic crops with improved tolerance against abiotic stresses. The major emphasis has been to introduce gene encoding compatible organic osmolytes, heat shock proteins, plant growth regulators, late embryogenesis abundant proteins and transcription factors responsible in activating gene expression (Ashraf et al., 2010).

Many genes play an important role in the synthesis of osmoprotectants in stress tolerant plant like proline, glycinebetaine and polyamines, mannitol, trehalose and galactinol which accumulate during osmotic adjustment (Vincour and Altman, 2005). Now it is well evident that these organic solutes play an important role in induction of drought tolerance (Ashraf and Foolad, 2007). Certain major crop plants such as rice, soybean, and potato lack significant amounts of betaines or any other osmoprotectant (reviewed by McNeil et al., 1999; Bhatnagar-Mathur et al., 2008); therefore, osmoregulatory genes have been first target of the researchers to introduce in crops. Also the overexpression of the genes encoding synthesis of organic solutes has been carried out. Among many organic osmolytes having substantial role in stress tolerance, glycine betaine (GB) is richly synthesized under dehydration stress (Mansour, 2000; Mohanty et al., 2002; Yang et al., 2003; Ashraf and Foolad, 2007).



Plant response to abiotic stress to develop tolerance or resistance

Drought tolerance

Water becomes an increasingly scarce and precious commodity. It is thus essential to improve water use efficiency in agriculture. The development of crop varieties with increase tolerance to drought, both by conventional breeding methods and genetic engineering is important to meet global food demand with less water. Conventional breeding and marker assisted selection are being used to develop varieties more tolerant to water stress. These methods are time taking and resource consuming and germplasm dependent. Genetic engineering is alternative because of its potential to improve abiotic stress tolerance more rapidly. The techniques for gene transformation of crop plants have been applied for identification of genes responsible for drought resistance and their transfer. Genes expressed during stress are anticipated to promote cellular tolerance to dehydration through protective functions in the cytoplasm, cell membrane, alternations of cellular water potential to promote water uptake, control of ion accumulation and further regulation of other gene(s). A comprehensive study on transgenic plants summarized and nature of tolerance has been listed in Table.

Table 1: List of transgenic research for salt stress tolerance

Gene	Gene action	Species	Phenotypic expression by transgenic plant
Mt1D	Manitol-1-phosphate dehydrogenase (manitol synthesis)	Tobacco	Increased plant height and fresh weight under salinity stress
p5cs	Pyrroline carboxylase synthetase (proline synthesis)	Tobacco	Enhanced biomass and flowering under salt stress
betA	Choline dehydrogenase (glycine betaine synthesis)	Tobacco	Increased tolerant to salinity stress
HVA1	Group 3 LEA protein gene	*Oryza sativa*	Increased tolerance to drought and salinity
IMT1	Myo-inositol-O-methyl transferase (D-ononitol synthesis)	Tobacco	Performed better under drought and salinity stress
Nt107	Glutathione S-transferase	Tobacco	Sustained growth under cold and salinity stress
p5cs	Pyrroline carboxylate synthetase (proline synthesis)	*Oryza sativa*	Increased biomass production under drought and salinity stress
DREB	Transcription factor	Arabidopsis	Increased tolerance to cold, drought and salinity
NHX1	-	Arabidopsis	Increased salt tolerance
DnaK	-	Tobacco	Increased salt tolerance
MsPRP2	Transcription factor	Alfalfa	Increased salinity tolerance
Osmotin	-	*Solanum tuberosum*	Increased salt tolerance
proDH	Proline dehydrogenase	Arabidopsis	Tolerant to freezing and high salinity
AtHAL3a	Phosphoprotein phosphatase	Arabidopsis	Regulates salinity, osmotic tolerance and plant growth
Glycolase	-	Tobacco	Increased salt tolerance
AtNHX1	Over expression of Na^+/H^+ antiport	Arabidopsis	Transgenic plants grow in 200 mM NaCl
Mn-SOD	Expression of superoxide dismutase	*Oryza sativa*	Increased salt tolerance
GS2	Chloroplastic glutamine synthetase	*Oryza sativa*	Increased salinity resistance and chilling tolerance
HAL1	-	*Solanum esculentum*	Increased salt tolerance
OsCDPK7	Transcription factor	*Oryza sativa*	Increased tolerance of cold, salinity and drought
codA	Glycine betaine synthesis	Brassica	Increased salt tolerance
p5csF	Proline synthesis (feed back inhibition removed)	Tobacco	Survived 200 mM NaCl, reduced oxidative stress induced by osmotic stress
ApoInv	Invertase (Sucrose break down)	Tobacco	Salt tolerance to high osmotic pressure increase in cell sap
AtNHX1	Over expression of Na^+/H^+ antiport	Tomato	Transgenic plants grow in 200 mM NaCl
DtNHX	Over expression of Na^+/H^+ antiport	Brassica	Transgenic plants grow in 200 mM NaCl

Trihelose biosynthesis: Bacterial trehalose-6-phosphate (OtsB) genes introduced in tobacco showed better growth under drought stress (Pilon-Smits et al., 1995). Transgenic plants also showed a better capacity to retain water and preformed more efficient photosynthesis under stress. The gene TPS1 found in yeast encodes for trehalose-6-phosphate synthetase and is involved in biosynthesis of trehalose. The transgenic tobacco plants containing the yeast TPS1 gene exhibited multiple alteration and improved drought tolerance (Romero et al., 1997). By determining the effect of withholding irrigation on the death and damage of leaf (Romero et al., 1997), it has been shown that the transgenic plants have increased drought resistance. Further, Holmstrom (1998) transferred tobacco, resulting in the accumulation of trehalose and improved drought resistance. It is suggested that the improved drought tolerance is due to enhanced water retention capacity. Serrano et al. (1998) reported that yeast regulatory gene involved in stress tolerance, TPS1, have been expressed in transgenic plants. Almeida et al. (2004) transformed Nicotiana tabaccum with Arbidopsis thaliana gene (AtTPS1), which is involved in trehalose biosynthesis, by Agrobacterium-mediated genetic transformation. They observed high germination rates at higher levels of manitol than did wild type plants.

LEA protein: Transgenic rice carrying barley HVA1 gene had shown drought resistance (Xu et al., 1996). Gene HVA1 encodes for a group of three LEA proteins which get accumulated in vegetative organs during drought condition. Transgenic rice showed enhanced accumulation of the HVA1 protein, which led to higher growth rates, delayed stress-related damage systems and improved recovery from the removal of stress conditions (Xu et al., 1996). The transgenic rice plants exhibited constitutive high expression of HVA1 protein ranging from 0.3-2.5 and 0.3-1.0% of the total soluble protein in leaf and root, respectively. Transgenic wheat plants containing the HVA1 gene showed consecutive expression of the transgene resulting in improvement of growth characteristics under water deficient conditions, more biomass and more efficient water use (Sivamani et al., 2000). Rohila et al. (2002) transformed Pusa Basmati 1 with HVA1 to increase tolerance against abiotic stresses. They developed transgenic lines which showed increased stress tolerance in terms of cell integrity and growth after imposed salt-and water stresses. Their findings exhibited high levels of LEA3 accumulation in the leaves of transgenic Pusa Basmati 1 rice plants might have conferred the significant increase in tolerance against drought and salt stresses.

Fructan synthesis: The bacterial gene sacB found in Bacillus subtilis encodes for levan sucrase, which takes part in fructan synthesis. Fructan promotes the process of root branching, thus increasing root surface and water uptake. Pilon-Smits et al. (1995) have shown that over-expression of scaB gene from Bacillus subtilis leads to high level of fructans in transgenic tobacco cells, leading in higher capacity for osmotic adjustment and this is associated with increased drought tolerance. The additional carbohydrate gained may lead to deeper rooting and greater water-uptake (Pilon-Smits et al. 1995; Blum et al., 1996; Schellenbaum et al., 1999). The transgenic tobacco that produces bacterial fructans was produced and examined for growth of the transgenic plants was significantly higher both on fresh weight and dry weight basis under drought stress compared to the wild type tobacco. The transgenic tobacco exhibited significantly more biomass accumulation in roots under drought stress and higher non-structural carbohydrate content under all conditions.

Proline accumulators: Proline accumulation has been demonstrated to be associated with abiotic stress (Delauney and Verma, 1993). Some genes related to proline synthesis, transport and accumulation have been identified (Delauney and Verma, 1993). Transgenic tobacco overexpressing P5CS gene transferred from mothbean exhibited a high level of enzyme and produced 10-18 fold more proline than control plant (Kishor et al., 1995). Over-production of proline enhanced root biomass and flower development under drought condition. Zhu et al. (1998) also found that transgenic rice with P5CS gene enhanced root biomass and flower development under water stress. Transgenic soybean plants transferred with antisense P5CR (L-Δ'-pyrroline-5-carboxylate reductase) gene showed increased proline accumulation, leading to higher tolerance to water stress (De Ronde et al., 2000).

Polyamine biosynthesis genes: In plants, polyamine, accumulate under several abiotic stress stimuli, including drought and salt. It has been suggested that this increase in polyamine concentration could be considered as an indicator of plant stress. With the availability of genes responsible for polyamine biosynthesis such as ADC (encodes for arginine decarboxylase), it is now possible to manipulate polyamine content using sense and antisense constructs of these genes in transgenic plants.

Engineering of the plant polyamine biosynthesis pathway has concentrated mostly on two species, tobacco and rice (Kumar and Minocha, 1998; Capell and Christou, 2004). They suggested that further studies are required to understand the tolerance ability of these genes. The increase in putrescine levels in plants under stress might be the cause of stress-induced injury or alternatively a mean of protection against stress. Roy and Wu (2001) expressing oat adc cDNA in rice under control of an ABA-inducible promoter resulted in transgenic rice plants with increased biomass when grown under salt stress. Capell and Christou (2004) have generated a diverse rice germplasm with altered polyamine content. Transgenic rice plant expressing the SAMDC DNA accumulated spermidine and spermine in seed at 2-3 fold higher levels compared to wild type. In another set of experiment, they have obtained ten fold putrescine accumulation in transgenic rice plants laboring oat adc cDNA compared to wild type. Spermidine and spermine de-novo synthesis in transgenic plants under drought stress is corroborated by the activation of the rice SAMDC gene. Transcript levels for rice SAMDC reach their maximum levels at 6 days after stress induction. Such increase in the endogenous spermidine and spermine pools of transgenic plants not only regulates the putrescine response, but also exerts an anti-senescence effect at the whole plant level, resulting in phenotypically normal plants. Wild type plants, however, are not able to raise their spermidine and spermine levels after 6 days of drought stress and consequently exhibit the classical drought stress response (Capell and Christou, 2004).

Choline monooxygenase (CMO) catalyzes the committed step of glycinebetaine (GlyBet) biosynthesis in many flowering plants. Over-expression of AhCMO improved drought tolerance in transgenic tobacco when cultured in medium containing PEG6000 (Shen et al., 2002).

Dehydration responsive transcription factor: Plant genomes contain a large number of Transcription Factors (TFs). TFs are key regulatory proteins that enhance or repress the transcriptional rate of their target genes by binding to specific promoter regions upon activation or deactivation of upstream signaling cascade. Most of these transcriptional factors belong to a few large multigene families. Individual members of the same family often respond differently to various stress stimuli, on the other hand, some stress responsive may share the same transcriptional factor (Chen et al., 2002; Kreps et al., 2002). The Dehydration-responsive Element (DRE) and C-repeat binding factors (CBF) was identified as a cis-acting element regulating gene expression in response to dehydration (salt, drought and cold stresses) in Arabidopsis (Van Rensburg and Kruger, 1994). The dehydration-responsive transcription factors DREB and C-repeat binding factors (CBF) bind to DRE and CRT cis-acting elements that contain the same motif (CCGAC). Members of the CBF/DREB1 family, such as CBF1, CBF2 and CBF3 (or DREB1B, DREB1C and DREB1A, respectively) are themselves stress-inducible. DREB/CBF proteins are encoded by AP2/EREBP multigene families and mediate the transcription of several genes. DREB1A, a transcription factor that recognizes dehydration response elements, has been shown in Arbidopsis thaliana to play a crucial role in promoting the expression of drought tolerant genes (Pellegrineschi et al., 2003, 2004). They have transformed DREB1A gene into wheat. Plant expressing the gene demonstrated substantial resistant to water stress compared with the control under stress condition. Recently, overexpression of CBF4 from barley has been shown to confer salinity, drought and low temperature tolerance in transgenic rice (Oh et al., 2007).

The NAC gene family members encode one of the largest families of plant specific TFs and are expressed in various developmental stages, tissues and environmental factors. Hu et al. (2006) isolated and characterized NAC gene SNAC1. SNAC1 over-expressing transgenic rice plants showed significantly improved drought resistance under field conditions and strong tolerance to salt stress (Hu et al., 2006).

The phosphoinositide pathway and inositol-1,4,5-trisphosphate (InsP3) are implicated in plant responses to stress. To determine the downstream consequences of altered InsP3-mediated signaling, Perera et al. (2008) generated transgenic Arabidopsis thaliana plants expressing the mammalian type I inositol polyphosphate 5-phosphatase (InsP 5-ptase), which specifically hydrolyzes soluble inositol phosphates and terminates the signal. Rapid transient Ca2+ responses to a cold or salt stimulus were reduced by ~30% in these transgenic plants. Drought stress studies revealed, surprisingly, that the InsP 5-ptase plants lost less water and exhibited increased drought tolerance. The onset of the drought stress was delayed in the transgenic plants and abscisic acid (ABA) levels increased less than in the

wild-type plants. Stomatal bioassays showed that transgenic guard cells were less responsive to the inhibition of opening by ABA but showed an increased sensitivity to ABA-induced closure. Transcript profiling revealed that the drought-inducible ABA-independent transcription factor DREB2A and a subset of DREB2A-regulated genes were basally upregulated in the InsP 5-ptase plants, suggesting that InsP3 is a negative regulator of these DREB2A-regulated genes. These results indicated that the drought tolerance of the InsP 5-ptase plants is mediated in part via a DREB2A-dependent pathway and that constitutive dampening of the InsP3 signal reveals unanticipated interconnections between signaling pathways (Perera et al., 2008).

Super oxidedismutase: Within a cell, the SODs constitute the first line of the defense against ROS. It is found in all subcellular locations, like mitochondria, chloroplast microsomes, glyoxysomes, peroxysomes, apoplast and the cytosol. SODs are metaloproteins found in various compartments of plant cells and contain Cu and Zn, Fe or Mn cofactors. Transgenic tobacco plants containing oxidative stress- related genes showed elevated levels of glutathione reductase, superoxide dismutase and ascorbate peroxidase, resulting in enhance drought tolerance (Van Rensburg and Kruger, 1994).

Wax production: The gene designated WXP1, is able to activate wax production and confer drought tolerance in alfalfa (Medicago sativa). Overexpression of WXP1 under the control of CaMV355 promoter led to a significant increase in cuticular wax loading on leaves of transgenic alfalfa (Zhang et al., 2005). WXP1 over expression induced a number of wax-related genes.

Transgenic leaves showed reduced water loss and chlorophyll leaching- transgenic alfalfa plants with increased cuticular wax showed enhanced drought tolerance demonstrated by delayed wilting after watering has been ceased and quicker and better recovery when the dehydrated plants were re-watered.

A full length rDNA of dehydrin BcDh2 from *Boea crassifolia* and its antisense nucleotide sequence was transferred into tobacco, var. NC89 under the control of CMV promoter (Ye et al., 2004). Under progressive water stress, the photosynthetic rate, transpiration rate and stomatal conductance of sense and antisense plants decreased. However, those parameters increased after 24 h of watering and the enhanced was higher in sense and antisense plants than the control.

Mitogen-activated protein: Expression of mitogen-activated protein kinase gene (MAPK) genes activates an oxidative signal cascade and lead to the tolerance of freezing, drought, heat and salinity stressing transgenic tobacco. MAPKs perform their function as a part of kinase modules, which is mainly composed of MAPKs, MAPKKs and MAPKKKs. Shou et al. (2004) transformed maize with a tobacco MAPKKK (NPK1). They found that the NPK1 expression enhanced drought tolerance in transgenic maize. Under drought stress, it maintained significantly higher photosynthesis rates than did the non-transgenic control, suggesting that protected photosynthesis machinery is protective from dehydration damage.

ABA Signaling: Protecting crop yield under drought stress is a major challenge for modern agriculture. One biotechnological target for improving plant drought tolerance is the genetic manipulation of the stress response to the hormone abscisic acid (ABA). In response of water stress, ABA levels in plant greatly increase resulting closure of stomata, thereby reducing the level of water loss through transpiration from leaves and activate response of genes. ERA1, a gene identified in Arabidopsis, encodes β-subunit of a farenesyl-transfarase and involves in ABA signaling. Wang et al. (2005) developed transgenic plants which performed significantly better under water stress, with consistently higher yield over conventional varieties of canola, whereas they observed no difference in performance of transgenic and control under sufficient water.

Salt tolerance

Salt tolerance is an important trait that requires overcoming salinity induced reduction in plant productivity. The genetic response of plants to abiotic stresses is complex involving simultaneous expression of a number of genes. Plant genetic engineering techniques could be effectively utilized to exploit some of the untapped potentials to increase the harvestable crop yield. It involves specific gene manipulation either through over expression or silencing of alien/native genes. A number of genes induced in response to salinity have been identified from a range of organisms adapted to stressful environment. If a salt tolerant gene is identified which can lead to betterment of the crops, it

is possible to transfer that progress in transgenic research for inclusion salinity stress tolerance, which has been presented in Table 1.

Transgenic research has made significant progress in crop genetic improvement with the advent of modern rDNA technologies. A large number of transgenics in diverse crops are on large-scale cultivation. Moreover, multiple genes can be stacked or transformed to a stock of interest through genetic transformation. There are a large number of genes found to be instrumental and there are many functional targets for engineering tolerance to salinity. Few of the genes of importance are briefed below.

Table 1: List of transgenic research for salt stress tolerance

Gene	Gene action	Species	Phenotypic expression by transgenic plant
Mt1D	Manitol-1-phosphate dehydrogenase (manitol synthesis)	Tobacco	Increased plant height and fresh weight unde salinity stress
p5cs	Pyrroline carboxylase synthetase (proline synthesis)	Tobacco	Enhanced biomass and flowering under salt stress
betA	Choline dehydrogenase (glycine betaine synthesis)	Tobacco	Increased tolerant to salinity stress
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Nt107	Glutathione S-transferase	Tobacco	Sustained growth under cold and salinity stress
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DREB	Transcription factor	Arabidopsis	Increased tolerance to cold, drought and salinity
NHX1	-	Arabidopsis	Increased salt tolerance
DnaK	-	Tobacco	Increased salt tolerance
MsPRP2	Transcription factor	Alfalfa	Increased salinity tolerance
Osmotin	-	*Solanum tuberosum*	Increased salt tolerance
proDH	Proline dehydrogenase	Arabidopsis	Tolerant to freezing and high salinity
AtHAL3a	Phosphoprotein phosphatase	Arabidopsis	Regulates salinity, osmotic tolerance and plant growth
Glycolase	-	Tobacco	Increased salt tolerance
AtNHX1	Over expression of Na^+/H^+ antiport	Arabidopsis	Transgenic plants grow in 200 mM NaCl
Mn-SOD	Expression of superoxide dismutase	*Oryza sativa*	Increased salt tolerance
GS2	Chloroplastic glutamine synthetase	*Oryza sativa*	Increased salinity resistance and chilling tolerance
HAL1	-	*Solanum esculentum*	Increased salt tolerance
OsCDPK7	Transcription factor	*Oryza sativa*	Increased tolerance of cold, salinity and drought
codA	Glycine betaine synthesis	Brassica	Increased salt tolerance
p5csF	Proline synthesis (feed back inhibition removed)	Tobacco	Survived 200 mM NaCl, reduced oxidative stress induced by osmotic stress
ApoInv	Invertase (Sucrose break down)	Tobacco	Salt tolerance to high osmotic pressure increase in cell sap
AtNHX1	Over expression of Na^+/H^+ antiport	Tomato	Transgenic plants grow in 200 mM NaCl

Ion transporter and Antiporter genes: A salt concentration of 200 mM is equivalent to 40% of the salt concentration of sea water and will inhibit growth of almost all crop plants.

Ion trans porters selectively transport ions and maintain them at physiologically relevant concentrations while Na+/H+ antiporters also play a crucial role in maintaining cellular ion homeostasis, thus permitting plant survival and growth under saline conditions. The Na+/H+ antiporters catalyze the exchange of Na+ for cytoplasmic pH, sodium levels and cell turgour (Serrano et al., 1998).

A construct containing the DtNHX gene, coding for a vacuolar Na+/H+ antiport from Arabidopsis thaliana, was introduced into the genome of Brassica napus cv. Westar by Zhang et al. (2001). Over expression of the vacuolar Na+/H+ antiport did not affect the growth of transgenic plants since similar growth was observed when wild type and transgenic plants were grown in the presence of 10 mM NaCl. While growth of wild type plants was severely affected by the presence of 200 mM NaCl in the growth solution, transgenic plants grew, flowered and produced seeds. They noted that the transgenic plants grown at 200 mM NaCl produced number of seeds similar to those of wild-type plants grown at low salinity. Moreover, qualitative and quantitative analysis of oil content showed no significant differences between seeds from wild type plants grown at low salinity and transgenic plants grown at high salinity. They also observed that the transgenic plants accumulated up to 6% Na without altering the yield and oil content. Transgenic Brassica juncia plants over expressing pgNHX1 withstand 300 mM salt stress till the seed setting stage and exhibited normal growth phenotype without much loss in seed yield (Rajgopal et al., 2007).

Glenn et al. (1999) have engineered transgenic Arabidopsis plants that overexpress AtNHX1, a vacuolar Na+/H+ antiport, which allowed the plants to grow in 200 mM NaCl. Zhang and Bhumwald (2001) reported the genetic modification of tomato plants to overexpress the Arabidopsis thaliana AtNHX1 antiport, which likewise allowed those plants to grow in the presence of 200 mM NaCl. Besides providing farmers with a cash crop for salted lands, such, plants may also pull salt out of soils, enabling other crops to thrive again. Wheat productivity is severely affected by soil salinity due to Na+ toxicity to plant cells. Xue et al. (2004) generated transgenic wheat expressing a vacuolar Na+/H+ antiport gene AtNHX1. The transgenic wheat lines exhibited improved biomass production. The field trial revealed that the transgenic wheat lines produced higher grain yield and heavier and larger grains in the field of saline soil. The transgenic rice accumulated a lower level of Na+ and higher level of K+ in the leaves than the non-transgenic plants under saline environment.

The *Escherchia coli* nahA gene encodes a Na+/H+ antiporter, which plays critical role in ion homeostasis has been transferred into rice (Oryza sativa L. sp. Japonica) by Wu et al. (2005). The transgenic plants showed better germination rate, growth and average yield per plant than control. They also reported higher sodium and proline content in transgenic lines, implying that nhaA over-expression enhance osmoregulation by activating the bio-synthesis of proline.

Compatible solutes: Compatible solutes accumulate in organisms in response of osmotic stress. The primary function of compatible solute is to maintain cell turgour and thus the driving gradient for water uptake. Studies also indicated that compatible solutes can act as free radical scavengers or chemical chaperon by directly stabilizing membrane and/or proteins (Diamant et al., 2001). The Δ'adi (Choline oxidase) gene isolated from the soil bacterium Arthrobacter globiformis converts choline to glycine betaine via betain aldehyde (Deshnium et al., 1995). This gene encodes choline oxidase, the enzyme that converts choline to glycinebetaine. Transgenic potato plants expressing the oxalate oxidase enzyme were produced by Turhan (2005) using Agrobacterium-mediated genetic transformation. His findings revealed a relatively higher salt tolerance ability of transgenic than the non-transgenic genotypes in vitro. However, the glasshouse results were less consistent, but some transgenic genotypes showed superior yield characteristics to the non-transgenic under salinity.

Glycinebetaine is an extremely efficient osmoprotectant widely distributed among plants. It is a quaternary ammonium compound belonging to the organic solutes, which accumulate in higher plants in response to osmotic stress induced by drought, high salinity or low temperature (McNeil et al., 2000). The ability to synthesize and accumulate glycinebetaine contributes to salt and drought tolerance (Grumet and Hanson, 1986). Transformation of Arabidopsis thaliana with Δ'adi gene resulted in enhanced salt tolerance of transgenic Arabidopsis plants (Hayashi et al., 1997). Thus synthesis of glycinebetaine in transgenic plants in vitro, as a result of the expression of Δ'adi gene, might be very useful in improving the ability of crop plants to tolerate salt stress (Hayashi et al., 2001). Sakamoto et al. (1998) reported transgenic rice expressing the Δ'adi gene in the chloroplast and the cytosol recovered to normal growth at a faster rate than the wild type after an initial growth inhibition under salt and low temperature stress.

Lilius et al. (1996) transformed tobacco plants with Δ'adi gene, which encodes for choline dehydrogenase protein (responsible for conversion of choline to betaine aldehyde) from E. coli. The Δ'adi gene introduction rendered the transgenic tobacco plants tolerant to high concentration of salt.

Betaine aldehyde dehydrogenease (BADH) is the second enzyme in this pathway responsible for conversion of betaine aldehyde to glycinebetaine.
Tobacco has been transformed with the Δ'adi and betB genes, involved in the glycinebetaine (betaine) pathway of Escherichia coli (Holmstrom, 1998). The transgenic plants produced and accumulated osmolyte and exhibited enhanced stress tolerance as shown by enhanced growth under salt stress and improved recovery of PS-II after salt stress. High tolerance to NaCl was also obtained transferring bacterial beta gene to cabbage (Brassica oleracea var. capitata) cultivar Golden Acre through Agrobacterium-mediated genetic transformation of hypocotyl explants (Bhattacharya et al., 2004).
Manitol gene: Osmotically shocked cells synthesize and accumulate massive amount of osmoprotectory compounds. Such compounds possibly help the cells to lower their osmotic potential and to draw water from the outside medium. Manitol as an osmoprotectory compound is primarily found in microbes. By introducing manitol-1-phosphate dehydrogenease gene (mt1D) isolated from E. coli (Tarezynski et al., 1993) showed over-expression of manitol in tobacco plants. These transgenic plants showed tolerance to high NaCl levels (250 mM). Seeds of transgenic Arabidopsis transformed with mt1D gene under control of CaMV 35 promoter over produced manitol and germinated in a medium supplemented with high amount of NaCl (Thomas et al., 1995). Li et al. (2004) introduced mt1D gene into upland rice (Oryza sativa var. japonica) by microprojectile bombardment. Growth rate of transgenic plants was significantly higher than the control on MS medium containing 1% NaCl. Non-transgenic plants died after 35 days. They reported less membrane damage and low Na+/K+ ratio than the control under salt stress.
LEA protein: Osmotic stresses induce Late-embryogenesis-abundant (LEA) proteins in vegetative tissues of plants. The functions of LEA proteins are largely unknown. Nevertheless, their considerable synthesis during the late embryogenesis, their induction by stress and their structural characteristics permit the prediction of some of their functions. It has been suggested that LEA type proteins act as water binding molecules, in ion sequestration and membrane stabilization. LEA-proteins are encoded by Responsive to Dehydration (RD), Early Responsive to Dehydration (ERD), cold inducible (KIN), cold regulated (COR) and responsive to ABA (RAB) genes in different plant species. Xu et al. (1996) found that the hva1 gene, which encodes for a specific class of LEA proteins, when overexpressed in rice leads to increased salt tolerance. This study demonstrated that subcellular compartmentalization of the biosynthesis of glycinebetaine was a critical step in attaining enhancement of tolerance for salinity and water stress. Tansformation of Chinese cabagge (Brassica campestris ssp. pekinensis) by overexpression a B. napus Group 3 LEA gene enhanced tolerance to salinity and drought (Park et al., 2005).
Trehalose: Trehalose is a non-reducing disaccharide of glucose that functions as a protectant in the stabilization of biological structure and enhances the tolerance of organisms to abiotic stress. Zhang et al. (2005) transformed tobacco plants with trehalose synthetase (Tsase) gene for manipulating abiotic stress tolerance. They reported higher trehalose accumulation in transgenic plants as compared to non-transgenics. The finding suggested that the transgenic plants transformed with Tsase gene can accumulate higher levels of trehalose and have enhanced tolerance to drought and salt stresses.
H+-pyrophosphatase (H+-Ppase) gene: An H+-Ppase gene named TsVP involved in basic biochemical and physiological mechanisms was cloned from Thellungiella halophila. Transgenic tobacco overexpressing TsVP had 60% greater dry weight than wild-type tobacco at 300 mM NaCl (Gao et al., 2006). Their findings suggested that over expression of H+-Ppase causes the accumulation of Na+ in vacuoles instead of in the cytoplasm and avoids the toxicity of excess Na+ in plant cells.

Heat tolerance
The successful development of transgenics have been presented in Table. The biochemical basis of heat tolerance was investigated by comparing the response of antisense and sense transgenic soybean plant containing L-Delta-1-pyrroline-5-carboxylate reductase gene (De Ronde et al., 2004) with non-transgenic wild-type plant. Upon stress, NADP+ levels decreased in sense plants. Sense plants had the highest ability to metabolize proline after rewatering. They reported dissociation of Oxygen-Evolving Complex (OEC) upon stress. In sense plants, which best resisted the stress, OEC dissociation was by passed by proline feeding electrons into PS II, maintaining an acceptable NADPH level, preventing further damage.

Table 4: Gene encoding for molecular chaperones and transgenic development

Gene	Gene action	Species	Phenotypic expression
Hsp70	Heat-inducible anti-sense HSP70	*Arabidopsis thaliana*	Increased thermotolerance in transgenic plants
Hsp17.7	Heat shock protein	*Daccus carota* L.	Increased or decreased thermotolerance
P5CR	Inducible heat shock promoter (IHSP)	*Glycin max*	Increased proline accumulation
Hsp101	Heat shock protein	*Arabidopsis thaliana*	Decreased thermotolerance in Hsp101-deficient (hot1) mutant
Hsp101	Heat shock protein	*Arabidopsis thaliana*	Manipulated thermotolerance in transgenic plants
DcHSP17.7	Heat shock protein	*Solanum tubersum* L.	Improved cellular membrane stability and enhanced *in vitro* tuberization

Heat shock proteins: It is important to maintain protein in their functional conformations and preventing aggregation of non-native proteins under stress. Many stress responsive proteins, particularly Heat Shock Proteins (HSPs) have been shown to act as molecular chaperones, which are responsible for protein synthesis, targeting, maturation and degradation in a broad array of normal cellular process. Furthermore, molecular chaperones function in the stabilization of proteins and membranes and in assisting protein folding under stress conditions.

Ahn and Zimmerman (2006) fused DcHSP17.7 gene (a carrot heat shock protein gene encoding HSP17.1) to a 6XHistidine (His) tag to distinguish the engineered protein from endogenous potato proteins and it was introduced into the potato cultivar Desiree under the control of the cauliflower mosaic virus (CaMV) 35S promoter. The integration was confirmed by Western Blot, which showed constitutive integration of DcHSP17.7 in transgenic potato lines before heat stress. They observed improved cellular membrane stability at high temperature, compared with wild type and vector controlled plants. Transgenic potato lines also exhibited enhanced tuberization in vitro.

Cold tolerance

Classical plant breeding has limited success in imparting cold hardiness to crop plants. Biotechnology with its powerful tolls however may provide answer through the isolation of cold fighting genes and thus may help in the development of crop plants that can withstand freezing temperature. Some of the heat tolerant genes/transgenic plants have been listed in Table 5.

Several comprehensive genomic studies based on DNA micro-array performed in the field of plant cold acclimation, identified large number of cold responsive genes (Seki et al., 2001; Fowler and Thomashow, 2002; Kreps et al., 2002; Seki et al., 2002), such as COR, lipid transfer protein and β-amylase.

Table 5: Cold tolerance gens/transgenic plants

Genes	Gene action	Species	Phenotypic Expression
Sod	Cu/Zn-SOD	Tobacco	Retained 90% photosynthesis under chilling and heat stress
Socl	Mn-SOD	Alfalfa	Increased tolerance to freezing stress
Nt107	Glutathion Stransferase	Tobacco	Sustained growth under cold and salinity stress
Wx	Controls amylase synthesis	Rice	Increased amylose content at low temperature
CBF1	Transcription factor	*Arabidopsis*	Increased cold tolerance
Cod A	Choline oxised (Glycine betaine synthesis)	*Arabidopsis*	Seedling tolerant of salinity stress and increased germination under cold
COR15a	Cold induced gene	*Arabidopsis*	Increased freezing tolerance
DREB	Transcription factor	*Arabidopsis*	Increased tolerance to cold, drought and salinity
AB13	Transcription factor	*Arabidopsis*	Increased freezing tolerance
Gs2	Chloroplastic glutamine synthetase	Rice	Increased salinity resistance and chilling tolerance
OsCDPK7	Transcription factor	Rice	Increased tolerance to cold, salinity and drought

A large number of these genes encode proteins with known enzyme activities, that potentioally contribute to freezing tolerance. Studies revealed that the cold acclimation is controlled by many genes and that cell membranes are particularly vulnerable to cold damage. Lee and Lee (2003) also identified many cold inducible alcohol dehydrogenase, β-amylase and many novel genes.

Dehydration responsive element: Many of the known cold regulated genes were under control of a primary master regulator, CBF/DREB1. Dehydration Response Element (DRE) plays an important role in the response to low temperature. The transcription factor DREB1A specifically interacts with DRE and induces the expression of stress tolerance genes in plant.

Over expression of DREB1A in transgenic Ar*a*bidopsis plants activated the expression of many of these stress tolerance genes and resulted in tolerance to freezing (Liu et al., 1998; Kasuga et al., 1999; Seki et al., 2001; Urao et al., 2000). Liu et al. (1998) and Jaglo-Ottosen et al. (1998) activated gene expression by overexpressing a homolog of CBF1, designated DREB1A. Their result indicated that the expression of the CRT/DRE regulation also increase drought tolerance. Liu et al. (1998) observed that the overexpression of DREB1A in transgenic Arabidosis resulted in dwarf phenotype. Fan et al. (2002) cloned an antifreeze gene from carrot and successfully transferred it into tobacco.

Cold regulated LEA protein: It was found that the COR15am the mature COR15a polypeptide, acts directly as cryoprotective protein by inhibiting the formation of hexagonal II phase lipid, a major type of freeze-induced membrane lession in non-acclimated plants (Steponkus et al., 1998). The COR15a gene enhanced the freezing tolerance of chloroplasts in engineered plants by almost 2C, which was nearly one third of the increase seen due to cold acclimation. While, this might not appear as large increase, an improvement of freeze tolerance by 2°C could potentially benefit certain crop plants (Artus et al., 1996). COR85, a group-II LEA protein was shown to be involved in cryoprotection of freezing-sensitive enzymes (Kazuaka and Oeda, 1994).

Overexpression of atRZ-1a did not affect the expression of various cold-responsive genes such as COR6.6, COR15a, COR47, RD29A, RD29B and LTI29 (Kim and Hunseung, 2006). Proteome analyses revealed that overexpression of atRZ-1a modulated the expression of several stress-responsive genes and the transcript levels and RNA stability of these target genes were not affected by atRZ-1a. atRZ-1a successfully complements the cold sensitivity of Escherichia coli lacking four cold shock proteins. These results strongly suggest that atRZ-1a plays a role as an RNA chaperone during the cold adaptation process.

Osmotin: Sarad et al. (2004) developed transgenic tomato with osmotin gene. Their preliminary tests revealed that the transgenic plants are more tolerant to cold than wild types.

Dehydrin: To elucidate the contribution of dehydrins (DHNs) to freezing stress tolerance, Puhakainen et al. (2004) developed transgenic Arabidopsis plants over-expressing multiple DHN genes. The transgenic plants exhibited lower LT50 values and improved survival when express to freezing stress compared to control plants. Thus they concluded that dehydrines contribute to freezing stress tolerance in plants and this could be partially due to dehydrins protective effect on membrane.

Compatible solutes: Parvanova et al. (2004) transformed tobacco to accumulate different compatible solutes (proline, fructans or glycine-betaine) in order to improve its tolerance to low temperature. The elevated parameters of transgenic plants successfully survived againsed freezing stress. Mukhoopadhyay et al. (2004) developed transgenic with OSISAP1 gene confering tolerance to cold, dehydration and salt stress. The gene OSISAP1 was isolated from rice encoding a zinc-finger protein induced under abiotic stresses. Hur et al. (2004) also developed transgenic rice with OsP5CS2 gene. This gene encodes for a protein that is highly homologous to δ-1-proline-5-caboxylate synthetase (P5CS), a proline biosynthesis enzyme. Their result indicated that the OsP5CS2 gene is necessary for plant tolerance to salt and cold tolerance.

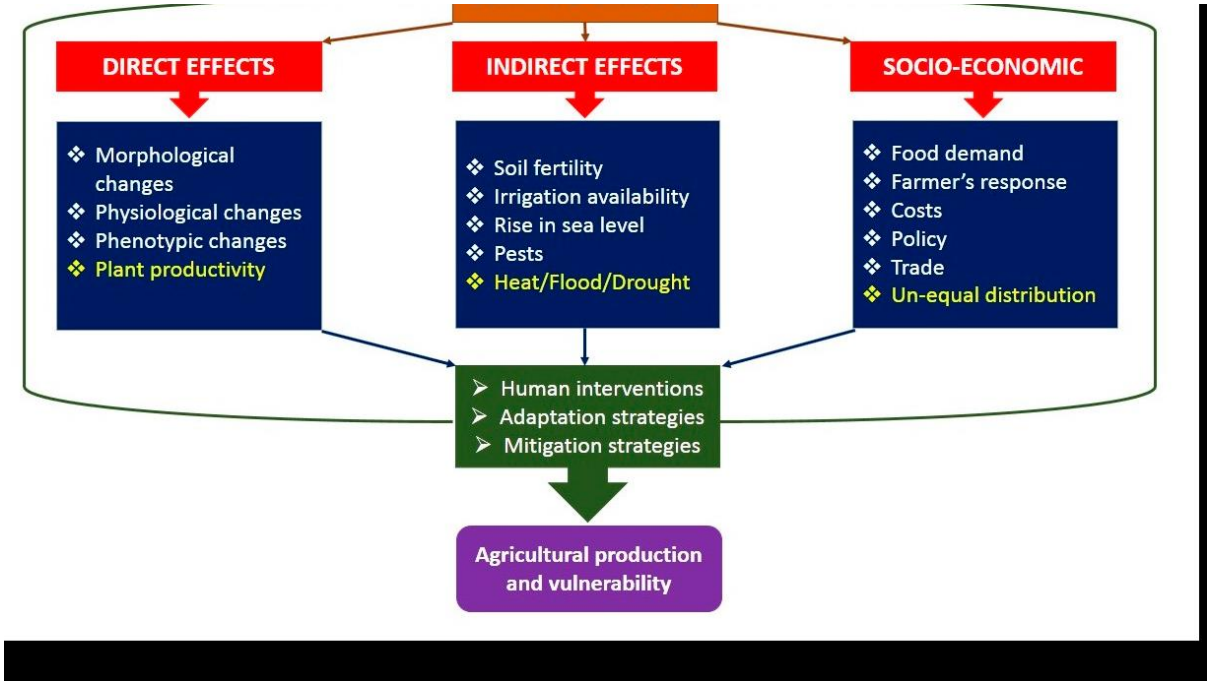
Potato plants (*Solanum tuberosum* cv. Desiree) transformed with yeast invertase gene acquired a higher tolerance to cold temperature as compared to the control plants, apparently due to the changes in sugar ratio produced by the foreign invertase. The expression of Osmyb4 in Arabidopsis thaliana plants showed a significant increase cold and freezing tolerance, measured as membrane or photosystem-II stability and as a whole plant tolerance (Vannini et al., 2004). They demonstrated by transient expression of Myb4 transactivater the PAL2, ScD9, SAD and COR15a cold inducible promoters.

Heat shock factors: Various studies showed that plant heat shock proteins are not only express in response to heat shock, but also under water, salt, oxidative stress and at low temperature. Li et al. (2003) suggested that Hsf (heat shock factor) gene may play a pivotal role in heat-shock-induced chilling tolerance and constitutive expression of the transcriopton regulated gene in chilling sensitive crops may be useful in improving tolerance against chilling stress. They transferred Arabidopsis thaliana Hsf1b (AtHsf1a) gene into tomato. The transgenic tomato plants harbouring this gene showed increased chilling tolerance.

10. Crop designing for stress tolerance and climate resilient plants.

Introduction:

Plant physiology has been greatly influenced by climate variability by several means. Environmental extremes and climate variability enhanced the chances of numerous stresses on plants. Climate change affects crop production by means of direct, indirect, and socio-economic effects as described in Figure 1. Furthermore, climate change (drought, flood, high temperature, storm etc.) events are increased dramatically as reported by Food and Agriculture Organization (FAO).



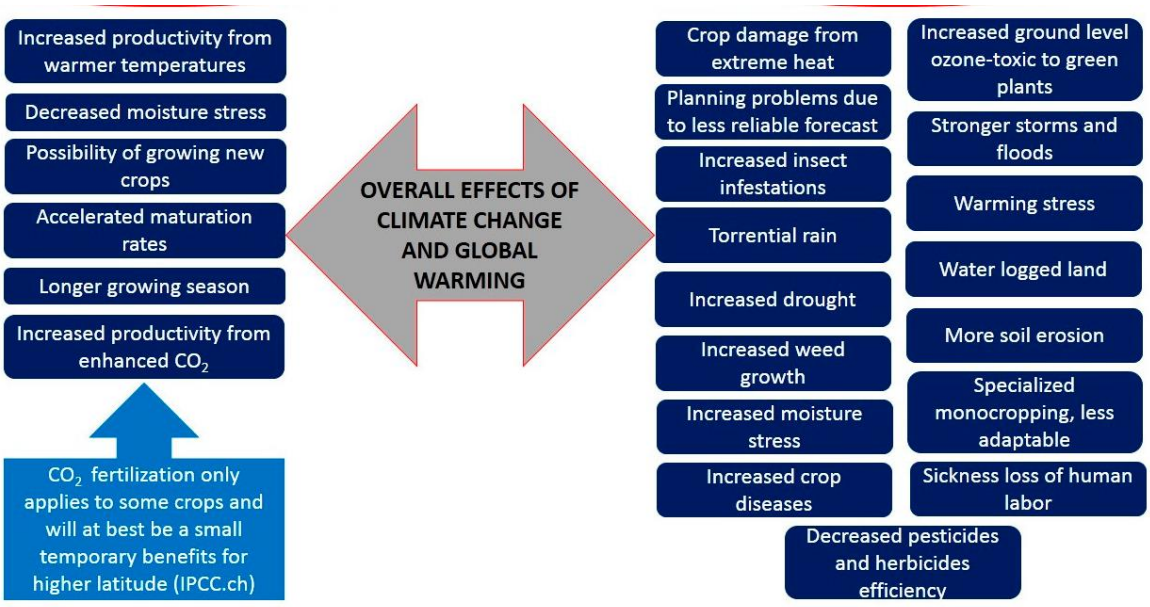
Boyer reported that the climate changes have reduced the crop yield up to 70% since 1982. According to the study of FAO 2007, all cultivated areas in the world are affected by climatic changes and only 3.5% of areas are safe from environmental limitations. Whereas the outcomes of abiotic stresses on crop yield are hard to calculate accurately, it is believed that abiotic stresses have a substantial influence on crop production depending upon the extent of damage to the total area under cultivation. In future, the productivity of the major crops is estimated to drop in many countries of the world due to global warming, water shortage, and other environmental impacts.

Due to climate change, water deficit and temperature extremes influence the reproductive phase of plant growth. It was described that the flower initiation and inflorescence is badly affected by the water stress in cereals. Similarly, if the temperature increase of about 30ºC during floret development it can cause sterility in cereals. During the meiotic phase, wheat and rice suffered from the 35–75% reduction in grain set due to water deficit. In rice, drought stress greatly disturbs the process of fertilization and anthesis. Due to water deficit, the harvest index is reduced to 60% and decreases the

grain set. The cocoa yield has been significantly reduced by the major drought spells in West Africa during the 1980s El Niño years. It has been estimated that agricultural production could reduce to 25.7% by 2080 due to climate change and maize will be the most affected crop in Mexico.
A study based on ECHAM6 climate data was analyzed for North German Plains during two different time durations: 1981–2010 and 2041–2070. The results showed that if the yield for winter wheat is to be sustained, water availability must be guaranteed. Zhao et al (2017) carried an experiment to analyze the climate change impact on major crop yields and showed considerable yield reductions of 6%, 3.2%, 3.1%, and 7.4% in wheat, rice, soybean, and maize respectively. To tackle the climate change new discoveries in genomics are enabling climate-smart agriculture by developing climate resilient crops.

Crop Adaptation to Overall Extreme Climate Stresses

With the increase of the Earth's temperature, the climate undergoes severe alterations and becomes abiotically stressful. Environmental changes are very damaging and pose various threats to naturally prevailing crop species. Under field circumstances, drought and heat are the most predominant stresses and have a significant influence on plants. It is reported that plants require an optimum temperature for their normal growth and blooming. Plant physiology is heavily influenced by temperature fluctuations. As heat stress affects the grain production and yield, cold stress results in sterility, and drought stress negatively influences the morpho-physiology of plants. These climatic problems severely distress plant development and yield, produce enormous responses, comprising molecular, biochemical, physiological, and morphological modifications. Overall, global warming and climate change both have some negative and positive effects on agricultural crops as well as on humans as explained in Figure.



In this context, understanding the stress-resistance processes in plants has emerged as a very difficult task for plant scientists in order to develop stress-resistant plants. The chief cereal crops around the world, such as maize, rice, and wheat, are crucial to meet the daily food demand. Out of them, wheat was the leading staple crop which has been cultivated on a large scale. Wheat is harvested on 38.8% of total agricultural land worldwide and provides a considerably high concentration of proteins: 15% per gram as compared to maize or rice which only supplies 2 to 3% . Regardless of large growing land globally, its productivity has been predominantly less than the maize and rice . Reasonable reduction was anticipated in wheat productivity with a 2ºC increase in temperature. Related research on environmental variability expected a 6% reduction in wheat yield. Challinor and his colleagues described that due to the increase in temperature, the grain filling phase decrease is the major reason of crop productivity reduction in changing climatic conditions. Therefore, sustaining crop yield is an important task in current agriculture, and to produce stress-tolerant crop plants

Approaches to Combat environmental stresses:

Variation in the environment has a long-lasting influence on agriculture and food security globally. Food security and safety are threatened by the severe weather conditions and it is not a recent problem. But formerly, no consideration was adopted to tackle this problem. Therefore, to cope with these weather variations is the most urgent demand worldwide. For crops to adapt to changing environmental stresses subsequent approaches are required.

Cultural Methodologies

Recently some experiments reported investigations of the strategies trialled by farmers to tackle the climatic variation for plant adaptation. There are many useful approaches adopted by farmers, including abiotic factors such as altering planting and harvesting time, a collection of crops with short life cycles, crop rotation, irrigation techniques, and variation in cropping schemes. Under climatic stress conditions, all of these approaches are very beneficial for crop adaptability. Modification in sowing time, application of drought resistant cultivars, and the cultivation of new crops are some important strategies to lessen the climatic variability danger and provide better adaptability to crop plants for assuring food safety and security. Another plant adaptability approach is by means of crop-management techniques that have the ability to enhance crop development under various environmental stresses. The choice of sowing time, planting density, and optimum irrigation practices are crucial techniques to tackle weather stresses. Fertilizers are also very vital to reduce the effect of global warming and support the plant for better adaptability. It provides substantial energy to plants and is beneficial to maintain the fertility of the soil and increase productivity. Hence, the importance of fertilizer in nourishing the world is undeniable.

Conventional Techniques

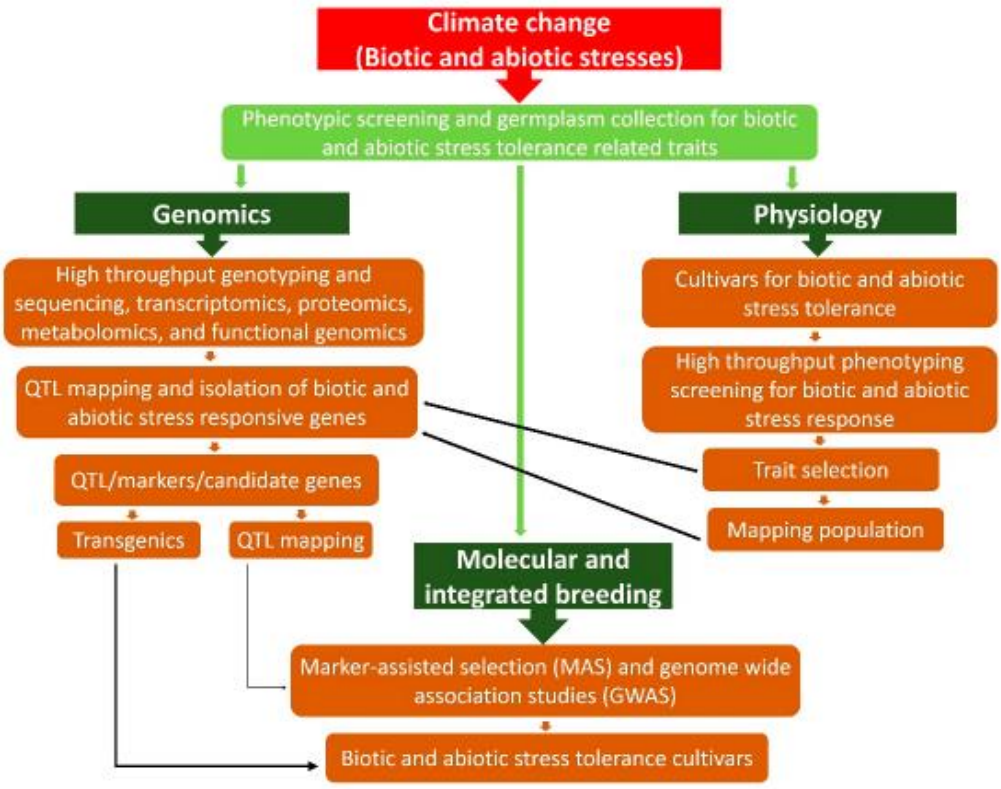
Under various environmental stresses, plant breeding shows dynamic techniques in crop development and betterment. It gives a way to potentially guarantee food security and safety under harsh weather variations and help plants escape from various stresses through a crucial phase of plant growth by developing stress resistant cultivars. Genetic divergence analysis is used for polymorphism, inbreeding, assessment, assortment, and recombination to attain plant perfection, and is amongst the main aspects for defining accomplished inbreeding. Genetic divergence analysis is considered a very important method for development of new cultivars based on genetic distance and similarities. For genetic studies landraces are a significant source, for example, a wheat landrace kept in data bank comprises broader genetic variance and is a valuable basis for stress resistance as it contains cultivars adjustable to diverse environmental stress. Figure 6 demonstrates how molecular and integrated plant breeding are useful to develop the biotic and abiotic stress tolerance cultivars using genomics approaches like marker-assisted selection (MAS) and genome wide associated studies (GWAS).

Genetics and Genomics Strategies

Omics-Led Breeding and Marker-Assisted Selection (MAS)

Omics approaches provide beneficial resources to elucidate biological functions of any genetic information for crop upgrading and development. Different molecular markers are studied in population genomics across the environment in many individuals to find out novel variation patterns and help to find if the genes have functions in significant ecological traits. In many crops, the breeding program is coupled with genomic approaches to achieve great heights in molecular breeding and to screen elite germplasms with multi-trait assembly For the identification of phenotypes under different environmental variation associations, genetics and transcriptomic analysis are used Genomics also enables investigation of the molecular mechanisms underlying the abiotic stress resistance. These approaches aid in the development of climate smart crops for better yield and production under different climate changes. With the advent of high throughput sequencing and phenotyping, genomic-led breeding paved the way for identifying different stresses that are expected to adversely affect crop yield. Furthermore, the data available on environmental extremes, DNA fingerprinting, and quantitative trait loci (QTL) mapping allows the screening of elite germplasm under abiotic stresses. QTL dissection of yield-related traits in crops under stress conditions permits the development of novel cultivars with better adaptability in abiotic stress. Molecular plant breeding is an essential approach to enhancing crop yield and production in the presence of various biotic and abiotic stresses. For speedy breeding progression marker-assisted selection (MAS) presents a crucial

part in the betterment of crop traits and yield. With the advancement in crop genomics, DNA markers have been identified which are valuable for marker-assisted breeding.
The introduction of novel sequencing tools greatly eased the difficulty in researching genomic variants and lead towards the identification of huge amounts of DNA polymorphism, particularly single nucleotide polymorphism (SNPs) markers Precision of QTL mapping enhanced on average from 10–30 centimogran to <1 cM with the advancement of linkage maps.
The high-throughput phenomics approach is also contributing to increasing the accuracy of QTL mapping. The association among genotypic and phenotypic data is crucial for enlightening the genetic basis of multiple traits. By applying QTL mapping Haley and his colleagues successfully developed a wheat variety called "Ripper" which has the ability to withstand the drought conditions of Colorado, without affecting its grain yield and quality. In 2009 Badu-Aparku and Yallou performed QTL mapping to screen elite maize germplasm with high yield under drought stress. Merchuk-Ovant et al. (2016) conducted marker assisted selection studies on bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum turgidum* L.) to identify the QTLs related to drought stress. Barley is cultivated on a wide range of land across the world but it is severely affected by drought stresses globally. QTL mapping of two novel barley cultivars that have been totally different in their response to drought stresses were selected and QTL mapping study performed for malting characters in double haploid. QTL investigation showed that MSA are specific reliable genomic sections regulating the malting feature which can be helpful. Similarly, a recent QTL study was carried out to explore the epistatic mechanism and physiology of QTL for the elucidation of the targeted gene. Under drought stresses, 3 QTL were recognized such as qDTY6.2, qDTY6.1, and qDTY3.1, which have a considerable impact on grain productivity. To be resistant under hyper temperature conditions three vital points on the genome of bread wheat have been predicted: 7D, 7B, and 2B. Tahmasebi et al. (2016) performed QTL mapping for a recombinant inbred lines (RILs) population of wheat under different stress conditions of flooding, drought, heat, and a combination of both heat and drought simultaneously.



GenomeWide Association Studies (GWAS) for Stress Tolerance
Genome wide association studies (GWAS) is a powerful tool for understanding the complete set of genetic variants in different crop cultivars to recognize allelic variant linked with any specific trait.

GWAS generally highlight linkage among SNPs and traits and based on GWAS design, genotyping tools, statistical models for examination, and results interpretation. In many crops GWAS has been carried out to exploit the genetic process responsible for genetic resistance under climate change. In plants, GWAS has widespread applications related to biotic and abiotic stresses. GWAS have been applied to describe drought tolerance, salt tolerance, and heat tolerance.

In *Arabidopsis thaliana* GWAS study was carried out by Verslues et al. (2013) aided by reverse genetic approaches to elucidate unique genes that accumulate proline under drought stress. The linkage among SNPs of both genotypic and phenotypic data were examined, and specific regions regulating proline accumulation were recognized. Similarly, different proteins controlling the pro-accumulation such as aMADS box protein, Universal Stress Protein A domain proteins, protein phosphatase 2A subunit A3, thioredoxins, ribosomal protein RPL24A, and mitochondrial protease LON1 were identified by using reverse genetics. This research gave insights for proline accumulation under drought stress conditions. *Aegilops tauschii* is reported to have many resistance genes regulating the abiotic stresses. A significant knowledge is required for the breeders to understand the genetic architecture of Aegilops tauschii to improve drought resilience. Qin et al. (2016) investigated different varieties of A. tauschii to examine 13 traits controlling drought stress. For GWAS 7185 SNPs were designated to study the phenotypic behavior and carried out mixed linear model and general linear model to find the association between SNPs with phenotypic traits.

QTLs related to salinity resistance in plants were studied by using GWAS. Kumar et al. (2015) reported various genes regulating the salinity tolerance in rice by using infinium high-throughput SNPs arrays. Six thousand genotype-based SNP were constructed for genes related to stress and linkage among SNPs and phenotypic data were interpreted. QTLs for salt tolerance by genomic regions were mapped on chromosome numbers 1, 4, 6, and 7. A novel QTL present on chromosome number 1 was reported and was called “Saltol” which is associated with salt tolerance at seedling stage. Chopra et al. (2017) reported various stress-tolerant genes in *Sorghum bicolor* associated with heat and cold stresses. GWAS was conducted for genotyping and phenotyping analysis. Thirty SNPs were identified for genes related to anthocyanin expression and carbohydrate metabolism, which are powerfully associated with cold stress at the seedling growth phase of sorghum. Similarly, 12 SNPs were discovered for heat stress at the seedling stage and controlled by the genes having functions in ion transport mechanism and sugar metabolism.

Genome Selection (GS) for Crop Improvement

Genomic selection (GS) is the exciting tool to revolutionize the crop improvement by using high-throughput phenotyping and marker densities to screen the elite germplasm, improving the polygenic traits and economical breeding line development. Currently, the prospective of genomic selection (GS) to fast-track the speed of genetic achievements in main crops has stimulated the development of multi-environment designs for genomic estimation. Burgueño et al. (2012) proposed the first statistical design by applying a linear mixed model to G E model. Jarquín et al. (2014) suggested a system of modeling connections among an elevated dimensional combination of markers and environment that integrates with each other (G E). Another model (GBLUP-type model) was proposed by Lopez-Cruz et al. (2015) in which regression of phenotypes was used for the interaction of marker environment (M E). The modern multi-environment model for genomic prediction was proposed by Cuevas et al. (2017) based on Bayesian model. These methods are applied on 4 wheat and 1 maize cultivars and CIMMYT data bank revealed that the G E model have high significance rates and better genomic predictions as compared to other models.

Around 40 research studies based on GS have been published so far. Wheat is the most studied crop with 29 genomic selection studies. Moreover barley, oat, and durum wheat have 5, 2, and 1 research paper published. Diversity Array Technology (DArT) was the most promising maker used in GS followed by single nucleotide polymorphism (SNP) and genotyping by sequencing (GBS). These experiments showed that GS could be magnificently used in cereal breeding. Genomic Selection (GS) designs were extensively developed for wheat to reveal the germplasms that have better ability to adapt in climate changes. Crain et al. (2018) studied the different GS techniques to detect phenotypic data from high throughput phenotyping. At CIMMYT, heat and drought stresses were examined in 1000 elite wheat cultivars by using a high throughput phenomics approach.

Genetic Engineered Plants for Stress Tolerance

The genetic modification through biotechnology is a powerful strategy. Encouraging data is collected from genetics which can be exploited significantly to various biotic and abiotic stresses such as salinity, drought, heat, and cold. Identification of stress-responsive TFs are powerful findings to develop stress-resistant crop cultivars. These TFs can control the phenotypes of genes in genetic engineered crops associated with various stresses. There are numerous transgenic plants which have been established by genetic engineering to tackle the biotic and abiotic stresses. These genetically engineered plants demonstrate significant resistance against climatic variations compared to normal plants.

Various transcriptions factor (TFs) are recognized as plant-specific TFs which includes AP2/ERFBP group. This family of AP2/ERFBP TFs is responsible for many plant growth pathways and has functions in biotic and abiotic stress responses. AP2/EREBP TFs are categorized into 4 sub-groups based on their similarity and numbers. The subfamilies consist of ERF TFs, DREB (dehydration-responsive element-binding protein), AP2 (Apetala 2), TFs, and RAV (related to ABI3/VP1). DREB and ERF are two major subfamilies which have been widely examined due to their role in plant biotic and abiotic responses. The DREB TFs have significant regulating ability in various water deficit and cold stress conditions. DREB TFs have been investigated in response to stresses in various plants species such as wheat, barley, maize, soybean, rice, tomato, and *Arabidopsis*. In numerous experiments DREB1 has been studied in rice and *Arabidopsis* for its controlling mechanism in cold stress while DREB2 functions in drought, salinity, and high temperature stresses.

Chen et al. (2007) revealed that the transgenic plants with over-expression of GmDREB2 gene extracted from soybean showed significant tolerance against salt and drought resistance. Mallikarjuna et al. (2011) successfully developed transgenic rice with the improved resistance against salinity and drought stresses by over-expressing the OsDREB2A gene.

The larger subfamily of AP2/EREBP TFs are ERF and are responsible to regulate stress-tolerance genes in plants. Under abiotic stresses these ERF genes are induced to hyper-express results in the better tolerance against stresses in transgenic plants. Additionally, some ERF TFs are also involved both in biotic and abiotic stress tolerance due to their ability to regulate numerous hormonal biosynthesis pathways. Further study was carried out to develop transgenic tobacco by an over-expressed GmERF3 gene. This transgenic tobacco has increased resistance against TMV, dehydration, and also toleration of salinity stress.

MYB TFs family called the myeloblastosis oncogene is a huge group of TFs discovered in eukaryotes and is extensively distributed in plants. Various MYB TFs have been identified to regulate numerous biochemical and physiological pathways such as the cell cycle, hormonal biosynthesis, and primary and secondary metabolism. These TFs are also known to have functions in biotic and abiotic stress responses. Li et al. (2015) have summarized various MYB TFs related to abiotic stress tolerance in plants and *Arabidopsis*. Some of the MYB TFs such as AtMYB61, AtMYB60, and AtMYB44 were identified to enhance the drought resistance in transgenic *Arabidopsis* by controlling the movement of stomata. Yang et al. (2012) developed transgenic rice by over-expressing the OsMYB2 gene to improve the resistance of rice against chilling, salinity, and dehydration. The GmMYB76 gene isolated from soybean was successfully transformed into *Arabidopsis* for salinity and freeze resistance. MdMYB121 from apple was significantly transformed into apple and tomato to develop transgenic plants with enhanced drought and salt tolerance. Similarly, the MdSIMYB1 gene from apple was used to developed transgenic tobacco and apple with improved resistance against cold, drought, and salt stresses. The TaPIMP1 gene was expressed to develop transgenic wheat and it showed remarkable tolerance against drought and fungal pathogen *Bipolaris sorokiniana*.

Hyper-expression of TaPIMP1 was confirmed by microarray analysis. In another experiment TaPIMP1 TF was investigated for its regulating ability to tackle biotic and abiotic stresses in transgenic tobacco. Transgenic tobacco showed resistance against *Ralstonia solanacearum*, salinity, and drought stresses.

Another important family of TFs are WRKY which are extensively distributed in relation to abiotic and biotic stresses in plants. In transgenic plants WRKY genes were over-expressed to increase the abiotic stress tolerance such as in transgenic rice OsWRKY11 gene was introduced to enhance its tolerance to heat and drought stresses. ZmWRKY33 genes in maize have been induced for salinity,

drought, freeze, and ABA stresses. Under salinity stress conditions transgenic Arabidopsis with over-expression of ZmWRKY33 genes showed significant tolerance.

NAC TFs have significant importance in many processes, such as flower growth, cell division, and stress-responsive regulation in the plant due to biotic and abiotic stresses. Numerous NAC TFs have been discovered in a wide range of plants with sequenced genomes such as in rice with 151, in Arabidopsis, in maize, and in soybean NAC TFs have been identified. Additionally, a large number of NAC TFs have been reported to have direct association in abiotic stresses, such as in transgenic Arabidopsis 31 NAC genes, which have been identified for salinity tolerance, and in rice 40 NAC genes were identified against drought tolerance.

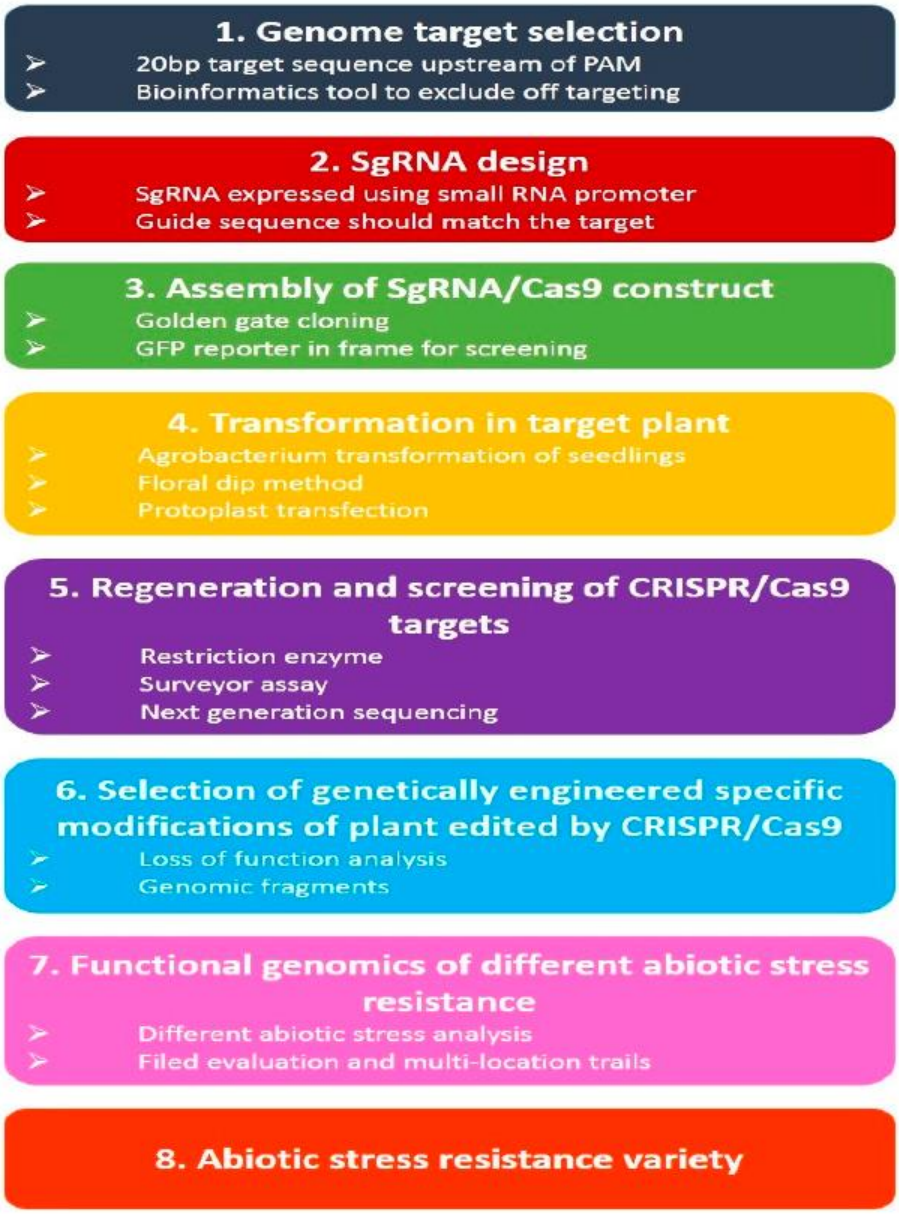
Genome Editing Strategies

Genome editing (GE) is the most powerful strategy to manipulate the plant genome by means of sequence-specific nucleases. GE for crop improvement has the remarkable ability to tackle food insecurity and develop a climate-smart agriculture system globally. In traditional plant breeding approaches, genes are discovered to be associated with various important traits by means of mutation and conventional breeding strategies, which has recognized as a significant technique for the development of elite and high yielding germplasm. Genetic diversity of various elite varieties has been substantially decreased due to the exploitation of important crops extensively that has, in various circumstances, been associated with the enhancement of the susceptibility to several abiotic and biotic stresses. Developing the novel modifications in the gene pool of various plant germplasm is required under abiotic and biotic stresses for the improvement of elite crop varieties with great ability to produce high yielding crops. In genome editing technology site specific endonucleases are used comprising of zinc-finger nucleases (ZFNs), transcription activator like effector nucleases (TALENs), and CRISPR-Cas9. In contrast to the ZENs and TALENs genome editing tools, the CRISPR/Cas9 system is emerging as the most powerful GE strategy because it is economical, rapid, accurate, and enables multiple site specific editing within the genome.

CRISPR/Cas9 System for Crop Advancement

CRISPR/Cas9 is a modern genome editing strategy based on the prokaryotic defense mechanism triggered by type II RNA organization that offers protection to prokaryotes against attacking viruses. Genome editing has been modernized by CRISPR-Cas9 assembly, by producing candidate gene mutants and knock down single nucleotides in a genome. As compared to other genome editing tools like TALENs/ZFNs, CRISPR-based strategies have been tremendously exploited in plant genomes. Moreover, it has great potential to aid crop breeding to establish high yielding and stress-resistant varieties. Most significantly, the CRISPR/Cas9 tool is converting into a comprehensible environmentally friendly technique for the establishment of genome edited non-transgenic plants to tackle environmental extremes and guarantee food security. A model of CRISPR/Cas9 based genome engineering to develop transgenic plants or abiotic stress tolerance cultivars is explained in Figure 7.

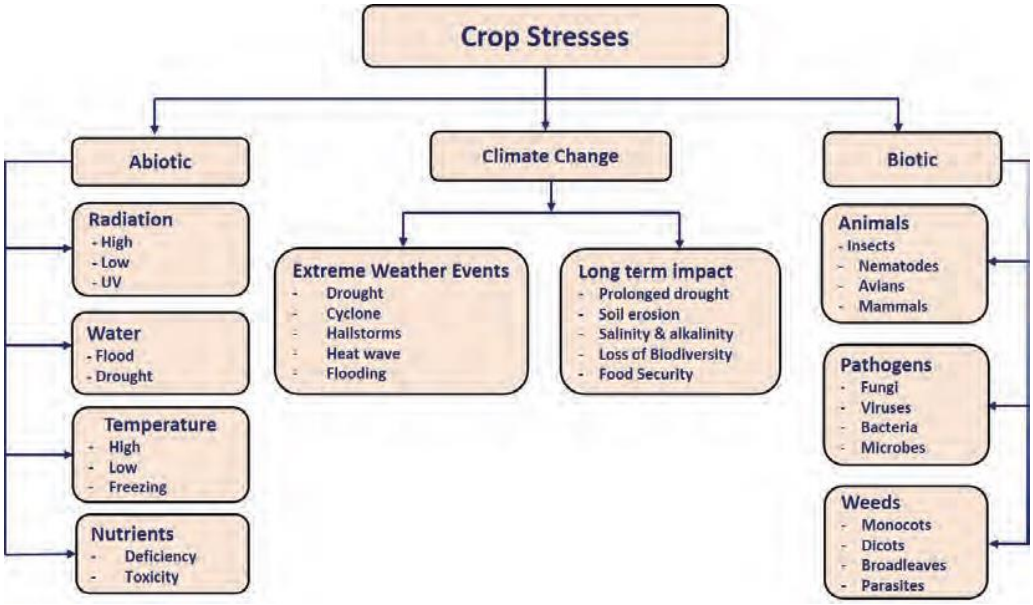
CRISPR/Cas9 has been extensively carried out for plant genome editing to cope with abiotic and biotic stresses. A study was conducted to disrupt the gene TaERF3 and TaDREB2 to produce abiotic stress resistance by using the CRISPR genome editing tool. Similarly, 21 KUP genes have been identified in cassava which were hyper-expressed under abiotic stresses. Differential expression analysis of KUP genes revealed that they induced drought resistance. For drought tolerance studies MAPKKK genes have been investigated by means of genome-wide analysis. CRISPR/Cas9 was applied in rice for producing triplet mutants. Genes TGW6, GW5, and GW2 have a function in regulating the seed size. By mutating this gene, the size of the seed was increased by 30%. CRISPR technology has been adopted for a mutation in *Brassica napus* by knocking down the gene CLVTA3 which resulted in more seed production.



A model of CRISPR/Cas9 based genome engineering to develop transgenic plants or abiotic stress tolerance cultivars

Climate resilient crop varieties for different abiotic stresses

The development and identification of climate resilient crop varieties, with enhanced tolerance to heat, drought, flooding, chilling and salinity stresses are essential in order to sustain and improve crop yields to cope with the challenges of climate change. It is essential to bridge the yield gaps, enhance the productivity and profitability, minimize risk and improve the livelihoods of millions of people dependent on agriculture. While, abiotic stresses such drought, heat or cold may trigger a series of responses in plants that include changes in gene expression, signal transduction pathways, metabolic and molecular mechanisms as well as cumulative manifestations of these in terms of source and sink relations for adaptation. The major biotic and abiotic stresses affecting crops that limit crop productivity is given in the following figure. Among various abiotic stresses, drought, heat, salinity, cold and flooding are the major factors that adversely affect plant growth and productivity (Maheswari et al., 2012).



All these adverse environmental conditions have potential to drastically reduce yields in warmer regions. To develop stress tolerant cultivars, it is essential to identify the traits that maintain and promote the growth and development of

Crop vareties suitable for cultivation under different abiotic stresses

Drought

There have been tremendous advances in understanding the physiology, biochemistry and molecular genetics of plant response to different abiotic stresses. Number of adaptive traits have been studied and used for improvement of drought tolerance like early vigour, osmotic adjustment, leaf senescence, stay green etc. Stay-green trait in plants, usually refer to tolerance against drought-induced postflowering senescence. Roots also play an important role in the adaptation of several crops to drought stress. Various ICAR institutes and state agricultural universities are making concerted efforts to develop high yielding cultivars of different crops with enhanced tolerance to delayed monsoon and drought over the years which can be utilized by the farming communities. Major food, vegetable and horticultural crop varieties with tolerance to drought stress and delayed monsoon are given in the following tables.

Table 1: Crop varieties suitable for cultivation under drought stress

Sl. No.	Varieties	Zone	Sub-Zone	State	Source of seed availability
			Cereals		
Rice					
1	Ashoka-200F	ACZ-IV B	ARS Banswara	Rajasthan	RSSC, Rajasthan
2	Gujarat Nagli-4	ACZ	Very Heavy Rainfall Zone	Gujarat	DRNAU, Gujarat
3	Kalinga, Sahabhagi, IR-36	ACZ-II	Red and Laterite Zone	West Bengal	BCKV, Kalyani
4	Kalinga-2, Kalyani-3, Narendar dhan 97	ACZ-I, ACZ-III	New Alluvial & Coastal Saline Zone	West Bengal	BCKV, Kalyani
5	Pant Dhan 16, Barani, Dhan-1, Aditya, Kalinga-3	ACZ-III & II	NEPZ	Uttarakhand, Chhattisgarh	GBPUA&T, Pantnagar; IGKV, Jabalpur
Wheat					
9	HD2888, K8027	ACZ-I, II, III	North Eastern Plain Zone	Bihar, Eastern UP, West Bengal	BAU, Sabour
10	Sujata, C-306, JWS-17, HI-8627, HI-1531	ACZ-II	Northern Hills	Chhattisgarh	IGKV, Jabalpur
11	HPW-155, HPW-236	ACZ-III	High Hills Temperate Wet Zone	Himachal Pradesh	CSK HPKVV, Palampur
12	VL421, HS277, VL Gehun 829, HPW249, VL907, HS420, HPW236	ACZ-I &II	Northern Hill Zone	Himachal Pradesh	CSK HPKV, Palampur
13	PBW 644, PBW 527, PBW 175	ACZ-I, II, III, IV, V	North Western Plain Zone	Punjab	PAU, Ludhana

Heat stress

The continuous exposure of plants to high temperatures or heat stress during crop growth cycle is a major impediment to agricultural production and cause an array of morpho-anatomical, physiological and biochemical changes in plants, which affect plant growth and development eventually reducing economic yield. Heat stress is often defined as the rise in temperature beyond a threshold level for a period of time sufficient to cause irreversible damage to plant growth and development. In general, a transient elevation in temperature, usually 10–15°C above ambient, is considered heat shock or heat stress. The adverse effects of heat stress can be mitigated by developing thermo-tolerant crop varieties through genetic improvement and when coupled with various adaptation and mitigation strategies can counter production losses. Heat tolerance is generally defined as the ability of the plant to grow and produce economic yield closest to its genetic potential under high temperatures. The heat-threshold level that the plant can withstand without adverse effects varies considerably at different developmental stages in different crops. For instance, during seed germination, high temperature may slow down or totally inhibit germination. High temperature, in general adversely affects photosynthesis, respiration, water relations and membrane stability production of ROS (Reactive Oxygen Species) and anti-oxidants, accumulation and adjustment of compatible solutes etc. In addition, plants intrinsically respond to high temperature stress by triggering a cascade of events and adapt by switching on numerous stress-responsive genes.

The genetic resources, especially land races and wild relatives from areas where past climates mimicked the projected future climates for agriculturally prime areas, could serve as the starting genotypes for breeding crops for heat tolerance. Some successful attempts have been made by conventional breeding methods in evolving plants with improved heat tolerance. However, the key to evolve heat tolerant crops lies in an integrated approach combining both traditional and molecular breeding techniques. Marker-assisted selection for heat tolerance as well as cloning and characterization of underlying genetic factors could be highly useful in this context. Major food, vegetable and horticultural crop varieties with tolerance to heat stress are given in Table 3.

Table 3: Crop varieties suitable for cultivation under heat stress

Sl. No.	Varieties	Zone	Sub-Zone	State	Source of seed availability
			Cereals		
Wheat					
1	Lok-1, Vidisha, GW-173, Arpa	ACZ-II	Northern Hills	Chhattisgarh	IGKV Jabalpur / Private sector
2	RSP 561	ACZ-II	Intermediate Hill Zone	Jammu & Kashmir	SKUA&T-Jammu
3	MP 4010, RVW 4106	ACZ-I	Vindhya Plateau Zone, Bundelkhand, Malwa Plateau Zone, Nimar Valley, Jhabua Hill Zone	Madhya Pradesh	RVSKVV, Gwalior
4	PBW 658, PBW 590	ACZ-I, II, III, IV, V	NWPZ	Punjab	PAU, Ludhiana
5	RAJ 3777, RAJ 3765, RaJ 3077	ACZ-I, IVA	North West Plain Zone-1B of Rajasthan	Rajasthan	NSC, RSSC, NSP, ARS, Durgapura
6	RAJ 3777, RAJ 3765, RAJ 3077, RAJ 4037, RAJ 4083	ACZ-I-B	North Western Plane	Rajasthan	NSC, RSSC, NSP
7	RAJ-3777, Raj-3765, MP-3288, HI-1500	ACZ-IV B	ARS Banswara	Rajasthan	RSSC, RSSC, MPSSC, MPSSC

Sl. No.	Varieties	Zone	Sub-Zone	State	Source of seed availability
9	Raj-4037, Raj-4083, RAJ 3765, RAJ 3077	ACZ-III-A	Semi-arid Eastern Plains, Jaipur	Rajasthan	RSSC, Rajasthan
	NIAW 34		Penninsular Zone	Karnataka	UAS, Dharwad
Maize					
10	Suwan	ACZ-I	North eastern plain zone	Bihar	BAU, Sabour / RAU, Pusa
11	Suwan	ACZ-II, III	North western plain zone, Southern alluvial zone	Bihar	BAU, Sabour / RAU, Pusa
12	PMH-7	ACZ-III	Central Plain Zone	Punjab	PAU, Ludhaiana
Pearl millet					
13	GHB-558, GHB-732 and GHB-538	ACZ-III, IV, V, VI, VII & VIII	Middle Gujarat, North Gujarat, South Shurash-tra, North Shurashtra	Gujarat	JAU, Jamnagar

Cold stress

Major food crops, maize (*Zea mays*) and rice (*Oryza sativa*) are very sensitive to low temperatures, the growth of these crops are severely affected in terms of their growth and development by temperatures below 10ºC resulting in considerable yield loss or even crop failure. When the temperature decreases to less than 5°C for more than three consecutive days it is considered as cold wave/stress in areas where normal temperature remains 10°C or above, while in areas where normal temperature is below 10°C, if temperature goes below 3°C for more than three days it is considered as cold wave (Venkateswarlu et al., 2011). Many plants, especially those, which are native to warm habitat, exhibit symptoms of injury when subjected to low non-freezing temperatures. These plants including maize, soybean, cotton, tomato and banana are in particular sensitive to temperatures below10–15°C. Various symptoms in response to cold/chilling stress include reduction of leaf expansion, wilting, chlorosis and necrosis. In chilling stress, primary injury is the initial rapid response that causes a dysfunction in the plant, but is readily reversible if the temperature is raised to non-chilling conditions (Kratsch and Wise 2000). Major food, vegetable and horticultural crop varieties with tolerance to cold stress released are given in the Table 4.

Table 4: Crop varieties suitable for cultivation under cold stress

Sl. No.	Varieties	Zone	Sub-Zone	State	Source of seed availability
Cereals					
Pearl millet					
1	GHB-538	ACZ-III, IV, V, VI, VII & VIII	Middle Gujarat, North Gujarat, South Shurashtra, North Shurashtra & Bhal costal region	Gujarat	JAU, Jamnagar
Rice					
2	Bhrigudhan, Varun Dhan	ACZ-III	High Hills Temperate Wet Zone	Himachal Pradesh	CSK HPKVV, Palampur
3	HPR2143, HPR1068, RP2421, Palam Basmati-1, Bhrigudhan, Varun Dhan	ACZ-II	Mid Hills Sub-Humid Zone	Himachal Pradesh	CSKHPKV, Palampur

Salinity stress

Salinity is a major environmental stress and is one of the chief constraints to crop production. Increased salinization of arable land is expected to have devastating effects on agricultural production in many countries including India. High salinity causes both hyper-ionic and hyper-osmotic stress and can lead to plant death.

Salinity in a given land area depends on the amount of evaporation in relation to the amount of precipitation leading to increase in salt concentration. Intrusion of sea water also is another major cause for increase in salinity. Sodicity is a secondary result of salinity in clay soils, where leaching washes soluble salts into the subsoil, while sodium is left bound to the negative charges of the clay (Wang et al., 2003). Agricultural lands that have been heavily irrigated are becoming highly saline, while in drier areas there is extensive water loss through a combination of both evaporation as well as transpiration. High salt concentration (Na+) deposited in the soil alter the basic texture of the soil resulting in decreased soil porosity, reduced soil aeration and water conductance. While, several crops are sensitive to salinity, rice can thrive relatively better on salt-affected soils as standing water helps in leaching salts from topsoil. A number of mapping studies have been attempted to identify QTLs located on different chromosomes for salinity tolerance in rice. A major QTL designated as 'SALTOL' was mapped on chromosome 1 which accounts for more than 40 per cent of the variation in salt uptake. Several lines containing SALTOL QTL were developed through marker assisted breeding. Major cereals varieties with tolerance to salinity stress are given in the Table 5.

Table 5: Crop varieties suitable for cultivation under salinity stress

Sl. No.	Varieties	Zone	Sub-Zone	State	Source of seed availability
			Cereals		
Rice					
1	Basmati CSR 30, CSR 36, CSR 27		Inland saline	Haryana	CSSRI, Karnal
2	CSR 23		Inland Saline	Uttar Pradesh	CSSRI, Karnal
3	CST 7-1	ACZ-III	Coastal Saline Zone	West Bengal	CSSRI Station, Canning Town
4	Jarva		Coastal Saline Zone	West Bengal, Kerala	DRR, Hyderabad
5	VTY-8		Coastal Saline Zone	Kerala	RRS(KAU), Vyttila
6	Panvel 3		Coastal Saline Zone	Maharashtra	Kharland Research

11. Let's sum up

- 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 (CO2 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.
- Abiotic stress is defined as the negative impact of non-living factors on the living organisms in a specific environment. The non-living variable must influence the environment beyond its normal range of variation to adversely affect the population performance or individual physiology of the organism in a significant way.

- 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.
- The accumulation of compatible solutes in response to osmotic stress requires the activation of the metabolic pathways that biosynthesize these solutes. Several genes coding for enzymes associated with osmotic adjustment are turned on (up-regulated) by osmotic stress and/or salinity, and cold stress. These genes encode enzymes such as the following Δ′1-Pyrroline-5-carboxylate synthase, Betaine aldehyde dehydrogenase and myo-Inositol 6-O-methyltransferase.
- In discussing the effects of salts in the soil, we distinguish between high concentrations of Na+, referred to as sodicity, and high concentrations of total salts, referred to as salinity. The two concepts are often related, but in some areas Ca2+, Mg2+ and SO42+, as well as NaCl, can contribute substantially to salinity.
- Halophytes are native to saline soils and complete their life cycles in that environment and Glycophytes (literally "sweet plants"), or nonhalophytes, are not able to resist salts to the same degree as halophytes.
- Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Oxidative stress from oxidative metabolism causes base damage, as well as strand breaks in DNA. Base damage is mostly indirect and caused by reactive oxygen species (ROS) generated, e.g. O^{2-} (superoxide radical), OH^{o} (hydroxyl radical) and H_2O_2 (hydrogen peroxide). Further, some reactive oxidative species act as cellular messengers in redox signaling. Thus, oxidative stress can cause disruptions in normal mechanisms of cellular signaling.
- The ROS defense mechanism consists of the antioxidant machinery which helps to mitigate the above mentioned oxidative stress-induced damages.The antioxidant machinery has two arms with the enzymatic components and non-enzymatic antioxidants.
- Transcriptomics is often considered as a step next to genomics in the study of biological systems. The transcriptome is the set of all mRNA molecules, or "transcripts", produced in one organism or cell type under a given set of conditions.
- Many transcription factors involved in stress responses have been identified. As an example, we describe here the transcriptional regulatory system for cold and drought (or osmotic) stresses. The DREB1/CBF family comprises APETALA2 (AP2) type transcription factors that recognize DRE/CRT and function in cold stress responses.
- Breeding objectives, however, may require more complex situations, such as the pyramiding of multiple QTLs having more subtle effects that are effective during different developmental stages, or the combining of QTLs for different abiotic stress tolerance into the same genetic background. Up to now, MABC has been successful in transferring traits whose expression is controlled by a single gene or by a gene that controls most of the phenotypic variance of the trait. However, the effective use of MABC in combining several genomic regions which control a single trait or a few independent traits required for a desired phenotype still awaits further development of more efficient technologies and innovative strategies.
- Most tissues of higher plants are unable to survive extended exposure to temperatures above 45°C. Nongrowing 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 thermotolerance.

- 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.
- 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.
- Transgenic techniques are being used to introduce biotic and abiotic stress related genes in crop plants. The major emphasis has been to introduce gene encoding compatible organic osmolytes, heat shock proteins, plant growth regulators, late embryogenesis abundant proteins and transcription factors responsible in activating gene expression.
- Variation in the environment has a long-lasting influence on agriculture and food security globally. Food security and safety are threatened by the severe weather conditions and it is not a recent problem. But formerly, no consideration was adopted to tackle this problem. Therefore, to cope with these weather variations is the most urgent demand worldwide. For crops to adapt to changing environmental stresses subsequent approaches are required.

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13. Assignment

1. Define drought-enduring species.
2. What do you mean by compatible solutes? Give an example.
3. Name to ion that induces salinity stress.
4. What is salt gland?
5. What are halophytes? Explain various mechanisms of salinity tolerance in halophytes.
6. Comment on the role of proline, polyols and stress induced proteins in the water stressed conditions
7. Write a short note on effects of drought in plant metabolisms.
8. Differentiate between saline and sodic soils.
9. What is ROS generation? Add a note on its effect on plants and scavenging.
10. What are the full form of PUFA and LPO?
11. How Antioxidation strategies helps to overcome oxidative stress.
12. Why is osmotic stress related to salinity stress?
13. Name two salt stress tolerant genes. Mention its expression.
14. How transcriptome analysis help in stress mediated responses to plant?
15. What is SRAP?
16. Explain structure and function of HSPs in plant.
17. What is GABA?
18. What is chilling injury? How do you overcome it?
19. Explain crop designing for climate resilient plants.
20. What is hydroactive closure?
21. Discuss the roles of Ca^{2+}, reactive oxygen species (ROS), and the MAP Kinase cascade during signaling in response to abiotic stress.
22. Explain the application of MAS for inducing abiotic stress tolerant plants.