



GOVERNMENT OF TAMIL NADU

BOTANY

HIGHER SECONDARY SECOND YEAR

Untouchability is Inhuman and a Crime

A publication under Free Textbook Programme of Government of Tamil Nadu

Department of School Education





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E-book



Assessment



DIGI links

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HOW TO USE THE BOOK

Career corner

List of professions related to the subject



Learning Objectives:

Learning objectives are brief statements that describe what students will be expected to learn by the end of school year, course, unit, lesson or class period.



Chapter Outline

Illustrate the complete overview of chapter



Activity

Amazing facts, Rhetorical questions to lead students to biological inquiry

Infographics

Visual representation of the lesson to enrich learning .

Evaluation

Assess students to pause, think and check their understanding



ICT

To motivate the students to further explore the content digitally and take them in to virtual world

Concept Map

Conceptual diagram that depicts relationships between concepts to enable students to learn the content schematically

Glossary

Explanation of scientific terms

English - Tamil Terminology

Tamil terminology for Botanical terms given for easy understanding

References

List of related books for further details of the topic

Web links

List of digital resources

Competitive Exam questions

Model questions to face various competitive exams



Scope of Botany

Higher Studies and Career Opportunities

List of Medical Courses

1 M. B. B. S. (Bachelor of Medicine and Bachelor of Surgery) – 5.5 years

MBBS is the bachelor degree in medical field for cure & diagnose, awarded in many countries.

2 B. D. S. (Bachelor of Dental Surgery) – 4 years

BDS is a professional degree programme in dentistry.

3 B. H. M. S. (Bachelor of Homeopathic Medicine & Surgery) – 5.5 years

BHMS is a bachelor degree in Homeopathic Education in India regulated by the National Institute of Homeopathy.

4 B. A. M. S. (Bachelor of Ayurvedic Medicine and Surgery) – 5.5 years

BAMS is a bachelor degree in ayurvedic system of medical field. In India, the Ayurvedic Education is regulated by the Central Council of Indian Medicine (CCIM).

5 B.Pharm (Bachelor of Pharmacy) – 4 years

This degree involves the knowledge of pharmacy.

6 B.Sc Nursing – 4 years

The motive of B.Sc. Nursing programme is to produce the qualified nurses, as a member of the health care team.

7 B.P.T (Physiotherapy) – 4.5 years

Physiotherapy helps the temporary disabled people in their rehabilitation.

8 B.O.T (Occupational Therapy) – 3 years

The Occupational Therapy helps the people to enable in their everyday life and treats the emotionally and physically challenged people.

9 B.U.M.S (Unani Medicine) – 5.5 years

BUMS degree is equivalent to the BAMS, but in Unani medicines.

Naturopathy & Yogic Science is one of the trusted fields after Allopathy in India.
Duration: 4 Years

10 D.Pharm (Ayurvedic, Siddha Medicine) – 2 years

It is a medical diploma course in pharmacy of ayurvedic medicines.

11 BMLT (Bachelor of Medical Lab Technicians) – 3 year

It is a bachelor degree of medical laboratory technology, contains the entire laboratory practices of the medical system. Various institutions are offering the B.Sc. programme in medical laboratory technology in India.

12 DMLT (Diploma of Medical Lab Technicians) – 1 year

It is a medical diploma course of medical laboratory technology, contains the laboratory practices of the medical field. Candidate must have cleared the 10+2 exam with PCB.



Agricultural Courses

- ❖ B.Sc in Agriculture
- ❖ B.Sc in Genetics and Plant Breeding
- ❖ B.Sc in Agriculture Economics and Farm Management
- ❖ B.Sc in Animal Husbandry
- ❖ B.Sc in Fisheries
- ❖ B.Sc in Forestry
- ❖ B.Sc Soil and water management
- ❖ B.Sc in Horticulture
- ❖ B.Sc Agriculture and Food Business
- ❖ M.Sc in Agronomy
- ❖ M.Sc Agricultural Economics
- ❖ M.Sc Seed science and Technology
- ❖ M.Sc Agricultural Entomology
- ❖ M.Sc Agricultural Statistics
- ❖ Diploma in Agriculture

Various Botany courses

- ❖ Bachelor of Science in Botany
- ❖ Bachelor of Science (Hons) in Botany
- ❖ Master of Science in Botany
- ❖ Master of Science in Botany and Forestry
- ❖ Master of Science in Applied Botany
- ❖ Master of Science in Herbal Science
- ❖ Post Graduate Diploma in Medico botany
- ❖ Post Graduate Diploma in Plant Biodiversity

Specializations available for botany are:

- | | |
|--------------------|--------------------|
| ❖ Cytology | ❖ Genetics |
| ❖ Lichenology | ❖ Economic botany |
| ❖ Palynology | ❖ Palaeobotany |
| ❖ Bryology | ❖ Ethnobotany |
| ❖ Phycology | ❖ Phytochemistry |
| ❖ Forestry | ❖ Plant morphology |
| ❖ Phytopathology | ❖ Plant anatomy |
| ❖ Plant physiology | ❖ Plant genetics |
| ❖ Agronomy | ❖ Horticulture |
| ❖ Plant ecology | ❖ Plant systematic |

Veterinary Science

Bachelor of Veterinary Science or B.V.Sc. is an undergraduate program in veterinary

Botany Career Opportunities and Job Prospects

The amount of diversity in the field of Botany gives it students to choose their specializations as per their choice, aptitude and interests. One can be a part of any reputed organization as a

Plant explorer: Botanist with a passion for plants who could be a photographer, writer, expeditioner, etc

Conservationist: Is an individual who works for the conservation of the environment and is often linked to organisations working for the cause.

Ecologist: A person who works for the ecosystem and a balanced environment.

Environment consultant: Some botanists qualify to work as environmental consultants, providing inputs and advice for the conservation of the environment.

Horticulturist: A horticulturist knows the science behind different plants, flowers, and greenery. They conduct research in gardening and landscaping, plant propagation, crop production, plant breeding, genetic engineering, plant biochemistry, and plant physiology.

Plant biochemist: Biochemists study the chemical and physical principles of living things and of biological processes, such as cell development, growth, heredity, and disease.

Molecular biologist: Molecular biologists conduct research and academic activities. The research component involves the study of biological structures in well-equipped laboratories with advanced technology to help them explore complex molecular structures and their particular functions. The equipment



may include microscopes, lab centrifuges, computers with specific software that allows them to analyze obtained data, and many more.

The knowledge of plant sciences is essential for development and management of forests, parks, waste lands, sea wealth etc.

Few of the industries which one can work with are:

- ❖ Chemical Industry
- ❖ Food Companies
- ❖ Arboretum
- ❖ Forest Services
- ❖ Biotechnology Firms
- ❖ Oil Industry
- ❖ Land Management Agencies
- ❖ Seed And Nursery Companies
- ❖ Plant Health Inspection Services
- ❖ Biological Supply Houses
- ❖ Plant Resources Laboratory
- ❖ Educational Institutions
- ❖ National Park Service
- ❖ Departments of Conservation and Land Management
- ❖ Public Health Service
- ❖ Department of Agriculture
- ❖ Forest Service
- ❖ Departments of Environmental Protection
- ❖ Departments of Agriculture and Water
- ❖ Nature Conservancy
- ❖ Environmental Protection Agency
- ❖ Medical Plant Resources Laboratory
- ❖ Several foreign countries provide platforms for Msc in Botany graduates to build up a good career in the field of Botany. Several undertakings functioning abroad demand the service of Msc in Botany graduates.

Research Institutes involved in Plant Science

a. Affiliated to Ministry of Environment and Forests

- ❖ Centre for Environment Education, Ahmedabad
- ❖ Forest Survey of India, Dehra Dun
- ❖ Indian Institute of Forest Management, Bhopal
- ❖ Institute of Forest Genetics and Tree Breeding, Jorhat

b. Affiliated to Indian Council of Agricultural Research

- ❖ Central Agricultural Research Institute, Port Blair
- ❖ Central Research Institute for Jute and Allied fibres, Barrackpore
- ❖ Directorate of Oilseeds Research, Hyderabad
- ❖ Indian Grassland and Fodder Research Institute, Jhansi
- ❖ Jute Technological Research Laboratories, Calcutta
- ❖ National Centre for Mushroom Research and Training, Solan

c. Affiliated to Council of Scientific and Industrial Research

- ❖ Central Drug Research Institute, Lucknow
- ❖ Central Food Technological Research Institute, Mysore
- ❖ National Environment Engineering Research Institute, Nagpur

d. Affiliated to Indian Council of Medical Research

- ❖ Central Council for Research in Unani Medicine, Delhi
- ❖ Central Council for Research in Ayurveda and Siddha, Delhi

e. Research Academics

- ❖ Indian Academy of Science, Bangalore
- ❖ Indian Botanical Society, Delhi
- ❖ Indian Mycological Society, Delhi
- ❖ Indian National Science Academy, Delhi
- ❖ Indian Society for Plantation Crops, Kasargod.





Chapter

1



UNIT VI: Reproduction in Plants

Asexual and Sexual Reproduction in Plants



Learning Objectives

The learner will be able to

- ❖ Recall various types of reproduction in lower and higher organisms.
- ❖ Discuss different methods of vegetative reproduction in plants.
- ❖ Recognise modern methods of reproduction.
- ❖ Recall the parts of a flower.
- ❖ Describe the steps involved in microsporogenesis.
- ❖ Recognise the structure of mature anther.
- ❖ Describe the structure and types of ovules.
- ❖ Explain the stages in megasporogenesis.
- ❖ Discuss the structure of embryo sac.
- ❖ Recognise different types of pollination.
- ❖ Identify the types of endosperms.
- ❖ Describe the development of Dicot embryo.
- ❖ Differentiate the structure of Dicot and Monocot seed.



Chapter outline

- 1.1 Asexual reproduction
- 1.2 Vegetative reproduction
- 1.3 Sexual Reproduction
- 1.4 Pre-fertilization structure and events
- 1.5 Fertilization
- 1.6 Post fertilization structure and events
- 1.7 Apomixis
- 1.8 Polyembryony
- 1.9 Parthenocarpy



4ATHKN

One of the essential features of all living things on the earth is reproduction. Reproduction is a vital process for the existence of a species and it also brings suitable changes through variation in the offsprings for their survival on earth. Plant reproduction is important not only for its own survival but also for the continuation and existence of all other organisms since the latter directly or indirectly depend on plants. Reproduction also plays an important role in evolution.

In this unit let us learn in detail about reproduction in plants.

Milestones in Plant Embryology

- 1682** - Nehemiah Grew mentioned stamens as the male organ of a flower.
- 1694** - R.J.Camerarius described the structure of a flower, anther, pollen and ovule
- 1761** - J.G. Kolreuter gave a detailed account on the importance of insects in pollination
- 1824** - G.B.Amici discovered the pollen tube.
- 1848** - Hofmeister described the structure of pollen tetrad
- 1870** - Hanstein described the development of embryo in *Capsella* and *Alisma*
- 1878** - E.Strasburger reported polyembryony
- 1884** - E.Strasburger discovered the process of Syngamy.
- 1898** - S.G.Nawaschin and L. Guignard & independently discovered Double fertilization
- 1904** - E.Hanning initiated embryo culture.
- 1950** - D.A. Johansen proposed classification for embryo development



- 1964** - S.Guha and S.C.Maheswari raised haploids from *Datura* pollen grains
- 1991** - E.S.Coen and E. M. Meyerowitz proposed the ABC model to describe the genetics of initiation and development of floral parts
- 2015** - K.V.Krishnamurthy summarized the molecular aspects of pre and post fertilization reproductive development in flowering plants

Panchanan Maheswari (1904-1966)

Professor P. Maheswari was an eminent Botanist who specialised in plant embryology, morphology and anatomy. In 1934, he became the Fellow of Indian Academy of Science. He published the book titled "An introduction to the Embryology of Angiosperms" in 1950. He established the International Society for Plant Morphologists, in 1951.



Basically reproduction occurring in organisms fall under two major categories

1. Asexual reproduction
2. Sexual reproduction.

1.1 Asexual Reproduction

The reproduction method which helps to perpetuate its own species without the involvement of gametes is referred to as asexual reproduction. From Unit I of Class XI we know that reproduction is one of the attributes of living things and the different types of reproduction have also been discussed. Lower plants, fungi and animals show different methods of asexual reproduction. Some of the methods include, formation of Conidia (*Aspergillus* and *Penicillium*); Budding (Yeast and *Hydra*); Fragmentation (*Spirogyra*); production of Gemma (*Marchantia*); Regeneration (*Planaria*)

and Binary fission (Bacteria) (Refer chapter 1 of Unit I of class XI). The individuals formed by this method is morphologically and genetically identical and are called **clones**. Higher plants also reproduce asexually by different methods which are given below:

1.2 Vegetative reproduction

1.2.1 Natural methods

Natural vegetative reproduction is a form of asexual reproduction in which a bud grows and develops into a new plant. The buds may be formed in organs such as root, stem and leaf. At some stage, the new plant gets detached from the parent plant and starts to develop into a new plant. Some of the organs involved in the vegetative reproduction also serve as the organs of storage and perennation. The unit of reproductive structure used in propagation is called **reproductive propagules or diaspores**. Some of the organs that help in vegetative reproduction are given in Figure 1.1.

A. Vegetative reproduction in root

The roots of some plants develop vegetative or adventitious buds on them. Example *Murraya*, *Dalbergia* and *Millingtonia*. Some tuberous adventitious roots apart from developing buds also store food. Example *Ipomoea batatas* and *Dahlia*. Roots possessing buds become detached from the parent plant and grow into independent plant under suitable condition.



Scourge of water bodies / Water hyacinth (*Eichhornia crassipes*) is an invasive weed on water bodies like ponds, lakes and reservoirs. It is popularly called "Terror of Bengal". It spreads rapidly through offset all over the water body and depletes the dissolved oxygen and causes death of other aquatic organisms.



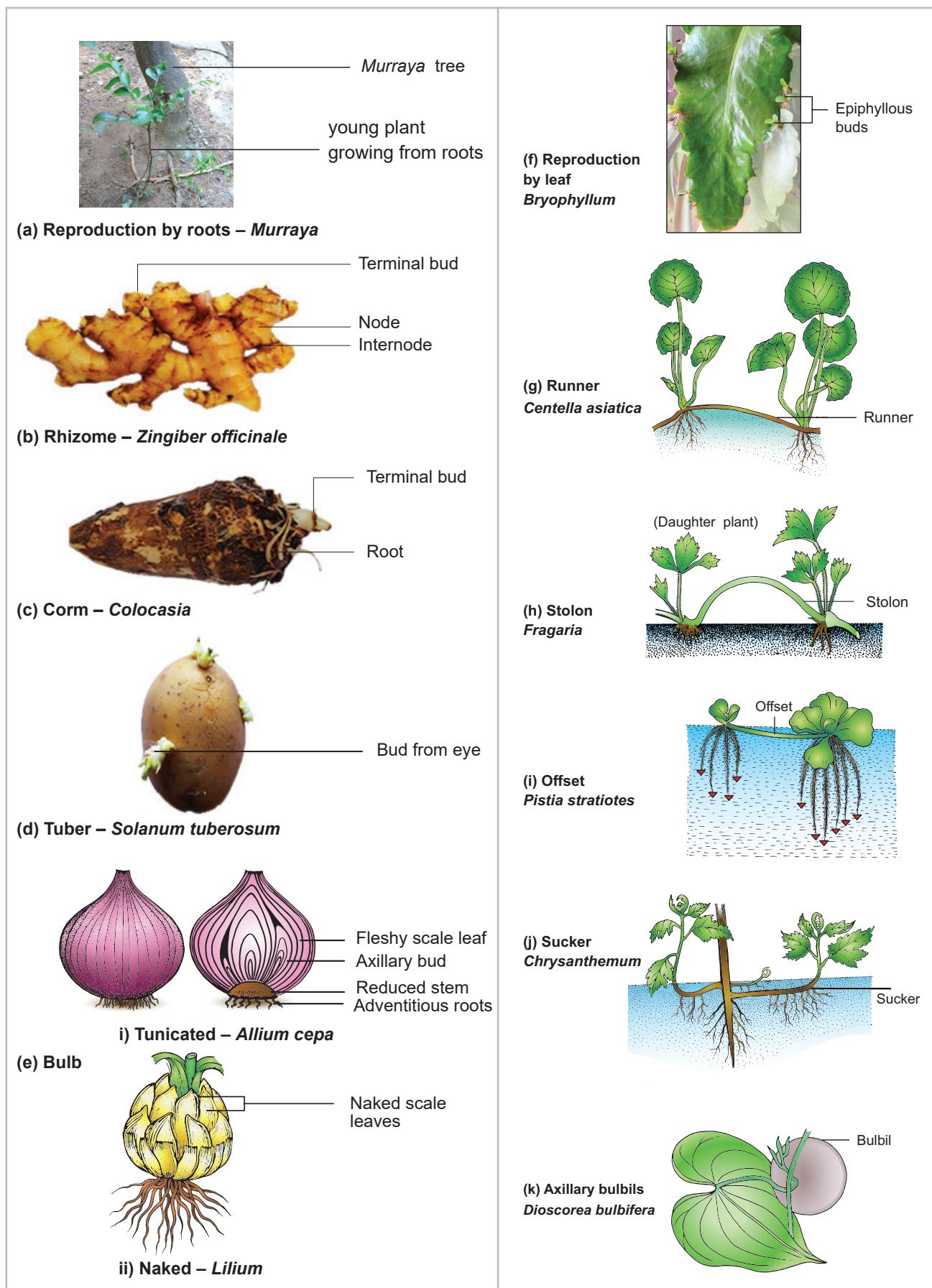


Figure 1.1 a-k: Natural methods of vegetative reproduction in plants.



Activity

Visit to a vegetable market and classify the vegetables into root, stem or leaf based on their utility and identify how many of them can be propagated through asexual methods.

B. Vegetative reproduction in stem

From the Unit 3 of class XI (Vegetative morphology) you are familiar with the structure of various underground stem and sub aerial stem modifications. These include rhizome (*Musa paradisiaca*, *Zingiber officinale* and *Curcuma longa*); corm (*Amorphophallus* and *Colocasia*); tuber (*Solanum tuberosum*); bulb (*Allium cepa* and *Lilium*) runner (*Centella asiatica*); stolon (*Mentha*, and *Fragaria*); offset (*Pistia*, and *Eichhornia*); sucker (*Chrysanthemum*) and bulbils (*Dioscorea* and *Agave*). The axillary buds from the nodes of rhizome and eyes of tuber give rise to new plants.

C. Vegetative reproduction in leaf

In some plants adventitious buds are developed on their leaves. When they are detached from the parent plant they grow into new individual plants. Examples: *Bryophyllum*, *Scilla*, and *Begonia*. In *Bryophyllum*, the leaf is succulent and notched on its margin. Adventitious buds develop at these notches and are called **epiphyllous buds**. They develop into new plants forming a root system and become independent plants when the leaf gets decayed. *Scilla* is a bulbous plant and grows in sandy soils. The foliage leaves are long and narrow and epiphyllous buds develop at their tips. These buds develop into new plants when they touch the soil.

Advantages of natural vegetative reproduction

- Only one parent is required for propagation.
- The new individual plants produced are genetically identical.
- In some plants, this enables to spread rapidly. Example: *Spinifex*

- Horticulturists and farmers utilize these organs of natural vegetative reproduction for cultivation and to harvest plants in large scale.

Disadvantage of natural vegetative reproduction

- New plants produced have no genetic variation.

1.2.2 Artificial Methods

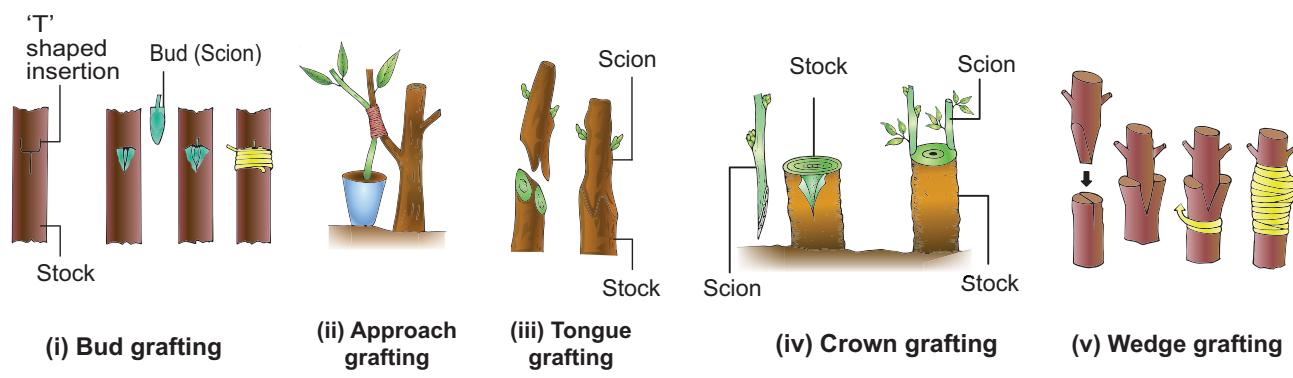
Apart from the above mentioned natural methods of vegetative reproduction, a number of methods are used in agriculture and horticulture to propagate plants from their parts. Such methods are said to be artificial propagation. Some of the artificial propagation methods have been used by man for a long time and are called **conventional methods**. Now-a-days, technology is being used for propagation to produce large number of plants in a short period of time. Such methods are said to be **modern methods**.

A. Conventional methods

The common methods of conventional propagation are cutting, grafting and layering.

a. Cutting: It is the method of producing a new plant by cutting the plant parts such as root, stem and leaf from the parent plant. The cut part is placed in a suitable medium for growth. It produces root and grows into a new plant. Depending upon the part used it is called as root cutting (*Malus*), stem cutting (*Hibiscus*, *Bougainvillea* and *Moringa*) and leaf cutting (*Begonia*, *Bryophyllum*). Stem cutting is widely used for propagation.

b. Grafting: In this, parts of two different plants are joined so that they continue to grow as one plant. Of the two plants, the plant which is in contact with the soil is called **stock** and the plant used for grafting is called **scion** (Figure 1.2 a). Examples are Citrus, Mango and Apple. There are different types of grafting based on the method of uniting the scion and stock. They are bud grafting, approach grafting, tongue grafting, crown grafting and wedge grafting.



a) Types of Grafting

Figure 1.2(a): Artificial methods of vegetative reproduction in plants

i. **Bud grafting:** A T-shaped incision is made in the stock and the bark is lifted. The scion bud with little wood is placed in the incision beneath the bark and properly bandaged with a tape.

ii. **Approach grafting:** In this method both the scion and stock remain rooted. The stock is grown in a pot and it is brought close to the scion. Both of them should have the same thickness. A small slice is cut from both and the cut surfaces are brought near and tied together and held by a tape. After 1-4 weeks the tip of the stock and base of the scion are cut off and detached and grown in a separate pot.

iii. Tongue grafting

A scion and stock having the same thickness is cut obliquely and the scion is fit into the stock and bound with a tape.

iv. Crown grafting.

When the stock is large in size scions are cut into wedge shape and are inserted on the slits or clefts of the stock and fixed in position using graft wax.

v. Wedge grafting

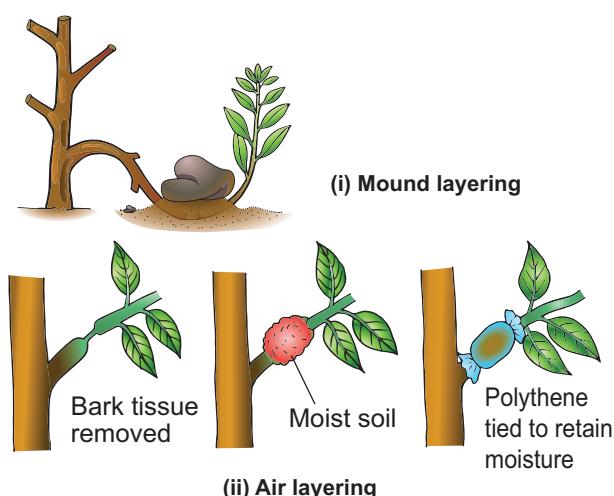
In this method a slit is made in the stock or the bark is cut. A twig of scion is inserted and tightly bound so that the cambium of the two is joined.

Activity

Visit a nursery, observe the method of grafting, layering and do these techniques with plants growing in your school or home

c. **Layering:** In this method, the stem of a parent plant is allowed to develop roots while still intact. When the root develops, the rooted part is cut and planted to grow as a new plant. Examples: *Ixora* and *Jasminum*. Mound layering and Air layering are few types of layering (Figure 1.2 b).

i. **Mound layering:** This method is applied for the plants having flexible branches. The lower branch with leaves is bent to the ground and part of the stem is buried in the soil and tip of the branch is exposed above the soil. After the roots emerge from the part of the stem buried in the soil, a cut is made in parent plant so that the buried part grow into a new plant.



b) Types of Layering

Figure 1.2 (b): Artificial methods of vegetative reproduction in plants



ii. Air layering: In this method the stem is girdled at nodal region and hormones are applied to this region which promotes rooting. This portion is covered with damp or moist soil using a polythene sheet. Roots emerge in these branches after 2-4 months. Such branches are removed from the parent plant and grown in a separate pot or ground.

Advantages of conventional methods

- The plants produced are genetically uniform.
- Many plants can be produced quickly by this method.
- Some plants produce little or no seeds; in others, the seeds produced do not germinate. In such cases, plants can be produced in a short period by this method.
- Some plants can be propagated more economically by vegetative propagation. Example: *Solanum tuberosum*.
- Two different plants with desirable characters such as disease resistant and high yield can be grafted and grown as a new plant with the same desirable characters.

Disadvantages of conventional methods

- Use of virus infected plants as parents produces viral infected new plants.
- Vegetative structures used for propagation are bulky and so they are difficult to handle and store.

B. Modern Method

Professor F.C. Steward(1932) of Cornell University showed that the mature phloem parenchyma cells removed from the carrot were placed in a suitable medium under controlled conditions, could be stimulated to start dividing again to produce a new carrot plant. These cells were described as **totipotent**. The genetic ability of a plant cell to produce the entire plant under suitable conditions is said to be totipotency. This characteristic feature of a cell is utilized in horticulture, forestry and industries to propagate plants. The growth of

plant tissue in special culture medium under suitable controlled conditions is known as **tissue culture**.

Micropropagation

The regeneration of a whole plant from single cell, tissue or small pieces of vegetative structures through tissue culture is called **micropropagation**. This is one of the modern methods used to propagate plants. The detailed steps involved in the micropropagation are given in Unit VIII.

Advantages of modern methods

- Plants with desired characteristics can be multiplied rapidly in a short duration.
- Plants produced are genetically identical.
- Tissue culture can be carried out in any season to produce plants.
- Plants which do not produce viable seeds and seeds that are difficult to germinate can be propagated by tissue culture.
- Rare and **endangered** plants can be propagated.
- Disease free plants can be produced by **meristem culture**.
- Cells can be genetically modified and transformed using tissue culture.

Disadvantages of modern methods

- It is labour intensive and requires skilled workers.
- Sterile condition must be maintained which adds to the cost.
- Since the clones are genetically identical, the entire crop is susceptible to new diseases or changes in environmental conditions will wipe out the species.



- Sometimes, **callus** undergoes genetical changes which are undesirable for commercial use.



1.3 Sexual Reproduction

In previous classes reproduction in lower plants like algae and bryophytes was discussed in detail. Sexual reproduction involves the production and fusion of male and female gametes. The former is called gametogenesis and the latter is the process of fertilization. Let us recall the sexual reproduction in algae and bryophytes. They reproduce by the production of gametes which may be motile or non motile depending upon the species. The gametic fusion is of three types (Isogamy, Anisogamy and Oogamy). In algae external fertilization takes place whereas in higher plants internal fertilization occurs.

Flower

A flower is viewed in multidimensional perspectives from time immemorial. It is an inspirational tool for the poets. It is a decorative material for all the celebrations. In Tamil literature the five lands are denoted by different flowers. The flags of some countries are embedded with flowers. Flowers are used in the preparation of perfumes. For a Morphologist, a flower is a highly condensed shoot meant for reproduction. As you have already learned about the parts of a flower in Unit II of Class XI, let us recall the parts of a flower. A Flower possesses four whorls- Calyx, Corolla, Androecium and Gynoecium. Androecium and Gynoecium are essential organs(Figure 1.3). The process or changes involved in sexual reproduction of higher plants include three stages .They are Pre-fertilization, Fertilization and Post fertilization changes. Let us discuss these events in detail.

1.4 Pre-fertilization structure and events

The hormonal and structural changes in plant lead to the differentiation and development of floral primordium. The

structures and events involved in pre-fertilization are given below

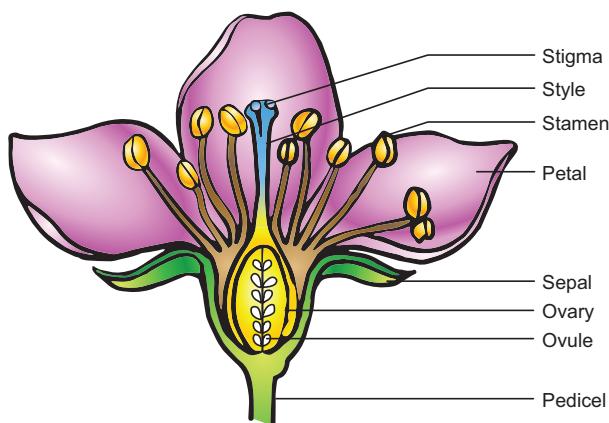


Figure 1.3 Parts of a Flower

1.4.1 Male Reproductive part - Androecium

Androecium is made up of stamens. Each stamen possesses an anther and a filament. Anther bears pollen grains which represent the male gametophyte. In this chapter we shall discuss the structure and development of anther in detail.

Development of anther: A very young anther develops as a homogenous mass of cells surrounded by an epidermis. During its development, the anther assumes a four-lobed structure. In each lobe, a row or a few rows of hypodermal cells becomes enlarged with conspicuous nuclei. This functions as archesporium. The archesporial cells divide by periclinal divisions to form primary parietal cells towards the epidermis and primary sporogenous cells towards the inner side of the anther. The primary parietal cells undergo a series of periclinal and anticlinal division and form 2-5 layers of anther walls composed of endothecium, middle layers and tapetum, from periphery to centre.

Microsporogenesis: The stages involved in the formation of haploid microspores from diploid microspore mother cell through meiosis is called **Microsporogenesis**. The primary sporogeneous cells directly, or may undergo a few mitotic divisions to form **sporogenous tissue**. The last

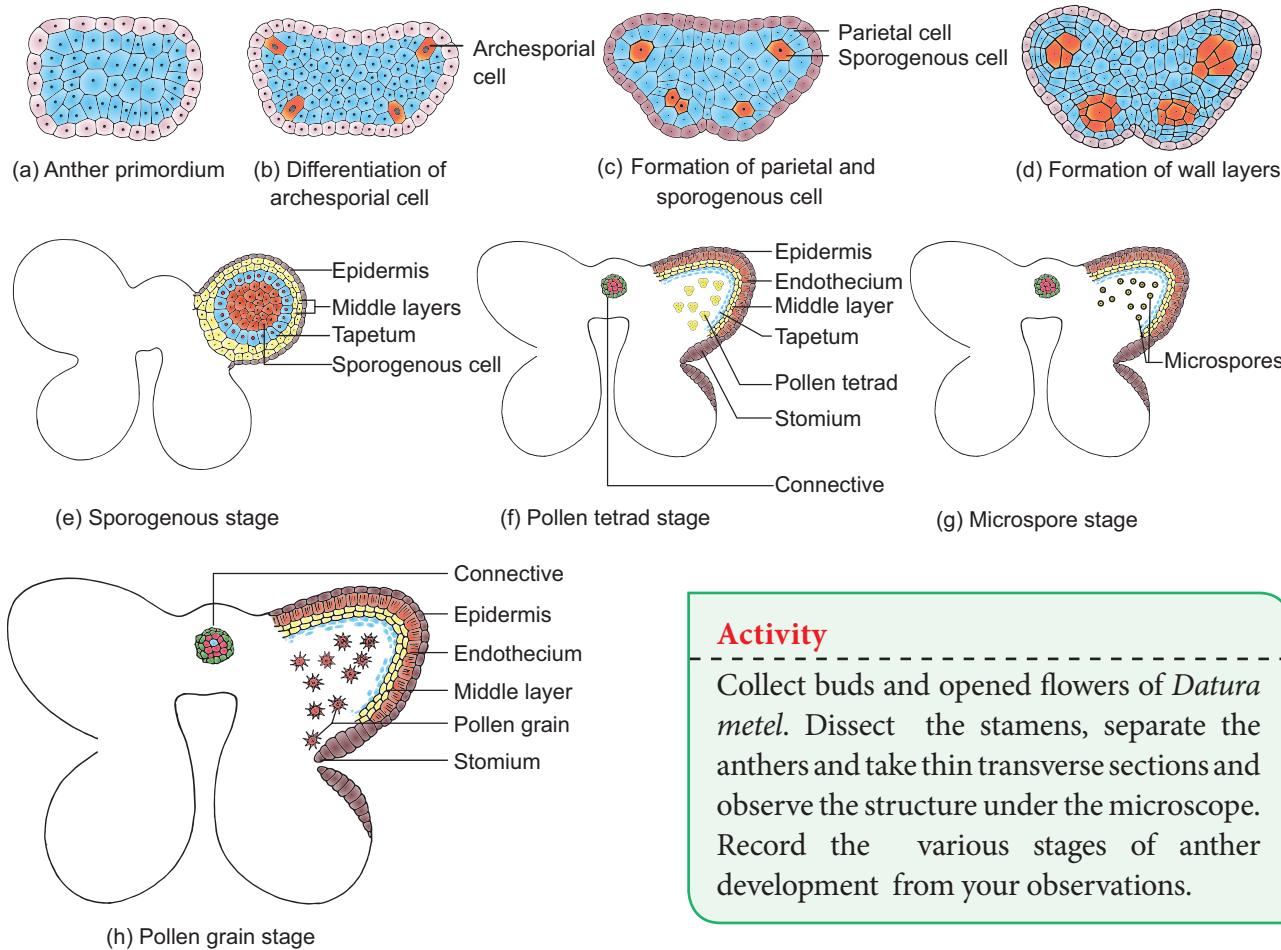


Figure 1.4 Stages in the development of anther

generation of sporogenous tissue functions as microspore mother cells. Each microspore mother cell divides meiotically to form a tetrad of four haploid microspores (microspore tetrad). The microspore tetrad may be arranged in a tetrahedral, decussate, linear, T shaped or isobilateral manner. Microspores soon separate from one another and remain free in the anther locule and develop into pollen grains. The stages in the development of microsporangia is given in Figure 1.4. In some plants, all the microspores in a microsporangium remain held together called **pollinium**. Example: *Calotropis*. Compound pollen grains are found in *Drosera* and *Drymis*.

T.S. of Mature anther

Transverse section of mature anther reveals the presence of anther cavity surrounded by an anther wall. It is bilobed, each lobe having 2 theca (dithecos). A typical anther is tetrasporangiate. The T.S. of Mature anther is given in Figure 1.5.

Activity

Collect buds and opened flowers of *Datura metel*. Dissect the stamens, separate the anthers and take thin transverse sections and observe the structure under the microscope. Record the various stages of anther development from your observations.

1. Anther wall

The mature anther wall consists of the following layers **a. Epidermis** **b. Endothecium** **c. Middle layers** **d. Tapetum**.

a. Epidermis: It is single layered and protective in function. The cells undergo repeated anticlinal divisions to cope up with the rapidly enlarging internal tissues.

b. Endothecium: It is generally a single layer of radially elongated cells found below the epidermis. The inner tangential wall develops bands (sometimes radial walls also) of cellulose (sometimes also slightly lignified). The cells are **hygroscopic**. In the anthers of aquatic plants, saprophytes, cleistogamous flowers and extreme parasites endothelial differentiation is absent. The cells along the junction of the two thecae of an anther lobe lack these thickenings. This region is called **stomium**. This region along with the hygroscopic nature of endothecium helps in the dehiscence of anther at maturity.

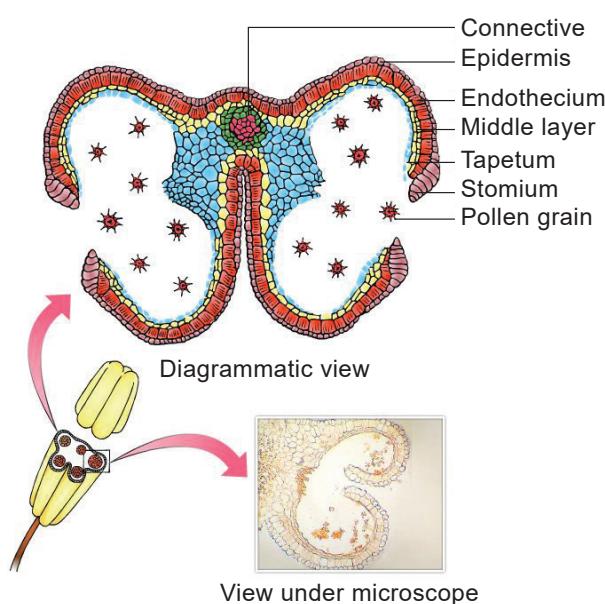


Figure 1.5 T.S. of Mature anther

c. **Middle layers:** Two to three layers of cells next to endothecium constitute middle layers. They are generally ephemeral. They disintegrate or get crushed during maturity.

d. **Tapetum:** It is the innermost layer of anther wall and attains its maximum development at the tetrad stage of microsporogenesis. It is derived partly from the peripheral wall layer and partly from the connective tissue of the anther lining the anther locule. Thus, the tapetum is dual in origin. It nourishes the developing sporogenous tissue, microspore mother cells and microspores. The cells of the tapetum may remain uninucleate or may contain more than one nucleus or the nucleus may become polyploid. It also contributes to the wall materials, sporopollenin, pollenkitt, tryphine and number of proteins that control incompatibility reaction. Tapetum also controls the fertility or sterility of the microspores or pollen grains.

There are two types of tapetum based on its behaviour. They are:

Secretory tapetum (parietal/glandular/cellular): The tapetum retains the original position and cellular integrity and nourishes the developing microspores.

Invasive tapetum (periplasmoidal): The cells loose their inner tangential and radial

walls and the protoplast of all tapetal cells coalesces to form a periplasmodium.

Functions of Tapetum:

- It supplies nutrition to the developing microspores.
- It contributes sporopollenin through **ubisch bodies** thus plays an important role in pollen wall formation.
- The pollenkitt material is contributed by tapetal cells and is later transferred to the pollen surface.
- Exine proteins responsible for '**rejection reaction**' of the stigma are present in the cavities of the exine. These proteins are derived from tapetal cells.

Many botanists speak of a third type of tapetum called amoeboid, where the cell wall is not lost. The cells protrude into the anther cavity through an amoeboid movement. This type is often associated with male sterility and should not be confused with periplasmoidal type.

2. Anther Cavity: The anther cavity is filled with microspores in young stages or with pollen grains at maturity. The meiotic division of microspore mother cells gives rise to microspores which are haploid in nature.

3. Connective: It is the column of sterile tissue surrounded by the anther lobe. It possesses vascular tissues. It also contributes to the inner tapetum.

Microspores and pollen grains

Microspores are the immediate product of meiosis of the microspore mother cell whereas the pollen grain is derived from the microspore. The microspores have protoplast surrounded by a wall which is yet to be fully developed. The pollen protoplast consists of dense cytoplasm with a centrally located nucleus. The wall is differentiated into two layers, namely, inner layer called **intine** and outer layer called **exine**. Intine is thin, uniform and is made up of pectin,



hemicellulose, cellulose and callose together with proteins. Exine is thick and is made up of cellulose, sporopollenin and pollenkitt. The exine is not uniform and is thin at certain areas. When these thin areas are small and round it is called germ pores or when elongated it is called furrows. It is associated with germination of pollen grains. The sporopollenin is generally absent in germ pores. The surface of the exine is either smooth or sculptured in various patterns (rod like, grooved, warty, punctuate etc.) The sculpturing pattern is used in the plant identification and classification.

Shape of a pollen grain varies from species to species. It may be globose, ellipsoid, fusiform, lobed, angular or crescent shaped. The size of the pollen varies from 10 micrometers in *Myosotis* to 200 micrometers in members of the family Cucurbitaceae and Nyctaginaceae



Palynology is the study of pollen grains. It helps to identify the distribution of coal and to locate oil fields. Pollen grains reflect the vegetation of an area.

Liquid nitrogen (-196°C) is used to preserve pollen in viable condition for prolonged duration. This technique is called **cryopreservation** and is used to store pollen grains (pollen banks) of economically important crops for breeding programmes..

The wall material sporopollenin is contributed by both pollen cytoplasm and tapetum. It is derived from carotenoids. It is resistant to physical and biological decomposition. It helps to withstand high temperature and is resistant to strong acid, alkali and enzyme action. Hence, it preserves the pollen for long periods in fossil deposits, and it also protects pollen during its journey from anther to stigma.

Pollenkitt is contributed by the tapetum and coloured yellow or orange and is chiefly made of carotenoids or flavonoids. It is an oily layer forming a thick viscous coating over pollen

surface. It attracts insects and protects damage from UV radiation.

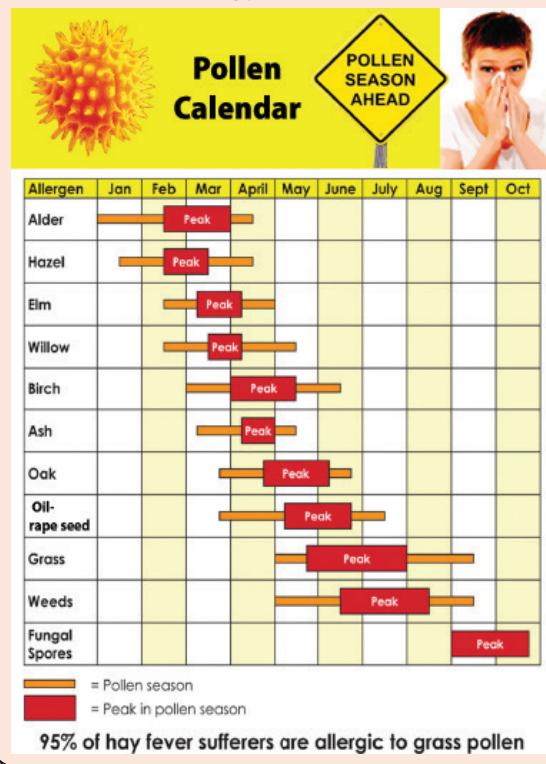


Beepollen is a natural substance and contains high protein, carbohydrate, trace amount of minerals and vitamins. Therefore, it is used as dietary supplement and is sold as pollen tablets and syrups. Further, it increases the performance of athletes, race horses and also heals the wounds caused by burns. The study of honey pollen is called **Mellitopalynology**.



Pollen calendar shows the production of pollen by plants during different seasons. This benefits the allergic persons. Pollen grains cause allergic reactions like asthma, bronchitis, hay fever, allergic rhinitis etc.,

Parthenium hysterophorus L. (Family-Asteraceae) is commonly called Carrot grass is a native of tropical America and was introduced into India as a contaminant along with cereal wheat. The pollen of this plant cause Allergy.





Development of Male gametophyte:

The microspore is the first cell of the male gametophyte and is haploid. The development of male gametophyte takes place while they are still in the microsporangium. The nucleus of the microspore divides to form a **vegetative** and a **generative** nucleus. A wall is laid around the generative nucleus resulting in the formation of two unequal cells, a large irregular nucleus bearing with abundant food reserve called vegetative cell and a small generative cell. At this 2 celled stage, the pollens are liberated from the anther. In some plants the generative cell again undergoes a division to form two male gametes. In these plants, the pollen is liberated at 3 celled stage. In 60% of the angiosperms pollen is liberated in 2 celled stage. Further, the growth of the male gametophyte occurs only if the pollen reaches the right stigma. The pollen on reaching the stigma absorbs moisture and swells. The intine grows as pollen tube through the germ pore. In case the pollen is liberated at 2 celled stage the generative cell divides in the pollen into 2 male cells (sperms) after reaching the stigma or in the pollen tube before reaching the embryo sac. The stages in the development of male gametophyte is given in Figure 1.6.

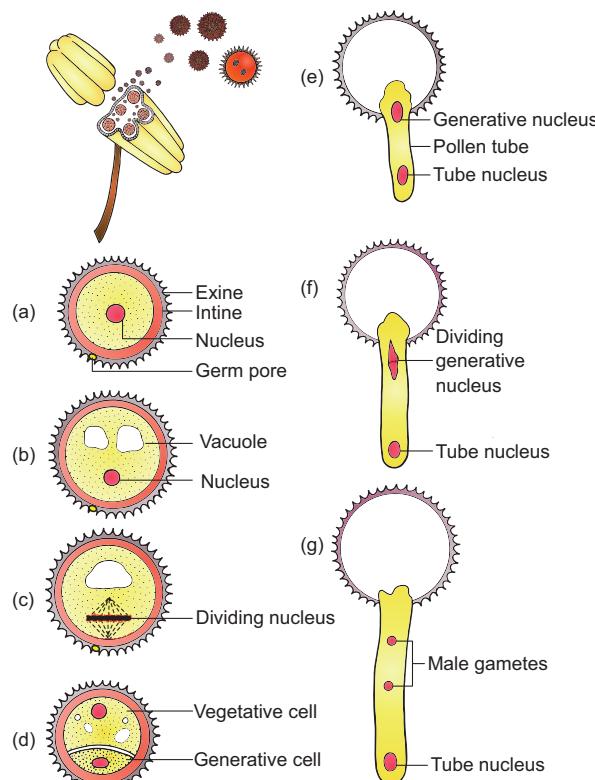


Figure 1.6 Development of male gametophyte

1.4.2 Female reproductive part - Gynoecium

The **gynoecium** represents the female reproductive part of the flower. The word gynoecium represents one or more pistils of a flower. The word pistil refers to the ovary, style and stigma. A pistil is derived from a carpel. The word ovary represents the part that contains the ovules. The stigma serves as a landing platform for pollen grains. The style is an elongated slender part beneath the stigma. The basal swollen part of the pistil is the ovary. The ovules are present inside the ovary cavity (locule) on the placenta. Gynoecium (carpel) arises as a small papillate outgrowth of meristematic tissue from the growing tip of the floral primordium. It grows actively and soon gets differentiated into ovary, style and stigma. The ovules or megasporangia arise from the placenta. The number of ovules in an ovary may be one (paddy, wheat and mango) or many (papaya, water melon and orchids).

Structure of ovule(Megasporangium):

Ovule is also called megasporangium and is protected by one or two covering called **integuments**. A mature ovule consists of a stalk and a body. The stalk or the **funiculus** (also called funicle) is present at the base and it attaches the ovule to the placenta. The point of attachment of funicle to the body of the ovule is known as **hilum**. It represents the junction between ovule and funicle. In an inverted ovule, the funicle is adnate to the body of the ovule forming a ridge called **raphe**. The body of the ovule is made up of a central mass of parenchymatous tissue called **nucellus** which has large reserve food materials. The nucellus is enveloped by one or two protective coverings called **integuments**. Integument encloses the nucellus completely except at the top where it is free and forms a pore called micropyle. The ovule with one or two integuments are said to be **unitegmic** or **bitegmic** ovules respectively. The basal region of the body of the ovule where the nucellus, the integument and the funicle meet or merge is called as **chalaza**. There is a large,

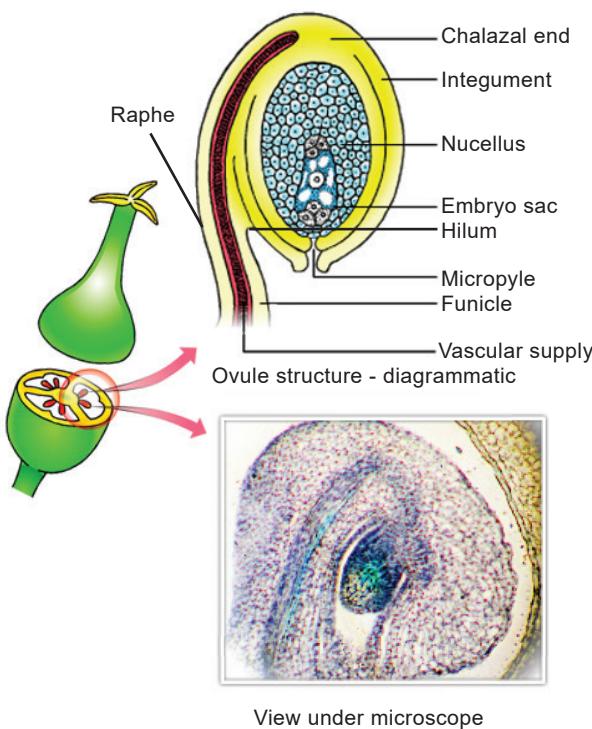


Figure 1.7 Structure of an ovule

oval, sac-like structure in the nucellus toward the micropylar end called **embryo sac** or female gametophyte. It develops from the functional megasporangium formed within the nucellus. In some species (unitegmic tenuinucellate) the inner layer of the integument may become specialized to perform the nutritive function for the embryo sac and is called as **endothelium** or **integumentary tapetum** (Example : Asteraceae). There are two types of ovule based on the position of the sporogenous cell. If the sporogenous cell is hypodermal with a single layer of nucellar tissue around it is called **tenuinucellate** type. Normally tenuinucellate ovules have very small nucellus. Ovules with subhypodermal sporogenous cell is called **crassinucellate** type. Normally these ovules have fairly large nucellus. Group of cells found at the base of the ovule between the chalaza and

embryo sac is called **hypostase** and the thick-walled cells found above the micropylar end above the embryo sac is called **epistase**. The structure of ovule is given in Figure 1.7.

Types of Ovules

The ovules are classified into six main types based on the orientation, form and position of the micropyle with respect to funicle and chalaza. Most important ovule types are orthotropous, anatropous, hemianatropous and campylotropous. The types of ovule is given in Figure 1.8.

Orthotropous: In this type of ovule, the micropyle is at the distal end and the micropyle, the funicle and the chalaza lie in one straight vertical line. Examples: Piperaceae, Polygonaceae

Anatropous: The body of the ovule becomes completely inverted so that the micropyle and funiculus come to lie very close to each other. This is the common type of ovules found in dicots and monocots.

Hemianatropous: In this, the body of the ovule is placed transversely and at right angles to the funicle. Example: Primulaceae.

Campylotropous: The body of the ovule at the micropylar end is curved and more or less bean shaped. The embryo sac is slightly curved. All the three, hilum, micropyle and chalaza are adjacent to one another, with the micropyle oriented towards the placenta. Example: Leguminosae

In addition to the above main types there are two more types of ovules they are,

Amphitropous: The distance between hilum and chalaza is less. The curvature of the ovule leads to horse-shoe shaped nucellus. Example: some Alismataceae.

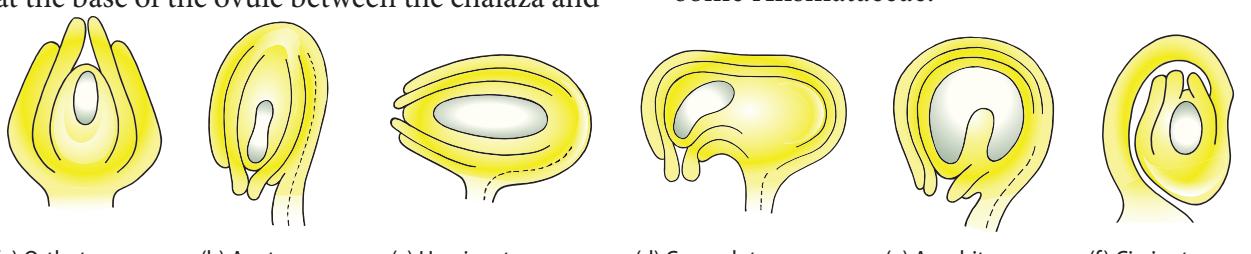


Figure 1.8 Types of ovule



Circinotropous: Funiculus is very long and surrounds the ovule. Example: Cactaceae

Megasporogenesis

The process of development of a megasporangium from a megasporangium mother cell is called **megasporogenesis**.

As the ovule develops, a single hypodermal cell in the nucellus becomes enlarged and functions as **archesporium**. In some plants, the archesporial cell may directly function as megasporangium mother cell. In others, it may undergo a transverse division to form outer primary parietal cell and inner primary sporogenous cell. The parietal cell may remain undivided or divide by few periclinal and anticlinal divisions to embed the primary sporogenous cell deep into the nucellus. The primary sporogenous cell functions as a megasporangium mother cell. The megasporangium mother cell undergoes meiotic division to form four haploid megasporangia. Based on the number of megasporangia that develop into the Embryo sac, we have three basic types of development: **monosporic**, **bisporic** and **tetrasporic**. The megasporangia are usually arranged in a linear tetrad. Of the four megasporangia formed, usually the chalazal one is functional and other

three degenerate. The functional megasporangium forms the female gametophyte or embryo sac. This type of development is called **monosporic** development (Example: *Polygonum*). Of the four megasporangia formed if two are involved in Embryo sac formation the development is called **bisporic** (Example: *Allium*). If all the four megasporangia are involved in Embryo sac formation the development is called **tetrasporic** (Example: *Peperomia*). An ovule generally has a single embryo sac. The development of monosporic embryo sac (*Polygonum* type) is given in Figure 1.9.

Development of Monosporic embryo sac.

To describe the stages in embryo sac development and organization the simplest monosporic type of development is given below.

The functional megasporangium is the first cell of the embryo sac or female gametophyte. The megasporangium elongates along micropylar-chalazal axis. The nucleus undergoes a mitotic division. Wall formation does not follow the nuclear division. A large central vacuole now appears between the two daughter nuclei. The vacuole expands and pushes the nuclei towards the opposite poles of the embryo sac. Both the nuclei divide twice mitotically, forming

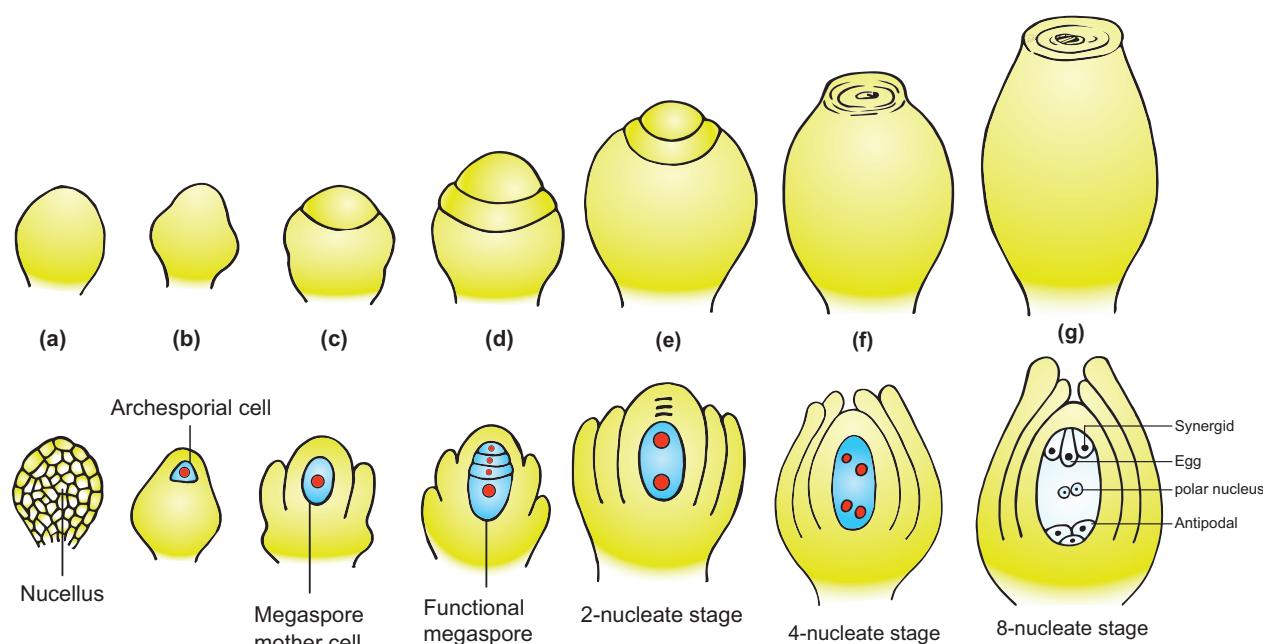


Figure 1.9 Development of ovule and embryo sac (*Polygonum* type).

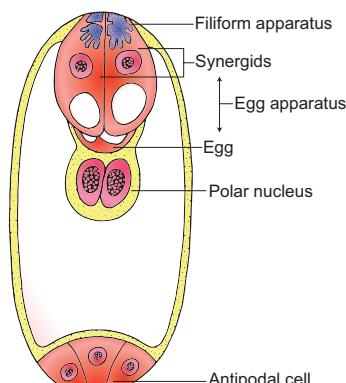


Figure 1.10 Structure of Embryo sac

four nuclei at each pole. At this stage all the eight nuclei are present in a common cytoplasm (free nuclear division). After the last nuclear division the cell undergoes a appreciable elongation,

assuming a sac-like appearance. This is followed by cellular organization of the embryo sac. Of the four nuclei at the micropylar end of the embryo sac, three organize into an **egg apparatus**, the fourth one is left free in the cytoplasm of the central cell as the upper polar nucleus. Three nuclei of the chalazal end form three **antipodal cells** whereas the fourth one functions as the lower polar nucleus. Depending on the plant the **2 polar nuclei** may remain free or may fuse to form a **secondary nucleus** (central cell). The egg apparatus is made up of a central egg cell and two synergids, one on each side of the egg cell. Synergids secrete chemotropic substances that help to attract the pollen tube. The special cellular thickening called filiform apparatus of synergids help in the absorption, conduction of nutrients from the nucellus to embryo sac. It also guides the pollen tube into the egg. Thus, a 7 celled with 8 nucleated embryo sac is formed. The structure of embryo sac is given in Figure 1.10.

1.4.3 Pollination

Pollination is a wonderful mechanism which provides food, shelter etc., for the pollinating animals. Many plants are pollinated by a particular



animal species and the flowers are modified accordingly and thus there exists a co-evolution between plants and animals. Let us imagine if pollination fails. Do you think there will be any seed and fruit formation? If not what happens to pollinating organisms and those that depend on these pollinating organism for the food? Here lies the significance of the process of pollination.

The pollen grains produced in the anther will germinate only when they reach the stigma of the pistil. The reproductive organs, stamens and pistil of the flower are spatially separated, a mechanism which is essential for pollen grains to reach the stigma is needed. This process of transfer of pollen grains from the anther to a stigma of a flower is called **pollination**.

Pollination is a characteristic feature of spermatophyte (Gymnosperms and Angiosperms). Pollination in gymnosperms is said to be direct as the pollens are deposited directly on the exposed ovules, whereas in angiosperms it is said to be indirect, as the pollens are deposited on the stigma of the pistil. In majority of angiosperms, the flower opens and exposes its mature anthers and stigma for pollination. Such flowers are called **chasmogamous** and the phenomenon is **chasmogamy**. In other plants, pollination occurs without opening and exposing their sex organs. Such flowers are called **cleistogamous** and the phenomenon is **cleistogamy**.

Based upon the flower on which the pollen of a flower reaches, the pollination is classified into two kinds, namely, **self-pollination (Autogamy)** and **cross-pollination (Allogamy)**.

A. Self-pollination or Autogamy (Greek Auto = self, gamos = marriage):

According to a majority of Botanists, the transfer of pollen on the stigma of the same flower is called **self-pollination or Autogamy**. Self-pollination is possible only in those plants which bear bisexual flowers. In order



to promote self-pollination the flowers of the plants have several adaptations or mechanisms. They are:

1. Cleistogamy: In cleistogamy (Greek Kleisto = closed. Gamos = marriage) flowers never open and expose the reproductive organs and thus the pollination is carried out within the closed flower. *Commelina*, *Viola*, *Oxalis* are some examples for cleistogamous flowers. In *Commelina benghalensis*, two types of flowers are produced-aerial and underground flowers. The aerial flowers are brightly coloured, chasmogamous and insect pollinated. The underground flowers are borne on the subterranean branches of the rhizome that are dull, cleistogamous and self-pollinated and are not depended on pollinators for pollination. (Figure 1.11).

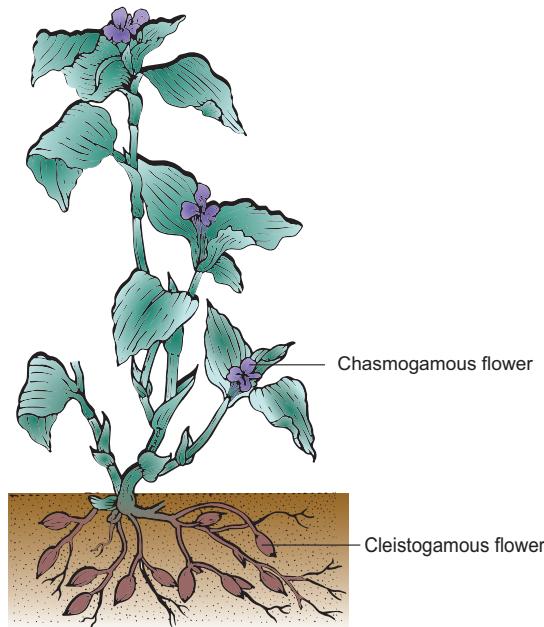


Figure 1.11 *Commelina* with Cleistogamous and Chasmogamous flowers

2. Homogamy: When the stamens and stigma of a flower mature at the same time it is said to be homogamy. It favours self-pollination to occur. Example: *Mirabilis jalapa*, *Catharanthus roseus*

3. Incomplete dichogamy: In dichogamous flowers the stamen and stigma of a flower mature at different times. Sometimes, the time of maturation of these essential organs overlap so that it becomes favourable for self-pollination.

B. Cross - pollination

It refers to the transfer of pollens on the stigma of another flower. The cross-pollination is of two types:

i. Geitonogamy: When the pollen deposits on another flower of the same individual plant, it is said to be geitonogamy. It usually occurs in plants which show monoecious condition. It is functionally cross-pollination but is similar to autogamy because the pollen comes from same plant.

ii. Xenogamy: When the pollen (genetically different) deposits on another flower of a different plant of the same species, it is called as xenogamy.

Contrivances of cross-pollination

The flowers of the plants have also several mechanisms that promote cross-pollination which are also called **contrivances of cross-pollination or outbreeding devices**. It includes the following.

1. Dicliny or Unisexuality

When the flowers are unisexual only cross-pollination is possible. There are two types.

i. Monoecious: Male and female flowers on the same plant. Coconut, Bitter gourd. In plants like castor and maize, autogamy is prevented but geitonogamy takes place.

ii. Dioecious : Male and female flowers on different plants. *Borassus*, *Carica papaya* and date palm. Here both autogamy and geitonogamy are prevented.

2. Monocliny or Bisexuality

Flowers are bisexual and the special adaptation of the flowers prevents self-pollination.

i. Dichogamy: In bisexual flowers anthers and stigmas mature at different times, thus checking self-pollination. It is of two types.

a. Protandry: The stamens mature earlier than the stigmas of the flowers. Examples: *Helianthus*, *Clerodendrum* (Figure 1.12 a).



b. Protogyny: The stigmas mature earlier than the stamens of the flower. Examples: *Scrophularia nodosa* and *Aristolochia bracteata* (Figure 1.12 b).

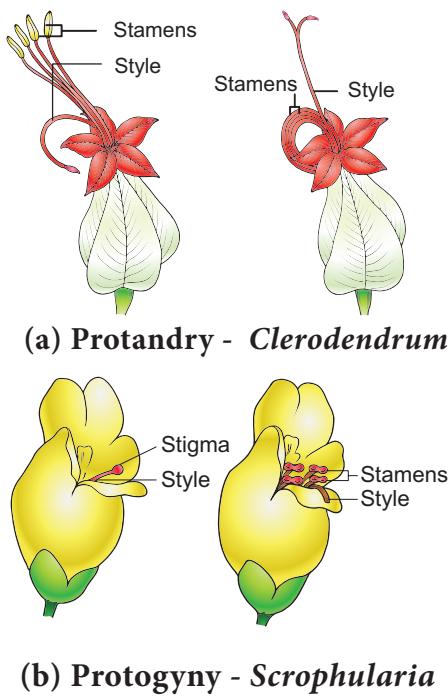


Figure 1.12 Dichogamy

ii. Herkogamy: In bisexual flowers the essential organs, the stamens and stigmas, are arranged in such a way that self-pollination becomes impossible. For example in *Gloriosa superba*, the style is reflexed away from the stamens and in *Hibiscus* the stigmas project far above the stamens (Figure 1.13).



Figure 1.13 Herkogamy - *Gloriosa*

iii. Heterostyly: Some plants produce two or three different forms of flowers that are different in their length of stamens and style. Pollination will take place only between organs of the same length. (Figure 1.14)

a. Distyly: The plant produces two forms of flowers, Pin or long style, long stigmatic papillae, short stamens and small pollen grains;

Thrum-eyed or short style, small stigmatic papillae, long stamens and large pollen grains. Example: *Primula* (Figure 1.14a). The stigma of the Thrum-eyed flowers and the anther of the pin lie in same level to bring out pollination. Similarly the anther of Thrum-eyed and stigma of pin ones is found in same height. This helps in effective pollination.

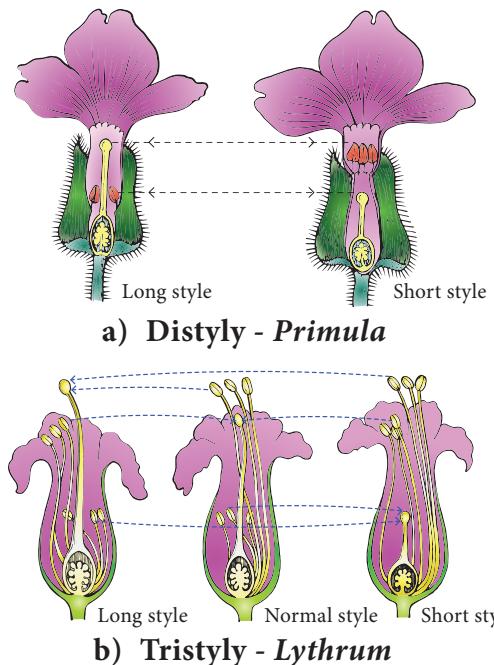


Figure 1.14 Heterostyly

b. Tristyly: The plant produces three kinds of flowers, with respect to the length of the style and stamens. Here, the pollen from flowers of one type can pollinate only the other two types but not their own type. Example : *Lythrum* (Figure 1.14b).

iv. Self sterility/ Self- incompatibility: In some plants, when the pollen grain of a flower reaches the stigma of the same, it is unable to germinate or prevented to germinate on its own stigma. Examples: *Abutilon*, *Passiflora*. It is a genetic mechanism.

Agents of pollination

Pollination is effected by many agents like wind, water, insects etc. On the basis of the agents that bring about pollination, the mode of pollination is divided into abiotic and biotic. The latter type is used by majority of plants.



Abiotic agents

1. Anemophily - pollination by Wind
2. Hydrophily - pollination by Water

Biotic agents

3. Zoophily

Zoophily refers to pollination through animals and pollination through insects is called Entomophily.

1. Anemophily: Pollination by wind. The wind pollinated flowers are called **anemophilous**. The wind pollinated plants are generally situated in wind exposed regions. Anemophily is a chance event. Therefore, the pollen may not reach the target flower effectively and are wasted during the transit from one flower to another. The common examples of wind pollinated flowers are - grasses, sugarcane, bamboo, coconut, palm, maize etc.,

Anemophilous plants have the following characteristic features:

- The flowers are produced in pendulous, catkin-like or spike inflorescence.
- The axis of inflorescence elongates so that the flowers are brought well above the leaves.
- The perianth is absent or highly reduced.
- The flowers are small, inconspicuous, colourless, not scented, do not secrete nectar.
- The stamens are numerous, filaments are long, exerted and versatile.
- Anthers produce enormous quantities of pollen grains compared to number of ovules available for pollination. They are minute, light and dry so that they can be carried to long distances by wind.
- In some plants anthers burst violently and release the pollen into the air. Example: *Urtica*.
- Stigmas are comparatively large, protruding, sometimes branched and feathery, adapted to catch the pollen grains. Generally single ovule is present.

- Plant produces flowers before the new leaves appear, so the pollen can be carried without hindrance of leaves.

Pollination in Maize (*Zea mays*): The maize is monoecious and unisexual. The male inflorescence (tassel) is borne terminally and female inflorescence (cob) laterally at lower levels. Maize pollens are large and heavy and cannot be carried by light breeze. However, the mild wind shakes the male inflorescence to release the pollen which falls vertically below. The female inflorescence has long stigma (silk) measuring upto 23 cm in length, which projects beyond leaves. The pollens drop from the tassel is caught by the stigma (Figure 1.15).

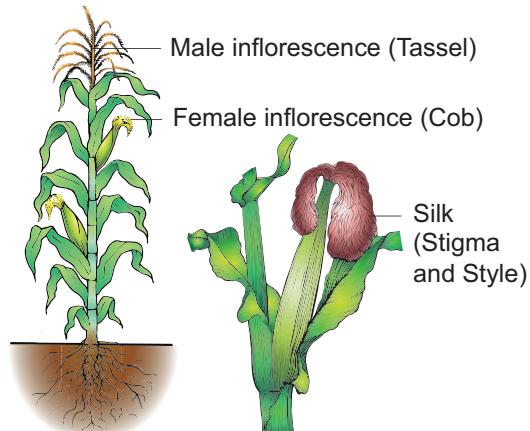


Figure 1.15 Pollination in *Zea mays*

2. Hydrophily: Pollination by water is called hydrophily and the flowers pollinated by water are said to be **hydrophilous** (Example: *Vallisneria*, *Hydrilla*). Though there are a number of aquatic plants, only in few plants pollination takes place by water. The floral envelop of hydrophilous plants are reduced or absent. In water plants like *Eichhornia* and water lilly pollination takes place through wind or by insects. There are two types of hydrophily, Epiphydrophily and Hypohydrophily. In most of the hydrophilous flowers, the pollen grains possesses mucilage covering which protects them from wetting.

a. Epiphydrophily: Pollination occurs at the water level. Examples: *Vallisneria spiralis*, *Elodea*.



Pollination in *Vallisneria spiralis*: It is a dioecious, submerged and rooted hydrophyte. The female plant bears solitary flowers which rise to the surface of water level using a long coiled stalk at the time of pollination. A small cup shaped depression is formed around the female flower on the surface of the water. The male plant produces male flowers which get detached and float on the surface of the water. As soon as a male flower comes in closer to a female flower, it gets settled in the depression and contacts with the stigma thus bringing out pollination. Later the stalk of the female flower coils and brings back the flower from surface to under water where fruits are produced. (Figure 1.16).

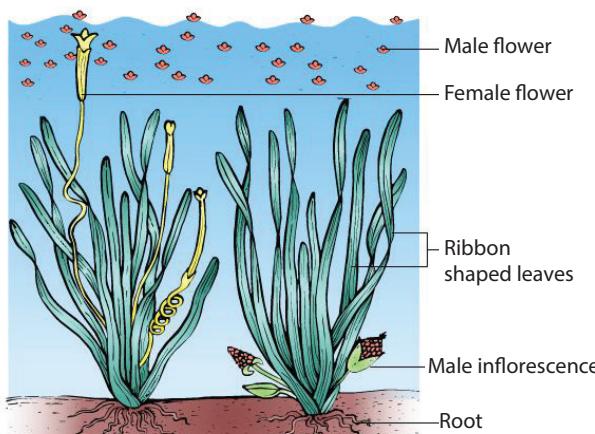


Figure 1.16 Pollination in *Vallisneria*

Activity

Visit to a nearby park and observe the different flowers. Record the adaptations or modifications found in the flowers for different types of pollination.

b. Hypohydrophily: Pollination occurs inside the water. Examples: *Zostera marina* and *Ceratophyllum*.

Zostera marina is a submerged marine sea grass and pollination takes place under water. The pollen grains are long, needle like. They are shed under water. The specific gravity of the pollen is same as that of sea water, so that, the pollen floats freely at any depth. The stigma is very large

and long. The pollen comes in contact with the stigma and gets coiled around the stigma thus effecting pollination.

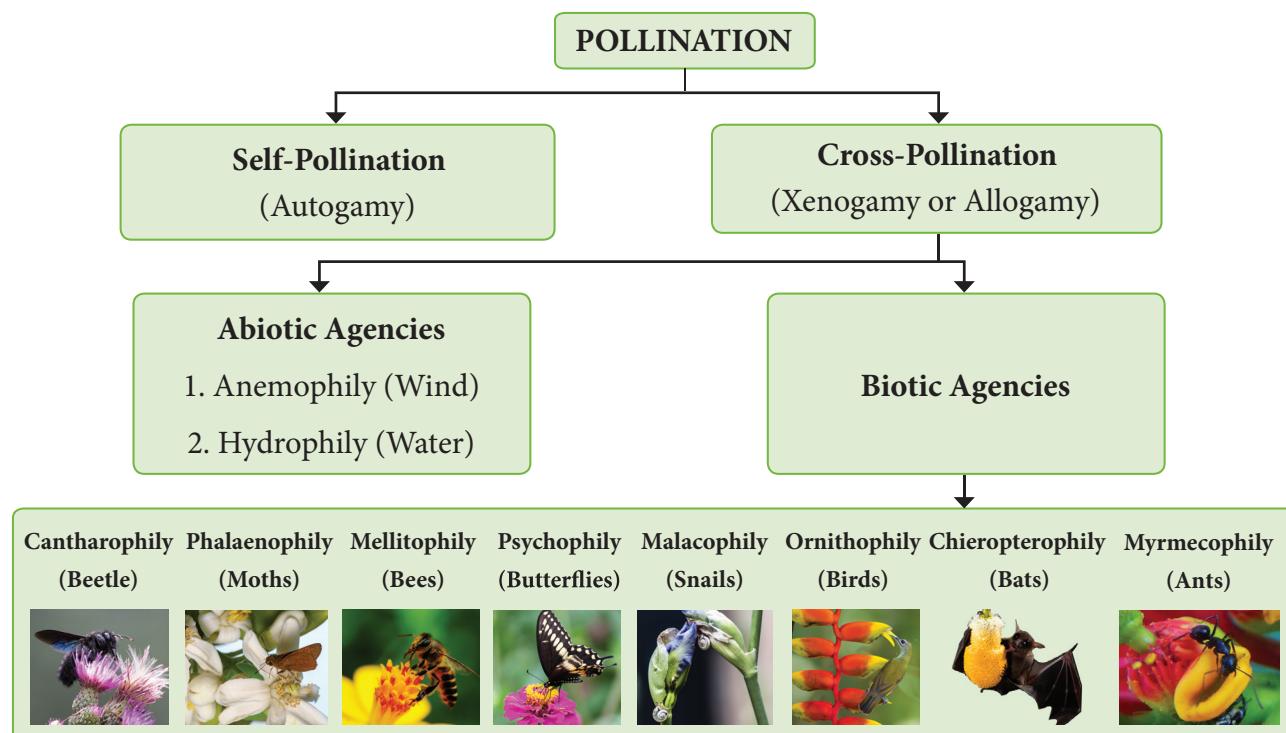
3. Zoophily: Pollination by the agency of animals is called zoophily and flowers are said to be zoophilous. Animals that bring about pollination may be birds, bats, snails and insects. Of these, insects are well adapted to bring pollination. Larger animals like primates (lemurs), arboreal rodents, reptiles (gecko lizard and garden lizard) have also been reported as pollinators.

A. Ornithophily: Pollination by birds is called Ornithophily. Some common plants that are pollinated by birds are *Erythrina*, *Bombax*, *Syzygium*, *Bignonia*, *Sterlitzia* etc., Humming birds, sun birds, and honey eaters are some of the birds which regularly visit flowers and bring about pollination.

The ornithophilous flowers have the following characteristic features:

- The flowers are usually large in size.
- The flowers are tubular, cup shaped or urn-shaped.
- The flowers are brightly coloured, red, scarlet, pink, orange, blue and yellow which attracts the birds.
- The flowers are scentless and produce nectar in large quantities. Pollen and nectar form the floral rewards for the birds visiting the flowers.
- The floral parts are tough and leathery to withstand the powerful impact of the visitors.

B. Cheiropterophily: Pollination carried out by bats is called cheiropterophily. Some of the common cheiropterophilous plants are *Kigelia africana*, *Adansonia digitata*, etc., Bats are nocturnal and are attracted by the odour of the flowers that open at or after dusk. The cheiropterophilous plants have flowers borne singly or in clusters quite away from the leaves and branches either on the long peduncle or on the trunk or branches. The flowers produce large quantities of nectar.



Pollination in *Adansonia digitata*: In this plant, the ball of stamens and the stigma project beyond the floral envelope. The bat holds the flower by clasping the stamen ball to its breast. While taking nectar its breast becomes laden with numerous pollen grains, some of which get deposited on the stigma of the flower when it visits next.

C. Malacophily: Pollination by slugs and snails is called malacophily. Some plants of Araceae are pollinated by snails. Water snails crawling among *Lemna* pollinate them.

D. Entomophily: Pollination by insects is called **Entomophily**. Pollination by ant is called **myrmecophily**. Insects that are well adapted to bring pollination are bees, moths, butterflies, flies, wasps and beetles. Of the insects, bees are the main flower visitors and dominant pollinators. Insects are chief pollinating agents and majority of angiosperms are adapted for insect pollination. It is the most common type of pollination.

The characteristic features of entomophilous flowers are as follows:

- Flowers are generally large or if small they are aggregated in dense inflorescence.

Example: Asteraceae flowers.

- Flowers are brightly coloured. The adjacent parts of the flowers may also be brightly coloured to attract insect. For example in *Poinsettia* and *Bougainvillea* the bracts become coloured.
- Flowers are scented and produce nectar.
- Flowers in which there is no secretion of nectar, the pollen is either consumed as food or used in building up of its hive by the honeybees. Pollen and nectar are the floral rewards for the visitors.
- Flowers pollinated by flies and beetles produce foul odour to attract pollinators.
- In some flowers juicy cells are present which are pierced and the contents are sucked by the insects.

Pollination in *Salvia* (Lever mechanism):

The flower of *Salvia* is adapted for Bee pollination. The flower is protandrous and the corolla is bilabiate with 2 stamens. A lever mechanism helps in pollination. Each anther has an upper fertile lobe and lower sterile lobe which is separated by a long connective which helps the anthers to swing freely. When a bee



visits a flower, it sits on the lower lip which acts as a platform. It enters the flower to suck the nectar by pushing its head into the corolla. During the entry of the bee into the flower the body strikes against the sterile end of the connective. This makes the fertile part of the stamen to descend and strike at the back of the bee. The pollen gets deposited on the back of the bee. When it visits another flower, the pollen gets rubbed against the stigma and completes the act of pollination in *Salvia* (Figure 1.17a). Some of the other interesting pollination mechanisms found in plants are a) Trap mechanism (*Aristolochia*); Pit fall mechanism (*Arum*); Clip or translator mechanism (*Asclepiadaceae*) and Piston mechanism (*Papilionaceae*).

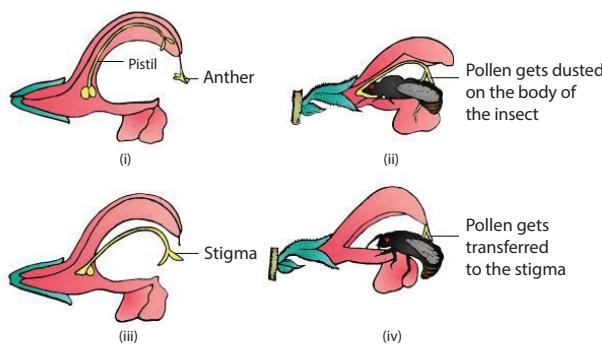


Figure 1.17 (a) Pollination in *Salvia* - Lever mechanism

Pollination in *Calotropis* (Translator mechanism)

This mechanism is found in members of Asclepiadaceae (Apocynaceae as per APG system of classification). The flowers are bisexual with 5 stamens forming **gynostegium** (union of stigma with the androecium). The stigma is large and 5-angled and is receptive on the underside. Each stamen at its back possesses a brightly coloured hood like outgrowth enclosing horn shaped nectar. The pollen in each anther lobe of a stamen unites into a mass, forming a pollinium. Pollinia are attached to a clamp or clip like sticky structure called **corpusculum**. The filamentous or thread like part arising from each pollinium is called **retinaculum**. The

whole structure looks like inverted letter 'Y' and is called **translator**. When the insect visits the flower for nectar, the translator gets attached to the proboscis or leg of the visitor. During the visit to the next flower the pollinia come in contact with the receptive stigma carrying out pollination.

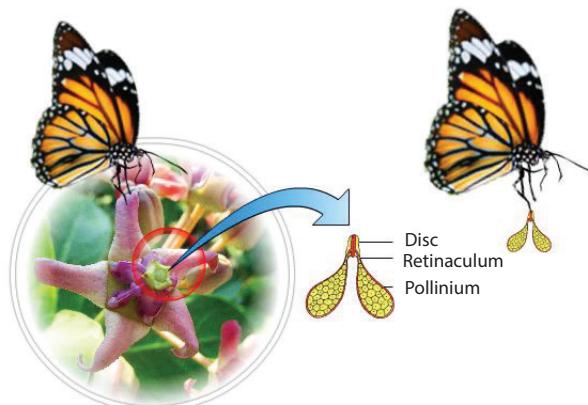


Figure 1.17 (b) Pollination in *Calotropis* - Translator mechanism

Pollination in *Aristolochia* (Trap mechanism)

A special mechanism to accomplish pollination called trap mechanism is found in *Aristolochia*. The flowers are axillary and perianth is tubular with a hood at the top. The basal region is swollen and possesses gynostegium. The gynostegium has six anthers.

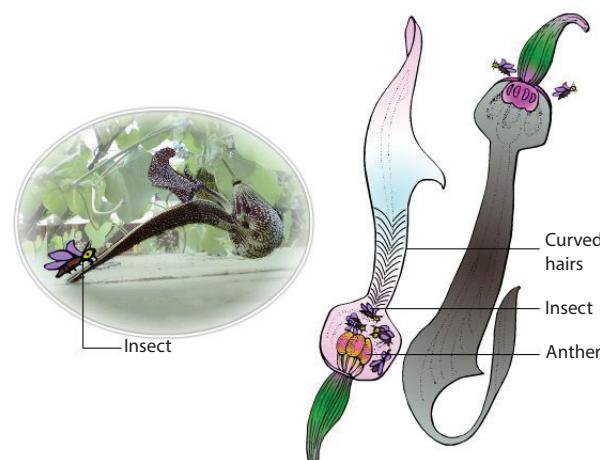


Figure 1.17 (c) Pollination in *Aristolochia* - Trap mechanism

The inner wall of tubular middle part of the perianth is slippery and lined with stiff hairs which are pointed downwards. The young flowers are erect. During this stage small flies



enter and could not escape because they are trapped by the hairs. As soon as the anthers of the flower ripe, the hairs wither and flower bends down. The flies escape with pollen and enter another flower where it dusts pollen on the stigma bringing out pollination.

Advantages of self-pollination:

- Pollination is almost certain in bisexual flowers.
- When the members of the species are uncommon and are separated by large distances, the plant has to depend on self-pollination.
- If all the chances of cross-pollination fails, self-pollination will take place and prevent the extinction of the species.

Disadvantages of self-pollination:

- Continuous self-pollination, generation after generation results in weaker progeny.
- Chances of producing new species and varieties are meager.

Advantages of cross-pollination:

- It always results in bringing out much healthier offsprings.
- Germination capacity is much better.
- New varieties may be produced.
- The adaptability of the plants to their environment is better.

Disadvantages of cross-pollination:

- Depend on external agencies for the pollination and the process is uncertain.
- Various devices have to be adopted to attract pollinating agents.

Significance of Pollination

- Pollination is a pre-requisite for the process of fertilisation. Fertilisation helps in the formation of fruits and seeds.
- It brings the male and female gametes closer for the process of fertilisation.
- Cross-pollination introduces variations in plants due to the mixing up of different genes. These variations help the plants to adapt to the environment and results in speciation.



Pollination – A composite event

Pollination provides information about evolution, ecology, animal learning and foraging behaviour. Flowers not only supply nectar but also provide microclimate, site and shelter for egg laying insects. The association of insects benefits the flower by getting pollinated and ensures the propagation of its own progeny. The floral parts are well modified in shape, size to attract the pollinators to accomplish pollination.

The relationship between *Yucca* and moth (*Tegeticula yuccasella*) is an example for obligate mutualism. The moth bores a hole in the ovary of the flower and lays eggs in it. Then it collects pollen and pushes it in the form of balls down the hollow end of the stigma. Fertilization takes place and seeds develop. Larvae feed on developing seeds. Some seeds remain unconsumed for the propagation of the plant species. It is interesting that the moth cannot survive without *Yucca* flowers and the plant fails to reproduce sexually without the moth.



Bee Orchid

Similarly in *Amorphophallus*, flowers apart from providing floral rewards, also forms safe site for laying eggs. Many visitors consume pollen and nectar and do not help in pollination. They are called pollen / nectar robbers.

In Bee orchid (*Ophrys*) the morphology of the flower mimics that of female wasp (*Colpa*). The male wasp mistakes the flowers for a female wasp and tries to copulate. This act of pseudocopulation helps in pollination. The pollination in Fig (*Ficus carica*) by the Wasp (*Blastophaga psenes*) is also an example for similar Plant – insect interaction.



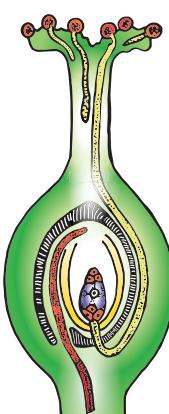
1.5 Fertilization

The fusion of male and female gamete is called **fertilization**. Fertilization in angiosperms is **double fertilization** type.

Events of fertilization

The stages involved in double fertilization are:- germination of pollen to form pollen tube in the stigma; growth of pollen tube in the style; direction

of pollen tube towards the micropyle of the ovule; entry of the pollen tube into one of the synergids of the embryo sac, discharge of male gametes; syngamy and triple fusion. The events from pollen deposition on the stigma to the entry of pollen tube in to the ovule is called **pollen-pistil interaction**. It is a dynamic process which involves recognition of pollen and to promote or inhibit its germination and growth.



Pollen on the stigma

In nature, a variety of pollens fall on the receptive stigma, but all of them do not germinate and bring out fertilization. The receptive surface of the stigma receives the pollen. If the pollen is compatible with the stigma it germinates to form a tube. This is facilitated by the stigmatic fluid in **wet stigma** and pellicle in **dry stigma**. These two also decide the incompatibility and compatibility of the pollen through **recognition-rejection protein reaction** between the pollen and stigma surface. Sexual incompatibility may exist between different species (interspecific) or between members of the same species (intraspecific). The latter is called self-incompatibility. The first visible change in the pollen, soon after it lands on stigma is hydration. The pollen wall proteins are released from the surface. During the germination of pollen its entire content moves into the pollen tube. The growth is restricted to the tip of the tube and all the cytoplasmic contents move to the tip

region. The remaining part of the pollen tube is occupied by a vacuole which is cut off from the tip by callose plug. The extreme tip of pollen tube appears hemispherical and transparent when viewed through the microscope. This is called **cap block**. As soon as the cap block disappear the growth of the pollen tube stops.

Pollen tube in the style

After the germination the pollen tube enters into the style from the stigma. The growth of the pollen tube in the style depends on the type of style.

Types of style

There are three types of style a) Hollow or open style b)solid style or closed style c) semi-solid or half closed style.

Hollow style (Open style): It is common among monocots. A hollow canal running from the stigma to the base of the style is present. The canal is lined by a single layer of glandular canal cells (Transmitting tissue).They secrete mucilaginous substances. The pollen tube grows on the surface of the cells lining the stylar canal. The canal is filled with secretions which serve as nutrition for growing pollen tubes and also controlling incompatibility reaction between the style and pollen tube. The secretions contain carbohydrates, lipids and some enzymes like esterases, acid phosphatases as well as compatibility controlling proteins.

Solid style (Closed type): It is common among dicots. It is characterized by the presence of central core of elongated, highly specialised cells called transmitting tissue.This is equivalent to the lining cells of hollow style and does the same function. Its contents are also similar to the content of those cells. The pollen tube grows through the intercellular spaces of the transmitting tissue.

Semi-solid style (half closed type): This is intermediate between solid and open type.

There is a difference of opinion on the nature of transmitting tissue. Some authors



consider that it is found only in solid styles while others consider the lining cells of hollow style also has transmitting tissue.

Entry of pollen tube into the ovule: There are three types of pollen tube entry into the ovule (Figure 1.18).

Porogamy: when the pollen tube enters through the micropyle.

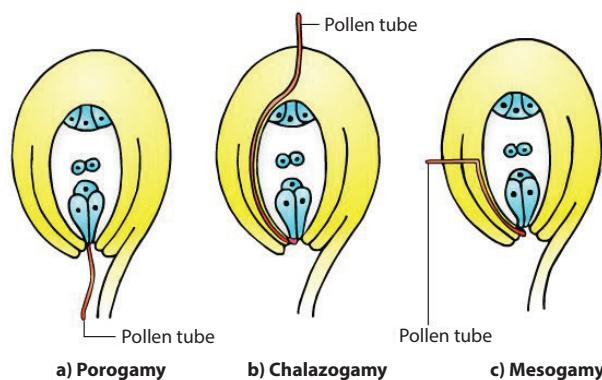


Figure 1.18 Path of pollen tube entry into the ovule

Chalazogamy: when the pollen tube enters through the chalaza.

Mesogamy: when the pollen tube enters through the integument.

Entry of pollen tube into embryo sac: Irrespective of the place of entry of pollen tube into ovule, it enters the embryo sac at the micropylar end. The pollen enters into embryo sac directly into one of the synergids.

The growth of pollen tube towards the ovary, ovule and embryo sac is due to the presence of chemotropic substances. The pollen tube after travelling the whole length of the style enters into the ovary locule where it is guided towards the micropyle of the ovule by a structure called **obturator** (See Do you know). After reaching the embryo sac, a pore is formed in pollen tube wall at its apex or just behind the apex. The content of the pollen tube (two male gametes, vegetative nucleus and cytoplasm) are discharged into the synergids into which pollen tube enters. The pollen tube does not grow beyond it, in the embryo sac. The tube nucleus disorganizes.



Obturator may originate from different regions of the ovule (Placenta – Euphorbiaceae, Funiculus – Anacardiaceae, Style – Thymelaeaceae and Ovary wall – Ottelia alismoides)

1.5.1 Double fertilization and triple fusion

S.G. Nawaschin and L. Guignard in 1898 and 1899, observed in *Lilium* and *Fritillaria* that both the male gametes released from a male gametophyte are involved in the fertilization. They fertilize two different components of the embryo sac. Since both the male gametes are involved in fertilization, the phenomenon is called **double fertilization** and is unique to angiosperms. One of the male gametes fuses with the egg nucleus (syngamy) to form **Zygote**. (Figure 1.19)

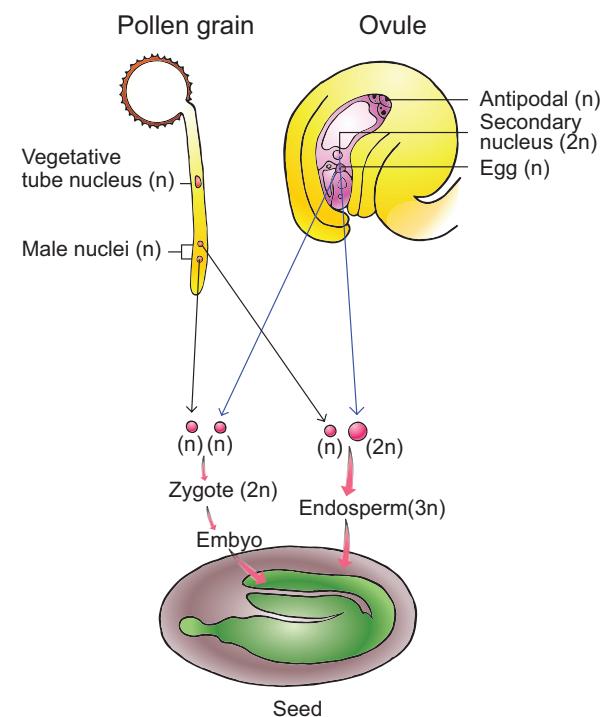


Figure 1.19 Fertilization in Angiosperms

The second gamete migrates to the central cell where it fuses with the **polar nuclei** or their fusion product, the secondary nucleus and forms the **primary endosperm nucleus (PEN)**.



Since this involves the fusion of three nuclei, this phenomenon is called **triple fusion**. This act results in endosperm formation which forms the nutritive tissue for the embryo.

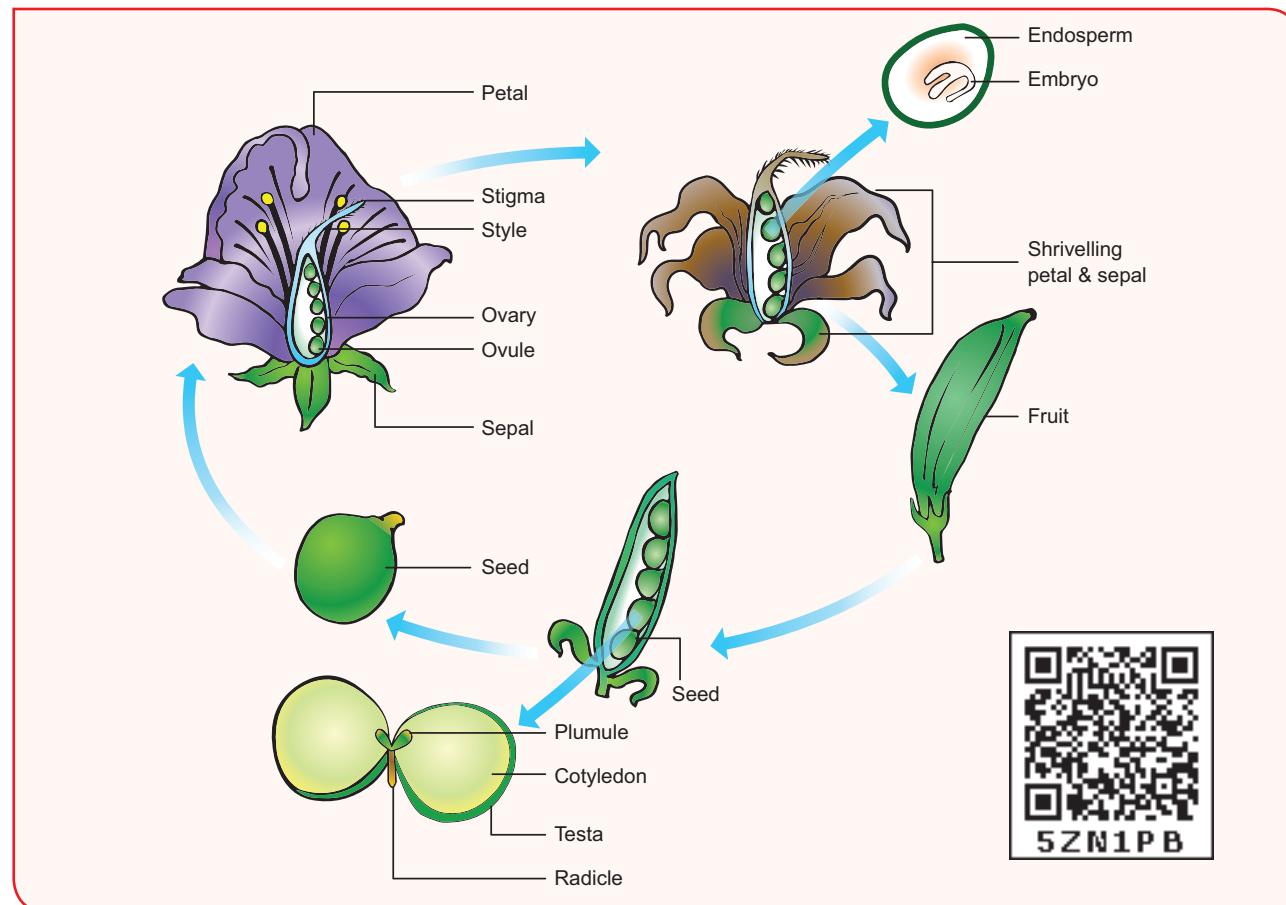
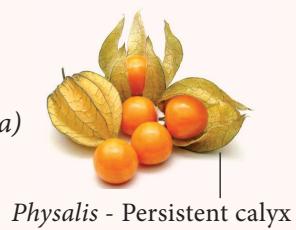


Figure 1.20 Post Fertilization changes in the flower of an angiosperms

More to Know

- The receptacle becomes fleshy and edible around the fruit enclosing the seeds as in *Pyrus malus* (apple)
- The calyx may persist and enlarge (*Solanum melongena*) or may cover the fruit (*Physalis minima*)
- The flower stalk or axis below the gynoecium enlarges into a juicy pear shaped body which is edible (*Anacardium occidentale*). The Perianth becomes fleshy as in Jack fruit.
- The cells present at the tip of the outer integument around the micropyle develop



Anacardium - pedicel (edible)

into a fleshy structure called **caruncle**. (*Ricinus communis*).



Ricinus - Caruncle

- The funiculus develops into a fleshy structure which is often very colourful and called **aril**. (*Myristica* and *Pithecellobium*)



Myristica



Pithecellobium

- The nucellar tissue is either absorbed completely by the developing embryo sac and embryo or small portion may remain as storage tissue. Thus the remnant of nucellar tissue in the seed is called **perisperm**. Example: Black pepper and beet root



1.6 Post Fertilization structure and events

After fertilization, several changes take place in the floral parts up to the formation of the seed (Figure 1.20).

The events after fertilization (endosperm, embryo development, formation of seed, fruits) are called post fertilization changes.

Parts before fertilization	Transformation after fertilization
Sepals, petals, stamens, style and stigma	Usually wither and fall off
Ovary	Fruit
Ovule	Seed
Egg	Zygote
Funicle	Stalk of the seed
Micropyle (ovule)	Micropyle of the seed(facilitates O ₂ and water uptake)
Nucellus	Perisperm
Outer integument of ovule	Testa (outer seed coat)
Inner integument	Tegmen (inner seed coat)
Synergid cells	Degenerate
Secondary nucleus	Endosperm
Antipodal cells	Degenerate

Endosperm

The primary endosperm nucleus (PEN) divides immediately after fertilization but before the zygote starts to divide, into an endosperm. The primary endosperm nucleus is the result of triple fusion (two polar nuclei and one sperm nucleus) and thus has $3n$ number of chromosomes. It is a nutritive tissue and regulatory structure that nourishes the developing embryo. Depending upon the mode of development three types of endosperm are recognized in angiosperms. They are nuclear endosperm, cellular endosperm and helobial endosperm (Figure 1.21).

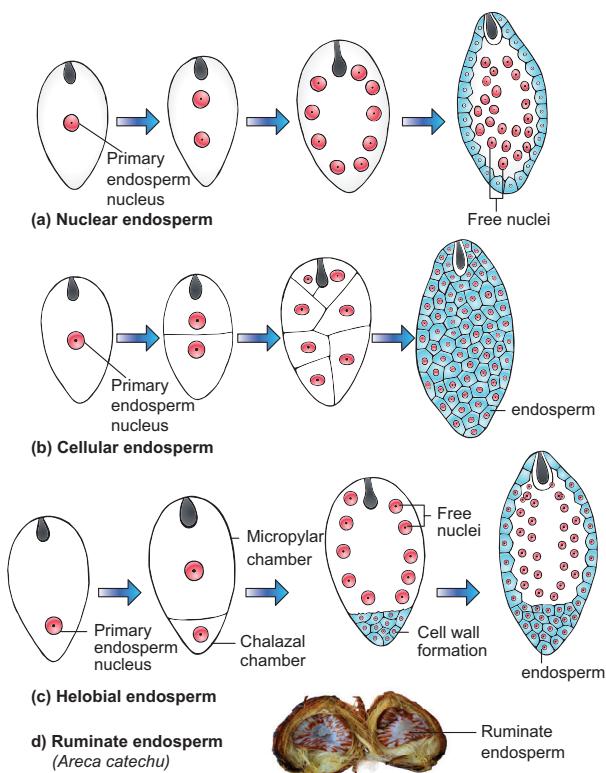


Figure 1.21 Types of Endosperm

Nuclear endosperm: In the nuclear type, the Primary endosperm nucleus (PEN) divides into two without any wall formation. The subsequent division of these two nuclei are free nuclear so that the endosperm consists of only free nuclei and cytoplasm around them. The nuclei may either remain free or may become separate by walls in later stages. Examples: *Coccinia*, *Capsella* and *Arachis*.



Aleurone tissue consists of highly specialised cells of one or few layers which are found around the endosperm of cereals (barley and maize). Aleurone grain contains sphaerosomes. During seed germination cells secrete certain hydrolytic enzymes like amylases, proteases which digest reserved food material present in the endosperm cells.

Cellular endosperm: In this type the primary endosperm nucleus(PEN) divides into 2 nuclei which are immediately followed by a wall formation. Subsequent divisions are also followed by walls. Examples: *Adoxa*, *Helianthus* and *Scoparia*.



Helobial endosperm: In helobial type, the primary endosperm nucleus (PEN) moves towards the base of the embryo sac where it divides into two nuclei. These 2 nuclei are separated by a wall to form a large micropylar chamber and a small chalazal chamber. The nucleus of the micropylar chamber undergoes several free nuclear divisions whereas that of the chalazal chamber may or may not divide. Examples : *Hydrilla* and *Vallisneria*

The endosperms may either be completely consumed by the developing embryo or it may persist in the mature seeds. Those seeds without endosperms are called non- endospermous or ex- albuminous seeds. Examples: Pea, Groundnut and Beans. Those seeds with endosperms are called endospermous or albuminous seeds. The endosperms in these seeds supply nutrition to the embryo during seed germination. Examples: Paddy, Coconut and Castor.

Ruminate endosperm: The endosperm with irregularity and unevenness in its surface forms ruminate endosperm (Example: *Areca catechu*). The activity of the seed coat or the endosperm itself results in this type of endosperm. The unequal radial elongation of the layer of seed coat results in the rumination of endosperm in *Passiflora*. In Annonaceae and Aristolochiaceae definite ingrowth or infolding of the seed coat produces ruminate endosperm. The irregular surface of the seed coat makes endosperm ruminate in *Myristica*.

Functions of endosperm:

- It is the nutritive tissue for the developing embryo.
- In majority of angiosperms, the zygote divides only after the development of endosperm.
- Endosperm regulates the precise mode of embryo development.

Endosperm haustoria

Another interesting feature of the endosperm is the presence of haustoria. In the case of helobial endosperm the chalazal chamber itself acts as a haustorial structure.

In cellular and nuclear endosperm special structures are produced towards the micropylar, chalazal, both micropylar and chalazal which may be in lateral direction depending on the species. These absorb nutrients from other outer tissue or from ovary tissue and supply them to the growing embryo.



Coconut milk is a basic nutrient medium which induces the differentiation of embryo (embryoids) and plantlets from various plant tissues. Coconut water from tender coconut is free-nuclear endosperm and white kernel part is cellular.

Embryogenesis

Development of Dicot embryo

The development of Dicot embryo (*Capsella bursa-pastoris*) is of Onagrad or crucifer type. The embryo develops at micropylar end of embryo sac.

The Zygote divides by a transverse division forming **upper or terminal cell** and **lower or basal cell**. The basal cell divides transversely and the terminal cell divides vertically to form a four celled proembryo. A second vertical division right angle to the first one takes place in terminal cell forming a four celled stage called **quadrant**. A transverse division in the quadrant results in eight cells arranged in two tiers of four each called **octant stage**.

The upper tier of four cells of the octant is called **epibasal** or anterior octant and the lower tier of four cells constitute **hypobasal** or posterior octants. A periclinal division in the octants results in the formation of 16 celled stage with eight cells in the outer and eight in the inner.

The outer eight cells represent the **dermatogen** and undergoes anticlinal division to produce epidermis. The inner eight cells divide by vertical and transverse division to form outer layer of **periblem** which give rise to cortex and a central region of **pleurome** which forms stele.

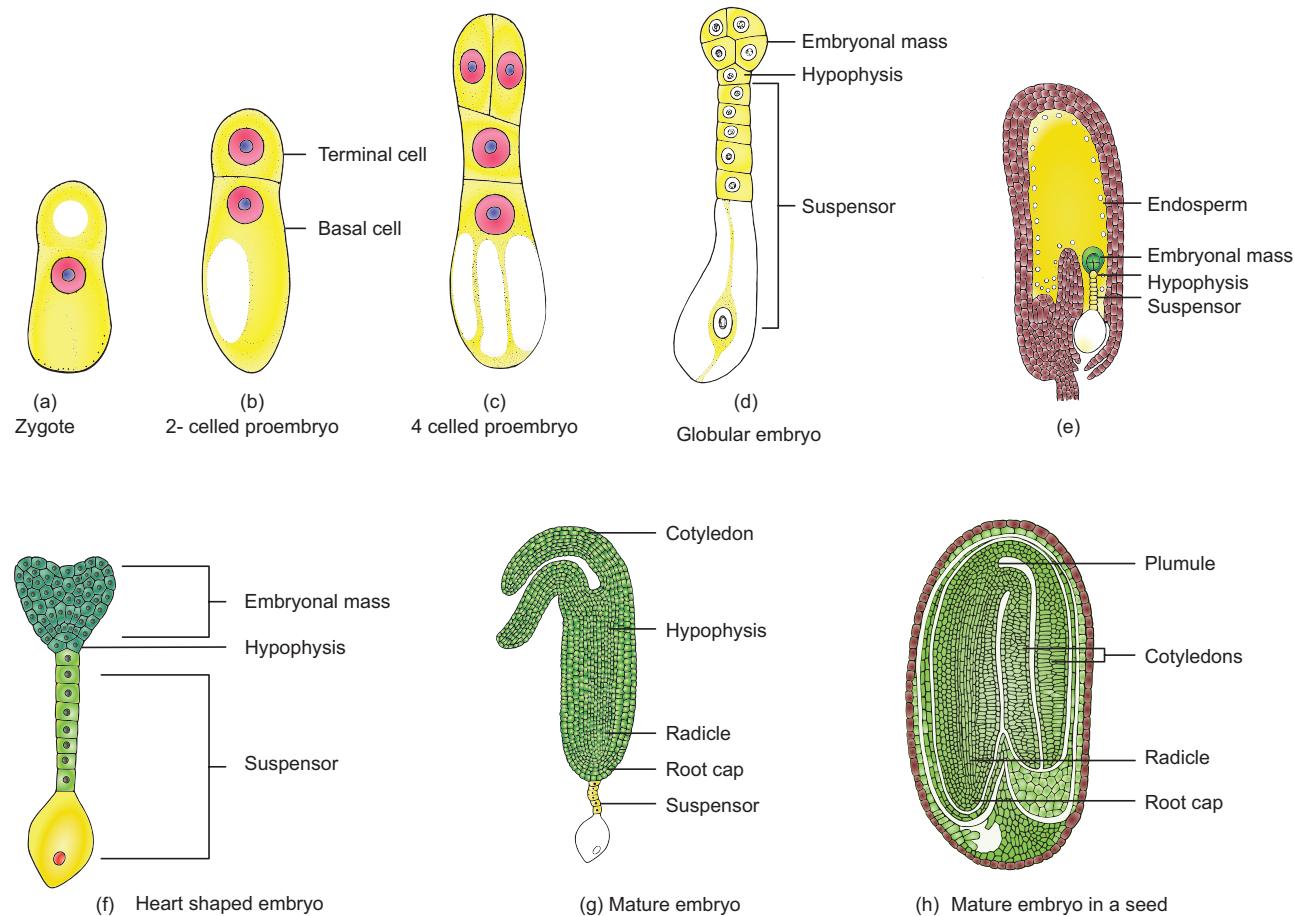


Figure 1.22 Development of Dicot embryo (*Capsella bursa-pastoris*)

During the development, the two cells of the basal cell undergoes several transverse division to form a six to ten celled **suspensor**. The embryo at this stage become **globular** and the suspensor helps to push the embryo deep into the endosperm. The uppermost cell of the suspensor enlarge to form a **haustorium**. The lowermost cell of the suspensor is called **hypophysis**. A transverse division and two vertical division right angle to each other of hypophysis results in the formation of eight cells. The eight cells are arranged in two tiers of four cells each. The upper tier give rise to root cap and epidermis. At this stage embryo proper appears heart shaped, cell divisions in the **hypocotyl** and cotyledon regions of the embryo proper results in elongation. Further development results in curved horse shoe shaped embryo in the embryo sac. The mature embryo has a **radicle**, two **cotyledons** and a **plumule** (Figure 1.22).

Activity

Collect the fruits of *Tridax* (*Cypsella*). Using a needle dissect out the content, separate the embryo and observe different stages of dicot embryo – globular, torpedo, heart shaped under a dissection microscope.

Seed

The fertilized ovule is called seed and possesses an embryo, endosperm and a protective coat. Seeds may be endospermous (wheat, maize, barley and sunflower) or non endospermous. (Bean, Mango, Orchids and cucurbits).



Fresh weight of an orchid seed may be 20.33 microgram and that of double coconut (*Lodoicea maldivica*) is about 6 kg.



Structure of a *Cicer* seed as an example for Dicot seed

The mature seeds are attached to the fruit wall by a stalk called **funiculus**. The funiculus disappears leaving a scar called **hilum**. Below the hilum a small pore called **micropyle** is present. It facilitates entry of oxygen and water into the seeds during germination. Each seed has a thick outer covering called seed coat. The seed coat is developed from integuments of the ovule. The outer coat is called **testa** and is hard whereas the inner coat is thin, membranous and is called **tegmen**. In Pea plant the tegmen and testa are fused. Two cotyledons laterally attached to the embryonic axis are present. It stores the food materials in pea whereas in other seeds like castor the endosperm contains reserve food and the cotyledons are thin. The portion of embryonal

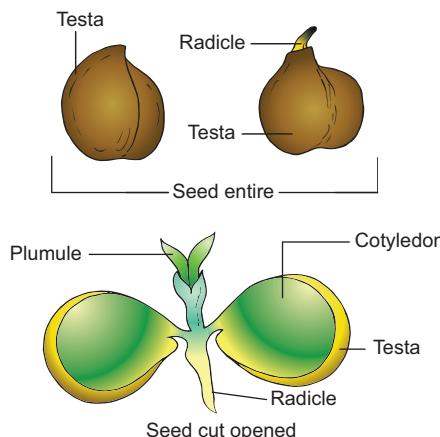


Figure 1.23(a) Dicot seed - *Cicer arietinum*

axis projecting beyond the cotyledons is called **radicle** or embryonic root. The other end of the axis called embryonic shoot is the **plumule**. Embryonal axis above the level of cotyledon is called **epicotyl** whereas the cylindrical region between the level of cotyledon is called **hypocotyl** (Figure 1.23 a). The epicotyl terminates in plumule whereas the hypocotyl ends in radicle.

Structure of *Oryza* seed as an example for Monocot seed

The seed of paddy is one seeded and is called

Caryopsis. Each seed remains enclosed by a brownish husk which consists of glumes arranged in two rows. The seed coat is a brownish, membranous layer closely adhered to the grain. Endosperm forms the bulk of the grain and is the storage tissue. It is separated from embryo by a definite layer called **epithelium**. The embryo is small and consists of one shield-shaped cotyledon known as **scutellum** present towards lateral side of embryonal axis. A short axis with plumule and radicle protected by the **root cap** is present. The plumule is surrounded by a protective sheath called **coleoptile**. The radicle including root cap is also covered by a protective sheath called **coleorrhiza**. The scutellum supplies the growing embryo with food material absorbed from the endosperm with the help of the epithelium (Figure 1.23 b).

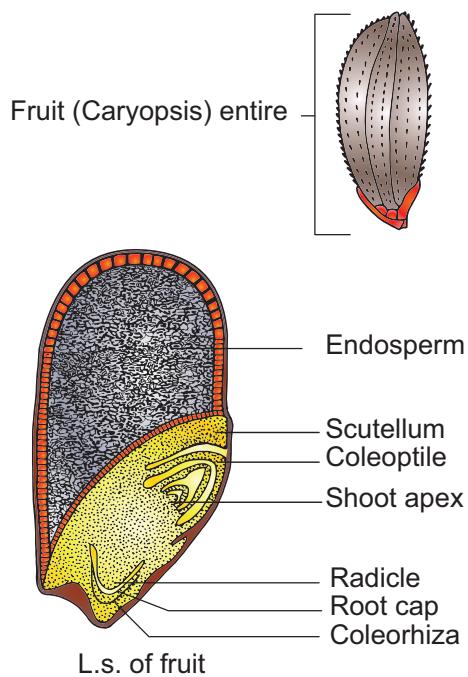


Figure 1.23(b) Monocot seed - *Oryza sativa*

Activity

Soak seeds of green gram for three hours. Drain the water and place few seeds in a clean tray containing moist cotton or filter paper. Allow the seeds to sprout. Collect the sprouted seeds, cut open and observe the parts. Record your observation.



1.7 Apomixis

Reproduction involving fertilization in flowering plants is called amphimixis and wherever reproduction does not involve union of male and female gametes is called apomixis.

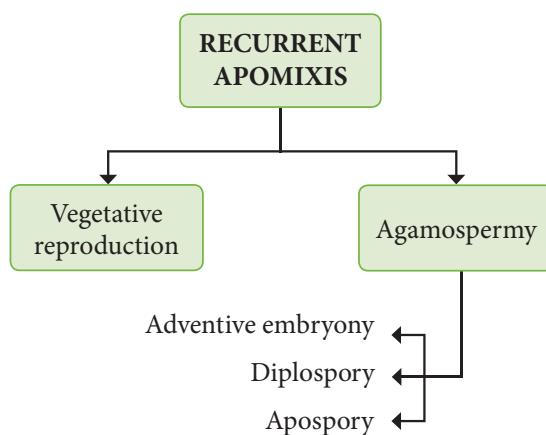
The term Apomixis was introduced by Winkler in the year 1908. It is defined as the substitution of the usual sexual system (Amphimixis) by a form of reproduction which does not involve meiosis and syngamy.

Maheswari (1950) classified Apomixis into two types - Recurrent and Non recurrent

Recurrent apomixis: It includes vegetative reproduction and agamospermy

Non recurrent apomixis: Haploid embryo sac developed after meiosis, develops into a embryo without fertilization.

The outline classification of Recurrent apomixis is given below.



Vegetative reproduction: Plants propagate by any part other than seeds

Bulbils – *Fritillaria imperialis*; Bulbs – *Allium*; Runner – *Mentha arvensis*; Sucker – *Chrysanthemum*

Agamospermy: It refers to processes by which Embryos are formed by eliminating meiosis and syngamy.

Adventive embryony

An Embryo arises directly from the diploid

sporophytic cells either from nucellus or integument. It is also called **sporophytic budding** because gametophytic phase is completely absent. Adventive embryos are found in *Citrus* and *Mangifera*

Diplosropy (Generative apospory): A diploid embryo sac is formed from megasporangium mother cell without a regular meiotic division Examples. *Eupatorium* and *Aerva*.

Apospory: Megasporangium mother cell undergoes the normal meiosis and four megasporangia formed gradually disappear. A nucellar cell becomes activated and develops into a diploid embryo sac. This type of apospory is also called somatic apospory. Examples *Hieracium* and *Parthenium*.

1.8 Polyembryony

Occurrence of more than one embryo in a seed is called polyembryony (Figure 1.24). The first case of polyembryony was reported in certain oranges by Anton van Leeuwenhoek in the year 1719. Polyembryony is divided into four categories based on its origin.

- Cleavage polyembryony** (Example: Orchids)
- Formation of embryo by cells of the Embryo sac other than egg** (Synergids – *Aristolochia*; antipodals – *Ulmus* and endosperm – *Balanophora*)

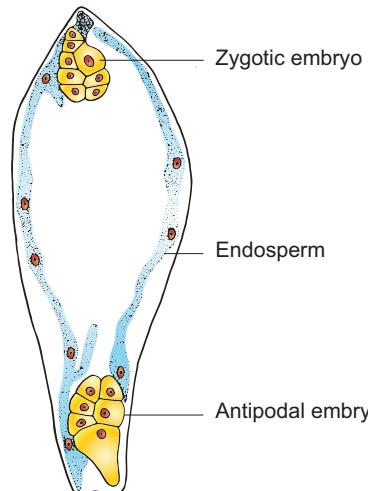


Figure 1.24 : Polyembryony – Embryo sac of *Ulmus glabra* showing zygotic and antipodal embryo



- c. **Development of more than one Embryo sac within the same ovule.** (Derivatives of same MMC, derivatives of two or more MMC – *Casuarina*)
- d. **Activation of some sporophytic cells of the ovule** (Nucellus/ integuments-*Citrus* and *Syzygium*).

Practical applications

The seedlings formed from the nucellar tissue in *Citrus* are found better clones for Orchards. Embryos derived through polyembryony are found virus free.

1.9 Parthenocarpy

As mentioned earlier, the ovary becomes the fruit and the ovule becomes the seed after fertilization. However in a number of cases, fruit like structures may develop from the ovary without the act of fertilization. Such fruits are called **parthenocarpic fruits**. Invariably they will not have true seeds. Many commercial fruits are made seedless. Examples: Banana, Grapes and Papaya.

Nitsch in 1963 classified the parthenocarpy into following types:

Genetic Parthenocarpy: Parthenocarpy arises due to hybridization or mutation
Examples: *Citrus*, *Cucurbita*.

Environmental Parthenocarpy:

Environmental conditions like frost, fog, low temperature, high temperature etc., induce Parthenocarpy. For example, low temperature for 3-19 hours induces parthenocarpy in Pear.

Chemically induced Parthenocarpy:
Application of growth promoting substances like Auxins and Gibberellins induces parthenocarpy.

Significance

- The seedless fruits have great significance in horticulture.
- The seedless fruits have great commercial importance.

- Seedless fruits are useful for the preparation of jams, jellies, sauces, fruit drinks etc.
- High proportion of edible part is available in parthenocarpic fruits due to the absence of seeds.

Summary

Reproduction is one of the attributes of living things. Lower plants, microbes and animals reproduce by different methods (fragmentation, gemma, binary fission, budding, regeneration). Organisms reproduce through asexual and sexual methods. Asexual methods in angiosperms occur through natural or artificial methods. The natural methods take place through vegetative propagules or diaspores. Artificial method of reproduction involves cutting, layering and grafting. Micropropagation is a modern method used to raise new plants.

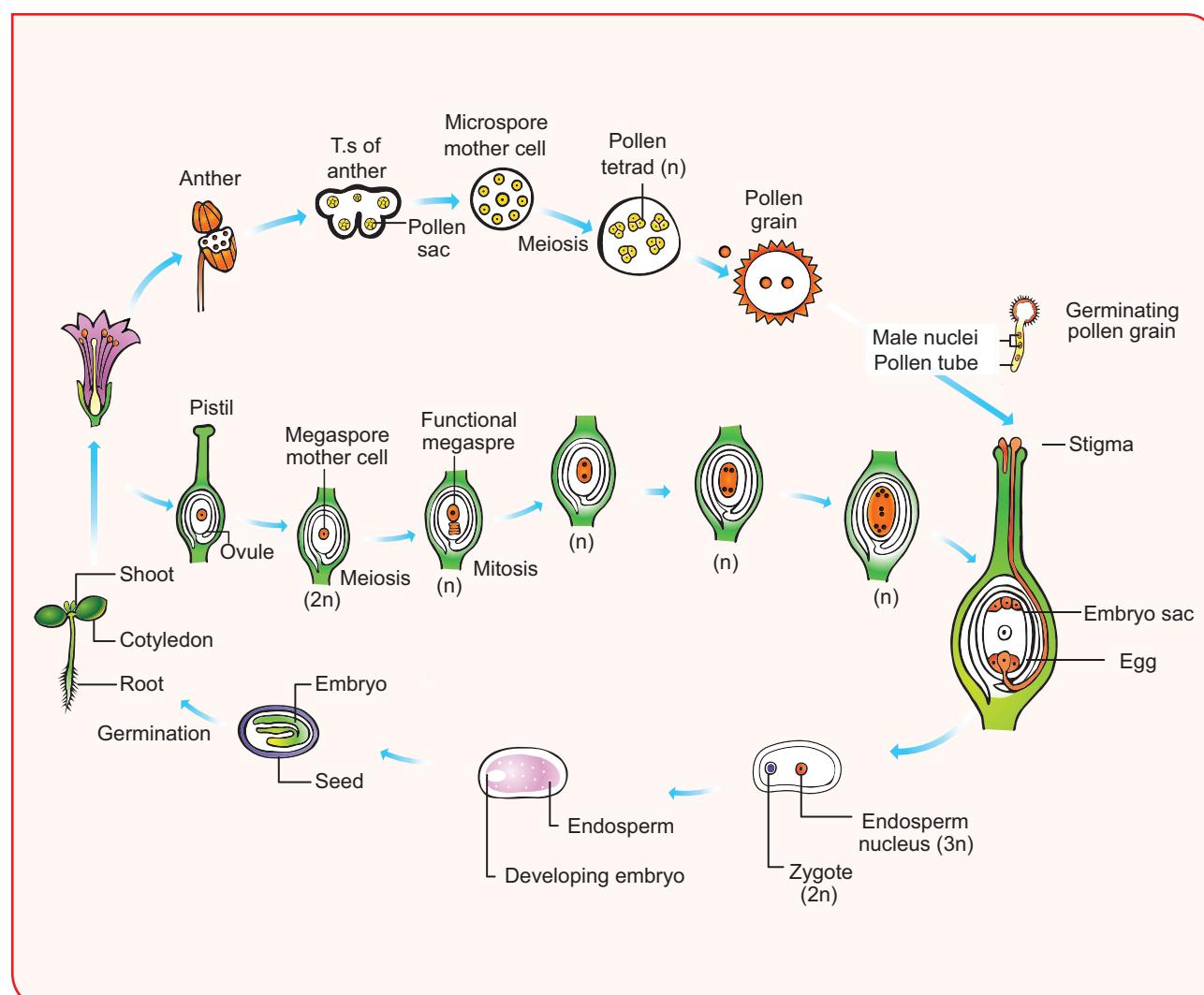
Sexual reproduction includes gametogenesis and fertilization. External fertilization occurs in lower plants like algae but in higher plants internal fertilization takes place. A flower is a modified shoot meant for reproduction. Stamen is the male reproductive part and produces pollen grains. The development of microspore is called microsporogenesis. The microspore mother cell undergoes meiotic division to produce four haploid microspores. In majority of Angiosperms the anther is dithecos and are tetrasporangiate. It possesses epidermis, endothecium, middle layers and tapetum. The hygroscopic nature of endothelial cell along with thin walled stomium helps in the dehiscence of anther. Tapetum nourishes the microspores and also contributes to the wall materials of the pollen grain. Pollen grain is derived from the microspore and possesses thin inner intine and thick outer exine. Sporopollenin is present in exine and is resistant to physiological and biological decomposition. Microspore is the first cell of male gametophyte.



The nucleus of the microspore divides to form a vegetative nucleus and a generative nucleus. The generative nucleus divides to form two male nuclei. Gynoecium is the female reproductive part of a flower and it represents one or more pistils. The ovary bears ovules which are attached to the placenta. There are six major types of ovules. The development of megasporangium from megasporangium mother cell is called megasporogenesis. A monosporic embryo sac (*Polygonum* type) possesses three antipodal cells in chalazal end, Three cells in the micropylar end constituting egg apparatus(1 egg and 2 Synergids) and two polar nucleus fused to form secondary nucleus. Thus, a

7 celled 8 nucleated Embryo sac is present.

The transfer of pollen grains to the stigma of a flower is called pollination. Self-pollination and cross-pollination are two types of pollination. Double fertilization and triple fusion are characteristic features of angiosperms. After fertilization the ovary transforms into a fruit and the ovule becomes a seed. Endosperm is triploid in angiosperms and is of three types – Nuclear, cellular, helobial. Reproduction which doesn't involve meiosis and syngamy is called apomixis. Occurrence of more than one embryo in a seed is called polyembryony. Formation of fruit without the act of fertilization is called parthenocarpy.





Evaluation

1. Choose the correct statement from the following
 - a) Gametes are involved in asexual reproduction
 - b) Bacteria reproduce asexually by budding
 - c) Conidia formation is a method of sexual reproduction
 - d) Yeast reproduce by budding
2. An eminent Indian embryologist is
 - a) S.R.Kashyap
 - b) P.Maheswari
 - c) M.S. Swaminathan
 - d) K.C.Mehta
3. Identify the correctly matched pair
 - a) Tuber - *Allium cepa*
 - b) Sucker - *Pistia*
 - c) Rhizome - *Musa*
 - d) Stolon - *Zingiber*
4. Pollen tube was discovered by
 - a) J.G.Kolreuter
 - b) G.B.Amici
 - c) E.Strasburger
 - d) E.Hanning
5. Size of pollen grain in *Myosotis*
 - a) 10 micrometer
 - b) 20 micrometer
 - c) 200 micrometer
 - d) 2000 micrometer
6. First cell of male gametophyte in angiosperm is
 - a) Microspore
 - b) megaspore
 - c) Nucleus
 - d) Primary Endosperm Nucleus
7. Match the following
 - I) External fertilization i) pollen grain
 - II) Androecium ii)anther wall
 - III) Male gametophyte iii)algae
 - IV) Primary parietal layer iv)stamens
 - a)I-iv;II-i;III-ii;IV-iii
 - b)I-iii;II-iv;III-i;IV-ii
 - c)I-iii;II-iv;III-ii,IV-i
 - d)I-iii;II-i;III-iv;IV-ii
8. Arrange the layers of anther wall from locus to periphery



- a) Epidermis,middle layers, tapetum, endothecium
 - b) Tapetum, middle layers, epidermis, endothecium
 - c) Endothecium, epidermis, middle layers, tapetum
 - d) Tapetum, middle layers endothecium epidermis
9. Identify the incorrect pair
 - a) sporopollenin - exine of pollen grain
 - b) tapetum – nutritive tissue for developing microspores
 - c) Nucellus – nutritive tissue for developing embryo
 - d) obturator – directs the pollen tube into micropyle
10. Assertion : Sporopollenin preserves pollen in fossil deposits
Reason : Sporopollenin is resistant to physical and biological decomposition
 - a) assertion is true; reason is false
 - b) assertion is false; reason is true
 - c) Both Assertion and reason are not true
 - d) Both Assertion and reason are true.
11. Choose the correct statement(s) about tenuinucellate ovule
 - a) Sporogenous cell is hypodermal
 - b) Ovules have fairly large nucellus
 - c) sporogenous cell is epidermal
 - d) ovules have single layer of nucellus tissue
12. The correct order of haploid, diploid and triploid structure in fertilized embryo sac is
 - a) synergid, zygote and PEN
 - b) synergid, antipodal and polar nuclei
 - c) antipodal, synergid and PEN
 - d) synergid, polar nuclei and zygote
13. Which of the following represent megagametophyte
 - a) Ovule
 - b)Embryo sac
 - c)Nucellus
 - d)Endosperm



14. In *Haplopappus gracilis*, number of chromosomes in cells of nucellus is 4. What will be the chromosome number in Primary endosperm cell?
- a) 8 b) 12 c) 6 d) 2
15. Transmitting tissue is found in
- a) Micropylar region of ovule
b) Pollen tube wall
c) Stylar region of gynoecium
d) Integument
16. The scar left by funiculus in the seed is
- a) tegmen b) radicle
c) epicotyl d) hilum
17. A Plant called X possesses small flower with reduced perianth and versatile anther. The probable agent for pollination would be
- a) water b) air
c) butterflies d) beetles
18. Consider the following statement(s)
- i) In Protandrous flowers pistil matures earlier
ii) In Protogynous flowers pistil matures earlier
iii) Herkogamy is noticed in unisexual flowers
iv) Distyly is present in *Primula*
- a) i and ii are correct
b) ii and iv are correct
c) ii and iii are correct
d) i and iv are correct
19. Ruminant endosperm is found in
- a) *Cocos* b) *Areca*
c) *Vallisneria* d) *Arachis*
20. Coelorrhiza is found in
- a) Paddy b) Bean
c) Pea d) *Tridax*
21. Caruncle develops from
- a) funicle b) nucellus
c) integument d) embryo sac
22. Parthenocarpic fruits lack
- a) Endocarp b) Epicarp
c) Mesocarp d) seed
23. In majority of plants pollen is liberated at
- a) 1 celled stage b) 2 celled stage
c) 3 celled stage d) 4 celled stage
24. What is reproduction?
25. Mention the contribution of Hofmeister towards Embryology.
26. List out two sub-aerial stem modifications with example.
27. What is layering?
28. What are clones?
29. How do *Dioscorea* reproduce vegetatively?
30. A detached leaf of *Bryophyllum* produces new plants. How?
31. Differentiate Grafting and Layering.
32. Write short notes on approach grafting.
33. "Tissue culture is the best method for propagating rare and endangered plant species" - Discuss.
34. Distinguish mound layering and air layering.
35. List down the advantages of conventional methods.
36. Explain the conventional methods adopted in vegetative propagation of higher plants.
37. Highlight the milestones from the history of plant embryology.
38. Discuss the importance of Modern methods in reproduction of plants.
39. Differentiate Secretary and invasive tapetum.
40. What is Cantharophily.
41. List any two strategy adopted by bisexual flowers to prevent self-pollination.
42. What is endothelium.
43. Name the cell which divides to form male nuclei.
44. 'The endosperm of angiosperm is different from gymnosperm'. Do you agree. Justify your answer.



45. Define the term Diplospory.
46. What is polyembryony. How it can commercially exploited.
47. Do you think parthenocarpy and apomixis are different process. Justify?
48. Why does the zygote divides only after the division of Primary endosperm cell.
49. What is Mellitophily?
50. Give examples for Helobial endosperm.
51. 'Endothecium is associated with dehiscence of anther' Justify the statement.
52. List out the functions of tapetum.
53. Write short note on Pollen kitt.
54. Distinguish tenuinucellate and crassinucellate ovules.
55. Give short notes on types of ovules.
56. 'Pollination in Gymnosperms is different from Angiosperms' – Give reasons.
57. Write short note on Heterostyly.
58. Enumerate the characteristic features of Entomophilous flowers
59. Explain the pollination mechanism in *Salvia*.
60. Discuss the steps involved in Microsporogenesis.
61. With a suitable diagram explain the structure of an ovule.
62. Give a concise account on steps involved in fertilization of an angiosperm plant.
63. What is endosperm. Explain the types.
64. Explain the development of a Dicot embryo
65. Differentiate the structure of Dicot and Monocot seed.
66. Give a detailed account on parthenocarpy. Add a note on its significance.

Glossary

Apospory: The process of embryo sac formation from diploid cells of nucellus as a result of mitosis

Budding: A method of asexual reproduction where small outgrowth(Bud) from a parent cell are produced

Callus: Undifferentiated mass of cells obtained through tissue culture.

Clone: Genetically identical individuals.

Endothecium: A single layer of hygroscopic, radially elongated cells found below the epidermis of anther which helps in dehiscence of anther.

Fertilization: The act of fusion of male and female gamete

Grafting: Conventional method of reproduction where stock and scion are joined to produce new plant.

Horticulture: Branch of plant science that deals with the art of growing fruits, vegetables, flowers and ornamental plants.

Nucellus: The diploid tissue found on the inner part of ovule next to the integuments.

Pollenkitt: A sticky covering found on the surface of the pollen that helps to attract insects.

Regeneration: Ability of organisms to replace or restore the lost parts.

Sporopollenin: Pollen wall material derived from carotenoids and is resistant to physical and biological decomposition.

Tapetum: Nutritive tissue for the developing sporogenous tissue

Transmitting tissue: A single layer of glandular canal cells lining the inner part of style.



Chapter

2



UNIT VII: Genetics

Classical Genetics



Learning Objectives

The Learner will be able to

- ❖ Differentiate classical and modern genetics.
- ❖ Understand the concepts of principles of inheritance.
- ❖ Describe the extensions of Mendelism.
- ❖ Explain polygenic inheritance and Pleiotropy.
- ❖ Analyze extra chromosomal inheritance in cytoplasmic organelles.



BJADTY



Chapter outline

- 2.1 Heredity and Variation
- 2.2 Mendelism
- 2.3 Laws of Mendelian Inheritance
- 2.4 Monohybrid, Dihybrid, Trihybrid cross, Backcross and Testcross
- 2.5 Interaction of Genes -Intragenic and Intergenic Incomplete dominance, Lethal genes, Epistasis
- 2.6 Polygenic inheritance in Wheat kernel colour, Pleiotropy – *Pisum sativum*
- 2.7 Extra chromosomal inheritance- Cytoplasmic inheritance in Mitochondria and Chloroplast.

Genetics is the study of how living things receive common traits from previous generations. No field of science has changed the world more, in the past 50 years than genetics. The scientific and technological advances in genetics have transformed agriculture, medicine and forensic science etc.

Genetics – The Science of heredity (Inheritance)

- “Genetics” is the branch of biological science which deals with the mechanism of transmission of characters from parents to offsprings. The term **Genetics** was introduced by **W. Bateson** in 1906.

The four major subdisciplines of genetics are

1. **Transmission Genetics / Classical Genetics**
– Deals with the transmission of genes from parents to offsprings. The foundation of classical genetics came from the study of hereditary behaviour of seven genes by Gregor Mendel.
2. **Molecular Genetics** – Deals with the structure and function of a gene at molecular level.
3. **Population Genetics** – Deals with heredity in groups of individuals for traits which is determined by a few genes.
4. **Quantitative Genetics** – Deals with heredity of traits in groups of individuals where the traits are governed by many genes simultaneously.

What is the reason for similarities, differences of appearance and skipping of generations?

Genes – Functional Units of inheritance: The basic unit of heredity (biological information) which transmits biochemical, anatomical and behavioural traits from parents to offsprings.



2.1 Heredity and variation

Genetics is often described as a science which deals with heredity and variation.

Heredity: Heredity is the transmission of characters from parents to offsprings.

Variation: The organisms belonging to the same natural population or species that shows a difference in the characteristics is called variation. Variation is of two types (i) Discontinuous variation and (ii) Continuous variation

1. Discontinuous Variation:

Within a population there are some characteristics which show a limited form of variation. Example: Style length in *Primula*, plant height of garden pea. In discontinuous variation, the characteristics are controlled by one or two major genes which may have two or more allelic forms. These variations are genetically determined by inheritance factors. Individuals produced by this variation show differences without any intermediate form between them and there is no overlapping between the two phenotypes. The phenotypic expression is unaffected by environmental conditions. This is also called as qualitative inheritance.

2. Continuous Variation:

This variation may be due to the combining effects of environmental and genetic factors. In a population most of the characteristics exhibit a complete gradation, from one extreme to the other without any break. Inheritance of phenotype is determined by the combined effects of many genes, (polygenes) and environmental factors. This is also known as quantitative inheritance. Example: Human height and skin color.

Importance of variations

- Variations make some individuals better fitted in the struggle for existence.

- They help the individuals to adapt themselves to the changing environment.
- It provides the genetic material for natural selection
- Variations allow breeders to improve better yield, quicker growth, increased resistance and lesser input.
- They constitute the raw materials for evolution.

2.2 Mendelism

The contribution of Mendel to Genetics is called Mendelism. It includes all concepts brought out by Mendel through his original research on plant hybridization. Mendelian genetic concepts are basic to modern genetics. Therefore, Mendel is called as **Father of Genetics**.

2.2.1 Father of Genetics – Gregor Johann Mendel (1822 – 1884)

The first Geneticist, Gregor Johann Mendel unraveled the mystery of heredity. He was born on 22nd July 1822 in Heinzendorf Silesia (now Hyncice, Czechoslovakia), Austria. After school

education, later he studied botany, physics and mathematics at the University of Vienna. He then entered a monastery of St. Thomas at Brunn in Austria and continued his interest in plant hybridization. In 1849 Mendel got a temporary position in a school as a teacher and he performed a series of elegant experiments with pea plants in his garden. In 1856, he started his historic studies on pea plants. 1856 to 1863 was the period of Mendel's hybridization experiments on pea plants. Mendel discovered the principles of heredity by studying the inheritance of seven pairs of contrasting traits of pea plant in his garden. Mendel crossed and catalogued 24,034



Figure 2.1: Gregor Johann Mendel



plants through many generations. His paper entitled “**Experiments on Plant Hybrids**” was presented and published in The Proceedings of the Brunn Society of Natural History in 1866. Mendel was the first systematic researcher in the field of genetics.

Mendel was successful because:

- He applied mathematics and statistical methods to biology and laws of probability to his breeding experiments.
- He followed scientific methods and kept accurate and detailed records that include quantitative data of the outcome of his crosses.
- His experiments were carefully planned and he used large samples.
- The pairs of contrasting characters which were controlled by factor (genes) were present on separate chromosomes.
- The parents selected by Mendel were pure breed lines and the purity was tested by self crossing the progeny for many generations.

Mendel’s Experimental System – The Garden pea.

He chose pea plant because,

- It is an annual plant and has clear contrasting characters that are controlled by a single gene separately.
- Self-fertilization occurred under normal conditions in garden pea plants. Mendel used both self-fertilization and cross-fertilization.
- The flowers are large hence emasculation and pollination are very easy for hybridization.

2.2.2 Mendel’s experiments on pea plant

Mendel’s theory of inheritance, known as the Particulate theory, establishes the existence of minute particles or hereditary units or factors, which are now called as **genes**. He performed artificial pollination or cross pollination

experiments with several true-breeding lines of pea plants. A true breeding lines (Pure-breeding strains) means it has undergone continuous self pollination having stable trait inheritance from parent to offspring. Matings within pure breeding lines produce offsprings having specific parental traits that are constant in inheritance and expression for many generations. Pure line breed refers to homozygosity only. Fusion of male and female gametes produced by the same individual i.e pollen and egg are

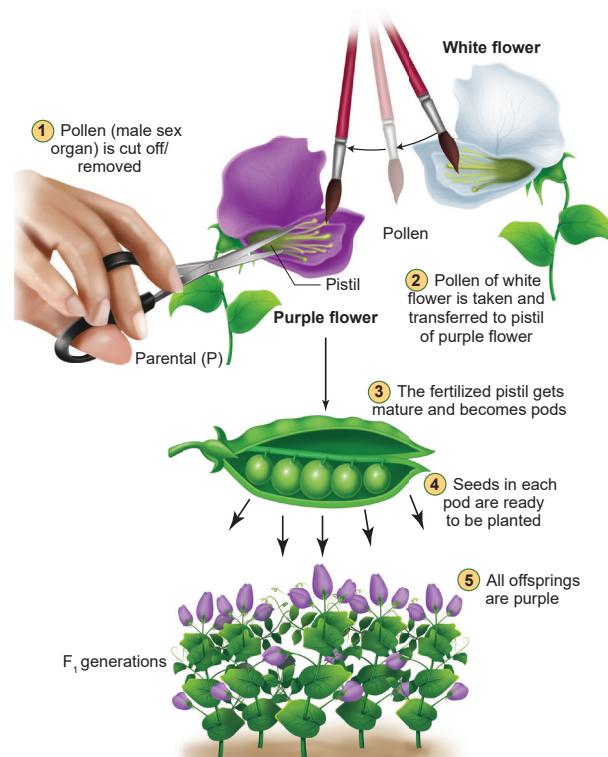


Figure 2.2: Steps in cross pollination of pea flowers

derived from the same plant is known as self-fertilization. Self pollination takes place in Mendel’s peas. The experimenter can remove the anthers (Emasculation) before fertilization and transfer the pollen from another variety of pea to the stigma of flowers where the anthers are removed. This results in cross-fertilization, which leads to the creation of hybrid varieties with different traits. Mendel’s work on the study of the pattern of inheritance and the principles or laws formulated, now constitute the Mendelian Genetics.



The First Model Organism in Genetics – Garden Peas (*Pisum sativum*) – Seven characters studied by Mendel.

Character	Dominant Trait	Recessive Trait
Stem length	Tall	Dwarf
Pod shape	Inflated	Constricted
Seed shape	Round	Wrinkled
Seed colour	Yellow	Green
Flower position	Axial	Terminal
Flower colour	Purple	White
Pod colour	Green	Yellow

Figure 2.3: Seven characters of *Pisum sativum* studied by Mendel.

Character	Gene	Dominant Trait	Recessive Trait
Plant Height	Le	Tall	Dwarf
Seed Shape	R	Round	Wrinkled
Cotyledon colour	I	Yellow	Green
Flower colour	A	Purple	White
Pod colour	GP	Green	Yellow
Pod form	V	Inflated	Constricted
Flower position	Fa	Axial	Terminal

Table 2.1 Seven characters of *Pisum sativum* with genes

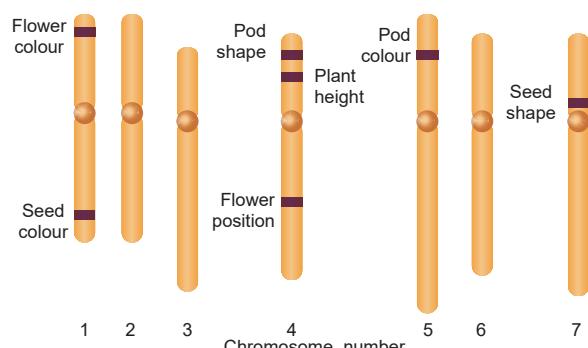


Figure 2.4: Mendel's seven characters in Garden Peas, shown on the plant's seven chromosomes

Can you identify Mendel's gene for regulating white colour in peas? Let us find the molecular answer to understand the gene function. Now the genetic mystery of Mendel's white flowers is solved.

It is quite fascinating to trace the Mendel's genes. In 2010, the gene responsible for regulating flower colour in peas were identified by an international team of researchers. It was called **Pea Gene A** which encodes a **protein** that functions as a transcription factor which is responsible for the production of **anthocyanin pigment**. So the flowers are purple. Pea plants with white flowers do not have anthocyanin, even though they have the gene that encodes the enzyme involved in anthocyanin synthesis.

Researchers delivered normal copies of gene A into the cells of the petals of white flowers by the gene gun method. When Gene A entered in a small percentage of cells of white flowers it is expressed in those particular cells, accumulated anthocyanin pigments and became purple.

In white flowers the gene A sequence showed a single-nucleotide change that makes the transcription factor inactive. So the mutant form of gene A do not accumulate anthocyanin and hence they are white.



Figure 2.5: Purple flower of Pea with Pea Gene A and White flower of Pea

Mendel worked at the rules of inheritance and arrived at the correct mechanism before any knowledge of cellular mechanism, DNA, genes, chromosomes became available. Mendel insights and meticulous work into the mechanism of inheritance played an important role which led



to the development of improved crop varieties and a revolution in crop hybridization.

Mendel died in 1884. In 1900 the work of Mendel's experiments were rediscovered by three biologists, **Hugo de Vries** of Holland, **Carl Correns** of Germany and **Erich von Tschermak** of Austria.

2.2.3 Terminology related to Mendelism

Mendel noticed two different expressions of a trait – Example: Tall and dwarf. Traits are expressed in different ways due to the fact that a gene can exist in alternate forms (versions) for the same trait is called **alleles**.

If an individual has two identical alleles of a gene, it is called as **homozygous**(TT). An individual with two different alleles is called **heterozygous**(Tt). Mendel's non-true breeding plants are heterozygous, called as **hybrids**.

When the gene has two alleles the dominant allele is symbolized with capital letter and the recessive with small letter. When both alleles are recessive the individual is called **homozygous recessive** (tt) dwarf pea plants. An individual with two dominant alleles is called **homozygous dominant** (TT) tall pea plants. One dominant allele and one recessive allele (Tt) denotes non-true breeding tall pea plants **heterozygous tall**.

2.2.4 Mendelian inheritance – Mendel's Laws of Heredity

Mendel proposed two rules based on his observations on monohybrid cross, today these rules are called laws of inheritance. The first law is The Law of Dominance and the second law is The Law of Segregation. These scientific laws play an important role in the history of evolution.

The Law of Dominance: The characters are controlled by discrete units called factors which occur in pairs. In a dissimilar pair of factors one member of the pair is dominant and the other is recessive. This law gives an explanation to the monohybrid cross (a) the expression of only one

of the parental characters in F₁ generation and (b) the expression of both in the F₂ generation. It also explains the proportion of 3:1 obtained at the F₂

The Law of Segregation (Law of Purity of gametes): Alleles do not show any blending, both characters are seen as such in the F₂ generation although one of the characters is not seen in the F₁ generation. During the formation of gametes, the factors or alleles of a pair separate and segregate from each other such that each gamete receives only one of the two factors. A homozygous parent produces similar gametes and a heterozygous parent produces two kinds of gametes each having one allele with equal proportion. **Gametes are never hybrid.**

2.3 Monohybrid cross

Monohybrid inheritance is the inheritance of a single character i.e. plant height. It involves the inheritance of two alleles of a single gene. When the F₁ generation was selfed Mendel noticed that 787 of 1064 F₂ plants were tall, while 277 of 1064 were dwarf. The dwarf trait disappeared in the F₁ generation only to reappear in the F₂ generation. The term **genotype** is the genetic constitution of an individual. The term **phenotype** refers to the observable characteristic of an organism. In a genetic cross the genotypes and phenotypes of offspring, resulting from combining gametes during fertilization can be easily understood with the help of a diagram called Punnett's Square named after a British Geneticist Reginald C. Punnett. It is a graphical representation to calculate the probability of all possible genotypes of offsprings in a genetic cross. The Law of Dominance and the Law of Segregation give suitable explanation to Mendel's monohybrid cross.

Reciprocal cross – In one experiment, the tall pea plants were pollinated with the pollens from a true-breeding dwarf plants, the result was all tall plants. When the parental types were reversed, the pollen from a tall plant was used to pollinate a dwarf pea plant which gave only tall plants. The result was the same - All tall plants.



Tall (♀) x Dwarf (♂) and Tall (♂) x Dwarf (♀) matings are done in both ways which are called reciprocal crosses. The results of the reciprocal crosses are the same. So it was concluded that the trait is not sex dependent. The results of Mendel's monohybrid crosses were not sex dependent.

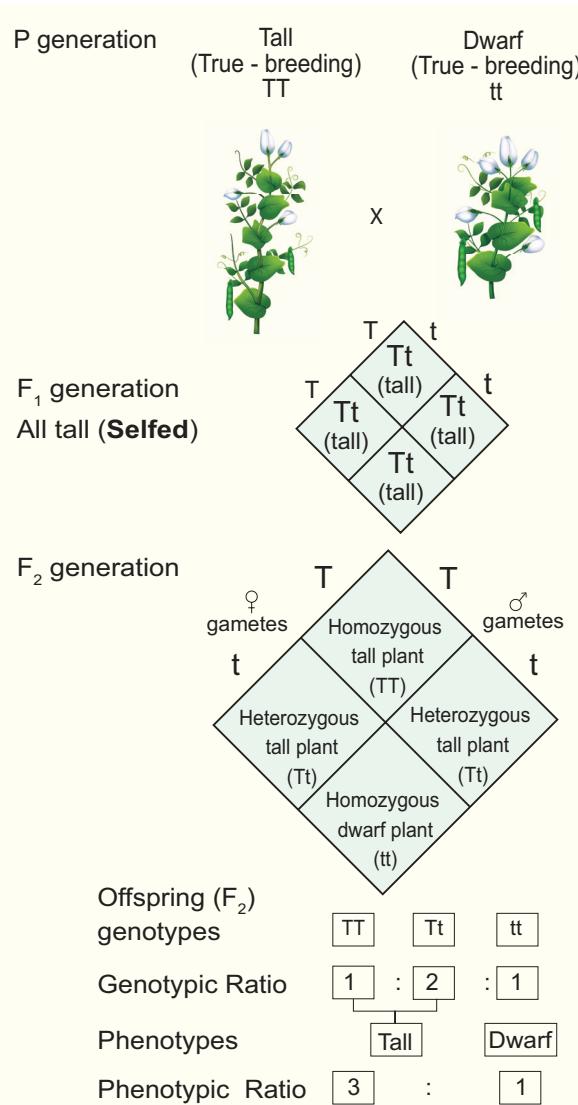


Figure 2.6: Monohybrid Cross

The gene for plant height has two alleles: Tall (T) x Dwarf (t). The phenotypic and genotypic analysis of the crosses has been shown by Checker board method or by Forkline method.

2.3.1 Mendel's analytical and empirical approach

Mendel chose two contrasting traits for each character. So it seemed logical that two distinct factors exist. In F₁ the recessive trait and its factors do not disappear and they are hidden

or masked only to reappear in $\frac{1}{4}$ of the F₂ generation. He concluded that tall and dwarf alleles of F₁ heterozygote segregate randomly into gametes. Mendel got 3:1 ratio in F₂ between the dominant and recessive trait. He was the first scientist to use this type of quantitative analysis in a biological experiment. Mendel's data is concerned with the proportions of offspring.

Mendel's analytical approach is truly an outstanding scientific achievement. His meticulous work and precisely executed breeding experiments proposed that discrete particulate units of heredity are present and they are transmitted from one generation to the other. Now they are called as genes. Mendel's experiments were well planned to determine the relationships which govern hereditary traits. This rationale is called an empirical approach. Laws that were arrived from an empirical approach is known as empirical laws.

2.3.2 Test cross

Test cross is crossing an individual of unknown genotype with a homozygous recessive.

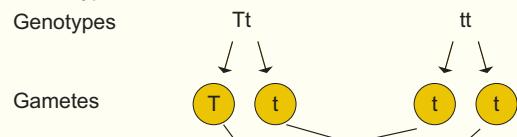
In Mendel's monohybrid cross all the plants are tall in F₁ generation. In F₂ tall and dwarf plants were in the ratio of 3:1. Mendel self pollinated dwarf F₂ plants and got dwarf plants in F₃ and F₄ generations. So he concluded that the genotype of dwarf was homozygous (tt). The genotypes of tall plants TT or Tt from F₁ and F₂ cannot be predicted. But how we can tell if a tall plant is homozygous or heterozygous? To determine the genotype of a tall plant Mendel crossed the plants from F₂ with the homozygous recessive dwarf plant. This he called a test cross. The progenies of the test cross can be easily analysed to predict the genotype of the plant or the test organism. Thus in a typical test cross an organism (pea plants) showing dominant phenotype (whose genotype is to be determined) is crossed with the recessive parent instead of self crossing. Test cross is used to identify whether an individual is homozygous or heterozygous for dominant character.



If heterozygous tall test cross

Parental (P) F₁ Heterozygous tall X Homozygous dwarf
Phenotypes

Genotypes

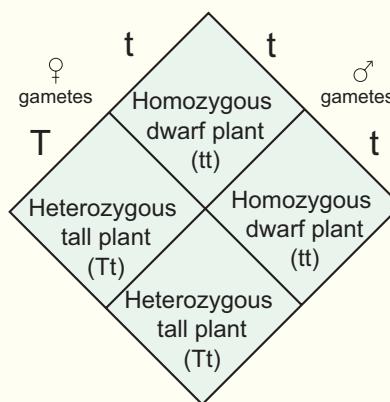


Offspring (F₁) genotypes

Genotypic Ratio

Phenotypes

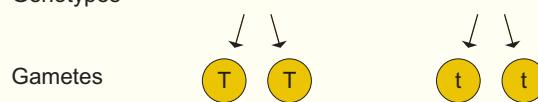
Phenotypic Ratio



If homozygous tall test cross

Parental (P) F₁ Homozygous tall X Homozygous dwarf
Phenotypes

Genotypes



Offspring (F₁) genotypes

Phenotypes

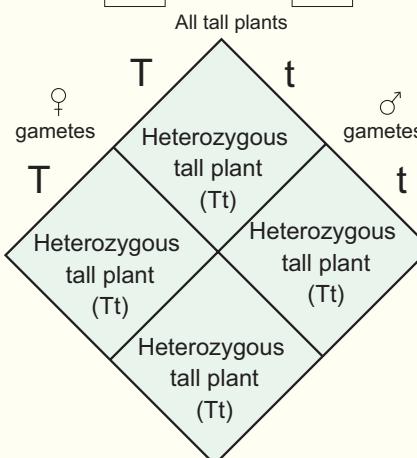


Figure 2.7: Test cross

Why Mendel's pea plants are tall and dwarf? Find out the molecular explanation.

Molecular characterization of Mendel's gene for plant height.

The plant height is controlled by a single gene with two alleles. The reason for this difference in plant height is due to the following facts: (i) the cells of the pea plant have the ability to convert a precursor molecule of gibberellins into an active form (GA1) (ii) Tall pea plants have one allele (Le) that codes for a protein (functional enzyme) which functions normally in the gibberellin-synthesis pathway and catalyzes the formation of gibberellins (GA1). The allele is dominant even if it is two (Le Le) or single (Le le), it produces gibberellins and the pea plants are tall. Dwarf pea plants have two recessive alleles (le le) which code for non-functional protein, hence they are dwarf.

Gene for plant height in Peas



Tall pea plants



Dwarf pea plant

(Le Le / Le le)

(le le)

Gibberellin
Precursor
molecule

Le allele codes for
functional enzyme GA1

Gibberellin
Precursor
molecule

le allele codes for
nonfunctional enzyme

Gibberellins
are not produced

Figure 2.8: Gene for plant height in Peas

2.3.3 Back cross

- Back cross is a cross of F₁ hybrid with any one of the parental genotypes. The back cross is of two types; they are dominant back cross and recessive back cross.
- It involves the cross between the F₁ offspring with either of the two parents.



- When the F_1 offsprings are crossed with the dominant parents all the F_2 develop dominant character and no recessive individuals are obtained in the progeny.
- If the F_1 hybrid is crossed with the recessive parent individuals of both the phenotypes appear in equal proportion and this cross is specified as test cross.
- The recessive back cross helps to identify the heterozygosity of the hybrid.

2.3.4 Dihybrid cross

It is a genetic cross which involves individuals differing in two characters. Dihybrid inheritance is the inheritance of two separate genes each with two alleles.

Law of Independent Assortment – When two pairs of traits are combined in a hybrid, segregation of one pair of characters is independent to the other pair of characters. Genes that are located in different chromosomes assort independently during meiosis. Many possible combinations of factors can occur in the gametes.

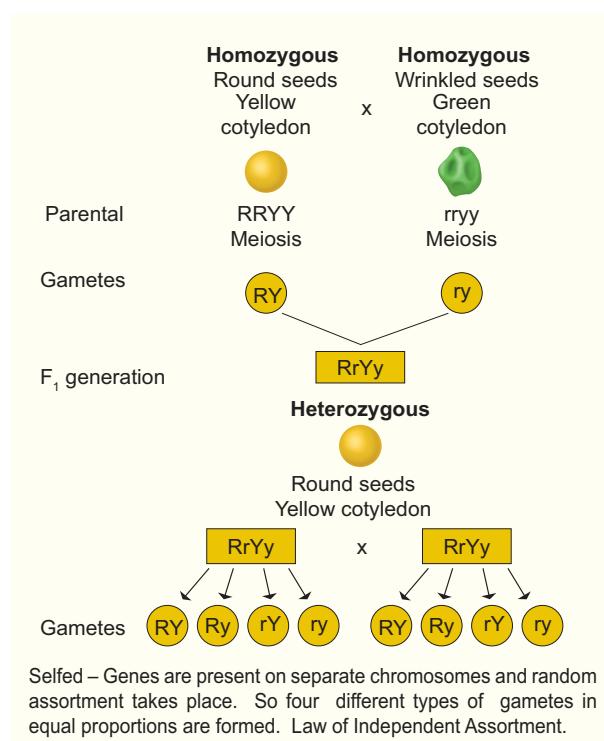


Figure 2.9: Dihybrid cross – Segregation of gametes

Independent assortment leads to genetic diversity. If an individual produces genetically dissimilar gametes it is the consequence of independent assortment. Through independent assortment, the maternal and paternal members of all pairs were distributed to gametes, so all possible chromosomal combinations were produced leading to genetic variation. In sexually reproducing plants/organisms, due to independent assortment, genetic variation takes place which is important in the process of evolution. The Law of Segregation is concerned with alleles of one gene but the Law of Independent Assortment deals with the relationship between genes.

The crossing of two plants differing in two pairs of contrasting traits is called dihybrid cross. In dihybrid cross, two characters (colour and shape) are considered at a time. Mendel considered the seed shape (round and wrinkled) and cotyledon colour (yellow & green) as the two characters. In seed shape round (R) is dominant over wrinkled (r); in cotyledon colour yellow (Y) is dominant over green (y). Hence the pure breeding round yellow parent is represented by the genotype RRYY and the pure breeding green wrinkled parent is represented by the genotype rryy. During gamete formation the paired genes of a character assort out independently of the other pair. During the $F_1 \times F_1$ fertilization each zygote with an equal probability receives one of the four combinations from each parent. The resultant gametes thus will be genetically different and they are of the following four types:

- Yellow round (YR) - 9/16
- Yellow wrinkled (Yr) - 3/16
- Green round (yR) - 3/16
- Green wrinkled (yr) - 1/16

These four types of gametes of F_1 dihybrids unite randomly in the process of fertilization and produce sixteen types of individuals in F_2 in the ratio of 9:3:3:1 as shown in the figure. Mendel's 9:3:3:1 dihybrid ratio is an ideal ratio based on the probability including segregation, independent assortment and random



fertilization. In sexually reproducing organism / plants from the garden peas to human beings, Mendel's findings laid the foundation for understanding inheritance and revolutionized the field of biology. The dihybrid cross and its result led Mendel to propose a second set of generalisations that we called Mendel's Law of independent assortment.

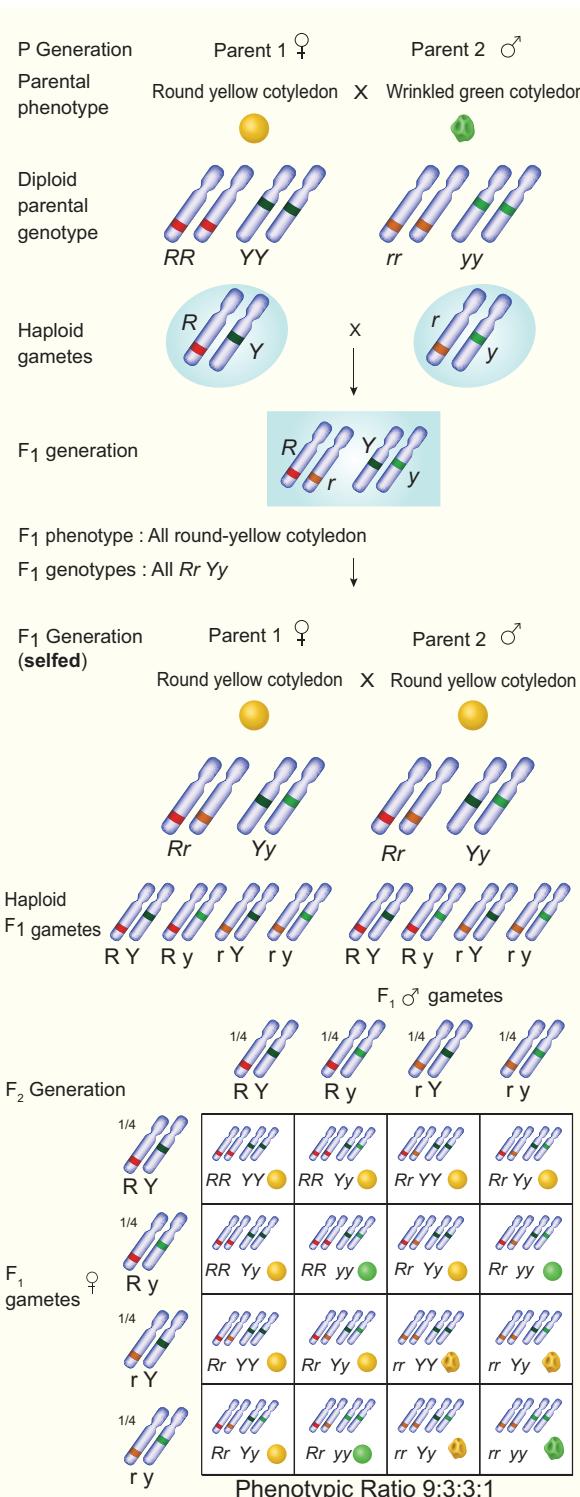
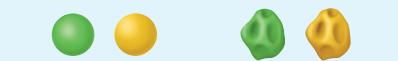


Figure 2.10: Dihybrid Cross in Garden peas

How does the wrinkled gene make Mendel's peas wrinkled? Find out the molecular explanation.

The protein called starch branching enzyme (SBEI) is encoded by the wild-type allele of the gene (RR) which is dominant. When the seed matures, this enzyme SBEI catalyzes the formation of highly branched starch molecules. Normal gene (R) has become interrupted by the insertion of extra piece of DNA (0.8 kb) into the gene, resulting in r allele. In the homozygous mutant form of the gene (rr) which is recessive, the activity of the enzyme SBEI is lost resulting in wrinkled peas. The wrinkled seed accumulates more sucrose and high water content. Hence the osmotic pressure inside the seed rises. As a result, the seed absorbs more water and when it matures it loses water as it dries. So it becomes wrinkled at maturation. When the seed has atleast one copy of normal dominant gene heterozygous, the dominant allele helps to synthesize starch, amylopectin an insoluble carbohydrate, with the osmotic balance which minimises the loss of water resulting in smooth structured round seed.

The wrinkled gene make Mendel's peas wrinkled



Round Peas & Wrinkled Peas

RR rr

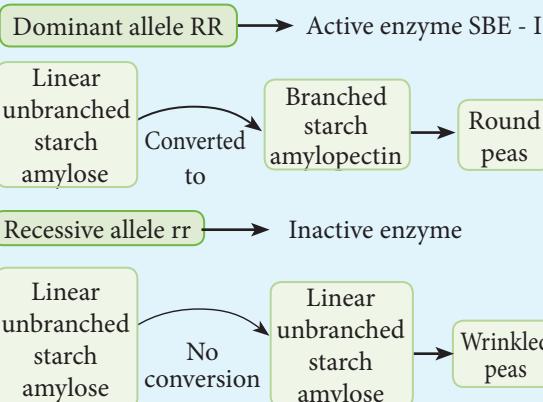


Figure 2.11: Molecular explanation of round and wrinkled peas.



2.3.5 The Dihybrid test cross

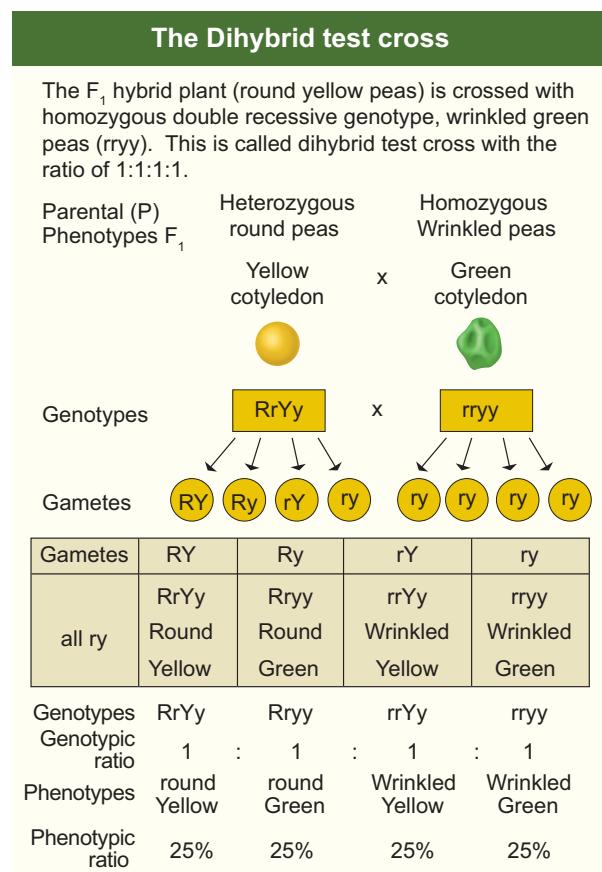


Figure 2.12: Dihybrid test cross

2.3.6. Trihybrid cross

The trihybrid cross demonstrates that Mendel's laws are applicable to the inheritance of multiple traits. Mendel Laws of segregation and independent assortment are also applicable to three pairs of contrasting characteristic traits called trihybrid cross.

A cross between homozygous parents that differ in three gene pairs (i.e. producing trihybrids) is called trihybrid cross. A self fertilizing trihybrid plant forms 8 different gametes and 64 different zygotes. In this a combination of three single pair crosses operating together. The three contrasting characters of a trihybrid cross are

Tall, Yellow, Round x Dwarf, Green, Wrinkled
 $TTYYRR \downarrow ttyyrr$
 F_1 Tall, Yellow, Round (Selfed)
 $TtYyRr$
 F_2 Phenotypic ratio - 27 : 9 : 9 : 9 : 3 : 3 : 3 : 1

2.3.7 Extensions of Mendelian Genetics

Apart from monohybrid, dihybrid and trihybrid crosses, there are exceptions to Mendelian principles, i.e. the occurrence of different phenotypic ratios. The more complex patterns of inheritance are the extensions of Mendelian Genetics. There are examples where phenotype of the organism is the result of the interactions among genes.

Gene interaction – A single phenotype is controlled by more than one set of genes, each of which has two or more alleles. This phenomenon is called Gene Interaction. Many characteristics of the organism including structural and chemical which constitute the phenotype are the result of interaction between two or more genes.

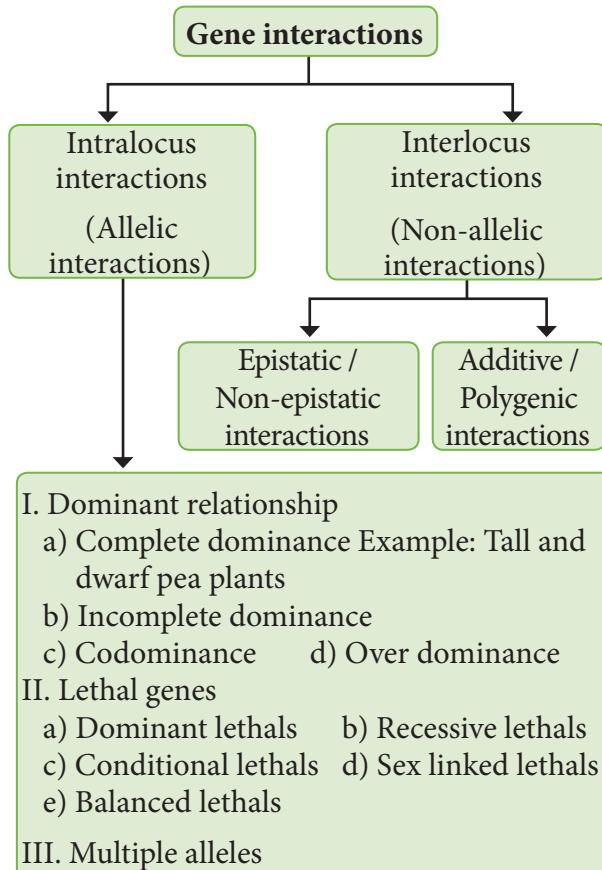


Figure 2.13: Gene Interaction



Mendelian experiments prove that a single gene controls one character. But in the post Mendelian findings, various exception have been noticed, in which different types of interactions are possible between the genes. This gene interaction concept was introduced and explained by W. Bateson. This concept is otherwise known as Factor hypothesis or Bateson's factor hypothesis. According to Bateson's factor hypothesis, the gene interactions can be classified as

- Intragenic gene interactions or Intra allelic or allelic interactions
- Intergenic gene interactions or inter allelic or non-allelic interactions

2.4 Intragenic gene interactions

Interactions take place between the alleles of the same gene i.e., alleles at the same locus is called intragenic or intralocus gene interaction. It includes the following:

- 1) Incomplete dominance (2) Codominance (3) Multiple alleles (4) Pleiotropic genes are common examples for intragenic interaction.

2.4.1. Incomplete dominance – No blending of genes

The German Botanist Carl Correns's (1905) Experiment - In 4 O' clock plant, *Mirabilis jalapa* when the pure breeding homozygous red (R^1R^1) parent is crossed with homozygous white (R^2R^2), the phenotype of the F_1 hybrid is heterozygous pink (R^1R^2). The F_1 heterozygous phenotype differs from both the parental homozygous phenotype. This cross did not exhibit the character of the dominant parent but an intermediate colour pink. When one allele is not completely dominant to another allele it shows incomplete dominance. Such allelic interaction is known as incomplete dominance. F_1 generation produces intermediate phenotype pink coloured flower. When pink coloured plants of F_1 generation

were interbred in F_2 both phenotypic and genotypic ratios were found to be identical as 1 : 2 : 1 (1 red : 2 pink : 1 white). Genotypic ratio is $1 R^1R^1 : 2 R^1R^2 : 1 R^2R^2$. From this we conclude that the alleles themselves remain discrete and unaltered proving the Mendel's Law of Segregation. The phenotypic and genotypic ratios are the same. There is no blending of genes. In the F_2 generation R^1 and R^2 genes segregate and recombine to produce red, pink and white in the ratio of 1 : 2 : 1. R^1 allele codes for an enzyme responsible for the formation of red pigment. R^2 allele codes for defective enzyme. R^1 and R^2 genotypes produce only enough red pigments to make the flower pink. Two R^1R^1 are needed for producing red flowers. Two R^2R^2 genes are needed for white flowers. If blending had taken place, the original pure traits would not have appeared and all F_2 plants would have pink flowers. It is very clear that Mendel's particulate inheritance takes place in this cross which is confirmed by the reappearance of original phenotype in F_2 .

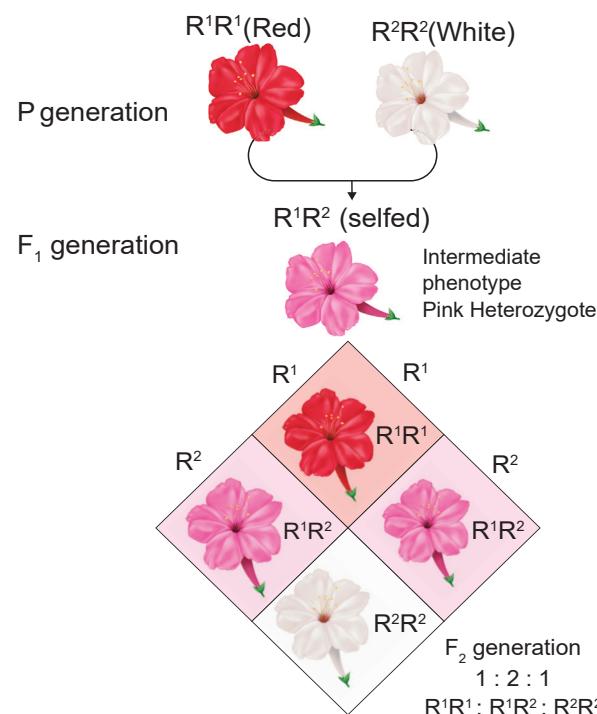


Figure 2.14: Incomplete dominance in 4 O' clock plant



How are we going to interpret the lack of dominance and give explanation to the intermediate heterozygote phenotype?

How will you explain incomplete dominance at the molecular level?

Gene expression is explained in a quantitative way. Wild-type allele which is a functional allele when present in two copies ($R^1 R^1$) produces an functional enzyme which synthesizes red pigments. The mutant allele which is a defective allele in two copies ($R^2 R^2$) produces an enzyme which cannot synthesize necessary red pigments. The white flower is due to the mutation causing complete loss of function. The F_1 intermediate phenotype heterozygote ($R^1 R^2$) has one copy of the allele R^1 . R^1 produces 50% of the functional protein resulting in half of the pigment of red flowered plant and so it is pink. The intermediate phenotype pink heterozygote with 50% of functional protein is not enough to create the red phenotype homozygous, which makes 100% of the functional protein.

2.4.2. Codominance (1 : 2 : 1)

This pattern occurs due to simultaneous (joint) expression of both alleles in the heterozygote - The phenomenon in which two alleles are both expressed in the heterozygous individual is known as codominance. Example: Red and white flowers of *Camellia*, inheritance of sickle cell haemoglobin, ABO blood group system in humanbeings. In humanbeings, I^A and I^B alleles of I gene are codominant which follows Mendels law of segregation. The codominance was demonstrated in plants with the help of electrophoresis or chromatography for protein or flavonoid substance. Example: *Gossypium hirsutum* and *Gossypium sturtianum*, their F_1 hybrid (amphiploid) was tested for seed proteins by electrophoresis. Both the parents have

different banding patterns for their seed proteins. In hybrids, additive banding pattern was noticed. Their hybrid shows the presence of both the types of proteins similar to their parents.

The heterozygote genotype gives rise to a phenotype distinctly different from either of the homozygous genotypes. The F_1 heterozygotes produce a F_2 progeny in a phenotypic and genotypic ratios of 1 : 2 : 1.

2.4.3. Lethal genes

An allele which has the potential to cause the death of an organism is called a "Lethal Allele". In 1907, E. Baur reported a lethal gene in snapdragon (*Antirrhinum sp.*). It is an example for recessive lethality. In snapdragon there are three kinds of plants.

1. Green plants with chlorophyll. (CC)
2. Yellowish green plants with carotenoids are referred to as pale green, golden or aurea plants (Cc)
3. White plants without any chlorophyll. (cc)

The genotype of the homozygous green plants is CC. The genotype of the homozygous white plant is cc.

The aurea plants have the genotype Cc because they are heterozygous of green and white plants. When two such aurea plants are crossed the F_1 progeny has identical phenotypic and genotypic ratio of 1 : 2 : 1 (viz. 1 Green (CC) : 2 Aurea (Cc) : 1 White (cc))

Since the white plants lack chlorophyll pigment, they will not survive. So the F_2 ratio is modified into 1 : 2. In this case the homozygous recessive genotype (cc) is lethal.

F_1	Heterozygote	\times	$Antirrhinum$	\times	$Antirrhinum$
			aurea		aurea
		x		x	
F_2					
			1 CC	:	2 Cc
			Green	:	Aurea
					1 cc
					White (lethal)

Figure: 2.15: Lethal genes



The term “lethal” is applied to those changes in the genome of an organism which produces effects severe enough to cause death. Lethality is a condition in which the death of certain genotype occurs prematurely. The fully dominant or fully recessive lethal allele kills the carrier individual only in its homozygous condition. So the F_2 genotypic ratio will be 2 : 1 or 1 : 2 respectively.

2.4.4. Pleiotropy – A single gene affects multiple traits

In Pleiotropy, the single gene affects multiple traits and alter the phenotype of the organism. The Pleiotropic gene influences a number of characters simultaneously and such genes are called pleiotropic gene. Mendel noticed pleiotropy while performing breeding experiment with peas (*Pisum sativum*). Peas with purple flowers, brown seeds and dark spot on the axils of the leaves were crossed with a variety of peas having white flowers, light coloured seeds and no spot on the axils of the leaves, the three traits for flower colour, seed colour and a leaf axil spot all were inherited together as a single unit. This is due to the pattern of inheritance where the three traits were controlled by a single gene with dominant and recessive alleles. Example: sickle cell anemia.

2.5 Intergenic gene interactions

Interlocus interactions take place between the alleles at different loci i.e between alleles of different genes. It includes the following:



Dominant Epistasis – It is a gene interaction in which two alleles of a gene at one locus interfere and suppress or mask the phenotypic expression of a different pair of alleles of another gene at another locus. The gene that suppresses or masks the phenotypic expression of a gene at another locus is known as **epistatic**. The gene whose expression is interfered by non-allelic genes and

prevents from exhibiting its character is known as **hypostatic**. When both the genes are present together, the phenotype is determined by the epistatic gene and not by the hypostatic gene.

In the summer squash the fruit colour locus has a dominant allele ‘W’ for white colour and a recessive allele ‘w’ for coloured fruit. ‘W’ allele is dominant that masks the expression of any colour. In another locus hypostatic allele ‘G’ is for yellow fruit and its recessive allele ‘g’ for green fruit. In the first locus the white is dominant to colour where as in the second locus yellow is dominant to green. When the white fruit with genotype WWgg is crossed with yellow fruit with genotype wwGG, the F_1 plants have white fruit and are heterozygous (WwGg). When F_1 heterozygous plants are crossed they give rise to F_2 with the phenotypic ratio of 12 white : 3 yellow : 1 green.

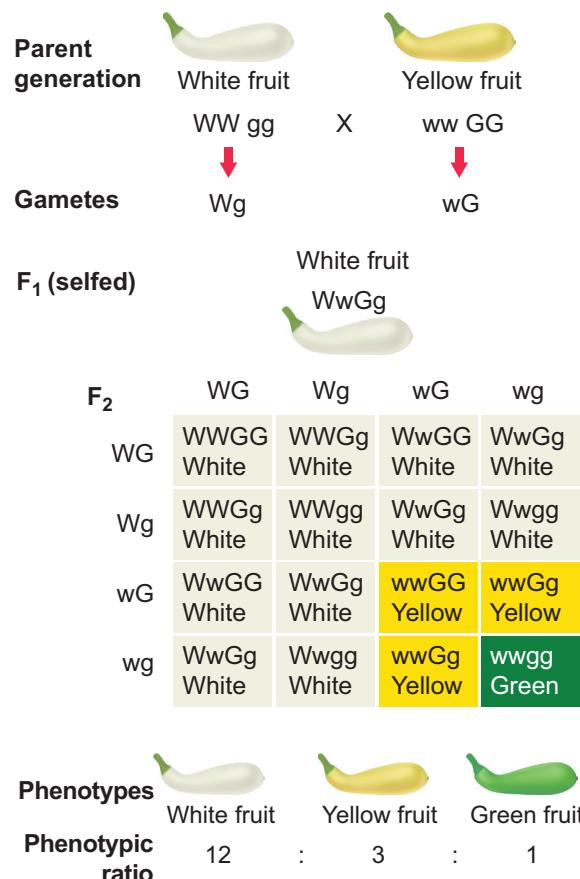


Figure 2.16: Dominant epistasis in summer squash

Since W is epistatic to the alleles ‘G’ and ‘g’, the white which is dominant, masks the effect of yellow or green. Homozygous



recessive ww genotypes only can give the coloured fruits (4/16). Double recessive 'wwgg' will give green fruit (1/16). The Plants having only 'G' in its genotype ($wwGg$ or $wwGG$) will give the yellow fruit(3/16).

Intra -genic or allelic interaction

S. No.	Gene interaction	Example	F_2 Phenotypic ratio
1	Incomplete Dominance	Flower colour in <i>Mirabilis jalapa</i> .	1 : 2 : 1
		Flower colour in snapdragon (<i>Antirrhinum spp.</i>)	1 : 2 : 1
2	Codominance	ABO Blood group system in humans	1 : 2 : 1

Table 2.2: Intra- genic interaction

Inter-genic or non-allelic interaction

S. No.	Epistatic interaction	Example	F_2 Ratio Phenotypic ratio
1	Dominant epistasis	Fruit colour in summer squash	12 : 3 : 1
2	Recessive epistasis	Flower colour of <i>Antirrhinum spp.</i>	9 : 3 : 4
3	Duplicate genes with cumulative effect	Fruit shape in summer squash	9 : 6 : 1
4	Complementary genes	Flower colour in sweet peas	9 : 7
5	Supplementary genes	Grain colour in Maize	9 : 3 : 4
6	Inhibitor genes	Leaf colour in rice plants	13 : 3
7	Duplicate genes	Seed capsule shape (fruit shape) in shepherd's purse <i>Bursa pastoris</i>	15 : 1

Table 2.3: Inter-genic interaction

2.6 Polygenic Inheritance in Wheat (Kernel colour)

Polygenic inheritance - Several genes combine to affect a single trait.

A group of genes that together determine (contribute) a characteristic of an organism is called polygenic inheritance. It gives explanations to the inheritance of continuous traits which are compatible with Mendel's Law.

The first experiment on polygenic inheritance was demonstrated by Swedish Geneticist H. Nilsson - Ehle (1909) in wheat kernels. Kernel colour is controlled by two genes each with two alleles, one with red kernel colour was dominant to white. He crossed the two pure breeding wheat varieties dark red and a white. Dark red genotypes $R_1R_1R_2R_2$ and white genotypes are $r_1r_1r_2r_2$. In the F_1 generation medium red were obtained with the genotype $R_1r_1R_2r_2$. F_1 wheat plant produces four types of gametes R_1R_2 , R_1r_2 , r_1R_2 , r_1r_2 . The intensity of the red colour is determined by the number of R genes in the F_2 generation.

Four R genes: A dark red kernel colour is obtained.
Three R genes: Medium - dark red kernel colour is obtained.
Two R genes: Medium-red kernel colour is obtained.
One R gene: Light red kernel colour is obtained.
Absence of R gene: Results in White kernel colour.

The R gene in an additive manner produces the red kernel colour. The number of each phenotype is plotted against the intensity of red kernel colour which produces a bell shaped curve. This represents the distribution of phenotype. Other example: Height and skin colour in humans are controlled by three pairs of genes.

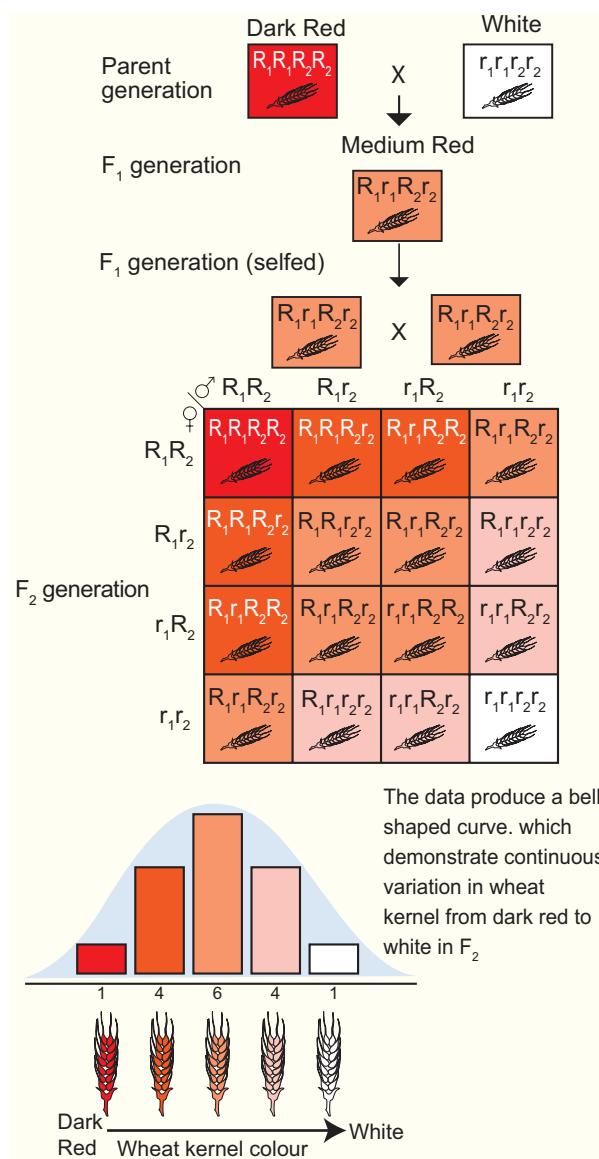


Figure 2.17 (a): Polygenic inheritance in wheat kernel colour

Parents	$R_1 R_1 R_2 R_2$	\times	$r_1 r_1 r_2 r_2$	
	Dark red		White	
F_1	$R_1 r_1 R_2 r_2$		Medium red	
F_2	Genotype		Phenotype	
1	$R_1 R_1 R_2 R_2$		Dark red	
2	$R_1 R_1 R_2 r_2$		Medium-dark red	
4	$R_1 r_1 R_2 R_2$		Medium-dark red	
1	$R_1 r_1 R_2 r_2$		Medium red	
6	$R_1 R_1 r_2 r_2$		Medium red	
1	$r_1 r_1 R_2 R_2$		Medium red	
2	$R_1 r_1 r_2 r_2$		Light red	
4	$r_1 r_1 R_2 r_2$		Light red	
1	$r_1 r_1 r_2 r_2$		White	

Figure 2.17 (b) : The genetic control of colour in wheat kernels.

Conclusion:

Finally the loci that was studied by Nilsson – Ehle were not linked and the genes assorted independently.

Later, researchers discovered the third gene that also affect the kernel colour of wheat. The three independent pairs of alleles were involved in wheat kernel colour. Nilsson – Ehle found the ratio of 63 red : 1 white in F_2 generation – 1 : 6 : 15 : 20 : 15 : 6 : 1 in F_2 generation.

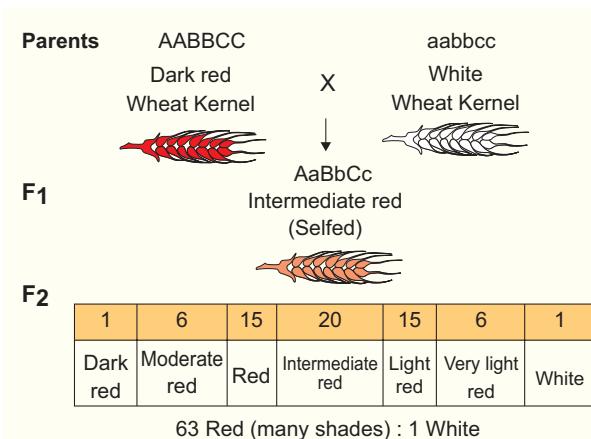


Figure 2.18: Polygenic inheritance in Wheat kernel

From the above results Nilsson – Ehle showed that the blending inheritance was not taking place in the kernel of wheat. In F_2 generation plants have kernels with wide range of colour variation. This is due to the fact that the genes are segregating and recombination takes place. Another evidence for the absence of blending inheritance is that the parental phenotypes dark red and white appear again in F_2 . There is no blending of genes, only the phenotype. The cumulative effect of several pairs of gene interaction gives rise to many shades of kernel colour. He hypothesized that the two loci must contribute additively to the kernel colour of wheat. The contribution of each red allele to the kernel colour of wheat is additive.



2.7 Extra Chromosomal Inheritance or Extra Nuclear Inheritance (Cytoplasmic Inheritance)

DNA is the universal genetic material. Genes located in nuclear chromosomes follow Mendelian inheritance. But certain traits are governed either by the chloroplast or mitochondrial genes. This phenomenon is known as extra nuclear inheritance. It is a kind of Non-Mendelian inheritance. Since it involves cytoplasmic organelles such as chloroplast and mitochondrion that act as inheritance vectors, it is also called Cytoplasmic inheritance. It is based on independent, self-replicating extra chromosomal unit called plasmogene located in the cytoplasmic organelles, chloroplast and mitochondrion.

Chloroplast Inheritance

It is found in 4 O' Clock plant (*Mirabilis jalapa*). In this, there are two types of variegated leaves namely dark green leaved plants and pale green leaved plants. When the pollen of dark green leaved plant (male) is transferred to the stigma of pale green leaved plant (female) and pollen of pale green leaved plant is transferred to the stigma of dark green leaved plant, the F₁ generation of both the crosses must be identical as per Mendelian inheritance. But in the reciprocal cross the F₁ plant differs from each other. In each cross, the F₁ plant reveals the character of the plant which is used as female plant.

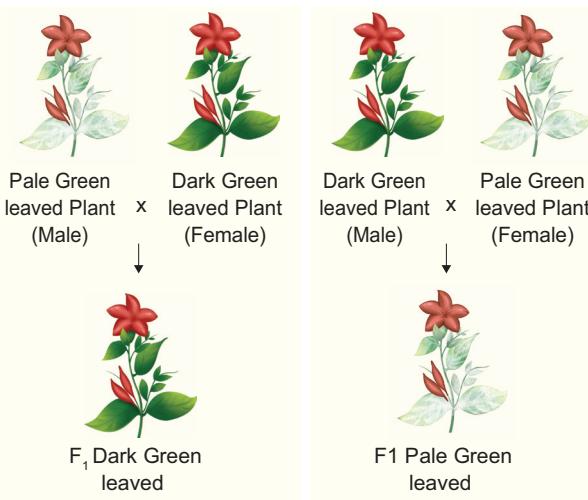


Figure 2.19: Chloroplast inheritance

This inheritance is not through nuclear gene. It is due to the chloroplast gene found in the ovum of the female plant which contributes the cytoplasm during fertilization since the male gamete contribute only the nucleus but not cytoplasm.

Mitochondrial Inheritance

Male sterility found in pearl maize (*Sorghum vulgare*) is the best example for mitochondrial cytoplasmic inheritance. So it is called **cytoplasmic male sterility**. In this, male sterility is inherited maternally. The gene for cytoplasmic male sterility is found in the mitochondrial DNA.

In this plant there are two types, one with normal cytoplasm (N) which is male fertile and the other one with aberrant cytoplasm (S) which is male sterile. These types also exhibit reciprocal differences as found in *Mirabilis jalapa*.

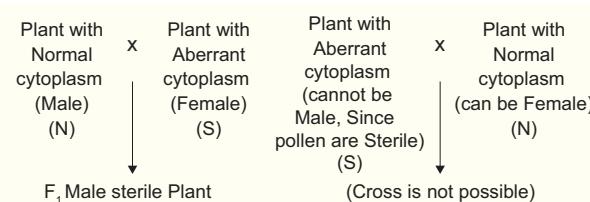


Figure 2.20: Mitochondrial Inheritance

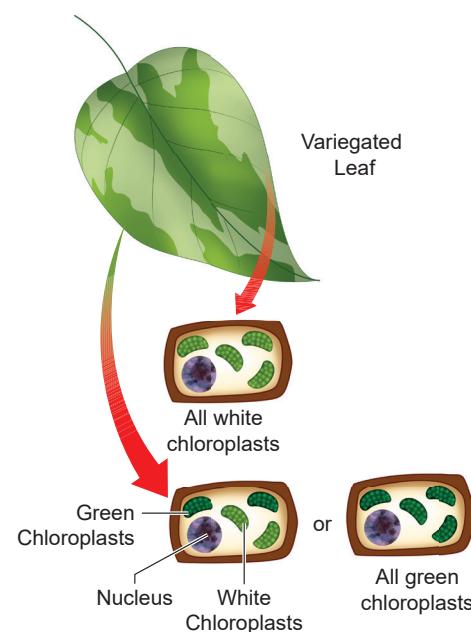


Figure 2.21: A cellular explanation of the variegated phenotype of the leaves in *Mirabilis jalapa*



Recently it has been discovered that cytoplasmic genetic male sterility is common in many plant species. This sterility is maintained by the influence of both nuclear and cytoplasmic genes. There are commonly two types of cytoplasm N (normal) and S (sterile). The genes for these are found in mitochondrion. There are also restores of fertility (Rf) genes. Even though these genes are nuclear genes, they are distinct from genetic male sterility genes of other plants. Because the Rf genes do not have any expression of their own, unless the sterile cytoplasm is present. Rf genes are required to restore fertility in S cytoplasm which is responsible for sterility. So the combination of N cytoplasm with rfrf and S cytoplasm with RfRf produces plants with fertile pollens, while S cytoplasm with rfrf produces only male sterile plants.

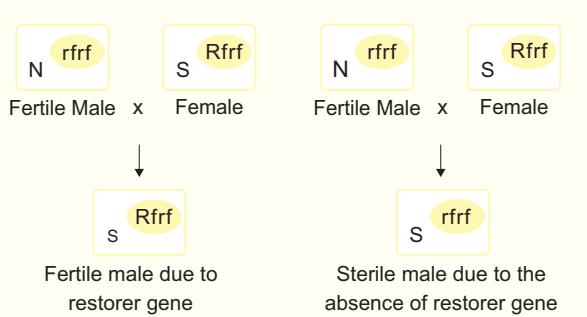


Figure 2.22: Cytoplasmic genetic male sterility

Atavism

Atavism is a modification of a biological structure whereby an ancestral trait reappears after having been lost through evolutionary changes in the previous generations. Evolutionary traits that have disappeared phenotypically do not necessarily disappear from an organism's DNA. The gene sequence often remains, but is inactive. Such an unused gene may remain in the genome for many generations. As long as the gene remains intact, a fault in the genetic control suppressing the gene can lead to the reappearance of that character again. Reemergence of sexual reproduction in the flowering plant *Hieracium pilosella* is the best example for Atavism in plants.

Summary

Gregor Johann Mendel, father of Genetics unraveled the mystery of heredity through his experiments on garden peas. Mendel's laws, analytical and empirical reasoning endure till now guiding geneticists to study variation. The monohybrid cross of Mendel proved his particulate theory of inheritance. In F_2 the alternative traits were expressed in the ratio of 3 dominant and 1 recessive. The characteristic 3 : 1 segregation is referred to as Mendelian ratio. Parents transmit discrete information about the traits to their offspring which Mendel called it as "factors". To test his experimental results Mendel devised a powerful procedure called the test cross. Test cross is used to determine the genotype of an individual when two genes are involved. In Mendel's dihybrid cross, the two pairs of factors were inherited independently. From the results of dihybrid cross Mendel gave the Law of Independent Assortment. Mendel's dihybrid ratio of 9 : 3 : 3 : 1 with the representation of two new recombinations appeared in the progeny, i.e. round green peas or wrinkled yellow peas. Molecular explanation of Mendel's gene for monohybrid cross, dihybrid cross were explained. Extension of Mendelian Genetics was dealt with examples for interaction among genes. Incomplete dominance is not an example for blending inheritance. Incomplete dominance exhibits a phenotypic heterozygote intermediate between the two homozygous. In plants codominance can be demonstrated by the methods of electrophoresis or chromatography for protein or flavonoid substances. Lethal genes with an example are explained. Pleiotropy a single gene which affects multiple traits was explained with an example of *Pisum sativum*. Dominant epistasis in summer squash with 12 : 3 : 1 ratio was discussed. Polygenic inheritance is an example for inheritance of continuous traits which is compatible with Mendel's laws. The inheritance of mitochondrial and chloroplast genes were explained with examples which does not follow the rules of nuclear genes.



Evaluation

1. Extra nuclear inheritance is a consequence of presence of genes in
 - a) Mitochondria and chloroplasts
 - b) Endoplasmic reticulum and mitochondria
 - c) Ribosomes and chloroplast
 - d) Lysosomes and ribosomes
2. In order to find out the different types of gametes produced by a pea plant having the genotype AaBb, it should be crossed to a plant with the genotype
 - a) aaBB
 - b) AaBB
 - c) AABB
 - d) aabb
3. How many different kinds of gametes will be produced by a plant having the genotype AABbCC?
 - a) Three
 - b) Four
 - c) Nine
 - d) Two
4. Which one of the following is an example of polygenic inheritance?
 - a) Flower colour in *Mirabilis Jalapa*
 - b) Production of male honey bee
 - c) Pod shape in garden pea
 - d) Skin Colour in humans
5. In Mendel's experiments with garden pea, round seed shape (RR) was dominant over wrinkled seeds (rr), yellow cotyledon (YY) was dominant over green cotyledon (yy). What are the expected phenotypes in the F₂ generation of the cross RRYY x rryy?
 - a) Only round seeds with green cotyledons
 - b) Only wrinkled seeds with yellow cotyledons
 - c) Only wrinkled seeds with green cotyledons



- d) Round seeds with yellow cotyledons and wrinkled seeds with yellow cotyledons
6. Test cross involves
 - a) Crossing between two genotypes with recessive trait
 - b) Crossing between two F₁ hybrids
 - c) Crossing the F₁ hybrid with a double recessive genotype
 - d) Crossing between two genotypes with dominant trait
7. In pea plants, yellow seeds are dominant to green. If a heterozygous yellow seeded plant is crossed with a green seeded plant, what ratio of yellow and green seeded plants would you expect in F₁ generation?
 - a) 9:1
 - b) 1:3
 - c) 3:1
 - d) 50:50
8. The genotype of a plant showing the dominant phenotype can be determined by
 - a) Back cross
 - b) Test cross
 - c) Dihybrid cross
 - d) Pedigree analysis
9. Select the correct statement from the ones given below with respect to dihybrid cross
 - a) Tightly linked genes on the same chromosomes show very few combinations
 - b) Tightly linked genes on the same chromosomes show higher combinations
 - c) Genes far apart on the same chromosomes show very few recombinations
 - d) Genes loosely linked on the same chromosomes show similar recombinations as the tightly linked ones
10. Which Mendelian idea is depicted by a cross in which the F₁ generation resembles both the parents?
 - a) Incomplete dominance



- b) Law of dominance
c) Inheritance of one gene
d) Co-dominance
11. Fruit colour in squash is an example of
a) Recessive epistasis
b) Dominant epistasis
c) Complementary genes
d) Inhibitory genes
12. In his classic experiments on Pea plants, Mendel did not use
a) Flowering position b) Seed colour
c) Pod length d) Seed shape
13. The epistatic effect, in which the dihybrid cross 9:3:3:1 between AaBb Aabb is modified as
a) Dominance of one allele on another allele of both loci
b) Interaction between two alleles of different loci
c) Dominance of one allele to another alleles of same loci
d) Interaction between two alleles of some loci
14. In a test cross involving F₁ dihybrid flies, more parental type offspring were produced than the recombination type offspring. This indicates
a) The two genes are located on two different chromosomes
b) Chromosomes failed to separate during meiosis
c) The two genes are linked and present on the same chromosome
d) Both of the characters are controlled by more than one gene
15. The genes controlling the seven pea characters studied by Mendel are known to be located on how many different chromosomes?
a) Seven b) Six
c) Five d) Four
16. Which of the following explains how progeny can possess the combinations of traits that none of the parent possessed?
a) Law of segregation
b) Chromosome theory
c) Law of independent assortment
d) Polygenic inheritance
17. "Gametes are never hybrid". This is a statement of
a) Law of dominance
b) Law of independent assortment
c) Law of segregation
d) Law of random fertilization
18. Gene which suppresses other genes activity but does not lie on the same locus is called as
a) Epistatic b) Supplement only
c) Hypostatic d) Codominant
19. Pure tall plants are crossed with pure dwarf plants. In the F₁ generation, all plants were tall. These tall plants of F₁ generation were selfed and the ratio of tall to dwarf plants obtained was 3:1. This is called
a) Dominance b) Inheritance
c) Codominance d) Heredity
20. The dominant epistasis ratio is
a) 9:3:3:1 b) 12:3:1
c) 9:3:4 d) 9:6:1
21. Select the period for Mendel's hybridization experiments
a) 1856 - 1863 b) 1850 - 1870
c) 1857 - 1869 d) 1870 - 1877
22. Among the following characters which one was not considered by Mendel in his experimentation pea?
a) Stem – Tall or dwarf
b) Trichomal glandular or non-glandular
c) Seed – Green or yellow



- d) Pod – Inflated or constricted
- 23. Name the seven contrasting traits of Mendel.
- 24. What is meant by true breeding or pure breeding lines / strain?
- 25. Give the names of the scientists who rediscovered Mendelism.
- 26. What is back cross?
- 27. Define Genetics.
- 28. What are multiple alleles
- 29. What are the reasons for Mendel's successes in his breeding experiment?
- 30. Explain the law of dominance in monohybrid cross.
- 31. Differentiate incomplete dominance and codominance.
- 32. What is meant by cytoplasmic inheritance
- 33. Describe dominant epistasis with an example.
- 34. Explain polygenic inheritance with an example.
- 35. Differentiate continuous variation with discontinuous variation.
- 36. Explain with an example how single genes affect multiple traits and alleles the phenotype of an organism.
- 37. Bring out the inheritance of chloroplast gene with an example.

Glossary

Alleles: Alternative forms of a gene.

Back Cross: Crosses between F_1 off-springs with either of the two parents (hybrid) are known as back cross

F_1 / First Filial Generation: The second stage of Mendel's experiment is called F_1 generation

Gene: The determinant of a characteristic of an organism (Mendelian factor). Gene symbols are underlined or italicized.

Genetic Code: The set of 64 triplets of bases (codons) corresponding to the twenty amino acids in proteins and the signals for initiation and termination of polypeptide synthesis.

Genotype: The types of alleles in a single individual is called genotype

Genome: The total complement of genes contained in a cell.

Heterozygous: Diploid organisms that have two different alleles at a specific gene locus are said to be heterozygous.

Homozygous: A diploid organism in which both alleles are the same at a given gene locus is said to be homozygous.

Hybrid Vigour or Heterosis: The superiority of hybrid over either of its parents in one or more traits.

Locus: The site or position of a particular gene on a chromosome.

Phenotype: The physical expression of an individual's gene. The physical observable characteristics of an organism.

Punnett Square / Checkerboard: A sort of cross-multiplication matrix used in the prediction of the outcome of a genetic cross, in which male and female gametes and their frequencies are arranged along the edges.



Chapter

3



UNIT VII: Genetics

Chromosomal Basis of Inheritance



Learning Objectives

The Learner will be able to

- ❖ Understand chromosomal theory of inheritance.
- ❖ Analyze the three-point test crosses and appreciate results in linkage map construction.
- ❖ Describe the sex determination in plants.
- ❖ Observe and calculate recombination frequency.
- ❖ Differentiate mutation types with examples.
- ❖ Explain DNA metabolism in Plants



Chapter outline

- 3.1 Chromosomal theory of Inheritance
- 3.2 Linkage - Eye colour in *Drosophila* and Seed colour in Maize
- 3.3 Crossing over, Recombination and Gene mapping
- 3.4 Multiple alleles
- 3.5 Sex determination in plants.
- 3.6 Mutation-types, mutagenic agents and their significance.
- 3.7 DNA Metabolism in Plants

In the previous chapter you have learned about Mendelian genetics, now you are going to be study with deviations of concepts related to Mendelian genetics and chromosomal theory of inheritance. You must recall the structure of chromosome and cell division from eleventh standard.

3.1 Chromosomal Theory of Inheritance

G. J. Mendel (1865) studied the inheritance of well-defined characters of pea plant but for several reasons it was unrecognized till 1900. Three scientists (de Vries, Correns and Tschermark) independently rediscovered Mendel's results on the inheritance of characters. Various cytologists also observed cell division due to advancements in microscopy. This led to the discovery of structures inside nucleus. In eukaryotic cells, worm-shaped structures formed during cell division are called **chromosomes** (colored bodies, visualized by staining). An organism which possesses two complete basic sets of chromosomes are known as diploid. A chromosome consists of long, continuous coiled piece of DNA in which genes are arranged in linear order. Each gene has a definite position (locus) on a chromosome. These genes are hereditary units. Chromosomal theory of inheritance states that Mendelian factors (genes) have specific locus (position) on chromosomes and they carry information from one generation to the next generation.

3.1.1 Historical development of chromosome theory

The important cytological findings related to the chromosome theory of inheritance are given below.

- **Wilhelm Roux (1883)** postulated that the chromosomes of a cell are responsible for transferring heredity.



- **Montgomery (1901)** was first to suggest occurrence of distinct pairs of chromosomes and he also concluded that maternal chromosomes pair with paternal chromosomes only during meiosis.
- **T. Boveri (1902)** supported the idea that the chromosomes contain genetic determiners, and he was largely responsible for developing the chromosomal theory of inheritance.
- **W.S. Sutton (1902)**, a young American student independently recognized a parallelism (similarity) between the behaviour of chromosomes and Mendelian factors during gamete formation.

Sutton and Boveri (1903) independently proposed the chromosome theory of inheritance. Sutton united the knowledge of chromosomal segregation with Mendelian principles and called it chromosomal theory of inheritance.

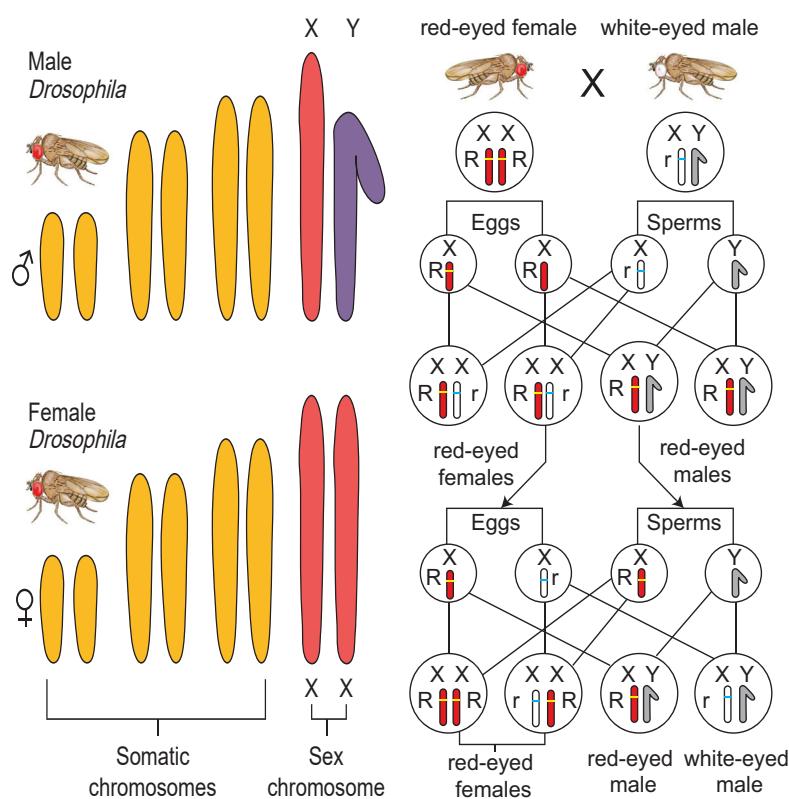


Figure 3.1: Structure of somatic and sex chromosomes in *Drosophila* and sex linkage

3.1.2 Salient features of the Chromosomal theory of inheritance

- Somatic cells of organisms are derived from the zygote by repeated cell division (mitosis). These consist of two identical sets of chromosomes. One set is received from female parent (maternal) and the other from male parent (paternal). These two chromosomes constitute the homologous pair.
- Chromosomes retain their structural uniqueness and individuality throughout the life cycle of an organism.
- Each chromosome carries specific determiners or Mendelian factors which are now termed as genes.
- The behaviour of chromosomes during the gamete formation (meiosis) provides evidence to the fact that genes or factors are located on chromosomes.

3.1.3 Support for chromosomal theory of heredity

This theory was widely discussed and controversies by scientists around the world. However, this debate has been finally cleared by the works of **Thomas Hunt Morgan (1910)** on the fruit fly *Drosophila melanogaster* ($2n=8$). This fruit fly completed their life cycle within two weeks. The alleles for red or white eye colour are present on the X chromosome but there is no counterpart for this gene on the Y chromosome. Thus, females have two alleles for this gene, whereas males have only one (Figure 3.1). The genetic results were completely based on meiotic behaviour of the X and Y chromosomes. Similarly, the genes for yellow body colour and miniature wings are also carried on the X chromosome. This study strongly supports the idea that genes are located on chromosomes. The linked genes connected together on sex chromosome is called **sex linkage**.



3.1. Comparison between gene and chromosome behaviour

Around twentieth century cytologists established that, generally the total number of chromosomes is constant in all cells of a species. A diploid eukaryotic cell has two haploid sets of chromosomes, one set from each parent. All somatic cells of an organism carry the same genetic complement. The behaviour of chromosomes during meiosis not only explains Mendel's principles but leads to new and different approaches to study about heredity.

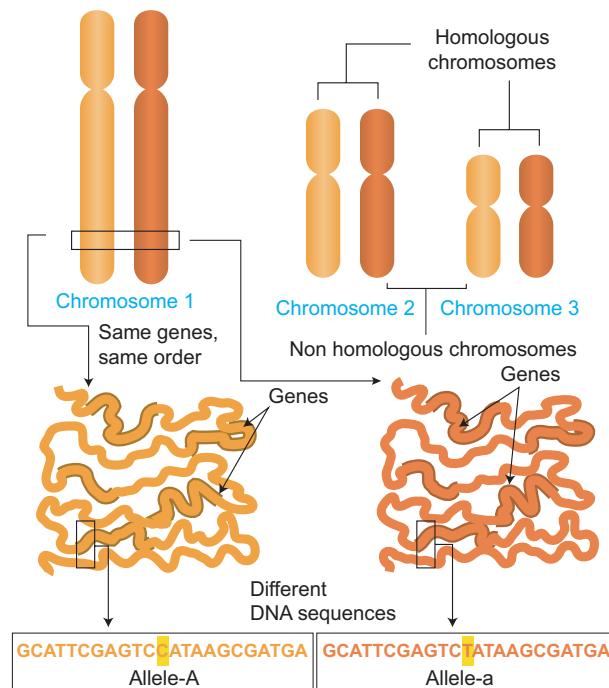


Figure 3.2: Comparison of chromosome and gene behaviour

Mendelian factors	Chromosomes behaviour
1. Alleles of a factor occur in pair	Chromosomes occur in pairs
2. Similar or dissimilar alleles of a factor separate during the gamete formation	The homologous chromosomes separate during meiosis
3. Mendelian factors can assort independently	The paired chromosomes can separate independently during meiosis but the linked genes in the same chromosome normally do not assort independently.

Table 3.1: Parallelism between Mendelian factors and chromosomal behaviour.

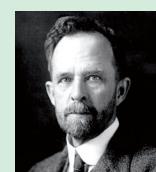
The important aspects to be remembered about the chromosome behaviour during cell division (meiosis) are as follows.

- The alleles of a genotype are found in the same locus of a homologous chromosome (A/a) (Figure 3.2).
- In the S phase of meiotic interphase each chromosome replicates forming two copies of each allele (AA/aa), one on each chromatid.
- The homologous chromosomes segregate in anaphase I, thereby separating two different alleles (AA) and (aa).
- In anaphase II of meiosis, separation of sister chromatids of homologous chromosomes takes place. Therefore, each daughter cell (gamete) carries only a single allele (gene) of a character (A), (A), (a) and (a).

Organism	Number of chromosomes (2n)
Adder's tongue fern (<i>Ophioglossum</i>)	1262
Horsetail (<i>Equisetum</i>)	216
Giant sequoia	22
<i>Arabidopsis</i>	10
Sugarcane	80
Apple	34
Rice	24
Potato	48
Maize	20
Onion	16
<i>Haplopappus gracilis</i>	4

Table 3.2 : Number of Chromosomes

Thomas Hunt Morgan (1933) received Nobel Prize in Physiology or Medicine for his discoveries concerning the role played by chromosomes in heredity.



Fossil Genes: Some of the junk DNA is made up of pseudo genes, the sequences presence in that was once working genes. They lost their ability to make proteins. They tell the story of evolution through fossilized parts.



DO YOU KNOW? Some of the junk DNA is made up of pseudo genes, the sequences presence in that was once working genes.

They lost their ability to make proteins. They tell the story of evolution through fossilized parts.



3.2 Linkage

The genes which determine the character of an individual are carried by the chromosomes. The genes for different characters may be present either in the same chromosome or in different chromosomes. When the genes are present in different chromosomes, they assort independently according to Mendel's Law of Independent Assortment. Biologists came across certain genetic characteristics that did not assort out independently in other organisms after Mendel's work. One such case was reported in Sweet pea (*Lathyrus odoratus*) by **William Bateson** and **Reginald C. Punnet** in 1906. They crossed one homozygous strain of sweet peas having **purple flowers and long pollen grains** with another homozygous strain having **red flowers and round pollen grains**. All the F₁ progenies had purple flower and long pollen grains indicating purple flower long pollen (PL/PL) was dominant over red flower round pollen (pl/pl). When they crossed the F₁ with double recessive parent (test cross) in results, F₂ progenies did not exhibit in 1:1:1:1 ratio as expected with independent assortment. A greater number of F₂ plants had purple flowers and long pollen or red flowers and round pollen. So they concluded that genes for purple colour and long pollen grain and the genes for red colour and round pollen grain were found close together in the same homologous pair of chromosomes. These genes do not allow themselves to be separated. So they do not assort independently. This type of tendency of genes to stay together during separation of chromosomes is called **Linkage**.

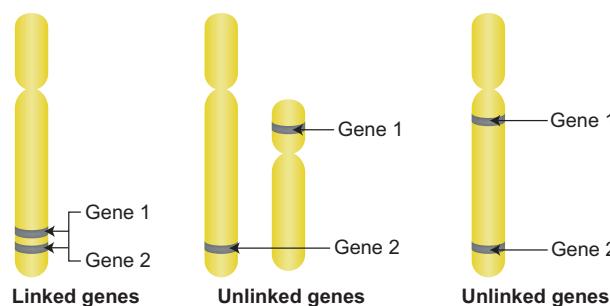


Figure 3.3: Arrangement of linked and unlinked genes on chromosome

Genes located close together on the same chromosome and inherited together are called **linked genes**. But the two genes that are sufficiently far apart on the same chromosome are called **unlinked genes or syntenic genes** (Figure 3.3). Such condition is known as **synteny**. It is to be differentiated by the value of recombination frequency. If the recombination frequency value is more than 50 % the two genes show unlinked. when the recombination frequency value is less than 50 %, they show linked. Closely located genes show strong linkage, while genes widely located show weak linkages.

3.2.1 Coupling and Repulsion theory

The two dominant alleles or recessive alleles occur in the same homologous chromosomes, tend to inherit together into same gamete are called **coupling or cis configuration** (Figure: 3.5). If dominant or recessive alleles are present on two different, but homologous chromosomes they inherit apart into different gamete are called **repulsion or trans configuration** (Figure: 3. 6).

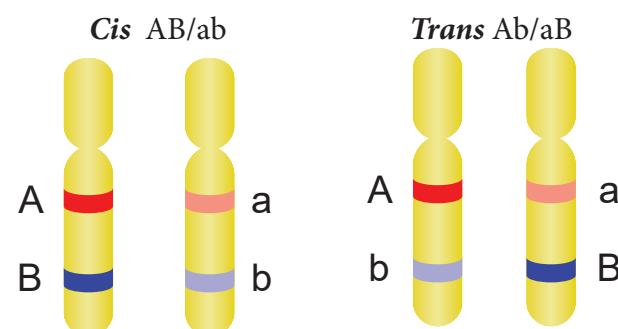


Figure 3.4: Cis-Trans arrangement of genes

3.2.2 Kinds of Linkage

T.H. Morgan found two types of linkage. They are complete linkage and incomplete linkage depending upon the absence or presence of new combination of linked genes.

Complete Linkage

If the chances of separation of two linked genes are not possible those genes always remain

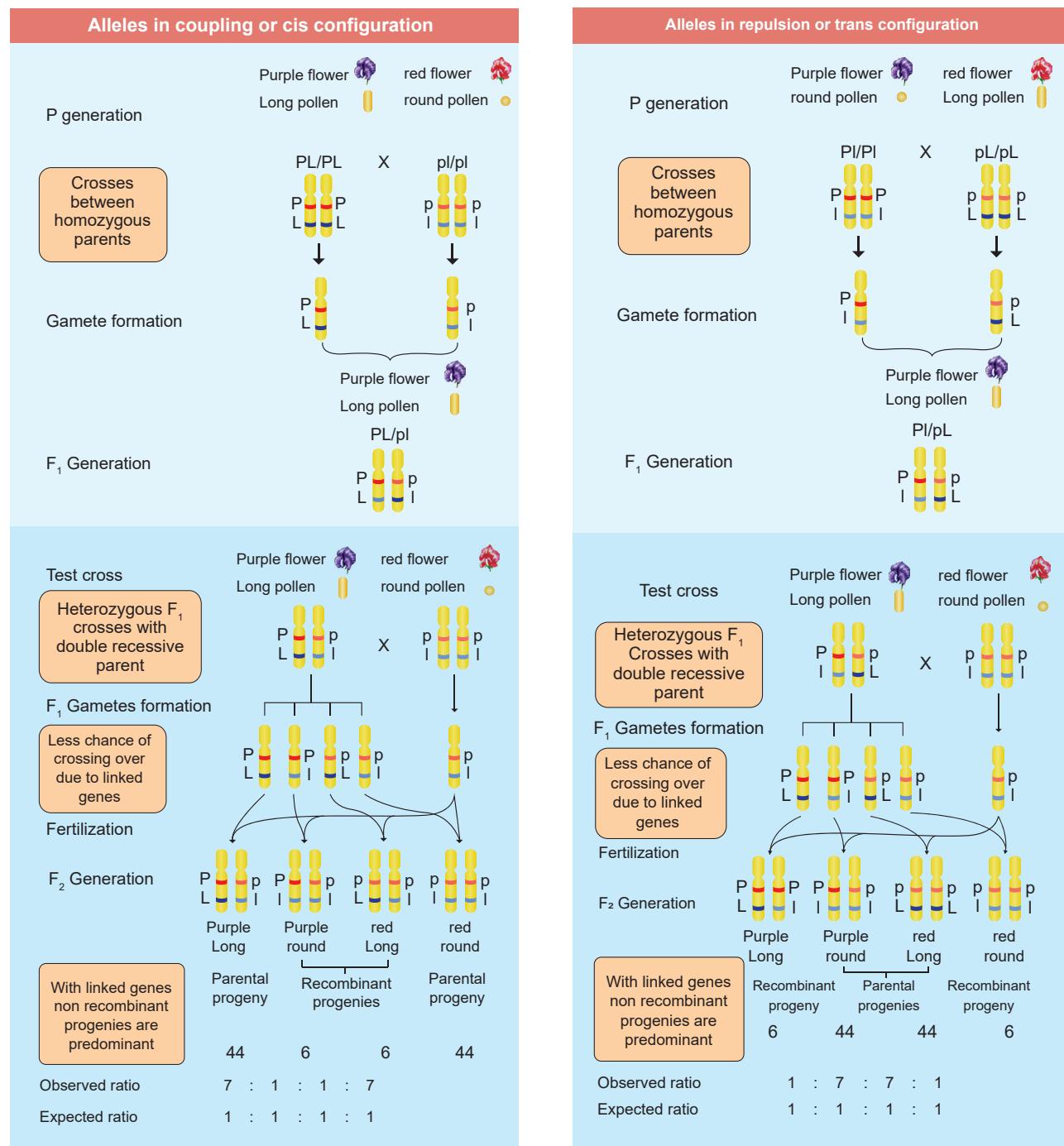


Figure 3.5: Alleles in coupling or cis configuration

together as a result, only parental combinations are observed. The linked genes are located very close together on the same chromosome such genes do not exhibit crossing over. This phenomenon is called **complete linkage**. It is rare but has been reported in male *Drosophila* (Figure 3.7). **C.B Bridges** (1919) discovered that crossing over is completely absent in some species of male *Drosophila*.

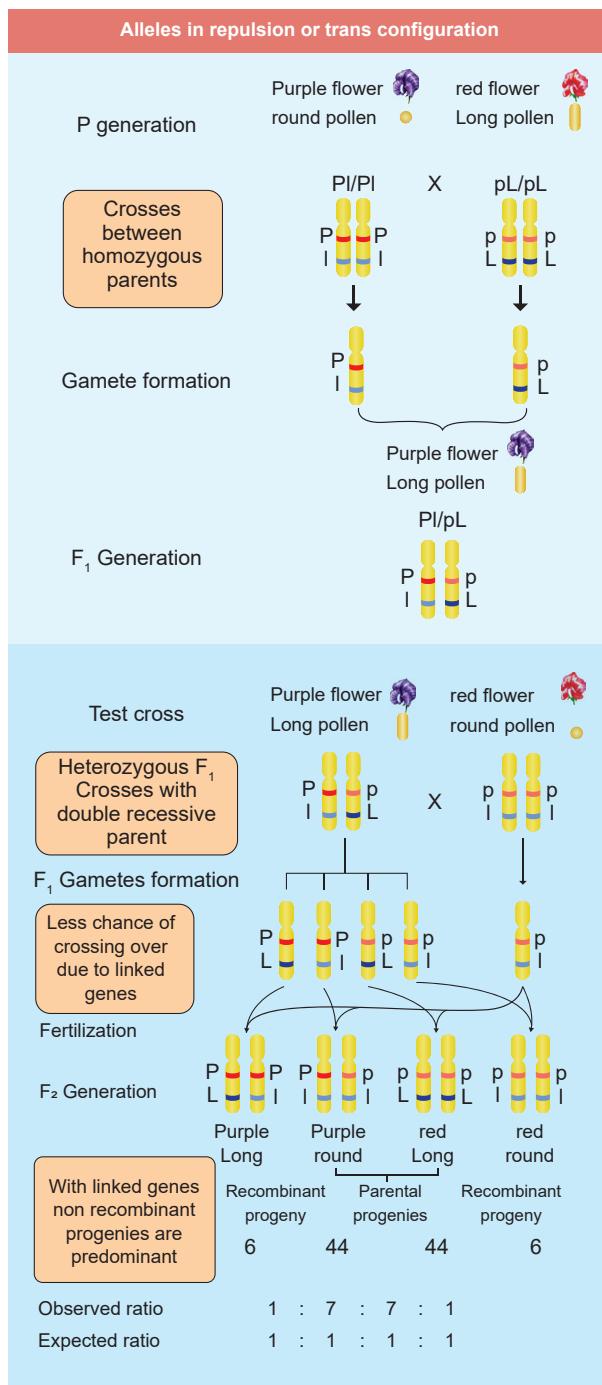


Figure 3.6: Alleles in repulsion or trans configuration

Incomplete Linkage

If two linked genes are sufficiently apart, the chances of their separation are possible. As a result, parental and non-parental combinations are observed. The linked genes exhibit some crossing over. This phenomenon is called **incomplete linkage**. This was observed in maize. (Figure 3.8) It was reported by Hutchinson.

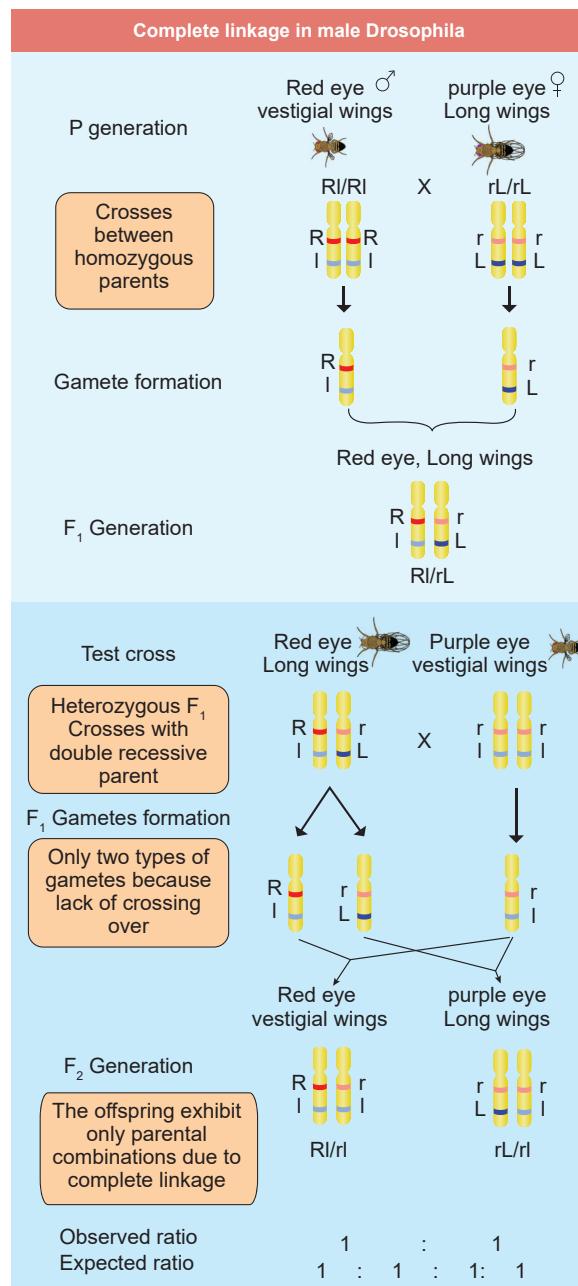


Figure 3.7: Complete linkage in male *Drosophila*

3.2.3 Linkage Groups

The groups of linearly arranged linked genes on a chromosome are called **Linkage groups**. In any species the number of linkage groups corresponds to the number haploid set of chromosomes. Example:

Name of organism	Linkage groups
<i>Mucor</i>	2
<i>Drosophila</i>	4
Sweet pea	7
<i>Neurospora</i>	7
Maize	10

Table 3.3 : Linkage groups in some organisms

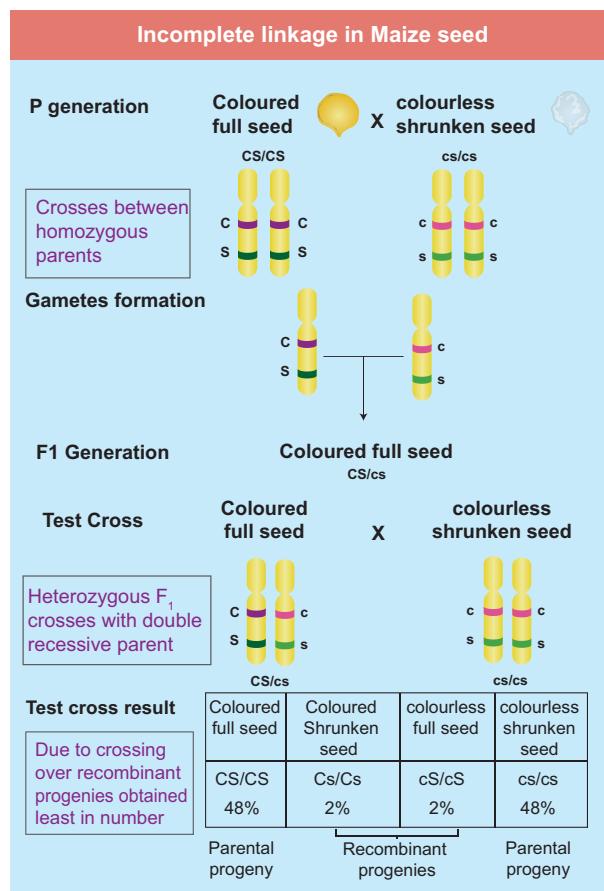


Figure 3.8: Incomplete linkage in Maize seed

Linkage and crossing over are two processes that have opposite effects. Linkage keeps particular genes together but crossing over mixes them. The differences are given below.

Linkage	Crossing over
1. The genes present on chromosome stay close together	It leads to separation of linked genes
2. It involves same chromosome of homologous chromosome	It involves exchange of segments between non-sister chromatids of homologous chromosome.
3. It reduces new gene combinations	It increases variability by forming new gene combinations. lead to formation of new organism

Table 3.4: Differences between linkage and crossing over



3.3 Crossing Over

Crossing over is a biological process that produces new combination of genes by inter-changing the corresponding segments between non-sister chromatids of homologous pair of chromosomes. The term 'crossing over' was coined by **Morgan (1912)**. It takes place during pachytene stage of prophase I of meiosis. Usually crossing over occurs in germinal cells during gametogenesis. It is called meiotic or germinal crossing over. It has universal occurrence and has great significance. Rarely, crossing over occurs in somatic cells during mitosis. It is called somatic or mitotic crossing over.

3.3.1 Mechanism of Crossing Over

Crossing over is a precise process that includes stages like synapsis, tetrad formation, cross over and terminalization.

(i) Synapsis

Intimate pairing between two homologous chromosomes is initiated during zygotene stage of prophase I of meiosis I. Homologous chromosomes are aligned side by side resulting in a pair of homologous chromosomes called **bivalents**. This pairing phenomenon is called **synapsis or syndesis**. It is of three types,

1. **Procentric synapsis:** Pairing starts from middle of the chromosome.
2. **Proterminal synapsis:** Pairing starts from the telomeres.
3. **Random synapsis:** Pairing may start from anywhere.

(ii) Tetrad Formation

Each homologous chromosome of a bivalent begin to form two identical sister chromatids, which remain held together by a centromere. At this stage each bivalent has four chromatids. This stage is called **tetrad stage**.

(iii) Cross Over

After tetrad formation, crossing over occurs in pachytene stage. The non-sister chromatids of homologous pair make a contact at one or more points. These points of contact between non-sister chromatids of homologous chromosomes

are called **Chiasmata** (singular-Chiasma). At chiasma, cross-shaped or X-shaped structures are formed, where breaking and rejoining of two chromatids occur. This results in reciprocal exchange of equal and corresponding segments between them. A recent study reveals that synapsis and chiasma formation are facilitated by a highly organised structure of filaments called **Synaptonemal Complex (SC)** (Figure 3.9). This synaptonemal complex formation is absent in some species of male *Drosophila* hence crossing over does not take place.

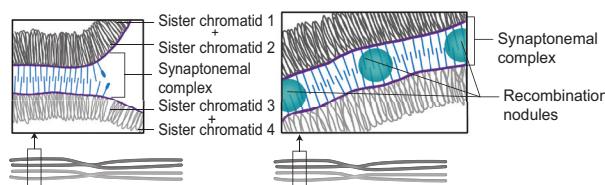


Figure 3.9: Structure of Synaptonemal Complex

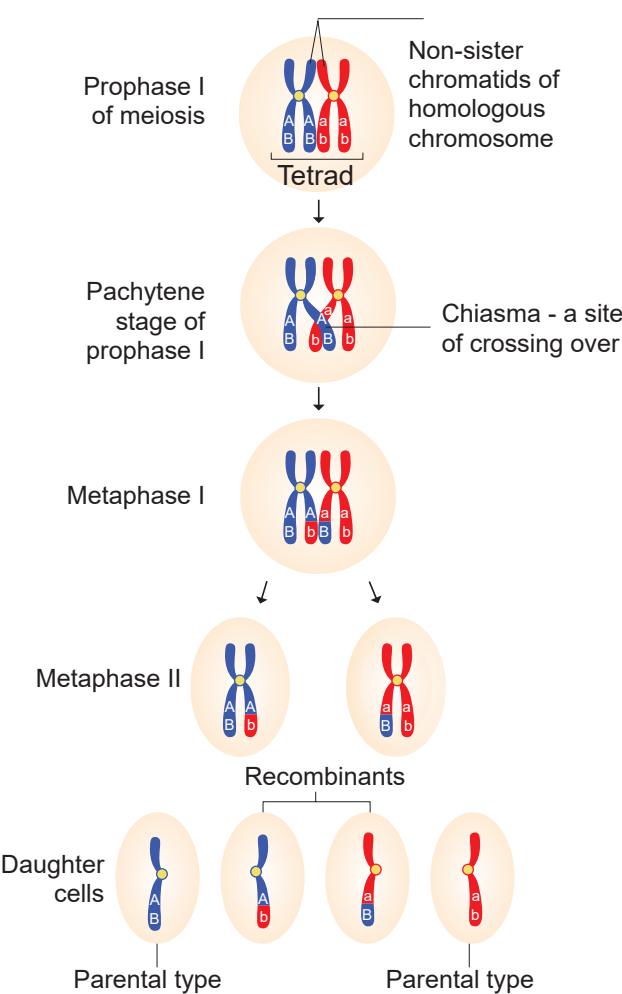


Figure 3.10: Mechanism of crossing over



(iv) Terminalisation

After crossing over, chiasma starts to move towards the terminal end of chromatids. This is known as **terminalisation**. As a result, complete separation of homologous chromosomes occurs. (Figure 4.10)

3.3.2 Types of Crossing Over

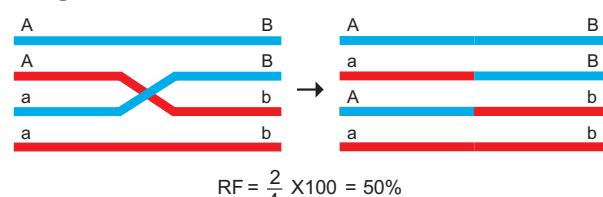
Depending upon the number of chiasmata formed crossing over may be classified into three types. (Figure 3.11)

1. **Single cross over:** Formation of single chiasma and involves only two chromatids out of four.

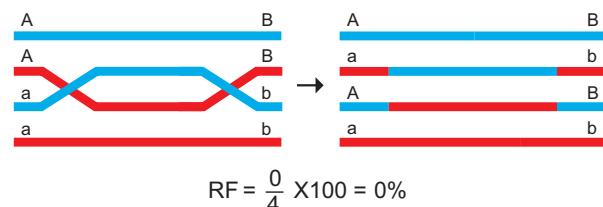
No cross over



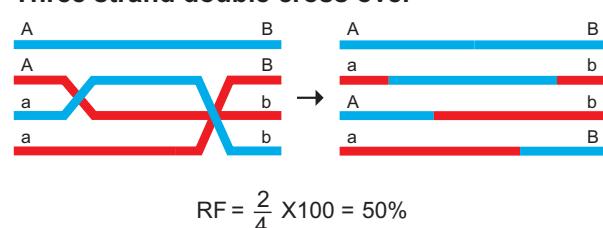
Single cross over



Two strand double cross over



Three strand double cross over



Four strand double cross over

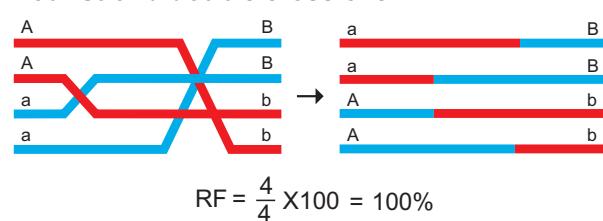


Figure 3.11: Types of crossing over and its Recombination Frequency (RF)

Activity: Solve this

Consider two hypothetical recessive autosomal genes *a* and *b*, where a heterozygote is testcrossed to a double homozygous mutant. Predict the phenotypic ratios under the following conditions:

- a* and *b* are located on separate autosomes.
- a* and *b* are linked on the same autosome but are so far apart that a crossover occurs between them.
- a* and *b* are linked on the same autosome but are so close together that a crossover almost never occurs.

2. **Double cross over:** Formation of two chiasmata and involves two or three or all four strands
3. **Multiple cross over:** Formation of more than two chiasmata and crossing over frequency is extremely low.

3.3.3 Importance of Crossing Over

Crossing over occurs in all organisms like bacteria, yeast, fungi, higher plants and animals. Its importance is

- Exchange of segments leads to new gene combinations which plays an important role in evolution.
- Studies of crossing over reveal that genes are arranged linearly on the chromosomes.
- Genetic maps are made based on the frequency of crossing over.
- Crossing over helps to understand the nature and mechanism of gene action.
- If a useful new combination is formed it can be used in plant breeding.

3.3.4 Recombination

Crossing over results in the formation of new combination of characters in an organism called recombinants. In this, segments of DNA are broken and recombined to produce new combinations of alleles. This process is called **Recombination**. (Figure 3.12)

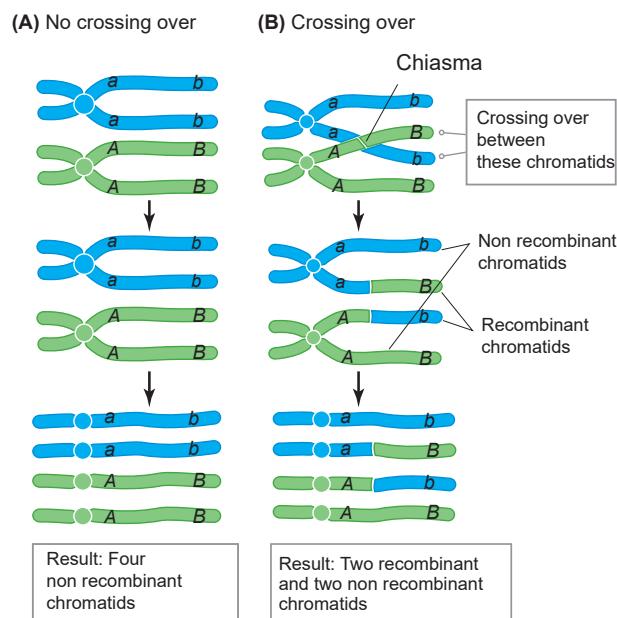


Figure 3.12 : Recombination

The widely accepted model of DNA recombination during crossing over is **Holliday's hybrid DNA model**. It was first proposed by **Robin Holliday** in 1964. It involves several steps. (Figure 3.13)

- Homologous DNA molecules are paired side by side with their duplicated copies of DNAs
- One strand of both DNAs cut in one place by the enzyme **endonuclease**.
- The cut strands cross and join the homologous strands forming the **Holliday structure or Holliday junction**.
- The Holliday junction migrates away from the original site, a process called **branch migration**, as a result heteroduplex region is formed.
- DNA strands may cut along through the vertical (V) line or horizontal (H) line.
- The vertical cut will result in heteroduplexes with recombinants.
- The horizontal cut will result in heteroduplex with non recombinants.

Calculation of Recombination Frequency (RF)

The percentage of recombinant progeny in a cross is called recombination frequency. The recombination frequency (cross over frequency) (RF) is calculated by using the following formula. The data is obtained from alleles in coupling configuration (Figure 3.14)

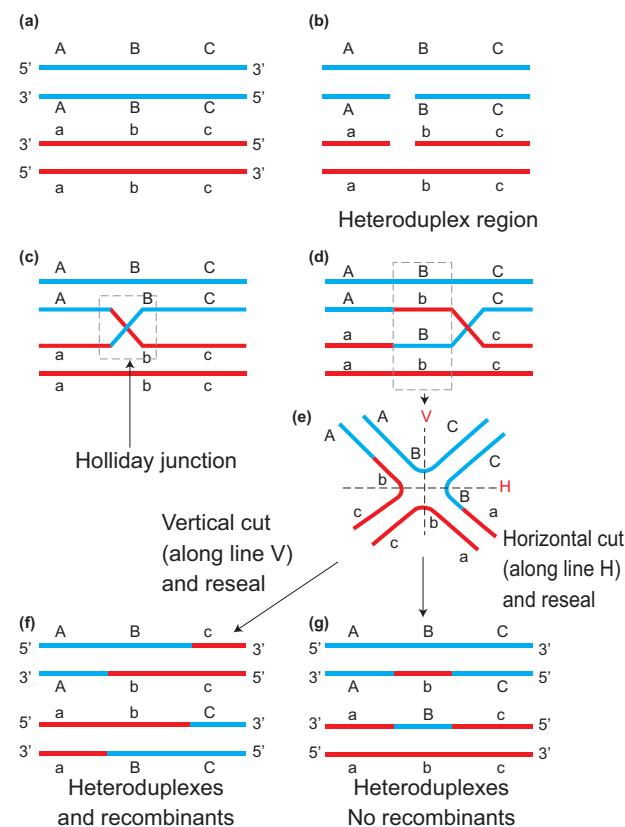


Figure 3.13: Holliday model showing Recombination

$$RF = \frac{\text{Number of recombinants}}{\text{Number of off springs}} \times 100$$

$$= \frac{6+6}{44+6+6+44} \times 100$$

$$= \frac{12}{100} \times 100$$

$$RF = 12\%$$

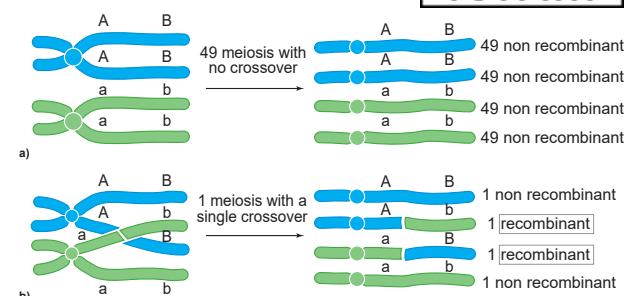


Figure 3.14 Recombination frequency observation

Check your Grasp

Find out Recombination frequency value from the above figure.

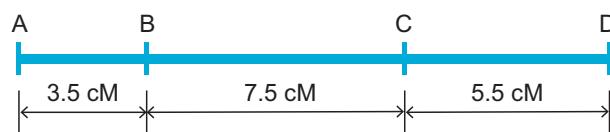


3.3.5 Genetic Mapping

Genes are present in a linear order along the chromosome. They are present in a specific location called **locus** (plural: loci). The diagrammatic representation of position of genes and related distances between the adjacent genes is called **genetic mapping**. It is directly proportional to the frequency of recombination between them. It is also called as **linkage map**. The concept of gene mapping was first developed by Morgan's student **Alfred H Sturtevant** in 1913. It provides clues about where the genes lies on that chromosome.

Map distance

The unit of distance in a genetic map is called a **map unit** (m.u). One map unit is equivalent to one percent of crossing over (Figure 4.). One map unit is also called a centimorgan (cM) in honour of **T.H. Morgan**. 100 centimorgan is equal to one Morgan (M). For example: A distance between A and B genes is estimated to be 3.5 map units. It is equal to 3.5 centimorgans or 3.5 % or 0.035 recombination frequency between the genes.



Genetic maps can be constructed from a series of test crosses for pairs of genes called **two point crosses**. But this is not efficient because double cross over is missed.

Three point test cross

A more efficient mapping technique is to construct based on the results of **three-point test cross**. It refers to analyzing the inheritance patterns of three alleles by test crossing a triple recessive heterozygote with a triple recessive homozygote. It enables to determine the distance between the three alleles and the order in which they are located on the chromosome. Double cross overs can be detected which will provide more accurate map distances.

Three-point test cross can be best understood by considering following an example.

In maize (corn), the three recessive alleles are

- l for lazy or prostrate growth habit
- g for glossy leaf
- s for sugary endosperm

These three recessive alleles (l g s) are crossed with wild type dominant alleles (L G S).

Parents	LGS / LGS	x	lgs / lgs
Gametes	LGS	x	lgs
F ₁ trihybrid	LGS / lgs		
Test cross			
(Heterozygous F ₁ crosses with triple recessive alleles)	LGS / lgs	x	lgs / lgs

This trihybrid test cross produces 8 different types ($2^3=8$) of gametes in which 740 progenies are observed. The following table shows the result obtained from a test cross of corn with three linked genes.

The analysis of a three-point cross:

S. no	Phenotype of test cross progeny	Gamete types	Number of progenies
1.	Normal (wild type)	L G S	286
2.	Lazy	l G S	33
3.	Glossy	L g S	59
4.	Sugary	L G s	4
5.	Lazy, glossy	l g S	2
6.	Lazy, sugary	l G s	44
7.	Glossy, sugary	L g s	40
8.	Lazy, glossy, sugary	l g s	272
Total			740

From the above result, we must be careful to observe parental (P) and recombinant (R) types. First note that parental genotypes for the triple homozygotes are L G S and l g s, then analyse two recombinant loci at a time orderly L G / l g, L S / l s and G S / g s. In this any combination other than these two constitutes a recombinant (R).

Let's analyse the loci of two alleles at a time starting with L and G Since the L G and l g parental genotypes the recombinants will be L g and l G. The Recombinant frequency (RF) for these two alleles can be calculated as follows



S.no	Phenotype of test cross progeny	Gamete types	Number of progenies	Recombinant for loci		
				L and G	L and S	G and S
1.	Normal (wild type)	L G S	286			
2.	Lazy	l G S	33	R	R	
3.	Glossy	L g S	59	R		R
4.	Sugary	L G s	4		R	R
5.	Lazy, glossy	l g S	2		R	R
6.	Lazy, sugary	l G s	44	R		R
7.	Glossy, sugary	L g s	40	R	R	
8.	Lazy, glossy, sugary	l g s	272			
Total			740	176	79	109

$$RF = \frac{\text{Total number of recombinants}}{\text{Total number of progenies}} \times 100$$

$$RF = \frac{33 + 59 + 44 + 40}{740} \times 100$$

$$RF = \frac{176}{740} \times 100$$

$$RF = 23.7\%$$

For L and S loci, the recombinants are L s and l S. The Recombinant frequency (RF) will be as follows

$$RF = \frac{33 + 4 + 2 + 40}{740} \times 100$$

$$RF = \frac{79}{740} \times 100$$

$$RF = 10.7\%$$

For G and S loci, the recombinants are G s and g S. The Recombinant frequency (RF) will be as follows

$$RF = \frac{59 + 4 + 2 + 44}{740} \times 100$$

$$RF = \frac{109}{740} \times 100$$

$$RF = 14.7\%$$

All the loci are linked, because all the RF values are considerably less than 50%. In this L G loci show highest RF value, they must be farthest apart. Therefore, the S locus must lie between them. The order of genes should be l s g. A genetic

map can be drawn as follows: (Figure 3.15)

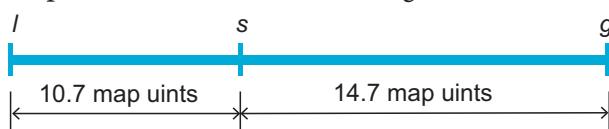


Figure: 3.15 Gene mapping

A final point note that two smaller map distances, 10.7 m.u and 14.7., is add up to 25.4 m.u., which is greater than 23.7 m.u., the distance calculated for l and g. we must identify the two least number of progenies (totaling 8) in relation to recombination of L and G. These two least progenies are double recombinants arising from double cross over. The two least progenies not only counted once should have counted each of them twice because each represents a double recombinant progeny. Hence, we can correct the value adding the numbers $33+59+44+40+4+4+2+2=188$. Of the total of 740, this number exactly 25.4 %, which is identical with the sum of two component values.

The test cross parental combination can be re written as follows:

$$\text{LSG / lsg} \quad \times \quad \text{lsg / lsg}$$

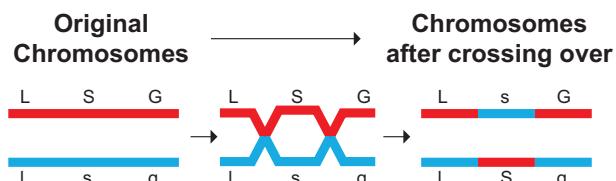


Figure: 3.16 Gene order showing double recombinant



Uses of genetic mapping

- It is used to determine gene order, identify the locus of a gene and calculate the distances between genes.
- They are useful in predicting results of dihybrid and trihybrid crosses.
- It allows the geneticists to understand the overall genetic complexity of particular organism.

3.4 Multiple alleles

A given phenotypic trait of an individual depends on a single pair of genes, each of which occupies a specific position called the locus on homologous chromosome. When any of the three or more allelic forms of a gene occupy the same locus in a given pair of homologous chromosomes, they are said to be called **multiple alleles**.

Check your Grasp

There may be multiple alleles within the population, but individuals have only two of those alleles. Why?

3.4.1 Characteristics of multiple alleles

- Multiple alleles of a series always occupy the same locus in the homologous chromosome. Therefore, no crossing over occurs within the alleles of a series.
- Multiple alleles are always responsible for the same character.
- The wild type alleles of a series exhibit dominant character whereas mutant type will influence dominance or an intermediate phenotypic effect.
- When any two of the mutant multiple alleles are crossed the phenotype is always mutant type and not the wild type

3.4.2 Self-sterility in *Nicotiana*

In plants, multiple alleles have been reported in association with self-sterility or self-incompatibility. Self-sterility means that the

pollen from a plant is unable to germinate on its own stigma and will not be able to bring about fertilization in the ovules of the same plant. East (1925) observed multiple alleles in *Nicotiana* which are responsible for self-incompatibility or self-sterility. The gene for self-incompatibility can be designated as S, which has allelic series S_1, S_2, S_3, S_4 and S_5 (Figure 3.17).

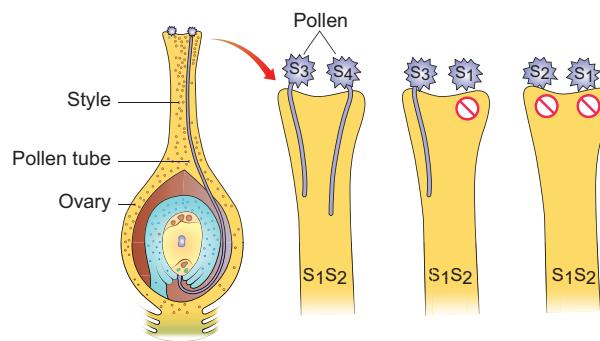


Figure: 3.17 The self-incompatibility in relation to its genotype in tobacco

The cross-fertilizing tobacco plants were not always homozygous as S_1S_1 or S_2S_2 , but all plants were heterozygous as S_1S_2, S_3S_4, S_5S_6 . When crosses were made between different S_1S_2 plants, the pollen tube did not develop normally. But effective pollen tube development was observed when crossing was made with other than S_1S_2 for example S_3S_4 .

Female parent (Stigma spot)	Male parent (Pollen source)		
	S_1S_2	S_2S_3	S_3S_4
S_1S_2	Self Sterile	S_3S_2 S_3S_1	S_3S_1 S_3S_2 S_4S_1 S_4S_2
S_2S_3	S_1S_2 S_1S_3	Self Sterile	S_4S_2 S_4S_3
S_3S_4	S_1S_3 S_1S_4 S_2S_3 S_2S_4	S_2S_3 S_2S_4	Self Sterile

Table: 3.5. Different combinations of progeny in self-incompatibility



When crosses were made between seed parents with S_1S_2 and pollen parents with S_2S_3 , two kinds of pollen tubes were distinguished. Pollen grains carrying S_2 were not effective, but the pollen grains carrying S_3 were capable of fertilization. Thus, from the cross $S_1S_2XS_3S_4$, all the pollens were effective and four kinds of progeny resulted: S_1S_3 , S_1S_4 , S_2S_3 and S_2S_4 . Some combinations are showed in the table-3.5.

3.5 Sex determination in plants

About 94% of all flowering plants have only one type of individual, which produces flowers with male organs (the stamens) and female organs (the carpels). Such plants are termed as sexually **monomorphic**. Some 6% of flowering plants which have two separate sexes are called **dimorphic**. Male plants produce flowers with stamens and female plants produce flowers with carpels only. Researchers are interested to study the mechanism of sex determination in plants. C.E. Allen (1917) discovered sex determination in plants. Sex determination is a complex process determined by genes, the environment and hormones.

Sex determination in *Silene latifolia* (*Melandrium album*) is of controlled by three distinct regions in a sex chromosome.

1. Y chromosome determines maleness
2. X specifies femaleness
3. X and Y show different segments

(I II III IV and V)



Does environment play a role on sex determination in plants?

Yes. Horsetail plant (*Equisetum*) grown under good conditions develop as female and those grown under stress condition develop into males.

3.5.1 Sex determination in papaya

Recently researchers in Hawaii discovered sex chromosomes in Papaya (*Carica papaya*, $2n=36$). Papaya has 17 pairs of autosomes and one pair of sex chromosomes. Male papaya plants have XY and female plants have XX. Unlike human sex chromosomes, papaya sex chromosomes look like autosomes and it is evolved from autosome. The sex chromosomes are functionally distinct because the Y chromosome carries the genes for male organ development and X bears the female organ developmental genes (Figure 3.18).

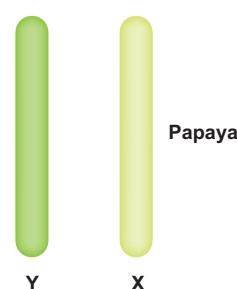


Figure 3.18 : Sex chromosome of papaya

In papaya sex determination is controlled by three alleles. They are m, M_1 and M_2 of a single gene.

Genotype	Dominant/recessive	Modification	Sex
mm	Homozygous recessive	Restrict maleness	Female
M_1m	Heterozygous	Induces maleness	Male
M_2m	Heterozygous	Induces both the sex	Bisexual (rare)
M_1M_1 or M_2M_2 or M_1M_2	Homozygous/ Heterozygous dominant	Inviable plants	Sterile

Table 3.6 : Sex determination in Papaya

3.5.2 Sex Determination in *Sphaerocarpos*

Sex determination was first described in the bryophyte *Sphaerocarpos donnellii* which has heteromorphic chromosomes. The gametophyte is haploid and heteromorphic. The male gametophyte as well as the female gametophyte is an haploid organism



Bottle liverwort-*Sphaerocarpos*

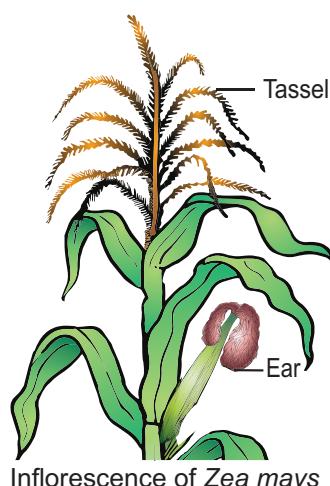


with 8 chromosome ($n=8$). The diploid sporophyte is always heterogametic. Seven autosomes are similar in both male and female gametophyte. But the eighth chromosome of female is X which is larger than the seven autosomes. The eighth chromosome of male is Y which is comparatively smaller than autosomes. The sporophyte containing XY combination produces two types of meiospores, that is some with X and others with Y chromosomes. The meiospores with X chromosomes produce female gametophyte and those with Y chromosome produces male gametophyte.

3.5.3 Sex determination in maize

Zea mays (maize) is an example for monoecious, which means male and female flowers are present on the same plant. There are two types of inflorescence. The terminal inflorescence which bears staminate florets develops from shoot apical meristem called **tassel**. The lateral inflorescence which develop pistillate florets from axillary bud is called **ear or cob**.

Unisexuality in maize occurs through the selective abortion of stamens in ear florets and pistils in tassel florets. A substitution of two single gene pairs '**ba**' for barren plant and '**ts**' for tassel seed makes the difference between monoecious and dioecious (rare) maize plants. The allele for barren plant (ba) when homozygous makes the stalk staminate by eliminating silk and ears. The allele for tassel seed (ts) transforms tassel into a pistillate structure that produce no pollen. The table-3.7 is the resultant sex expression based on the combination of these alleles. Most of these mutations are shown to be defects in



gibberellin biosynthesis. Gibberellins play an important role in the suppression of stamens in florets on the ears.

Genotype	Dominant/recessive	Modification	Sex
ba/ba ts/ts	Double recessive	Lacks silk on the stalk, but transformed tassel to pistil	Rudimentary female
ba/ba ts ⁺ /ts ⁺	Recessive and dominant	Lacks silk and have tassel	Male
ba ⁺ /ba ⁺ ts ⁺ /ts ⁺	Double dominant	Have both tassel and cob	Monoecious
ba ⁺ /ba ⁺ ts/ts	Dominant and recessive	Bears cob and lacks tassel	Normal female

Table 3.7: Sex determination in Maize (Superscript (+) denotes dominant character)

3.6 Mutation

Genetic variation among individuals provides the raw material for the ultimate source of evolutionary changes. Mutation and recombination are the two major processes responsible for genetic variation. A sudden change in the genetic material of an organisms is



Mutant Leaf

called **mutation**. The term mutation was introduced by **Hugo de Vries** (1901) while he has studying on the plant, evening primrose (*Oenothera lamarckiana*) and proposed '**Mutation theory**'. There are two broad types of changes in genetic material. They are point mutation and chromosomal mutations.





Mutational events that take place within individual genes are called gene mutations or point mutation, whereas the changes occur in structure and number of chromosomes is called chromosomal mutation. Agents which are responsible for mutation are called **mutagens**,

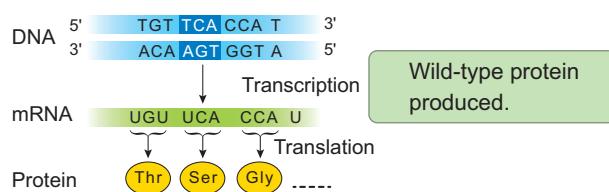
that increase the rate of mutation. Mutations can occur either spontaneously or induced. The production of mutants through exposure of mutagens is called mutagenesis, and the organism is said to be **mutagenized**.

S.No	Basis of classification	Major types of mutations	Major features
1.	Origin	Spontaneous	Occurs in the absence of known mutagen
		Induced	Occurs in the presence of known mutagen
2.	Cell type	Somatic	Occurs in non-reproductive cells
		Germ-line	Occurs in reproductive cells
3.	Effect on function	Loss-of-function (knockout, null)	Eliminates normal function
		Hypomorphic(leaky)	Reduces normal function
		Hypermorphic	Increases normal function
		Gain-of-function (ectopic expression)	Expressed at incorrect time or inappropriate cells
4.	Molecular change	Nucleotide substitution <ul style="list-style-type: none">• Transition• Transversion• Insertion• Deletion	A base pair in DNA duplex is replaced with a different base pair Purine to purine(A→G) or pyrimidine to pyrimidine(T→C) Purine to pyrimidine(A→T) or pyrimidine to purine(C→G) One or more extra nucleotides are present One or more nucleotides are missing
		<ul style="list-style-type: none">• Silent (synonymous)	No change in amino acid encoded
		<ul style="list-style-type: none">• Missense (non-synonymous)	Change in amino acid encoded
		<ul style="list-style-type: none">• Nonsense(termination)	Creates translational termination codon (UAA, UAG, or UGA)
		<ul style="list-style-type: none">• Frameshift	Shifts triplet reading of codons out of correct phase
5.	Effect on translation		

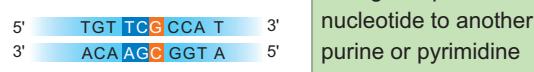
Table 3.8: Major types of mutations



a) No mutation

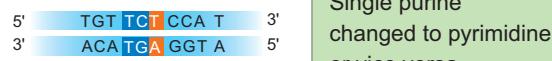


b) Transition mutation



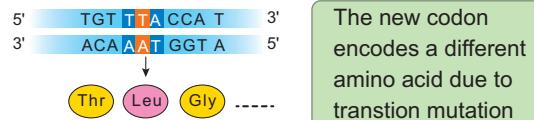
changes a purine nucleotide to another purine or pyrimidine to another pyrimidine

c) Transversion mutation



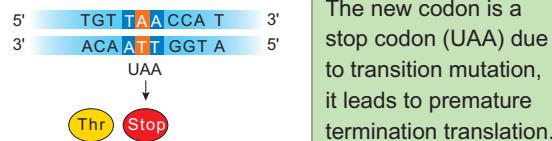
Single purine changed to pyrimidine or vice versa.

d) Missense mutation



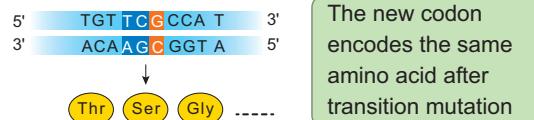
The new codon encodes a different amino acid due to transition mutation

e) Non-Sense mutation



The new codon is a stop codon (UAU) due to transition mutation, it leads to premature termination translation.

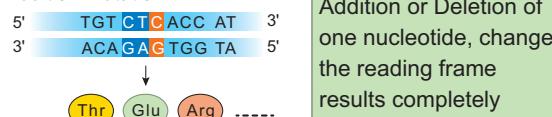
f) Silent mutation



The new codon encodes the same amino acid after transition mutation

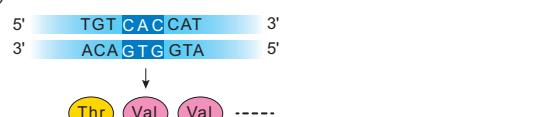
g) Frame shift mutation

i) Addition mutation



Addition or Deletion of one nucleotide, change the reading frame results completely different translation

ii) Deletion mutation



Thr Val Val ----

Figure: 3.19 Types of point mutation

3.6.1 Types of mutation

Let us see the two general classes of gene mutation:

- Mutations affecting single base or base pair of DNA are called point mutation
- Mutations altering the number of copies of a small repeated nucleotide sequence within a gene

Point mutation

It refers to alterations of single base pairs of DNA or of a small number of adjacent base pairs

Types of point mutations

Point mutation in DNA are categorised into two main types. They are base pair substitutions and base pair insertions or deletions. Base substitutions are mutations in which there is a change in the DNA such that one base pair is replaced by another (Figure: 3.17). It can be divided into two subtypes: transitions and transversions. Addition or deletion mutations are actually additions or deletions of nucleotide pairs and also called base pair addition or deletions. Collectively, they are termed **indel mutations** (for insertion-deletion).

Substitution mutations or indel mutations affect translation. Based on these different types of mutations are given below.

The mutation that changes one codon for an amino acid into another codon for that same amino acid are called **Synonymous or silent mutations**. The mutation where the codon for one amino acid is changed into a codon for another amino acid is called **Missense or non-synonymous mutations**. The mutations where codon for one amino acid is changed into a termination or stop codon is called **Nonsense mutation**. Mutations that result in the addition or deletion of a single base pair of DNA that changes the reading frame for the translation process as a result of which there is complete loss of normal protein structure and function are called **Frameshift mutations** (Figure: 3.19).

3.6.2 Mutagenic agents

The factors which cause genetic mutation are called **mutagenic agents or mutagens**. Mutagens are of two types, physical mutagen and chemical mutagen. **Muller** (1927) was the first to find out physical mutagen in *Drosophila*.



Physical mutagens:

Scientists are using temperature and radiations such as X rays, gamma rays, alfa rays, beta rays, neutron, cosmic rays, radioactive isotopes, ultraviolet rays as physical mutagen to produce mutation in various plants and animals.

Temperature: Increase in temperature increases the rate of mutation. While rise in temperature, breaks the hydrogen bonds between two DNA nucleotides which affects the process of replication and transcription.

Radiation: The electromagnetic spectrum contains shorter and longer wave length rays than the visible spectrum. These are classified into ionizing and non-ionizing radiation. Ionizing radiation are short wave length and carry enough higher energy to ionize electrons from atom. X rays, gamma rays, alfa rays, beta rays and cosmic rays which breaks the chromosomes (chromosomal mutation) and chromatids in irradiated cells. Non-ionizing radiation, UV rays have longer wavelengths and carry lower energy, so they have lower penetrating power than the ionizing radiations. It is used to treat unicellular microorganisms, spores, pollen grains which possess nuclei located near surface membrane.

Sharbati Sonora

Sharbati Sonora is a mutant variety of wheat, which is developed from Mexican variety (Sonora 64) by irradiating of gamma rays. It is the work of Dr. M.S.Swaminathan who is known as '**Father of Indian green revolution**' and his team.

Castor Aruna

Castor Aruna is mutant variety of castor which is developed by treatment of seeds with thermal neutrons in order to induce very early maturity (120 days instead of 270 days as original variety).

Chemical mutagens:

Chemicals which induce mutation are

called chemical mutagens. Some chemical mutagens are mustard gas, nitrous acid, ethyl and methyl methane sulphonate (EMS and MMS), ethyl urethane, magnous salt, formaldehyde, eosin and entrosine. Example: Nitrous oxide alters the nitrogen bases of DNA and disturb the replication and transcription that leads to the formation of incomplete and defective polypeptide during translation.

Comutagens

The compounds which are not having own mutagenic properties but can enhance the effects of known mutagens are called comutagens.

Example: Ascorbic acid increase the damage caused by hydrogen peroxide.

Caffeine increase the toxicity of methotrexate

Mustard gas (Dichloro ethyl sulphide) used as chemical weapon in world war I.

H J Muller (1928) first time used X rays to induce mutations in fruit fly.

L J Stadler reported induced mutations in plants by using X rays and gamma rays.

Chemical mutagenesis was first reported by C. Auerback (1944).

3.6.3 Chromosomal mutations

The genome can also be modified on a larger scale by altering the chromosome structure or by changing the number of chromosomes in a cell. These large-scale variations are termed as **chromosomal mutations** or **chromosomal aberrations**. Gene mutations are changes that take place within a gene, whereas chromosomal mutations are changes to a chromosome region consisting of many genes. It can be detected by microscopic examination, genetic analysis, or both. In contrast, gene mutations are never detectable microscopically. Chromosomal mutations are divided into two groups: changes in chromosome number and changes in chromosome structure.



I. Changes in chromosome number

Each cell of living organisms possesses fixed number of chromosomes. It varies in different species. Even though some species of plants and animals are having identical number of chromosomes, they will not be similar in character. Hence the number of chromosomes will not differentiate the character of species from one another but the nature of hereditary material (gene) in chromosome that determines the character of species.

Sometimes the chromosome number of somatic cells are changed due to addition or elimination of individual chromosome or basic set of chromosomes. This condition is known as **numerical chromosomal aberration** or **ploidy**. There are two types of ploidy.

- (i). Ploidy involving individual chromosomes within a diploid set (**Aneuploidy**)
- (ii). Ploidy involving entire sets of chromosomes (**Euploidy**) (Figure 3.20)

(i) Aneuploidy

It is a condition in which diploid number is altered either by addition or deletion of one or more chromosomes. Organisms

showing aneuploidy are known as **aneuploids** or **heteroploids**. They are of two types, Hyperploidy and Hypoploidy (Figure 3.21).

1. Hyperploidy

Addition of one or more chromosomes to diploid sets are called **hyperploidy**. Diploid set of chromosomes represented as Disomy. Hyperploidy can be divided into three types. They are as follows,

(a) Trisomy

Addition of single chromosome to diploid set is called **Simple trisomy** ($2n+1$). Trisomics were first reported by Blackeslee (1910) in *Datura stramonium* (Jimson weed). But later it was reported in *Nicotiana*, *Pisum* and *Oenothera*. Sometimes addition of two individual chromosome from different chromosomal pairs to normal diploid sets are called **Double trisomy** ($2n+1+1$).

(b) Tetrasomy

Addition of a pair or two individual pairs of chromosomes to diploid set is called **tetrasomy** ($2n+2$) and **Double tetrasomy** ($2n+2+2$) respectively. All possible tetrasomics are available in Wheat.

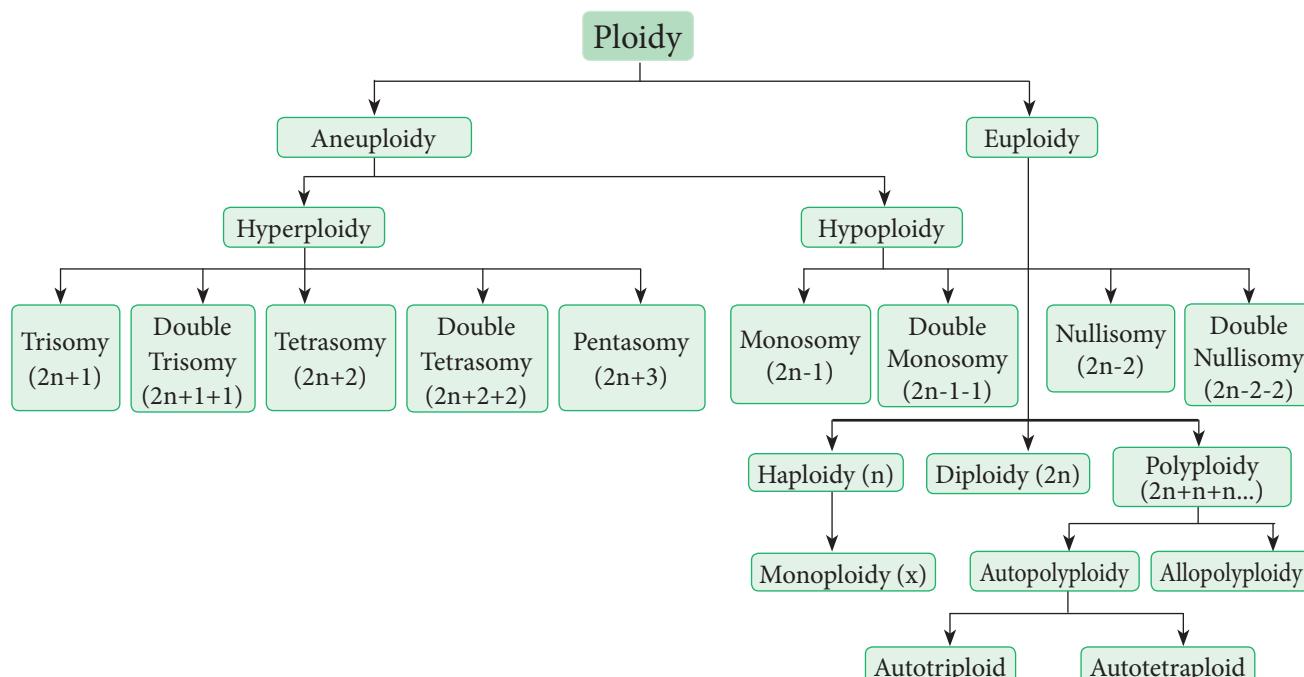


Figure 3.20 Types of Ploidy



(c) Pentasomy

Addition of three individual chromosome from different chromosomal pairs to normal diploid set are called pentasomy ($2n+3$).

2. Hypoploidy

Loss of one or more chromosome from the diploid set in the cell is called **hypoploidy**. It can be divided into two types. They are

(a) Monosomy

Loss of a single chromosome from the diploid set are called **monosomy** ($2n-1$). However loss of two individual or three individual chromosomes are called **double monosomy** ($2n-1-1$) and **triple monosomy** ($2n-1-1-1$) respectively. Double monosomics are observed in maize.

(b) Nullisomy

Loss of a pair of homologous chromosomes or two pairs of homologous chromosomes from the diploid set are called **Nullisomy** ($2n-2$) and **double Nullisomy** ($2n-2-2$) respectively. Selfing of monosomic plants produce nullisomics. They are usually lethal.

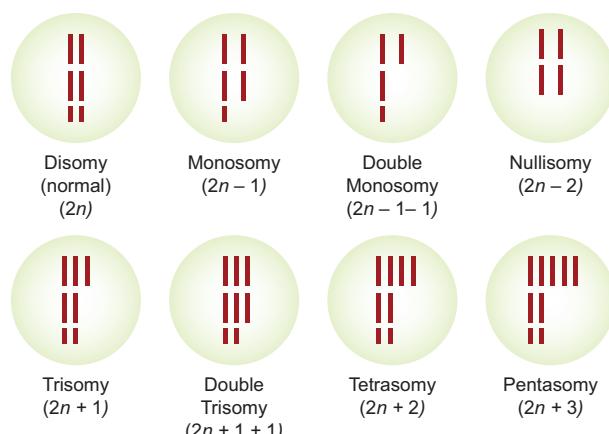


Figure 3.21 Types of aneuploidy

(ii) Euploidy

Euploidy is a condition where the organisms possess one or more basic sets of chromosomes. Euploidy is classified as monoploid, diploid and polyploid. The condition where an organism or somatic cell has two sets of chromosomes are

called diploid ($2n$). Half the number of somatic chromosomes is referred as gametic chromosome number called haploid (n). It should be noted that haplody (n) is different from a monoploid (x). For example, the common wheat plant is a polyploid (hexaploid) $2n=6x=42$ chromosomes. Its haploid number (n) is 21, but its monoploid (x) is 12. Therefore, the haploid and diploid condition came regularly one after another and the same number of chromosomes is maintained from generation to generation, but monoploid condition occurs when an organism is under polyploid condition. In a true diploid both the monoploid and haploid chromosome number are same. Thus a monoploid can be a haploid but all haploids cannot be a monoploid.

Polyploidy

Polyploidy is the condition where an organism possesses more than two basic sets of chromosomes. When there are three, four, five or six basic sets of chromosomes, they are called triploidy ($3x$), tetraploidy ($4x$), pentaploidy ($5x$) and hexaploidy ($6x$) respectively. Generally, polyploidy is very common in plants but rarer in animals. An increase in the number of chromosome sets has been an important factor in the origin of new plant species. But higher ploidy level leads to death. Polyploidy is of two types. They are autopolyploidy and allopolyploidy.

1. Autopolyploidy

The organism which possesses more than two haploid sets of chromosomes derived from within the same species is called autopolyploid. They are divided into two types. Autotriploids and autotetraploids.

Autotriploids have three set of its own genomes. They can be produced artificially by crossing between autotetraploid and diploid species. They are highly sterile due to defective gamete formation. Example: The cultivated banana are usually triploids and are seedless having larger fruits than diploids. Triploid sugar beets have higher sugar content than diploids



and are resistant to moulds. Common doob grass (*Cyanodon dactylon*) is a natural autotriploid. Seedless watermelon, apple, sugar beet, tomato, banana are man made autotriploids.

Autotetraploids have four copies of its own genome. They may be induced by doubling the chromosomes of a diploid species. Example: rye, grapes, alfalfa, groundnut, potato and coffee.

2. Allopolyploidy

An organism which possesses two or more basic sets of chromosomes derived from two different species is called allopolyploidy. It can be developed by interspecific crosses and fertility is restored by chromosome doubling with colchicine treatment. Allopolyploids are formed between closely related species only. (Figure 3.22)

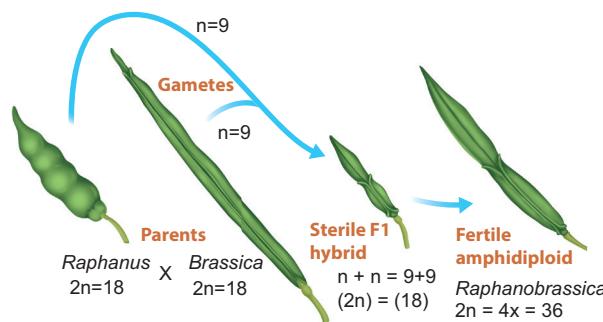


Figure 3. 22 Raphanobrassica

Example:1 **Raphanobrassica**, G.D. Karpechenko (1927) a Russian geneticist, crossed the radish (*Raphanus sativus*, 2n=18) and cabbage (*Brassica oleracea*, 2n=18) to produce F₁ hybrid which was sterile. When he doubled the chromosome of F₁ hybrid he got it fertile. He expected this plant to exhibit the root of radish and the leaves like cabbage, which would make the entire plant edible, but the case was vice versa, so he was greatly disappointed.

Example: 2 **Triticale**, the successful first man made cereal. Depending on the ploidy level Triticale can be divided into three main groups.

- Tetraploidy: Crosses between diploid wheat and rye.
- Hexaploidy: Crosses between tetraploid wheat (*Triticum durum*) (macaroni wheat) and rye

(iii). Octoploidy: Crosses between hexaploid wheat *T. aestivum* (bread wheat) and rye

Hexaploid Triticale hybrid plants demonstrate characteristics of both macaroni wheat and rye. For example, they combine the high-protein content of wheat with rye's high content of the amino acid lysine, which is low in wheat. It can be explained by chart below (Figure: 3.23).

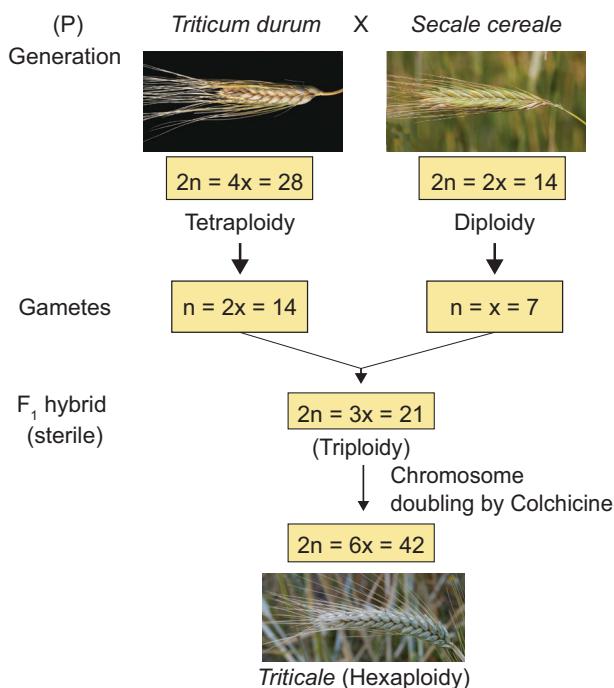


Figure 3.23 Triticale

Colchicine , an alkaloid is extracted from root and corms of *Colchicum autumnale*, when applied in low concentration to the growing tips of the plants it will induce polyploidy. Surprisingly it does not affect the source plant *Colchicum*, due to presence of anticolchicine.



Activity: Solve this

When two plants (A and B) belonging to the same genus but different species are crossed, the F₁ hybrid is viable and has more ornate flowers. Unfortunately, this hybrid is sterile and can only be propagated by vegetative cuttings. Explain the sterility of the hybrid and what would have to occur for the sterility of this hybrid to be reversed.



Significance of Ploidy

- Many polyploids are more vigorous and more adaptable than diploids.
- Many ornamental plants are autotetraploids and have larger flower and longer flowering duration than diploids.
- Autopolyploids usually have increase in fresh weight due to more water content.
- Aneuploids are useful to determine the phenotypic effects of loss or gain of different chromosomes.
- Many angiosperms are allopolyploids and they play a role in an evolution of plants.

II Structural changes in chromosome (Structural chromosomal aberration)

Structural variations caused by addition or deletion of a part of chromosome leading to rearrangement of genes is called **structural chromosomal aberration**. It occurs due to ionizing radiation or chemical compounds. On the basis of breaks and reunion in chromosomes, there are four types of aberrations. They are classified under two groups.

A. Changes in the number of the gene loci

- Deletion or Deficiency
- Duplication or Repeat

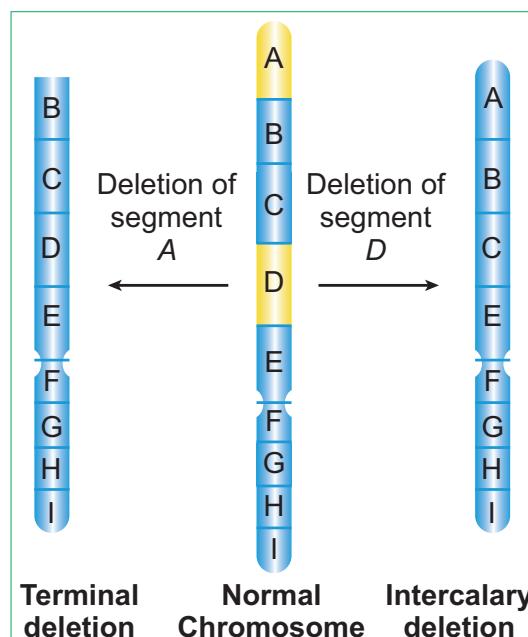


Figure 3.24 Deletion

B. Changes in the arrangement of gene loci

- Inversion
- Translocation

1. Deletion or Deficiency

Loss of a portion of chromosome is called deletion. On the basis of location of breakage on chromosome, it is divided into terminal deletion and intercalary deletion. It occurs due to chemicals, drugs and radiations. It is observed in *Drosophila* and Maize. (Figure 3.24)

There are two types of deletion:

- Terminal deletion:** Single break in any one end of the chromosome.

- Intercalary deletion or interstitial deletion:** It is caused by two breaks and reunion of terminal parts leaving the middle.

Both deletions are observable during meiotic pachytene stage and polytene chromosome. The unpaired loop formed in the normal chromosomal part at the time of chromosomal pairing. Such loops are called as **deficiency loops** and it can be seen in meiotic prophase. Larger deletions may lead to lethal effect.

2. Duplication or Repeat

The process of arrangement of the same order of genes repeated more than once in the same

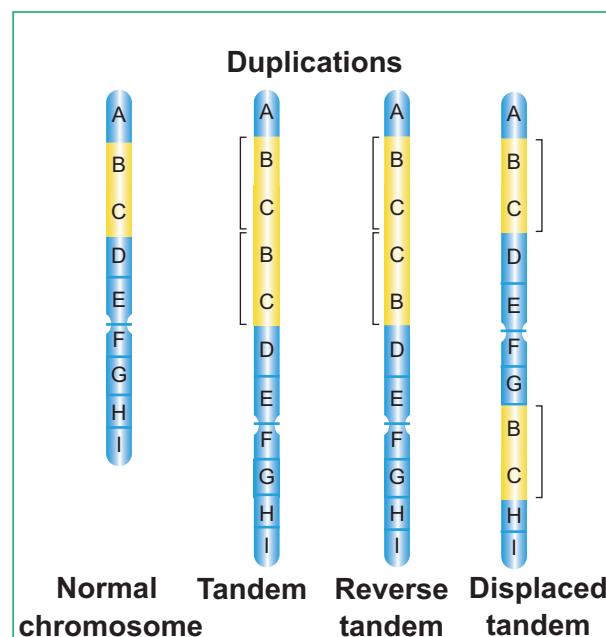


Figure 3.25 Duplication



chromosome is known as **duplication**. Due to duplication some genes are present in more than two copies. It was first reported in *Drosophila* by Bridges (1919) and other examples are Maize and Pea. It is three types.

i. Tandem duplication

The duplicated segment is located immediately after the normal segment of the chromosome in the same order.

ii. Reverse tandem duplication

The duplicated segment is located immediately after the normal segment but the gene sequence order will be reversed.

iii. Displaced duplication

The duplicated segment is located in the same chromosome, but away from the normal segment. (Figure 3.25)

Duplications play a major role in evolution.

3. Inversion

A rearrangement of order of genes in a chromosome by reversal by an angle 180°. This involves two chromosomal breaks and reunion. During this process there is neither gain nor loss but the gene sequences are rearranged. Inversion was first reported in *Drosophila* by Sturtevant (1926). There are two types of inversion, paracentric and pericentric (Figure 3.26).

i. Paracentric inversion: An inversion which takes place apart from the centromere

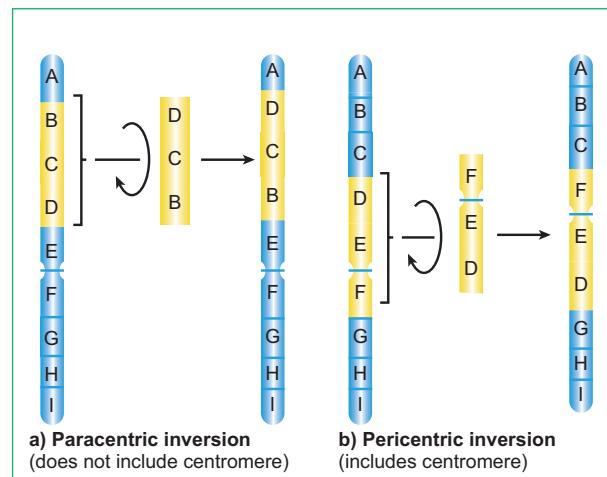


Figure 3.26 Inversion

ii. Pericentric inversion: An inversion that includes the centromere.

Inversions lead to evolution of a new species.

4. Translocation

The transfer of a segment of chromosome to a non-homologous chromosome is called translocation. Translocation should not be confused with crossing over, in which an exchange of genetic material between homologous chromosomes takes place. Translocation occurs as a result of interchange of chromosome segments in non-homologous chromosomes. There are three types

- Simple translocation
- Shift translocation
- Reciprocal translocation

i. Simple translocation

A single break is made in only one chromosome. The broken segment gets attached to one end of a non-homologous chromosome. It occurs very rarely in nature.

ii. Shift translocation

Broken segment of one chromosome gets inserted interstitially in a non-homologous chromosome.

iii. Reciprocal translocations

It involves mutual exchange of chromosomal segments between two non-homologous

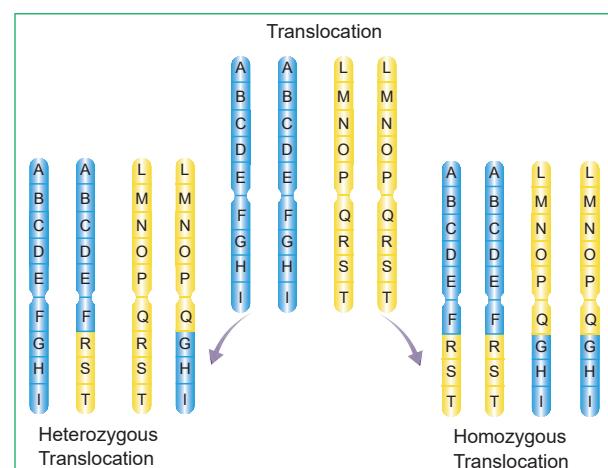


Figure: 3.27 Translocation



chromosomes. It is also called **illegitimate crossing over**. It is further divided into two types (Figure 3.27).

- Homozygous translocation:** Both the chromosomes of two pairs are involved in translocation. Two homologous of each translocated chromosomes are identical.
- Heterozygous translocation:** Only one of the chromosome from each pair of two homologous are involved in translocation, while the remaining chromosome is normal.

Translocations play a major role in the formation of species.

3.7. DNA Metabolism in Plants

As the repository of genetic information, DNA occupies a unique and central place among biological macromolecules. The structure of DNA is a marvelous device for the storage of genetic information. The term “DNA Metabolism” can be used to describe process by which copies of DNA molecules are made (replication) along with repair and recombination.

In this chapter we briefly discuss about the DNA metabolism in plants

DNA Replication: In the double helix the two parental strands of DNA separate and each parental strand synthesizes a new complementary strand. DNA replication is semiconservative, i.e each new DNA molecule conserves one original strand.

DNA Repair: How is genomic stability maintained in all living organisms? How do organisms on earth survive? What is essential for their survival?

DNA is unique because it is the only macromolecule where the repair system exists, which recognises and removes mutations. DNA is subjected to various types of damaging reactions such as spontaneous or environmental agents or natural endogenous threats. Such

damages are corrected by repair enzymes and proteins, immediately after the damage has taken place. DNA repair system plays a major role in maintaining the genomic / genetic integrity of the organism. DNA repair systems protect the integrity of genomes from genotoxic stresses.



Plants are sessile. How do they protect themselves from the exposure of sunlight throughout the day?

Plants have effective DNA repair mechanism to prevent UV damage from sunlight. They produce an enzyme called photolyase, which can repair the thymine dimers and restore the structure of DNA.

Recombination: In cells the genetic information within and among DNA molecule are re-arranged by a process called genetic recombination. Recombination is the result of crossing over between the pairs of homologous chromosomes during meiosis. In earlier classes you have learnt chromosomal recombination. In molecular level it involves breakage and reunion of polynucleotides.

3.7.1 Eukaryotic DNA replication

Replication starts at a specific site on a DNA sequence known as the Origin of replication. There are more than one origin of replication in eukaryotes. *Saccharomyces cerevisiae* (yeast) has approximately 400 origins of replication. DNA replication in eukaryotes starts with the assembly of a prereplication complex (preRC) consisting of 14 different proteins. Part of a preRC is a group of 6 proteins called the origin recognition complex (ORC) which acts as initiator in eukaryotic DNA replication. The origin of replication in yeast is called as **ARS sites (Autonomously Replicating Sequences)**. In yeast, ORC was identified as a protein complex which binds directly to ARS elements.

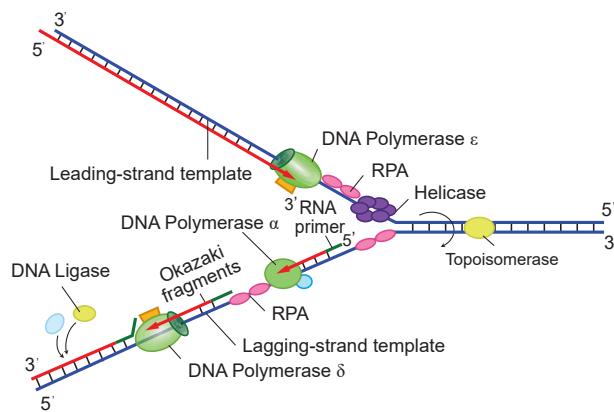


Figure: 3.28 Eukaryotic replication fork

Replication fork is the site (point of unwinding) of separation of parental DNA strands where new daughter strands are formed. Multiple replication forks are found in eukaryotes. The enzyme **helicases** are involved in unwinding of DNA by breaking hydrogen bonds holding the two strands of DNA and replication protein A (RPA) prevents the separated polynucleotide strand from getting reattached.

Topoisomerase is an enzyme which breaks DNAs covalent bonds and removes positive supercoiling ahead of replication fork. It eliminates the torsional stress caused by unwinding of DNA double helix.

DNA replication is initiated by an enzyme **DNA polymerase α** / primase which synthesizes short stretch of RNA primers on both leading strand (continuous DNA strand) and lagging strands (discontinuous DNA strand). Primers are needed because DNA polymerase requires a free 3' OH to initiate synthesis. DNA polymerase covalently connects the nucleotides at the growing end of the new DNA strand.

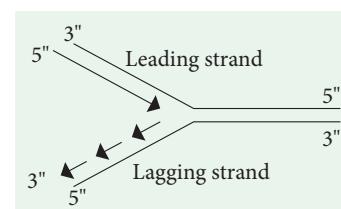
DNA Pol α (alpha), DNA Pol δ (delta) and DNA Pol ε (Epsilon) are the 3 enzymes involved in nuclear DNA replication.

DNA Pol α – Synthesizes short primers of RNA
DNA Pol δ – Main Replicating enzyme of cell nucleus

DNA Pol ε – Extend the DNA Strands in replication fork

DNA Polymerase β does not play any role in the replication of normal DNA. Function - Removing incorrect bases from damaged DNA. It is involved in Base excision repair.

DNA Synthesis takes place in 5' → 3' direction and it is semidiscontinuous. When DNA is synthesized in 5' → 3' direction, only in the free 3' end (OH end) DNA is elongated. In 1960s Reiji Okazaki and his colleagues found out that one of the new DNA strands is synthesized in short pieces called **Okazaki fragments**. In discontinuous strand where the Okazaki fragments are united by ligase is called Lagging strand where the replication direction is 5' → 3' which is opposite to the direction of fork movement. . The continuous strand is called Leading strand where the replication direction is 5' → 3' which is same to the direction to that of the replication fork movement. DNA ligase joins any nicks in the DNA by forming a phosphodiester bond between 3' hydroxyl and 5' phosphate group.



Arabidopsis telomere sequence - TTTAGGG

Plants Lacks Telomere Clock

Plant meristematic cells produces telomerase so the meristematic cells has an unlimited ability to divide. You have already studied about the telomeres in Chapter 6 and 8 of Class XI. In plants telomeres do not shrink as in somatic cells of vertebrates. Telomerase levels are higher in root tips and seedlings (renewable tissue) which has a higher amount of meristematic cells than proliferative structures like leaves.

What is the special mechanism which replicates chromosomal ends?

After the replication of the chromosomes, the enzyme **telomerase** adds several more repeats of DNA sequences to the telomeres. Telomerase



use short RNA molecules as a template and add repeat sequences on to telomeres (DNA nucleotide polymerisation).

The Energetics of DNA Replication - Deoxyribonucleotides such as deoxyadenosine triphosphate dATP, dGTP, dCTP and dTTP provide energy for the synthesis of DNA. Purpose of Deoxyribonucleotides (1) acts as a substrate (2) provide energy for polymerisation.

3.7.2 Experimental evidence of DNA replication: Taylors Experiment

J. Herbert Taylor, Philip Woods and Walter Hughes demonstrated the semiconservative replication of DNA in the root cells of *Vicia faba*. They labelled DNA with ^3H Thymidine, a radioactive precursor of DNA and performed autoradiography. They grew root tips in a medium in the presence of radioactive labelled thymidine, so that the radioactivity was incorporated into the DNA of these cells. The outline of this labelled chromosomes appears in the form of scattered black dots of silver grains on a photographic film.

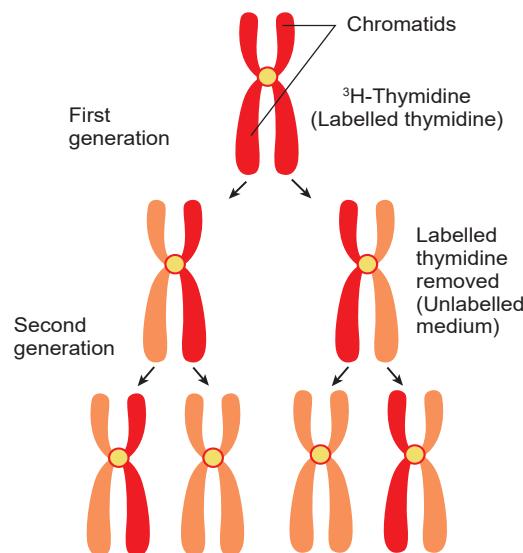


Figure: 3.29 Taylors Experiment on *Vicia faba*

The root tips with labelled chromosomes were placed in an unlabelled medium containing colchicine to arrest the culture at the metaphase and examine the chromosome by autoradiography. The observations were,

1. In the chromosome of **first generation** the radioactivity was found to be distributed to **both the chromatids** because in the original strand of DNA double helix was labelled with radioactivity and the new strand was unlabelled.
2. In the chromosome of the **second generation** **only one of the two chromatids** in each chromosome was radioactive (labelled).

The results proved the semiconservative method of DNA replication.

3.8 Protein synthesis in plants

The process of protein synthesis consists of two major steps, they are Transcription and Translation.

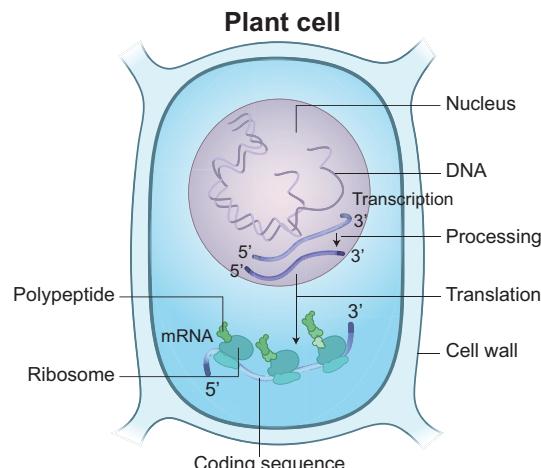


Figure: 3.30 Protein synthesis in plants

3.8.1 Transcription

Transcription is the process in which one strand of DNA acts as a template to generate mRNA with the bases complementary to the template strand. It is catalyzed by the enzymes called RNA polymerases.

Transcription and processing of RNA takes place in the **nucleus**, whereas the **translation** occurs in the ribosomes found in **cytoplasm**. In Eukaryotes, mRNA molecules are **monocistronic** with only one protein being derived from each mRNA.



The transcription begins with unwinding of DNA double helix and the hydrogen bonds are broken at the site of the gene being transcribed.

Template Strand / Non-Coding Strand / Antisense Strand

The strand of DNA which is oriented in 3' → 5' direction that serves as a template for the synthesis of mRNA is called template strand.

Coding strand / Non-template strand / Sense Strand

The other strand of DNA which is not transcribed is called the Coding Strand.

A specific sequence of DNA nucleotides called the **Promoter** is necessary for transcription to take place. It consists of TATA box and transcription start site where transcription begins.

Termination sequences are the DNA sequences which tell when the RNA polymerase should stop producing RNA molecule.

Eukaryotic structural gene has 3 features in promoter

1. Regulatory elements
2. TATA box
3. A transcriptional start site

The transcription start site contains about 25 bp (basepairs) upstream, the sequence is TATAAT known as **TATA or Hogness box** which is present in core promoter. **General transcriptional factors**

are the proteins which recognise base sequences of DNA and controls transcription. Some transcription factors bind directly to the promoter.

Some transcription factors recognize the regulatory elements and bind to them to increase the rate of transcription, others inhibits transcription.

To start the process of transcription the Regulatory elements help the RNA polymerase to recognize core promoter. The two categories of regulatory elements are

1. **Enhancer sequences** – they are DNA sequences (activating sequences) which help to influence transcription.
2. **Silencer sequence** – DNA sequences that inhibit transcription or decrease transcription.

Consensus sequence – An ideal sequence in which each position represents the base which is found most often.

In addition to General transcription factors (GTF) and **RNA Pol II**, a **mediator** is required for transcription. The interactions between RNA polymerase II and regulatory TF that bind to enhancers or silencers are mediated by a mediator.

RNA Polymerases cannot bind directly to the DNA, first it binds to the transcription factor which recognizes the promoter sequences which helps to find the protein coding regions of DNA.

RNA Polymerase with the promoter sequence will transcribe the gene. Transcription factor plays an important role in guiding RNA Polymerases to the promoter sequence. RNA Polymerases bind RNA nucleotides together

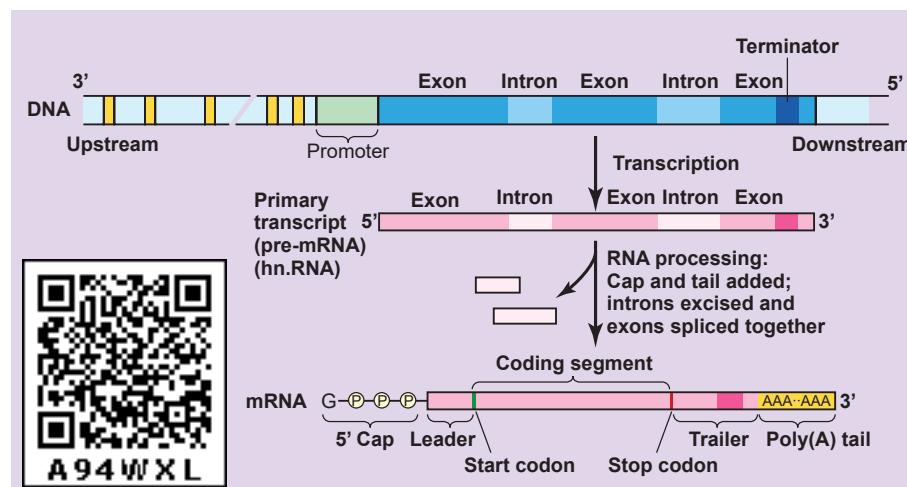


Figure: 3.31 Transcription



forming a growing strand in the $5' \rightarrow 3'$ direction. Transcription occurs in $5' \rightarrow 3'$ direction, RNA Polymerase catalyses the addition of nucleotides at the $3'$ end of the growing chain of RNA.

In Eukaryotes, 3 different RNA Polymerases called RNA Polymerases I, II and III are found.

Enzyme	Synthesis
RNA Polymerase I	Large Ribosome RNAs except 5S rRNA
RNA Polymerase II	Precursors of mRNAs (hnRNAs)
RNA Polymerase III	tRNAs, 5S ribosomal RNA, snRNAs (small nuclear RNA)

The processing of pre-mRNA to mature mRNA / Molecular mechanism of RNA modification

In eukaryotes three major types of RNA, mRNA, tRNA and rRNA are produced from a precursor RNA molecule termed as the primary transcript or preRNA. The RNA polymerase II transcribes the precursor of mRNA, which are also called the **heterogenous nuclear RNA** or hnRNA which are processed in the nucleus before they are transported into the cytoplasm.

Capping

Modification at the $5'$ end of the primary RNA transcript (hn RNA) with methylguanosine triphosphate is called capping.



Internal methylation

Apart from capping, the internal nucleotides in mRNA are also methylated. Methylated sites are present in translated, untranslated regions, introns and exons.

Purpose of Capping

- Protects RNA from degradation.
- Capping plays an important role in removal of first intron in pre mRNA.

- It regulates the mRNA export from the nucleus into the cytoplasm.
- It helps in binding of mRNA to the ribosome.

Tailing / Polyadenylation

The $3'$ end of hnRNA is cleaved by an endonuclease and a string of adenine nucleotides is added to the $3'$ end of hnRNA (pre mRNA) is known as Poly (A) tail - Polyadenylation. This process is called tailing or polyadenylation.

Purpose of Tailing

- Translation of RNA transcript is facilitated.
- Helps in the synthesis of Polypeptides.
- It enhances the mRNA stability in the cytoplasm.

The protein coding regions are not continuous in eukaryotes. Split genes were independently discovered by Richard J Roberts and Phillip A. Sharp in 1977 and was awarded Nobel Prize in 1993. **Exons** are the coding sequences or expressed sequences contain biological informations in the matured processed mRNA. **Introns** are intervening sequences, which are non-coding sequences (non-amino acid-coding sequences) that should be removed from a gene before the mRNA product is made. Introns do not code for any enzyme or structural protein or polypeptides. These exons and introns are known as Split Genes.

3.8.2 RNA Splicing in plants

RNA Splicing is a process which involves the cutting or removing out of introns and knitting of exons. This process takes place in spherical particles which is a multiprotein complex called **SPLICISOMES**. It is approximately 40 – 60 nm in diameter. The spliceosomes have many small nuclear ribonucleic acids (snRNAs) and small nuclear ribonuclear protein particles (snRNPs) which identify and helps in the removal of introns.

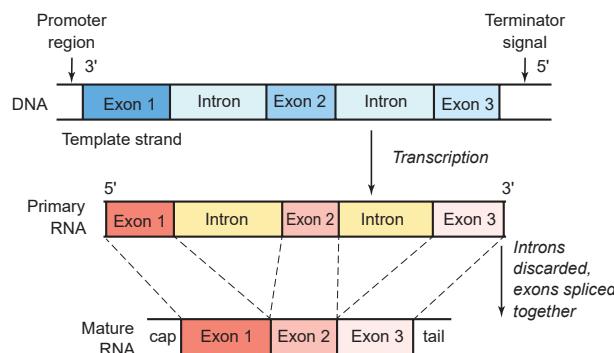


Figure: 3.32 RNA Splicing in plants

A spliceosome removes the introns with an enzyme ribozyme. Now the mature mRNA comes away from the spliceosomes through the nuclear pore and is transported out from the nucleus into cytoplasm, and gets attached to ribosome to carry out translation. The RNA and many proteins are transported through a nuclear pore complex by an energy dependent process.

3.8.3 Translation

The genetic information in the DNA code is copied onto mRNA bound in ribosomes for making polypeptides. The mRNA nucleotide sequence is decoded into amino acid sequence of the protein which is catalyzed by the ribosome. This process is called translation.

Terminology in Protein synthesis

Codon – DNA codes are referred to as triplet codes and those in mRNA is called as Codons. Each triplet specifies a particular amino acid. Codons present in mRNA are read in 5' → 3' direction. There are 64 codons of which 61 codons codes for amino acids.

Start codon – AUG specified methionine

Stop or Termination codon – UAA – Ochre UAG – amber and UGA – Opal.

Anticodons – The triplet of bases in a tRNA molecule is known as anticodon. In tRNA sequence of three bases which is complementary to codons of mRNA are called anticodon. The codons of mRNA are recognized by the anticodons of tRNA which are oriented in 3' → 5' direction.

Process of translation

The following are major steps in translation process

1. Initiation

The translation begins with the AUG codon (start codon) of mRNA. Translation occurs on the surface of the macromolecular arena called the ribosome. It is a nonmembranous organelle. During the process of translation the two subunits of ribosomes unite (combine) together and hold mRNA between them. The protein synthesis begins with the reading of codons of mRNA. The tRNA brings amino acid to the ribosome, a molecular machine which unites amino acids into a chain according to the information given by mRNA. rRNA plays the structural and catalytic role during translation.

A ribosome has one binding site for mRNA and two for tRNA. The two binding sites of tRNA are

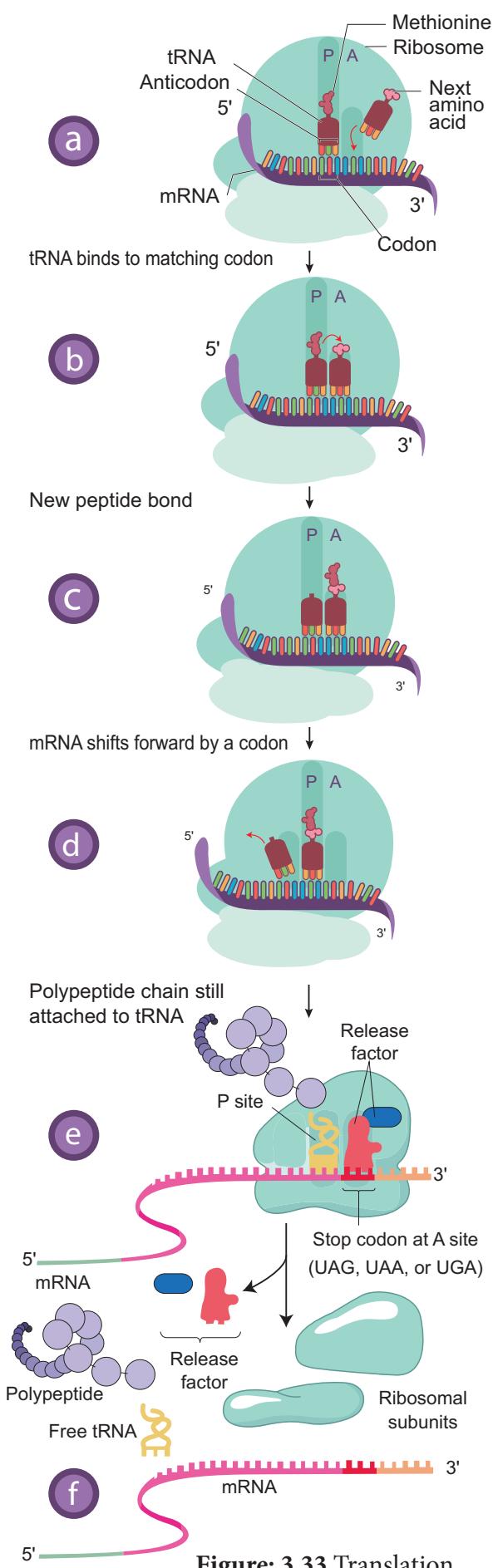
- P-Site – The peptidyl – tRNA binding site** is one of the tRNA binding site. At this site tRNA is held and linked to the growing end of the polypeptide chain.
- A-Site – The Aminoacyl – tRNA binding site.** This is another tRNA binding site which holds the incoming amino acids called aminoacyl tRNA. The anticodons of tRNA pair with the codons of the mRNA in these sites.

2. Elongation of polypeptide chain

The P and A sites are nearby, so that two tRNA form base pairs with adjacent codon. The polypeptide chain is formed by the pairing of codons and anticodons according to the nucleotide sequence of the mRNA.

Translators of the genetic code - tRNA

The tRNA translates the genetic code from the nucleic acid sequence to the amino acid sequence i.e from gene – Polypeptide. When an amino acid is attached to tRNA it is called **aminoacylated or charged**. This is an energy requiring process which



uses the ATP for its energy requirement. Protein synthesis takes place as the next aminoacyl tRNA binds to the A-Site.

The translation begins with the AUG codon (start codon) of mRNA. The tRNA which carries first amino acid **methionine** attach itself to P-site of ribosome. The ribosome adds new amino acids to the growing polypeptides. The second tRNA molecules has anticodons which carries amino acid **alanine** pairs with the mRNA codon in the A-site of the ribosome. The aminoacids **methionine** and **alanine** are close enough so that a peptide bond is formed between them.

The bond between the first tRNA and **methionine** now breaks. The first tRNA leaves the ribosome and the P-site is vacant. The ribosome now moves one codon along the mRNA strand. The second t-RNA molecule now occupies the P-site. The third t-RNA comes and fills the A site (serine). Now a peptide bond is formed between **alanine** and **serine**. The mRNA then moves through the ribosome by three bases. This expels deacylated / uncharged tRNA from P-site and moves peptidyl tRNA into the P-site and empties the A-site. This movement of tRNA from A-site to P-site is said to be translocation. The translocation requires the hydrolysis of GTP.

The **ribosome (ribozyme - peptidyl transferase)** catalyses the formation of peptide bond by adding amino acid to the growing polypeptide chain.

The ribosome moves from codon to codon along the mRNA in the 5' to 3' direction. Amino acids are added one by one translated into polypeptide as dictated by the mRNA. Translation is an energy intensive process. A cluster of ribosomes are linked together by a molecule of mRNA and forming the site of protein synthesis is called as **polysomes** or **polyribosomes**.



3. Termination of polypeptide synthesis

Eukaryotes have cytosolic proteins called **release factors** which recognize the termination codon, UAA, UAG, or UGA when it is in the A site. When the ribosome reaches a stop codon the protein synthesis comes to an end. So ribosomes are the protein making factories of a cell. When the polypeptide is completed the ribosome releases the polypeptide and detaches from the mRNA molecule. Now the ribosome splits into small and large subunits after the release of mRNA.

3.8.4 Alternative Splicing in plants

It is very useful in regulating gene expression to overcome the environmental stress in plants.

Alternative splicing is an important mechanism / process by which multiple mRNA's and multiple proteins products can be generated from a single gene. The different proteins generated are called isoforms. There are various modes of alternative splicing. When multiple introns are present in a gene, they are removed separately or as a unit. In certain cases one or more exons which is present between the introns are also removed.

Significance of alternative splicing

1. The protein transcribed from alternatively spliced mRNA containing different amino acid sequence lead to the generation of protein diversity and biological functions.

acid sequence lead to the generation of protein diversity and biological functions.

2. Multiple protein isoforms are formed.
3. It creates multiple mRNA transcripts from a single gene. A process of producing related proteins from a single gene thereby the number of gene products are increased.
4. It plays an important role in plant functions such as stress response and trait selection. The plant adapts or regulates itself to the changing environment.

3.8.5. RNA Editing – Post Transcriptional RNA Processing in plants

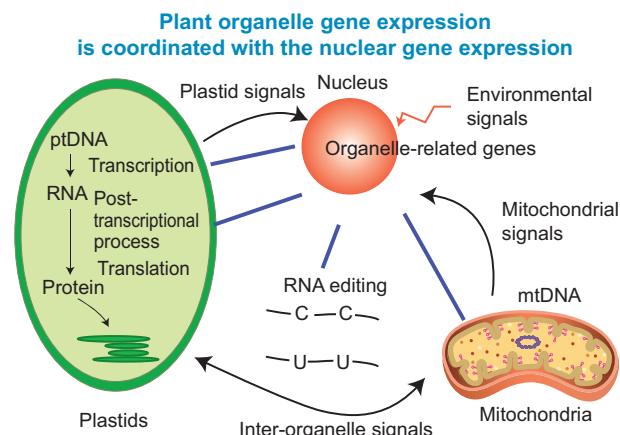


Figure: 3.35 RNA Editing – Post Transcriptional RNA Processing in plants

Chemical modification such as base modification, nucleotide insertion or deletions and nucleotide replacements of mRNA results in the alteration of amino acid sequence of protein that is specified is called RNA editing. This results in the change in the protein coding sequence of RNA following transcription. The coding properties of the RNA transcript is changed. The genetic information encoded in the chloroplast genome is altered by post transcriptional

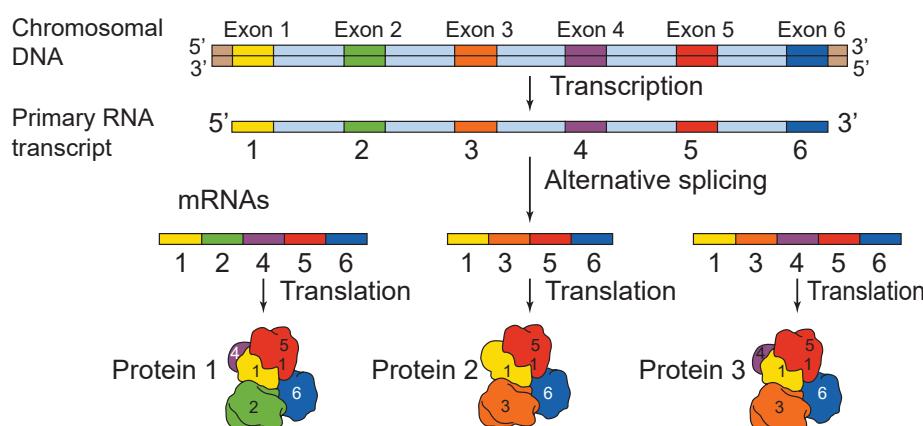


Figure: 3.34 Alternative splicing in plants



Year	Editing type	Organelle in Plant cell	Target	Inference / Result
1989	C → U	Plant mitochondria	mRNA	For conserved amino acids, multiple changes in codon takes place
1990	U → C	Plant mitochondria	mRNA	First report on editing (U → C)
1991	C → U	Plant chloroplast	mRNA	First report in chloroplast

Table 3.9: RNA Editing types

phenomenon which is site – specific (C → U) in chloroplast of higher plants – RNA editing occurs in plant mitochondria and chloroplast.

In plant cells RNA editing by pyrimidine transitions occurs in mitochondria and plastids (chloroplast). There are two main types of RNA editing. (1) Substitution editing – Alteration of individual nucleotide bases. Mitochondria and chloroplast RNA in plants. (2) Insertion / Deletion editing – Nucleotides are added or deleted from the total number of bases.

Significance of RNA Editing

1. In higher plant chloroplast, it helps to restore the codons for conserved amino acids which include initiation and termination codon.
2. It regulates Organellar gene expression in plants.
3. RNA editing results in the restoration of codons for phylogenetically conserved amino acid residues.

3.8.6 Jumping Genes



Figure: 3.36 Barbara McClintock

Have you heard of Jumping Genes or Hopping Genes?

This is the nick name of transposable genetic elements. Transposons are the DNA sequences which can move from one position to another position in a genome. This was first reported in 1948 by American Geneticist Barbara McClintock as “mobile controlling element” in Maize. One of the most significant scientists of 20th century was Barbara McClintock because she gave a shift in gene organization. McClintock was awarded Nobel prize in 1983 for her work on transposons. Barbara McClintock when studying aleurone of single maize kernels, noted the unstable inheritance of the mosaic pattern of blue, brown and red spots due to the differential production of vacuolar anthocyanins.

In maize plant genome has AC / Ds transposon (AC = Activator, Ds = Dissociation). The activity of AC element is very distinct in maize plant. The transposition in somatic cells results in the changes in gene expression such as variegated pigmentation in maize kernels. Maize genome has transposable elements which regulated the different colour pattern of kernels.

McClintock's findings concluded that Ds and AC genes were mobile controlling elements. We now call it as transposable elements, a term coined by maize geneticist, Alexander Brink. McClintock gave the first direct experimental evidence that genomes are not static but are highly plastic entities.



Significance of transposons

1. They contribute to many visible mutations and mutation rate in an Organism.
2. In evolution, they contribute to genetic diversity.
3. In genetic research transposons are valuable tools which are used as mutagens, as cloning tags, vehicles for inserting foreign DNA into model organism.

Plant genome – The word genome is defined as the full complement of DNA (including all the genes and the intergenic regions) present in an organism. It specifies the entire biological information of an organism. There are three distinct genomes in eukaryotic cells and they are (1) The nuclear genome (2) The mitochondrial genome and (3) The chloroplast genome present only in plants.

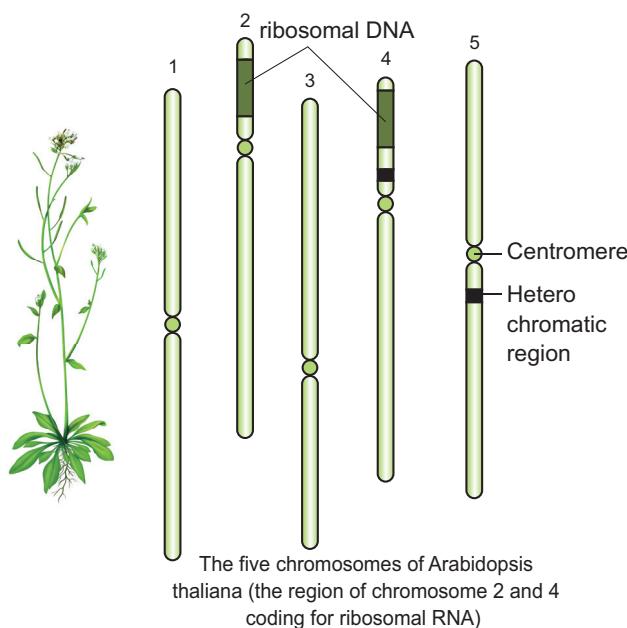


Figure: 3.37 *Arabidopsis thaliana*

Arabidopsis thaliana – Thale cress, Mouse-ear cress

1. It is a model plant for the study of genetic and molecular aspects of plant development.
2. It belongs to mustard family and it is

the first flowering plant, where its entire genome is sequenced.

3. The two regions of the nucleolar organiser ribosomal DNA which codes for the ribosomal RNA are present at the extremity of chromosomes 2 and 4
4. It is Diploid plant having small genome with $2n = 10$ chromosomes. Several generations can be produced in one year. So it facilitates rapid genetic analysis. The genome has low repetitive DNA, over 60% of the nuclear DNA have protein coding functions.
5. The plant is small, self fertilizes, annual long-day plant with short-life cycle (only 6 weeks), large numbers of seeds are produced and they are easy to be grown in laboratory. It is easy to induce mutations. It has many genomic resources and the transformation can be done easily.
6. In 1982, *Arabidopsis* has successfully completed its life cycle in Microgravity i.e. space. This shows that Human Space Missions with plant companions may be possible.

SUMMARY

Chromosomal theory of inheritance states that Mendelian factors have specific locus on chromosomes and they carry information from one generation to the next generation. Genes located close together on the same chromosome and inherited together are called linked genes the phenomenon is called Linkage. Two types of linkage are complete linkage and incomplete linkage. The groups of linearly arranged linked genes are called Linkage groups. Crossing over is biological a process that produces new combination of genes by inter-changing the corresponding



segments between non-sister chromatids of homologous pair of chromosomes. In this segment of DNA are broken and recombined to produce new combinations of alleles a process is called Recombination. The diagrammatic representation of distances between the adjacent genes which is directly proportional to the frequency of recombination between them is called genetic mapping. When any of the three or more allelic forms of a gene occupy the same locus in a given pair of homologous chromosomes, they are said to be multiple alleles. Papaya sex determination is controlled by three alleles. They are m, M₁ and M₂ of a single gene. Mutational events that take place within individual genes are called gene mutations or point mutation, whereas the changes occur in structure and number of chromosomes is called chromosomal mutation. The agents which are responsible for mutation is called mutagens.

DNA metabolism includes replication, repair and recombination. Protein synthesis in eukaryotes is unique due to the capping, tailing and splicing. Transcription takes place in nucleus and translation in cytoplasm. AUG codes for methionine and it is monocistronic. Alternative splicing is a mechanism to overcome stress in plants. RNA editing takes place in chloroplast and mitochondria of plants which is of phylogenetic importance. Controlling elements gave a major shift in the gene organisation in plants to prove that DNA is not static but plastic entities. The first genome sequenced is *Arabidopsis thaliana*, which is a potential genetic tool to study the development and metabolism in plants.

Evaluation

1. An allohexaploid contains
 - a) Six different genomes
 - b) Six copies of three different genomes
 - c) Two copies of three different genomes
 - d) Six copies of one genome
2. The A and B genes are 10 cM apart on a chromosome. If an AB/ab heterozygote is testcrossed to ab/ab, how many of each progeny class would you expect out of 100 total progeny?
 - a) 25 AB, 25 ab, 25 Ab, 25 aB
 - b) 10 AB, 10 ab
 - c) 45 AB, 45 ab
 - d) 45 AB, 45 ab, 5 Ab, 5 aB
3. Match list I with list II

List I	List II
A. A pair of chromosomes extra with diploid	i) monosomy
B. One chromosome extra to the diploid	ii) tetrasomy
C. One chromosome loses from diploid	iii) trisomy
D. Two individual chromosomes lose from diploid	iv) double monosomy

5. a) A-i, B-iii, C-ii, D-iv b) A-ii, B-iii, C-iv, D-i
c) A-ii, B-iii, C-i, D-iv d) A-iii, B-ii, C-i, D-iv
4. Which of the following sentences are correct?
 1. The offspring exhibit only parental combinations due to incomplete linkage
 2. The linked genes exhibit some crossing over in complete linkage
 3. The separation of two linked genes are possible in incomplete linkage
 4. Crossing over is absent in complete linkage





- a) 1 and 2 b) 2 and 3
c) 3 and 4 d) 1 and 4
5. Accurate mapping of genes can be done by three point test cross because increases
a) Possibility of single cross over
b) Possibility of double cross over
c) Possibility of multiple cross over
d) Possibility of recombination frequency
6. Due to incomplete linkage in maize, the ratio of parental and recombinants are
a) 50:50 b) 7:1:1:7 c) 96:4:3:6 d) 1:7:7:1
7. Genes **G S L H** are located on same chromosome. The recombination percentage is between L and G is 15%, S and L is 50%, H and S are 20%. The correct order of genes is
a) GHSL b) SHGL c) SGHL d) HSLG
8. The point mutation sequence for transition, transition, transversion and transversion in DNA are
a) A to T, T to A, C to G and G to C
b) A to G, C to T, C to G and T to A
c) C to G, A to G, T to A and G to A
d) G to C, A to T, T to A and C to G
9. If haploid number in a cell is 18. The double monosomic and trisomic number will be
a) 35 and 37 b) 34 and 35
c) 37 and 35 d) 17 and 19
10. Changing the codon AGC to AGA represents
a) missense mutation b) nonsense mutation
c) frameshift mutation d) deletion mutation
11. **Assertion (A):** Gamma rays are generally used to induce mutation in wheat varieties.
Reason (R): Because they carry lower energy to non-ionize electrons from atom
a) A is correct. R is correct explanation of A
b) A is correct. R is not correct explanation of A
c) A is correct. R is wrong explanation of A
- d) A and R is wrong
12. How many map units separate two alleles A and B if the recombination frequency is 0.09?
a) 900 cM b) 90 cM c) 9 cM d) 0.9 cM
13. Which one of the following pairs of codons is correctly matched with their function or the signal for the particular amino acid?
a) UUA, UCA - Leucine
b) GUU, GCU - Alanine
c) UAG, UGA - Stop
d) AUG, ACG – Start / Methionine
14. Removal of introns and joining of exons in a defined order during transcription is called
a) Splicing b) Looping
c) Inducing d) Slicing
15. If one strand of DNA has the nitrogenous base sequence as ATCTS, what would be the complementary RNA strand sequence?
a) ATCGU b) TTAGU
c) UAGAC d) AACTG
16. Removal of RNA polymerase III nucleoplasm will affect the synthesis of
a) rRNA b) tRNA
c) hnRNA d) mRNA
17. DNA dependent RNA polymerase catalyzes transcription on one strand of the DNA which is called the
a) Alpha strand b) Anti strand
c) Template strand d) Coding strand
18. Which of the following correctly represents the flow of genetic information?
a) DNA → RNA → Protein
b) RNA → DNA → Protein
c) RNA → Protein → DNA
d) Protein → RNA → DNA
19. Initiation codon is
a) UUU b) UGA
c) AUG d) UAG
20. A eukaryotic gene contains two kinds of base



- sequences which of these plays an important role in protein synthesis?
- Introns
 - Exons
 - Both a and b
 - None of the above
21. Codon – anticodon interactions occur by
- Covalent bond
 - Electrostatic interactions
 - Hydrogen bonds
 - Hydrophobic interaction
22. Which of the following RNA polymerases is responsible for the transcription of protein coding genes in eukaryotes?
- RNA Pol I
 - RNA Poly II
 - RNA Pol III
 - RNA Pol IV
23. How are RNA molecules transported out of the nucleus
- Passive diffusion through the membrane
 - Through membrane pores in an energy independent process
 - Through membrane pores in an energy dependent process
 - Through a channel in the membrane that leads to the endoplasmic reticulation
24. During translation the codon in mRNA is actually “read” by
- The A site in the ribosomes
 - The P site in the ribosomes
 - The anticodon in at RNA
 - The anticodon is an amino acid
25. A complex of ribosome attached to a single strand of RNA is known as
- Polysome
 - Polymer
 - Polypeptide
 - Okazaki fragment
26. Which of the following is the start codon
- AUG
 - UGA
 - UAA
 - UAG
27. What is true about tRNA?
- It binds with an amino acid at its 3' end
 - It has 5 double stranded regions
- c) It has a codon at one end which recognizes the anticodon of mRNA
- d) It looks like clover leaf in the three D structure
28. Which one of the following hydrolyses internal phosphodiester bonds in a polynucleotide chain?
- Lipase
 - Exonuclease
 - Endonuclease
 - Protease
29. DNA element with ability to change position is called
- Cistron
 - Transposon
 - Intron
 - Recon
30. Spliceosomes are not found in cells of
- Plants
 - Fungi
 - Animals
 - Bacteria
31. During DNA replication Okazaki fragments are used to elongate
- The leading strand towards replication fork
 - The lagging strand towards replication fork
 - The leading strand away from replication fork
 - The lagging strand away from replication fork
32. When two different genes came from same parent they tend to remain together.
- What is the name of this phenomenon?
 - Draw the cross with suitable example.
 - Write the observed phenotypic ratio.
33. If you cross dominant genotype PV/PV male *Drosophila* with double recessive female and obtain F₁ hybrid. Now you cross F₁ male with double recessive female.
- What type of linkage is seen?
 - Draw the cross with correct genotype.
 - What is the possible genotype in F₂ generation?



34.

S. no	Gamete types	Number of progenies
1.	ABC	349
2.	Abc	114
3.	abC	124
4.	AbC	5
5.	aBc	4
6.	aBC	116
7.	ABc	128
8.	abc	360

- i) What is the name of this test cross?
 - ii) How will you construct gene mapping from the above given data?
 - iii) Find out the correct order of genes.
35. What is the difference between missense and nonsense mutation?
- 36.
- From the above figure identify the type of mutation and explain it.
37. Write the salient features of Sutton and Boveri concept.
38. Explain the mechanism of crossing over.
39. Write the steps involved in molecular mechanism of DNA recombination with diagram.
40. How is *Nicotiana* exhibit self-incompatibility. Explain its mechanism.
41. How sex is determined in monoecious plants. write their genes involved in it.
42. What is gene mapping? Write its uses.
43. Draw the diagram of different types of aneuploidy.
44. Mention the name of man-made cereal. How it is formed?
45. What is DNA repair?

46. What is replication fork?

47. Write about the energetics of DNA replication.

48. What is TATA box?

49. What is alternative splicing?

50. What is coding strand?

51. What are the enzymes involved in DNA replication in eukaryotes?

52. Differentiate coding and non coding strand.

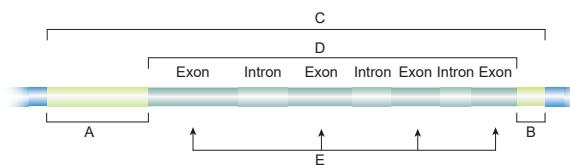
53. What are splicesomes?

54. What is meant by capping and tailing?

55. What is RNA editing?

56. Explain the DNA replication in eukaryotes.

57. With reference to the given diagram correctly match the following pairs.



Column I Column II

- | | |
|---|---|
| A | Transcribed region |
| B | Regulation of initiation of transcription |
| C | Protein-encoding sequence |
| D | Termination of transcription |
| E | Gene |
58. What attributes make Arabidopsis a suitable model plant for molecular genetic research?
59. Describe the molecular mechanism of RNA modification.
60. Explain ribosomal translocation in protein synthesis.
61. Describe transposons.
62. Describe RNA editing in plants.



Glossary

Antisense strand: It is also called Template strand. It is the strand of DNA that is used as template for RNA synthesis

Branch Migration: The process in which base pairs on homologous strands are consequently exchanged at a Holliday junction, moving the branch up or down the DNA sequence.

Cis configuration: The presence of dominant alleles of two or more pairs on one chromosome and the recessive alleles on the homologous chromosome.

Exons: A segment of DNA that is both transcribed into RNA and translated into protein.

Feminizing Masculinizing: To induce female characteristics in male To induce male characteristics in female

Heteroduplex: A double stranded molecule of nucleic acid originated through genetic recombination from different sources

Introns: Eukaryotes have non-amino acid coding sequences called Introns.

Monocistronic: Eukaryotic mRNAs contain amino acid coding information from just one gene.

Okazaki fragment: A short segment of DNA produced by discontinuous replication elongating in the $5' \rightarrow 3'$ direction away from the replication.

Primase: It is a type of RNA polymerase an enzyme that catalyzes the polymerization of ribonucleotides to RNA. It creates a primer for DNA synthesis.

Promoter: A specific nucleotide sequence to which RNA polymerase attaches to initiate transcription of mRNA from a gene.

Self incompatibility: A genetic mechanism which prevent self fertilization thus encourage outcross.

Synapsis: The pairing of two homologous chromosomes that occurs during meiosis.

Tassel seed: Feminization of the tassel

Terminalisation: The movement of transverse bonds between paired chromosomes in meiosis from their points of origin toward the ends of the chromosomes.

Termination codon: A stop codon

Trans configuration: An arrangement in which the dominant allele of one pair of genes and the recessive allele of another pair are on the same chromosome

Transcript: The DNA is said to be transcribed into RNA and RNA is call as Transcript.

Transesterification: A reaction that breaks and makes chemical bonds in a coordinated transfer, so that no energy is required.

Transposon: A DNA sequence capable of transposition.

Vestigial: Rudimentary organ of body become functionless in the course of evolution



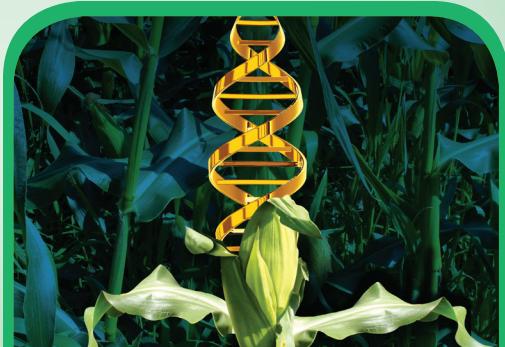
Chapter

4



UNIT VIII: Biotechnology

Principles and Processes of Biotechnology



Learning Objectives

The learner will be able to

- ❖ Apply the knowledge of traditional and modern biotechnology in day to day life.
- ❖ Appreciate the uses of fermentation process.
- ❖ Acquire the knowledge on the process of genetic engineering
- ❖ Analyse the uses and limitations of genetically modified plants
- ❖ Cognize the terms of bio prospecting and bio piracy.



Biotechnology is the science of applied biological process. In other words it is science of development and utilization of biological processes, forms and systems for the benefit of mankind and other life forms. The term biotechnology was coined by Karl Ereky, a Hungarian Engineer in 1919. Biotechnology has been extended to include any process in which organisms, tissues, cells, organelles or isolated molecules such as enzymes are used to convert biological or other raw materials to products of greater value.



Karl Ereky



Chapter outline

- 4.1 Development of Biotechnology
- 4.2 Historical Perspective
- 4.3 Traditional Biotechnology
- 4.4 Advancements in Modern Biotechnology
- 4.5 Tools for Genetic Engineering
- 4.6 Methods of Gene transfer
- 4.7 Screening for Recombinants
- 4.8 Transgenic Plants / Genetically Modified Crops
- 4.9 Applications of Biotechnology.

4.1 Development of Biotechnology

Biotechnology has developed by leaps and bounds during the past century. The development of the biotechnology can be well understood under two main heads namely **conventional or traditional biotechnology** and **modern biotechnology**

1. Conventional or traditional biotechnology: This is the kitchen technology developed by our ancestors, it is as old as human civilization. This technology uses bacteria and other microbes in the daily usage for preparation of dairy products like curd, ghee, cheese and in preparation of foods like idli, dosa, nan, bread and pizza. This conventional biotechnology also extends to preparation of alcoholic beverages like beer, wine, etc.



With the advancement of the science and technology during the 18th century, these kitchen technologies gained scientific validation.

2. Modern biotechnology

There are two main features of this technology, that differentiated it from the conventional technology are its i) ability to change the genetic material for getting new products with specific requirement through recombinant DNA technology ii) ownership of the newly developed technology and its social impact. Today, biotechnology is a billion dollar business around the world, pharmaceutical companies, breweries, agro industries and other biotechnology based industries apply biotechnological tools for their product improvement.

Modern biotechnology embraces all methods of genetic modification by recombinant DNA and cell fusion technology. The major focus of biotechnology are

- **Fermentation** for production of acids, enzymes, alcohols, antibiotics, fine chemicals, vitamins and toxins

- **Biomass** for bulk production of single cell protein , alcohol, and biofuel
- **Enzymes** as biosensors, in processing industry
- **Biofuels** for production of hydrogen, alcohol, methane
- **Microbial inoculants** as biofertiliser, and nitrogen fixers
- **Plant and animal cell culture** for production of secondary metabolites, monoclonal antibodies
- **Recombinant DNA technology** for production of fine chemicals, enzymes, vaccines, growth hormones, antibiotics, and interferon
- **Process engineering** – tools of biotechnology is used for effluent treatment, water recycling.

This unit will reveal the various aspects of modern biotechnology, its products and applications.

Interdisciplinarity Fields of Biotechnology

Biotechnology is one of the most important applied interdisciplinary sciences of the **21st century**. It is the trusted area that enables us to find the beneficial way of life. Biotechnology has wide applications in various sectors like agriculture, medicine, environment and commercial industries.

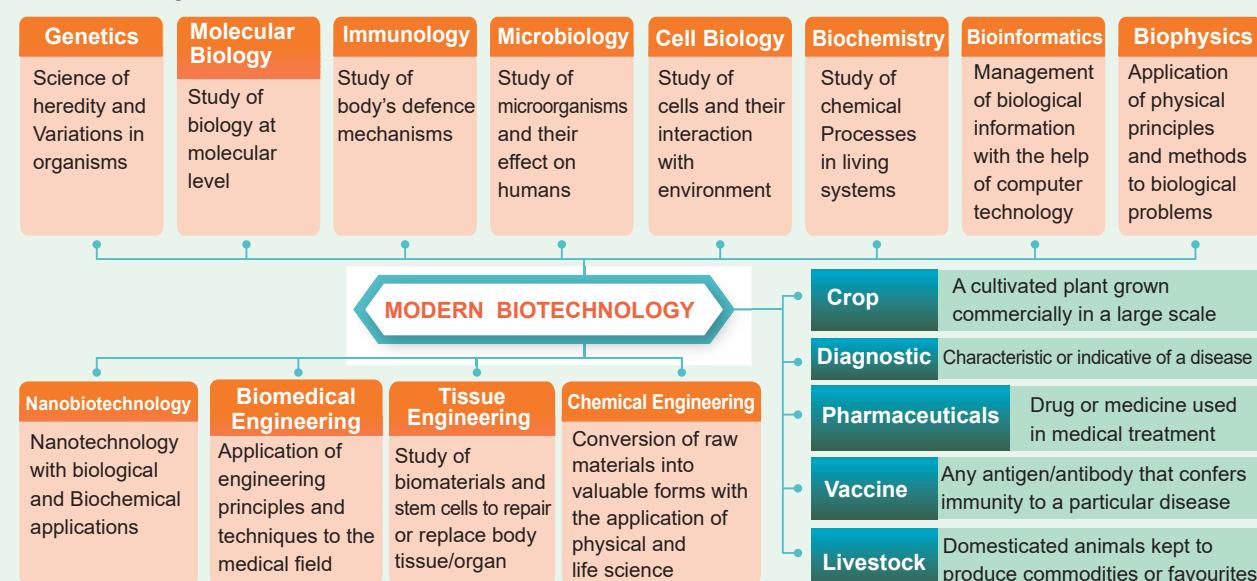


Figure 4.1: Interdisciplinarity Fields of Biotechnology



4.2 Historical Perspective

The major historical events for the development of Biotechnology, as an interdisciplinary field with multidisciplinary applications are listed below:

Before Common Era

6000 BC – 3000 BC – Bread making, fermentation of fruit juices and plant exudates to produce alcoholic beverages using yeast.

Pre – 20th Century

- 1770** – Antoine Lavoisier gave chemical **basis of alcoholic fermentation**.
- 1798** – Edward Jenner uses **first viral vaccine** to inoculate a child from smallpox.
- 1838** – **Protein** discovered, named and recorded by Gerardus Johannes Mulder and Jons Jacob Berzelius.
- 1871** – Ernst Hoppe, Seyler discovered **enzyme invertase**, which is still used for making artificial sweeteners.
- 1876** – Louis Pasteur identified **role of microorganisms in fermentation**.

20th Century

- 1919** – The **term biotechnology** was coined by Karl Ereky
- 1928** – **Discovery of Penicillin** by Alexander Fleming
- 1941** – Experiment with *Neurospora crassa* resulting in **one gene one enzyme hypothesis** by George Beadle and Edward Tatum.
- 1944** – Identification of **DNA as the genetic material** Avery-MacLeod-McCarty
- 1953** – Discovery of **double helix structure of DNA** by James Watson and Francis Crick.
- 1972** – Discovery of **Restriction enzymes** by Arber, Smith and Nathans.
- 1973** – Fragmentation of DNA-combined with Plasmid DNA, **r-DNA technology** - Genetic engineering -Modified gene by Stanley Cohen, Annie Chang, Robert Helling and Herbert Boyer.
- 1975** – Production of **Monoclonal antibodies** by Kohler and Milstein
- 1976** – Sanger and Gilbert developed **techniques to sequence DNA**

1978 – Production of **human insulin** in E.Coli

1979 – **Development of Artificial gene** – functioning within the living cells by H.G. Khorana

1982 – U.S approved **humulin** (human insulin) the first pharmaceutical product of rDNA technology, for human use.

1983 – Use of **Ti plasmids** to genetically transform plants

1986 – Development of **Polymerase Chain Reaction (PCR)** technology by Kary Mullis.

1987 – Gene transfer by **biostatic transformation**

1992 – First chromosomes of yeast is sequenced

1994 – U.S approved the first **Genetically Modified food: Flavr Savr tomato**.

1997 – The first **transgenic animal**, mammalian sheep, Dolly developed by **nuclear cloning** by Ian Wilmet.

2000 – First **plant Genome** of *Arabidopsis thaliana* sequenced

21st Century

2001 – Human genome Project creates a **draft of the human genome sequence**.

2002 – First **crop plant genome** sequenced in *Oryza sativa*

2003 – **Human genome project is completed**, providing information on the locations and sequence of human genes on all 46 chromosomes.

2010 – Sir Robert G. Edwards developed ***in vitro* fertilization in animal**.

2016 – Stem cells injected into stroke patients re-enable patient to walk – **Stem cell therapy**

2017 – **Blood stem cells** grown in lab.

2018 – James Allison and TasukuHonjo **discovered protein found in immune cells**. This found a new role in cancer therapy.



4.3. Traditional Biotechnology

As described earlier, it is the kitchen technology developed by our ancestors that was using the fermenting bacteria. Thus it includes the process that is based on the natural capabilities of organisms.

4.3.1 Fermentation

The word fermentation is derived from the Latin verb 'fervere' which means 'to boil'. Fermentation refers to the metabolic process in which organic molecules (normally glucose) are converted into acids, gases, or alcohol in the absence of oxygen or any electron transport chain. The study of fermentation, its practical uses is called zymology and originated in 1856, when French chemist Louis Pasteur demonstrated that fermentation was caused by yeast. Fermentation occurs in certain types of bacteria and fungi that require an oxygen-free environment to live. The processes of fermentation are valuable to the food and beverage industries, with the conversion of sugar into ethanol to produce alcoholic beverages, the release of CO₂ by yeast used in the leavening of bread, and with the production of organic acids to preserve and flavor vegetables and dairy products.

Bioreactor (Fermentor)

Bioreactor (Fermentor) is a vessel or a container that is designed in such a way that it can provide an optimum environment in which microorganisms or their enzymes interact with a substrate to produce the required product. In the bioreactor aeration, agitation, temperature and pH are controlled. Fermentation involves two process namely upstream and downstream process.

i. Upstream process

All the process before starting of the fermenter such as sterilization of the fermenter, preparation and sterilization of culture medium and growth of the suitable inoculum are called upstream process.

ii. Downstream process

All the process after the fermentation process is known as the downstream process. This process includes distillation, centrifuging, filtration and solvent extraction. Mostly this process involves the purification of the desired product.

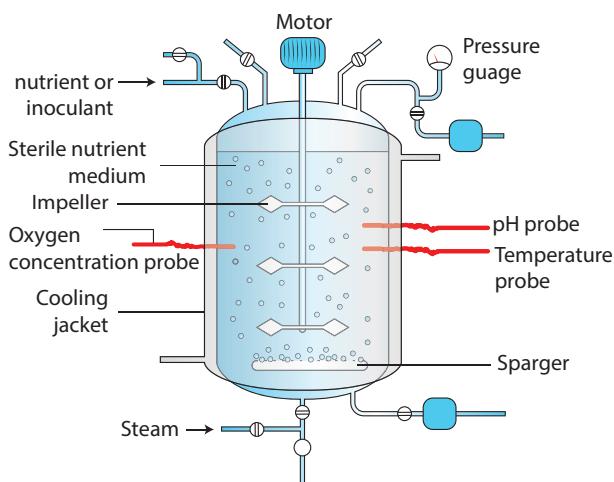


Figure 4.2: Bioreactor

Procedure of Fermentation

- Depending upon the type of product, bioreactor is selected.
- A suitable substrate in liquid media is added at a specific temperature, pH and then diluted.
- The organism (microbe, animal/plant cell, sub-cellular organelle or enzyme) is added to it.
- Then it is incubated at a specific temperature for the specified time.
- The incubation may either be aerobic or anaerobic.
- Withdrawal of product using downstream processing methods

Application of fermentation in industries

Fermentation has industrial application such as:

1. Microbial biomass production

Microbial cells (biomass) like algae, bacteria, yeast, fungi are grown, dried and used as source of a complete protein called



'single cell protein (SCP)' which serves as human food or animal feed.

2. Microbial metabolites

Microbes produce compounds that are very useful to man and animals. These compounds are called metabolites, can be grouped into two categories:

- Primary metabolites:** Metabolites produced for the maintenance of life process of microbes are known as primary metabolites Eg. Ethanol, citric acid, lactic acid, acetic acid.
- Secondary metabolites:** Secondary metabolites are those which are not required for the vital life process of microbes, but have value added nature, this includes antibiotics e.g -Amphotericin-B (*Streptomyces nodosus*), Penicillin (*Penicillium chrysogenum*) Streptomycin (*S. grises*), Tetracycline (*S. aureofaciens*), alkaloids, toxic pigments, vitamins etc.

3. Microbial enzymes

When microbes are cultured, they secrete some enzymes into the growth media. These enzymes are industrially used in detergents, food processing, brewing and pharmaceuticals. Eg. protease, amylase, isomerase, and lipase.

4. Bioconversion, biotransformation or modification of the substrate

The fermenting microbes has the capacity to produce valuable products, eg. conversion of ethanol to acetic acid (vinegar), isopropanol to acetone, sorbitol to sorbose (this is used in the manufacture of vitamin C), sterols to steroids.

4.3.2 Single Cell Protein (SCP)

Single cell proteins are dried cells of microorganism that are used as protein supplement in human foods or animal feeds. Single Cell Protein (SCP) offers an unconventional but plausible solution to protein deficiency faced by the entire humanity. Although single cell protein has

high nutritive value due to their higher protein, vitamin, essential amino acids and lipid content, there are doubts on whether it could replace conventional protein sources due to their high nucleic acid content and slower in digestibility. Microorganisms used for the production of Single Cell Protein are as follows:

- Bacteria - *Methylophilus methylotrophus*, *Cellulomonas*, *Alcaligenes*
- Fungi - *Agaricus campestris*, *Saccharomyces cerevisiae* (yeast), *Candida utilis*
- Algae - *Spirulina*, *Chlorella*, *Chlamydomonas*

The single cell proteins forms an important source of food because of their protein content, carbohydrates, fats, vitamins and minerals. It is used by Astronauts and Antarctica expedition scientists.

Spirulina can be grown easily on materials like waste water from potato processing plants (containing starch), straw, molasses, animal manure and even sewage, to produce large quantities and can serve as food rich in protein, minerals, fats, carbohydrate and vitamins. Such utilization also reduces environmental pollution. 250 g of *Methylophilus methylotrophus*, as its high rate of biomass production and growth, can be expected to produce 25 tonnes of protein.



Figure 4.3: *Spirulina* products

Applications of Single-Cell Protein

- It is used as protein supplement
- It is used in cosmetics products for healthy hair and skin
- It is used in poultry as the excellent source of proteins and other nutrients, it is widely used for feeding cattle, birds, fishes etc.



- It is used in food industry as aroma carriers, vitamin carrier, emulsifying agents to improve the nutritive value of baked products, in soups, in ready-to-serve-meals, in diet recipes
- It is used in industries like paper processing, leather processing as foam stabilizers.

4.4 Advancements in Modern Biotechnology

The modern biotechnology embraces all the genetic manipulations, protoplasmic fusion techniques and the improvements made in the old biotechnological processes. Some of the major advancements in modern biotechnology are described below.

4.4.1 Genetic Engineering

Genetic engineering or recombinant DNA technology or gene cloning is a collective term that includes different experimental protocols resulting in the modification and transfer of DNA from one organism to another.

The definition for conventional recombination was already given in Unit II. Conventional recombination involves exchange or recombination of genes between homologous chromosomes during meiosis. Recombination carried out artificially using modern technology is called recombinant DNA technology (r-DNA technology). It is also known as gene manipulation technique. This technique involves the transfer of DNA coding for a specific gene from one organism into another organism using specific agents like vectors or using instruments

like electroporation, gene gun, liposome mediated, chemical mediated transfers and microinjection.

4.4.2 Steps involved in Recombinant DNA Technology

The steps involved in recombinant DNA technology are:

- Isolation of a DNA fragment containing a gene of interest that needs to be cloned. This is called an **insert**.
- Generation of recombinant DNA (rDNA) molecule by insertion of the DNA fragment into a carrier molecule called a **vector** that can self-replicate within the host cell.
- Selection of the transformed host cells that is carrying the rDNA and allowing them to multiply thereby multiplying the rDNA molecule.

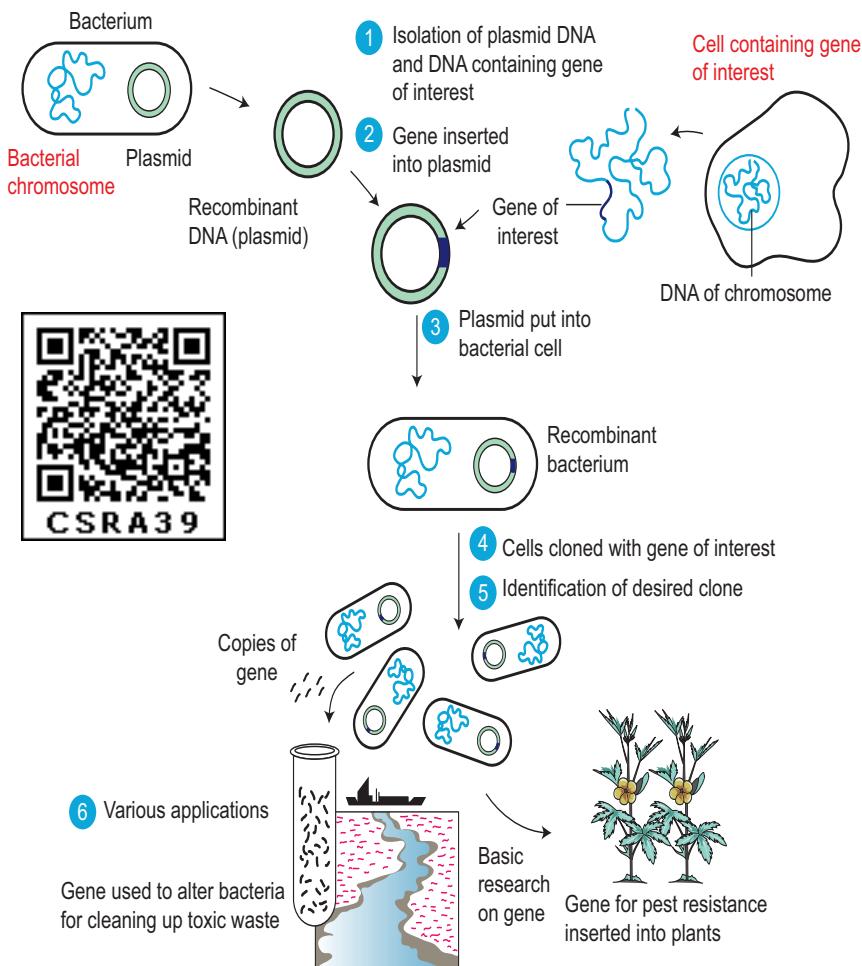


Figure 4.4: Steps involved in r-DNA Technology



- The entire process thus generates either a large amount of rDNA or a large amount of protein expressed by the insert.
- Wherever vectors are not involved the desired gene is multiplied by PCR technique. The multiple copies are injected into the host cell protoplast or it is shot into the host cell protoplast by shot gun method.

PCR: Polymerase Chain Reaction is a common laboratory technique used to make copies (millions) of a particular region of DNA.

4.5 Tools for Genetic Engineering

Now we know from the foregoing discussion that in order to generate recombinant DNA molecule, certain basic tools are necessary for the process. The basic tools are enzymes, vectors and host organisms. The most important enzymes required for genetic engineering are the restriction enzymes, DNA ligase and alkaline phosphatase.

4.5.1 Restriction Enzymes

The two enzymes responsible for restricting the growth of bacteriophage in *Escherichia coli* were isolated in the year 1963. One was the enzyme which added methyl groups to DNA, while the other cut DNA. The later was called restriction endonuclease. A **restriction enzyme** or **restriction endonuclease** is an enzyme that

cleaves DNA into fragments at or near specific recognition sites within the molecule known as **restriction** sites. Based on their mode of action restriction enzymes are classified into Exonucleases and Endonucleases.

- Exonucleases are enzymes which remove nucleotides one at a time from the end of a DNA molecule. e.g. Bal 31, Exonuclease III.
- Endonucleases are enzymes which break the internal phosphodiester bonds within a DNA molecule. e.g. Hind II, EcoRI, PvuI, BamHI, TaqI.

Restriction endonuclease: Molecular scissors

The restriction enzymes are called as molecular scissors. These act as foundation of recombinant DNA technology. These enzymes exist in many bacteria where they function as a part of their defence mechanism called restriction-modification system.

There are three main classes of restriction endonuclease : Type I, Type II and Type III, which differ slightly by their mode of action. Only type II enzyme is preferred for use in recombinant DNA technology as they recognise and cut DNA within a specific sequence typically consisting of 4-8 bp. Examples of certain enzymes are given in table 5.1.

The restriction enzyme **Hind II** always cut DNA molecules at a point of recognising a specific sequence of six base pairs. This sequence is known as recognition sequence. Today more

than 900 restriction enzymes that have been isolated from over 230 strains of bacteria with different recognition sequences.

Restriction endonucleases are named by a standard procedure. The first letter of the enzymes indicates the genus name, followed by the first two letters of the species, then comes the strain of the organism and finally a roman numeral indicating the order of discovery. For example, **EcoRI** is from *Escherichia* (E)

Restriction enzyme	Microbial source	Recognition sequence	Fragments	
Alu I	<i>Arthrobacter luteus</i>	5'AG/CT3' 3'TC/GA5'	A-G C-T T-C G-A	Blunt ends
BamHI	<i>Bacillus amyloliquefaciens</i>	5'G/GATCC3' 3'CCTAG/G5'	G G-A-T-C-C C-C-T-A-G G	Sticky ends
EcoRI	<i>Escherichia coli</i>	5'G/AATT3' 3'CCTAG/G5'	G A-A-T-T-C C-T-T-A-A G	Sticky ends
HaeIII	<i>Haemophilus aegyptus</i>	5'GG/CC3' 3'CC/GG5'	G-G C-C C-C G-G	Blunt ends
HindIII	<i>Haemophilus influenza</i>	5'A/AGCTT3' 3'TTCGA/A5'	A A-G-C-T-T T-T-C-G-A A	Sticky ends

Table 4.1: Type II restriction enzyme with source, recognition and cleavage site.



coli (**co**), strain RY 13 (**R**) and first endonuclease (**I**) to be discovered.

It contains 2 different antibiotic resistance genes and recognition site for several restriction enzymes. This sequence is referred to as a restriction site and is generally -palindromic which means that the sequence in both DNA strands at this site read same in 5' – 3' direction and in the 3'-5' direction

Example: MALAYALAM: This phrase is read the same in either of the directions.

Palindromic repeats: A symmetrical repeated sequence in DNA strands

5' ... CATTATATAATG ... 3'

3' ... GTAATATATTAC ... 5'

Note: That the sequence of the base pairs in the reverse direction when compare to the first sequence.

The exact kind of cleavage produced by a restriction enzyme is important in the design of a gene cloning experiment. Some cleave both strands of DNA through the centre resulting in **blunt** or **flush end**. These are known as symmetric cuts. Some enzymes cut in a way producing protruding and recessed ends known as **sticky** or **cohesive end**. Such cut are called staggered or asymmetric cuts.

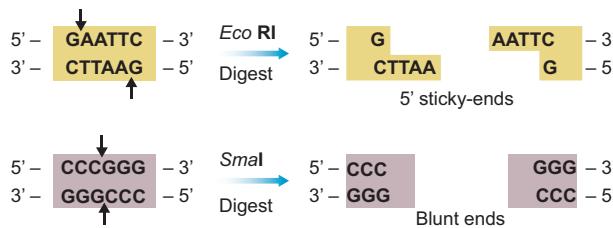


Figure 4.5: Sticky and Blunt ends

Two other enzymes that play an important role in recombinant DNA technology are DNA ligase and alkaline phosphatase

4.5.2 DNA Ligase

DNA ligase enzyme joins the sugar and phosphate molecules of double stranded DNA (dsDNA) with 5'-PO₄ and a 3'-OH in

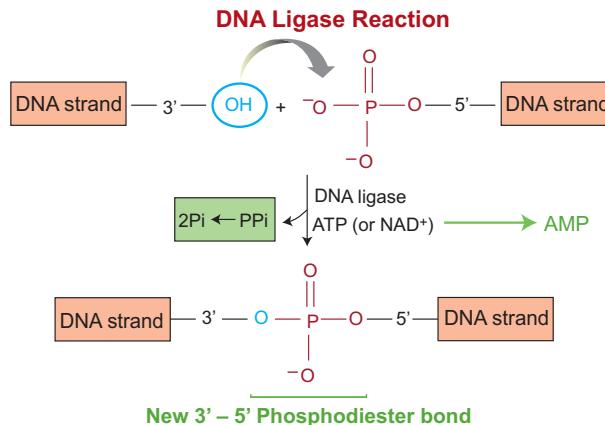


Figure 4.6: DNA ligase reaction

an Adenosine Triphosphate (ATP) dependent reaction. This is isolated from T4 phage.

4.5.3 Alkaline Phosphatase

It is a DNA modifying enzymes and adds or removes specific phosphate group at 5' terminus of double stranded DNA (dsDNA) or single stranded DNA (ssDNA) or RNA. Thus it prevents self ligation. This enzyme is purified from bacteria and calf intestine.

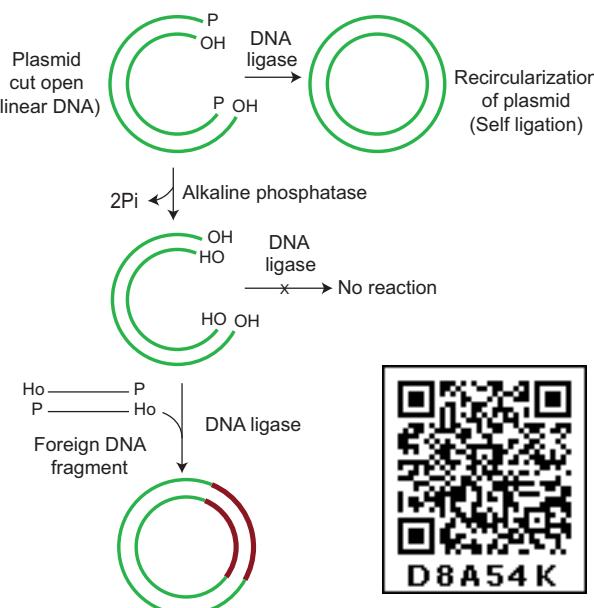
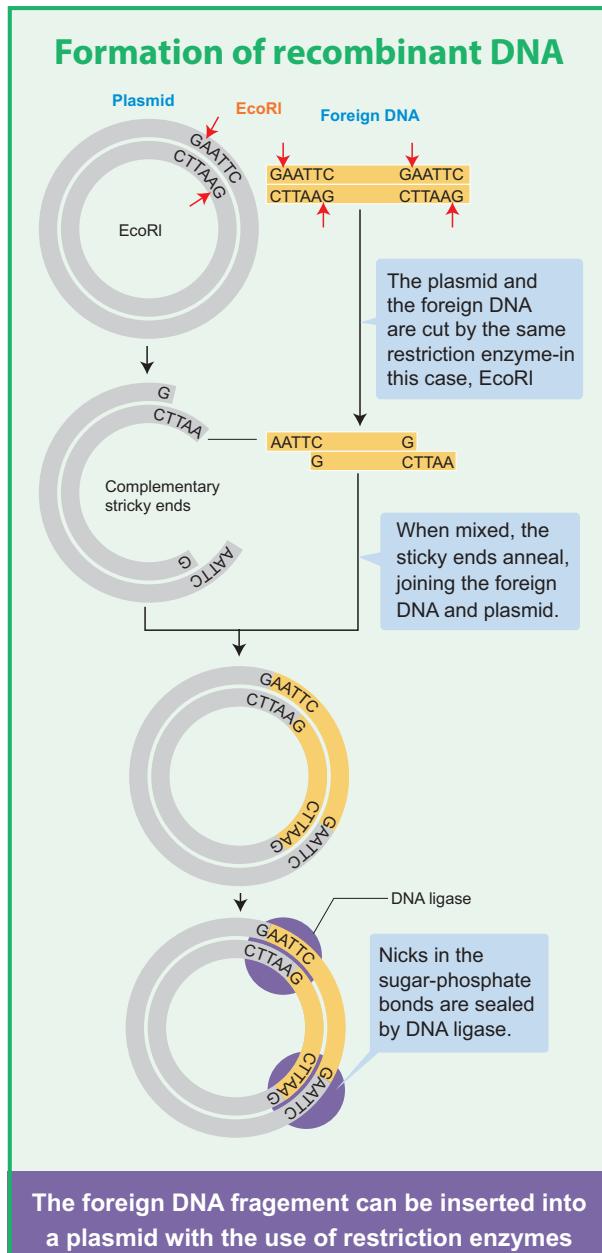


Figure 4.7: Action of Alkaline Phosphatase

4.5.4 Vectors

Another major component of a gene cloning experiment is a vector such as a plasmid. A Vector is a small DNA molecule capable of self-replication and is used as a carrier and transporter of DNA fragment which is inserted



into it for cloning experiments. Vector is also called **cloning vehicle** or **cloning DNA**. Vectors are of two types: i) Cloning Vector, and ii) Expression Vector. Cloning vector is used for the cloning of DNA insert inside the suitable host cell. Expression vector is used to express the DNA insert for producing specific protein inside the host.

Properties of Vectors

Vectors are able to replicate autonomously to produce multiple copies of them along with their DNA insert in the host cell.

- It should be small in size and of low molecular weight, less than 10 Kb (kilo base pair) in size so that entry/transfer into host cell is easy.

- Vector must contain an origin of replication so that it can independently replicate within the host.
- It should contain a suitable marker such as antibiotic resistance, to permit its detection in transformed host cell.
- Vector should have unique target sites for integration with DNA insert and should have the ability to integrate with DNA insert it carries into the genome of the host cell. Most of the commonly used cloning vectors have more than one restriction site. These are Multiple Cloning Site (MCS) or polylinker. Presence of MCS facilitates the use of restriction enzyme of choice.

The following are the features that are required to facilitate cloning into a vector.

- Origin of replication (ori):** This is a sequence from where replication starts and piece of DNA when linked to this sequence can be made to replicate within the host cells.

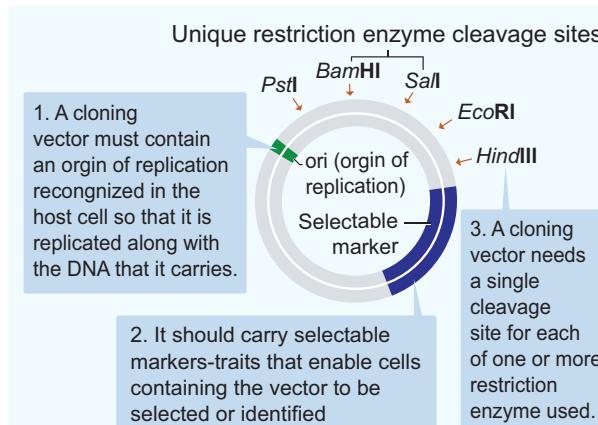


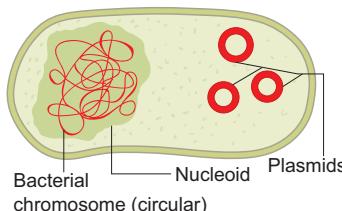
Figure 4.8: Properties of Vector

- Selectable marker:** In addition to **ori** the vector requires a selectable marker, which helps in identifying and eliminating non transformants and selectively permitting the growth of the transformants.
- Cloning sites:** In order to link the alien DNA, the vector needs to have very few, preferably single, recognition sites for the commonly used restriction enzymes.



Types of vector

Few types of vectors are discussed in detail below:



Plasmid

Plasmids are extra chromosomal, self replicating ds circular DNA molecules, found in the bacterial cells in addition to the bacterial chromosome. Plasmids contain Genetic information for their own replication.

pBR 322 Plasmid

pBR 322 plasmid is a reconstructed plasmid and most widely used as cloning vector; it contains 4361 base pairs. In pBR, *p* denotes plasmid, *B*and *R* respectively the names of scientist Boliver and Rodriguez who developed this plasmid. The number 322 is the number of plasmid developed from their laboratory. It contains amp^R and tet^R two different antibiotic resistance genes and recognition sites for several restriction enzymes. (*Hind III*, *EcoRI*, *BamH I*, *Sal I*, *Pvu II*, *Pst I*, *Cla I*), ori and antibiotic resistance genes. Rop codes for the proteins involved in the replication of the plasmid.

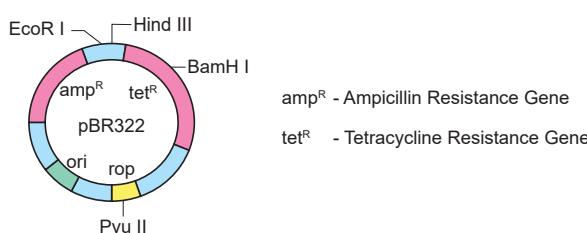


Figure 4.10: pBR 322

Ti Plasmid

Ti plasmid is found in *Agrobacterium tumefaciens*, a bacteria responsible for inducing tumours in several dicot plants. The plasmid carries transfer (tra) gene which help to transfer T- DNA from one bacterium to other bacterial or plant cell. It has Onc gene for oncogenecity,

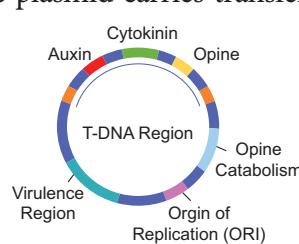


Figure 4.11: Ti Plasmid

ori gene for origin for replication and inc gene for incompatibility. T-DNA of Ti-Plasmid is stably integrated with plant DNA. Agrobacterium plasmids have been used for introduction of genes of desirable traits into plants.

Transposon as Vector

Transposons (Transposable elements or mobile elements) are DNA sequence able to insert itself at a new location in the genome without having any sequence relationship with the target locus and hence transposons are called **walking genes** or **jumping genes**. They are used as genetic tools for analysis of gene and protein functions, that produce new phenotype on host cell. The use of transposons is well studied in *Arabidopsis thaliana* and bacteria such as *Escherichia coli*.

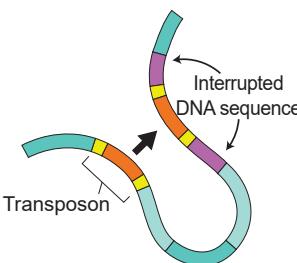


Figure 4.12: Transposon *thaliana* and bacteria such as *Escherichia coli*.

Walking Genes - Gene walking involves the complete sequencing of large more than 1 kb stretches of DNA.

Expression vectors

Vectors which are suitable for expressing foreign proteins are called expression vectors. This vector consists of signals necessary for transcription and translation of proteins in the host. This helps the host to produce foreign protein in large amounts. Example: pUC 19.

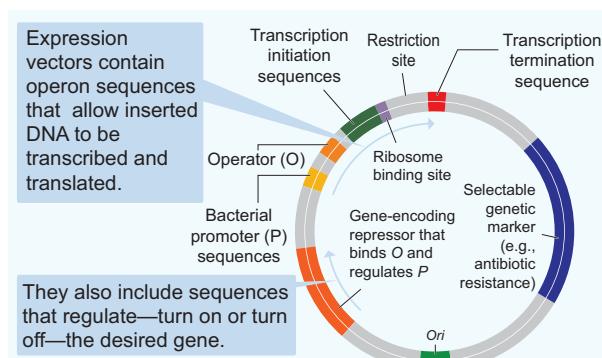
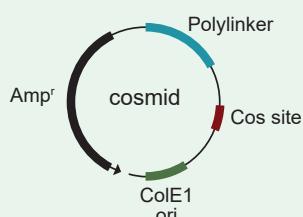


Figure 4.13: E.Coli Expression vector



More vectors to know



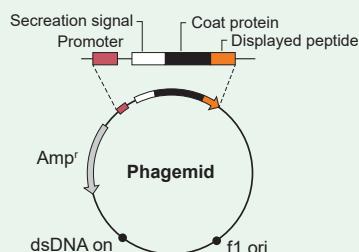
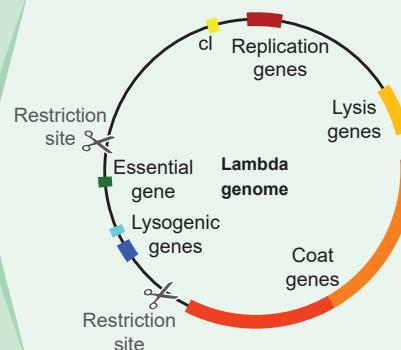
Cosmid

Cosmids are plasmids containing the 'cos' - Cohesive Terminus, the sequence having cohesive ends. They are hybrid vectors derived from plasmids having a fragment of lambda phage DNA with its Cos site and a bacterial plasmid.

Bacteriophage Vectors

Bacteriophages are viruses that infect bacteria. The most commonly used *E. coli* phages are λ phage (Lambda phage) and M13 phage. Phage vectors are more efficient than plasmids - DNA upto 25 Kb can be inserted into phage vector.

Lambda genome: Lambda phage is a temperate bacteriophage that infects *Escherichia coli*. The genome of lambda-Phage is 48502 bp long, i.e. 49Kb and has 50 genes.

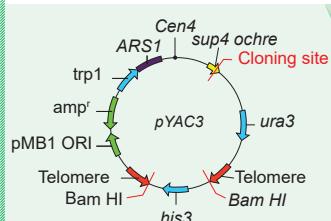
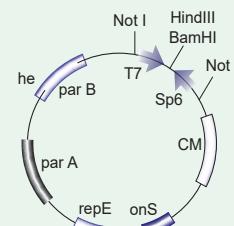


Phagemid Vectors

Phagemids are reconstructed plasmid vectors, which contain their own origin - 'ori' gene and also contain origin of replication from a phage. pBluescript SK (+/-) is an example of phagemid vector.

Bacterial Artificial Chromosome (BAC) Vector

BAC is a shuttle plasmid vector, created for cloning large-sized foreign DNA. BAC vector is one of the most useful cloning vector in r-DNA technology they can clone DNA inserts of upto 300 Kb and they are stable and more user-friendly.

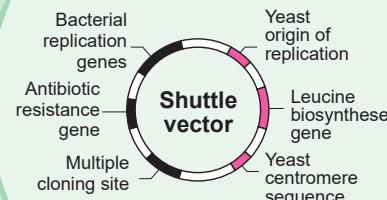


Yeast Artificial chromosome (YAC vector)

YAC plasmid vector behaves like a yeast chromosome, which occurs in two forms, i.e. circular and linear. The circular YAC multiplies in Bacteria and linear YAC multiplies in Yeast Cells.

Shuttle Vectors

The shuttle vectors are plasmids designed to replicate in cells of two different species. These vectors are created by recombinant techniques. The shuttle vectors can propagate in one host and then move into another host without any extra manipulation. Most of the Eukaryotic vectors are Shuttle Vectors.





4.5.5 Competent Host (For Transformation with Recombinant DNA)

The propagation of the recombinant DNA molecules must occur inside a living system or host. Many types of host cells are available for gene cloning which includes E.coli, yeast, animal or plant cells. The type of host cell depends upon the cloning experiment. E.coli is the most widely used organism as its genetic make-up has been extensively studied, it is easy to handle and grow, can accept a range of vectors and has also been studied for safety. One more important feature of E.coli to be preferred as a host cell is that under optimal growing conditions the cells divide every 20 minutes.

Since the DNA is a hydrophilic molecule, it cannot pass through cell membranes. In order to force bacteria to take up the plasmid, the bacterial cells must first be made competent to take up DNA. This is done by treating them with a specific concentration of a divalent cation such as calcium. Recombinant DNA can then be forced into such cells by incubating the cells with recombinant DNA on ice, followed by placing them briefly at 42°C (heatshock) and then putting them back on ice. This enables bacteria to take up the Recombinant DNA.

For the expression of eukaryotic proteins, eukaryotic cells are preferred because to produce a functionally active protein it should fold properly and post translational modifications should also occur, which is not possible by prokaryotic cell (E.coli).

4.6 Methods of Gene Transfer

The next step after a recombinant DNA molecule has been generated is to introduce it into a suitable host cell. There are many methods to introduce recombinant vectors and these are dependent on several factors such as the vector type and host cell.

For achieving genetic transformation in plants, the basic pre-requisite is the construction of a vector which carries the gene of interest

flanked by the necessary controlling sequences, i.e., the promoter and terminator, and deliver the genes into the host plant. There are two kinds of gene transfer methods in plants. It includes:

- Direct or vectorless gene transfer
- Indirect or vector – mediated gene transfer

4.6.1 Direct or Vectorless Gene Transfer

In the direct gene transfer methods, the foreign gene of interest is delivered into the host plant without the help of a vector. The following are some of the common methods of direct gene transfer in plants.

- a. **Chemical mediated gene transfer:** Certain chemicals like polyethylene glycol (PEG) and dextran sulphate induce DNA uptake into plant protoplasts.
- b. **Microinjection:** The DNA is directly injected into the nucleus using fine tipped glass needle or micro pipette to transform plant cells. The protoplasts are immobilised on a solid support (agarose on a microscopic slide) or held with a holding pipette under suction.
- c. **Electroporation Methods of Gene Transfer:** A pulse of high voltage is applied to protoplasts, cells or tissues which makes transient pores in the plasma membrane through which uptake of foreign DNA occurs.

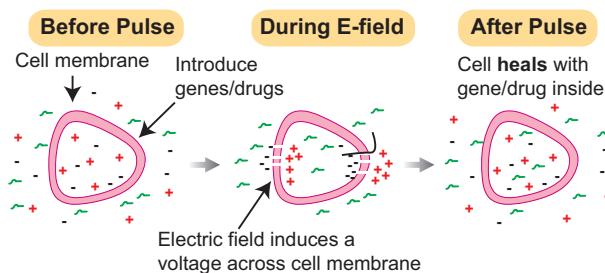


Figure 4.14: Electroporation Methods of Gene Transfer

- d. **Liposome mediated method of Gene Transfer:** Liposomes the artificial phospholipid vesicles are useful in gene transfer. The gene or DNA is transferred from liposome into vacuole of plant

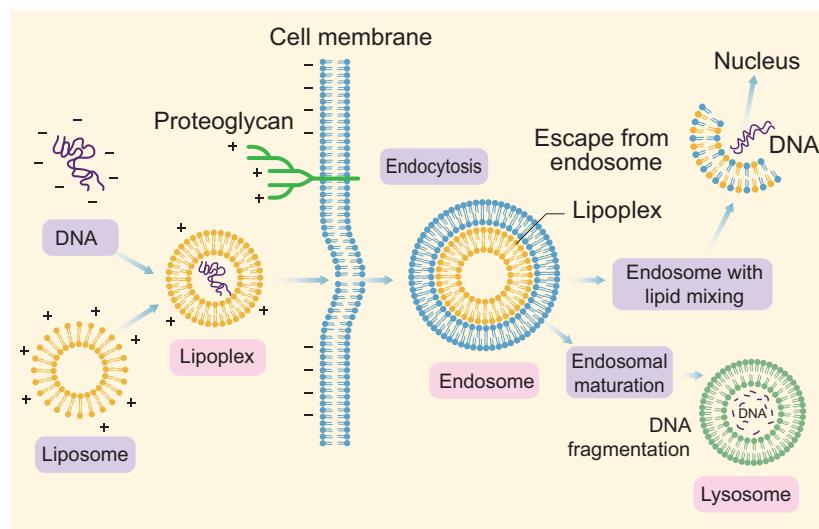


Figure 4.15: Liposome mediated method of Gene Transfer

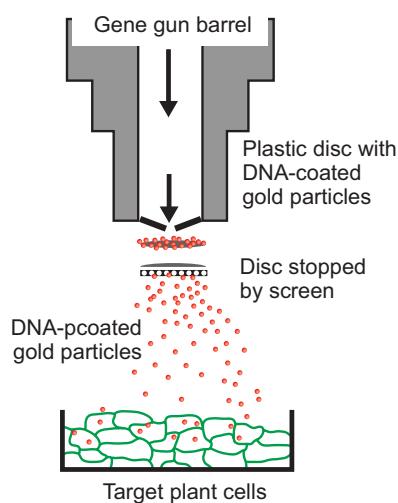


Figure 4.16: Gene gun method of Gene Transfer

cells. It is carried out by encapsulated DNA into the vacuole. This technique is advantageous because the liposome protects the introduced DNA from being damaged by the acidic pH and protease enzymes present in the vacuole. Liposome and tonoplast of vacuole fusion resulted in gene transfer. This process is called lipofection.

- e. **Biostatics:** The foreign DNA is coated onto the surface of minute gold or tungsten particles (1-3 μm) and bombarded onto the target tissue or cells using a particle gun (also called as **gene gun/micro projectile gun/shotgun**). Then the bombarded cells or tissues are cultured on selected medium to regenerate plants from the transformed cells.(Figure 4.16)

4.6.2 Indirect or Vector-Mediated Gene Transfer

Gene transfer is mediated with the help of a plasmid vector is known as indirect or vector mediated gene transfer. Among the various vectors used for plant transformation, the Ti-plasmid from *Agrobacterium tumefaciens* has been used extensively. This bacterium has a large size plasmid, known as Ti plasmid (Tumor inducing) and a portion of it referred as T-DNA (transfer DNA) is transferred to plant genome in the infected cells and cause plant tumors (crown gall). Since this bacterium has the natural ability to transfer T-DNA region of its plasmid into plant genome, upon infection of cells at the wound site, it is also known as the natural genetic engineer of plants.

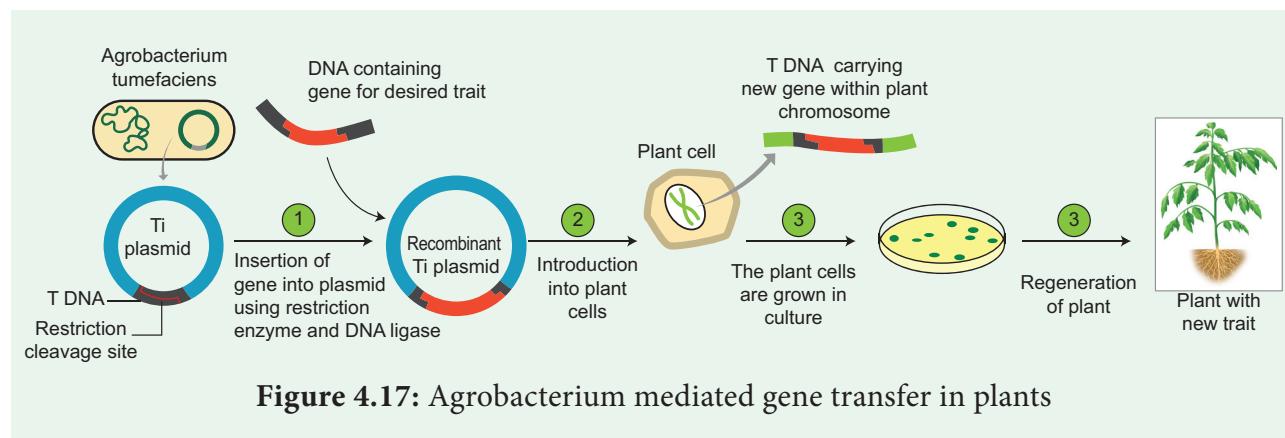


Figure 4.17: Agrobacterium mediated gene transfer in plants



The foreign gene (e.g. Bt gene for insect resistance) and plant selection marker gene, usually an antibiotic gene like *npt II* which confers resistance to antibiotic kanamycin are cloned in the T DNA region of Ti-plasmid in place of unwanted DNA sequences.(Figure 4.17)

4.7 Screening for Recombinants

After the introduction of r-DNA into a suitable host cell, it is essential to identify those cells which have received the r-DNA molecule. This process is called screening. The vector or foreign DNA present in recombinant cells expresses the characters, while the non-recombinants do not express the characters or traits. For this some of the methods are used and one such method is Blue-White Selection method.

4.7.1 Insertional Inactivation - Blue-White Colony Selection Method

It is a powerful method used for screening of recombinant plasmid. In this method, a reporter gene *lacZ* is inserted in the vector. The *lacZ* encodes the enzyme β -galactosidase and contains several recognition sites for restriction enzyme.

β -galactosidase breaks a synthetic substrates called X-gal (5-bromo-4-chloro-indolyl- β -D-galacto-pyranoside) into an insoluble blue coloured product. If a foreign gene is inserted into *lacZ*, this gene will be inactivated. Therefore, no-blue colour will develop (white) because β -galactosidase is not synthesized due to inactivation of *lacZ*. Therefore, the host cell containing r-DNA form white coloured colonies on the

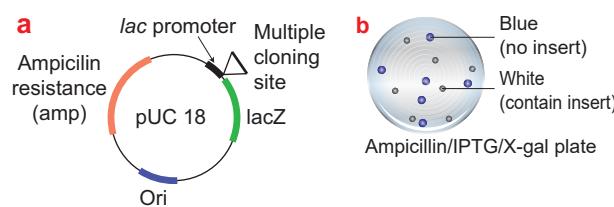


Figure 4.18: a. Plasmid vector designed for blue-white screening b. Blue-white colony selection method

medium contain X-gal, whereas the other cells containing non-recombinant DNA will develop the blue coloured colonies. On the basis of colony colour, the recombinants can be selected.

4.7.2 Antibiotic resistant markers

An antibiotic resistance marker is a gene that produces a protein that provides cells with resistance to an antibiotic. Bacteria with transformed DNA can be identified by growing on a medium containing an antibiotic. Recombinants will grow on these medium as they contain genes encoding resistance to antibiotics such as ampicillin, chloro amphenicol, tetracycline or kanamycin, etc., while others may not be able to grow in these media, hence it is considered useful selectable marker.

4.7.3. Replica plating technique

A technique in which the pattern of colonies growing on a culture plate is copied. A sterile filter plate is pressed against the culture plate and then lifted. Then the filter is pressed against a second sterile culture plate. This results in the new plate being infected with cell in the same relative positions as the colonies in the original plate. Usually, the medium used in the second plate will differ from that used in the first. It may include an antibiotic or without a growth factor. In this way, transformed cells can be selected.

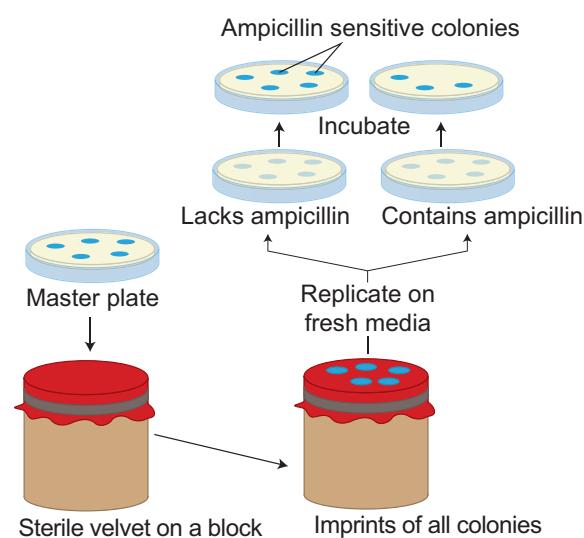


Figure 4.19: Replica plating technique



4.7.4 Molecular Techniques - Isolation of Genetic Material and Gel Electrophoresis

Electrophoresis is a separating technique used to separate different biomolecules with positive and negative charges.

Principle

By applying electricity (DC) the molecules migrate according to the type of charges they have. The electrical charges on different molecules are variable.

+ve charged	Cations	will move towards	-ve Cathode
-ve charged	Anions	will move towards	+ve Anode

Agarose GEL Electrophoresis

It is used mainly for the purification of specific DNA fragments. Agarose is convenient for separating DNA fragments ranging in size from a few hundred to about 20000 base pairs. Polyacrylamide is preferred for the purification of smaller DNA fragments. The gel is complex network of polymeric molecules. DNA molecule is negatively charged molecule - under an electric field DNA molecule migrates through the gel. The electrophoresis is frequently performed with marker DNA fragments of known size which allow accurate size determination of an unknown DNA molecule by interpolation. The advantages of agarose gel electrophoresis are that the DNA bands can be readily detected at high sensitivity. The bands of DNA in the gel are stained with the dye **Ethidium Bromide** and DNA can be detected as visible fluorescence illuminated in UV light will give orange fluorescence, which can be photographed.

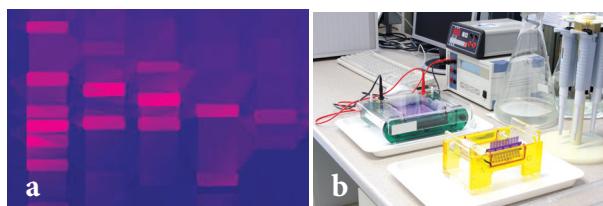


Figure 4.20: a. Bands of DNA in Agarose gel
b. Gel Electrophoresis Instrument

Agricultural diagnostics refers to a variety of tests that are used for detection of pathogens in plant tissues. Two of the most efficient methods are

1. ELISA (Enzyme Linked Immuno Sorbent Assay)

Elisa is a diagnostic tool for identification of pathogen species by using antibodies and diagnostic agents. Use of ELISA in plant pathology especially for weeding out virus infected plants from large scale planting is well known.

2. DNA Probes

DNA Probes, isotopic and non-isotopic (Northern and Southern blotting) are popular tools for identification of viruses and other pathogens

4.7.5 Nucleic Acid Hybridization - Blotting Techniques

Blotting techniques are widely used analytical tools for the specific identification of desired DNA or RNA fragments from larger number of molecules. Blotting refers to the process of immobilization of sample nucleic acids or solid support (nitrocellulose or nylon membranes.) The blotted nucleic acids are then used as target in the hybridization experiments for their specific detection.

Types of Blotting Techniques

Southern Blotting: The transfer of DNA from agarose gels to nitrocellulose membrane.

Northern Blotting: The transfer of RNA to nitrocellulose membrane.

Western Blotting: Electrophoretic transfer of Proteins to nitrocellulose membrane.

Southern Blotting Techniques - DNA

The transfer of denatured DNA from Agarose gel to Nitrocellulose Blotting or Filter Paper technique was introduced by Southern in 1975 and this technique is called Southern Blotting Technique.



Steps

The transfer of DNA from agarose gel to nitrocellulose filter paper is achieved by Capillary Action.

A buffer Sodium Saline Citrate (SSC) is used, in which DNA is highly soluble, it can be drawn up through the gel into the Nitrocellulose membrane.

By this process ss-DNA becomes '**Trapped**' in the membrane matrix.

This DNA is hybridized with a nucleic acid and can be detected by autoradiography.

Autoradiography - A technique that captures the image formed in a photographic emulsion due to emission of light or radioactivity from a labelled component placed together with unexposed film.

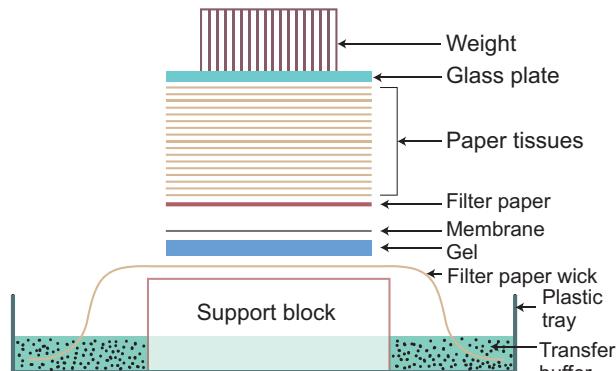


Figure 4.21: Diagrammatic representation of a typical blotting apparatus

Northern Blot

It was found that RNA is not binding to cellulose nitrate. Therefore, Alwin *et al.* (1979) devised a procedure in which RNA bands are transferred from the agarose gel into nitrocellulose filter paper. This transfer of RNA from gel to special filter paper is called Northern Blot hybridization. The filter paper used for Northern blot is Amino Benzyloxymethyl Paper which can be prepared from Whatman 540 paper.

Western Blot

Refers to the electrophoretic transfer of proteins to blotting papers. Nitrocellulose filter paper can be used for western blot technique. A particular

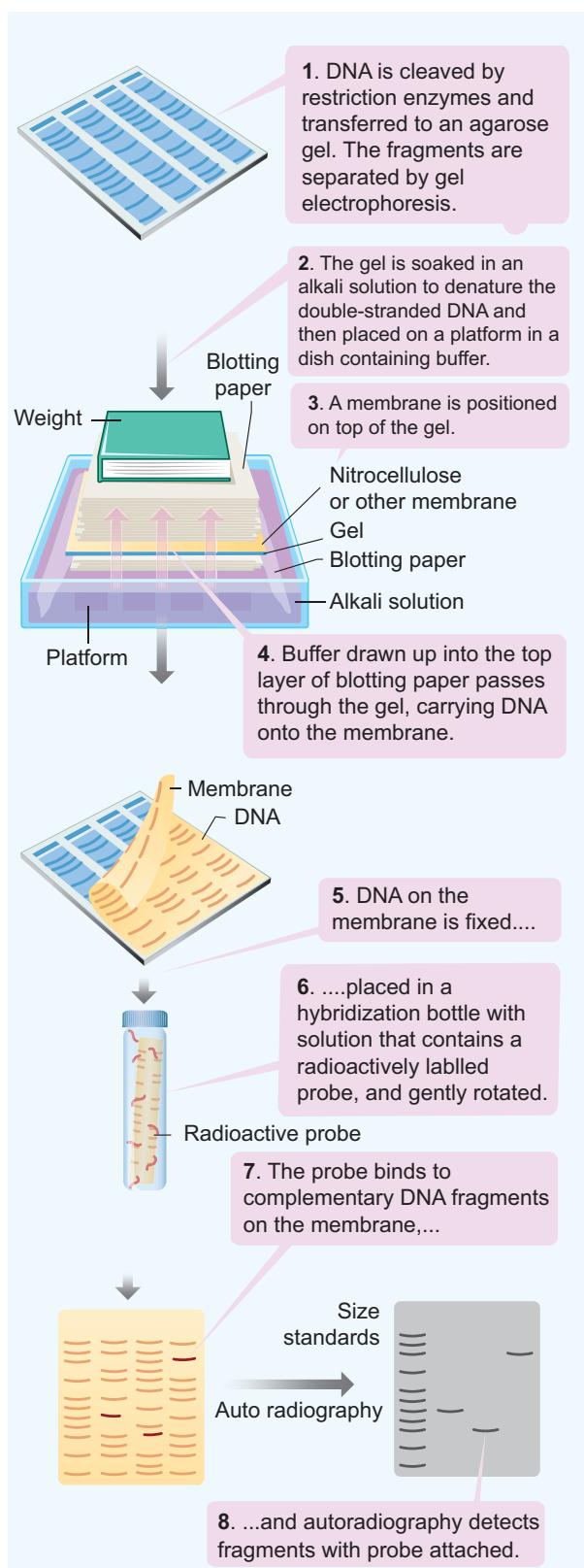


Figure 4.22: Steps involved in southern blotting technique

protein is then identified by probing the blot with a radio-labelled antibody which binds on the specific protein to which the antibody was prepared.



Differences between Blotting Techniques

	Southern blotting	Northern blotting	Western blotting
Name	Southern name of the inventor	Northern a misnomer	Western a misnomer
Separation of	DNA	RNA	Proteins
Denaturation	Needed	Not needed	Needed
Membrane	Nitrocellulose/ nylon	Amino benzyloxymethyl	Nitrocellulose
Hybridisation	DNA-DNA	RNA-DNA	Protein-antibody
Visualising	Autoradiogram	Autoradiogram	Dark room

Table 4.2: Difference between Blotting Techniques

4.7.6 Bioassay for Target Gene Effect

Target gene is target DNA, foreign DNA, passenger DNA, exogenous DNA, gene of interest or insert DNA that is to be either cloned or specifically mutated. Gene targeting experiments have been targeting the nuclei and this leads to 'gene knock-out'. For this purpose, two types of targeting vectors are used. They are insertion vectors and replacement or transplacement vectors.

1. Insertion vectors are entirely inserted into targeted locus as the vectors are linearized within the homology region. Initially, these vectors are circular but during insertion, become linear. It leads to duplication of sequences adjacent to selectable markers.
2. The replacement vector has the homology region and it is co-linear with target. This vector is linearized prior to transfection outside the homology region and then consequently a crossing over occurs to replace the endogenous DNA with the incoming DNA.

Transfection: Introduction of foreign nucleic acids into cells by non-viral methods.

4.7.7. Genome Sequencing and Plant Genome Projects

The whole complement of gene that determine all characteristic of an organism is called genome. The genome may be nuclear genome,

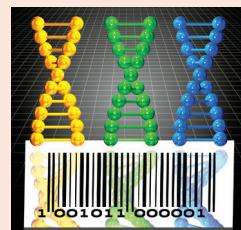
mitochondrial genome or plastid genome. Genome of many plants contain both functional and non-expressive DNA proteins. Genome project refer to a project in which the whole genome of plant is analysed using sequence analysis and sequence homology with other plants. Such genome projects have so far been undertaken in *Chlamydomonas*(algae), *Arabidopsis thaliana*, rice and maize plants.

Genome content of an organism is expressed in terms of number of base pairs or in terms of the content of DNA is expressed in c-value.

Genome sequencing: The location of genes on the entire diploid chromosome of an organism.



Barcode: You might have seen in all books barcoding and also in items you buy in supermarket. This will reveal the identity of the book or item as well the details like prize. Similarly, Barcode in genetic term refer to the identify of the taxon based on its genetic makeup. In practice, it is an optical, machine-readable representation of data which describes about the characters of any plants or any objects.





4.7.8 Evolutionary pattern assessed using DNA.

In recent years the evolutionary relationship between different plant taxa is assessed using DNA content as well as the similarities and differences in the DNA sequence (sequence homology). Based on such analysis the taxa and their relationship are indicated in cladogram. Such cladogram will show the genetic distance between two taxa. It is also showed antiquity or modernity of any taxon with respect to one another (See also Unit-2, Chapter-5 of XI Std.)

4.7.9 Genome editing and CRISPR - Cas9

Genome editing or gene editing is a group of technologies that has the ability to change an organism's DNA. These technologies allow genetic material to be added, removed, or altered at particular locations in the genome. Several approaches to genome editing have been developed. A recent one is known as CRISPR-Cas9, which is short form of **Clustered Regularly Interspaced Short Palindromic Repeats** and CRISPR-associated protein 9. The CRISPR-Cas9 system has generated a lot of excitement in the scientific community because it is faster, cheaper, more accurate, and more efficient than other existing genome editing methods.

Rice, was among the first plants to be used to demonstrate the feasibility of CRISPR-mediated targeted mutagenesis and gene replacement. The gene editing tool CRISPR can be used to make hybrid rice plants that can clone their seed. Imtiyaz

Khand and Venkatesan Sundaresan and colleagues reported in a new study which clearly shows one can re-engineer rice to switch it from a sexual to an asexual mode.



4.7.10 RNA Interference (RNAi)

All characters of organism are the result of expression of different genes which are regions of nuclear DNA. This expression involves transcription and translation. Transcription refers to the copying of genetic information from one strand of the DNA (called sense strand) by RNA. This RNA, as soon as it formed cannot be straight away sent to the cytoplasm to undertake the process of translation. It has to be edited and made suitable for translation which brings about protein synthesis. One of the main items removed from the RNA strand are the introns. All these changes before translation normally take place whereby certain regions of DNA are silence. However, there is an (RNAi) pathway. RNA interference is a biological process in which RNA molecules inhibit gene expression or translation. This is done by neutralising targetd mRNA molecules.

A simplified model for the RNAi pathway is based on two steps, each involving ribonuclease enzyme. In the first step, the trigger RNA (either dsRNA or miRNA primary transcript) is processed into a short interfering RNA (siRNA) by the RNase II enzymes called Dicer and Drosha. In the second step, siRNAs are loaded into the effector complex RNA-induced silencing complex (RISC). The siRNA is unwound during RISC assembly and the single-stranded RNA hybridizes with mRNA target. This RNAi is seen in plant feeding nematodes.

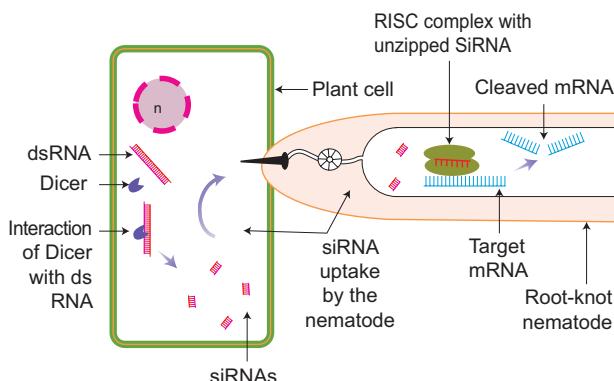


Figure 4.23: RNA Interference



4.8 Transgenic Plants / Genetically Modified Crops (Gm Crops)

4.8.1 Herbicide Tolerant – Glyphosate

Weeds are a constant problem in crop fields. Weeds not only compete with crops for sunlight, water, nutrients and space but also a carrier for insects and diseases. If left uncontrolled, weeds can reduce crop yields significantly.

Transgenic plants contain a novel DNA introduced into its genome.

Glyphosate herbicide produced by Monsanto, USA company under the trade name 'Round up' kills plants by blocking the 5-enopyruvate shikimate-3 phosphate synthase (EPSPS) enzyme, an enzyme involved in the biosynthesis of aromatic amino acids, vitamins and many secondary plant metabolites. There are several ways by which crops can be modified to be glyphosate-tolerant.

Protocol for Glyphosate tolerant Potato Plant

Introduction of 'bar' gene through vector
↓
Cell culture of potato with 'bar' gene
↓
Herbicide tolerant potato cells
↓
In vitro culture
↓
Callus → Organogenesis
↓
Development of Herbicide tolerant transgenic plants

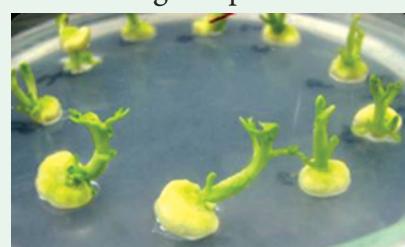


Figure 4.24: Glyphosate Tolerant Potato Plant

One strategy is to incorporate a soil bacterium gene that produces a glyphosate tolerant form of EPSPS. Another way is to incorporate a different soil bacterium gene that produces a glyphosate degrading enzyme.

Advantages of Herbicide Tolerant Crops

- Weed control improves higher crop yields;
- Reduces spray of herbicide;
- Reduces competition between crop plant and weed;
- Use of low toxicity compounds which do not remain active in the soil; and
- The ability to conserve soil structure and microbes.

4.8.2 Herbicide Tolerant - Basta

Trade name 'Basta' refers to a non-selective herbicide containing the chemical compound phosphinothrinicin. Basta herbicide tolerant gene PPT (*L*-phosphinothrinicin) was isolated from *Medicago sativa* plant. It inhibits the enzyme glutamine synthase which is involved in ammonia assimilation. The PPT gene was introduced into tobacco and transgenic tobacco produced was resistant to PPT. Similar enzyme was also isolated from *Streptomyces hygroscopicus* with bar gene encodes for PAT (Phosphinothrinicin acetyl transferase) and was introduced into crop plants like potato and sugar-beet and transgenic crops have been developed.

4.8.3 Insect resistance - Bt Crops:

i. Bt Cotton

Bt cotton is a genetically modified organism (GMO) or genetically modified pest resistant plant cotton variety, which produces an insecticide activity to bollworm.

Strains of the bacterium *Bacillus thuringiensis* produce over 200 different Bt toxins, each harmful to different insects. Most Bt toxins are insecticidal to the larvae of moths and butterflies, beetles, cotton bollworms and gnatflies but are harmless to other forms of life.



The genes are encoded for toxic crystals in the Cry group of endotoxin. When insects attack and eat the cotton plant the Cry toxins are dissolved in the insect's stomach.

The epithelial membranes of the gut block certain vital nutrients thereby sufficient regulation of potassium ions are lost in the insects and results in the death of epithelial cells in the intestine membrane which leads to the death of the larvae.



Figure 4.25: Bt Cotton

Advantages

The advantages of Bt cotton are:

- Yield of cotton is increased due to effective control of bollworms.
- Reduction in insecticide use in the cultivation of Bt cotton
- Potential reduction in the cost of cultivation.

Disadvantages

Bt cotton has some limitations:

- Cost of Bt cotton seed is high.
- Effectiveness up to 120 days after that efficiency is reduced
- Ineffective against sucking pests like jassids, aphids and whitefly.
- Affects pollinating insects and thus yield.

ii. Bt Brinjal

The Bt brinjal is another transgenic brinjal created by inserting a crystal protein gene (Cry1Ac) from the soil bacterium *Bacillus thuringiensis* into the genome of various brinjal cultivars. The insertion of the gene, along with other genetic elements such as promoters, terminators and an antibiotic resistance marker gene into the brinjal plant is accomplished using *Agrobacterium*-mediated genetic transformation. The Bt brinjal has been developed to give resistance against



Figure 4.26: Bt Brinjal

Lepidopteron insects, in particular the Brinjal Fruit and Shoot Borer (*Leucinodes orbonalis*).

iii. Dhara Mustard Hybrid (DMH)

DMH -11 is transgenic mustard developed by a team of scientists Centre for Genetic Manipulation of Crop Plants at Delhi University under Government sponsored project. It is genetically modified variety of Herbicide Tolerant (HT) mustard. It was created by using "barnase/barstar" technology for genetic modification by adding genes from soil bacterium that makes mustard, a self-pollinating plant. DMH -11 contains three genes viz. Bar gene, Barnase and Barstar sourced from soil bacterium. The bar gene had made plant resistant to herbicide named Basta.



Figure 4.27:
Dhara Mustard

4.8.4 Virus Resistance

Many plants are affected by virus attack resulting in series loss in yield and even death. Biotechnological intervention is used to introduce viral resistant genes into the host plant so that they can resist the attack by virus. This is by introducing genes that produce resistant enzymes which can deactivate viral DNA.

4.8.5 FlavrSavr Tomato

Agrobacterium mediated genetic engineering technique was followed to produce Flavr-Savr tomato, i.e., retaining the natural colour and flavor of tomato.

Through genetic engineering, the ripening process of the tomato is slowed down and thus prevent it from softening and to increase the shelf life. The tomato was made more resistant to rotting by *Agrobacterium* mediated gene transfer mechanism of introducing an antisense gene which interferes with the production of



Figure 4.28:
FlavrSavr Tomato



the enzyme polygalacturonase, which help in delaying the ripening process of tomato during long storage and transportation.

4.8.6 Golden rice - Biofortification

Golden rice is a variety of *Oryza sativa* (rice) produced through genetic engineering of biosynthesized beta-carotene, a precursor of Vitamin-A in the edible parts of rice developed by Ingo Potrykus and his group. The aim is to produce a fortified food to be grown and consumed in areas with a shortage of dietary Vitamin-A, which kills so many children under five year age. Golden rice differs from its parental strain by the addition of three beta-carotene biosynthesis genes namely 'psy' (phytoene synthase) from daffodil plant *Narcissus pseudonarcissus* and 'crt-1' gene from the soil bacterium *Erwinia auroedorora* and 'lyc' (lycopene cyclase) gene from wild-type rice endosperm.

The endosperm of normal rice, does not contain beta-carotene. Golden-rice has been genetically altered so that the endosperm now accumulates Beta-carotene. This has been done using Recombinant DNA technology. Golden rice can control childhood blindness - Xerophthalmia.



Figure 4.29: Golden rice

GM Food - Benefits

- High yield without pest
- 70% reduction of pesticide usage
- Reduce soil pollution problem
- Conserve microbial population in soil

Risks - believed to

- Affect liver, kidney function and cancer
- Hormonal imbalance and physical disorder

- Anaphylactic shock (sudden hypersensitive reaction) and allergies.
- Adverse effect in immune system because of bacterial protein.
- Loss of viability of seeds show in terminator seed technology of GM crops.

4.8.7 Polyhydroxybutyrate (PHB)

Synthetic polymers are non-degradable and pollute the soil and when burnt add dioxin in the environment which cause cancer. So, efforts were taken to provide an alternative eco-friendly biopolymers. Polyhydroxyalkanoates (PHAs) and polyhydroxybutyrate (PHB) are group of degradable biopolymers which have several medical applications such as drug delivery, scaffold and heart valves. PHAs are biological macromolecules and thermoplastics which are biodegradable and biocompatible.

Several microorganisms have been utilized to produce different types of PHAs including Gram-positive like *Bacillus megaterium*, *Bacillus subtilis* and *Corynebacterium glutamicum*, Gram-negative bacteria like group of *Pseudomonas* sp. and *Alcaligenes eutrophus*.

4.8.8 Polylactic acid (PLA)

Polylactic acid or polylactide (PLA) is a biodegradable and bioactive thermoplastic. It is an aliphatic polyester derived from renewable resources, such as corn starch, cassava root, chips or starch or sugarcane. For the production of PLA, two main monomers are used: lactic acid, and the cyclic diester, lactide. The most common route is the ring-opening polymerization of lactide with metal catalysts like tin octoate in solution. The metal-catalyzed reaction results in equal amount of *d* and polylactic acid.

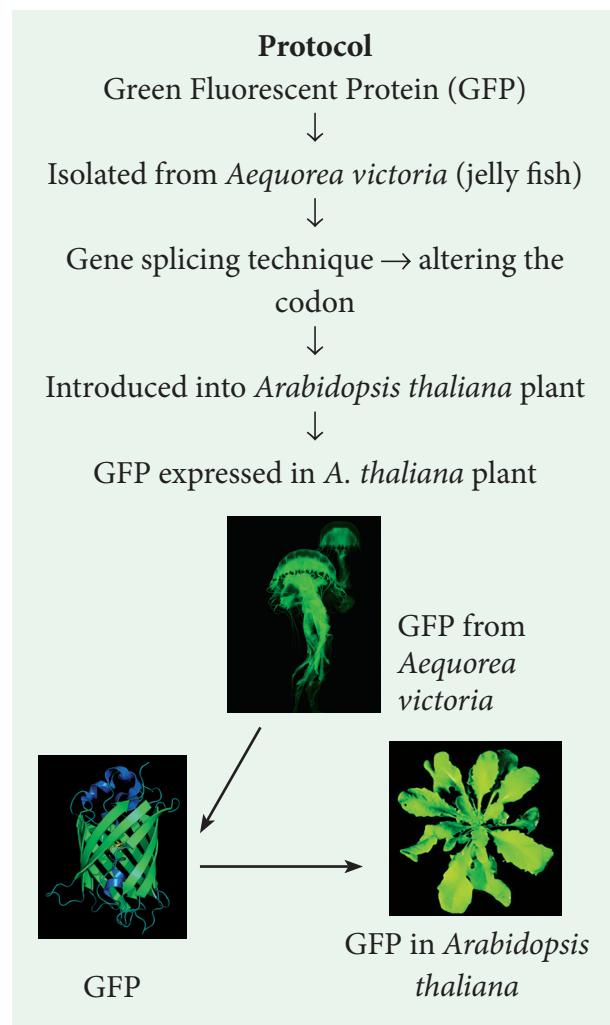


Figure 4.30: Polylactic acid product



4.8.9 Green Fluorescent Protein (GFP)

The green fluorescent protein (GFP) is a protein containing 238 amino acid residues of 26.9 kDa that exhibits bright green fluorescence when exposed to blue to ultraviolet range (395 nm). GFP refers to the protein first isolated from the jellyfish *Aequorea victoria*. GFP is an excellent tool in biology due to its ability to form internal chromophore without requiring any accessory cofactors, gene products, enzymes or substrates other than molecular oxygen. In cell and molecular biology, the GFP gene is frequently used as a reporter of expression. It has been used in modified forms to make biosensors.



4.8.10 Biopharming

Biopharming also known as molecular pharming is the production and use of transgenic plants genetically engineered to

produce pharmaceutical substances for use of human beings. This is also called "**molecular farming or pharming**". These plants are different from medicinal plants which are naturally available. The use of plant systems as bioreactors is gaining more significance in modern biotechnology. Many pharmaceutical substances can be produced using transgenic plants. Example: Golden rice

4.8.11 Bioremediation

It is defined as the use of microorganisms or plants to clean up environmental pollution. It is an approach used to treat wastes including wastewater, industrial waste and solid waste. Bioremediation process is applied to the removal of oil, petrochemical residues, pesticides or heavy metals from soil or ground water. In many cases, bioremediation is less expensive and more sustainable than other physical and chemical methods of remediation. Bioremediation process is a cheaper and eco-friendly approach and can deal with lower concentrations of contaminants more effectively. The strategies for bioremediation in soil and water can be as follows:

- Use of indigenous microbial population as indicator species for bioremediation process.
 - Bioremediation with the addition of adapted or designed microbial inoculants.
 - Use of plants for bioremediation - green technology.

Some examples of bioremediation technologies are:

- **Phytoremediation** - use of plants to bring about remediation of environmental pollutants.
 - **Mycoremediation** - use of fungi to bring about remediation of environmental pollutants.
 - **Bioventing** is the process that increases the oxygen or air flow to accelerate the



degradation of environmental pollutants.

- **Bioleaching** is the use of microorganisms in solution to recover metal pollutants from contaminated sites.
- **Bioaugmentation** is the addition of selected microbes to speed up degradation process.
- **Composting** is the process by which the solid waste is composted by the use of microbes into manure which acts as a nutrient for plant growth.
- **Rhizofiltration** is the uptake of metals or degradation of organic compounds by rhizosphere microorganisms.
- **Rhizostimulation** is the stimulation of plant growth by the rhizosphere by providing better growth condition or reduction in toxic materials.

Limitations

- Only biodegradable contaminants can be transformed using bioremediation processes.
- Bioremediation processes must be specifically made in accordance to the conditions at the contaminated site.
- Small-scale tests on a pilot scale must be performed before carrying out the procedure at the contaminated site.
- The use of genetic engineering technology to create genetically modified microorganism or a consortium of microbes for bioremediation process has great potential.

4.8.12 Biofuel: Algal Biofuel

Algal fuel, also known as algal biofuel, or algal oil is an alternative to liquid fossil fuels, the petroleum products. This use algae as a source of energy-rich oils. Also, algal fuels are an alternative to commonly known biofuel sources obtained from corn and sugarcane. The energy crisis and the world food crisis have initiated interest in algal culture (farming algae) for making biodiesel and other biofuels using land unsuitable for agriculture. *Botryococcus braunii* is normally used to produce algal biofuel.

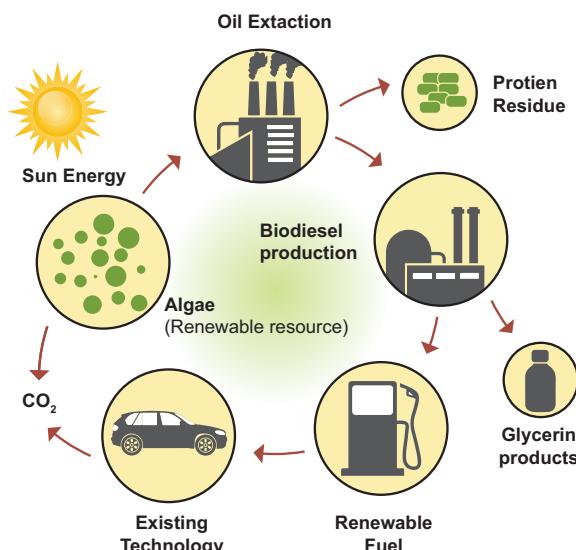


Figure 4.31: Algal Biofuel

Biological hydrogen production by algae

The biological hydrogen production with algae is a method of photo biological water splitting. In normal photosynthesis the alga, *Chlamydomonas reinhardtii* releases oxygen. When it is deprived of sulfur, it switches to the production of hydrogen during photosynthesis and the electrons are transported to ferredoxins. [Fe]-hydrogenase enzymes combine them into the production of hydrogen gas.

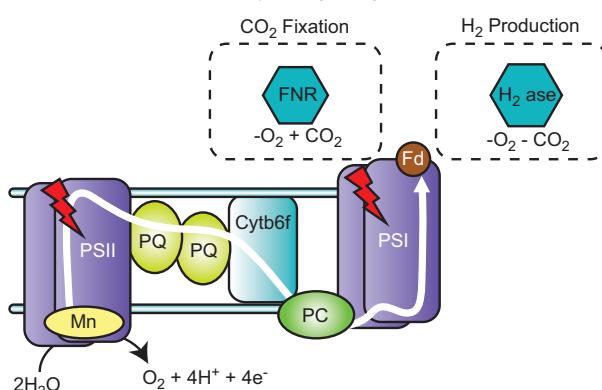


Figure 4.32: Hydrogen production by algae

4.8.13 Bioprospecting

Bioprospecting is the process of discovery and commercialization of new products obtained from biological resources. Bioprospecting may involve biopiracy, in which indigenous knowledge of nature, originating with indigenous people, is used by others for profit, without authorization or compensation to the indigenous people themselves.



Biopiracy

Biopiracy can be defined as the manipulation of intellectual property rights laws by corporations to gain exclusive control over national genetic resources, without giving adequate recognition or remuneration to the original possessors of those resources. Examples of biopiracy include recent patents granted by the U.S. Patent and Trademarks Office to American companies on turmeric, 'neem' and, most notably, 'basmati' rice. All three products are indigenous to the Indo-Pak subcontinent.

Biopiracy of Neem

The people of India used neem and its oil in many ways to controlling fungal and bacterial skin infections. Indians have shared the knowledge of the properties of the neem with the entire world. Pirating this knowledge, the United States Department of Agriculture (USDA) and an American MNC (Multi Nation Corporation) W.R.Grace in the early 90's sought a patent from the European Patent Office (EPO) on the "method for controlling of diseases on plants by the aid of extracted hydrophobic neem oil". The patenting of the fungicidal and antibacterial properties of Neem was an example of biopiracy but the traditional knowledge of the Indians was protected in the end.

Biopiracy of Turmeric

The United States Patent and Trademark Office, in the year 1995 granted patent to the method of use of turmeric as an antiseptic agent. Turmeric has been used by the Indians as a home remedy for the quick healing of the wounds and also for purpose of healing rashes. The journal article published by the Indian Medical Association, in the year 1953 wherein this remedy was mentioned. Therefore, in this way it was proved that the use of turmeric as an antiseptic is not new to the world and is not a new invention, but formed a part of the traditional knowledge of the Indians. The objection in this case US patent and trademark office was upheld and traditional knowledge of the Indians was protected. It is another example of Biopiracy.

Biopiracy of Basmati

On September 2, 1997, the U.S. Patent and Trademarks Office granted Patent on "basmati rice lines and grains" to the Texas-based company RiceTec. This broad patent gives the company several rights, including exclusive use of the term 'basmati', as well proprietary rights on the seeds and grains from any crosses. The patent also covers the process of breeding RiceTec's novel rice lines and the method to determine the cooking properties and starch content of the rice grains.

India had periled the United States to take the matter to the WTO as an infringement of the TRIPS agreement, which could have resulted in major embarrassment for the US. Hence voluntarily and due to few decisions taken by the US patent office, Rice Tec had no choice but to lose most of the claims and most importantly the right to call the rice "Basmati". In the year 2002, the final decision was taken. Rice Tec dropped down 15 claims, resulting in clearing the path of Indian Basmati rice exports to the foreign countries. The Patent Office ordered the patent name to be changed to 'Rice lines 867'.

4.9 Applications of Biotechnology

- Biotechnology is one of the most important applied interdisciplinary sciences of the **21st century**. It is the trusted area that enables us to find the beneficial way of life.
- Biotechnology has wide applications in various sectors like agriculture, medicine, environment and commercial industries.
- This science has an invaluable outcome like **transgenic varieties** of plants e.g. transgenic cotton (Bt-cotton), rice, tomato, tobacco, cauliflower, potato and banana.
- The development of transgenics as pesticide resistant, stress resistant and disease resistant varieties of agricultural crops is the immense outcome of biotechnology.
- The synthesis of **human insulin** and blood protein in *E.coli* and utilized for



insulin deficiency disorder in human is a breakthrough in biotech industries in medicine.

- The synthesis of vaccines, enzymes, antibiotics, dairy products and beverages are the products of biotech industries.
- **Biochip** based biological computer is one of the successes of biotechnology.
- Genetic engineering involves genetic manipulation, tissue culture involves aseptic cultivation of totipotent plant cell into plant clones under controlled atmospheric conditions.
- **Single cell protein** from *Spirulina* is utilized in food industries.
- Production of **secondary metabolites**, biofertilizers, biopesticides and enzymes.
- Biomass energy, biofuel, Bioremediation, phytoremediation for environmental biotechnology.

Summary

Biotechnology is the science of applied biological process in which there is a controlled use of biological agents such as microorganisms or cellular components for beneficial use. A Hungarian Engineer, Karl Ereky (1919) coined the term biotechnology. Biotechnology broadly categorized into traditional practices and modern practices. Traditional biotechnology includes our ancient practices such as fermentation. Single Cell Protein (SCP) organisms are grown in large quantities to produce goods rich in protein, minerals, fats, carbohydrates and vitamins. The modern biotechnology embraces all the genetic manipulations. The recombinant DNA technology is a technique of modern biotechnology in which transfer of DNA coding for a specific gene from one organism is introduced into another organism using specific agents like vectors or using instruments like electroporation, gene gun, liposome mediated, chemical mediated and micro injection. Other tools are enzymes and host

organisms. The enzyme restriction endonuclease is a molecular scissor that cleaves DNA into fragments at or near specific recognition sites with the molecule known as restriction sites. Other enzymes are DNA ligase and alkaline phosphatase. DNA ligase enzyme joins the sugar and phosphate molecules of double stranded DNA. Alkaline phosphatase is an enzyme which adds or removes specific phosphate group of double stranded DNA.

A vector is a small DNA molecule capable of self replication and used as a carrier of DNA inserted in the host cell. Few examples of vectors are plasmid – pBR 322, cosmid – Lambda phage, M13, Phagemid , BAC, YAC, transposon, shuttle vector and expression vector.

After production of recombinant DNA molecule has been generated is introduced into a suitable host cell. Type of host cell depends upon the cloning experiment. E.coli is the most widely used host organism. There are two kinds of gene transfer methods in plants. They are direct or vectorless gene transfer and indirect or vector mediated gene transfer. Direct gene transfer includes chemical mediated gene transfer, micro injection, electroporation. Gene gun method and Liposome mediated method of gene transfer. Indirect or vector mediated gene transfer is a method of gene transfer with the help of a plasmid vector. In this method Ti-plasmid from *Agrobacterium tumefaciens* has been used extensively for vector mediated gene transfer.

After the introduction of rDNA into a host cell, it is essential to identify those cells which have received the rDNA molecule. This process is called screening. One of the method of recombinant screening is blue white selection method Replica plating technique in which the pattern of colonies growing on a culture plate is copied. Electrophoresis is a separating technique used to separate different biomolecules.

Blotting techniques are widely used tools for identification of desired DNA or RNA fragments from larger number of molecules. Some of the genetically modified crops are herbicide tolerant



- Basta, Dhara mustard, insects resistance
 - Bt crops, flavrSavr – Tomato, Golden rice.
- Biopolymers are polyhydroxybutyrate (PHB), polylactic acid (PLA) and green fluorescent protein (GFP) is used to make biosensors. Other applications are biopharming, bioprospecting, biomedication and biofuel, etc.

Evaluation

1. Restriction enzymes are

- a. Not always required in genetic engineering
- b. Essential tools in genetic engineering
- c. Nucleases that cleave DNA at specific sites
- d. both b and c

2. Plasmids are

- a. circular protein molecules
- b. required by bacteria
- c. tiny bacteria
- d. confer resistance to antibiotics

3. EcoRI cleaves DNA at

- a. AGGGTT b. GTATATC
- c. GAATTTC d. TATAGC

4. Genetic engineering is

- a. making artificial genes.
- b. hybridization of DNA of one organism to that of the others.
- c. production of alcohol by using micro organisms.
- d. making artificial limbs, diagnostic instruments such as ECG, EEG etc.,

5. Consider the following statements:

- I. Recombinant DNA technology is popularly known as genetic engineering is a stream of biotechnology which deals with the manipulation of genetic materials by man invitro
- II. pBR322 is the first artificial cloning vector developed in 1977 by Boliver and Rodriguez from E.coli plasmid
- III. Restriction enzymes belongs to a class



of enzymes called nucleases.
Choose the correct option regarding above statements

- a. I & II b. I & III
 - c. II & III d. I,II & III
6. The process of recombinant DNA technology has the following steps
- I. amplification of the gene
 - II. Insertion of recombinant DNA into the host cells
 - III. Cutting of DNA at specific location using restriction enzyme .
 - IV. Isolation of genetic material (DNA)
- Pick out the correct sequence of step for recombinant DNA technology.
- a. II, III, IV, I b. IV, II, III, I
 - c. I, II, III, IV d. IV, III, I, II
7. Which one of the following palindromic base sequence in DNA can be easily cut at about the middle by some particular restriction enzymes?
- a. 5' CGTTCG 3' 3' ATCGTA 5'
 - b. 5' GATATG 3' 3' CTACTA 5'
 - c. 5' GAATTTC 3' 3' CTTAAG 5'
 - d. 5' CACGTA 3' 3' CTCAGT 5'
8. pBR 322, BR stands for
- a. Plasmid Bacterial Recombination
 - b. Plasmid Bacterial Replication
 - c. Plasmid Boliver and Rodriguez
 - d. Plasmid Baltimore and Rodriguez
9. Which of the following one is used as a Biosensors?
- a. Electrophoresis b. Bioreactors
 - c. Vectors d. Electroporation

10. Match the following :

Column A	Column B
1 Exonuclease	a. add or remove phosphate
2 Endonuclease	b. binding the DNA fragments
3 Alkaline Phosphatase	c. cut the DNA at terminus
4 Ligase	d. cut the DNA at middle



- | | | | | | |
|----|---|---|---|---|--|
| | 1 | 2 | 3 | 4 | |
| A) | a | b | c | d | |
| B) | c | d | b | a | |
| C) | a | c | b | d | |
| D) | c | d | a | b | |
- 11 In which techniques Ethidium Bromide is used?
- Southern Blotting techniques
 - Western Blotting techniques
 - Polymerase Chain Reaction
 - Agrose Gel Electroporosis
- 12 **Assertion :** Agrobacterium tumifaciens is popular in genetic engineering because this bacterium is associated with the root nodules of all cereals and pulse crops
- Reason:** A gene incorporated in the bacterial chromosomal genome gets automatically transferred to the cross with which bacterium is associated.
- Both assertion and reason are true. But reason is correct explanation of assertion.
 - Both assertion and reason are true. But reason is not correct explanation of assertion.
 - Assertion is true, but reason is false.
 - Assertion is false, but reason is true.
 - Both assertion and reason are false.
- 13 Which one of the following is not correct statement.
- Ti plasmid causes the bunchy top disease
 - Multiple cloning site is known as Polylinker
 - Non viral method transfection of Nucleic acid in cell
 - Polylactic acid is a kind of biodegradable and bioactive thermoplastic.
- 14 An analysis of chromosomal DNA using the southern hybridisation technique does not use
- Electrophoresis
 - Blotting
 - Autoradiography
 - Polymerase Chain Reaction
- 15 An antibiotic gene in a vector usually helps in the selection of
- Competent cells
 - Transformed cells
 - Recombinant cells
 - None of the above
- 16 Some of the characteristics of Bt cotton are
- Long fibre and resistant to aphids
 - Medium yield, long fibre and resistant to beetle pests
 - high yield and production of toxic protein crystals which kill dipteran pests.
 - High yield and resistant to ball worms
- 17 How do you use the biotechnology in modern practice?
- 18 What are the materials used to grow microorganism like Spirulina?
- 19 You are working in a biotechnology lab with a bacterium namely E.coli. How will you cut the nucleotide sequence? explain it.
- 20 What are the enzymes you can use to cut terminal end and internal phospho di ester bond of nucleotide sequence?
- 21 Name the chemicals used in gene transfer.
- 22 What do you know about the word pBR322?
- 23 Mention the application of Biotechnology.
- 24 What are restriction enzyme. Mention their type with role in Biotechnology.
- 25 Is there any possibilities to transfer a suitable desirable gene to host plant without vector? Justify your answer.
- 26 How will you identify a vectors?
- 27 Compare the various types of Blotting techniques.
- 28 Write the advantages of herbicide tolerant crops.
- 29 Write the advantages and disadvantages of Bt cotton.
- 30 What is bioremediation? give some examples of bioremediation.
- 31 Write the benefits and risk of Genetically Modified Foods.



Glossary

3' Hydroxy end: The hydroxyl group attached to 3' carbon atom of sugar of the terminal nucleotide of a nucleic acid.

Bacterial artificial chromosomes (BAC): A cloning vector for isolation of genomic DNA constructed on the basis of F-factor.

Chimeric DNA: A recombinant DNA molecule containing unrelated genes.

Cleave: To break phosphodiester bonds of dsDNA, usually with a restriction enzyme.

Cloning site: A location on a cloning vector into which DNA can be inserted.

Cloning: Incorporation of a DNA molecule into a chromosomal site or a cloning vector.

Cloning Vector: A small, self-replicating DNA inserted in a cloning gene.

COS sites: The 12-base, single strand, complementary extension of phage lambda (λ) DNA.

DNA Polymerase: An enzyme that catalyses the phosphodiester bond in the formation of DNA.

Endonucleases: An enzyme that catalyses the cleavage of DNA at internal position, cutting DNA at specific sites.

Genome: The entire complement of genetic material of an organism.

Insert DNA: A DNA molecule incorporated into a cloning vector.

Ligase: An enzyme used in genetic engineering experiment to join the cut ends of dsDNA.

M-13: AssDNA bacteriophage used as vector for DNA sequencing.

Phagemid: A cloning vector that contains components derived from both phage DNA and plasmid.

Plasmid: Extrachromosomal, self-replicating, circular dsDNA containing some non-essential genes.

Restriction map: A linear array of sites on DNA cleaved by various restriction enzymes.

Shuttle Vector: A plasmid cloning vector that can replicate in two different organisms due to the presence of two different origin of replication Ori^{EUK} and Ori^{E. coli}

Taq polymerase: A heat stable DNA polymerase isolated from a thermophilic bacterium *Thermus aquaticus*.

Vectors: Vehicles for transferring DNA from one cell to another.

Biofuel: Fuels like hydrogen, ethanol and methanol produced from a biological source by the action of microorganisms.

Bioleaching: Process of using microorganisms to recover metals from their ores or contaminant environment

Bioremediation: Process of using organisms to remove or reduce pollutants from the environment.

Green Technology: Pollution-free technology in which pollution is controlled at source.

Phytoremediation: Use of certain plants to remove contaminants or pollutants from the environment (soil, water or air).

Recombinant: Cell / Organism formed by a recombination of genes.

Transformation: Process of transferring a foreign DNA into a cell and changing its genome.

Vector: Agent used in recombinant DNA technique to carry new genes into foreign cells.

Wild Type: Natural form of organisms.

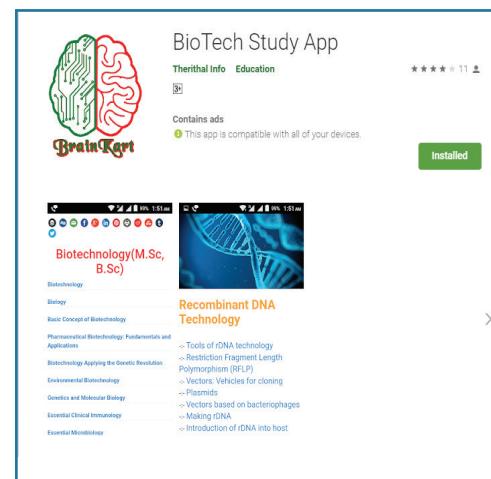


ICT Corner

Principles and Processes of Biotechnology

BIO TECH STUDY APP

Let us know about the information Bio Technology through this activity.



Steps

- Type the URL or scan the QR code to open the activity page.
 - Click on the topic to know in detail.
 - To know the sub topics in detail click on the dots in top right corner.

<h2>Biotechnology(M.Sc, B.Sc)</h2>		<p>Biotechnology: Protein Structure And Engineering</p> <h2>Purification of Proteins</h2> <p>Posted On : 01.08.2017 11:47 am</p> <p>Isolation of a protein from a microbial culture, plant and animal sources involves various separation techniques.</p>	<h3>Genome Sequencing Projects</h3> <p>There are several reasons for completely sequencing a genome.</p> <ul style="list-style-type: none">First it provides a means for the discovery of all the genes and thus provides an inventory of genes.Second, the sequence shows the relationships between genes.Third, it provides a set of tools for future experimentation
Biotechnology	<h2>Recombinant DNA Technology</h2>	<h3>Purification of Proteins</h3>	
Biology	<ul style="list-style-type: none">- Tools of rDNA technology- Restriction Fragment Length Polymorphism (RFLP)- Vectors: Vehicles for cloning- Plasmids- Vectors based on bacteriophages- Making rDNA- Introduction of rDNA into host		
Basic Concept of Biotechnology			
Pharmaceutical Biotechnology: Fundamentals and Applications			
Biotechnology Applying the Genetic Revolution			
Environmental Biotechnology			
Genetics and Molecular Biology			
Essential Clinical Immunology			
Essential Microbiology			
Step 1	Step 2	Step 3	Step 4
URL:	https://play.google.com/store/apps/details?id=info.therithal.brainkart.biotechstudyapp		 B266_12_BOT_EM
* Pictures are indicative only			



Chapter

5



UNIT VIII: Biotechnology

Plant Tissue Culture



Learning Objectives

The learner will be able to

- ❖ Perceive the concepts of tissue culture.
- ❖ Cognize the steps of tissue culture techniques and its types.
- ❖ Understand the protoplast culture in detail.
- ❖ Elicit the list of secondary metabolites obtained through cell suspension culture.
- ❖ Learn plant regeneration pathway.
- ❖ Appreciate the uses of micro propagation, somatic hybridization, shoot meristem culture and germplasm conservation.
- ❖ Acquire the knowledge of patenting Biosafety and Bioethics.

Growing plant protoplasts, cells, tissues or organs away from their natural or normal environment, under artificial condition, is known as Tissue Culture.

It is also known as *in vitro* (*In vitro* is a Latin word, it means that - in glass or in test-tube) growth of plant protoplasts, cells, tissues and organs. A single explant can be multiplied into several thousand plants in short time period and space under controlled conditions.



Gottlieb
Haberlandt

Tissue culture techniques are often used for commercial production of plants as well as for plant research. Plant tissue culture serves as an indispensable tool for regeneration of transgenic plants. Apart from this some of the main applications of Plant tissue culture are clonal propagation of elite varieties, conservation of endangered plants, production of virus-free plants, germplasm preservation, industrial production of secondary metabolites. etc., In this chapter let us discuss the history , techniques, types , applications of plant tissue culture and get aware on ethical issues.

Gottlieb Haberlandt (1902) the German Botanist proposed the concept **Totipotency** and he was also the first person to culture plant cells in artificial conditions using the mesophyll cells of *Lamium purpureum* in culture medium and obtained cell proliferation. He is regarded as the father of tissue culture.



Chapter outline

- 5.1 Milesones in plant tissue culture
- 5.2 Basic concepts in plant disuse culture
- 5.3 Plant tissue culture techniques and types
- 5.4 Plant regeneration pathway
- 5.5 Applications of plant tissue culture
- 5.6 Conservation of plant genetic resources
- 5.7 Intellectual rights of property (IPR), Biosafety and Bioethics
- 5.8 Future Biotechnology



FRWH98



5.1 Milestones in Plant Tissue Culture

Haberlandt (1902)

cultured plant cells in artificial condition called *in vitro* (inside glass) in culture medium (Knop's salt solution) containing glucose and peptone and developed callus (unorganized growth of cells and tissue) and proposed the concept Totipotency, it means the development of whole plant from isolated cells or tissue in *in vitro* condition.

P.R.White (1934)

developed root cultures, used Knop's solution along with three vitamins like pyridoxine, thiamine and nicotinic acid

F.C. Steward (1948)

used coconut water in plant tissue culture work and obtained cell proliferation from carrot explants (Cellular totipotency).

Morel and Martin (1952, 1955)

developed virus-free *Dahlia* and potato plants using shoot meristem culture.

Murashige and Skoog (1962)

formulated tissue culture medium, a landmark in plant tissue culture and it is the most frequently used medium for all kinds of tissue culture work.

Kanta et al. (1962)

produced test-tube fertilization in flowering plants.

Yamada et al. (1963)

produced *calli* and free cells in tissue culture of *Tradescantia reflexa*.

Guha and Maheshwari (1964)

developed *in vitro* production of haploid embryos from anthers of *Datura*.

Vasil and Hildbrandt (1965)

achieved differentiation of tobacco plants from single, isolated cells in micro propagation.

Takebe et al. (1971)

regenerated tobacco plants from isolated mesophyll protoplasts.

Carlson

and co-workers obtained protoplast fusion between *Nicotiana glauca* and *Nicotiana longsdorffii* and developed first interspecific somatic hybrid in 1971.

Melchers and co-workers in 1978

developed intergenic hybrid between potato and tomato called pomato.

Chilton (1983)

produced transformed tobacco plants from single cell transformation and gene insertion.

Horsh et al. (1984)

developed transgenic tobacco by Agrobacterium mediated gene transfer.

Knop's solution: Nutrient solution used in growth experiments of plants which contains:

Calcium nitrate 3.0 g

Potassium nitrate 1.0 g

Sucrose 50.0 g (optimal)

Magnesium sulfate 1.0 g

Dibasic Potassium phosphate 1.0 g

Deionized water 1000.0 ml



5.2 Basic concepts of Tissue Culture

Basic concepts of plant tissue culture are totipotency, differentiation, dedifferentiation and redifferentiation.

Totipotency

The property of live plant cells that they have the genetic potential when cultured in nutrient medium to give rise to a complete individual plant.

Differentiation

The process of biochemical and structural changes by which cells become specialized in form and function.

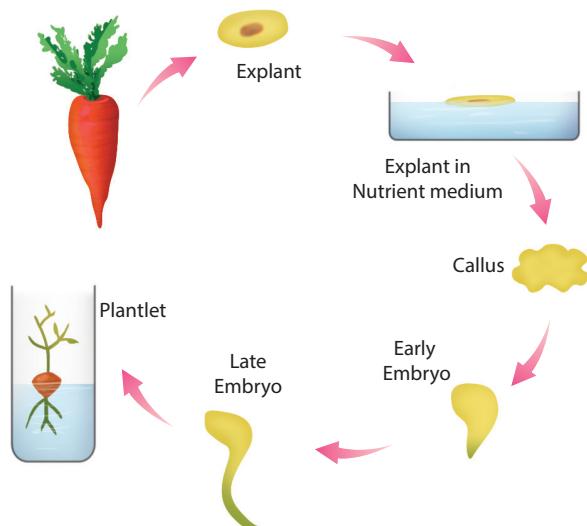


Figure 5.1: Totipotency

Redifferentiation

The further differentiation of already differentiated cell into another type of cell. For example, when the component cells of callus have the ability to form a whole plant in a nutrient medium, the phenomenon is called redifferentiation.

Dedifferentiation

The phenomenon of the reversion of mature cells to the meristematic state leading to the formation of callus is called dedifferentiation. These two phenomena of redifferentiation and dedifferentiation are the inherent capacities of living plant cells or tissue. This is described as totipotency.

5.3 Plant Tissue Culture (PTC)

Plant tissue culture is used to describe the *in vitro* and aseptic growth of any plant part on a tissue culture medium. This technology is based on three fundamental principles:

- The plant part or explant must be selected and isolated from the rest of plant body.
- The explant must be maintained in controlled physically (environmental) and chemically defined (nutrient medium) conditions.

Explant: The tissue taken from a selected plant transferred to a culture medium often to establish a new plant.

5.3.1 Laboratory Facilities for PTC

For PTC, the laboratory must have the following facilities:



Figure 5.2: Tissue culture lab

- Washing facility for glassware and ovens for drying glassware.
- Medium preparation room with autoclave, electronic balance and pH meter.
- Transfer area sterile room with laminar air-flow bench and a positive pressure ventilation unit called High Efficiency Particulate Air (HEPA) filter to maintain aseptic condition.
- Culture facility: Growing the explant inoculated into culture tubes at 22-28° C with illumination of light 2400 lux, with a photoperiod of 8-16 hours and a relative humidity of about 60%.



5.3.2 Technique Involved in PTC

1. Sterilization:

Sterilization is the technique employed to get rid of microbes such as bacteria and fungi in the culture medium, vessels and explants.

i. Maintenance of Aseptic Environment:

During in vitro tissue culture maintenance of aseptic environmental condition should be followed, i.e., sterilization of glassware, forceps, scalpels, and all accessories in wet steam sterilization by autoclaving at 15 psi (121°C) for 15 to 30 minutes or dipping in 70% ethanol followed by flaming and cooling.

ii. **Sterilization of culture room:** Floor and walls are washed first with detergent and then with 2% sodium hypochlorite or 95% ethanol. The cabinet of laminar airflow is sterilized by clearing the work surface with 95% ethanol and then exposure of UV radiation for 15 minutes.

iii. **Sterilization of Nutrient Media:** Culture media are dispensed in glass containers, plugged with non-absorbent cotton or sealed with plastic closures and then sterilized using autoclave at 15 psi (121°C) for 15 to 30 minutes. The plant extracts, vitamins, amino acids and hormones are sterilized by passing through Millipore filter with 0.2 mm pore diameter and then added to sterilized culture medium inside Laminar Airflow Chamber under sterile condition.

iv. **Sterilization of Explants:** The plant materials to be used for tissue culture should be surface sterilized by first exposing the material in running tap water and then treating it in surface sterilization agents like 0.1% mercuric chloride, 70% ethanol under aseptic condition inside the Laminar Air Flow Chamber.

2. Media Preparation

The success of tissue culture lies in the composition of the growth medium, plant growth regulators and culture conditions such as temperature, pH, light and humidity. No single medium is capable of maintaining optimum growth of all plant tissues. Suitable nutrient medium as per the principle of tissue culture is prepared and used.

MS nutrient medium (Murashige and Skoog 1962) is commonly used. It has carbon sources, with suitable vitamins and hormones. The media formulations available for plant tissue culture other than MS are B5 medium (Gamborg et.al 1968), White medium (white 1943), Nitsch's medium (Nitsch & Nitsch 1969). A medium may be solid or semisolid or liquid. For solidification, a gelling agent such as agar is added.

Agar: A complex mucilaginous polysaccharide obtained from marine algae (sea weeds) used as solidifying agent in media preparation.

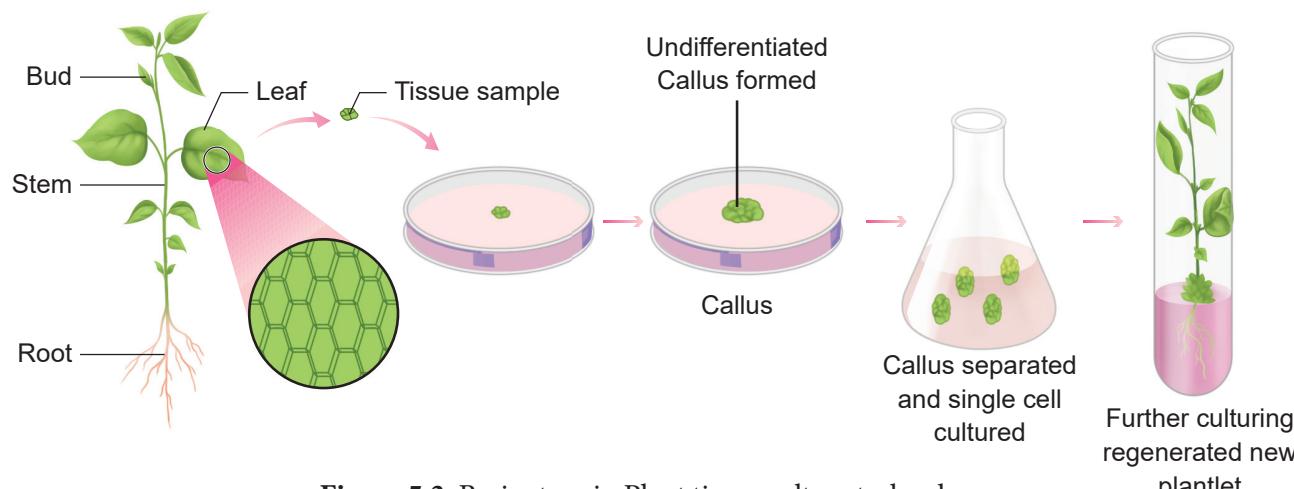


Figure 5.3: Basic steps in Plant tissue culture technology



Composition of MS (Murashige and Skoog) Medium	
Macronutrients:	
Ammonium nitrate (NH_4NO_3)	1650.0 mg/l
Potassium nitrate (KNO_3)	1900.0 mg/l
Calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$)	440.0 mg/l
Magnesium sulphate ($\text{MgSO}_4 \cdot 6\text{H}_2\text{O}$)	370.0 mg/l
Potassium dihydrogen phosphate (KH_2PO_4)	170.0 mg/l
Micronutrients:	
Manganese sulphate ($\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$)	22.3 mg/l
Zinc sulphate ($\text{ZnSO}_4 \cdot 4\text{H}_2\text{O}$)	8.6 mg/l
Boric acid (H_3BO_3)	6.2 mg/l
Potassium iodide (KI)	0.83 mg/l
Minor nutrient:	
Sodium molybdate ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$)	0.250 mg/l
Cupric sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$)	0.025 mg/l
Cobaltous chloride ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$)	0.025 mg/l
Iron stock	
Na EDTA	37.25 mg/l
Ferrous Sulphate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$)	27.85 mg/l
Vitamins	
Glycine	2.0 mg/l
Nicotinic acid	0.5 mg/l
Pyridoxin HCl	0.5 mg/l
Thiamine HCl	0.1 mg/l
Growth Hormones	
IAA	1.30 mg/l
Kinetin	0.4–10.0 mg/l
Myo-inositol	100.0 mg/l
Sucrose	30.0 g/l
Solidifying Agent	
Agar	8.0 g/l

3. Culture condition

pH

The pH of medium is normally adjusted between 5.6 to 6.0 for the best result.

Temperature

The cultures should be incubated normally at constant temperature of $25^\circ\text{C} \pm 2^\circ\text{C}$ for optimal growth.

Humidity and Light Intensity

The cultures require 50-60% relative humidity and 16 hours of photoperiod by the illumination of cool white fluorescent tubes of approximately 1000 lux.

Aeration

Aeration to the culture can be provided by shaking the flasks or tubes of liquid culture on automatic shaker or aeration of the medium by passing with filter-sterilized air.

4. Induction of Callus

Explant of 1-2 cm sterile segment selected from leaf, stem, tuber or root is inoculated (transferring the explants to sterile glass tube containing nutrient medium)



Figure 5.4:
Induction of callus

in the MS nutrient medium supplemented with auxins and incubated at $25^\circ\text{C} \pm 2^\circ\text{C}$ in an alternate light and dark period of 12 hours to induce cell division and soon the upper surface of explant develops into callus. Callus is a mass of unorganized growth of plant cells or tissues in *in vitro* culture medium.

5. Embryogenesis

The callus cells undergoes differentiation and produces somatic embryos, known as **Embryoids**. The embryoids are sub-cultured to produce plantlets.



Figure 5.5:
Embryogenesis



6. Hardening

The plantlets developed *in vitro* require a hardening period and so are transferred to greenhouse or hardening chamber and then to normal environmental conditions.

Hardening is the gradual exposure of *in vitro* developed plantlets in humid chambers in diffused light for acclimatization so as to enable them to grow under normal field conditions.

5.3.3 Types of Plant tissue cultures

Based on the explants some other plant tissue culture types are

1. Organ culture
2. Meristem culture
3. Protoplast culture
4. Cell culture.

1. Organ culture

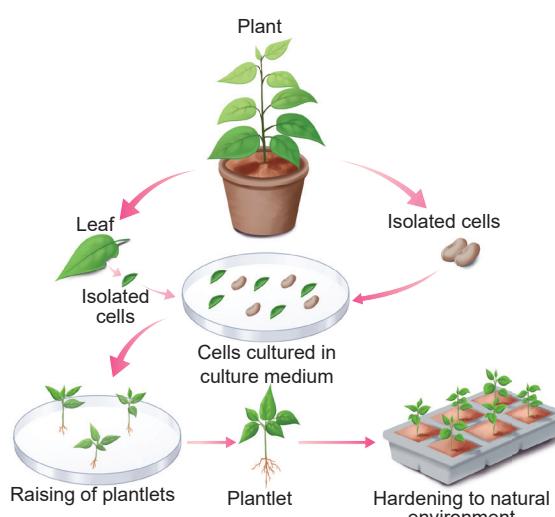


Figure 5.6: Organ Culture

The culture of embryos, anthers, ovaries, roots, shoots or other organs of plants on culture media.

2. Meristem Culture:

The culture of any plant meristematic tissue on culture media.

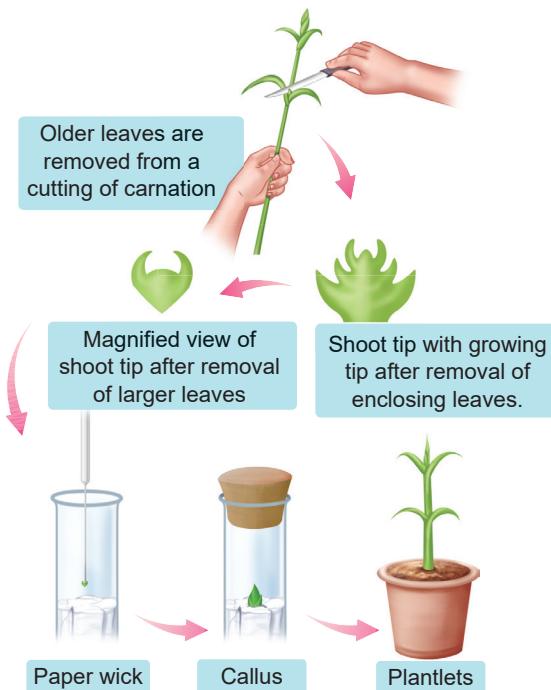


Figure 5.7: Meristem Culture

3. Protoplast Culture:

Protoplasts are cells without a cell wall, but bounded by a cell membrane or plasma membrane. Using protoplasts, it is possible to regenerate whole plants from single cells and also develop somatic hybrids. The steps involved in protoplast culture.

i. **Isolation of protoplast:** Small bits of plant tissue like leaf tissue are used for isolation of protoplast. The leaf tissue is immersed in 0.5% Macrozyme and 2% Onozuka cellulase enzymes dissolved in 13% sorbitol or mannitol at pH 5.4. It is then incubated over-night at 25°C. After a gentle teasing of cells, protoplasts are obtained, and these are then transferred to 20% sucrose solution to retain their viability. They are then centrifuged to get pure protoplasts as different from debris of cell walls.

ii. **Fusion of protoplast:** It is done through the use of a suitable fusogen. This is normally PEG (Polyethylene Glycol). The isolated protoplast are incubated in 25 to 30% concentration of PEG with Ca++ ions and the protoplast shows agglutination (the formation of clumps of cells) and fusion.

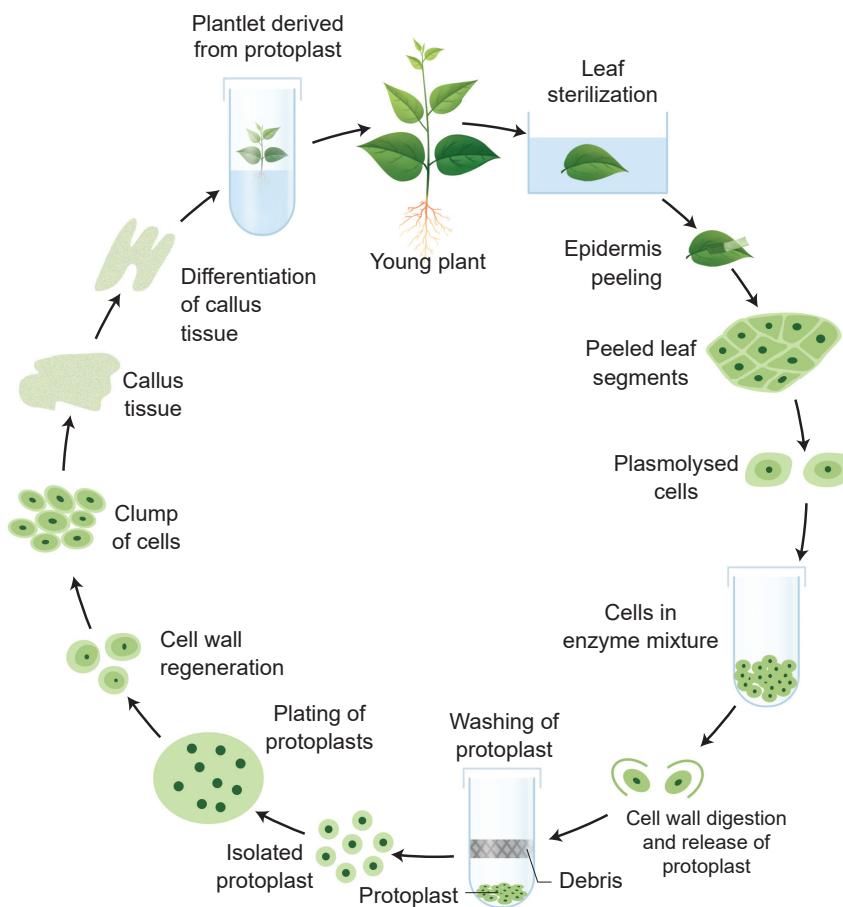


Figure 5.8: Protoplast Culture

iii. **Culture of protoplast:** MS liquid medium is used with some modification in droplet, plating or micro-drop array techniques. Protoplast viability is tested with fluorescein diacetate before the culture. The cultures are incubated in continuous light 1000-2000 lux at 25°C. The cell wall formation occurs within 24-48 hours and the first division of new cells occurs between 2-7 days of culture.

iv. **Selection of somatic hybrid cells:** The fusion product of protoplasts without nucleus of different cells is called a cybrid. Following this nuclear fusion happens. This process is called somatic hybridization.

4. Cell Suspension Culture

The growing of cells including the culture of single cells or small aggregates of cells *in vitro* in liquid medium is known as cell suspension culture. The cell suspension is prepared by transferring a portion of callus to

the liquid medium and agitated using rotary shaker instrument. The cells are separated from the callus tissue and used for cell suspension culture.

Production of Secondary Metabolites

Cell suspension culture can be useful for the production of secondary metabolites like alkaloids, flavonoids, terpenoids, phenolic compounds and recombinant proteins. Secondary metabolites are chemical compounds that are not required by the plant for normal growth and development but are produced in the plant as 'byproducts' of cell metabolism. For Example: Biosynthesis and isolation of indole alkaloids from *Catharanthus roseus* plant cell culture.

The process of production of secondary metabolites can be scaled up and automated using bio-reactors for commercial production. Many strategies such as biotransformation, elicitation and immobilization have been used to make cell suspension cultures more efficient in the production of secondary metabolites. Few examples of industrially important plant secondary metabolites are listed below in the table:

Secondary metabolites	Plant source	Uses
Digoxin	<i>Digitalis purpurina</i>	Cardiac tonic
Codeine	<i>Papaver somniferum</i>	Analgesic
Capsaicin	<i>Capsicum annum</i>	Rheumatic pain treatment
Vincristine	<i>Catharanthus roseus</i>	Anti-carcinogenic
Quinine	<i>Cinchona officinalis</i>	Antimalarial

Table 5.1: Secondary metabolites and its plant resources



5.4 Plant Regeneration Pathway

From the explants, plants can be regenerated by somatic embryogenesis or organogenesis.

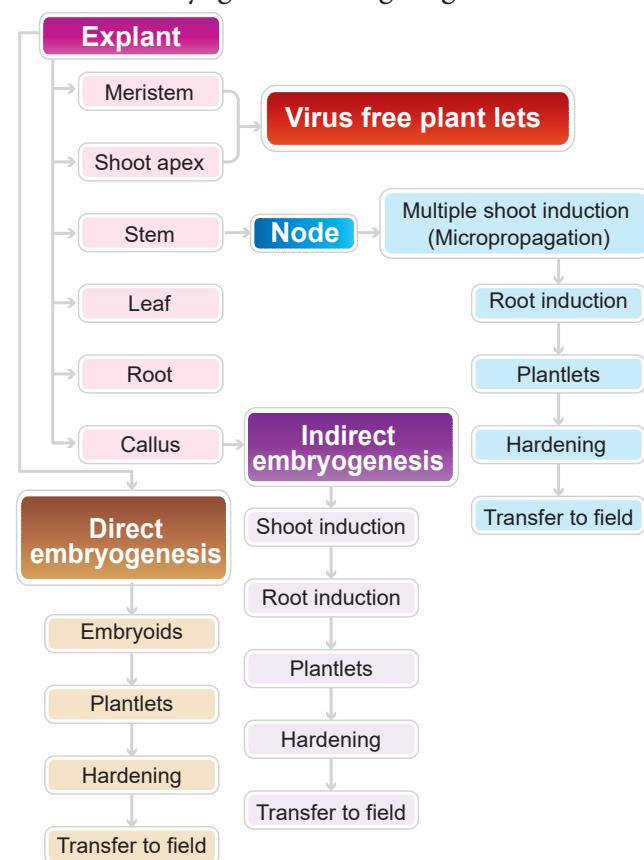


Figure 5.9: Flow chart of Plant regeneration pathway

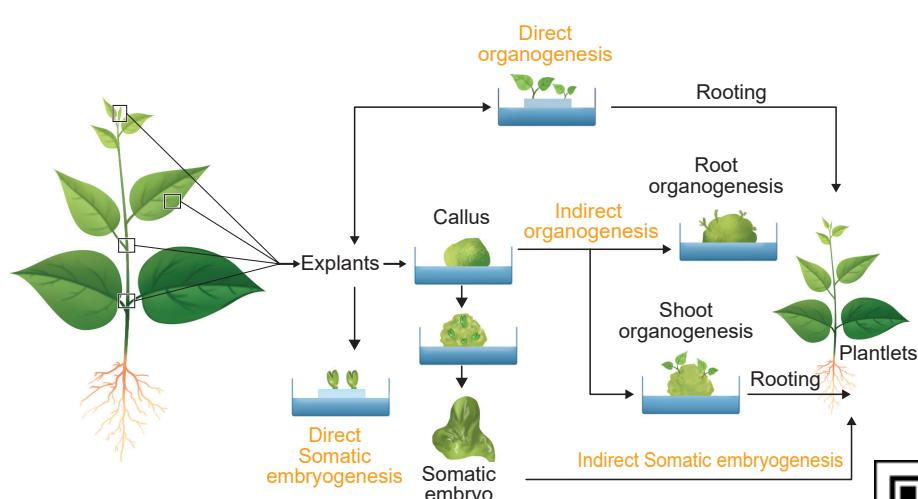


Figure 5.10: Plant Regeneration Pathway

5.4.1 Somatic Embryogenesis

Somatic embryogenesis is the formation of embryos from the callus tissue directly and these embryos are called **Embryoids** or from the *in vitro* cells directly form pre-embryonic cells which differentiate into embryos.

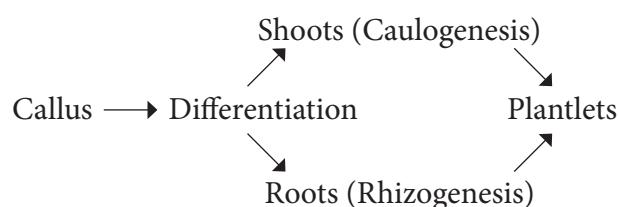
Applications

- Somatic embryogenesis provides potential plantlets which after hardening period can establish into plants.
- Somatic embryoids can be used for the production of synthetic seeds.
- Somatic embryogenesis is now reported in many plants such as *Allium sativum*, *Hordeum vulgare*, *Oryza sativa*, *Zea mays* and this possible in any plant.

Synthetic seeds are produced by encapsulation of embryoids in agarose gel or calcium alginate.

5.4.2 Organogenesis

The morphological changes occur in the callus leading to the formation of shoot and roots is called organogenesis.



- Organogenesis can be induced *in vitro* by introducing plant growth regulators in the MS medium.
- Auxin and cytokinins induce shoot and root formation.





5.5 Applications of Plant Tissue Culture

Plant tissue culture techniques have several applications such as:

- i. Improved hybrids production through somatic hybridization.
- ii. Somatic embryos can be encapsulated into synthetic seeds (synseeds). These encapsulated seeds or synthetic seeds help in conservation of plant biodiversity.
- iii. Production of disease resistant plants through meristem and shoot tip culture.
- iv. Production of stress resistant plants like herbicide tolerant, heat tolerant plants.
- v. Micropropagation technique to obtain large numbers of plantlets of both crop and tree species useful in forestry within a short span of time and all through the year.
- vi. Production of secondary metabolites from cell culture utilized in pharmaceutical, cosmetic and food industries.

Somaclonal variations: Somatic variations found in plants regenerated in vitro (i.e. variations found in leaf, stem, root, tuber or propagule)

Gametoclonal variations: Gametophytic variations found in plants regenerated in vitro gametic origin (i.e. variations found in gametes and gametophytes)

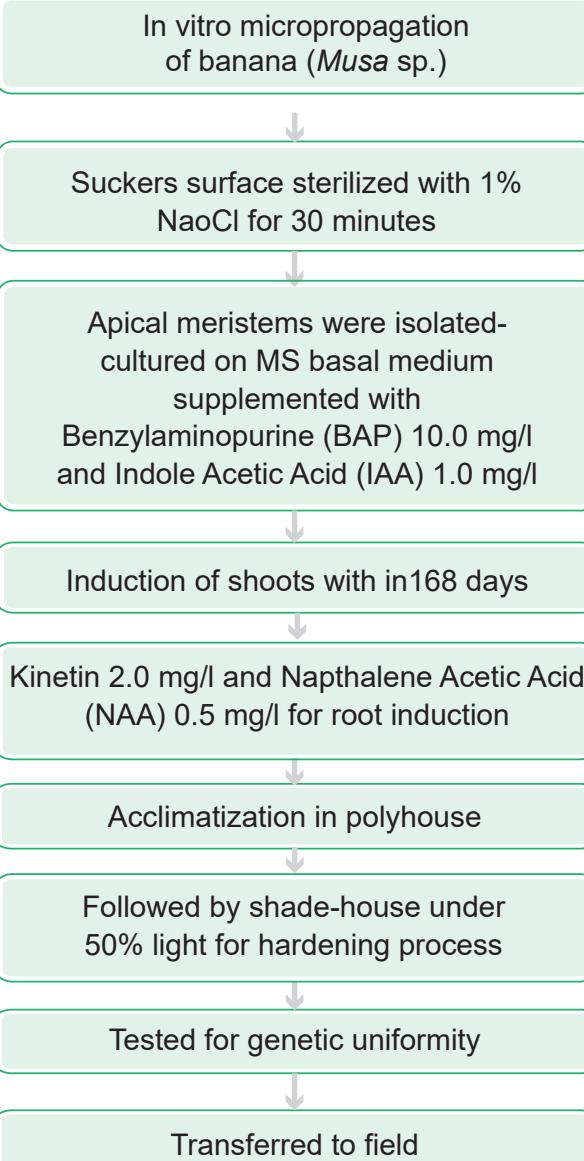
5.5.1 Micropropagation of Banana

Micropropagation of plants at industrial level maintains high standards of homogeneity in plants like pineapple, banana, strawberry and potato.



Figure 5.11: Micropropagation of Banana

Micropropagation protocol for banana



5.5.2 Artificial Seed

Artificial seeds or synthetic seeds (synseeds) are produced by using embryos (somatic embryos) obtained through in vitro culture. They may even be derived from single cells from any part of the plant that later divide to form cell mass containing dense cytoplasm, large nucleus, starch grains, proteins, and oils etc., To prepare the artificial seeds different inert materials are used for coating the somatic embryos like agarose and sodium alginate.

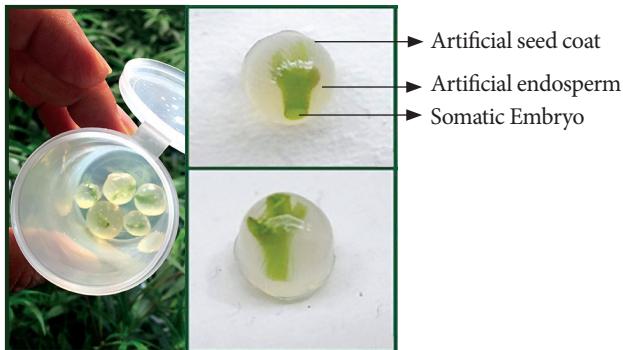


Figure 5.12: Artificial seeds

Advantages of Artificial seeds

Artificial seeds have many advantages over the true seeds

- Millions of artificial seeds can be produced at any time at low cost.
- They provide an easy method to produce genetically engineered plants with desirable traits.
- It is easy to test the genotype of plants.
- They can potentially stored for long time under cryopreservation method.
- Artificial seeds produce identical plants
- The period of dormancy of artificial seeds is greatly reduced, hence growth is faster with a shortened life cycle.

5.5.3 Virus-free plants

The field grown plants like perennial crops, usually are infected by variety of pathogens like fungi, bacteria, mycoplasma, viruses which cause considerable economic losses. Chemical methods can be used to control fungal and bacterial pathogens, but not viruses generally.

Shoot meristem tip culture is the method to produce virus-free plants, because the shoot meristem tip is always free from viruses.



Figure 5.13: Shoot tip - Apical Meristem

Protocol for virus free meristem tip culture

Apical meristem tip with 1 or 2 leaf primordia are excised in sterile condition from the explant

In vitro culture in 10ml of solid MS medium supplemented with growth hormones

Cultures are maintained at $24\pm1^{\circ}\text{C}$ in dark for 3 days followed by normal illumination of 2400Lux

Plantlets developed from meristem-tip culture after organogenesis process, transferred to hardening process

Transferred to field condition

Figure 5.14: Protocol for virus free meristem culture

5.6 Conservation of plant genetic resources

5.6.1 Germplasm Conservation

Germplasm conservation refers to the conservation of living genetic resources like pollen, seeds or tissue of plant material maintained for the purpose of selective plant breeding, preservation in live condition and used for many research works.

Germplasm conservation resources is a part of collection of seeds and pollen that are stored in seed or pollen banks, so as to maintain their viability and fertility for any later use such as hybridization and crop improvement.

Germplasm conservation may also involve a gene bank, DNA bank of elite breeding lines of plant resources for the maintenance of biological diversity and also for food security.



Figure 5.15: Seed bank



5.6.2 Cryopreservation (-195.C)

Cryopreservation, also known as Cryo-conservation, is a process by which protoplasts, cells, tissues, organelles, organs, extracellular matrix, enzymes or any other biological materials are subjected to preservation by cooling to very low temperature of -196°C using liquid nitrogen.



At this extreme low temperature any enzymatic or chemical activity of the biological material will be totally stopped and this leads to preservation of material in dormant status. Later these materials can be activated by bringing to room temperature slowly for any experimental work.

Protective agents like dimethyl sulphoxide, glycerol or sucrose are added before cryopreservation process. These protective agents are called cryoprotectants, since they protect the cells, or tissues from the stress of freezing temperature.

5.7 Intellectual Property Right (IPR)

Intellectual property right (IPR) is a category of property that includes intangible creation of the human intellect, and primarily consists of copyrights, patents, and trademarks. It also includes other types of rights, such as trade secrets, publicity rights, moral rights, and rights against unfair competition.

- In biotechnology, the transformed microorganisms and plants and technologies for the production of commercial products are exclusively the property of the discoverer.
- The discoverer has the full rights on his property. It should not be neglected by the others without legal permission.
- The right of discoverer must be protected and it does by certain laws framed by a country.
- The IPR is protected by different ways

like patents, copyrights, trade secrets and trademarks, designs and geographical indications.

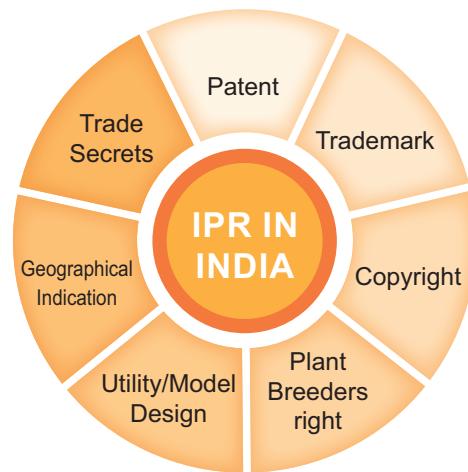


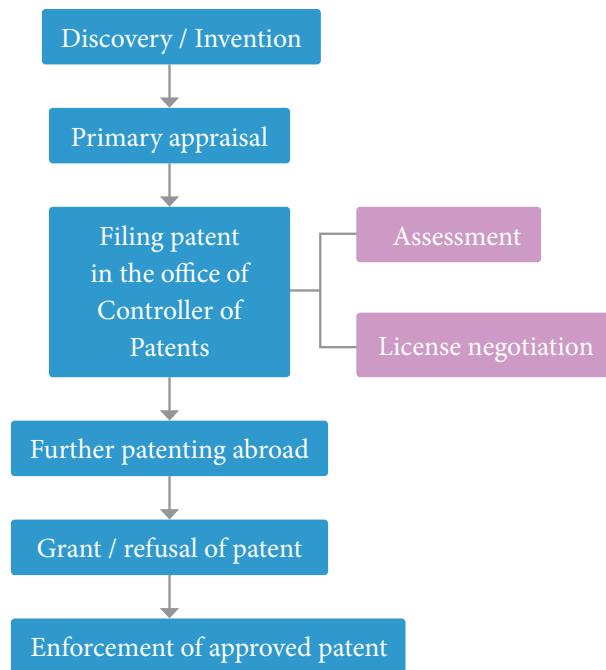
Figure 5.17: IPR in India

5.7.1 Patents

- It is a special right to the discoverer/inventor that has been granted by the government through legislation for trading new articles.
- A patent is a personal property which can be licensed or sold by the person or organisation just like any other property.
- Patent terms give the inventor the rights to exclude others from making, using or selling his invention.
- It is difficult to keep secret certain inventions and therefore, guidance should be obtained from a qualified patent attorney.
- A patent consists of three parts: the grant, specifications and claims.
- **The grant** is filed at the patent office which is not published. It is a signed document, actually the agreement that grants patent right to the inventor.
- **The specification** and claims are published as a single document which is made public from the patent office. The specification part is narrative in which the subject matter of invention is described as how the invention was carried out.
- **The claim** specifically defines the scope of the invention to be protected by the patent which the others may not practice.



General Steps in Patenting



5.7.2 Biosafety and Bioethics

Advances in biotechnology and their applications are mostly associated with controversies. This is because the major part of the modern biotechnology deals with genetic manipulations. ELSI which represents **ethical, legal and social implications** of biotechnology broadly covers the relationship between biotechnology and society with particular reference to ethical and legal aspects.

Biosafety

Biosafety is the prevention of large-scale loss of biological integrity, focusing both on ecology and human health. These prevention mechanisms include conduction of regular reviews of the biosafety in laboratory settings, as well as strict guidelines to follow. Biosafety is used to protect from harmful incidents. Many laboratories handling biohazards employ an ongoing risk management assessment and enforcement process for biosafety. Failures to follow such protocols can lead to increased risk of exposure to biohazards or pathogens. Human error and poor techniques contribute to unnecessary exposure to hazards and compromise the best safeguards set into place for protection.

Potential risks and consideration for safety aspects

- Pathogenicity of living organisms and viruses - natural and genetically modified - to infect humans, animals and plants to cause diseases.
- Toxicity of allergy associated with microbial production.
- Increasing number of antibiotic resistant pathogenic microorganisms.
- Problems associated with the disposal of spent microbial biomass and purification of effluent from biotechnological process.
- Safety aspects associated with contamination, infection or mutation of process strains.
- Safety aspects associated with the industrial use of microorganisms containing *in vitro* recombinants.

Biosafety guidelines are being implemented by:

- The Institutional Bio-safety Committees (IBSCs) monitor the research activity at institutional level.
- The Review Committee on Genetic Manipulation (RCGM) functioning in the Department of Biotechnology (DBT) monitors the risky research activities in the laboratories.
- The Genetic Engineering Approval Committee (GEAC) of Ministry of Environment and Forest has the power to permit the use of Genetically Modified Organism (GMO) at commercial level and open field trials of transgenic materials including agricultural crops, industrial products and health care products.

Bioethics - Ethical, Legal and Social Implications (ELSI)

Bioethics refers to the study of ethical issues emerging from advances in biology and medicine. It is also a moral discernment as it relates to medical policy and practice. Bioethicists are concerned with the ethical questions that arise in the relationships among



life sciences, biotechnology and medicine. It includes the study of values relating to primary care and other branches of medicine.

The scope of bioethics is directly related to biotechnology, including cloning, gene therapy, life extension, human genetic engineering, astroethics life in space, and manipulation of basic biology through altered DNA, RNA and proteins. These developments in biotechnology will affect future evolution, and may require new principles, such as biotic ethics, that values life and its basic biological characters and structures.

The Ethical, Legal, and Social Implications (ELSI) program was founded in 1990 as an integral part of the Human Genome Project. The mission of the ELSI program was to identify and address issues raised by genomic research that would affect individuals, families, and society. A percentage of the Human Genome Project budget at the National Institutes of Health and the U.S. Department of Energy was devoted to ELSI research.

Ethical issues in Genomic Research

- Privacy and fairness in the use of genetic information, including the potential for genetic discrimination in employment and insurance.
- The integration of new genetic technologies, such as genetic testing, into the practice of clinical medicine.
- Ethical issues surrounding the design and conduct of genetic research with people, including the process of informed consent.

Genetic Engineering Appraisal Committee (GEAC)

GEAC is an apex body under Ministry of Environment, Forests and Climate change for regulating manufacturing, use, import, export and storage of hazardous microbes or genetically modified organisms (GMOs) and cells in the country. It was established as an apex body to accord approval of activities involving large scale use of hazardous microorganisms

and recombinants in research and industrial production. The GEAC is also responsible for approval of proposals relating to release of genetically engineered organisms and products into the environment including experimental field trials (Biosafety Research Level trial-I and II known as BRL-I and BRL-II).

5.8 Future of Biotechnology

Biotechnology has become a comprehensive scientific venture from the point of academic and commercial angles, within a short time with the sequencing of human genome and genome of some important organisms. The future developments in biotechnology will be exciting. Thus the development in biotechnology will lead to a new scientific revolution that would change the lives and future of people. Like industrial and computer revolution, biotechnological revolution will also promise major changes in many aspects of modern life.

Summary

Tissue culture is the in vitro aseptic culture of cells, tissues or organs into whole plants under controlled nutritional and environmental conditions. A German physiologist Gotlieb Haberlant in 1902 for the first time attempted to culture plant cells in artificial medium, hence he was regarded as father of Tissue culture. Tissue culture mainly based on the concepts totipotency, differentiation, redifferentiation and dedifferentiation. Plant tissue culture technique involves selection of explants, sterilization, media preparation, maintaining culture condition, callus formation, embryogenesis or organogenesis and hardening. Based on the explants chosen the types of tissue culture are organ culture, meristem culture, protoplast culture and cell suspension culture. From the explants, plants can be regenerated by somatic embryogenesis or organogenesis is said to be plant regeneration pathway. Some of the main applications of tissue culture are



production of somatic hybrids, artificial seeds, disease resistant and stress resistant plants, germplasm conservation, micropropagation and production of secondary metabolites. Intellectual Property Right (IPR) is primarily aimed at patents, copyrights, trade secret and trademark given to the discoverer / inventor for the commercial production of transformed micro organisms or plants. Biosafety is the prevention mechanism to protect harmful incidents due to biohazards or pathogens. Bioethics dealt with ethical issue emerging from biotechnological advancement. ELSI program addresses issues related to genomic research. GEAC (Genetic Engineering Appraisal Committee) is a regulatory authority for release of genetically modified products or organisms into the environment.

Evaluation

Choose the correct answer from the given option:

1. Totipotency refers to
 - a) capacity to generate genetically identical plants.
 - b) capacity to generate a whole plant from any plant cell / explant.
 - c) capacity to generate hybrid protoplasts.
 - d) recovery of healthy plants from diseased plants.
2. Micro propagation involves
 - a) vegetative multiplication of plants by using micro-organisms.
 - b) vegetative multiplication of plants by using small explants.
 - c) vegetative multiplication of plants by using microspores.
 - d) Non-vegetative multiplication of plants by using microspores and megasporangia.



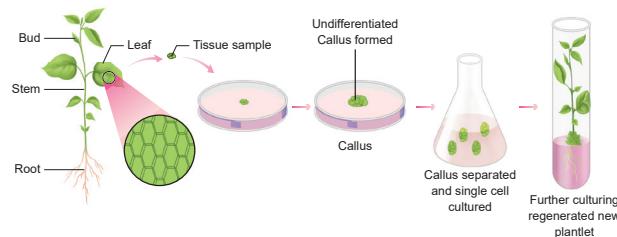
3. Match the following :

	Column A	Column B
1)	Totipotency	A) Reversion of mature cells into meristem
2)	Dedifferentiation	B) Biochemical and structural changes of cells
3)	Explant	C) Properties of living cells develops into entire plant
4)	Differentiation	D) Selected plant tissue transferred to culture medium

- 1 2 3 4
- a) C A D B
- b) A C B D
- c) B A D C
- d) D B C A
4. The time duration for sterilization process by using autoclave is _____ minutes and the temperature is _____
 - a) 10 to 30 minutes and 125° C
 - b) 15 to 30 minutes and 121° C
 - c) 15 to 20 minutes and 125° C
 - d) 10 to 20 minutes and 121° C
5. Which of the following statement is correct
 - a) Agar is not extracted from marine algae such as seaweeds.
 - b) Callus undergoes differentiation and produces somatic embryos.
 - c) Surface sterilization of explants is done by using mercuric bromide
 - d) PH of the culture medium is 5.0 to 6.0
6. Select the incorrect statement from given statement
 - a) A tonic used for cardiac arrest is obtained from Digitalis purpurea
 - b) Medicine used to treat Rheumatic pain is extracted from Capsicum annum



- c) An anti malarial drug is isolated from *Cinchona officinalis*.
- d) Anti-carcinogenic property is seen in *Catharanthus roseus*.
7. Virus free plants are developed from
- Organ culture
 - Meristem culture
 - Protoplast culture
 - Cell suspension culture
8. The prevention of large scale loss of biological integrity
- Biopatent
 - Bioethics
 - Biosafety
 - Biofuel
9. Cryopreservation means it is a process to preserve plant cells, tissues or organs
- at very low temperature by using ether.
 - at very high temperature by using liquid nitrogen
 - at very low temperature of -196 by using liquid nitrogen
 - at very low temperature by using liquid nitrogen
10. Solidifying agent used in plant tissue culture is
- Nicotinic acid
 - Cobaltous chloride
 - EDTA
 - Agar
11. What is the name of the process given below? Write its 4 types.



12. How will you avoid the growing of microbes in nutrient medium during culture process? What are the techniques used to remove the microbes?

13. Write the various steps involved in cell suspension culture.
14. What do you mean by Embryoids? Write its application.
15. Give the examples for micro propagation performed on plants.
16. Explain the basic concepts involved in plant tissue culture.
17. Based on the material used, how will you classify the culture technology? Explain it.
18. Give an account on Cryopreservation.
19. What do you know about Germplasm conservation. Describe it.
20. Write the protocol for artificial seed preparation.

Glossary

Aseptic condition: Preparation of materials free from microbes in *in vitro* cultures.

Cell Culture: Growing of cells *in vitro*, including the culture of single cells or small aggregates of cells in a liquid medium.

Chemically defined medium: A nutritive medium used for culturing cells or tissue; each chemical of this medium is known and defined;

Cybrid: Cytoplasmic hybrid obtained by the fusion of cytoplasm of cells of different parental sources; a term applied to the fusion of cytoplasms of two different protoplasts;

Organogenesis: The process of initiation and development of shoot or root through *in vitro* culture particularly from callus



Chapter

6



UNIT IX: Plant Ecology

Principles of Ecology



Learning Objectives

The learner will be able to

- ❖ Understand the interaction between organisms and their environment.
- ❖ Describe biotic and abiotic factors that influence the dynamics of populations.
- ❖ Describe how organisms adapt themselves to environmental changes.
- ❖ Learn the structure of various fruits and seeds related to their dispersal mechanism.



IBIUD V



Chapter outline

- 6.1 Ecology
- 6.2 Ecological factors
- 6.3 Ecological adaptations
- 6.4 Dispersal of seeds and fruits

Ecology is a division of biology which deals with the study of environment in relation to organisms. It can be studied by considering individual organisms, population, community, biome or biosphere and their environment. While observing our different environments, one can ask questions like

- Why do plants or animals vary with places?
- What are the causes for variation in biological diversity of different places?

- How soil, climate and other physical features affect the flora and fauna or vice versa?

These questions can be better answered with the study of ecology.

Ecology is essentially a practical science involving experiments, continuous observations to predict how organisms react to particular environmental circumstances and understanding the principles involved in ecology.

6.1 Ecology

The term “ecology” (**oekologie**) is derived from two Greek words – **oikos** (meaning house or dwelling place and **logos** meaning study) It was first proposed by **Reiter** (1868). However, the most widely accepted definition of ecology was given by **Ernest Haeckel** (1869).



R. Misra

Alexander von Humbolt - Father of Ecology

Eugene P. Odum - Father of modern Ecology

R. Misra - Father of Indian Ecology

6.1.1 Definitions of ecology

“The study of living organisms, both plants and animals, in their natural habitats or homes.”

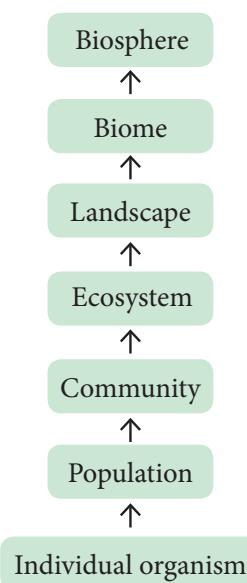
- **Reiter (1885)**

“Ecology is the study of the reciprocal relationship between living organisms and their environment.” - **Ernest Haeckel (1869)**



6.1.2 Ecological hierarchy

The interaction of organisms with their environment results in the establishment of grouping of organisms which is called **ecological hierarchy** or ecological levels of organization. The basic unit of ecological hierarchy is an individual organism. The different hierarchy of ecological systems is illustrated below:



6.1.3 Branches of Ecology:

Ecology is mainly divided into two branches, they are autecology and syncology.

- 1. Autecology** is the ecology of an individual species and is also called species ecology.
- 2. Syncology** is the ecology of a population or community with one or more species and also called as community ecology.

Many advances and developments in the field ecology resulted in various new dimensions and branches. Some of the advanced fields are Molecular ecology, Eco technology, Statistical ecology and Environmental toxicology.

6.1.4 Habitat and Niche

Habitat

Habitat is a specific physical place or locality occupied by an organism or any species which has a particular combination of abiotic or environmental factors. But the environment of any community is called **Biotope**.

Niche

An ecological niche refers to an organism's place in the biotic environment and its functional role in an ecosystem. The term was coined by the naturalist **Roswell Hill Johnson** but **Grinnell** (1917) was probably first to use this term. The

habitat and niche of any organism is called **Ecotope**

The differences between habitat and niche are as follows.

	Habitat	Niche
1.	A specific physical space occupied by an organism (species)	A functional space occupied by an organism in the same eco-system
2.	Same habitat may be shared by many organisms (species)	A single niche is occupied by a single species
3.	Habitat specificity is exhibited by organism.	Organisms may change their niche with time and season.

Table 6.1: Difference between habitat and niche



Applied ecology or environmental technology:

Application of the Science of ecology is otherwise called as **Applied ecology or Environmental technology**. It helps us to manage and conserve natural resources, particularly ecosystems, forest and wild life conservative and management. Environmental management involves Bio-diversity conservation, Ecosystem restoration, Habitat management, Invasive species management, Protected areas management and also help us plan landscapes and environmental impact designing for the futuristic ecology.

6.1.5 Ecological equivalents

Taxonomically different species occupying similar habitats (Niches) in different geographical regions are called **Ecological equivalents**.



Examples:

- Certain species of epiphytic orchids of Western Ghats of India differ from the epiphytic orchids of South America. But they are epiphytes.
- Species of the grass lands of Western Ghats of India differ from the grass species of temperate grass lands of Steppe in North America. But they are all ecologically primary producers and fulfilling similar roles in their respective communities.

6.2 Ecological factors

Many organisms co-exist in an environment. The environment (surrounding) includes physical, chemical and biological components. When a component surrounding an organism affects the life of an organism, it becomes a factor. All such factors together are called **environmental factors** or **ecological factors**. These factors can be classified into living (**biotic**) and non-living (**abiotic**) which make the environment of an organism. However the ecological factors are meaningfully grouped into four classes, which are as follows:

- i. Climatic factors
- ii. Edaphic factors
- iii. Topographic factors
- iv. Biotic factors

We will discuss the above factors in a concise manner.



Flowers of poppy, chicory, dog rose and many other plants, blossom before the break of dawn (4 – 5 am), evening primrose open up with the onset of dusk (5 – 6 pm) due to diurnal rhythm.

6.2.1 Climatic Factors

Climate is one of the important natural factors controlling the plant life. The climatic factors include light, temperature, water, wind and fire.

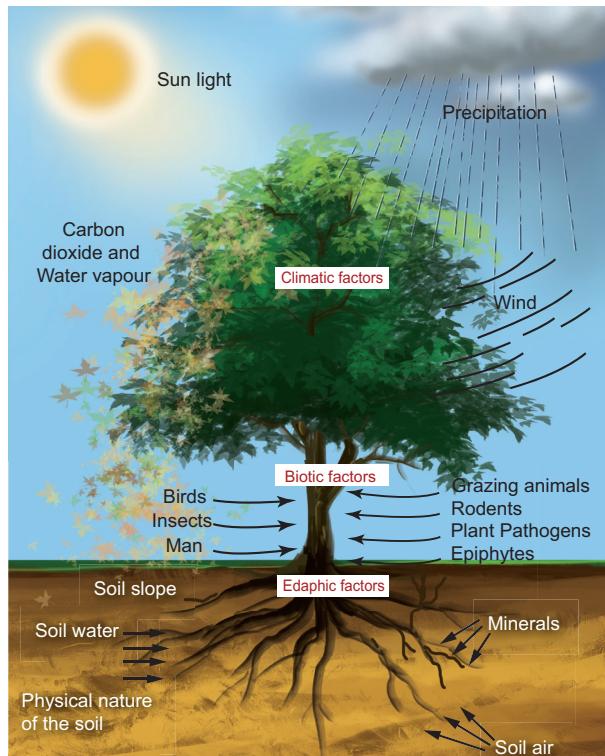


Figure 6.1: Environmental factors affecting a plant

a. Light

Light is a well known factor needed for the basic physiological processes of plants, such as photosynthesis, transpiration, seed germination and flowering. The portion of the sunlight which can be resolved by the human eye is called **visible light**. The visible part of light is made up of wavelength from about 400 nm (**violet**) to 700 nm (**red**). The rate of photosynthesis is maximum at **blue** (400 – 500 nm) and **red** (600 – 700 nm). The **green** (500 – 600 nm) wave length of spectrum is less strongly absorbed by plants.

Effects of light on plants

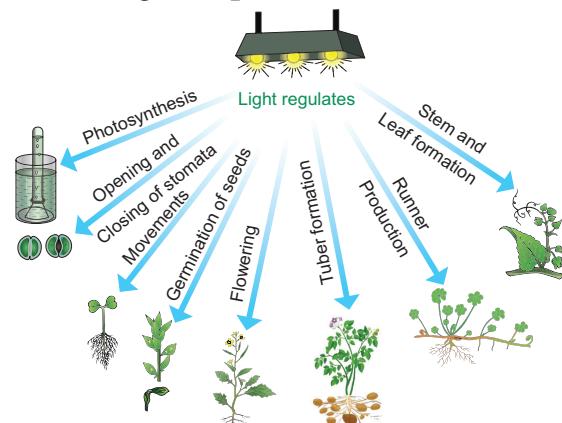


Figure 6.2: Various effects of light upon a green plant



Based on the tolerance to intensities of light, the plants are divided into two types. They are

1. **Heliophytes** - Light loving plants.
Example: Angiosperms.
2. **Sciophytes** - Shade loving plants.
Example: Bryophytes and Pteridophytes.

In deep sea (>500m), the environment is dark and its inhabitants are not aware of the existence of celestial source of energy called Sun. What, then is their source of energy?



Palaeoclimatology—Helps to reconstruct past climates of our planet and flora, fauna and ecosystem in which they lived. Example: Air bubbles trapped in ice for tens of thousands of years with fossilized pollen, coral, plant and animal debris.

b. Temperature

Temperature is one of the important factors which affect almost all the metabolic activities of an organism. Every physiological process in an organism requires an optimum temperature at which it shows the maximum metabolic rate. Three limits of temperature can be recognized for any organism. They are

1. **Minimum temperature** - Physiological activities are lowest.
2. **Optimum temperature** - Physiological activities are maximum.
3. **Maximum temperature** - Physiological activities will stop.

Based on the temperature prevailing in an area, **Raunkiaer** classified the world's vegetation into the following four types. They are megatherms, mesotherms, microtherms and hekistotherms. In thermal springs and deep sea hydrothermal vents where average temperature exceed 100°C.

Based on the range of **thermal tolerance**, organisms are divided into two types.

1. Eurythermal: Organisms which can tolerate a wide range of temperature fluctuations.

Example: *Zostera* (A marine Angiosperm) and *Artemisia tridentata*.

2. Stenothermal: Organisms which can tolerate only small range of temperature variations. Example: Mango and Palm (Terrestrial Angiosperms).

Mango plant do not grow in temperate countries like Canada and Germany.

Thermal Stratification

It is usually found in aquatic habitat. The change in the temperature profile with increasing depth in a water body is called **thermal stratification**. There are three kinds of thermal stratifications.

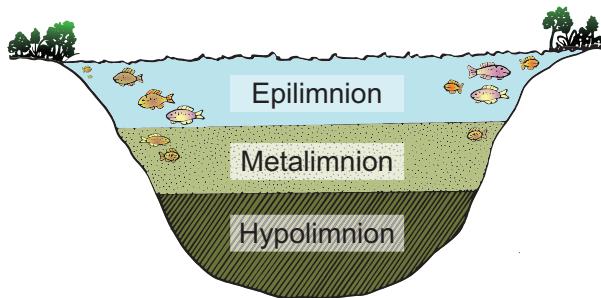


Figure 6.3: Thermal stratification of pond

1. **Epilimniotn** – The upper layer of warmer water.
2. **Metalimnion** – The middle layer with a zone of gradual decrease in temperature.
3. **Hypolimnion** - The bottom layer of colder water.

Temperature based zonation

Variations in **latitude** and **altitude** do affect the temperature and the vegetation on the earth surface. The latitudinal and altitudinal zonation of vegetation is illustrated below:

Latitude: Latitude is an angle which ranges from 0° at the equator to 90° at the poles.

Altitude: How high a place is located above the sea level is called the altitude of the place.

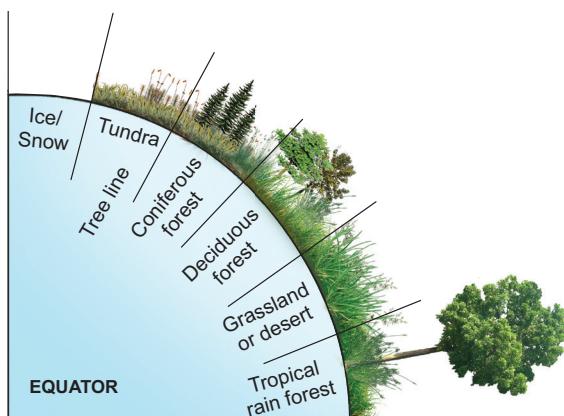


Figure 6.4: Latitudinal zonation of vegetation type

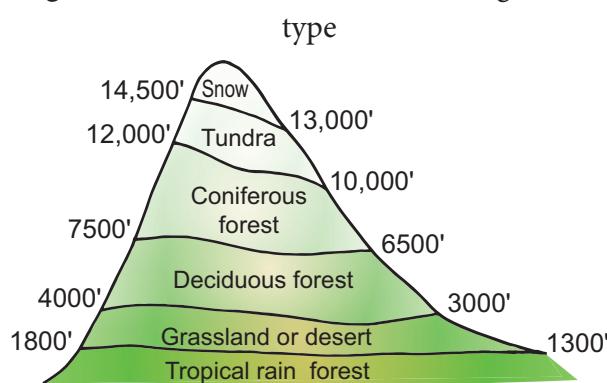


Figure 6.5: Altitudinal zonation of vegetation type

Timber line / Tree line : It is an imaginary line in a mountain or higher areas of land that marks the level above which trees do not grow. The altitudinal limit of normal tree growth is about 3000 to 4000m.

Effects of temperature

The following physiological processes are influenced by temperature:

- Temperature affects the enzymatic action of all the bio-chemical reactions in a plant body.
- It influences CO₂ and O₂ solubility in the biological systems. Increases respiration and stimulates growth of seedlings.
- Low temperature with high humidity can spread diseases to plants.
- The varying temperature with moisture determines the distribution of the vegetation types.

c. Water

Water is one of the most important climatic factors. It affects the vital processes of all living organisms. It is believed that even life had originated only in water during the evolution of Earth. Water covers more than 70% of the earth's surface. In nature, water is available to plants in three ways. They are **atmospheric moisture**, **precipitation** and **soil water**.



Evergreen forests – Found where heavy rainfall occurs throughout the year.

Sclerophyllous forests – Found where heavy rainfall occurs during winter and low rainfall during summer.

The productivity and distribution of plants depend upon the availability of water. Further the quality of water is also important especially for the aquatic organisms. The total amount of water salinity in different water bodies are : i).5% in inland water (Fresh water) ii).30 – 35% in sea water and iii). More than 100% in hypersaline water (**Lagoons**) Based on the range of tolerance of salinity, organisms are divided into two types.

1. Euryhaline: Organisms which can live in water with wide range of salinity. Examples: Marine algae and marina angiosperms

2. Stenohaline: Organisms which can withstand only small range of salinity. Example: Plants of estuaries.

Terminology	Environmental factor	
Stenothermal	Eurythermal	Temperature
Stenohaline	Euryhaline	Salinity
Stenoecious	Euryoecious	Habitat selection (niche)
Stenohydric	Euryhydric	Water
Stenophagic	Euryphagic	Food
Stenobathic	Eurybathic	Depth of water / habitat

Table 6.2: Tolerance of Environmental factor



Examples of tolerance to toxicity

- Soyabean and tomato manage to tolerate presence of cadmium poisoning by isolating cadmium and storing into few group of cells and prevent cadmium affecting other cells .
- Rice and *Eichhornia* (water hyacinth) tolerate cadmium by binding it to their proteins.

These plants otherwise can also be used to remove cadmium from contaminated soil ,this is known as **Phytoremediation**.

d. Wind

Air in motion is called wind. It is also a vital ecological factor. The atmospheric air contains a number of gases, particles and other constituents. The composition of gases in atmosphere is as follows: Nitrogen -78% , Oxygen -21%, Carbon-di-oxide -0.03%, Argon and other gases - 0.93%. The other components of wind are water vapour, gaseous pollutants, dust, smoke particles, microorganisms, pollen grains, spores, etc. **Anemometer** is the instrument used to measure the speed of wind.

DO YOU KNOW?

Green House Effect Albedo Effect

Gases let out to atmosphere causes climatic change.

Emission of dust and aerosols (small solids or liquid particles in suspension in the atmosphere) from industries, automobiles, forest fire, SO_2 and DMS (dimethyl sulphur) play an important role in disturbing the temperature level of any region. Aerosols with small particles is reflecting the solar radiation entering the atmosphere. This is known as **Albedo effect**. So it reduces the temperature (cooling) limits, photosynthesis and respiration. The sulphur compounds are responsible for **acid rain** due to acidification of rain water and destroy the ozone.

Effects of wind

- Wind is an important factor for the formation of rain
- Causes wave formation in lakes and ocean, which promotes aeration of water
- Strong wind causes soil erosion and reduces soil fertility
- Increases the rate of transpiration
- Helps in pollination in anemophilous plants
- It also helps in dispersal of many fruits, seeds, spores, etc.
- Strong wind may cause up-rooting of big trees
- Unidirectional wind stimulates the development of **flag forms** in trees.

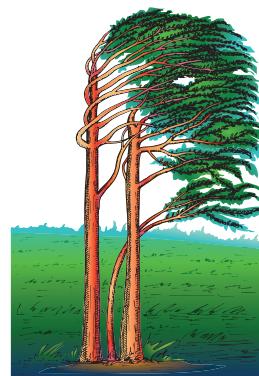


Figure 6.6: Flag form in trees

e. Fire

Fire is an exothermic factor caused due to the chemical process of combustion, releasing heat and light. It is mostly man-made and sometimes develops naturally due to the friction between the tree surfaces. Fire is generally divided into

- Ground fire** – Which is flameless and subterranean.
- Surface fire** – Which consumes the herbs and shrubs.
- Crown fire** – Which burns the forest canopy.

Effects of fire

- Fire has a direct lethal effect on plants
- Burning scars are the suitable places for the entry of parasitic fungi and insects
- It brings out the alteration of light, rainfall, nutrient cycle, fertility of soil, pH, soil flora and fauna
- Some fungi which grow in soil of burnt areas called pyrophilous.

Example: *Pyronema confluens*.



Indicators of fire – *Pteris* (fern) and *Pyronema* (fungus) indicates the burnt up and fire disturbed areas. So they are called indicators of fire.

Fire break – It is a gap made in the vegetation that acts as a barrier to slow down or stop the progress of fire.

A **natural fire break** may occur when there is a lack of vegetation such as River, lake and canyon found in between vegetation may act as a natural fire break.

Rhytidome: It is the structural defense by plants against fire. The outer bark of trees which extends to the last formed periderm is called Rhytidome. It is composed of multiple layers of suberized periderm, cortical and phloem tissues. It protects the stem against fire, water loss, invasion of insects and prevents infections by microorganisms.

6.2.2 Edaphic factors

Edaphic factors, the abiotic factors related to soil, include the physical and chemical composition of the soil formed in a particular area. The study of soils is called **Pedology**.

The soil

Soil is the weathered superficial layer of the Earth in which plants can grow. It is a complex composite mass consisting of soil constituents, soil water, soil air and soil organisms, etc.

Soil formation

Soil originates from rocks and develops gradually at different rates, depending upon the ecological and climatic conditions. Soil formation is initiated by the weathering process. Biological weathering takes place when organisms like bacteria, fungi, lichens and plants help in the breakdown of rocks through the production of acids and certain chemical substances.

Soil types

Based on soil formation (**pedogenesis**), the soils are divided into

- Residual soils** – These are soils formed by weathering and **pedogenesis** of the rock.
- Transported soils** – These are transported by various agencies.

The important edaphic factors which affect vegetation are as follows:

- Soil moisture:** Plants absorbs rain water and moisture directly from the air
- Soil water:** Soil water is more important than any other ecological factors affecting the distribution of plants. Rain is the main source of soil water. Capillary water held between pore spaces of soil particles and angles between them is the most important form of water available to the plants.
- Soil reactions:** Soil may be **acidic** or **alkaline** or **neutral** in their reaction. pH value of the soil solution determines the availability of plant nutrients. The best pH range of the soil for cultivation of crop plants is **5.5 to 6.8**.
- Soil nutrients:** Soil fertility and productivity is the ability of soil to provide all essential plant nutrients such as minerals and organic nutrients in the form of ions.
- Soil temperature:** Soil temperature of an area plays an important role in determining the geographical distribution of plants. Low temperature reduces use of water and solute absorption by roots.
- Soil atmosphere:** The spaces left between soil particles are called pore spaces which contains **oxygen** and **carbon-di-oxide**.
- Soil organisms:** Many organisms existing in the soil like bacteria, fungi, algae, protozoans, nematodes, insects, earthworms, etc. are called soil organisms.





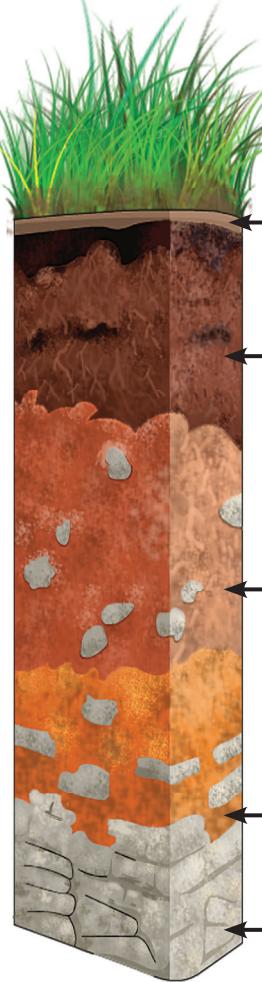
	Horizon	Description
O-Horizon (Organic horizon) Humus		It consists of fresh or partially decomposed organic matter. O1 – Freshly fallen leaves, twigs, flowers and fruits O2 – Dead plants, animals and their excreta decomposed by micro-organisms. Usually absent in agricultural and deserts.
A-Horizon (Leached horizon) Topsoil - Often rich in humus and minerals.		It consists of top soil with humus, living creatures and in-organic minerals. A1 – Dark and rich in organic matter because of mixture of organic and mineral matters. A2 – Light coloured layer with large sized mineral particles.
B-Horizon (Accumulation horizon) (Subsoil-Poor in humus, rich in minerals)		It consists of iron, aluminium and silica rich clay organic compounds.
C - Horizon (Partially weathered horizon) Weathered rock Fragments - Little or no plant or animal life.		It consists of parent materials of soil, composed of little amount of organic matters without life forms.
R – Horizon (Parent material) Bedrock		It is a parent bed rock upon which underground water is found .

Figure 6.7: Soil Profile

Soil Profile

Soil is commonly stratified into horizons at different depth. These layers differ in their physical, chemical and biological properties. This succession of super-imposed horizons is called soil profile.

Types of soil particles

Based on the relative proportion of soil particles, four types of soil are recognized.

	Soil type	Size	Relative proportion
1	Clayey soil	Less than 0.002 mm	50% clay and 50% silt (cold / heavy soil)
2	Silt soil	0.002 to 0.02mm	90% silt and 10% sand
3	Loamy soil	0.002 to 2mm	70% sand and 30 % clay / silt or both (Garden soil)
4	Sandy soil	0.2 to 2 mm	85% sand and 15% clay (light soil)

Table 6.3: Types of soil particles

Loamy soil is ideal soil for cultivation. It consists of 70% sand and 30% clay or silt or both. It ensures good retention and proper drainage of water. The porosity of soil provides adequate aeration and allows the penetration of roots.

Based on the water retention, aeration and mineral contents of soil, the distribution of vegetation is divided into following types.

- Halophytes:** Plants living in saline soils
- Psammophytes:** Plants living in sandy soils
- Lithophytes:** Plants living on rocky surface
- Chasmophytes:** Plants living in rocky crevices
- Cryptophytes:** Plants living below the soil surface
- Cryophytes:** Plants living in ice surface
- Oxylophytes:** Plants living in acidic soil
- Calciphytes:** Plants living in calcium rich alkaline soil.



Hollard – Total soil water content

Chresard – Water available to plants

Echard – Water not available to plants

6.2.3 Topographic factors

The surface features of earth are called **topography**. Topographic influence on the climate of any area is determined by the interaction of solar radiation, temperature, humidity, rainfall, latitude and altitude. It affects the vegetation through climatic variations in small areas (micro climate) and even changes the soil conditions. Topographic factors include latitude, altitude, direction of mountain, steepness of mountain etc.

a. Latitudes and altitudes

Latitudes represent distance from the equator. Temperature values are maximum at the equator and decrease gradually towards poles. Different types of vegetation occur from equator to poles which are illustrated below.

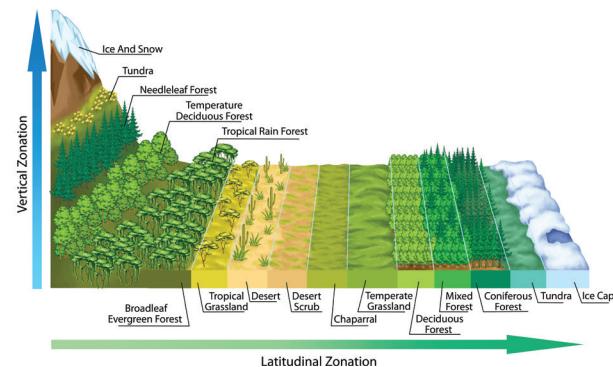


Figure 6.8: Latitudinal and Altitudinal Vegetation

Height above the sea level forms the **altitude**. At high altitudes, the velocity of wind remains high, temperature and air pressure decrease while humidity and intensity of light increases. Due to these factors, vegetation at different altitudes varies, showing distinct zonation.

b. Direction of Mountain

North and south faces of mountain or hill possess different types of flora and fauna because they differ in their humidity, rainfall, light intensity, light duration and temperature regions.

Ecotone - The transition zone between two ecosystems. Example: The border between forest and grassland.

Edge effect – Those species are found in the ecotone areas due to the effect of environment of the two habitats. This is called edge effect. Example: Owl in the ecotone area between forest and grassland.

The two faces of the mountain or hill receive different amount of solar radiation, wind action and rain. Of these two faces, the windward region possesses good vegetation due to heavy rains and the leeward region possesses poor vegetation due to rain shadows (rain deficit).

Similarly in the soil of aquatic bodies like ponds the center and edge possess different depth of water due to soil slope and different wave actions in the water body. Therefore, different parts of the same area may possess different species of organisms.

c. Steepness of the mountain

The steepness of the mountain or hill allows the rain to run off. As a result the loss of water causes water deficit and quick erosion of the top soil resulting in **poor vegetation**. On the other hand, the plains and valley are **rich in vegetation** due to the slow drain of surface water and better retention of water in the soil.

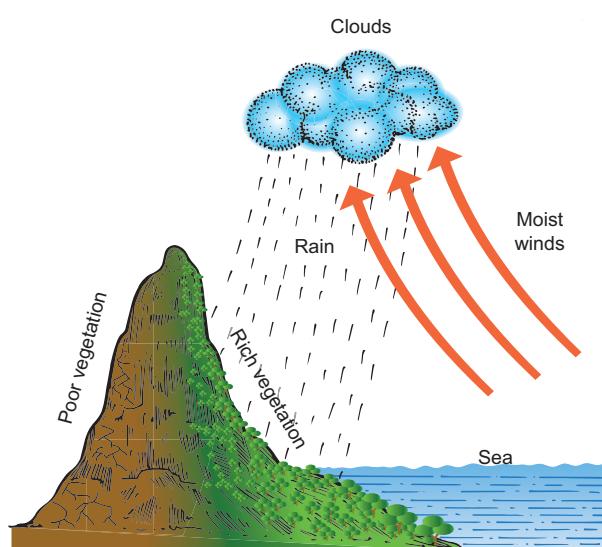


Figure 6.9: Steepness of mountain



6.2.4 Biotic factors

The interactions among living organisms such as plants and animals are called **biotic factors**, which may cause marked effects upon vegetation. The effects may be direct and indirect and modifies the environment. The plants mostly which lives together in a community and influence one another. Similarly, animals in association with plants also affect the plant life in one or several ways. The different interactions among them can be classified into following two types they are positive interaction and negative interaction

Positive interactions

When one or both the participating species are benefited, it is positive interaction. Examples; Mutualism and Commensalism.

a. **Mutualism:** It is an interaction between two species of organisms in which both are benefitted from the obligate association. The following are common examples of mutualism.

Nitrogen fixation

Rhizobium (Bacterium) forms nodules in the roots of leguminous plants and lives symbiotically. The *Rhizobium* obtains food from leguminous plant and in turn fixes atmospheric nitrogen into nitrate, making it available to host plants.

Other examples:

- Water fern (*Azolla*) and Nitrogen fixing Cyanobacterium (*Anabaena*).
- *Anabaena* present in coralloid roots of *Cycas*. (Gymnosperm)
- Cyanobacterium (*Nostoc*) found in the thalloid body of *Anthoceros*. (Bryophytes)
- Wasps present in fruits of fig.
- Lichen is a mutual association of an **alga** and a **fungus**.
- Roots of terrestrial plants and fungal hyphae- **Mycorrhiza**

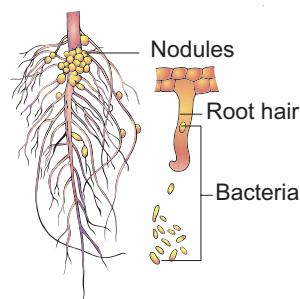


Figure 6.10:
A nodulated legume plant root with bacteria

b. **Commensalism:** It is an interaction between two organisms in which one is benefitted and the other is neither benefitted nor harmed. The species that derives benefit is called the **commensal**, while the other species is called the **host**. The common examples of commensalism are listed below:

	Interaction type	Combination	Effects		Examples
1. Positive interaction					
1	Mutualism	(+)	(+)	Both species benefitted	Lichen, Mycorrhiza etc.
2	Commensalism	(+)	(0)	One species is benefitted and the other species is neither benefitted nor harmed	orchids, Lianas etc.
2. Negative interaction					
4	Predation	(+)	(-)	One species benefitted, the other species are harmed	<i>Drosera, Nepenthes</i> etc.
5	Parasitism	(+)	(-)	One species benefitted, the other species are harmed	<i>Cuscuta, Duranta, Viscum</i> etc.
6	Competition	(-)	(-)	Harmful for both	Grassland species
7	Amensalism	(-)	(0)	Harmful for one, but the other species are unaffected	<i>Penicillium</i> and <i>Staphylo coccus</i>

(+) Benefitted, (-) Harmed (0)Unaffected

Table 6.4: Different interactions of plant



Epiphytes

The plants which are found growing on other plants without harming them are called epiphytes. They are commonly found in tropical rain forest.

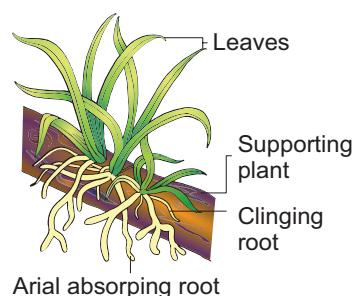


Figure 6.11:
An epiphytic plant- *Vanda*

The epiphytic higher plant (**Orchids**) gets its nutrients and water from the atmosphere with the help of their hygroscopic roots which contain special type of spongy tissue called **Velamen**. So it prepares its own food and does not depend on the host. They use the host plant only for support and does not harm it in any way.

- Many orchids, ferns, lianas, hanging mosses, *Peperomia*, money plant and *Usnea* (Lichen) are some of the examples of epiphytes.
- Spanish Moss – *Tillandsia* grows on the bark of Oak and Pine trees.



Proto Cooperation

An interaction between organisms of different species in which both organisms benefit but neither is dependent on the relationship. Example: Soil bacteria / fungi and plants growing in the soil.

Negative interactions

When one of the interacting species is benefitted and the other is harmed, it is called **negative interaction**. Examples: predation, parasitism, competition and amensalism.

a. **Predation:** It is an interaction between two species, one of which captures, kills and eats up the other. The species which kills is called a **predator** and the species which is killed is called a **prey**. The predator is benefitted while the prey is harmed.

Examples:

- A number of plants like *Drosera* (Sun dew Plant), *Nepenthes* (Pitcher Plant), *Diaonaea* (Venus fly trap), *Utricularia* (Bladder wort) and *Sarracenia* are predators which consume insects and other small animals for their food as a source of nitrogen. They are also called as **insectivorous plants**.

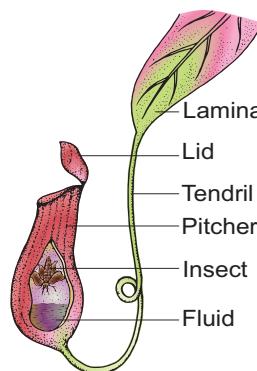


Figure 6.12: Pitcher plant – with insect

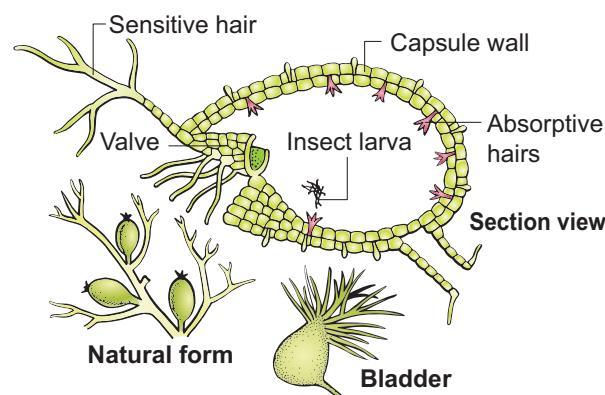


Figure 6.13: Insectivorous plant *Utricularia*

- Many herbivores are **predators**. Cattles, Camels, Goats etc., frequently browse on the tender shoots of herbs, shrubs and trees. Generally annuals suffer more than the perennials. Grazing and browsing may cause remarkable changes in vegetation. Nearly 25 percent of all insects are known as phytophagous(feeds on plant sap and other parts of plant)
- Many **defense mechanisms** are evolved to avoid their predations by plants. Examples: *Calotropis* produces highly poisonous cardiac glycosides, Tobacco produces nicotine, coffee plants produce caffeine, *Cinchona* plant produces quinine. Thorns of *Bougainvillea*, spines of *Opuntia*, and latex of cacti also protect them from predators.



b. Parasitism: It is an interaction between two different species in which the **smaller partner** (parasite) obtains food from the **larger partner** (host or plant). So the parasitic species is benefited while the host species is harmed. Based on the host-parasite relationship, parasitism is classified into two types they are holoparasite and hemiparasite.

Holoparasites

The organisms which are dependent upon the host plants for their entire nutrition are called **Holoparasites**. They are also called **total parasites**.

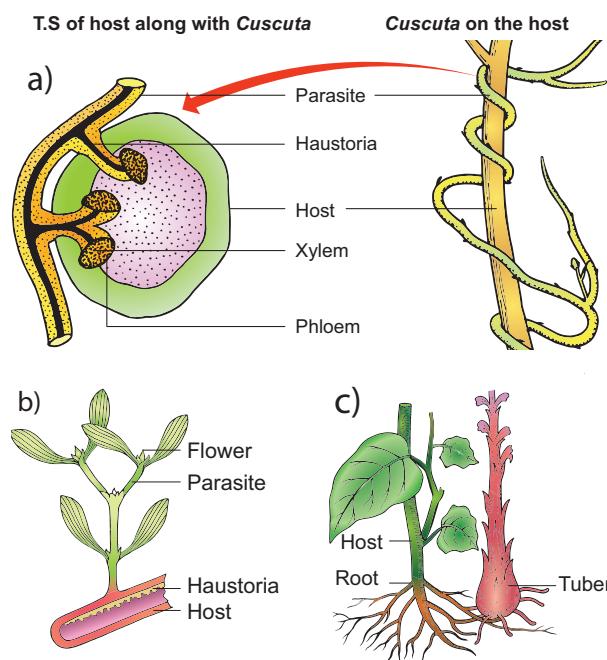


Figure 6.14: a) Holoparasite – *Cuscuta*
b) A Partial stem parasite – *Viscum*
c) Root parasite on the brinjal root *Orobanche* spp.

Examples:

- *Cuscuta* is a total stem parasite of the host plant *Acacia*, *Duranta* and many other plants. *Cuscuta* even gets flower inducing hormone from its host plant.
- *Balanophora*, *orobanche* and *Refflesia* are the total root parasites found on higher plants.

Hemiparasites

The organisms which derive only water and minerals from their host plant while synthesizing their own food by photosynthesis are called **Hemiparasites**. They are also called **partial parasites**.

Examples:

- *Viscum* and *Loranthus* are **partial stem parasites**.
- *Santalum* (Sandal Wood) is a **partial root parasite**.

The parasitic plants produce the **haustorial roots** inside the host plant to absorb nutrients from the vascular tissues of host plants.

c. Competition: It is an interaction between two organisms or species in which both the organisms or species are harmed. Competition is the severest in population that has irregular distribution. Competition is classified into intraspecific and interspecific.

1. **Intraspecific competition:** It is an interaction between individuals of the same species. This competition is very severe because all the members of species have similar requirements of food, habitat, pollination etc. and they also have similar adaptations to fulfill their needs.

2. **Interspecific competition:** It is an interaction between individuals of different species. In grassland, many species of grasses grow well as there is little competition when enough nutrients and water is available. During drought shortage of water occurs. A life and death competition starts among the different species of grass lands. Survival in both these competitions is determined by the quantity of nutrients, availability of water and migration to new areas. Different species of herbivores, larvae and grass hopper competing for fodder or forage plants. Trees, shrubs and herbs in a forest struggle for sunlight, water and nutrients and also for pollination and dispersal of fruits and seeds. The *Utricularia* (Bladderwort) competes with tiny fishes for small crustaceans and insects.



d. Amensalism: It is an interspecific interaction in which one species is inhibited while the other species is neither benefitted nor harmed. The inhibition is achieved by the secretion of certain chemicals called **allelopathic** substances. Amensalism is also called **antibiosis**.

- *Penicillium notatum* produces penicillin to inhibit the growth of a variety of bacteria especially *Staphylococcus*.
- *Trichoderma* inhibits the growth of fungus *Aspergillus*.
- Roots and hulls of Black Walnut *Juglans nigra* secretes an alkaloid **Junglone** which inhibits the growth of seedlings of Apple, Tomato and *Alfalfa* around it.

Interspecific interactions/ Co-evolutionary dynamics

i. Mimicry: It is a phenomenon in which living organism modifies its form, appearance, structure or behavior and looks like another living organism as a self defence and increases the chance of their survival. Floral mimicry is for usually inviting pollinators but animal mimicry is often protective. Mimicry is a result of evolutionary significance due to shape and sudden heritable mutation and preservation of natural selection.

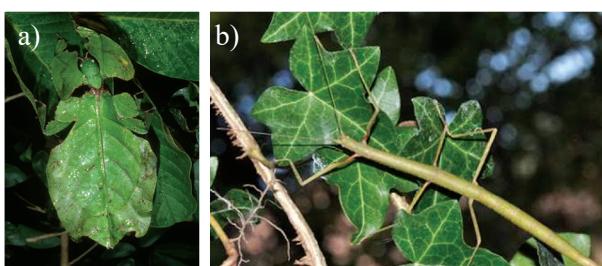


Figure 6.15: Mimicry

a) *Phyllium frondosum* b) *Carausium morosus*

Example:

- The plant, *Ophrys* an orchid, the flower looks like a female insect to attract the male insect to get pollinated by the male insect and it is otherwise called 'floral mimicry'.
- *Carausium morosus* – stick insect or walking stick. It is a protective mimicry.

- *Phyllium frondosum* – leaf insect, another example of protective mimicry.

ii. Myrmecophily: Sometimes, ants take their shelter on some trees such as Mango, Litchi, Jamun, *Acacia* etc.

These ants act as body guards of the plants against any disturbing agent and the plants in turn provide food and shelter to these ants. This phenomenon is known as Myrmecophily. Example: Acacia and acacia ants.

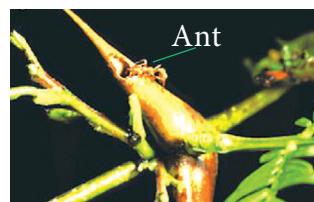


Figure 6.16: Myrmecophily

iii. Co-evolution: The interaction between organisms, when continues for generations,

involves reciprocal changes in genetic and morphological characters of both organisms.

This type of evolution is called Co-evolution. It is a kind of co-adaptation and mutual change among interactive species.

Examples:

- Corolla length and proboscis length of butterflies and moths (*Habenaria* and Moth).
- Bird's beak shape and flower shape and size.
- More examples: Horn bills and birds of Scrub jungles ,Slit size of pollinia of Apocynaceae members and leg size of insects.



Kairomone released from *Pieris rapae* caterpillar exposed to wild Radish gets the capacity to transmit defence induced by predator to progeny of wild radish. Transmission capacity of defence induced by predator to progeny of wild radish.



6.3 Ecological adaptations

The modifications in the structure of organisms to survive successfully in an environment are called **adaptations** of organisms. Adaptations help the organisms to exist under the prevailing ecological habitat. Based on the habitats and the corresponding adaptations of plants, they are classified as hydrophytes, xerophytes, mesophytes, epiphytes and halophytes.

Hydrophytes

The plants which are living in water or wet places are called hydrophytes. According to their relation to water and air, they are subdivided into following categories: i) Free floating hydrophytes, ii) Rooted- floating hydrophytes, iii) Submerged floating hydrophytes, iv) Rooted -submerged hydrophytes, v) Amphibious hydrophytes.

i. Free floating hydrophytes: These plants float freely on the surface of water. They remain in contact with water and air, but not with soil. Examples: *Eichhornia*, *Pistia* and *Wolffia* (smallest flowering plant).

ii. Rooted floating hydrophytes: In these plants, the roots are fixed in mud, but their leaves and flowers are floating on the surface of water. These plants are in contact with soil, water and air. Examples: *Nelumbo*, *Nymphaea*, *Potamogeton* and *Marsilea*.

Lotus seeds showing highest longevity in plant kingdom.

iii. Submerged floating hydrophytes: These plants are completely submerged in water and not in contact with soil and air. Examples: *Ceratophyllum* and *Utricularia*.

iv. Rooted- submerged hydrophytes: These plants are completely submerged in water and rooted in soil and not in contact with air. Examples: *Hydrilla*, *Vallisneria* and *Isoetes*.

v. Amphibious hydrophytes (Rooted emergent hydrophytes): These plants are adapted to both

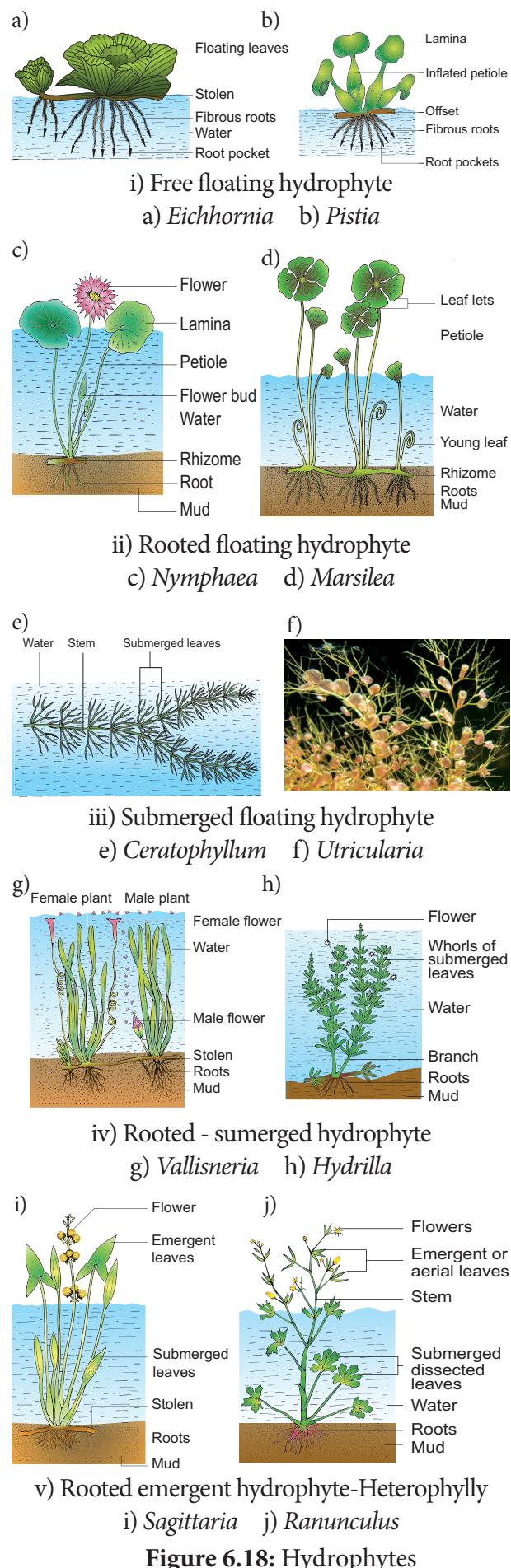


Figure 6.18: Hydrophytes



aquatic and terrestrial modes of life. They grow in shallow water. Examples: *Ranunculus*, *Typha* and *Sagittaria*.

Hygrophytes: The plants which can grow in moist damp and shady places are called hygrophytes. Examples: *Habenaria* (Orchid), Mosses (Bryophytes), etc.

Morphological adaptations of Hydrophytes:

In root

- Roots are totally absent in *Wolffia* and *Salvinia* or poorly developed in *Hydrilla* or well developed in *Ranunculus*.
- The root caps are replaced by **root pockets**. Example: *Eichhornia*

In stem

- The stem is long, slender, spongy and flexible in sub-merged forms.
- In free floating forms the stem is thick, short stoloniferous and spongy; and in rooted floating forms, it is a rhizome .
- Vegetative propagation is through runners, stolon, stem and root cuttings , tubers, dormant apices and offsets.

In leaves

- The leaves are thin, long and ribbon shaped in *Vallisneria* or long and linear in *Potamogeton* or finely dissected in *Ceratophyllum*
- The floating leaves are large and flat as in *Nymphaea* and *Nelumbo*. In *Eichhornia* and *Trapa* petioles become swollen and spongy.
- In emergent forms, the leaves show **heterophylly** (Submerged leaves are dissected and aerial leaves are entire). Example: *Ranunculus*, *Limnophila heterophylla* and *Sagittaria*

Anatomical adaptations

- Cuticle is either completely absent or if present it is thin and poorly developed
- Single layer of epidermis is present

- Cortex is well developed with aerenchyma
- Vascular tissues are poorly developed. In emergent forms vascular elements are well developed.
- Mechanical tissues are generally absent except in some emergent forms. Pith cells are sclerenchymatous.

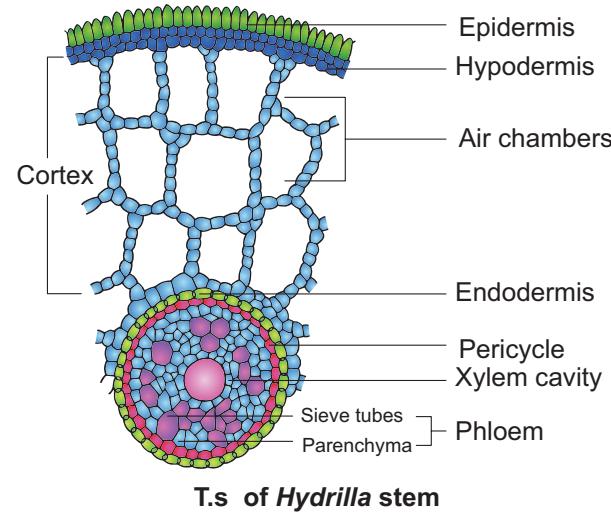


Figure 6.19: T.S. of *Hydrilla* stem

Physiological adaptations of Hydrophytes:

- Hydrophytes have the ability to withstand anaerobic conditions .
- They possess special aerating organs.

Xerophytes

The plants which are living in dry or xeric condition are known as **Xerophytes**. Xerophytic habitat can be of two different types. They are:

a. Physical dryness: In these habitats, soil has a little amount of water due to the inability of the soil to hold water because of low rainfall.

b. Physiological dryness: In these habitats, water is sufficiently present but plants are unable to absorb it because of the absence of capillary spaces. Example: Plants in salty and acidic soil.

Based on adaptive characters xerophytes are classified into three categories. They are Ephemerals, Succulents and Non succulent plants.



i. Ephemerals:

These are also called **drought escapers** or **drought evaders**.

These plants complete their life cycle within a short period (**single season**).

These are not true xerophytes. Examples: *Argemone*, *Mollugo*, *Tribulus* and *Tephrosia*.



Figure 6.20:

Argemone mexicana-Ephemerals

ii. Succulents: These are also called drought **enduring plants**. These plants store water in their plant parts during the dry period. These plants develop certain adaptive characters to resist extreme drought conditions. Examples: *Opuntia*, *Aloe*, *Bryophyllum* and *Begonia*.

iii. Non succulents: These are also called **drought resistant plants (true xerophytes)**. They face both external and internal dryness. They have many adaptations to resist dry conditions. Examples: *Casuarina*, *Nerium*, *Zizyphus* and *Acacia*.

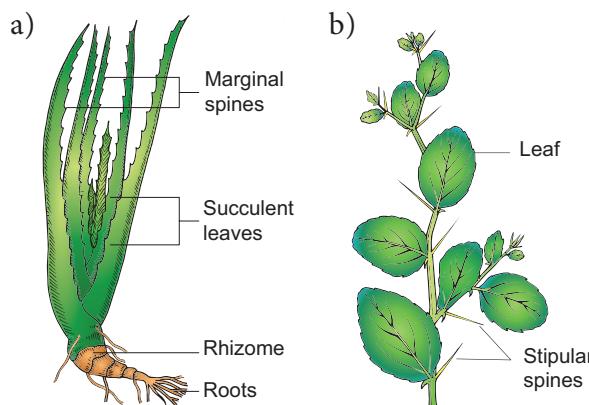


Figure 6.21: a) Succulent xerophyte – *Aloe*
b) Non succulent perennial - *Ziziphus*

Morphological Adaptations

In root

- Root system is well developed and is greater than that of shoot system.
- Root hairs and root caps are also well developed.

In Xerophytic plants with the leaves and stem are covered with hairs are called **trichophyllous plants**. Example: *Cucurbita* (*Melothria* and *Mukia*)

In stem

- Stems are mostly hard and woody. They may be aerial or underground
- The stems and leaves are covered with wax coating or covered with dense hairs.
- In some xerophytes all the internodes in the stem are modified into a fleshy leaf structure called **phylloclades** (*Opuntia*).
- In some of the others single or occasionally two internodes modified into fleshy green structure called **cladode** (*Asparagus*).

In some the petiole is modified into a fleshy leaf like structure called **phyllode** (*Acacia melanoxylon*).

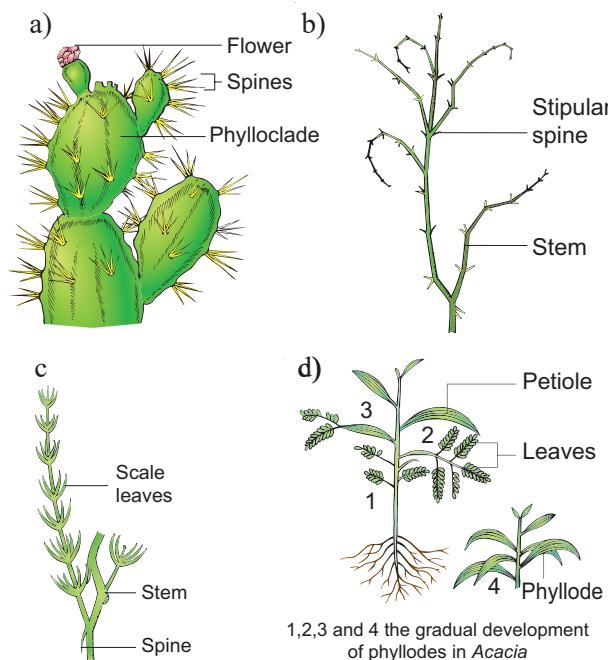


Figure 6.22: Xerophytes

- A succulent xerophyte: Phylloclade – *opuntia*
- Non succulent: Perennial - *Capparis*
- Cladode of *Asparagus*
- Phyllode – *Acacia*



In leaves

- Leaves are generally leathery and shiny to reflect light and heat.
- In some plants like *Euphorbia*, *Acacia*, *Ziziphus* and *Capparis*, the stipules are modified into spines.
- The entire leaves are modified into spines (*Opuntia*) or reduced to scales (*Asparagus*).

Anatomical adaptations

- Presence of multilayered epidermis with heavy cuticle to prevent water loss due to transpiration.
- Hypodermis is well developed with sclerenchymatous tissues.
- Sunken shaped stomata are present only in the lower epidermis with hairs in the sunken pits.
- Scotoactive type of stomata found in succulent plants.
- Vascular bundles are well developed with several layered bundle sheath.
- Mesophyll is well differentiated into palisade and spongy parenchyma.
- In succulents the stem possesses a water storage region.

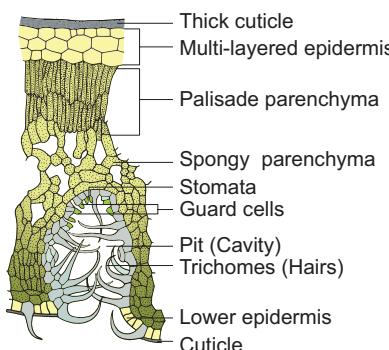


Figure 6.23: T.S. of *Nerium* leaf

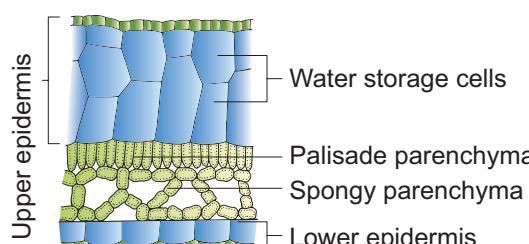


Figure 6.24: A Succulent leaf of *Pepronia* (T.S.)
(lateral wing portion only)

Physiological adaptations

- Most of the physiological processes are designed to reduce transpiration.
- Life cycle is completed within a short period (Ephemerals).

Mesophytes

The plants which are living in moderate conditions (neither too wet nor too dry) are known as **mesophytes**. These are common land plants. Example: Maize and *Hibiscus*.

Morphological adaptations

- Root system is well developed with root caps and root hairs
- Stems are generally aerial, stout and highly branched.
- Leaves are generally large, broad, thin with different shapes.

Anatomical adaptations

- Cuticle in aerial parts are moderately developed.
- Epidermis is well developed and stomata are generally present on both the epidermis.
- Mesophyll is well differentiated into palisade and spongy parenchyma.
- Vascular and mechanical tissues are fairly developed and well differentiated.

Physiological adaptations

- All physiological processes are normal.
- Temporary wilting takes place at room temperature when there is water scarcity.

Tropophytes are plants which behave as xerophytes at summer and behave as mesophytes (or) hydrophytes during rainy season.

Epiphytes

Epiphytes are plants which grow perched on other plants (Supporting plants). They use the supporting plants only as shelter and not for water or food supply. These epiphytes



are commonly seen in tropical rain forests. Examples: Orchids, Lianas, Hanging Mosses and Money plant.

Morphological adaptations

- Root system is extensively developed. These roots may be of two types. They are Clinging roots and Aerial roots.

Clinging roots fix the epiphytes firmly on the surface of the supporting objects.

Aerial roots are green coloured roots which may hang downwardly and absorb moisture from the atmosphere with the help of a spongy tissue called **velamen**.

- Stem of some epiphytes are succulent and develop pseudo bulb or tuber.
- Generally the leaves are lesser in number and may be fleshy and leathery
- **Myrmecophily** is a common occurrence in the epiphytic vegetation to prevent the predators.
- The fruits and seeds are very small and usually dispersed by wind, insects and birds.

Anatomical adaptations

- Multilayered epidermis is present. Inner to the velamen tissue, the peculiar exodermis layer is present.
- Presence of thick cuticle and sunken stomata greatly reduces transpiration.
- Succulent epiphytes contain well developed parenchymatous cells to store water.

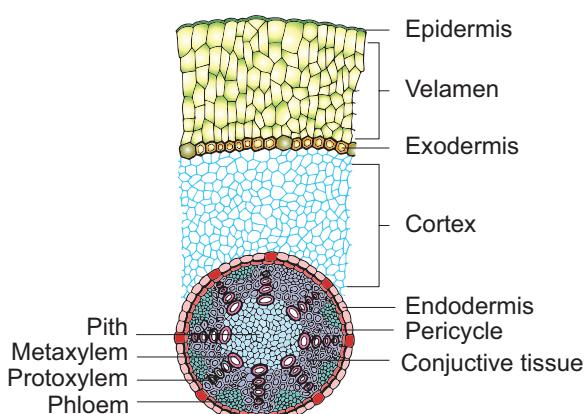


Figure 6.25: T.S. of an aerial root of orchid showing velamen tissue

Physiological adaptations

Special absorption processes of water by velamen tissue .

Halophytes

There are special type of **Halophytic plants** which grow on soils with high concentration of salts. Examples: *Rhizophora*, *Sonneratia* and *Avicennia*.

Halophytes are usually found near the sea-shores and Estuaries. The soils are physically wet but physiologically dry. As plants cannot use salt water directly they require filtration of salt using physiological processes. This vegetation is also known as **mangrove forest** and the plants are called **mangroves**.

Morphological adaptations

- The temperate halophytes are herbaceous but the tropical halophytes are mostly bushy
- In addition to the normal roots, many stilt roots are developed
- A special type of negatively geotropic roots called **pneumatophores** with **pneumatodes** to get sufficient aeration are also present. They are called breathing roots. Example: *Avicennia*

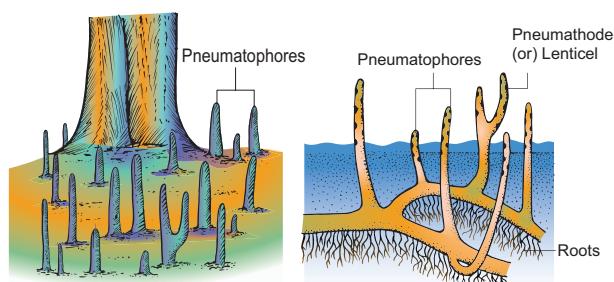


Figure 6.26a: Pneumatophores of mangrove plant

- Presence of thick cuticle on the aerial parts of the plant body
- Leaves are thick, entire, succulent and glossy. Some species are **aphyllous** (without leaves).

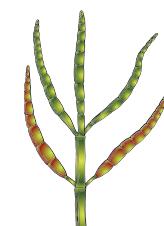


Figure 6.26b: Succulent halophyte - *Salicornia*



- Vivipary mode of seed germination is found in halophytes

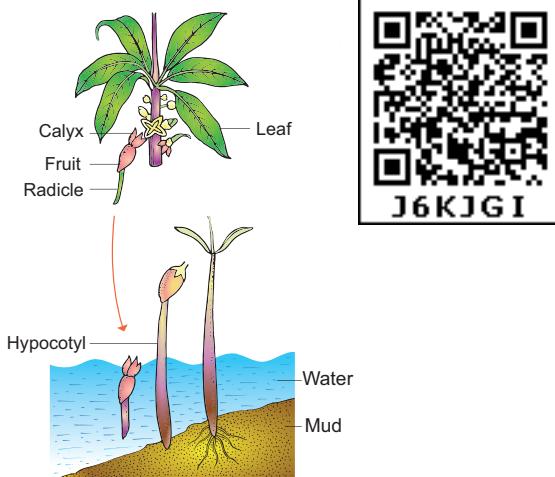


Figure 6.27: Vivipary germination

Anatomical adaptations

- Epidermal cells of stem is heavy cutinized, almost squarish and are filled with oil and tannins.
- ‘Star’ shaped sclereids and ‘H’ shaped heavy thickened spicules that provide mechanical strength to cortex are present in the stem.
- The leaves may be dorsiventral or isobilateral with salt secreting glands.

Physiological adaptations

- High osmotic pressure exists in some plants .
- Seeds germinate in the fruits of mother plant itself (**Vivipary**).



Out of three districts of Tamil Nadu (Nagapattinam, Thanjavur and Thiruvarur), Muthupet (Thiruvarur district) was less damaged by Gaja cyclone (November 2018) due to the presence of mangrove forest.

6.4 Dispersal of Fruits and Seeds

Both fruits and seeds possess attractive colour, odour, shape and taste needed for the dispersal by birds, mammals, reptiles, fish, ants and insects even earthworms. The seed consists of an embryo, stored food material and a

protective covering called **seed coat**. As seeds contain miniature but dormant future plants, their dispersal is an important criterion for distribution and establishment of plants over a wide geographical area. The dissemination of seeds and fruits to various distances from the parent plant is called seed and fruit dispersal. It takes place with the help of ecological factors such as wind, water and animals.

Seed dispersal is a regeneration process of plant populations and a common means of colonizing new areas to avoid seedling level competition and from natural enemies like herbivores, frugivores and pathogens.

Fruit maturation and seed dispersal is influenced by many ecologically favourable conditions such as Season (Example: Summer), suitable environment, and seasonal availability of dispersal agents like birds, insects etc.

Seeds require agents for dispersal which are crucial in plant community dynamics in many ecosystems around the globe. They offer many benefits to communities such as food and nutrients, migration of seeds across habitats and helps spreading plant genetic diversity.

6.4.1 Dispersal by Wind (Anemochory)

The individual seeds or the whole fruit may be modified to help for the dispersal by wind. Wind dispersal of fruits and seeds is quite common in tall trees. The adaptation of the wind dispersal plants are

- **Minute seeds:** Seeds are minute, very small, light and with inflated covering. Example: Orchids.
- **Wings:** Seeds or whole fruits are flattened to form a wing. Examples: Maple, *Gyrocarpus*, *Dipterocarpus* and *Terminalia*



Figure 6.28: Asclepias



Figure 6.29: Gyrocarpus



- **Feathery Appendages:** Seeds or fruits may have feathery appendages which greatly increase their buoyancy to disperse to high altitudes. Examples: *Vernonia* and *Asclepias*.
- **Censor mechanisms:** The fruits of many plants open in such a way that the seeds can escape only when the fruit is violently shaken by a strong wind. Examples: *Aristolochia* and Poppy.

Guess!! Who am I.....? I am dispersed by ant and I have caruncle.

6.4.2 Dispersal by Water (Hydrochory)

Dispersal of seeds and fruits by water usually occurs in those plants which grow in or near water bodies . Adaptation of hydrochory are

- Obconical receptacle with prominent air spaces. Example: *Nelumbo*.
- Presence of fibrous mesocarp and light pericarp. Example: Coconut.
- Seeds are light, small, provided with aril which encloses air.Example: *Nymphaea*.
- The fruit may be inflated. Examples: *Heritiera littoralis*.
- Seeds by themselves would not float may be carried by water current. Example: Coconut.



Figure 6.30: Nelumbo Figure 6.31: Coconut



beings and get dispersed.

ii. Sticky fruits and seeds:

- a. Some fruits have sticky glandular hairs by which they adhere to the fur of grazing animals. Example: *Boerhaavia* and *Cleome*.
- b. Some fruits have viscid layer which adhere to the beak of the bird which eat them and when they rub them on to the branch of the tree, they disperse and germinate. Example: *Cordia* and *Alangium*

iii. Fleshy fruits: Some fleshy fruits with conspicuous colours are dispersed by human beings to distant places after consumption. Example: Mango and *Diplocyclos*



Figure 6.32: Sunflower Figure 6.33: Papaya



6.4.3 Dispersal by Explosive Mechanism (Autochory)

Some fruits burst suddenly with a force enabling to throw seeds to a little distance away from the plant. Autochory shows the following adaptations.

- Mere touch of some plants causes the ripened fruit to explode suddenly and seeds are thrown out with great force. Example: *Impatiens* (Balsam), *Hura*.
- Some fruits when they come in contact with water particularly after a shower of rain, burst suddenly with a noise and scatter the seeds.Examples: *Ruellia* and *Crossandra*.
- Certain long pods explode with a loud noise like cracker, scattering the seeds in all directions. Example: *Bauhinia vahlii* (Camel's foot climber)
- As the fruit matures, tissues around seeds are converted into a mucilaginous fluid, due to which a **high turgor pressure** develops inside the fruit which leads to the dispersal of seeds.



Example: *Ecballium elatrium* (Squirting cucumber) *Gyrocarpus* and *Dipterocarpus*.



Figure 6.34: *Ecballium*



Figure 6.35: *Impatiens*

Human aided seed dispersal

Seed Ball : Seed ball is an ancient Japanese technique of encasing seeds in a mixture of clay and soil humus (also in cow dung) and scattering them on to suitable ground, not planting of trees manually. This method is suitable for barren and degraded lands for tree regeneration and vegetation before monsoon period where the suitable dispersal agents become rare.



Figure 6.36: Seed ball

Guess? what is atelochory or Achory?

Ecologically important days
March 21 - World forest day
April 22 - Earth day
May 22 - World bio diversity day
June 05 - World environment day
July 07 - Van Mohostav day
September 16 - International Ozone day

Advantages of seed dispersal:

- Seeds escape from mortality near the parent plants due to predation by animals or getting diseases and also avoiding competition.
- Dispersal also gives a chance to occupy favourable sites for growth.
- It is an important process in the movement of plant genes particularly this is the only method available for self-fertilized flowers and maternally transmitted genes in

outcrossing plants.

- Seed dispersal by animals help in conservation of many species even in human altered ecosystems.
- Understanding of fruits and seed dispersal acts as a key for proper functioning and establishment of many ecosystems from deserts to evergreen forests and also for the maintenance of biodiversity conservation and restoration of ecosystems.

Summary

Ecology is a division of biology and deals with the study of environment in relation to organisms. Ecology is mainly divided into two branches Autecology and Syncology. The environment (surrounding) includes physical, chemical and biological components. These factors can be classified into living (biotic) and non-living (abiotic), which make the environment of an organism. The ecological factors are meaningfully grouped into four classes, which are as follows: 1. Climatic factors 2. Edaphic factors 3. Topographic factors 4. Biotic factors.

Climate is one of the important natural factors controlling the plant life. The climatic factors includes light, temperature, water, wind, fire, etc. Edaphic factors, the abiotic factors related to soil, include the physical and chemical composition of the soil formed in a particular area. The surface features of earth are called topography. Topographic influence on the climate of any area is determined by the interaction of solar radiation, temperature, humidity ,rainfall, latitude and altitude. The interactions among living organisms, the plants and animals are called biotic factors, which may cause marked effects upon vegetation.

The modifications in the structure of organisms to survive successfully in an environment are called adaptations of organisms. Based on the habitats and the corresponding adaptations of plants, they are



classified into 1) Hydrophytes 2) Xerophytes 3) Mesophytes 4) Epiphytes and 5) Halophytes. The dissemination of seeds and fruits to various distances from the parent plant is called **seed and fruit dispersal**. It takes place with the help of ecological factors such as wind, water and animals.

Evaluation

1. Arrange the correct sequence of ecological hierarchy starting from lower to higher level.
 - a) Individual organism → Population Landscape → Ecosystem
 - b) Landscape → Ecosystem → Biome → Biosphere
 - c) community → Ecosystem → Landscape → Biome
 - d) Population → organism → Biome → Landscape
2. Ecology is the study of an individual species is called
 - i) Community ecology ii) Autecology
 - iii) Species ecology iv) Synecology
 - a) i only b) ii only
 - c) i and iv only d) ii and iii only
3. A specific place in an ecosystem, where an organism lives and performs its functions is
 - a) habitat b) niche
 - c) landscape d) biome
4. Read the given statements and select the correct option.
 - i) Hydrophytes possess aerenchyma to support themselves in water.
 - ii) Seeds of *Viscum* are positively photoblastic as they germinate only in presence of light.
 - iii) Hygroscopic water is the only soil water available to roots of plant growing in soil as it is present inside the micropores.
 - iv) High temperature reduces use of water and solute absorption by roots.



- a) i, ii, and iii only b) ii, iii and iv
- c) ii and iii only d) i and ii only
5. Which of the given plant produces cardiac glycosides?
 - a) *Calotropis*
 - b) *Acacia*
 - c) *Nepenthes*
 - d) *Utricularia*
6. Read the given statements and select the correct option.
 - i) Loamy soil is best suited for plant growth as it contains a mixture of silt, sand and clay.
 - ii) The process of humification is slow in case of organic remains containing a large amount of lignin and cellulose.
 - iii) Capillary water is the only water available to plant roots as it is present inside the micropores.
 - iv) Leaves of shade plant have more total chlorophyll per reaction centre, low ratio of chl *a* and chl *b* are usually thinner leaves.
 - a) i, ii and iii only b) ii, iii and iv only
 - c) i, ii and iv only d) ii and iii only
7. Read the given statements and select the correct option.
Statement A : Cattle do not graze on weeds of *Calotropis*.
Statement B : *Calotropis* have thorns and spines, as defense against herbivores.
 - a) Both statements A and B are incorrect.
 - b) Statement A is correct but statement B is incorrect.
 - c) Both statements A and B are correct but statement B is not the correct explanation of statement A.
 - d) Both statements A and B are correct and statement B is the correct explanation of statement A.
8. In soil water available for plants is
 - a) gravitational water
 - b) chemically bound water
 - c) capillary water
 - d) hygroscopic water



9. Read the following statements and fill up the blanks with correct option.
- Total soil water content in soil is called _____
 - Soil water not available to plants is called _____
 - Soil water available to plants is called _____

	(i)	(ii)	(iii)
(a)	Holard	Echard	Chresard
(b)	Echard	Holard	Chresard
(c)	Chresard	Echard	Holard
(d)	Holard	Chresard	Echard

10. Column I represent the size of the soil particles and Column II represents type of soil components. Which of the following is correct match for the Column I and Column II

Column - I	Column - II
I). 0.2 to 2.00 mm	i) Slit soil
II) Less than 0.002 mm	ii) Clayey soil
III) 0.002 to 0.02 mm	iii) Sandy soil
IV) 0.002 to 0.2 mm	iv) Loamy soil

	I	II	III	IV
a)	ii	iii	iv	i
b)	iv	i	iii	ii
c)	iii	ii	i	iv
d)	None of the above			

11. The plant of this group are adapted to live partly in water and partly above substratum and free from water
- Xerophytes
 - Mesophytes
 - Hydrophytes
 - Halophytes

- 12 . Identify the A, B, C and D in the given table

Interaction	Effects on species X	Effects on species Y
Mutualism	A	(+)
B	(+)	(-)
Competition	(-)	C
D	(-)	0

	A	B	C	D
a)	(+)	Parasitism	(-)	Amensalism
b)	(-)	Mutalism	(+)	Competition
c)	(+)	Competition	(0)	Mutalism
d)	(0)	Amensalism	(+)	Parasitism

13. *Ophrys* an orchid resembling the female of an insect so as to able to get pollinated is due to phenomenon of
a) Myrmecophily b) Ecological equivalents
c) Mimicry d) None of these
14. A free living nitrogen fixing cyanobacterium which can also form symbiotic association with the water fern *Azolla*
a) *Nostoc* b) *Anabaena*
c) *chlorella* d) *Rhizobium*
15. Pedogenesis refers to
a) Fossils b) Water c) Population d) Soil
16. Mycorrhiza promotes plant growth by
a) Serving as a plant growth regulators
b) Absorbing inorganic ions from soil
c) Helping the plant in utilizing atmospheric nitrogen
d) Protecting the plant from infection
17. Which of the following plant has a non-succulent xerophytic and thick leathery leaves with waxy coating
a) *Bryophyllum* b) *Ruscus*
c) *Nerium* d) *Calotropis*

18. In a fresh water environment like pond, rooted autotrophs are
a) *Nymphaea* and *typha*
b) *Ceratophyllum* and *Utricularia*
c) *Wolffia* and *pistia*
d) *Azolla* and *lemonia*



19. Match the following and choose the correct combination from the options given below:

Column I (Interaction)	Column II (Examples)
I. Mutualism	i). <i>Trichoderma</i> and <i>Penicillium</i>
II. Commensalism	ii). <i>Balanophora</i> , <i>Orobanche</i>
III. Parasitism	iii). <i>Orchids</i> and <i>Ferns</i>
IV. Predation	iv). <i>Lichen</i> and <i>Mycorrhiza</i>
V. Amensalism	v). <i>Nepenthes</i> and <i>Diaonaea</i>

	I	II	III	IV	V
a)	i	ii	iii	iv	v
b)	ii	iii	iv	v	i
c)	iii	iv	v	i	ii
d)	iv	iii	ii	v	i

20. Strong, sharp spines that get attached to animal's feet are found in the fruits of

- a) *Argemone*
- b) *Ecballium*
- c) *Heritier*
- d) *Crossandra*

21. Sticky glands of *Boerhaavia* and *Cleome* support

- a) Anemochory
- b) Zoothochory
- c) Autochory
- d) Hydrochory

22. Define ecology.

23. What is ecological hierarchy? Name the levels of ecological hierarchy.

24. What are ecological equivalents? Give one example .

25. Distinguish habitat and niche

26. Why are some organisms called as eurythermals and some others as stenohaline ?

27. 'Green algae are not likely to be found in the deepest strata of the ocean'. Give at least one reason.

28. What is Phytoremediation ?

29. What is Albedo effect and write their effects?

30. The organic horizon is generally absent from agricultural soils because tilling, e.g., plowing, buries organic matter. Why is an organic horizon generally absent in desert soils ?

31. Soil formation can be initiated by biological organisms. Explain how?

32. Sandy soil is not suitable for cultivation. Explain why?

33. Describe the mutual relationship between the fig and wasp and comment on the phenomenon that operates in this relationship.

34. *Lichen* is considered as a good example of obligate mutualism. Explain.

35. What is mutualism? Mention any two example where the organisms involved are commercially exploited in modern agriculture.

36. List any two adaptive features evolved in parasites enabling them to live successfully on their host?

37. Mention any two significant roles of predation plays in nature.

38. How does an orchid *ophrys* ensures its pollination by bees ?

39. Water is very essential for life. Write any three features for plants which enable them to survive in water scarce environment.

40. Why do submerged plants receive weak illumination than exposed floating plants in a lake?

41. What is vivipary? Name a plant group which exhibits vivipary.



42. What is thermal stratification? Mention their types.
43. How is rhytidome act as the structural defence by plants against fire?
44. What is myrmecophily?
45. What is seed ball?
46. How is anemochory differ from zoolochory?
47. What is co evolution?
48. Explain Raunkiaer classification in the world's vegetation based on the temperature.
49. List out the effects of fire to plants.
50. What is soil profile? Explain the characters of different soil horizons.
51. Give an account of various types of parasitism with examples.
52. Explain different types of hydrophytes with examples.
53. Enumerate the anatomical adaptations of xerophytes.
54. List out any five morphological adaptations of halophytes.
55. What are the advantages of seed dispersal?
56. Describe dispersal of fruit and seeds by animals.

Glossary

Antibiosis: An association of two organisms which is harmful to one of them.

Biome: A major regional community of plants and animals with similar life forms and environmental conditions.

Biosphere: The envelope containing all living organisms on earth.

Community: A group of organism living in the same place.

Flora: The kinds of plants in region

Frugivores: Fruit eating organisms

Hekistotherms: (Temperature less than 70°C) Where very low temperature prevails and the dominant vegetation is alpine vegetation.

Landscape: The visible features of an area of land.

Lianes: Twining vines with woody stems, common in forest of warm climate.

Megatherms: (Temperature more than 240°C) Where high temperature prevails throughout the year and the dominant vegetation is tropical rain forest.

Mesotherms: (Temperature ranges between 170°C and 240°C) Where high temperature alternates with low temperature and the dominant vegetation is tropical deciduous forest.

Microtherms: (Temperature ranges between 70°C and 170°C) Where low temperature prevails and the dominant vegetation is mixed coniferous forest.

Population: A group of individuals of a single species.

Scotoactive type of stomata: Stomata opens during night in succulent plants and closes during the day.

Vivipary: When seeds or embryos begin to develop before they detach from the parent.