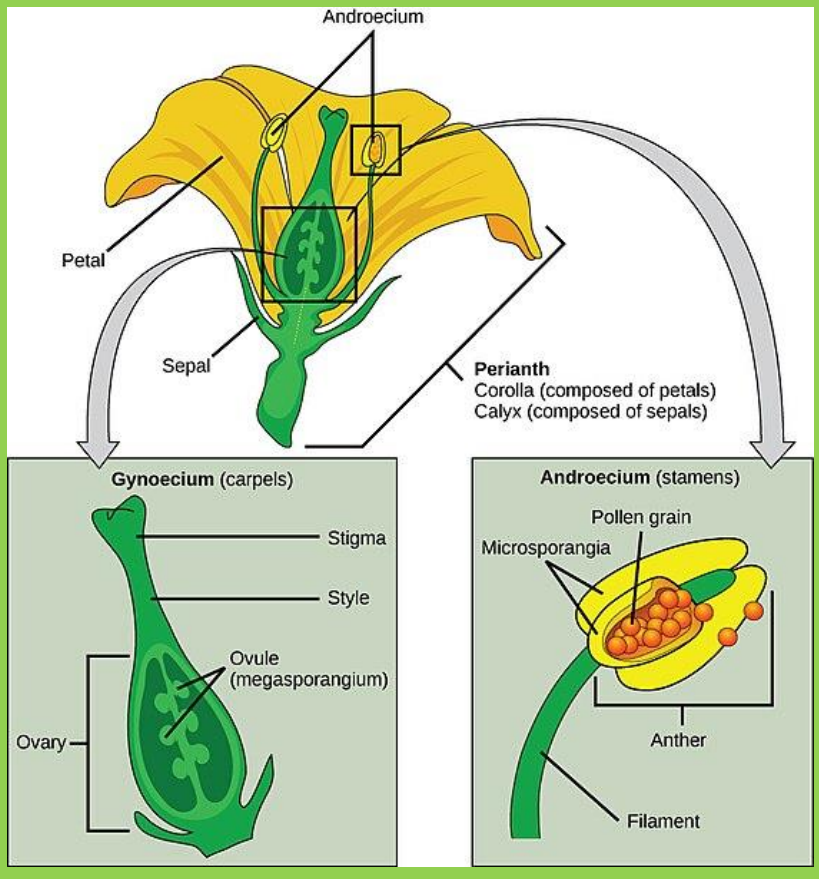
BOT(N)-202 & BOT(N)-202L

B.Sc. IV Semester

ANATOMY, EMBRYOLOGY AND ELEMENTARY MORPHOGENESIS



DEPARTMENT OF BOTANY

SCHOOL OF SCIENCES

UTTARAKHAND OPEN UNIVERSITY, HALDWANI

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BLOCK-1 GENERAL ANATOMY

UNIT-1 TOOLS AND TECHNIQUES IN PLANT ANATOMY

1.1 OBJECTIVES

After reading this unit students will be able -

- to familiar with the history of microscopy and different parts of compound microscopes.
- to learn different techniques of anatomy like sectioning and staining.
- to know Mounting media and mounting techniques.
- to explain the common stains for plant cells

1.2 INTRODUCTION

As in all experimental sciences, research in plant anatomy depends on the laboratory methods that can be used to study cell structure and function. Many important advances in understanding cells have directly followed the development of new methods that have opened novel avenues of investigation. An appreciation of the experimental tools available to the cell biologist is thus critical to understanding both the current status and future directions of this rapidly moving area of science. The elements of the plant cell are the membrane and the protoplast. The protoplast includes the cytoplasm, the nucleus, the plastids, the mitochondria, and other organelles.

In the past, the chief objects of study in plant anatomy were the vegetative organs (stem, root, and leaf); today, attention is also given to the structure of flowers, fruits, and seeds. Within the field of plant anatomy there is:

(1) Physiological plant anatomy, which is concerned with the links existing between plant structure and internal processes.
(2) Ecological plant anatomy, which is the study of environmental effects on plant structure.
(3) Pathological plant anatomy, which is the study of the effect of disease-producing agents of a biological, physical, and chemical character on plant structure, and
(4) Comparative or systematic plant anatomy, which introduces the comparative study of representatives of the different systematic groups (taxa) - species, genera, families, and so forth for the clarification of their phylogenetic bonds.

The basic method used in plant anatomy, or the study of internal plant structure, is the preparation of thin slices which are studied microscopically. From this the science “derives its name (in Greek, *anatome* means “dissection”). The emergence of the field of plant anatomy is closely related to the invention and perfection of the microscope. The English physicist R. Hooke observed in 1665 the cellular structure of thin slices of cork, elder pith, and wood from various plants, using a microscope of his own improved design.

The real founders of plant anatomy, however, are considered to be the Italian biologist M. Malpighi and the English botanist N. Grew, who published the first (1675–79) and the second

(1682) works on this subject; in these works the results of a systematic microscopic study of plant material were presented. Further development came only at the beginning of the 19th century. The German scientist J. Moldenhawer in 1812 and the French researcher R. Dutrochet in 1824 were able to divide plant tissue into its component cells through maceration (soaking). In 1831 the English botanist R. Brown observed the cell nucleus; this achievement, in combination with the studies of the German botanist M. J. Schleiden, played a great role in the founding of cellular theory, whose author was the German biologist T. Schwann (in 1839). Great contributions to the field of plant anatomy were made by the French biologist Edward. van Tieghem and the German biologists Antony de Bary, Carl Von Nageli, K. Sanio, J. Hanstein, and S. Schwendener.

1.3 TOOLS IN PLANT ANATOMY

The theoretical knowledge is incomplete without the practical work. Plants are easily available material for the lab studies and their study in the lab adds immense knowledge to the subject. The practical work develops the scientific outlook and makes the rational approach based on facts and figures. For a better observation and defining the anatomical features of the plants in the laboratory we use different tools and techniques.

Practical Microscopy: The cells of plants are quite minute and microscopic in size, so cannot be observed by naked eyes. Such objects are visible only under microscopes. Our eye has limited magnification or resolution power so unable to distinguish the objects smaller than 0.1 mm. Moreover the living cells are transparent in ordinary light and cannot be distinguished among various cellular components. The microscopes are the most important tools in the plant anatomy and their magnification power is achieved by lenses of various type. The fascinating world of microorganisms and different anatomical features would have remained unknown had the microscope not been invented.

Roger Bacon (1267) described a lens for the first time. However, his observation was not pursued immediately thereafter. In 1590 glass polishers Hans and Zacchrius Jensen constructed a crude type of simple microscope by placing two lenses together, which permitted them to see minute objects. In 1609-1610 Galileo made the first simple microscope with a focusing device and observed the water flea through his microscope. In 1617-1619 the first double lens microscope with a single convex objective and ocular appeared the inventor of which was thought to be the physicist C. Drebbel. This microscope was used to study the cells, plant and animal tissue, and also the minute living organisms. Till then, the name microscope had not been given to this device; the name ‘microscope’ was first proposed by Faber in 1625. The credit of developing a compound microscope with multiple lenses goes to Robert Hooke (1665) of

England. It was only after 1670 that a cloth merchant of Delft (Holland), Antony van Leeuwenhoek (1632-1723), started his hobby of making microscopes. Considerable progress was made in improving the microscope in nineteenth century.

Compound Microscope: A compound microscope is the primary tool in the anatomy. Therefore, a clear understanding of structure, use and manipulations of a compound microscope is a must for all students of anatomy (Fig.1.1).

a. Essential parts: The essential parts of usually used monocular compound microscope are the following:

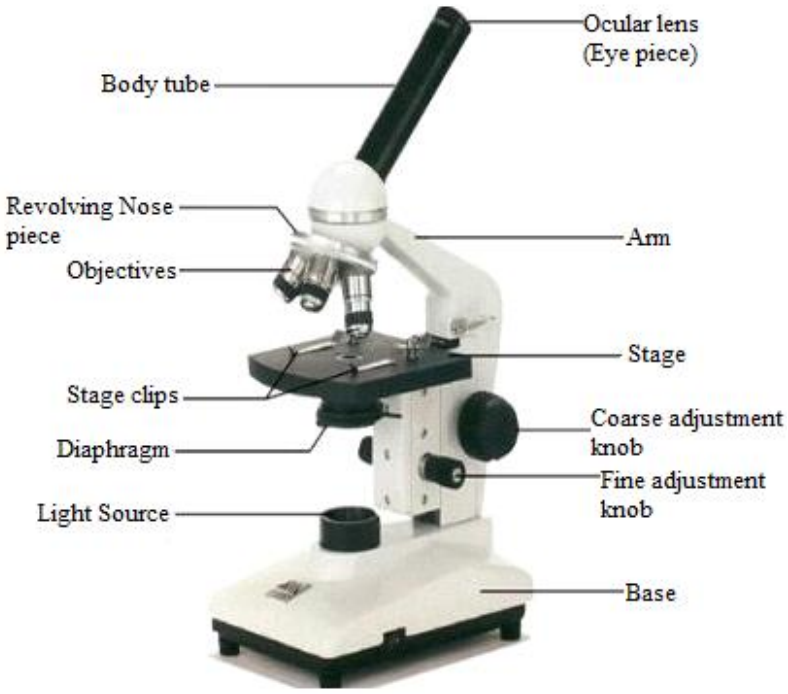


Fig. 1.1: Compound Microscope

Lenses: The **eye piece** with different magnifications (5-20 times). It has field lens towards the object and eye-lens close to the observer's eye. The **objectives** generally with three different magnifications viz., low (10 X), high (40 X) and oil-immersion (97 X). The focal lengths of these are 16 mm, 4mm, and 1.6 mm respectively. These objectives are mounted on a revolving nose piece for convenience. The eye piece and objectives are fitted at the two ends of a hollow tube called the 'body tube'.

Adjustment of objective lens: In some microscopes coarse arid fine focusing adjustment knobs are both provided in order to lower or raise the body tube with lenses for rendering image clear. This is done by rotation of the knobs. The coarse adjustment is meant to bring the object into vision whereas the fine adjustment is used for focusing finer details.

Stage: The object to be observed is kept on a glass slides and placed on the stage. It may have clips to keep the slide in desired position or a mechanical stage for horizontal movement of the object. In some microscopes the stage may be raised or lowered with coarse and fine adjustments for focusing the object.

Mirror: The mirror reflects light, which is transmitted through the object for observing it. The mirror has two planes, one concave and the other plane. When natural light is available the plane mirror may be used for reflection of light because concave mirror would form window images. However, with artificial illumination, the concave mirror is necessary for higher magnification whereas for lower, the plane mirror may be used.

Substage diaphragm: This is meant to control the amount of light transmitted through the object.

Substage condenser: The substage condenser consists of convex lenses which concentrate and intensify the light reflected by-the mirror. With objectives of magnification exceeding 10X, the use of condenser becomes necessary for narrowing the core of transmitted light, which would fill the smaller aperture of the objective. The condensers usually employed are called 'Abbe' condensers and these are used with plane mirrors.

1.4 TECHNIQUES IN PLANT ANATOMY

Solid material should be sectioned in several planes in order to discover the distribution of the various tissues within it. The complete investigation of axial structures, such as stem or root, normally requires a transverse (cross) section at one, or more, levels; and radial longitudinal, and tangential longitudinal sections at different depths from the surface to the center. Foliar structures generally require transverse, and paradermal sections; and vertical longitudinal sections may occasionally be necessary. For anatomical study different techniques are used for visualizing the cells. Some of the techniques are given below.

i) Epidermal peels: The superficial tissues of many plant parts (especially leaves) may be peeled away in strips thin enough for microscopic examination. To make such a peel, break or cut the surface of the plant apart. Then, grip the epidermis with forceps at one of the cut edges, and pull the outer tissue layer back away from the cut. The resulting epidermal peel should be mounted in water containing a wetting agent, or in alcohol if it is very hydrophobic.

ii) Macerations: The three-dimensional form of a cell is most easily seen when the cell is separated from the surrounding cells of the tissue. Macerating fluids accomplish this through a hydrolysis of the middle lamella. The following method is a gentle, but effective technique: Cut small pieces of the tissue into a mixture of 1 part Hydrogen peroxide, 4 parts distilled water, and 5 parts glacial acetic acid. Cook the mixture in a 56-60 degree oven for 24 hours.

If further macerating is needed, replace the old fluid with a fresh mixture and cook the tissues for another 24 hours. Repeat the process until the material is mostly colorless, and may be easily teased apart with a dissecting probe. When the maceration is complete, rinse the tissues in water in an uncovered container. Stain in 0.25% Safranin in water and mount in dilute glycerin.

iii) Squashes: Material can be squashed on a slide for cytological examination. This technique is most often used for chromosome counts and examination of mitotic structures. Dissect away non-meristematic tissues, chop the meristem with a scalpel, place a cover slip over the tissue, place paper towels on the cover slip and apply vertical pressure through your thumb.

iv) Free-Hand Sectioning: Material should be kept moist while sectioning. Liquid should be kept on the razor blade, so that the sections float as they are cut. In general, it is inadvisable to take particular care over individual sections. Better results are usually obtained by cutting a large number of slices rapidly, and sorting out the best ones. Sections of uniform thinness are usually not necessary. Wedge-shaped slices which taper from opaque, overly thick margins to ultra-thin edges will show useable areas of the proper thickness. When cutting longitudinal sections, it is important to use a short piece of material not much longer than wide. It is impossible to cut satisfactory longitudinal sections of any considerable length by the freehand technique. Flexible structures, such as leaves, require some support during sectioning. Many leaves will yield good transverse, and vertical longitudinal sections if rolled or folded so that 10 or more thicknesses are cut at each stroke. If some extra support is necessary, the material may be inserted into the cut and of a young carrot which has been pickled in alcohol. The material and the surrounding carrot tissue are then cut at the same time. This technique should produce results superior to those of the more classical elderberry pith method. To obtain paradermal sections of a leaf, bend it over a finger and cut small slices off the curved surface. Sections of dry material should first be soaked in alcohol or hot water to soften it and remove air from the cells.

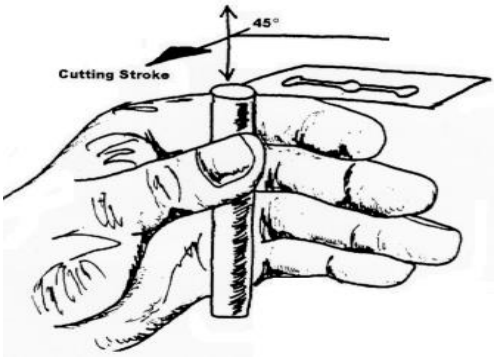


Fig.1.2: Hand sectioning

Steps in Sectioning

1. Obtain a new double edge razor blade. To minimize the risk of cutting oneself, cover one edge of the razor blade with masking tape. Rinse the blade with warm tap water to remove traces of grease from the surface of the blade if necessary.
2. Hold the plant material firmly. The material should be held against the side of the first finger of the left hand (or right hand) by means of the thumb (Fig.1.2). The first finger should be kept as straight as possible, while the thumb is kept well below the surface of the material out of the way of the razor edge.
3. Flood the razor blade with water this will reduce the friction during cutting as sections can float onto the surface of the blade. Take the razor blade in the right hand (or left hand) and place it on the first finger of the left hand (or right hand), more or less at a right angle to the specimen.
4. Draw the razor across the top of the material in such a way as to give the material a drawing cut (about 45^0 in the horizontal direction). This results in less friction as the razor blade passes through the specimen. Cut several sections at a time. Sections will certainly vary in thickness. However, there will be usable ones among the "thick" sections!
5. Transfer sections to water, always using a brush, not a forceps or needle.
6. Select and transfer the thinnest sections (the more transparent ones) onto a glass slide and stain.

Note: For cross sections, special care should be taken during sectioning to see that the material is not cut obliquely. During sectioning, a number of sections should be cut at the same time and one should not worry about the section thickness at this time. By slightly and progressively increasing the pressure with the razor blade on the first finger, and simultaneously exerting increasing pressure onto the specimen by the thumb, a number of sections can be cut without moving the material or the thumb. It is best to start cutting with the razor blade right at the surface of the specimen rather than against the side of the material. Since the root and stem usually have a radial symmetry, it is usually not necessary that a section should be complete, as long as it includes a portion of the tissues from the center to the outer edge of the specimen. For delicate and hard to hold specimens such as thin leaves and tiny roots, additional support can be used to facilitate hand sectioning. The following methods will allow for the sectioning of thin leaves and small, soft specimens such as roots. Tissue pieces can be inserted into a small piece of pith such as a carrot root. Once the tissue is firmly in place, the hand sectioning technique can be applied. Longitudinal sections are also difficult to obtain by hand without supporting material as small stem and root pieces are difficult to hold with one's finger. However, by cutting a v-shaped notch into the pith support, it is possible to hold the tissue firmly for free hand sections.

Treatment of Sections: The student should become familiar with the use of the following simple techniques and apply them in the study of laboratory materials: "Wet" water used for preparing wet mounts of specimens for general observations with a minimum of trapped air. Dilute glycerin used for preparing wet mounts for general observations when rapid drying of the mount is undesirable. Chloral hydrate is used to clear whole structures or sections which are not otherwise transparent. Mount the specimen in a few drops of chloral hydrate under a cover glass. Warm the mount over alcohol lamp until it seems more transparent. Do not allow the fluid to boil violently. Observe the mount directly, or rinse and remount in glycerin. This treatment renders cell walls visible, but removes most cell contents. Starch grains are dissolved, but crystals of calcium oxalate do not dissolve. Aniline blue used to stain callose in the phloem, and to stain nuclei and nucleoli.

When cut, sections of fresh material should be placed in water, and those of preserved materials into alcohol. However, it may be necessary to place sections of some fresh materials into alcohol to get rid of the air within them. In order to avoid unnecessary handling of the sections, all treatments should be carried out on the slides on which the sections will ultimately be mounted. When the liquid surrounding the section is to be changed, merely add a pool of the new liquid at one end of the slide. Pull the section into the new liquid with a water color brush, and pour the old liquid off the other end of the slide. Some techniques may require especially long rinses. In those cases, the sections may be rinsed more thoroughly in a shallow dish.

The best instrument for moving sections when using a dish is a small watercolor brush. Sections of preserved material should be mounted in a drop of dilute glycerin. Sections of fresh material should be mounted in water or dilute glycerin. A few air bubbles in the final mount are generally not a problem, as long as they can be recognized and do not obscure the areas of interest. The size and frequency of such bubbles may be reduced by mounting sections in water containing a wetting agent such as glycerin or detergent.

Clearing: This technique is especially useful for examination of the intact vascular systems of leaves and floral parts. However, it may also be resorted to as a means of making thick free hand sections more transparent. The easiest method is to clear tissue by incubating it in Ethyl Alcohol to remove hydrophobic pigments including chlorophyll. This may take several hours. Many such plant parts of either fresh, preserved or dry materials may be cleared sufficiently by warming them in chloral hydrate. Others may require treatment with NaOH as follows:

Clear in 5% NaOH in a petridish in an oven (the time varies from one to several days depending on the material). Wash 3 to 5 times in distilled water carefully with a pipette. If more clearing

appears to be necessary, use a saturated aqueous solution of chloral hydrate for 24 hours. Wash again in distilled water.

v) Staining

The most basic reason that cells are stained is to enhance visualization of the cell or certain cellular components under a microscope. Cells may also be stained to highlight metabolic processes or to differentiate between live and dead cells in a sample. Cells may also be enumerated by staining cells to determine biomass in an environment of interest. By using different stains, one can preferentially stain certain cell components, such as a nucleus or a cell wall, or the entire cell. Most stains can be used on fixed, or non-living cells, while only some can be used on living cells, some stains can be used on either living or non-living cells.

Cell staining techniques and preparation depend on the type of stain and analysis used. One or more of the following procedures may be required to prepare a sample:

a) Permeabilization: treatment of cells, generally with a mild surfactant, which dissolves cell membranes in order to allow larger dye molecules to enter inside the cell.

b) Fixation: serves to "fix" or preserve cell or tissue morphology through the preparation process. This process may involve several steps, but most fixation procedures involve adding a chemical fixative that creates chemical bonds between proteins to increase their rigidity. Common fixatives include formaldehyde, ethanol, methanol, and/or picric acid.

c) Mounting: involves attaching samples to a glass microscope slide for observation and analysis. Cells may either be grown directly to the slide or loose cells can be applied to a slide using a sterile technique. Thin sections (slices) of material such as tissue may also be applied to a microscope slide for observation.

d) Staining: application of stain to a sample to color cells, tissues, components, or metabolic processes. This process may involve immersing the sample (before or after fixation or mounting) in a dye solution and then rinsing and observing the sample under a microscope. Some dyes require the use of a mordant, which is a chemical compound that reacts with the stain to form an insoluble colored precipitate.

Botanical specimens from differing divisions (based on their taxonomy) respond to stains in a unique way. Stains that Bryophytes require might not be the same for Algae or Fungi, or even Pteridophytes. The most commons stains used in laboratory work are Aniline blue, Fast green, Safranin, Cotton blue, Methylene blue or Crystal violet. Media used for mounting may vary between Glycerine 10%, Glycerine jelly, Lactophenol, Erythrosine or Canada Balsam (or D.P.X. Mountant) depending on whether they are for temporary or permanent preparations.

Algae: Temporary preparations

- Single staining: Aniline blue - 0.1% aqueous, Fast green - 0.5% aqueous, Safranin - 0.5% aqueous
- Mounting media: Glycerine 10 % or glycerine jelly

Fungi: Temporary preparations

- Single staining: Cotton blue, Analine blue
- Mounting media: Lactophenol or glycerine 10%

Bryophytes: Temporary preparations

- Single staining: Safranin and Fast green
- Mounting media: Glycerine 10% or glycerine jelly

Pteridophytes: Temporary and permanent preparations

Double staining

- Primary stains: Safranin Fast green
- Secondary stains: Crystal violet Erythrosine
- Mounting media: Glycerine 10% for temporary preparations and Canada balsam or D.P.X. mountant for permanent preparations.

Gymnosperms: Temporary and permanent preparations

Double staining

- Primary stains: Safranin Fast green
- Secondary stains: Crystal violet Erythrosine
- Mounting media: Glycerine 10% for temporary preparations and Canada balsam or D.P.X. mountant for permanent preparations.

Angiosperms: Temporary and permanent preparations

Double staining

- Primary stains: Safranin Fast green
- Secondary stains: Crystal violet Erythrosine
- Mounting media: Glycerine 10% for temporary preparations and Canada balsam or D.P.X. mountant for permanent preparations.

Mixtures of Some Common Stains

Crystal violet:

- Crystal violet : 3 gms
- Distilled water : 80 ml
- Ethyl alcohol (95%) : 20 ml, dissolved and mixed with 0.8 gms of ammonium oxalate.

It is a violet dye and is used to stain the lignified tissues.

Methylene blue:

- Methylene blue : 0.3 gms
- (0.01%) distilled water - 100 ml
- Ethyl alcohol (95%) : 30 ml, dissolved and mixed with potassium hydroxide

It is used to stain cellulose walls.

Safranin:

- Safranin : 0.25 gms
- Alcohol (95%) : 10 ml
- Distilled water : 100 ml

This is mainly used to stain lignified tissues.

Fast Green:

- Fast Green: 0.5 gm
- Alcohol (95%): 100c.c.

Bacteria can be divided into gram negative and gram positive bacteria. Gram's stain is used for this purpose and help with microscopic studies of the same.

Gram's iodine solution: iodine - 1 gm, potassium iodide, 2 gms and distilled water 300 ml.

vi) Common Biological Stains

Different stains react or concentrate in different parts of a cell or tissue, and these properties are used to advantage to reveal specific parts or areas. Some of the most common biological stains are listed below. Unless otherwise marked, all of these dyes may be used with fixed cells and tissues; vital dyes (suitable for use with living organisms) are noted.

Carmine

Carmine is an intensely red dye used to stain glycogen, while Carmine alum is a nuclear stain. Carmine stains require the use of a mordant, usually aluminium.

Crystal violet

Crystal violet, when combined with a suitable mordant, stains cell walls purple. Crystal violet is the stain used in Gram staining. Crystal violet stains the acidic components of the neuronal cytoplasm a violet color, often used in brain research.

Eosin

Eosin is most often used as a counter stain to haematoxylin, imparting a pink or red color to cytoplasmic material, cell membranes, and some extracellular structures. It also imparts a strong red color to red blood cells. Eosin may also be used as a counter stain in some variants of Gram staining, and in many other protocols. There are actually two very closely related compounds commonly referred to as eosin. Most often used is eosin Y (also known as eosin Y was or eosin yellowish); it has a very slightly yellowish cast. The other eosin compound is eosin B (eosin bluish or imperial red); it has a very faint bluish cast. The two dyes are interchangeable, and the use of one or the other is more a matter of preference and tradition.

Acid fuchsine

Acid fuchsine may be used to stain collagen, smooth muscle, or mitochondria. Acid fuchsine is used as the nuclear and cytoplasmic stain in Mallory's trichrome method. Acid fuchsine stains cytoplasm in some variants of Masson's trichrome. In Van Gieson's picro-fuchsine, acid fuchsine imparts its red color to collagen fibers. Acid fuchsine is also a traditional stain for mitochondria (Altmann's method).

Haematoxylin

Haematoxylin (hematoxylin in North America) is a nuclear stain. Used with a mordant, haematoxylin stains nuclei blue-violet or brown. It is most often used with eosin in H & E (Haematoxylin and Eosin) staining-one of the most common procedures in histology.

Iodine

Iodine is used in chemistry as an indicator for starch. When starch is mixed with iodine in solution, an intensely dark blue color develops, representing a starch/iodine complex. Starch is a substance common to most plant cells and so a weak iodine solution will stain starch present in the cells. Iodine is one component in the staining technique known as Gram staining, used in microbiology. Iodine is also used as a mordant in Gram's staining; it enhances dye to enter through the pore present in the cell wall/membrane.

Methyl green

Methyl green is used commonly with bright-field microscopes to dye the chromatin of cells so that they are more easily viewed.

Methylene blue

Methylene blue is used to stain animal cells, such as human cheek cells, to make their nuclei more observable. Also used to stain the blood film and used in cytology.

Safranin

Safranin (or Safranin O) is a nuclear stain. It produces red nuclei, and is used primarily as a counter stain. Safranin may also be used to give a yellow color to collagen.

vii) Anatomical drawings

When you make anatomical drawings, you develop several useful skills including the ability to interpret complex information, identify diagnostic features that distinguish among similar structures, and represent and communicate this information in visual form. The purpose of a drawing is to convey information, and to provide you with a record of what you have seen for future reference. To best accomplish this, use the following steps when planning your drawings:

- Select the magnification and field according to what you are asked to illustrate. Given the same prepared slide, you might be asked to illustrate:
  a) a cell type, b) the arrangement of cells within a tissue, or c) an arrangement of tissues within an organ (i.e., a diagram). The resulting drawings would be very different.
- Include details that distinguish the subject from other similar structures. Given the assignments above, your drawings might include:
  a) details of the individual cells, b) outline of individual cells with enough detail to distinguish among cell types or c) you may not need to draw individual cells at all. If the point is to show how vascular bundles arranged, you need only outline the boundaries of vascular bundles.
- Label all distinguishing features. Each drawing should be labeled with the :
  - plant material used
  - type of sectioning and plane, if appropriate
  - fixation and staining
  - magnification and microscopy used

viii) Staining and Permanent Slide Preparation Procedure

- Deparaffinize in xylene and bring slides to 70% using a graded Ethyl alcohol series using 10, 20, 30, and 50 and than 70% solution. Coating is optional and is used only if test sections fall off the slides.
- Stain 2–24 h in safranin staining solution.
- Wash out excess stain for a few moments with distilled water. You may use running water but take care not to dislodge sections. A good technique is to run water through a flexible tube into the bottom of the staining dish.
- Dehydrate for 10 sec. in 95% Ethyl Alcohol plus 0.5% picric acid. Picric acid will cause safranin differentiation.
- Wash for 10 sec. to 1 min. (no longer) in 95% + 4 drops ammonium hydroxide per 100 ml to stop picric acid action. Excessive Ethyl Alcohol washing will completely remove Safranin staining.
- Dip briefly (10 s) in 100% Ethyl Alcohol to finish dehydration.
- Counter stain for 10-15 sec. in Fast Green staining solution. Test staining on a single slide and dilute the Fast Green solution if it is too concentrated.
- As the Fast Green staining solution evaporates with use, add additional solvent, not dye solution to maintain the correct dye concentration.
- Rinse excess Fast Green with “used clearing solution.” You can use either a Coplin jar for a few slides or a staining dish for many slides.

- Wash slides in clearing solution by dipping the sections for 5-10 sec.
- Remove clearing solution by dipping for a few moments into xylene plus 2-3 drops 100% Ethyl Alcohol (to remove residual water).
- Keep the slides in the final xylene solution while you mount the cover slip one slide at a time. Do not allow the slides to dry before mounting cover slip, because tissue damage may occur.

1.5 SUMMARY

Plant cells are quite minute and cannot be seen through naked eyes. For understanding the anatomical features of the plant different techniques are used such as microscopy, sectioning, staining etc. For anatomical analyses, the xylem and phloem of stems, branches, roots (root collar), rhizomes of dicots and monocots, needles, leaves and below-ground stems can be used. Microscopes are important tools for observation due to immense resolution power and the magnification of the microscope is determined by multiplying the magnification of the eyepiece by the magnification of the objective lens. In order to reveal the cellular structure plant material are being cut in various planes. Normally cross and longitudinal sections are taken for the study. These sections are stained through chemical stains and then after mounting we put them under microscope for the study. Staining is used for tissue differentiation with different types of stains which are normally chemical dyes. We can also preserve our sections by making permanent preparation of the slides.

1.6 GLOSSARY

Microscopy: Technique to see or visualize the microscopic objects through lenses
Aleuron: Protein grains found in general protoplasm used as reserve food material
Biometry: Application of mathematics to study living things
Clearing: Process of making thick sections more transparent
Cross section: Here the section passes at the right angle to the material
Cyanin: Blue pigment
Double staining: Use of two dyes for coloring the tissues of plants
Longitudinal section: Section is cut at the right angle to the transverse axis
Maceration: Process of separation of cells from the surrounding cells
Mounting: Attaching a section to the slide
Sectioning: Process of cutting plant material in thin slices
Staining: Technique to color the tissues by different chemicals
Squash: Technique for studying cell by crushing them over a slide

1.7 SELF ASSESSMENT QUESTION

1.7.1 Fill in the blanks with the appropriate answer.

1-Greek letter "*anatome*" means …….
2-…… and …… are considered as father of plant anatomy.
3-Name microscope was given by ……
4-Superficial tissue of plants are …….. in strips for microscopic study.
5-The best instrument for moving sections is ……..
6-…… means attaching sample to glass microscopic slides for observation.
7-…… is used to stain cells of fungi.
8-For temporary preparations …… is the most common mounting media.
9-…… is used to stain lignified tissues.
10-For staining bacteria we use ……..

Answer Key:

1.7.1. Fill in the blanks-

1-Dissection, 2- Malpighi and Grew, 3- Faber(1625), 4- Peeled (Peeling), 5- Coloring brush, 6-Mounting, 7- Aniline blue, 8- Glycerine, 9- Safranin, 10- Grahm stain

1.8 REFERENCES

- Carlquist, S. & Schneider, E.L. Origins and nature of vessels in Monocotyledons. I. *Acorus*. *International Journal of Plant Sciences*. 1997: 158:52–56.
- Craig, Richard and Vassilyev, Andrey. "*Plant Anatomy*". McGraw-Hill. Archived from the original on 24 July 2010.
- Esau, K. (1965). *Plant* A*natomy*. John Wiley & Sons Inc., New York.
- Esau, K. (1977). A*natomy of Seed Plants*. John Wiley & Sons Inc., New York.
- Esau's Plant Anatomy, Meristems, Cells, and Tissues of the Plant Body: their Structure, Function, and Development. 3rd edn.". *Annals of Botany* 99 (4):785–786.
- Fahn, A. (1990). *Plant Anatomy*. Pergamon Press, Oxford.
- Pandey, B. P. (2001). *Plant Anatomy*. S. Chand & Company Ltd., New Delhi.
- Pandey B.P. (2012). *Modern Practical Botan.* Vol II. S. Chand & Company Ltd., New Delhi.
- Pandey, S.N. (1997). *Plant Anatomy and Embryology*. Vikas Publishing House Pvt Ltd, New Delhi.
- Singh, V. 2010. *Plant Anatomy and Embryology of Angiosperms*. Global Media Publications. Meerut.

- Sharma A.K. & Sharma R. 2010. *Structure Development and Reproduction in Flowering Plants*. Jagdamba Publishing Co., New Delhi.

1.9 SUGGESTED READINGS

- Esau K. (1965). *Plant anatomy*. John Wiley & Sons. Inc., New York.
- Fahn, A. (1990). *Plant Anatomy*. Pergamon Press, Oxford.
- Pandey, B. P. (2001). *Plant Anatomy*. Published by S. Chand & Company Ltd., New Delhi.
- Pandey, S.N. (1997). *Plant Anatomy and Embryology.* Vikas Publishing House Pvt Ltd, New Delhi.
- Singh, V. (2010). *Plant Anatomy and Embryology of Angiosperms*. Global Media Publications, Meerut.
- Vasishta, P.C. (1968). *Plant Anatomy.* Pradeep Publication & Co., Chandigarh.

1.10 TERMINAL QUESTIONS

1.10.1 Answer the following questions in two or three sentences.

1- Define plant anatomy.
2- What is sectioning?
3- What is staining?
4- What is a transverse section?
5- Define single staining in plants.
6- What is mounting?
7- What do you understand by clearing?
8- What are the different tissues stained by biological stains?
9- What does the word "maceration" mean?

1.10.2 Answer the following questions in about 100 words.

1- How many types of anatomy are there in botany?
2- What are the different parts of compound microscopes?
3- Name different types of stains used for Algae, Fungi, Bryophytes, Pteridophytes and Gymnosperms.
4-Define sectioning technique.
5- What is double staining? Define it in Pteridophytes.
6- What is squash technique and why it is used?

1.10.3 Answer the following questions in about 200 words.

1- Draw a well labelled diagram of compound microscopes and define its different parts.

2- Describe in detail about the permanent preparation of slides.
3- Define the different types of stains and their nature used in plant anatomy?
4- Define the process of mounting.

UNIT-2 TYPES OF TISSUES, ANATOMY OF ROOT, SHOOT AND LEAF

2.1 OBJECTIVES

After reading this unit, students will be able to:

- Explain tissue and different types of tissue
- Discuss meristematic tissue
- Recall permanent tissue and their position
- Discuss epidermal tissue system
- Explain the root and shoot anatomy
- Compare the anatomy of dicot and monocot stem
- Discuss anatomical difference of monocot and dicot leaves

2.2 INTRODUCTION

Students as you know that Plant anatomy or Phytotomy is the general term for the study of the internal structure of plants. The science of the structure of the organized plant body learned by dissection is called Plant Anatomy (anatomy-dissection). In general, Plant Anatomy refers to study of internal morphology, pertaining to different tissues. The subject of this chapter is internal structure of Angiosperms, with emphasis on primary tissues. While originally it included plant morphology, which is the description of the physical form and external structure of plants, since the mid-20th century the investigations of plant anatomy are considered a separate, distinct field, and plant anatomy refers to *just* the internal plant structures.

In higher plants the plant body is more complex and the cells differ in their kind, form and origin. A study of internal structure shows that the vascular cryptogams and the spermatophyte have many types of cells that cluster together to form various types of tissue system. Plant body in Angiosperms is differentiated into root, stem, leaf and flower. All these parts are made up of different types of tissues containing different cell types. A tissue is a mass of similar or dissimilar cells performing a common function.

The first developed plat body is known as primary plant body and its tissue as primary tissue. In lower plants and monocotyledons the plant body remains primary throughout the life span. While in the Gymnosperms, in most dicots and in some monocots secondary thickening in stem and roots or secondary growth takes place and its tissues are known as secondary tissues. The secondary growth does not fundamentally change the primary structure. The primary plant structures root, stem and leaves are distinguished by relative distribution of vascular and ground tissue system.

2.3 TYPES OF TISSUES

Now we will discuss the different types of tissue, but before this what are the tissues? In broad sense, a tissue may be defined as, "*a group of similar or dissimilar cells that perform a common function and have a common origin*." Plant body comprises different functions and for this it has different tissue system. For example epidermal tissue system protect inner cells and help in gaseous exchange; ground tissue system performs photosynthesis and the vascular tissue system helps in conduction of water and food. The various types of tissues are classified into three groups - **meristematic, permanent and secretary tissues.**

2.3.1 Meristem or Meristematic Tissue

Initially all embryonic cells of an embryo have the capacity to divide and multiply but as the embryo develops into a plant body, this capacity for division is restricted to certain parts of the plant body called meristems which are active throughout the life of the plant body (unlike that of an animal body). When meristematic cells divide, a group of the daughter cells remain meristematic; the other daughter cells called derivatives differentiate into various tissue elements. Before the occurrence of any cell division, usually cells become enlarged accompanied with addition of protoplasmic and cell wall material. So a meristmatic tissue is a group of cells that are in continuous state of division or retain their power of division (Fig.2.1). Meristmatic cells have some characteristics like:

- Meristematic cells may be rounded, oval or isodiametric in shape.
- Compactly arranged i.e. no intercellular space and with dense cytoplasm
- Large nucleus, and small vacuoles or without vacuoles
- Cell wall is thin and donot store reserve food material
- Always in active state of division and divide in a plane

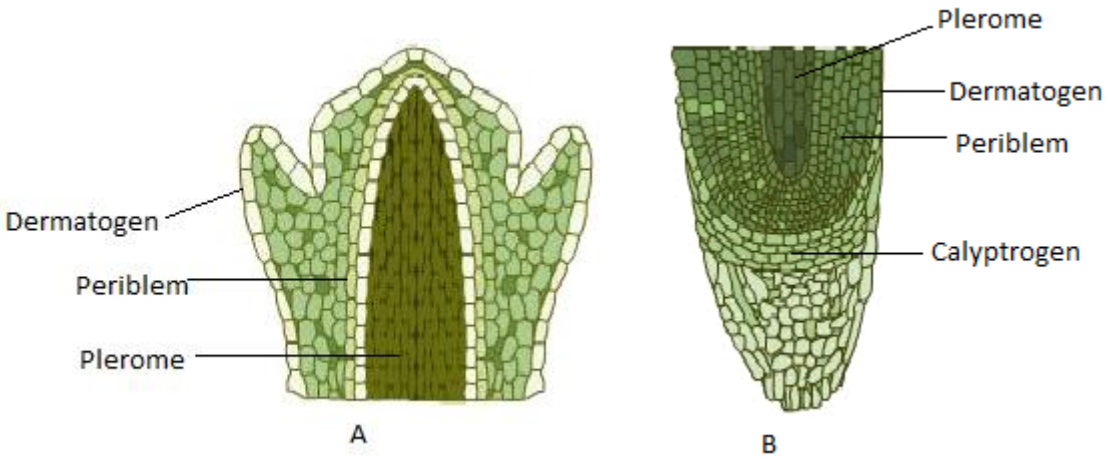


Fig.2.1: Meristematic tissue: A. Stem apex; B. Root apex

Meristems are located in the growing zones and after continuous division they regularly produce new cells, which later on after maturation forms the anatomical sections. This process is called differentiation in which the newly produced cells gets modify into the mature and permanent cells. Meristems which occur at the apices of stem, root and other branches are called apical meristems, which bring about primary growth of the plants, hence also called as primary meristems. On basis of origin it is classified as **primary meristem and secondary meristem.** Primary meristem develops at the stage of embryonic development and forms the primary plant body. The secondary meristem develops later on and forms the secondary tissue system of the plant body.

Types of Meristem: You are now well aware of meristems which are mainly responsible for the growth of the plants. Meristems are classified differently by workers like on basis of origin, position in plant body, plane of division and their function.

On basis of origin meristems are classified as:

- **Promeristem:** Group of cells which represent primary stage of meristematic cells. They are present in small region at the apices of shoots and roots. They give rise to primary meristem.
- **Primary Meristem:** The meristematic cells that originate from promeristem and these cells are always in active state of division and give rise to permanent tissue. In most monocots and herbaceous dicots only primary meristem is present.
- **Secondary Meristem:** They are the meristems developed from primary permanent tissue. They are not present from the very beginning of the organ but develop at a later stage and give rise to secondary permanent tissue. Secondary growth occurs due to these cells and plant increases in its diameter.

On the basis of position in plant body the meristem is of following type:

- **Apical Meristem:** It lies at the apices of root, stem and often in leaves as well. These are responsible for the growth of plants. These cells always maintain their position and capacity to divide. In higher vascular plants apical cells are found in groups but in vascular cryptogams it is found singly.
- **Intercalary Meristem:** This is also a primary meristem, found inserted between permanent tissues, in the bases of internodes and leaf sheaths of grasses. They originate from the apical meristem when their portions get portions get detached due to growth of the organ. Wherever stem is jointed, elongation of internodes is due to intercalary meristem as in Bamboos. Even prolonged growth of leaves, flowers and fruits may be regarded as an intercalary growth.
- **Lateral Meristem:** Found in the lateral zones of the plants and increase the diameter of the organ means they are responsible for growth in thickness. The vascular cambium and cork

cambium are referred to as secondary meristems because they produce secondary tissues, and increase the thickness of the plant body. This process is called secondary growth, seen in Dicotyledons and Gymnosperms.

On the basis of plane of division in plant body the meristem is of following type:

- **Mass Meristem:** In this cell division occurs in all planes so that irregular shaped structure is formed e.g. endosperm.
- **Plate Meristem:** It consists of parallel layers of cell which divide anticlinally in two planes so that a plate like structure is seen. This pattern is seen in development of leaf lamina.
- **Rib or File Meristem:** In this type of development cells divide at right angles or anticlinally in one plane. It is found in the development of lateral roots.

Meristems are also classified on the basis of their function. These are classified as **protoderm-** the outermost tissue develops into epidermis, **procambium-** develops into primary vascular tissue and **ground meristem -** develops into ground tissue like cortex, pericycle and pith.

2.3.2 Permanent or Mature Tissues

Cells derived from meristems gradually change in their structure, metabolism and chemistry and acquire specialized characters by their various modes of differentiation. Not all the cells totally differ from the meristems. Some cells retain the power of division and others cannot divide. In a strict sense only those cells which have lost the power for division must be regarded as permanent tissues, but in a broad sense, cells derived from meristem that have acquired a special function like photosynthesis, secretion, storage are treated as part of matured tissue. The cells of these tissues may be living or dead and thin or thick walled. The thin walled tissue are generally living whereas the thick walled may be dead or living.

There are two types of mature or permanent tissues: Simple and Complex.

1: Simple Tissue: These tissues or group of cells are similar and simple type having different structural entities and mainly forms the vegetative plant body. Simple tissues are made up of one type of cells forming a uniform system of cell. On basis of structural difference these are of following three types:

a) Parenchyma (Para-beside, enchyma-In poured)

Parenchyma is the fundamental tissue of the plant body. It is found in every part of the plant body like pith and cortex of stem and root, mesophyll of leaves, flesh of fruits, floral parts and even in xylem and Phloem. Cells have thin primary walls and polyhedral shapes. Cells are compactly arranged or more commonly spaciously arranged with intercellular spaces as in cortex and pith. Cells possess dense cytoplasm and are active metabolites.

Cells consist of isodiametric thin walled and equally expanded cells. Parenchyma cells are oval, rounded and polygonal in shape having well developed spaces among them. The cells are living and contain sufficient amount of cytoplasm in them. Usually each cell possesses one or more nuclei. Generally cells of parenchyma are involved in storage of starch, sucrose, protein, water, phenol derivatives, many mineral substances, etc. (Fig.2.2).

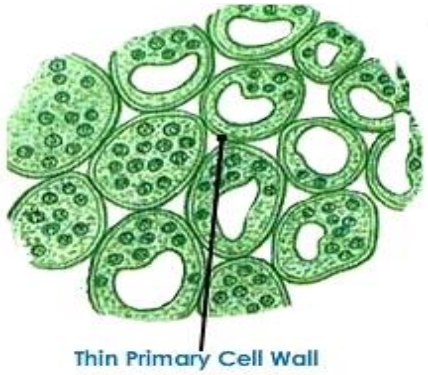


Fig. 2.2: Parenchyma

Parenchymatous cells may also perform specialized functions and are structurally modified. The turgid parenchyma helps in giving rigidity to the plat body. Partial conduction of water is also maintained through these cells. The following are the different types of parenchyma.

(i) Aerenchyma: Parenchyma in aquatic plants gets modified and the cortex cells possess well developed air spaces. Intercellular spaces filled with air are large in size and many in number and such tissue are called aerenchyma. Cells occupy a smaller area and the cells are smaller, still they provide the required strength to the aquatic plants. In these plants air spaces are common, helping in aeration and buoyancy. Air spaces are also seen in roots of grasses, petioles of canna, aroids etc.

(ii) Chlorenchyma: Cells of photosynthetic parenchyma contain numerous chloroplasts. When parenchymatous cells are exposed to sunlight they develop chloroplast in them and called chlorenchyma. These cells are commonly seen in leaves, sometimes in young shoots. The chlorenchyma cells also possess large intercellular spaces. Cells of Chlorenchyma are of two types:

1) Palisade cells that is elongated and compactly arranged.
2) Spongy cells that are spaciously arranged and irregularly shaped.

b) Collenchyma (Kolla - Glue)

Collenchyma is a simple, living tissue composed of elongated cells. Morphologically it is a simple tissue because of having one type of cells. Cell walls are thickened due to deposition of pectin. Collenchyma is the primary supporting tissue in stems, leaves and floral parts of dicots, where as in stems and leaves of monocots collenchyma is usually absent, (instead,

sclerenchyma is present in monocots). The most important character of this tissue is its early development and its adaptability to change in rapid growing organ (Fig.2.3).

Collenchyma is usually hypodermal in position just beneath the epidermis and typical supporting organ. Cells are more elongated and narrower than parenchyma. Like parenchyma, collenchyma may also contain chloroplasts or may regain the thickening. Intercellular spaces may or may not be present. Three forms of collenchyma are recognized based on the types of thickenings:

i. Thickening is on the tangential wall - **lamellar collenchyma**.

ii. Deposition of pectin is in the corners where several cells meet-**angular collenchyma**.

iii. Thickenings are around the intercellular spaces - **tubular or lacunar collenchyma**.

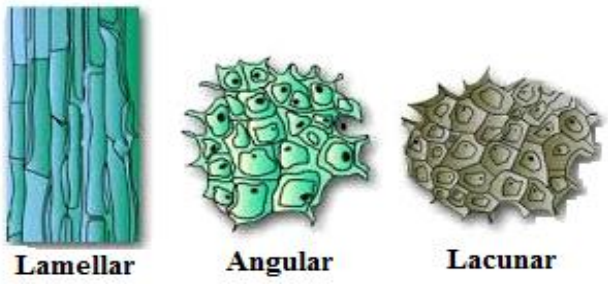


Fig. 2.3: Types of collenchyma

The chief primary function of the tissue is to provide support to plant body and due to presence in peripheral position in stem, petiole and leaf midrib it is very important.

c) Sclerenchyma (Scleros-hard)

Cells of sclerenchyma are thick walled and are usually lignified. The thickness is due to formation of secondary wall. At least initial the secondary wall is free from primary wall. At maturity, usually the cells are devoid of protoplast means they are dead one. The cell wall encloses a cavity lumen and on the cell wall, pits are usually present (simple type). This is supporting tissue that withstands various strains resulting from stretching and bending of plant organ without any damage. They are of various shape and size; even some of these cells are longest in plant kingdom. The primary function of this tissue is to provide mechanical support. Commonly sclerenchyma cells are classified into fibers and sclereids (Fig. 2.4).

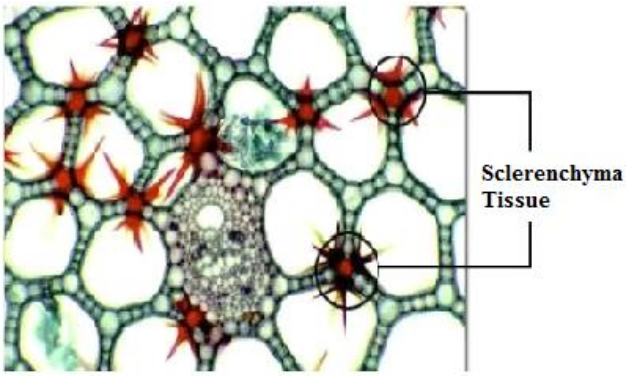


Fig. 2.4: Sclerenchyma

Fibers: These are usually long, spindle shaped structures, with tapering or blunt ends. Longest fiber is seen in *Boehmeria nivea* (55 cms). They are arranged in groups. Secondary thickening may account for 90% of the area of the cell; the lumen is narrow. Cells have pits which are very small, round or slit like and often oblique. Fibers are grouped into xylary fibers and extraxylary fibers. Xylary fibers, also called wood fibers are parts of xylem and are longest among xylem elements.

The fiber cells are classified according to their mode of origin. They can be classified into three groups: 1- Surface fibers, 2- Wood fibers and 3-Bast fibers.

1. **Surface fibers** are found in the testa of many seeds and covering of fruits in many plants like cotton (*Gossypium*), coconut, *Calotropis* sp. etc. These are made up of cellulose and used in stuffing pilose etc.
2. **Wood fibers** are present in xylem of the stem and roots and also known as xylary fibers. They are of two types: libriform fiber and fiber tracheids. The two differ from each other in their length, thickness of walls and type of pit chambers. The wall in libriform is thick and in tracheids it is thinner, libriform cells have simple pits and tracheids have bordered pits, in libriform cells the pit aperture does not exceed in diameter but exceeds in fiber tracheids.
3. **Bast Fibers or phloem fibers** are lignified or non-lignified and obtained from the phloem and pericycle of the plant. On account of their elasticity, fibers enable the plant body to withstand various strains. Plants yielding phloem fibers are *Corchorus capsularis, Hibiscus cannabis, Tilia* sp., *Nerium* sp., *Vinca* sp. and *Crotolaria juncea* etc. These fibers are used in manufacturing of coarse cloth, cordage, ropes, bags and carpets etc. Commercial fibers like jute, flax and ramie are also extraxylary fibers.

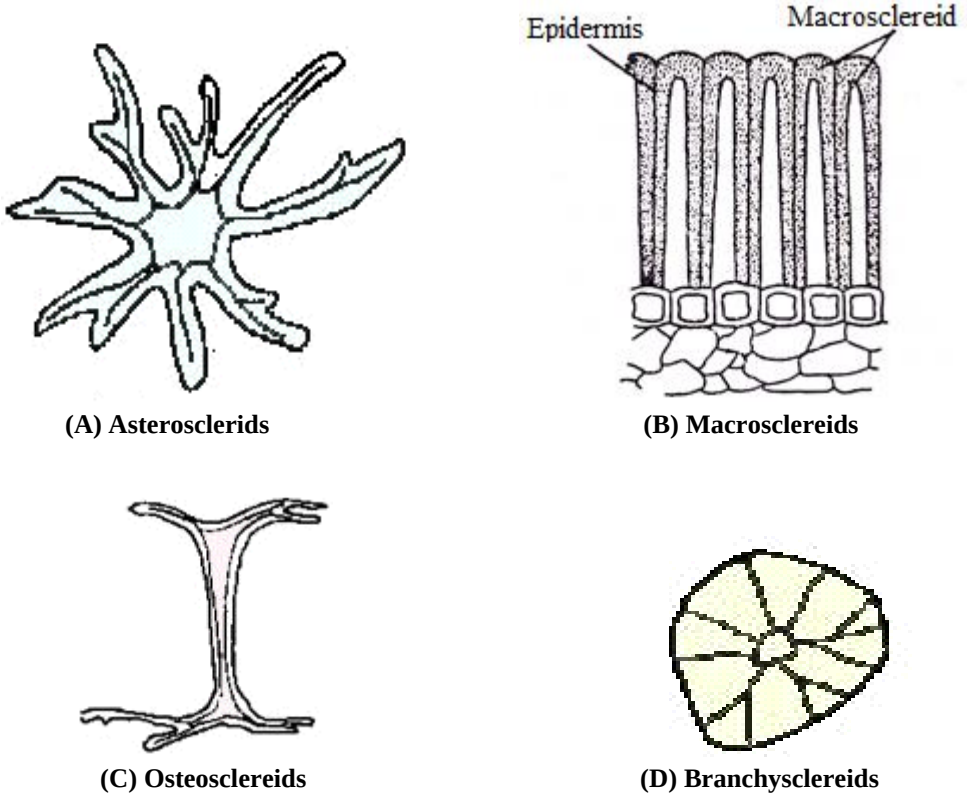
   Pericycle fibers are obtained from *Cannabis sativa* (Bhang or Hemp) and *Linum usitatissimum* (Alsi or Flex). These are used in making ropes, carpets, paper and twines etc.

   The chief function of the fibers to the plant is to give mechanical support and save them from various stresses of the nature like strong wind. Their presence gives rigidity to the plants and prevents them from collapsing.

Sclereids: These are shorter than fibers with thick wall and spherical, oval and cylindrical shape. Sclereids occur singly or in groups with lignified walls and cells devoid of living content. Sclereids are commonly found in fruit wall, seed coat, epidermal scales, and occasionally found in cortex, pith, mesophyll and petiole of submerged aquatics. They can be obtained from the endocarp of the almond and coconut and from the hard seed coats of some leguminous seeds (*Pisum*, *Phaseolus*) (Fig.2.5).

The cells devoid of living content and have tubular canals called simple pits. They may be simple or branched. On basis of structure there are many types of sclereids:

1. Asterosclereids are star shaped (Found in leaf)
2. Macrosclereids are similar to palisade cells or rod like shaped (Found in seed coat)
3. Osteosclereids are bone like that are enlarged at their ends (Found in leaf)
4. Brachysclereids are isodiametric like parenchyma (Found in bark, pith and cortex)



(A) Asterosclerids **(B) Macrosclereids**

(C) Osteosclereids **(D) Branchysclereids**

Fig.2.5: Types of Sclereids

2. Complex Tissue: A complex tissue means *collection of different types of cell that perform a common function.* Xylem and the phloem are examples of complex tissues because these transportation organs are made up of more than one type of cells. Both of these structures are

assemblage of living and nonliving cells, they constitute different shaped and sized cells. Xylem and phloem tissues are collectively known as vascular tissue.

Vascular Tissue

This tissue is a complex tissue and is heterogeneous in nature with different types of cells. Its role is conduction of food, water and minerals in the plant body and the chief elements are xylem and phloem.

(i) Xylem (Wood): Vascular plants have evolved a highly specialized tissue called xylem, which provides mechanical support and transports water, mineral nutrients and phytohormonal signals in the plant. Although it is the most abundant biological tissue on earth, much remains to be learned about the structure, function, development and evolution of xylem and of the genes that regulate the processes. Xylem is the water conducting tissue from roots to leaves. It consists of living cells like parenchyma and dead cells like tracheary elements, along with these fibers are also present (Fig.2.6).

Xylem is made up of four types of cells; i) tracheids, ii) vessels, iii) xylem fibers and iv) xylem parenchyma. This configuration is found in Angiosperms but in Pteridophytes and Gymnosperms tracheids are absent. Even in some Angiosperm tracheids or vessels are absent so this is not a universal combination of xylem.

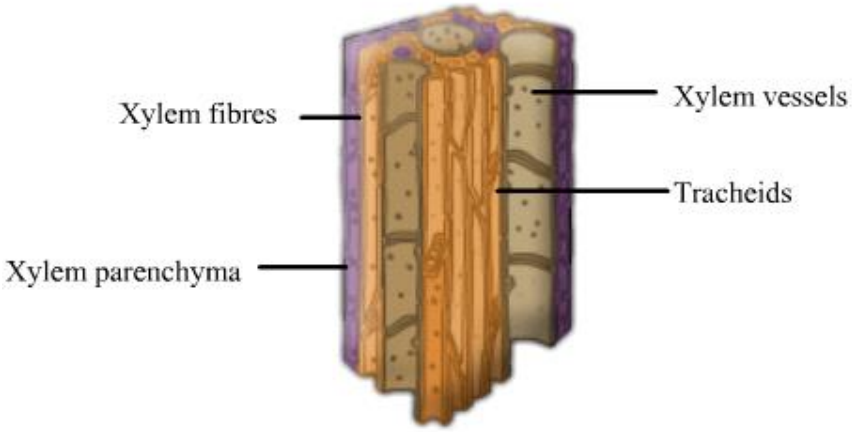


Fig. 2.6: Xylem structure

Tracheary elements are of two types - 1) Vessels and 2) Tracheids, Vessels are limited in their growth, are joined end to end to form continuous tubular structures with perforations in their cross walls. Water and minerals are efficiently conducted through these perforations. Vessels are present in most of the Angiosperms and also in certain lower plants like *Gnetum*, *Marsilea* and *Selaginella*.

Tracheids are generally elongated and non-perforated. They have only pit pairs, at the regions of union with other tracheids. Conduction of water and minerals is not as efficient as in vessels. Tracheids are found in Pteridophytes, most of the Gymnosperms and a few Angiosperms.

Secondary thickenings in tracheary elements are deposited in various forms:

- as rings called annular
- as continuous helices called helical or spiral
- as network called reticulate
- as transverse nets called scalariform
- as extensive thickenings except in the region of pits called pitted

Living cells responsible for most of the storage function of xylem and are called **xylem parenchyma** cells. Many xylem parenchyma cells have secondary lignified walls, particularly in wooden plants. In other cases, these cells have thin, primary walls with areas of plasmodesmata, called primary pit fields, through which cell-to-cell movement of water and mineral nutrients can take place. Mature xylem parenchyma cells in active xylem tissue retain functional protoplasm and can store carbohydrates in the form of starch. These cells also play an important role in wound healing by forming callus and can differentiate to regenerate functional xylem cells.

Xylem fibers are sclerenchymatous cells found in xylem tissue and found in both i.e. primary and secondary. Xylem elements differentiating from an apical meristem constitute primary xylem, whereas secondary xylem is differentiated from vascular cambium. Xylem is the most preserved tissue in fossils, due to the development of lignified secondary walls. Fibers add strength and rigidity to an organ.

Primary Xylem: This is differentiated into protoxylem and metaxylem. In stems, protoxylem is found nearest to central axis (endarch xylem) and in roots away from centre (exarch xylem). The protoxylem elements have annular, spiral and sometimes reticulate secondary thickenings.Fibers are absent in protoxylem. Metaxylem appears after protoxylem. Metaxylem has only pitted secondary walls. Metaxylem is more complex than protoxylem and its elements are wider.

Secondary Xylem: The vascular cambium (intrafascicular cambium and interfascicular cambium) producing secondary xylem consists of fusiform and ray initials. When compared with primary xylem, secondary xylem is more complex and shows orderly development. Pitted and scalariform secondary thickenings are developed.

In the water plants (Hydrophytes) it is not developed that much so it is difficult to differentiate between xylem and phloem. In this case both xylem and phloem is thin walled cortex like structures. The xylem is called the primary xylem if its elements are developed from apical meristem. Secondary xylem elements develop from the vascular cambium during the process of secondary growth.

The earlier formed cells are known as protoxylem and the later formed are called metaxylem. There are three main patterns to the arrangement of protoxylem and metaxylem in stems and roots.

- ***Exarch*** is used when there is more than one strand of primary xylem in a root, and the xylem develops from the outside inwards towards the center, i.e. centripetally. The metaxylem is thus closest to the center of the root and the protoxylem closest to the periphery. The roots of vascular plants are normally considered to have exarch development.
- ***Endarch*** is used when there is more than one strand of primary xylem in a stem or root, and the xylem develops from the inside outwards towards the periphery, i.e. centrifugally. The protoxylem is thus closest to the center of the stem or root and the metaxylem closest to the periphery. The stems of seed plants typically have endarch development.
- ***Mesarch*** is used when there is more than one strand of primary xylem in a stem or root, and the xylem develops from the middle of a strand in both directions. The metaxylem is thus on both the peripheral and central sides of the strand with the protoxylem between the metaxylem (possibly surrounded by it). The leaves and stems of many ferns have mesarch development.

(ii)Phloem (Bast)

Phloem is the complex food conducting tissue and also known as bast. It is composed of sieve elements, companion cells, parenchyma cells and fibers. Like xylem all these types of cells are not of universal occurrence. In Gymnosperms and the Pteridophytes the companion cells are absent. In some hydrophytes there is no evident differentiation of these cells. Phloem differentiated from procambium is called primary phloem; secondary phloem is initiated from vascular cambium (Fig. 2.7).

a) Sieve elements: These are classified into sieve cells and sieve tube members. Sieve cells are commonly long, slender, and taper at both ends. These cells overlapping, many sieve areas are seen. Sieve areas have cluster of pores through which adjacent sieve cells are interconnected by protoplasmic strands. Sieve cells are found in Pteridophytes and Gymnosperms. Sieve tube members are tubular, placed in long series and have specialized sieve areas called sieve plates occurring at end walls. These sieve plates are often inclined. In addition to sieve plates, there may be also sieve areas on lateral walls of sieve tube members. In sieve plate, sieve areas, having large pores are seen. Initially sieve tube members contain uninucleated protoplast.

Gradually the nucleus and its associated endoplasmic reticulum become disorganized. In the centre of the cell a mixture of vascular sap and disorganized cytoplasmic matter is seen. Tonoplast is not found surrounding the vacuole.

Sieve tube member is associated with one or more specialized parenchyma cells called companion cells are seen only in Angiosperms. Both sieve tube member and companion cell are derived from the same meristematic cell. Often, companion cells of sieve tube members form longitudinal series. Companion cell is nucleated. Probably these cells provide energy for food conduction.

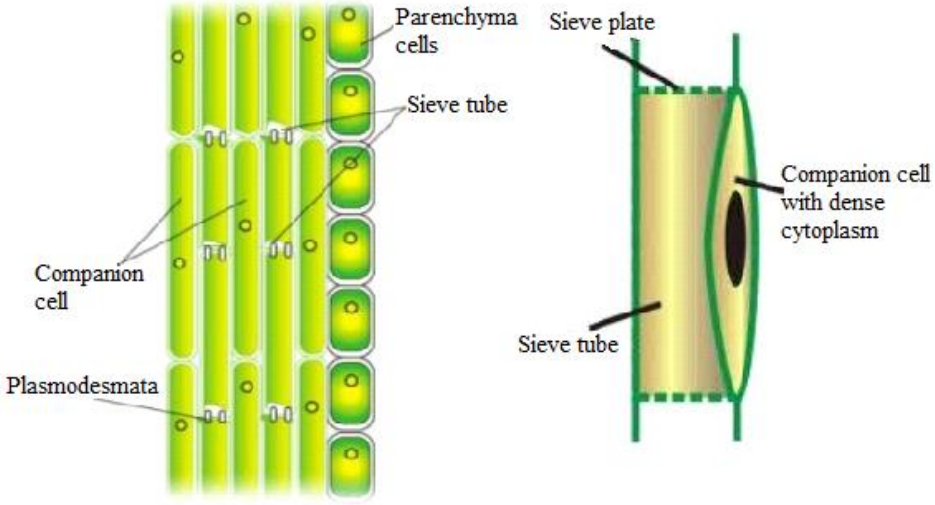


Fig. 2.7: Phloem structure

b) Companion cells: These are specialized type of parenchyma cells which closely associated in origin, position and function with sieve tube element. Each sieve-tube member has an associated specialized parenchyma cell called a companion cell. They are derived by mitosis from the same parent cell and remain connected with each other. Photosynthates are actively secreted into, and actively removed from sieve-tube members by their companion cells. Other unspecialized parenchyma cells also are present in primary phloem and provide storage.

c) Phloem parenchyma: Phloem parenchyma is living and has thin cell walls. Phloem parenchyma cells, called transfer cells and border parenchyma cells, are located near the finest branches and terminations of sieve tubes in leaf veinlets, where they also function in the transport of foods. These cells form the packing tissue between all the other types of cells. The phloem parenchyma *stores compounds* such as starch.

d) Phloem fiber or Bast fiber (also called **phloem fiber** or **skin fiber**) is plant fiber collected from the **bast** surrounding the stem of certain dicotyledonous plants. They support the conductive cells of the phloem and provide strength to the stem. Most of the economically important bast fibers are obtained from herbs cultivated in agriculture, as for instance flax, hemp, or ramie, but also bast fibers from wild plants, as stinging nettle, and trees such as lime or linden, wisteria, and mulberry have been used in the past. Bast fibers are

classified as soft fibers, and are flexible. Since the valuable fibers are located in the phloem, they must often be separated from the xylem material and sometimes also from epidermis. The process for this is called retting, and can be performed by micro-organisms either on land or in water, or by chemicals or by pectinolytic enzymes. In the phloem, bast fibers occur in bundles that are glued together by pectin and calcium ions. More intense retting separates the fiber bundles into elementary fibers that can be several centimetres long. Often bast fibers have higher tensile strength than other kinds, and are used in high-quality textiles (sometimes in blends with cotton or synthetic fibers), ropes, yarn, paper, composite materials and burlap.

Primary phloem: This is classified into protophloem and metaphloem. In protophloem, sieve tube members are without companion cells. Sieve tubes function for a brief period and soon they get crushed by the surrounding pressure. The crushed cells may disappear. Metaphloem tissue survives for a longer period. Its elements are longer and wider. Usually fibers are absent in dicotyledons, whereas in monocotyledons and herbaceous dicots, parenchyma cells are present.

Secondary phloem: Similar to secondary xylem, there are two systems of arrangements in secondary phloem:

1) Axial system producing sieve elements, phloem parenchyma and phloem fibers.
2) Transverse system producing ray parenchyma cells.

Pericycle (A Circle All Around)

Pericycle is the region, consisting of one - few layers of cells, found external to central cylinder (stele) e.g. roots and dicot stems. Pericycle may be composed of parenchyma cells or sclerenchyma cells or both. Lateral branches and phellogen may arise from pericycle.

2.3.3 Specialized tissue or Secretary Tissue

Cell or organizations of cells which produce a variety of secretions are called secretory tissues. The secreted substance may remain deposited within the secretory cell itself or may be excreted, that is, released from the cell. Substances may be excreted to the surface of the plant or into intercellular cavities or canals. Some of the many substances contained in the secretions are not further utilized by the plant (resins, rubber, tannins, and various crystals), while others take part in the functions of the plant (enzymes and hormones). Secretory structures range from single cells scattered among other kinds of cells to complex structures involving many cells; the latter are often called glands.

Epidermal hairs of many plants are secretory or glandular. Such hairs commonly have a head composed of one or more secretory cells borne on a stalk. The hair of a stinging needle is bulbous below and extends into a long, fine process above. If one touches the hair, its tip breaks

off, the sharp edge penetrates the skin, and the poisonous secretion is released. Glands secreting a sugary liquid-the nectar-in flowers pollinated by insects is called nectaries. Nectaries may occur on the floral stalk or on any floral organ like sepal, petal, stamen, or ovary.

The hydathode structures discharge water- a phenomenon called guttation through openings in margins or tips of leaves. The water flows through the xylem to its endings in the leaf and then through the intercellular spaces of the hydathode tissue toward the openings in the epidermis. Strictly speaking, such hydathodes are not glands because they are passive with regard to the flow of water.

As a result of cellular processes, substances that are left to accumulate within the cell can sometimes damage the protoplasm. Thus it is essential that these materials are either isolated from the protoplasm in which they originate, or be moved outside the plant body. Although most of these substances are waste products, some substances are vital to normal plant functions. Examples: oils in citrus, pine resin, latex, opium, nectar, perfumes and plant hormones. Generally, secretory cells are derived from parenchyma cells and may function on their own or as a tissue. They sometimes have great commercial value. There are a large number of plants in the world which have special cells or groups of cells that secrete or excrete products from the plant body. The tissues that are concerned with the secretion of gums, resins, volatile oils, nectar latex, and other substances in plants are called **secretory tissues.** These tissues are divided into two groups.

1. Laticiferous tissues

These consist of thin walled, greatly elongated and much branched ducts containing a milky or yellowish colored juice known as latex. This mixture storage, generally milky white, other colors rarely, form an emulsion which are active ingredients as protein, carbohydrates, enzymes, tannins, rubber, hormones and alkaloids and is called latex (Fig. 2.8). A plant rich in latex is the papaya. They irregularly distributed in the mass of parenchymatous cells. Laticiferous ducts, in which latex are found are again two types:

- Latex cell or non-articulate latex ducts
- Latex vessels or articulate latex

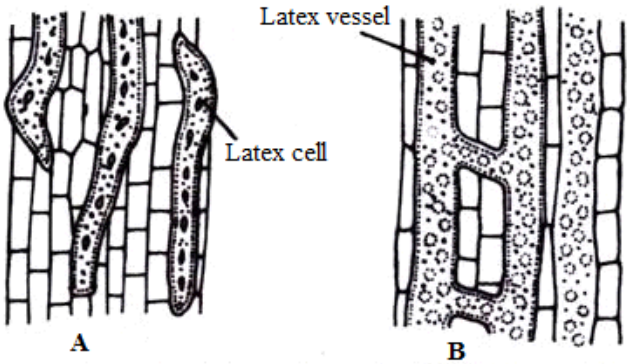


Fig. 2.8: A. Latex cells in madar; B. Latex vessels in rubber plant

a) Latex cells

Also called as "non-articulate latex ducts", these ducts are independent units which extend as branched structures for long distances in the plant body. They originate as minute structures, elongate quickly and by repeated branching ramify in all directions but do not fuse together. Thus a network is not formed as in latex vessels.

b) Latex vessel

Also called "articulate latex ducts", these ducts or vessels are the result of anastomosing of many cells together. They grow more or less as parallel ducts which by means of branching and frequent anastomose to form a complex network. Latex vessels are commonly found in many Angiosperm families Papaveraceae, Asteraceae, Euphorbiaceae, Moraceae etc.

2. Glandular tissues

This tissue consists of special structures called the glands. These glands contain some secretory or excretory products. A gland may consist of isolated cells or small group cells with or without a central cavity. They are of various kinds and may be internal or external.

Internal glands are:

- Oil-gland secreting essential oils, as in the fruits and leaves of orange, lemon.
- Mucilage secreting glands as in the betel leaf.
- Glands secreting gum, resin, tannin etc.
- Digestive glands secreting enzymes or digestive agents.
- Special water secreting glands at the tip of veins.

External glands are commonly short hairs tipped by glands. They are:

- water-secreting hairs or glands.
- Glandular hairs secreting gum like substances as in tobacco, plumbago etc.
- Glandular hairs secreting irritating, poisonous substances as in nettles.
- Honey glands as in carnivorous plants.

Gum ducts are similar to resin ducts and may contain resins, oils, and gums. Usually, the term gum duct is used with reference to the dicotyledons, although gum ducts also may occur in the gymnosperms. Oil ducts are intercellular canals whose secretory cells produce oils or similar substances. Such ducts may be seen, for example, in various parts of the plant of the carrot family (Apiaceae). Laticifers are cells or systems of cells containing latex, a milky or clear, colored or colorless liquid. Latex occurs under pressure and exudes from the plant when the latter is cut.

Lysigenous cavities are formed by cavities coming from cell groups which are loaded of secretory products and whose protoplasm membranes have been destroyed gradually (Fig. 2.9). This group of cells is also called "pockets" of secretion and can be found in fruits and young stems of citrus.

Schizogenous cavities are the cells or epithelial located inside the parenchyma tissue or inside other tissues. They always wrap rounded or irregular intercellular spaces, or intercellular ducts, simple or branched, which sometimes go through all the plant as communications ducts. These intercellular spaces are caused by separation of the glandular cells and are the containers of the developed products; they are the schizogenous cavities or secretion cavities (Fig. 2.9). According to its contents, these are distinguished ducts or cavities with lipids (essential oils), resins, gums and mucilage.

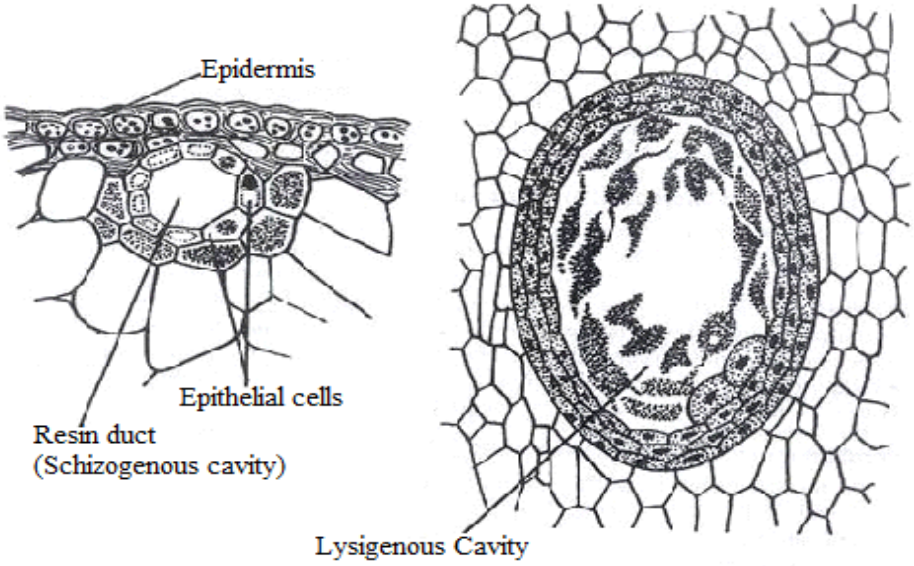


Fig. 2.9: Structure of Schizogenous and Lysigenous cavities

2.4 ROOT ANATOMY

Evolutionarily, the root seemed to be the last of the three main vegetative organs to evolve, perhaps since early land plants grew on or near the water and so much of their early innovations were geared toward maximizing photosynthesis through development of stems and leaves. There are generally two very different developmental and structural aspects to Angiosperm root systems. The primary root system is derived from the radicle and tends to be dominant in dicots, and gives rise to lateral roots with various degrees of branching. In monocots, the primary root is often ephemeral, and so adventitious roots (derived usually from stems and leaves) and seminal roots (derived from mesocotyl) comprise their root systems where they also produce lateral roots. The root system also has an apical meristem, known as the **root apical meristem**. This acts in much the same way as the shoot apical meristem, causing extension growth. The main difference is the growth goes down into the ground, and roots, not leaves and branches come from the root apical meristem. Roots have really important jobs, and they do not get due credit for their hard work because they remain underground all the time. Roots are responsible for:

- Anchoring the plant into the ground

- Absorbing water and nutrients
- Storing nutrients
- Associating with soil microbes in symbiotic relationships

As roots grow, they travel downward through the soil, dodging rocks and other obstacles that might be in their way. Just as you should wear a helmet when riding a motorcycle or playing hockey, roots have their own type of helmet: a **root cap**. The root cap protects the root apical meristem as the root pushes its way through the soil. It also secretes slimy ooze that lubricates the soil around the tip of the root, aiding the root on its journey through the harsh soil.

Anatomy of root is simpler than stem and show some characteristic features by which we can determine roots. They lack chlorophyll and are positively geotropic and they are not susceptible to light. Roots have root cap at the apex with root hairs near the apex. Vascular bundles are radial and exarch type i.e. xylem and phloem in different radii and protoxylem towards periphery and metaxylem towards center.

Anatomical Characters:

We can understand root anatomy as per the Angiospermic plant group i.e. monocot and dicot plants. Both of these plant groups has different anatomical features and given in detail here.

Anatomy of Dicot Root: The important anatomical features of a dicot root are visible in the cross section of a dicot plant and following tissues are seen (Fig. 2.10):

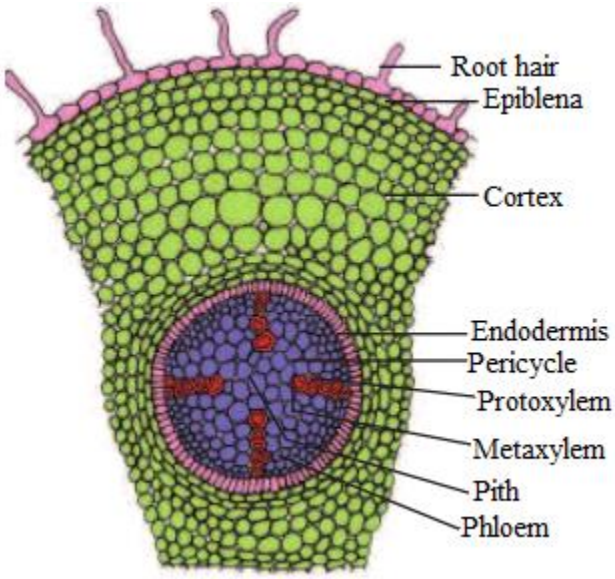


Fig. 2.10: T.S. of Dicot root

Epiblema (Epidermis): Epiblema is made up of thin walled living cells (parenchymatous) that are compactly arranged. This is the outermost layer in root and some of these cells form root hairs. Root hairs are outgrowth of epidermal cell and help in both absorption and anchorage.

Root hairs are unicellular, long and tubular. Root epidermis is devoid of stomata although stomata are found in some species. The breathing roots in the halophytes possess specialised pores in the epidermis. These are called lenticels and such roots are called pneumatophores. The main function of the epidermis is protection and absorption of water and solutes.

Cortex: Root cortex composed of thin walled cells with lots of intercellular spaces. Cells are usually oval, rounded and polygonal in shape with a distinct mode of arrangement. In some herbaceous dicots which lack secondary growth, the cortex permanently retained and develops various types of mechanical tissues. Even chloroplast is reported in the cortex of *Tinospora* sp. Tannin cells, mucilage cells and latex cells are also found in the cortical region of dicot roots. In most of the dicots the cortex is replaced by suberized cells as a result of secondary growth. Cortical cells help in gaseous exchange, passage of absorbed water, maintain root pressure and during secondary growth regain meristmatic activity and gives rise to cork cambium.

Endodermis: This is a uniseriate layer forming the central cylinder of the plant. Endodermis is a distinct layer having living cell with casparian strips in their radial and transverse wall. Casparian strips are actually bands of suberin deposited on the walls of endodermis. The endodermis is generally destroyed after secondary growth. The casparian strip maintains the movement of materials in the root and their passage into xylem cells. Endodermis not only regulates the movement of materials in the root and their passage into the xylem but also contains the starch grain, performing storage function as well.

Pericycle: This is a thin walled parenchymatous cell layer next to epidermis. It may be uni or multiseriate and responsible to give rise the lateral roots. It maintains its meristmatic activity so that it produces lateral roots, phellogen and some part of vascular cambium.

Vascular bundles: Vascular bundles are radial and tetrarch. There are four bundles each of xylem and phloem occurring alternately. Xylem is described as exarch i.e. metaxylem towards centre and protoxylem towards

Pith: Pith is absent in the older root.

Anatomy of Monocot Root: The important anatomical features of a monocot roots are visible in the cross section of a monocot plant and following tissues are seen (Fig. 2.11):

Epiblema (Epidermis) : Epiblema is the outermost covering of the root formed by a single layer of compactly arranged, barrel-shaped parenchyma cells. The cells are characteristically thin-walled since they are involved in absorption of water. A cuticle and stomata are absent. Some of the epiblema cells are produced into long unicellular projections called root hairs. Hence, epiblema is also known as piliferous layer.

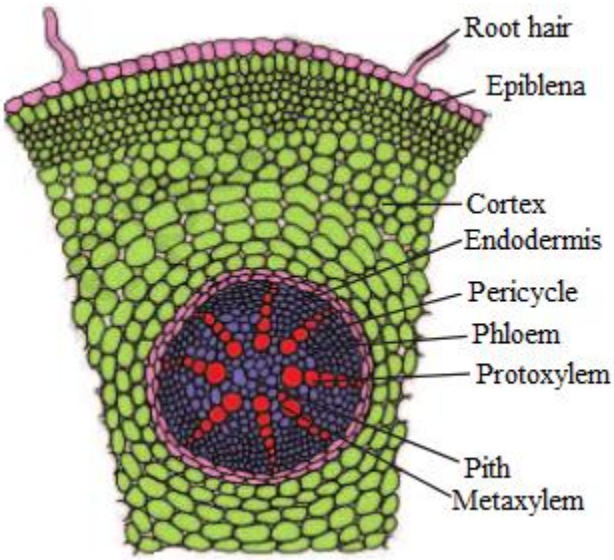


Fig. 2.11: T.S. of Monocot root

Cortex: Cortex is a major component of the ground tissue of root. It is represented by several layers of loosely arranged parenchyma cells. Intercellular spaces are prominent. The cortex is mainly meant for storage of water. The cells also allow a free movement of water into the xylem vessels.

Endodermis: It is the innermost layer of cortex formed by compactly arranged barrel-shaped cells. Some of the cells in the endodermis are thin-walled and are known as passage cells. The passage cells allow water to pass into the xylem vessels. The remaining cells in the endodermis are characterised by the presence of thickening on their radial walls. These thickenings are known as casparian thickenings. They are formed by the deposition of a waxy substance called suberin. The casparian thickenings play an important role in creating and maintaining a physical force called root pressure.

Stele: Stele is the central cylinder of the root consisting of pericycle, conjunctive tissue, pith and vascular bundles.

Pericycle: Pericycle is the outermost covering of the stele represented by a single layer of parenchyma cells.

Conjunctive tissue: It is represented by loosely arranged parenchyma cells found in between the vascular bundles. The cells are specialized for storage of water.

Pith: Pith is the innermost region of the root representing the central axis. It is composed of few loosely arranged parenchyma cells.

Vascular bundles: Vascular bundles are radial in arrangement. There are eight bundles each of xylem and phloem. Hence, the condition is described as polyarch. Xylem is described as exarch.

Dicot root

1. Cortex is comparatively narrow
2. Pericycle is single layered
3. Pericycle produces lateral roots cambium and cork cambium
4. Vascular bundles range from two to six in number
5. Xylem vessels are angular
6. Pith is not well developed or absent
7. Secondary growth takes place

Monocot root

1. Cortex is wide
2. Pericycle is often multilayered
3. Pericycle produces lateral roots
4. Vascular bundles are more than six in number
5. Xylem vessels are oval or rounded
6. Pith is well developed
7. Secondary growth does not take place

S.No.	Monocot Root	Dicot Root
1	A large number of vascular bundles	A limited number of vascular bundles.
2	The vascular bundles are scattered in the ground tissue	The vascular bundles are arranged in a ring
3	No cambium occurs between the xylem and phloem	Cambium occurs between the xylem and phloem
4	There is no distinction between the cortex and pith	The cortex and pith can be clearly distinguished
5	No Secondary thickening	Secondary thickening can occur
6	No annual rings are formed	Annual rings are formed due to secondary thickening

2.5 SHOOT ANATOMY

Stems are usually above ground organs and grow towards light (positively phototropic) and away from the ground (negatively geotropic), except in the case of certain metamorphic (modified) stems. The main stem develops from the plumule of the embryo, while lateral branches develop from auxillary buds or from adventitious buds. In normal stems clearly defined internodes and nodes can be distinguished, the latter being the regions where the leaves are attached. In younger stems stomata are found in the epidermis while in the mature

stems lenticels are evident. Depending on the hardness of the stem one can also distinguish between herbaceous and woody stems. In this section we will discuss the internal structures of young dicotyledonous and monocotyledonous stems, secondary thickening in the stems of dicot, and differences in the internal structures of dicots and monocots.

Stem is one of two main structural axes of a vascular plant, the other being the root. The stem is normally divided into nodes and internodes: The nodes hold one or more leaves, as well as buds which can grow into branches (with leaves, conifer cones, or inflorescences (flowers)). Adventitious roots may also be produced from the nodes.

The term "shoots" is often confused with "stems"; "shoots" generally refers to new fresh plant growth including both stems and other structures like leaves or flowers. In most plants stems are located above the soil surface but some plants have underground stems.

Stems have four main functions which are:

- Support for and the elevation of leaves, flowers and fruits
- The stems keep the leaves in the light and provide a place for the plant to keep its flowers and fruits
- Transport of fluid between the root and shoot by the xylem and phloem
- Storage of nutrients

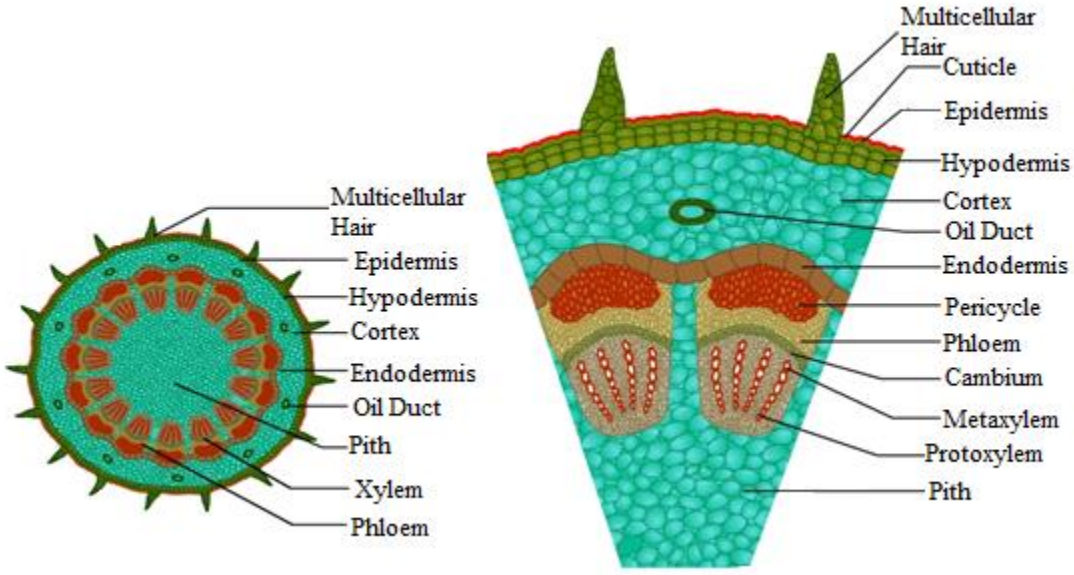
Production of new living tissue i.e. stems have cells called meristems that annually generate new living tissue. **Shoots** consist of stems including their appendages, the leaves and lateral buds, flowering stems and flower buds. The new growth from seed germination that grows upward is a **shoot** where leaves will develop. In the spring, perennial plant shoots are the new growth that grows from the ground in herbaceous plants or the new stem and/or flower growth that grows on woody plants.

In everyday speech, shoots are often synonymous with stems. Stems, which are an integral component of shoots, provide an axis for buds, fruits, and leaves. Young shoots are often eaten by animals because the fibers in the new growth have not yet completed secondary cell wall development, making the young shoots softer and easier to chew and digest. As shoots grow and age, the cells develop secondary cell walls that have a hard and tough structure. Some plants (e.g. bracken) produce toxins that make their shoots inedible or less palatable

Stem usually consist of three tissues, dermal tissue, ground tissue and vascular tissue. The dermal tissue covers the outer surface of the stem and usually functions to water proof, protect and control gas exchange. The ground tissue usually consists mainly of parenchyma cells and fills in around the vascular tissue. It sometimes functions in photosynthesis. Vascular tissue provides long distance transport and structural support. Most or all ground tissue may be lost in woody stems. The dermal tissue in aquatic plants, stems may lack the waterproofing as found in aerial stems. The arrangement of the vascular tissues varies widely among plant species.

Dicot stems

Dicot stems with primary growth have pith in the center, with vascular bundles forming a distinct ring visible when the stem is viewed in cross section. The outside of the stem is covered with an epidermis, which is covered by a waterproof cuticle. The epidermis also may contain stomata for gas exchange and multicellular stem hairs called trichomes. A cortex consisting of hypodermis (collenchyma cells) and endodermis (starch containing cells) is present above the pericycle and vascular bundles.



A **B**

Fig.2.12: T. S. of Dicot stem; A. Diagrammatic; B. A portion enlarged

From the study of the transverse section of the dicotyledonous stem you will identify the following three regions of tissues: epidermis, cortex and vascular cylinder or stele (Fig. 2.12).

Epidermis: The epidermis consists of a single layer of living cells which are closely packed. The walls are thickened and covered with a thin waterproof layer called the cuticle. Stomata with guard cells are found in the epidermis. In some stems either unicellular or multicellular hair-like outgrowths, trichomes, appear from the epidermis.

- The epidermis *protects the underlying tissues*
- The cuticle *prevents the desiccation of inner tissues* and thus *prevents water loss*
- The stomata allow *gaseous exchange* for the processes of respiration and photosynthesis

Cortex: This region comprises the cells of collenchyma, parenchyma and endodermis. It is situated to the inside of the epidermis. Collenchyma cells lie under the epidermis and constitute three to four layers of cells with cell walls thickened at the corners. The collenchyma

cells contain chloroplasts. This tissue serves to *strengthen* the young stem. The chloroplasts are responsible for the *synthesis of organic food* during photosynthesis. Beneath the collenchyma cells are a few layers of thin-walled cells, parenchyma, with intercellular spaces. The parenchyma cells make up the bulk of the cortex. They synthesized *organic food (mainly starch)* is *stored here*. The intercellular air spaces are responsible for *gaseous exchange*.

Endodermis: It is starch sheath which forms the innermost layer of the cortex. This is a single layer of tightly-packed rectangular cells bordering the stele of the stem. The cells of this tissue *store starch*. It allows *solutions to pass from the vascular bundles to the cortex*.

Vascular cylinder or Stele: This region comprises the pericycle, vascular bundles and pith (medulla). The pericycle is made up of sclerenchyma cells which are lignified, dead fiber cells. These cells have thick, woody walls and tapering ends. It *strengthens* the stem. It provides *protection* for the vascular bundles. The vascular bundles are situated in a ring on the inside of the pericycle of the plant. This distinct ring of vascular bundles is a distinguishing characteristic of dicotyledonous stems. A mature vascular bundle consists of three main tissues - xylem, phloem and cambium. The phloem is located towards the outside of the bundle and the xylem towards the center. The cambium separates the xylem and phloem which bring about secondary thickening.
The xylem provides a *passage for water and dissolved ions* from the root system to the leaves. The xylem also *strengthens and supports* the stem. The phloem *transports synthesized organic food* from the leaves to other parts of the plant. The cambium, divides to produce new xylem and phloem cells, making *secondary thickening* possible.

Pith (Medulla): It occupies the large central part of the stem. It consists of thin-walled parenchyma cells with intercellular air spaces. Between each vascular bundle is a band of parenchyma, the medullary rays, continuous with the cortex and the pith. The cells of the pith *store water and starch*. They *allow for the exchange of gases* through the intercellular air spaces. The medullary rays *transport substances* from the xylem and phloem to the inner and outer parts of the stem.

Monocot stems

Vascular bundles are present throughout the monocot stem, although concentrated towards the outside. This differs from the dicot stem that has a ring of vascular bundles and often none in the center. The shoot apex in monocot stems is more elongated. Leaf sheathes grow up around it to protect it. This is true to some extent of almost all monocots. Monocots rarely produce secondary growth and are therefore seldom woody, with Palms and Bamboos being notable exceptions. However, many monocot stems increase in diameter due to anomalous secondary growth (Fig. 2.13).

The tissues of dicots and monocots are basically the same as you will see. However, there are essential differences in the arrangement of the epidermis, ground tissue and vascular tissue (Fig. 2.14).

The structure and functions of **Epidermis** is same as the epidermis of the stem of a dicotyledonous plant. The epidermis consists of a single layer of living cells which are closely packed. The walls are thickened and covered with a thin waterproof layer called the cuticle. Stomata with guard cells are found in the epidermis. In some stems either unicellular or multicellular hair-like outgrowths, trichomes, appear from the epidermis. The epidermis *protects the underlying tissues*. The cuticle *prevents the desiccation of inner tissues* and thus *prevents water loss.* The stoma allows *gaseous exchange* for the processes of respiration and photosynthesis.

Ground Tissue composed of small, thick-walled sclerenchyma on the inside of the epidermis. These layers of cells are followed by larger thin-walled parenchyma cells. Intercellular air spaces are found in the parenchyma. Cortex and pith are absent. Sclerenchyma tissue *strengthens* the stem. Parenchyma tissue *stores synthesized organic food* such as starch. Intercellular air spaces allow the *exchange of gases*.

The vascular bundles are found scattered throughout the ground tissue. The vascular bundles occurring nearer the rind of the stem are smaller and are closer to one another. The vascular bundles contain no cambium and consequently secondary thickening does not occur. Thick-walled sclerenchyma fibers surround the vascular bundle. Sclerenchyma sheaths *protect the vascular* bundles and give *strength* to the stem. Large xylem vessels are found within an irregular intercellular air space called the lysigenous cavity. This space is surrounded by thin-walled parenchyma cells. Phloem is composed of thin-walled cells, viz. sieve tubes and companion cells.

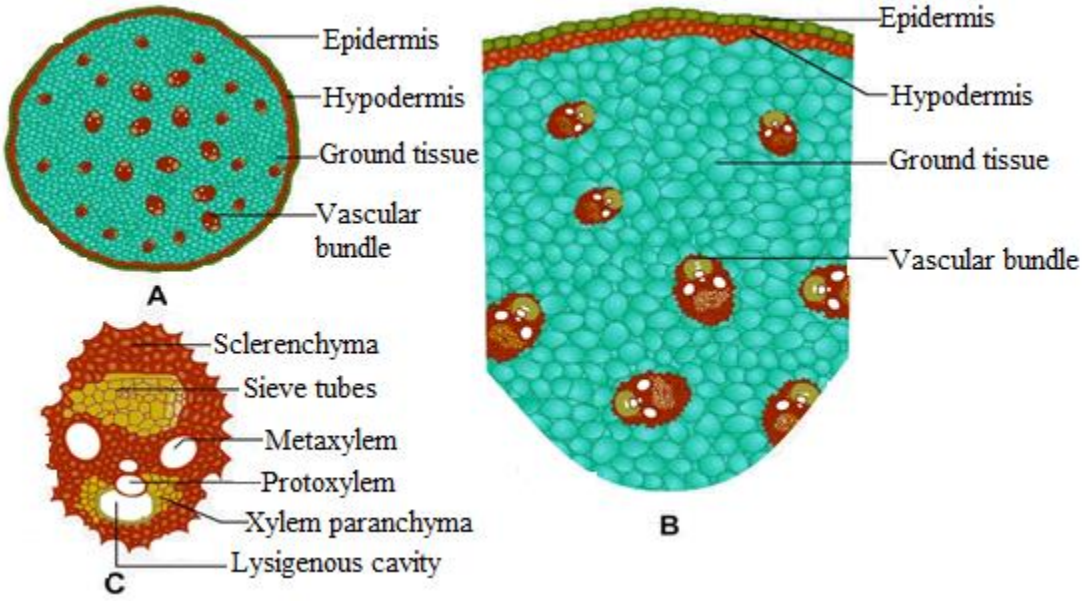


Fig.2.13: T.S. of Monocot stem; A. Diagrammatic; B. A portion enlarged; C.Single vascular bundle

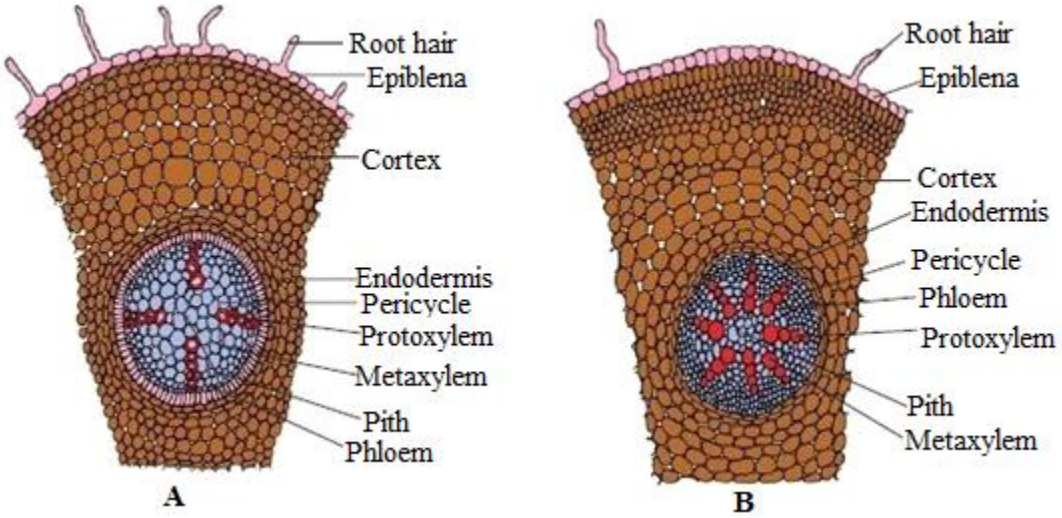


Fig.2.14: T.S. of root; A. Dicot root; B. Monocot root

Comparison of dicot and monocot stem anatomy

S.No.	**Dicot Stem**	**Monocot Stem**
1	Single layered epidermis with thick cuticle	Single layered epidermis with thick cuticle.
2	Multicellular epidermal hairs may or may not be present	Epidermal hairs absent

3	Hypodermis is generally collenchymatous	Hypodermis is generally sclerenchymatous
4	The different tissues are arranged in concentric fashion	Concentric arrangement is absent
5	Ground tissue is differentiated into hypodermis, cortex, endodermis, pericycle and pith	No differentiation except hypodermis
6	Always solid	Solid or hollow
7	Vascular bundles are of similar size	Vascular bundles are of different sizes
8	Vascular bundles are wedge shaped, definite and arranged in one or two rings	Oval or rounded, numerous and scattered in the ground tissue
9	Bundle sheath absent	Bundle sheath present
10	Vascular bundles conjoint, collateral or bicollateral and open	Conjoint, collateral and closed
11	No cavity in the vascular bundles	A protoxylem cavity present
12	Vessels are polygonal, numerous and arranged in chains	Vessels are oval, few and arranged like the letter V or Y
13	Phloem parenchyma present	Phloem parenchyma absent
14	Secondary growth occurs due to formation of lateral meristem	Secondary growth is generally absent

2.6 LEAF ANATOMY

A **leaf** is an organ of a vascular plant and is the principal lateral appendage of the stem. The leaves and stem together form the shoot. **Foliage** is a mass noun that refers to leaves collectively. Typically a leaf is a thin, dorsiventrally flattened organ, borne above ground and specialized for photosynthesis. Most leaves have distinctive upper (adaxial) and lower (abaxial) surfaces that differ in colour, hairiness, the number of stomata (pores that intake and output gases) and other features. In most plant species, leaves are broad and flat. Such species are referred to as broad-leaved plants. Many Gymnosperm species have thin needle-like leaves that can be advantageous in cold climates frequented by snow and frost. Leaves can also have other shapes and forms such as the scales in certain species of conifers. Some leaves are not above ground (such as bulb scales). Succulent plants often have thick juicy leaves, but some leaves are without major photosynthetic function and may be dead at maturity, as in some cataphylls, and spines). The primary site of photosynthesis in most leaves (palisade mesophyll) almost always occurs on the upper side of the blade or lamina of the leaf but in some species, including the mature foliage of *Eucalyptus* palisade occurs on both sides and the leaves are said to be isobilateral.

The leaf is the primary photosynthetic organ of the plant. It consists of a flattened portion, called the blade, which is attached to the plant by a structure called the petiole. Sometimes leaves are divided into two or more sections called leaflets. Leaves with a single undivided blade are called simple leaf, those with two or more leaflets are called compound leaf.

General Characteristics of Leaves

Leaves are the power house of plants. In most plants, leaves are the major site of food production for the plant. Structures within a leaf convert the energy in sunlight into chemical energy that the plant can use as food. Chlorophyll is the molecule in leaves that uses the energy in sunlight to turn water (H_2O) and carbon dioxide gas (CO_2) into sugar and oxygen gas (O_2). This process is called photosynthesis. The internal organisation of most kinds of leaves has evolved to maximize exposure of the photosynthetic organelles, the chloroplasts, to light and to increase the absorption of carbon dioxide. Gas exchange is controlled by stomata, which open or close to regulate the exchange of carbon dioxide, oxygen, and water vapour with the atmosphere. In a given square centimeter of a plant leaf there may be from 1,000 to 1,00,000 stomata. Some leaf forms are adapted to modulate the amount of light they absorb to avoid or mitigate excessive heat, ultraviolet damage, or desiccation, or to sacrifice light-absorption efficiency in favour of protection from herbivory.

Leaves can also store food and water, and are modified accordingly to meet these functions, for example in the leaves of succulent plants and in bulb scales. The concentration of photosynthetic structures in leaves requires that they be richer in protein, minerals, and sugars than woody stem tissues. Accordingly, leaves are prominent diet of many animals. This is true for humans, for whom leaf vegetables commonly are food staples.

Deciduous plants in frigid or cold temperate regions typically shed their leaves in autumn, whereas in areas with a severe dry season, some plants may shed their leaves until the dry season ends. In either case the shed leaves may be expected to contribute their retained nutrients to the soil where they fall.

The anatomy of leaf shows following cell composition.

Epidermis: A leaf is made of many layers that are sandwiched between two layers of tough skin cells (called the epidermis). The epidermis also secretes a waxy substance called the cuticle. These layers protect the leaf from insects, bacteria, and other pests. Among the epidermal cells are pairs of sausage-shaped guard cells. Each pair of guard cells forms a pore (called stoma; the plural is stomata). Gases enter and exit the leaf through the stomata. The epidermal cells are barrel-shaped, compactly arranged; upper epidermis is covered with thick cuticle and lacks stomata; lower epidermis is light green, covered with thin cuticle and is interrupted by stomata. Epidermis helps the plant by:

- The cuticle *prevents water loss*
- The epidermis *protects the internal tissues* from injury

- The stomata *allows for gaseous exchange for photosynthesis and respiration*

Since the epidermis is translucent it allows light to reach the mesophyll tissue for photosynthesis. Within the leaf, there is a layer of cells called the **mesophyll**. The word mesophyll is Greek and means "middle" (meso) "leaf" (phyllon). Mesophyll can then be divided into two layers, the **palisade layer** and the **spongy layer**. Palisade cells are more column-like, and lie just under the epidermis, the spongy cells are more loosely packed and lie between the palisade layer and the lower epidermis. The air spaces between the spongy cells allow for gas exchange. Mesophyll cells (both palisade and spongy) are packed with chloroplasts, and this is where photosynthesis actually occurs. This is the ground tissue. Palisade parenchyma is found immediately below the upper epidermis, 2 to 3 layered, with compactly arranged tubular cells, rich in parietal chloroplasts. Spongy parenchyma is found above the lower epidermis; these cells are varied in shapes and sizes, very loosely arranged enclosing air spaces some of which open into stomata. Chloroplasts are parietal in the parenchyma cells. Most food production takes place in elongated cells called palisade mesophyll. Gas exchange occurs in the air spaces between the oddly-shaped cells of the spongy mesophyll.

Vascular tissue: The vascular tissue, xylem and phloem are found within the veins of the leaf. Veins are actually extensions that run from to tips of the roots all the way up to the edges of the leaves. The outer layer of the vein is made of cells called **bundle sheath cells** (E), and they create a circle around the xylem and the phloem. On the picture, **xylem** is the upper layer of cells (G) and is shaded a little lighter than the lower layer of cells - **phloem** (H). Recall that xylem transports water and phloem transports sugar (food). Vascular bundles vary in size; each bundle is conjoint, collateral and closed. The vascular bundle is covered by a bundle sheath of parenchyma cells. Phloem is towards lower epidermis; xylem is towards upper epidermis, with metaxylem facing phloem. Fibers are absent in both xylem and phloem. The xylem and phloem elements are conspicuous only in large vascular bundles.

Dicot and monocot plants have different leaf morphology and anatomy and their description is given below.

Monocot leaf: Example: Maize.
The leaf of monocot plants is known as isobilateral and is vertically oriented.

Epidermis: This is uniseriate, with barrel-shaped, compactly arranged cells and is covered with thick cuticle. Stomata are found on both upper and lower epidermal layers hence it is called amphistomatic (more on the lower epidermis). Though the leaf is referred to as isobilateral, it is only in upper epidermis, a few large, empty and colorless bulliform or motor cells are present. During dry weather, these motor cells help the leaf to roll over, due to the changes in turgidity. This rolling of leaf reduces the rate of stomatal transpiration.

Mesophyll: There is no differentiation of mesophyll into spongy and palisade parenchyma. All the cells of chlorenchyma are alike, isodiametric, almost compactly arranged with numerous parietal chloroplasts.

Vascular tissue: The vascular bundles are numerous, arranged in parallel series (venation is palmate-parallel), conjoint, collateral and closed. Phloem is towards lower epidermis. Each vascular bundle is surrounded by chlorenchymatous bundle; this sheath also serves for temporary storage of starch. A few vascular bundles are larger in size, with more amounts of xylem and phloem and with large bundle sheath cells. A patch of sclerenchyma is present above and below the large sized vascular bundles (Fig. 2.15).

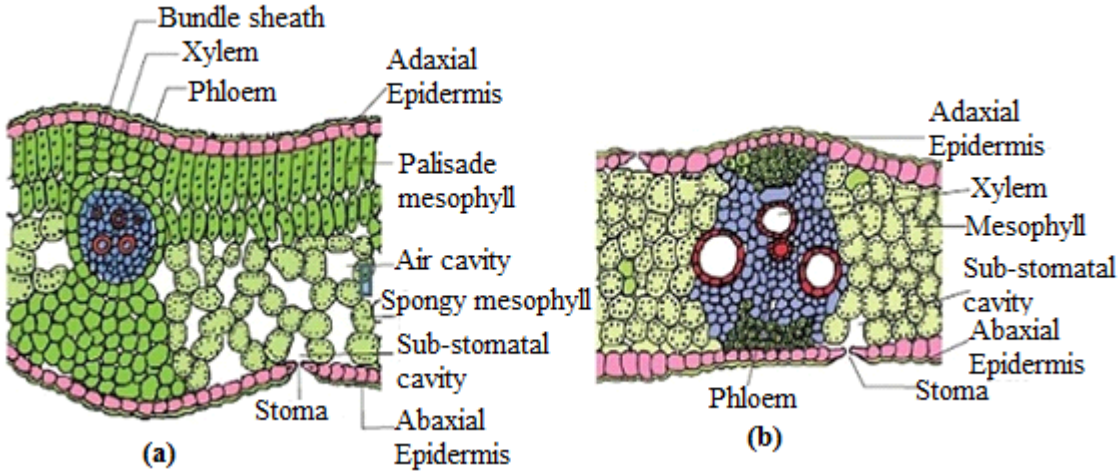


Fig.2.15: A.T.S. of Dicot leaf; B. T.S. of Monocot leaf

Dicot leaf: Example: Sunflower

It is more strongly illuminated on the upper surface than the lower. This unequal illumination induces a difference in the internal structure between upper and lower sides. A section made at right angle to one of the bigger veins reveals following internal structures.

Epidermis: It is in two layers, one on each surface of the leaf. Both the layers are composed of compactly arranged, barrel-shaped cells. Intercellular spaces are absent. A cuticle surrounds both the layers. Multicellular hairs called trichomes are present on both the layers. Stomata occur only in the lower epidermis. This condition is described as hypostomatic.

Mesophyll: The ground tissue that occurs between the two epidermal layers. It is exclusively composed of chlorenchyma cells. The mesophyll is characteristically differentiated into two regions namely, an upper palisade parenchyma and a lower spongy parenchyma.

a) Palisade parenchyma is composed of two or three layers of elongated, compactly arranged chlorenchyma cells. Intercellular spaces are absent. The cells contain a very large number of chloroplasts. Due to this fact the upper surface seems greener than the lower surface in dorsiventral leaf.

b) Spongy parenchyma is composed of a few layers of loosely arranged spherical or oval chlorenchyma cells with prominent intercellular spaces. These cells contain very few chloroplasts. They fit closely around the vein or vascular bundle. The cells contain few chloroplasts. Spongy cells help diffusion of gases through the empty spaces left between them. They manufacture sugar and starch to some extent only.

Vascular tissue: Veins represent the vascular bundles. They are found irregularly scattered in the mesophyll due to reticulate venation. The largest and the oldest vein is found in the centre. It is known as midrib vein. Each vein has a bundle sheath composed of single layer of compactly arranged barrel shaped parenchyma cells. The bundle sheath encloses both xylem and phloem. Xylem is found towards upper epidermis and phloem towards lower epidermis. In the xylem many protoxylem and metaxylem vessels are found. Protoxylem orients towards upper epidermis. Hence, the vascular bundles are described as conjoint and collateral with endarch xylem. The bundle sheath of the midrib vein is connected to the upper and the lower epidermal layers by many layers of collenchyma cells, representing bundle sheath extensions or hypodermal collenchymas (Fig. 2.15).

Comparison between the leaf structures of Dicot and Monocot:

Characters	**Dicot Leaf** e.g. Mango leaf	**Monocot Leaf** e.g. Maize leaf
1. Orientation of Leaf	Horizontal, dorsiventrally differentiated	Vertical, isobilateral
2. Stomata	Usually on lower epidermis	On both epidermal layers
3. Cuticle	Thick on upper epidermis	Thick on both epidermal layers
4. Motor cells	Absent	Present in upper epidermis
5. Mesophyll	Differentiated into upper palisade parenchyma and lower spongy parenchyma	No differentiation of mesophyll
6.Vascular bundles	All of them are not seen in parallel series and are supported by bundle sheath extension	Vascular bundles are in parallel series and are supported by sclerenchyma patches

How can you identify dorsiventral leaf?

- Presence of two epidermal layers
- Presence of cuticle and trichomes in both the epidermal layers
- Hypostomatic conditions
- Mesophyll differentiated into upper palisade parenchyma and lower spongy parenchyma.
- Veins irregularly scattered in the mesophyll
- Presence of a bundle sheath made up of parenchyma.
- Vascular bundles are conjoint, collateral with endarch xylem.
- Presence of bundle sheath extensions made up of collenchyma.
- Representing bundle sheath

How can you identify isobilateral leaf?

- Presence of two epidermal layers
- Presence of cuticle and trichomes in both the epidermal layers
- Amphistomatic condition
- Presence of motor cells in the upper epidermis
- Presence of undifferentiated mesophyll
- Vascular bundle parallel arranged
- Vascular bundle conjoint, collateral with endarch xylem
- Presence of hypodermal sclerenchyma

2.7 SUMMARY

Based on the types of cell structure and function, tissues are classified into meristematic, simple and complex tissues. Meristematic tissues are actively dividing cells, which are isodiametric in shape, rich in cytoplasm, with small vacuoles or no vacuoles and cells are actively metabolic. Simple tissues contain cells of similar structure, function and have common origin. They are divided in parenchyma, collenchyma and sclerenchyma. Complex tissues are the cells that are made up of more than one type of cells and differ in their structure, shape and function, and have different origin, but together they perform a common function e.g. vascular tissues, secretary tissues etc.

Roots have root cap at the apex with root hairs near the apex. Vascular bundles are radial and exarch type i.e. xylem and phloem in different radii and protoxylem towards periphery and metaxylem towards center. The stem is normally divided into nodes and internodes: The nodes hold one or more leaves, as well as buds which can grow into branches. Stem usually consist of three tissues, dermal tissue, ground tissue and vascular tissue. The dermal tissue covers the outer surface of the stem and usually functions as water proof layer, protect and control gas exchange. The ground tissue usually consists mainly of parenchyma cells and fills in around the vascular

tissue. Monocot and dicot stems are identified on basis of nature of vascular bundles. Monocots have scattered vascular bundles and dicots have vascular bundles arranged in a ring. Dicot leaves comprise mesophyll cells i.e. palisade and spongy parenchyma. While the monocot leaves only have spongy cells in their mesophyll.

2.8 GLOSSARY

Air space: Intercellular gaps within the spongy mesophyll. These gaps are filled with gas that the plant uses (Carbon dioxide - CO_2) and gases that the plant is expelling (Oxygen - O_2, and water vapor)

Chlorophyll: A molecule in leaves that can use light energy from sunlight to turn water and carbon dioxide gas into sugar and oxygen (this process is called photosynthesis). Chlorophyll is magnesium-based and is green

Cuticle : The waxy, water-repelling layer on the outer surface of a leaf that helps keep it from dying out (and protect it from invading bacteria, insects, and fungi). The cuticle is secreted by the epidermis (including the guard cells) and is often thinner on the underside of leaves. The cuticle is generally thicker on plants that live in dry environments

Epidermis: The protective, outer layer of cells on the surface of a leaf. The guard cells (and stoma) are part of the epidermis. The surface of many leaves is coated with a waxy cuticle which is secreted by the epidermis

Guard cell: One of a pair of sausage-shaped cells that surround a stoma (a pore in a leaf). Guard cells change shape (as light and humidity change), causing the stoma to open and close

Lamina: The blade of a leaf

Mesophyll: The chlorophyll-containing leaf tissue located between the upper and lower epidermis. These cells convert sunlight into usable chemical energy for the plant

Midrib: The central rib of a leaf - it is usually continuous with the petiole

Palisade mesophyll: A layer of elongated cells located under the upper epidermis. These cells contain most of the leaf's chlorophyll, converting sunlight into usable chemical energy for the plant

Petiole: A leaf stalk which attaches the leaf to the plant

Photosynthesis: The process in which plants convert sunlight, water, and carbon dioxide into food energy (sugars and starches), oxygen and water. Chlorophyll or closely-related pigments (substances that color the plant) are essential to the photosynthetic process

Pinnate: A compound leaf that is made up of many small leaflets arranged in pairs on either side of a long central midrib (the rachis). There is often a single terminal leaflet at the end of the midrib

Sclereid: A type of sclerenchyma, made up of gritty cells, often called "stone cells." Sclereids are what make a pear slightly gritty

Schlerenchyma: Tissue composed of thick-walled cells containing lignin for strength and support

Sieve element: Cell in the phloem tissue concerned with longitudinal conduction of food materials. In flowering plants, it is called a sieve-tube element

Sieve tube: A series of sieve-tube elements arranged end to end and interconnected through sieve plates

Spongy mesophyll: The layer below the palisade mesophyll; it has irregularly-shaped cells with many air spaces between the cells. These cells contain some chlorophyll. The spongy mesophyll cells communicate with the guard cells (stomata), causing them to open or close, depending on the concentration of gases

Stem: (Also called the axis) the main support of the plant

Stoma: (Plural stomata) a pore (or opening) in a plant's leaves where water vapor and other gases leave and enter the plant. Stomata are formed by two guard cells that regulate the opening and closing of the pore. Generally, many more stomata are on the lower side of a leaf than on the upper

Vascular bundle: Veins provide support for the leaf and transport both water and minerals (via xylem) and food energy (via phloem) through the leaf and on to the rest of the plant

2.9 SELF ASSESSMENT QUESTION

2.9.1 Choose and write the correct options:

1-The change from meristematic tissue to permanent tissue is called:

(a) Differentiation (b) Self perpetuating

(c) Photosynthesis (d) Cell division

2-The tissue generally present in all organs of plant is:

(a) Parenchyma (b) Chlorenchyma

(c) Collenchyma (d) Sclerenchyma

3-The root hairs are produced from:

(a) Rhizodermis (b) Trichomes

(c) Accessory cells (d) Trichoblasts

4-The osteosclereids are seen in:

(a) Seed coat of *Crotalaria* (b) Seed coat of *Pisum*

(c) Pulp of *Pyrus* (d) Petioles of banana

5-In root vascular bundles are:

(a) Endarch (b) Exarch
(c) Mesarch (d) None of these

6-Which meristem helps in increasing girth?
(a) Lateral meristem (b) Intercalary meristem
(c) Primary meristem (d) Apical meristem.

7-Pith and cortex do not differentiate in:
(a) Monocot stem (b) Dicot stem
(c) Monocot root (d) Dicot root.

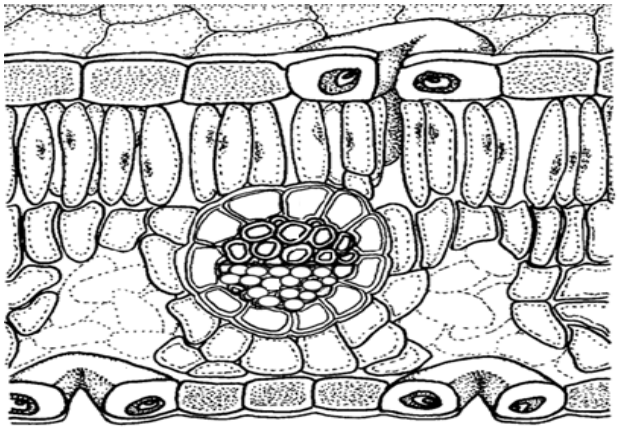
8-Sieve tubes are suited for translocation of food because they possess:
(a) Bordered pits (b) No ends walls
(c) Broader lumen and perforated cross walls (d) No protoplasm.

9-Where do the casparian bands occur:
(a) Epidermis (b) Endodermis
(c) Pericycle (d) Phloem

10-Bordered pits are found in:
(a) Sieve cells (b) Vessel wall
(c) Companion cells (d) Sieve tube wall.

2.9.2 Short answer type questions:

1. What two tissues are found within a vein?
2. What does the word "mesophyll" mean?
3. What types of vascular bundles are found in dicot stem?
4. Label the diagram of the leaf given below:



2.9.1: Answer Keys: 1- (a), 2- (a), 3-(a), 4-(b), 5- (b), 6-(b), 7- (a), 8- (c), 9-(b), 10-(b)

2.10 REFERENCES

- Ayensu E.S. *Anatomy of the Monocotyledons*. VI. Dioscoreales. Oxford: Clarendon Press; 1972.
- Barlow, P.W. Structure and function at the root apex - phylogenetic and ontogenetic perspectives on apical cells and quiescent centres. *Plant and Soil* 1994; 167:1–16.
- Byrne, M.E, Kidner CA, Martiennsen RA. Plant stem cells: divergent pathways and common themes in shoots and roots. *Current Opinion in Genetics and Development* 2003; 13:551–557.
- Carlquist, S. (1975). *Ecological strategies of xylem evolution*. Berkeley, CA: University of California Press.
- Carlquist, S., Schneider, E.L. Origins and nature of vessels in Monocotyledons. I. *Acorus. International Journal of Plant Sciences* 1997: 158: 52–56.
- Clowes, F.A.L. (1994). Origin of the epidermis in root meristems. *New Phytologist* 127: 335–347.
- Clowes, F.A.L.(2000). Patterns of root meristem development in Angiosperms. *New Phytologist* 146: 83–94.
- Craig, Richard and Vassilyev, Andrey. *Plant Anatomy*. McGraw-Hill. Archived from the original on 24 July 2010.
- Esau K. 1977. *Anatomy of Seed Plants*. John Wiley & Sons Inc., New York.
- Esau's Plant Anatomy, Meristems, Cells, and Tissues of the Plant Body: their Structure, Function, and Development. 3rd edition.". *Annals of Botany* **99** (4): 785–786.
- Fahn, A. 1990. *Plant Anatomy*. Pergamon Press, Oxford.
- Pandey, B. P. (2001). *Plant Anatomy*. S. Chand & Company Ltd., New Delhi.
- Pandey, S.N. (1997). *Plant Anatomy and Embryology.* Vikas PublishingHouse Pvt Ltd, New Delhi.
- Singh, V., 2010. *Plant Anatomy and Embryology of Angiosperms*. Global Media Publications, Meerut.
- Singh, V., Pande, P.C. and Jain, D.K. 2012, *Structure Development and Reproduction in Angiosperms*. Rastogi Publications, Meerut.

2.11 SUGGESTED READINGS

- Esau K. (1977). A*natomy of Seed Plants*. John Wiley & Son. Inc., New York.

- Esau's Plant Anatomy, Meristems, Cells, and Tissues of the Plant Body: their Structure, Function, and Development. 3rd edn.". *Annals of Botany* **99** (4): 785–786.
- Fahn, A. (1990). *Plant Anatomy*. Pergamon Press, Oxford.
- Pandey, B. P. (2001). *Plant Anatomy*. S. Chand & Company Ltd., New Delhi.
- Pandey, S.N. (1997). *Plant Anatomy and Embryology*. Vikas Publishing House Pvt Ltd, New Delhi.
- Singh, V. (2010). *Plant Anatomy and Embryology of Angiosperms.* Global Media Publications, Meerut
- Vasishta, P.C. (1968). *Plant Anatomy.* Pradeep Publication & Co., Chandigarh.

2.12 TERMINAL QUESTIONS

2.12.1. Answer the following questions in two or three sentences

1- Define plant anatomy.
2- Define a tissue.
3- What is differentiation?
4- What is an aerenchyma? State its function.
5- What are called guard cells?
6- What is a meristematic tissue?
7- What are called lateral meristems?
8- What are the types of simple tissues and complex tissues?
9-What is a chlorenchyma?
10- Differentiate angular collenchyma from lacunar collenchyma.

2.12.2 Answer the following questions in about 100 words.

1- Bring out the characters of meristematic cells.
2- Explain different types of meristems based on their positions.
3- Draw a labeled diagram of T.S. of dicot stem.
4- Compare the anatomical features of dicot and monocot root.
5- Cut a transverse section of young stem of a plant from your garden and observe it under the microscope. How would you ascertain whether it is a monocot stem or a dicot stem? Give reasons.

2.12.3 Answer the following questions in about 200 words.

1- Write an essay on the location, structure and functions of parenchyma.
2- Describe in detail with diagram about the anatomy of monocot stem.
3- Define the anatomy of dicot root in detail.

UNIT-3 STRUCTURE OF VASCULAR TISSUES

3.1 OBJECTIVES

After reading this unit students will be able to know

- What are vascular bundles?
- Detail structure of xylem and phloem
- The major differences between xylem and phloem
- Briefly explain different types of vascular bundle and their arrangement
- Functions of vascular bundle
- How vascular bundles can be used to identify different kinds of plants

3.2 INTRODUCTION

The human body requires a circulatory system for survival - and so do plant bodies. But while we are usually pretty familiar with our own arteries and veins, we tend to tune out when it comes to plant vessels. Not all plants have vascular tissue. Algae do not need it, since they are immersed in their source of nutrition and hydration. Mosses do not have vascular tissue, either. Water and other materials move throughout these plants by diffusion (this is a major reason they do not grow to be very tall). Ferns have vascular tissue, though, as do higher plants like fir trees and conifers (Gymnosperms) and seed-bearing plants (Angiosperms).

Think about the largest tree you have ever seen. How do you think the tree moves water and food through that very tall trunk? Some types of plants, known as vascular plants, have a system of vessels within them that carry water and food throughout the plant. These vessels are found in the roots, stems, and leaves of the plant. The vascular vessels are divided into two types based on what they transport. Not only do vascular vessels help plants move water and food more efficiently throughout the plant, they also make it possible for the plant to grow larger. By having these vessels, plants can move necessary supplies farther and therefore grow larger. These vascular vessels are similar to the closed circulatory system of humans, because both systems transport nutrients and allow the organisms to grow larger due to the ability to transport farther.

The general characteristics associated with vascular plants incorporate a broad range of plants, and therefore these plants can be divided further into more specific categories. Vascular plants can be divided by their method of reproduction. Vascular plants that reproduce by the use of spores are characterized as ferns. This type of vascular plant is often referred to as a seedless vascular plant. The majority of vascular plants reproduces by creating seeds rather than spores and are classified as either Gymnosperms or Angiosperms. Gymnosperms are vascular plants that create cones to house their seeds. Common Gymnosperms include large trees, such as cedars, hemlocks, pines, and spruces. Angiosperms are vascular plants that create their seeds inside fruits or flowers and are often referred to simply as flowering plants. Some common examples of Angiosperms include sunflowers, dogwood trees, elm trees, lilies, and maple trees.

Being that Angiosperms are a very large group of plants, with over 250,000 known species, they are often further classified into monocots and dicots.

The majority of tissue in a plant stem is called ground tissue and basically fills the space around the vascular tissue. We will look at vascular tissue after going over the three types of ground tissue: parenchyma, collenchyma and sclerenchyma. Parenchyma is the most common form of tissue in plants and provides a variety of functions, including the storage of food and water. Collenchyma tissue helps support young stems and roots. Lastly, sclerenchyma tissue provides rigid support and protection for the plant stem. The Greek term *sclera* means 'hard.' This can help you remember that sclerenchyma is hard tissue that provides rigid support.

Remember that vascular tissue is the tissue used to transport water and nutrients throughout a plant. It acts like roads and plumbing, moving around nutrients and water needed by the plant. There are two types of vascular tissue: xylem and phloem. Xylem transports water and dissolved minerals, while phloem transports food. Previously it was pointed out that an easy way to remember which vascular tissue is which is that 'phloem' and 'food' both start with the same sound. This may help you remember that phloem moves food and that the other tissue, xylem, moves water.

Vascular tissue is a complex conducting tissue, formed of more than one cell type, found in vascular plants. The primary components of vascular tissue are the xylem and phloem. These two tissues transport fluid and nutrients internally. There are also two meristems associated with vascular tissue: the vascular cambium and the cork cambium. All the vascular tissues within a particular plant together constitute the vascular tissue system of that plant. The cells in vascular tissue are typically long and slender. Since the xylem and phloem function in the conduction of water, minerals, and nutrients throughout the plant, it is not surprising that their form should be similar to pipes. The individual cells of phloem are connected end-to-end, just as the sections of a pipe might be. As the plant grows, new vascular tissue differentiates in the growing tips of the plant. The new tissue is aligned with existing vascular tissue, maintaining its connection throughout the plant. The vascular tissue in plants is arranged in long, discrete strands called vascular bundles. These bundles include xylem and phloem, as well as supporting and protective cells. In stems and roots, the xylem typically lies closer to the interior of the stem with phloem towards the exterior of the stem. In the stems of some Asteridae dicots, there may be phloem located inwardly from the xylem as well.

In leaves, the vascular bundles are located among the spongy mesophyll. The xylem is oriented toward the adaxial surface of the leaf (usually the upper side), and phloem is oriented toward the abaxial surface of the leaf (usually the underside). This is why aphids are typically found on the underside of the leaves rather than on the upper, since the phloem transports sugars manufactured by the plant and they are closer to the lower surface.

Now that we have the basic concepts of xylem and phloem, let us look at their structures a little more in depth. We will first look at xylem. Xylem is made of **tracheids**, which are non-living, elongated cells to allow for the transport of fluids. The xylem can sometimes help in the support of the stem. The movement of fluids in plants is generally from the roots up through the stem to the leaves. While xylem is made of non-living cells, phloem is always made of living cells and transports nutrients from the leaves down through the stem. Phloem is made of **sieve elements**, including sieve cells, plates and tubes that are specialized for the movement of food in plants. The **phloem** is vessels on the outer layer of the stem that transport food materials such as sugars from the leaves, where they are produced, or from storage tissues, to the rest of the plant. If a tree is cut, you can often see sap seep out of the tree, and this is the contents of the phloem. If you have ever had maple syrup, it is the processed form of the sap that is found in the phloem of maple trees. Botanists use the arrangement of vascular tissue in plant stems in order to help classify plants. We will focus on the differences seen between monocots and dicots.

Phloem's primary job is to conduct sucrose that is made in the leaves to the rest of the plant. It also carries molecules necessary for growth and defence. Unlike xylem, which conducts water up, phloem's contents, which we commonly call '**sap**' move as needed to different parts of the plant. For example, phloem might move sucrose from the leaves to the roots for storage during the summer, and then back up to the leaves to provide the plant with the energy necessary for budding during the spring. Unlike xylem cells, phloem cells are alive.

3.3 STRUCTURE OF XYLEM

Xylem is one of the two types of transport tissue in vascular plants, phloem being the other. The word xylem is derived from the Greek word meaning "wood"; the best-known xylem tissue is wood, though it is found throughout the plant. Xylem conducts water from the roots, through the shoots, and out of the plant. Most xylem cells are dead cells that form a hollow cylinder that indirectly travels through the entire plant, root to leaf. Water constantly leaves plants through leaves via transpiration, the process of water loss by evaporation. Because water molecules tend to stick together due to their molecular structure, the moisture that absorbed through the roots is carried up through xylem to the leaves in order to replace the water that has been lost. Xylem also functions by transporting dissolved minerals, and because the cells have thick cell walls, provides some means of support for the plant. The most distinctive xylem cells are the long tracheary elements that transport water. Tracheids and vessel elements are distinguished by their shape; vessel elements are shorter, and are connected together into long tubes that are called vessels (Fig. 3.1).

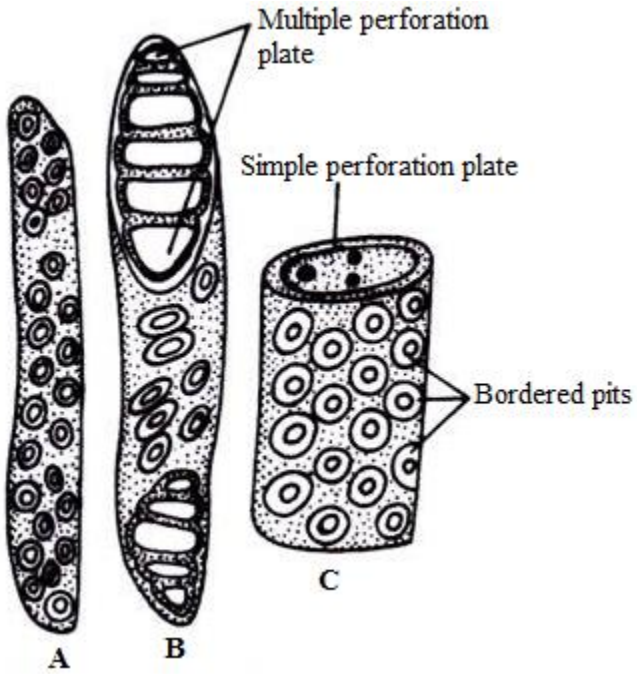


Fig.3.1: Xylem structure; A: Tracheids; B & C. Vessels

Xylem can be found:

- in vascular bundles, present in non-woody plants and non-woody parts of woody plants
- in secondary xylem, laid down by a meristem called the vascular cambium in woody plants as part of a stelar arrangement not divided into bundles, as in many ferns.

In transitional stages of plants with secondary growth, the first two categories are not mutually exclusive, although usually a vascular bundle will contain *primary xylem* only.

Primary and Secondary Xylem

Primary xylem is the xylem that is formed during primary growth from procambium. It includes protoxylem and metaxylem. Metaxylem develops after the protoxylem but before secondary xylem. Metaxylem has wider vessels and tracheids than protoxylem.

Secondary xylem is the xylem that is formed during secondary growth from vascular cambium. Although secondary xylem is also found in members of the "Gymnosperm" groups Gnetophyta and Ginkgophyta and to a lesser extent in members of the Cycadophyta, the two main groups in which secondary xylem can be found are:

- Conifers (*Coniferae*): there are some six hundred species of conifers. All species have secondary xylem, which is relatively uniform in structure throughout this group. Many conifers become tall trees: the secondary xylem of such trees is used and marketed as soft wood.

- Angiosperms: there are some quarters of a million to four hundred thousand species of Angiosperms. Within this group secondary xylem is rare in the monocots. Many monocot Angiosperms become trees, and the secondary xylem of these is used and marketed as hard wood.

To be free from the constraints of small size and constant moisture that the parenchymatic transport system inflicted, plants needed a more efficient water transport system. During the early Silurian, they developed specialized cells, which were lignified (or bore similar chemical compounds) to avoid implosion; this process coincided with cell death, allowing their innards to be emptied and water to be passed through them. These wider, dead, empty cells were a million times more conductive than the inter-cell method, giving the potential for transport over longer distances, and higher CO_2 diffusion rates.

Water transport requires regulation, and dynamic control is provided by stomata. By adjusting the amount of gas exchange, they can restrict the amount of water lost through transpiration. This is an important role where water supply is not constant, and indeed stomata appear to have evolved before tracheids, being present in the non-vascular hornworts.

An endodermis probably evolved during the Silu-Devonian, but the first fossil evidence for such a structure is Carboniferous. This structure in the roots covers the water transport tissue and regulates ion exchange (and prevents unwanted pathogens etc. from entering the water transport system). The endodermis can also provide an upwards pressure, forcing water out of the roots when transpiration is not enough of a driver. Once plants had evolved this level of controlled water transport, they were truly homoiohydric, able to extract water from their environment through root-like organs rather than relying on a film of surface moisture, enabling them to grow to much greater size. As a result of their independence from their surroundings, they lost their ability to survive desiccation-a costly trait to retain.

While wider tracheids with robust walls make it possible to achieve higher water transport pressures, this increases the problem of cavitation. Cavitation occurs when a bubble of air forms within a vessel, breaking the bonds between chains of water molecules and preventing them from pulling more water up with their cohesive tension. A tracheid once cavitated, cannot have its embolism removed and return to service (except in a few advanced Angiosperms which have developed a mechanism of doing so). Therefore it is well worth plants while to avoid cavitation occurrence. For this reason, pits in tracheid walls have very small diameters, to prevent air entering and allowing bubbles to nucleate. Damage to a tracheid's wall almost inevitably leads to air leaking in and cavitation, hence the importance of many tracheids working in parallel.

Cavitation is hard to avoid, but once it has occurred plants have a range of mechanisms to contain the damage. Small pits link adjacent conduits to allow fluid to flow between them, but not air-although ironically these pits, which prevent the spread of embolisms, are also a major cause of them. These pitted surfaces further reduce the flow of water through the xylem by as

much as 30%. Other plants simply accept cavitation; for instance, oaks grow a ring of wide vessels at the start of each spring, none of which survive the winter frosts. Maples use root pressure each spring to force sap upwards from the roots, squeezing out any air bubbles.

Growing to height also employed another trait of tracheids - the support offered by their lignified walls. Defunct tracheids were retained to form a strong, woody stem, produced in most instances by a secondary xylem. However, in early plants, tracheids were too mechanically vulnerable, and retained a central position, with a layer of tough sclerenchyma on the outer rim of the stems. Even when tracheids do take a structural role, they are supported by sclerenchymatic tissue.

Tracheids end with walls, which impose a great deal of resistance on flow; vessel members have perforated end walls, and are arranged in series to operate as if they were one continuous vessel. The function of end walls, which were the default state in the Devonian, was probably to avoid embolisms. An embolism is where an air bubble is created in a tracheid. This may happen as a result of freezing, or by gases dissolving out of solution. Once an embolism is formed, it usually cannot be removed (but see later); the affected cell cannot pull water up, and is rendered useless.

The size of tracheids is limited as they comprise a single cell; this limits their length, which in turn limits their maximum useful diameter to 80 μm. Conductivity grows with the fourth power of diameter, so increased diameter has huge rewards; **vessel elements**, consisting of a number of cells, joined at their ends, overcame this limit and allowed larger tubes to form, reaching diameters of up to 500μm, and lengths of up to 10μm.

Vessels first evolved during the dry, low CO_2 periods of the late Permian, in the horsetails, ferns and Selaginellales independently and later appeared in the mid Cretaceous in Angiosperms. Vessels allow the same cross-sectional area of wood to transport around a hundred times more water than tracheids! This allowed plants to fill more of their stems with structural fibers, and also opened a new niche to vines, which could transport water without being as thick as the tree they grew on. Despite these advantages, tracheid-based wood is a lot lighter, thus cheaper to make, as vessels need to be much more reinforced to avoid cavitation.

Development

Xylem development can be described by four terms: centrarch, exarch, endarch and mesarch. As it develops in young plants, its nature changes from *protoxylem* to *metaxylem* (i.e. from *first xylem* to *after xylem*). The patterns in which protoxylem and metaxylem are arranged are important in the study of plant morphology.

Protoxylem and metaxylem

As a young vascular plant grows, one or more strands of primary xylem form in its stems and roots. The first xylem to develop is called 'protoxylem'. In appearance protoxylem is usually distinguished by narrower vessels formed of smaller cells. Some of these cells have walls which

contain thickenings in the form of rings or helices. Functionally, protoxylem can extend: the cells are able to grow in size and develop while a stem or root is elongating. Later, 'metaxylem' develops in the strands of xylem. Metaxylem vessels and cells are usually larger; the cells have thickenings which are typically either in the form of ladder like transverse bars (scalariform) or continuous sheets except for holes or pits (pitted). Functionally, metaxylem completes its development after elongation ceases when the cells no longer need to grow in size.

Patterns of protoxylem and metaxylem

There are four main patterns to the arrangement of protoxylem and metaxylem in stems and roots (Fig. 3.2).

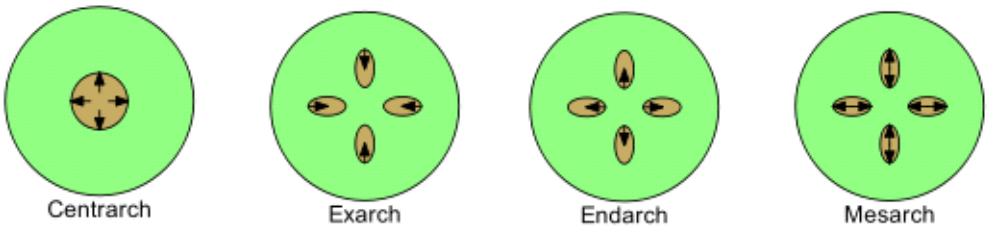


Fig. 3.2: Patterns of protoxylem and metaxylem

- *Centrarch* refers to the case in which the primary xylem forms a single cylinder in the center of the stem and develops from the center outwards. The protoxylem is thus found in the central core and the metaxylem in a cylinder around it. This pattern was common in early land plants, such as "Rhyniophytes", but is not present in any living plants.

  The other three terms are used where there is more than one strand of primary xylem.

- *Exarch* is used when there is more than one strand of primary xylem in a stem or root, and the xylem develops from the outside towards the center, i.e. centripetally. The metaxylem is thus closest to the center of the stem or root and the protoxylem closest to the periphery. The roots of vascular plants are normally considered to have exarch development.

- *Endarch* is used when there is more than one strand of primary xylem in a stem or root, and the xylem develops from the inside towards the periphery, i.e. centrifugally. The protoxylem is thus closest to the center of the stem or root and the metaxylem closest to the periphery. The stems of seed plants typically have endarch development.

- *Mesarch* is used when there is more than one strand of primary xylem in a stem or root, and the xylem develops from the middle of a strand in both directions. The metaxylem is thus on both the peripheral and central sides of the strand with the protoxylem between the metaxylem (possibly surrounded by it). The leaves and stems of many ferns have mesarch development.

3.4 STRUCTURE OF PHLOEM

In vascular plants, **phloem** is the living tissue that carries organic nutrients (known as photosynthate), in particular, sucrose, a sugar, to all parts of the plant where needed. In trees, the phloem is the innermost layer of the bark, hence the name, derived from the Greek word (*phloios*) meaning "bark". The phloem is concerned mainly with the transport of soluble organic material made during photosynthesis. This process of transportation is called translocation.

Phloem tissue consists of: conducting cells, generally called sieve elements; parenchyma cells, including both specialized companion cells or albuminous cells and unspecialized cells; and supportive cells, such as fibers and sclereids (Fig. 3.3).

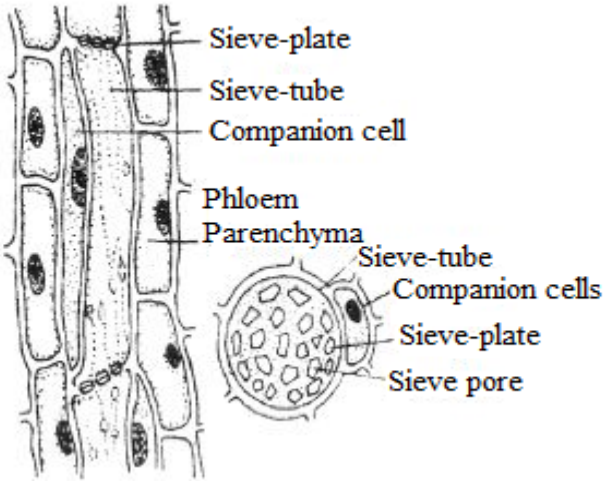


Fig. 3.3: A. L.S.; B. T.S. of different types of Phloem tissue

Conducting cells (Sieve elements)

Sieve elements are the type of cells that are responsible for transporting sugars throughout the plant. At maturity they lack a nucleus and have very few organelles, so they rely on companion cells or albuminous cells for most of their metabolic needs. Sieve tube cells do contain vacuoles and other organelles, such as ribosomes, before they mature, but these generally migrate to the cell wall and dissolve at maturity; this ensures there is little to impede the movement of fluids. One of the few organelles they do contain at maturity is the smooth endoplasmic reticulum, which can be found at the plasma membrane, often nearby the plasmodesmata that connect them to their companion or albuminous cells. All sieve cells have groups of pores at their ends that grow from modified and enlarged plasmodesmata, called sieve areas. The pores are reinforced by platelets of a polysaccharide called callose.

Companion cells

The metabolic functioning of sieve-tube members depends on a close association with the *companion cells*, a specialized form of parenchyma cell. All of the cellular functions of a sieve-tube element are carried out by the (much smaller) companion cell; a typical nucleate plant cell except the companion cell usually has a larger number of ribosomes and mitochondria.
The dense cytoplasm of a companion cell is connected to the sieve-tube element by plasmodesmata. The common sidewall shared by sieve tube elements and companion cell have large numbers of plasmodesmata which form sieve areas.

There are three types of companion cells.

- Ordinary companions cells, which have smooth walls and few or no plasmodesmata connections to cells other than the sieve tube.
- Transfer cells, which have much-folded walls that are adjacent to non-sieve cells, allowing for larger areas of transfer. They are specialized in scavenging solutes from those in the cell walls that are actively pumped requiring energy.
- Intermediary cells, which have smooth walls and numerous plasmodesmata connecting them to other cells.

The first two types of cell collect solutes through apoplastic (cell wall) transfers, whilst the third type can collect solutes via the symplast through the plasmodesmata connections.

Albuminous cells

Albuminous cells have a similar role to companion cells, but are associated with sieve cells only and are therefore found only in seedless vascular plants and Gymnosperms.

Other parenchyma cells

Other parenchyma cells within the phloem are generally undifferentiated and used for food storage.

Supportive cells

Although its primary function is transport of sugars, phloem may also contain cells that have a mechanical support function. These generally fall into two categories: fibers and sclereids. Both cell types have a secondary cell wall and are therefore dead at maturity. The secondary cell wall increases their rigidity and tensile strength.

Fibers

Fibers are the long, narrow supportive cells that provide tension strength without limiting flexibility. They are also found in xylem, and are the main component of many textiles such as paper, linen, and cotton.

Sclereids

Sclereids are irregularly shaped cells that add compression strength but may reduce flexibility to some extent. They also serve as anti-herbivory structures, as their irregular shape and hardness will increase wear on teeth as the herbivores chew. For example, they are responsible for the gritty texture in pears.

Unlike xylem (which is composed primarily of dead cells), the phloem is composed of still-living cells that transport sap. The sap is a water-based solution, but rich in sugars made by the photosynthetic areas. These sugars are transported to non-photosynthetic parts of the plant, such as the roots, or into storage structures, such as tubers or bulbs.

During the plant's growth period, usually during the spring, storage organs such as the roots are sugar sources, and the plant's many growing areas are sugar sinks. The movement in phloem is multidirectional, whereas, in xylem cells, it is unidirectional (upward). After the growth period, when the meristems are dormant, the leaves are sources, and storage organs are sinks. Developing seed-bearing organs (such as fruit) are always sinks. Because of this multi-directional flow, coupled with the fact that sap cannot move with ease between adjacent sieve-tubes, it is not unusual for sap in adjacent sieve-tubes to be flowing in opposite directions.

While movement of water and minerals through the xylem is driven by negative pressures (tension) most of the time, movement through the phloem is driven by positive hydrostatic pressures. This process is termed *translocation*, and is accomplished by a process called *phloem loading* and *unloading*. Cells in a sugar source "load" a sieve-tube element by actively transporting solute molecules into it. This causes water to move into the sieve-tube element by osmosis, creating pressure that pushes the sap down the tube. In sugar sinks, cells actively transport solutes *out* of the sieve-tube elements, producing the exactly opposite effect.

Girdling

Because phloem tubes sit on the outside of the xylem in most plants, a tree or other plant can be effectively killed by stripping away the bark in a ring on the trunk or stem. With the phloem destroyed, nutrients cannot reach the roots, and the tree/plant will die. Trees located in areas with animals such as beavers are vulnerable since beavers chew off the bark at a fairly precise height. This process is known as girdling, and can be used for agricultural purposes. For example, enormous fruits and vegetables seen at fairs and carnivals are produced via girdling. A farmer would place a girdle at the base of a large branch, and remove all but one fruit/vegetable from that branch. Thus, all the sugars manufactured by leaves on that branch have no sinks to go to but the one fruit/vegetable, which thus expands to many times normal size.

Types of vascular bundles

The vascular bundles are arranged differently in plants depending on their internal structure. Plants classified as dicots and monocots have their vascular bundles arranged in a circle within

the stem, with phloem on the outside and xylem on the inside. A layer of cambium in between each bundle is found in dicots and absent in monocots. It is xylem in these plants that can later become woody tissue, in many cases. According to relative position of xylem and phloem, the vascular bundles are classified variously.

(A) Radial vascular bundle

In these types of vascular bundle, xylem and phloem tissues occur in separate groups on alternate radial positions. Xylem is present in different radii and phloem in different alternating to each other and this type of arrangement is seen in roots (Fig. 3.4).

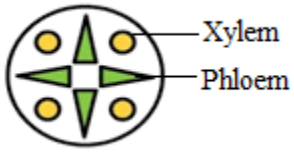


Fig.3.4: Radial vascular bundle

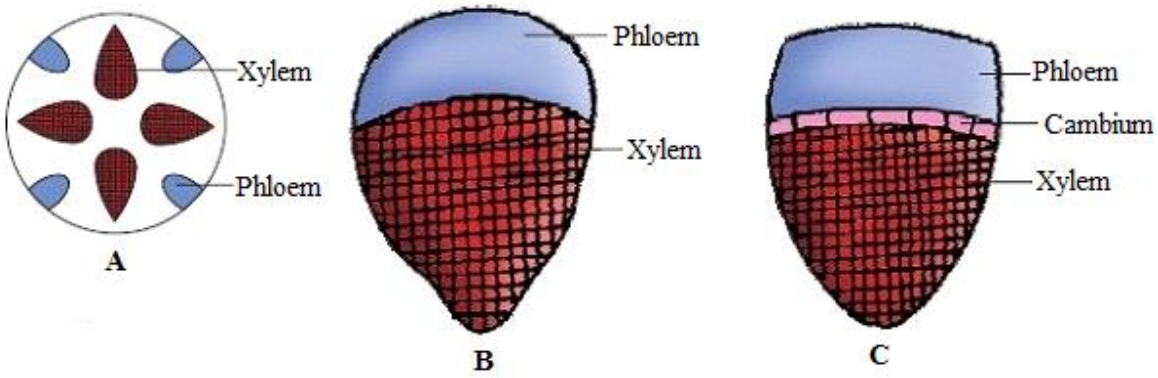


Fig. 3.5: Various types of vascular bundles (A) Radial; (B) Conjoint close; (C) Conjoint open

(B) Conjoint vascular bundles

When the xylem and phloem tissues are present on the same radius and just opposed to each other then it is known as conjoint vascular bundles. In this case generally xylem is present towards the center and phloem is found outside to the xylem cells. It is a common occurrence in dicot stems. Few meristmatic cells called cambial cells are also found in between xylem and phloem cells. Whenever cambial cells are found in between the vascular bundles are called open type and when the cambial cells are not found the vascular bundles are known as closed type. Depending on the number and position of phloem group, conjoint vascular bundles are of two types: **collateral type** and **bi-collateral type**.

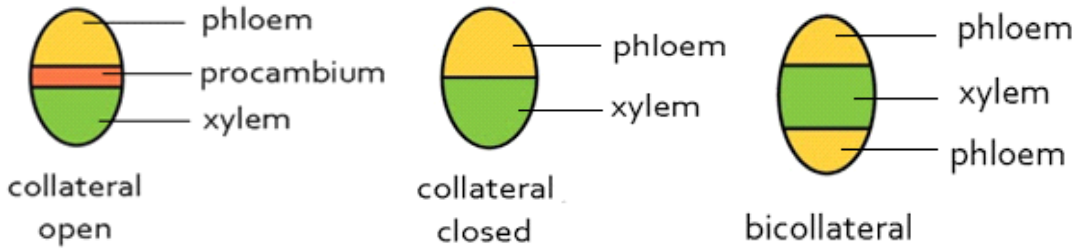


Fig. 3.6 Structure of types of conjoint vascular bundles

Collateral vascular bundles are very common type and seen in stems of dicotyledons except the members of Cucurbitaceae and some members of Convolvulaceae. In this condition xylem is present towards the center and phloem towards outward. Phloem surrounds the xylem within the bundle and cambial cells may be present or absent in between xylem and phloem patches. If the cambial cells are present in between the xylem and phloem then the vascular bundles are known as open type. But if the cambial cells are not present in between these two then it is known as closed type.

Bicollateral vascular bundles contain two patches of phloem on either sides of the xylem on the same radius. The outer phloem or external phloem remains towards the periphery of the central cylinder and inner or internal phloem remains towards the centre. However, there are two patches of cambium found in these vascular bundles. The outer cambium separates outer phloem and xylem; whereas the inner cambium separates the xylem and the inner phloem. The outer cambium is concave in shape and is more active than the inner cambium strip which is inactive or less active (Fig. 3.5, 3.6).

(C) Concentric vascular bundles

Sometimes, either xylem surrounds the phloem tissue or vice versa. In this case xylem and phloem cells are not found in separate bundles instead they completely surround each other. Such vascular bundles are called concentric vascular bundles (Fig. 3.7).

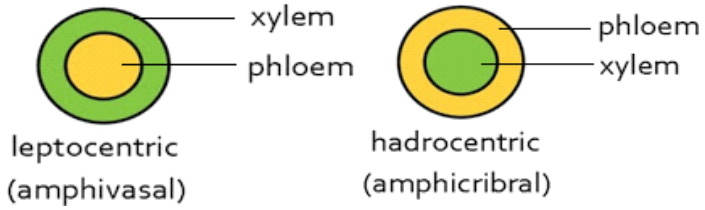


Fig. 3.7: Structure of types of concentric vascular bundles

When xylem surrounds the phloem tissue from all sides the vascular bundle is called **amphivasal** vascular bundle or **leptocentric** type. Such bundles are seen in monocot plant like *Dracaena* after secondary growth.
When phloem surrounds the xylem tissue entirely, the vascular bundle is called **amphicribal** vascular bundle or **hadrocentric** type. Such vascular bundles are seen in Pteridophytes like *Lycopodium*, *Selaginella*.

Function of Vascular Bundles

A **vascular bundle** is a part of the transport system in vascular plants. Just like you have veins, arteries, and capillaries, plants have several different tissue types that make up their vascular bundles. The transport itself happens in vascular tissue, which exists in two forms: xylem and phloem. Both these tissues are present in a vascular bundle, which in addition will include supporting and protective tissues.

The xylem typically lies adaxial with phloem positioned abaxial. In a stem or root this means that the xylem is closer to the centre of the stem or root while the phloem is closer to the exterior. In a leaf, the adaxial surface of the leaf will usually be the upper side, with the abaxial surface the lower side. This is why aphids are typically found on the underside of a leaf rather than on the upper side, since the sugars manufactured by the plant are transported by the phloem, which is closer to the lower surface. The position of vascular bundles relative to each other may vary considerably.

The xylem transports water and dissolved minerals from the roots to wherever it is needed in the plant, going up. Since water transportation relies on transpiration to function, hydrogen bonds of water and tension. Xylem cells are dead at functional maturity. The phloem transports sugar and any other photosynthetic products to the parts of that plant that need it. Phloem cells are alive when they function so it can transport its food-stuffs either upward or downward. The plant tissues that make up the bulk of the 'filling' spaces in plants are known as **parenchyma**, which grow with the plant and also help in the storage of various substances. Also involved in growth is the **cambium** tissue, which creates new xylem and phloem as the plant stems increase in girth. All of these tissues serve to ensure that critical substances are transported through the plant.

3.5 VASCULAR CAMBIUM

The vascular cambium is a plant tissue located between the xylem and the phloem in the stem and root of a vascular plant, and is the source of both the secondary xylem growth inwards, towards the pith and the secondary phloem growth outwards to the bark. It is a cylinder of unspecialized meristem cells that divide to give new cells which then specialize to form secondary vascular tissues.

Vascular cambia are found in dicots and Gymnosperms but not in monocots, which usually lack secondary growth. A few leaf types also have a vascular cambium. Vascular cambium does not transport water, minerals, or dissolved food through the plant. It does, however, produce the phloem and xylem, which do perform these functions. For successful grafting, the vascular

cambia of the rootstock and scion must be aligned so they can grow together. In wood, the vascular cambium is the obvious line separating the bark and wood.

The cambium present between primary xylem and primary phloem is called intrafascicular cambium. At the time of secondary growth, cells of medullary rays, in a line with intrafascicular cambium, become meristematic and form interfascicular cambium. The intrafascicular and interfascicular cambia, therefore, represent a continuous ring which bisects the primary xylem and primary phloem and is known as cambial ring. The vascular cambium then produces secondary xylem on the inside of the ring, and secondary phloem on the outside, pushing the primary xylem and phloem apart.

The normal vascular cambium usually consists of two types of cells:

- Fusiform initials (tall cells, axially oriented)
- Ray initials (almost isodiametric cells - smaller and round to angular in shape)

Fusiform cells are elongated with tapering ends and their size is variable as per the species. They are much longer then wide, broad in middle and tapering at both ends. While Ray initial are isodiametric and much smaller. They constitute the axial system and form radial system of vascular cambium.

The tracheary element fibers, xylem and phloem parenchyma, sieve elements develops from fusiform initials. The vascular rays developed from ray initials. Cambial cells are highly vacuolated and their thin cell walls possess primary pit fields with plasmodesmata. The radial walls of xylem and phloem mother cells are thicker than the tangential ones because cambial cells predominantly divide periclinally during which the thickening of the radial walls is continuous.

Plant vasculature forms a network of interconnected cells spanning the plant's body in an organized manner, from the root tip immersed deep within the soil to the highest tree-tops. The vascular system of multicellular land plants fulfills two main functions, long distance transport and mechanical support. Xylem cells, with thick secondary cell walls rich in lignin, cellulose and hemicellulose, are mainly responsible for providing support to the plant, as well as bulk transport of water, nutrients and minerals from the root system to the shoot. Phloem mediates the shoot-to-root transport of the autotrophic energy source, photoassimilates, as well as signaling molecules, such as plant hormones and peptides.

Plant growth arises from mitotic cell divisions taking place in growth foci called meristems. The earliest (primary) meristems are of embryonic origin, such as the root apical meristem (RAM)

and shoot apical meristem (SAM), which contributes to root and shoot elongation, respectively. These meristems produce the primary plant body, including the primary vasculature. In the primary shoot, the vasculature is located in separate collateral vascular bundles with primary xylem towards the pith parenchyma cells. In roots, the vascular tissue is arranged in a bisymmetric pattern; primary xylem forms a central axis flanked by two poles of primary phloem. Procambial cells intervene between the primary xylem and phloem in both root and shoot vasculature; at the onset of secondary growth, these begin to divide periclinally (parallel to the plant axis/surface), giving rise to secondary xylem (inwards), secondary phloem (outwards), and a secondary meristem called vascular cambium, which forms a continuous ring in an organ-specific manner, discussed in detail later). The vascular cambium is responsible for the lateral (secondary) growth of plants, a process which must be carefully regulated in order to ensure holistic development of the plant vasculature.

Meristematic cells are small, cytoplasmic and undifferentiated. As these cells divide, the outermost cells are pushed away from the meristem, where they cease division, initiate turgor-driven cell expansion and differentiate into specialized cell types. The balance between cell proliferation and differentiation into other cell types is crucial for meristem indeterminacy, and it is evident that both of these aspects of growth are under genetic control.

Vascular meristems generate cells which differentiate into xylem and phloem. The apical meristems in the shoot and root contain procambium, the primary vascular meristem. Vascular tissue in the primary root and hypocotyl originates from embryonic provascular tissue, whereas shoot vascular tissue, located in vascular bundles, is derived from the shoot apical meristem. In Arabidopsis and other species which undergo secondary growth, a lateral vascular meristem called cambium develops mainly from the procambium embedded between the differentiated xylem and phloem. In the shoot, the cambium between the vascular bundles arises from parenchyma and endodermis tissues. Consequently, the complete ring of vascular cambium is formed early on in root/hypocotyl, whereas in shoot (inflorescence stem) the formation of a closed cambial circle is a late event, occurring only after initiation of the interfascicular cambium between the vascular bundles.

Stem cells are located in the meristems, where they maintain the undifferentiated state of the other meristematic cells. A classical stem cell niche consists of a group of cells called an organizing center which keeps the adjacent stem cells from differentiating.

3.5.1 Normal Activity

The secondary growth in dicot stem occurs due to activity of cambium at stellar region and activity of cork cambium at extrastelar region. Stelar secondary growth leads to formation of annual ring. Generally, dicot stem contains conjoint, collateral, open and endarch vascular bundles arranged in a ring. The fascicular cambium is present between xylem and phloem of

each vascular bundle. At the time of secondary growth, the parenchymatous cells of primary medullary rays lying between the edges of fascicular cambium become meristmatic. They divide, redivide, and form a strip interfascicular cambium. The interfascicular cambium joins with fascicular cambium of either side and forms a complete ring of cambium. Secondly, growth begins with the activity of this cambial ring. Cambium cuts down secondary phloem towards the periphery and secondary xylem towards the center. Normally, cambium produces more secondary xylem than secondary phloem. Due to formation of more secondary tissues, the primary tissues are pushed apart from each other and remain small patches or the completely crushed.

The secondary growth starts when the vascular cambial cells starts dividing. This leads to the production of two cells. Among which one can turn into either secondary phloem cell or secondary xylem cell. The other cell of vascular bundle remains undifferentiated (same as vascular bundle cell). This leads to the division of this undifferentiated cell again, so the process repeats continuously.

- The vascular cambium cell divides produces secondary phloem to outside of the dicot stem and secondary xylem inside thus increasing the diameter.
- During secondary growth, as the secondary phloem increases in its thickness, the primary phloem decreases in its thickness. An outer layer of it is nothing but cork cambium, another type of cambium apart from vascular cambium which plays role in secondary growth.
- Cork cambium gives rise to cork cells and the epidermis is gradually replaced by periderm.
- This leads to the growth of bark.
- Bark consists of all the tissue layers outside the vascular cambium. Like secondary phloem, cork, cork cambium and periderm.

The secondary xylem consists of vessels, tracheids, wood fibers and wood parenchyma. Vessels are more abundant. The secondary phloem consists of sieve tubes, companion cells, phloem parenchyma and phloem fibers or bast fibers. The cells of secondary phloem are arranged in radial rows. The cambial cells between vascular bundles cut down secondary phloem. The activity of cambium is influenced by the variations of climate. It produces more vessels with inner cavity during spring called the spring wood and less vessels with narrow cavities, more tracheids and wood fibers in autumn called autumn wood or late wood. These two types together form a growth ring or annual ring. By counting the total number of annual ring, the approximate age of the plant can be determined. After a long period of secondary growth, two types of woods appear in the stem such as, sapwood, heartwood. Sapwood is recently formed wood and heartwood is earlier formed wood.

If we look closely at the cells of the vascular cambium we see two patterns of division. Initial cells can undergo multiplicative divisions or they can undergo additive divisions. Multiplicative

divisions produce more initial cells and result in the increased circumference of the vascular cambium. Of the two cells produced from an additive division one is retained as an initial cell that will divide again and the other will become a phloem mother cell or a xylem mother cell. These mother cells will differentiate into their respective cell types.

The cambial ring exhibits mitotic activity on both the sides. The mitotic activity on the inner surface results in the formation of cells, which differentiate into a set of xylem. It represents the secondary xylem. Similarly, the mitotic activity on the outer surface results in the formation of cells, which differentiate into a set of phloem. It represents the secondary phloem. Due to the formation of secondary xylem, the primary xylem becomes pushed more towards the pith and the pith gets slightly reduced. However, the secondary phloem grows and completely masks the primary phloem. Hence, it is not visible.

The mitotic activity of the cambial ring is purely seasonal. It occurs only twice during every year, once in the spring and once in the autumn. Thus, every year two sets of secondary xylem and two sets of secondary phloem are formed. Each year, the mitotic division of the cambial ring usually begins in the spring season. The secondary xylem that is formed in the spring season is therefore known as springwood or early wood, while the secondary xylem formed in the autumn is known as autumn wood or late wood. The springwood is generally characterized by the presence of xylem vessels having wider lumen. This is because, spring is the ideal season for growth and the water requirement of the plant is more in the spring. The autumn wood has xylem vessels with narrow lumen, since water requirement in the winter is less.

The two distinct layers of secondary xylem, the inner springwood and the outer autumn wood together represent the (or annual ring). One such annual ring is added every year due to secondary growth. Thus, it is possible to ascertain the age of a dicot tree by counting the number of annual rings. While every year two sets of secondary xylem and two sets of secondary phloem are formed, only one set is visible because the secondary phloem formed later (in the autumn) grows over and masks the secondary phloem formed earlier (in the spring).

This is however applicable only to the temperate regions. The growth seasons in the tropics are different, with the growing season coinciding with the rains, in true rainforest areas where seasonality is least pronounced, many trees show continuous growth and growth rings are not seen at all.

3.5.2 Products

Due to the activity of cambial cells wood is produced and the plant increases in the girth. The wood which we use for timber, furniture, fuel and other purposes from trees is the result of activity of cambium. During the spring growing season, cells of the secondary xylem have a large internal diameter; their primary cell walls are not extensively thickened. This is known as early wood, or spring wood. During the fall season, the secondary xylem develops thickened cell

walls, forming late wood, or autumn wood, which is denser than early wood. This alternation of early and late wood is due largely to a seasonal decrease in the number of vessel elements and a seasonal increase in the number of tracheids. It results in the formation of an annual ring, which can be seen as a circular ring in the cross section of the stem. Along with this in the event of an injury the cambium rapidly forms a parenchymatous tissue callus or wound tissue. Even in grafting there is union of cambium of two plants i.e. stock and scion. If these two are incompatible their cambia do not fuse and normal xylem and phloem are not formed and parenchymatous mass is produced indicating weak union and slow conduction. Normally cambium is not present in monocots but in *Dracaena* and *Yucca* a ring of meristmatic tissue originates within parenchymatous tissue just outside primary vascular bundles.

3.6 SUMMARY

A **vascular bundle** is a part of the transport system in vascular plants. The transport itself happens in vascular tissue, which exists in two forms as xylem and phloem. Vascular tissue is made of xylem and phloem tissue which transports water and nutrients; they always lie next to each other, forming a structure called a vascular bundle in stems. Vascular tissue is made of xylem tissue which transports water and nutrients from the roots to different parts of the plant and phloem tissue which transports organic compounds from the site of photosynthesis to other parts of the plant. Xylem transports and stores water and water-soluble nutrients in vascular plants. Phloem is responsible for transporting sugars, proteins, and other organic molecules in plants. Vascular bundles are the criteria to identify the plant anatomy. By the help of vascular bundles we can determine whether the section is of stem or root. Along with this by the position of vascular bundles we can also determine whether the plant is monocot or dicot. In between the vascular bundles the cambial cells help to produce the wood and by counting the annual rings produced due to the activity of cambium we determine the age of the trees.

According to relative position of xylem and phloem, the vascular bundles are classified variously. When xylem and phloem tissues occur in separate groups on alternate radial positions are known as **Radial vascular bundle**. This is seen in roots. When xylem and phloem tissues are present on the same radius and just opposed to each other in conjoint vascular bundles they are called c**onjoint vascular bundles**. It is a common occurrence in dicot stems.

In between xylem and phloem cambial cells are present so that vascular bundles are called open type of vascular bundles. Cambium divides and increases the plant in girth called secondary growth and in the peripheral region forms cork cambium which gives rise to the bark.

3.7 GLOSSARY

Amphiphloic: Having phloem on both sides of the xylem

Analogous, analogy: Opposite to homologous, correspondence in function between anatomical parts of different structure and origin; analogous: exhibit analogy (synonym: similar)

Apical meristem: Embryonic, totipotent tissue in the tips of the roots and shoots of plants

Bifacial (vascular cambium) : Having two "faces" i.e. a vascular cambium that produces cells on both sides; in seed plants phloem is produced to the outside and xylem to the inside; compare to unifacial (vascular cambium); see cambium

Cambium: A lateral meristem that produces secondary growth

Collenchyma: Tissue composed of unevenly thickened cell walls; collenchyma cells are flexible and support young parts of the plant without hindering growth; collenchyma cells are composed of cellulose

Companion cell : A specialized cell of phloem, derived from the same parent cell as the closely associated sieve-tube element immediately adjacent to it; the nucleus of the companion cell supports both its own cell and the cell of its associated the sieve-tube element

Complex tissue: Tissue that consists of more than one cell type e.g. phloem

Cork: A plant tissue composed of cells whose walls are impregnated with suberin and are non-living at maturity; cork is produced by the cork cambium

Cortex: A primary tissue composed mainly of parenchyma cells, which extends between the epidermis and the vascular tissue

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

Cutin: The waxy substance of which a cuticle is composed

Determinate growth: A type of growth in which the axis ceases growing, usually after the apical meristem differentiates into a reproductive organ, such as a flower or a cone

Dictyostele: A dissected siphonostele with two or more overlapping leaf bases

Ectophloic: Having phloem only on the outer side of the stele; compare to amphiphloic

Endarch: A type of xylem maturation in which protoxylem is internal to metaxylem and development proceeds centrifugally (from the inside out)

Epidermis: The exterior tissue, usually on cell thick, of leaves and young stems and roots

Exarch: A type of xylem maturation in which protoxylem is external to metaxylem and development proceeds centripetally (from the outside in)

Fiber: A long-walled plant cell which is often dead at maturity; fibers impart elasticity, flexibility and tensile strength to plant structure

Ground tissue: A tissue consisting mostly of parenchyma cells that makes up the bulk of a young plant

Growth form: A general description of the type of growth exhibited by a plant such as herbaceous, shrubby (bush-like) and arborescent (tree-like)

Leaf gap: A parenchyma filled interruption in a stem's cylinder of vascular tissue immediately above the point at which a branch of vascular tissue (**leaf trace**) leading to a leaf occurs.

Meristem: Region of totipotent cells in which cell division and initiation of tissues and organs takes place; see apical meristem, vascular cambium and cork cambium

Mesarch: A type of xylem maturation in which the protoxylem is embedded in the metaxylem and development proceeds both centripetally (from the outside in) and centrifugally (from the inside out); compare to endarch and exarch

Mesophyll: Parenchyma tissue between the upper and lower epidermis of a leaf

Metaxylem: **A** type of primary xylem that differentiates and matures later than the protoxylem; generally metaxylem tracheids are longer than protoxylem

Parenchyma: the most common type of plant cell; thin-walled cells varying in size, shape, and function

Phloem: A vascular tissue in land plants primarily responsible for the distribution of sugars and nutrients manufactured in the shoot

Pith: The central parenchymatous tissue in a vascular plant axis

Polystelic: Having more than one stele

Primary growth: **G**rowth in length, controlled by the apical meristem

Protostele: A type of stele with a solid core of primary xylem

Protoxylem: The first primary xylem to differentiate and mature, usually before and during elongation of the axis; protoxylem cells are generally smaller in diameter than metaxylem

Sclereids: **A** short, irregular sclerenchyma cell with pits; sclereids function as tissue support

Sclerenchyma: Tissue composed of cells with walls thickened with lignin; sclerenchyma tissue functions primarily in strengthening and support

Secondary growth: Growth in width initiated and maintained by the vascular cambium and cork cambium

Secondary xylem: Xylem produced by the vascular cambium

Sieve cell: A phloem conducting cell type in all vascular plants except Angiosperms

Sieve plate: Area of the wall of a sieve tube element that contains several to many perforations that permits cytoplasmic connections between sieve tube cells

Sieve tube cell : In Angiosperms, a specialized cell derived from the same parent cell as the closely associated companion cell immediately adjacent to it; sieve tube cells are elongated cells with sieve plates; sieve tube cells form sieve tubes through which photosynthate is transported

Siphonostele: A type of stele that consists of a ring of vascular tissue surrounding pith

Stele: The central vascular cylinder in stems and roots where the vascular tissue is located

Tracheid: A water conducting and supportive cell type of xylem composed of long, thin cells with tapered ends and walls hardened with lignin

Transverse section: Cross section; a section perpendicular to the longitudinal axis of the plant organ

Triarch: Consisting of three; for example a triarch stele has three lobes

Unifacial (Vascular cambium): Having one "face", i.e. a cambium that produces cells only on one side; a unifacial vascular cambium that produces only secondary xylem is found in some fossil non-seed plants.

Vascular bundle: A strand of tissue composed mostly of xylem and of phloem

Vascular cambium: **A** lateral meristem that produces secondary vascular tissue in stems and roots

Vascular tissue: Tissue composed of conducting cells, i.e. xylem and phloem

Xylem: A vascular tissue in land plants primarily responsible for the distribution of water and minerals taken up by the roots; also the primary component of wood

3.8 SELF ASSESSMENT QUESTION

3.8.1: Multiple choice questions

1. The formation of distinct annual rings during secondary growth mainly depends on:

(a) Contrasting seasonal variation (b) Uniform climate

(c) Phellogen formation (d) Xylem quantity

2. The waxy substance associated with the wall of cork cells is:

(a) Cutin (b) Lignin

(c) Hemicellulose (d) Suberin

3. A simple mechanical tissue devoid of lignin is:

(a) Parenchyma (b) Sclerenchyma

(c) Collenchyma (d) Chlorenchyma

4. Vascular bundles in a dicot stem are:

(a) Open, collateral, exarch (b) Closed, collateral, endarch

(c) Closed, collateral, exarch (d) Open, collateral, endarch

5. Abnormal secondary growth is found in :

(a) *Dracaena* (b) *Triticum*

(c) *Helianthus* (d) *Cucurbita*

6. Presence of casparian strips is characteristic feature of:

(a) Endodermis (b) Exodermis

(c) Epidermis (d) Pericycle

7. External protective tissue of plants are:
(a) Cork and cortex (b) Cortex and epidermis
(c) Epidermis and cork (d) Pericycle and cortex

8. Annual rings are distinct in plants growing in :
(a) Temperate regions (b) Tropical regions
(c) Grasslands (d) Arctic region
9. Cambium causes growth in:
(a) Circumference (b) Width
(c) Length (d) All the above

10. A dicot root differs from monocot root in the presence of:
(a) Piliferous layer (b) Exodermis
(c) Ill developed pith (d) Radial vascular bundle

3.8.1: Answer Key: 1-(a), 2-(d), 3-(a), 4-(d), 5-(a), 6-(a), 7-(a), 8-(a), 9-(a), 10-(c)

3.9 REFERENCES

- Cutler, Botha and Stevenson, Plant Anatomy, an Applied Approach and Raven, Evert and Eichhorn, Biology of Plants (6^{th} or later edition) is highly recommended for additional background information.
- Esau, K. and Cheadle, V.I. (1969). Secondary growth in *Bougainvillea*. *Annals of Botany* 33: 807-819.
- Esau, K. (1977). *Anatomy of Seed Plants*. John Wiley & Sons. Inc., New York.
- Ewers, F.W. 1982. Secondary growth in needle leaves of *Pinus longaeva* (bristle-cone pine) and other conifers: Quantitative data. *American Journal of Botany* 69: 1552-1559.
- Fahn, A. (1967). *Plant Anatomy*. Pergamon Press, Oxford.
- Foster, A.S. (1951). *Practical Plant Anatomy*. Van Nostrand, Princeton.
- Pandey, B.P. (2012). *Plant Anatomy*. S. Chand & Company Ltd., New Delhi.
- Pande and Chaddha (1993). *A Text Book of Botany: Plant Anatomy and Economic Botany*. Vol. III. Vikas Publishing House Pvt Ltd, New Delhi.
- Tayal, M.S. (2015-16). *Plant Anatomy.* Rastogi Publications, Meerut.
- Thompson, N.P. and Heimsch, C. (1964). Stem anatomy and aspects of development in tomato. *American Journal of Botany* 51: 7-19.

3.10 SUGGESTED READINGS

- *Integrative Plant Anatomy*. 26 Apr 2000, by William C. Dickison Academic Press Inc.
- Pandey, S.N. (1997). *Plant Anatomy and Embryology.* Vikas Publication House Pvt Ltd, New Delhi.
- Pandey, B.P. (2012). *Plant Anatomy.* S. Chand & Company Ltd., New Delhi
- *Roy, P. (2010). Plant Anatomy.* New Central Book Agency, Kolkata.
- Plant Anatomy: Tissue By: Dr. Manisha Majumdar, Book Rix Edition, 2011.
- Singh, V., Pande, P.C. and Jain, D.K. (2012-13). *Structure Development and Reproduction in Angiosperm.* Rastogi Publications, Meerut.
- An Introduction to Plant Structure and Development: Plant Anatomy for the Twenty by Charles B. Beck, Cambridge Press.
- Esau's Plant Anatomy: Meristems, Cells, and Tissues of the Plant Body: Their Structure, Function, and Development, 3rd Edition by Ray F. Evert (Author), Susan E. Eichhorn ISBN-13: 978-0471738435

3.11 TERMINAL QUESTIONS

3.11.1 Answer the following questions in two or three sentences.

1- Name two plants which possess conjoint, collateral vascular bundle.
2-What are radial types of vascular bundles?
3-What is xylem fiber and how it is important?
4-Differentiate between open and closed types of vascular bundles.
5-Phloem comprises how many types of cells?
6-Define amphivasal type of vascular bundle.
7-Why phloem is important for plants?
8-Differentiate between radial and conjoint vascular bundles?
9-What is the role of xylem?
10-How we can identify the anatomy of root and stem?

3.11.2 Answer the following questions in about 100 words.

1-Define radial types of vascular bundle with diagram.
2-Explain the detailed structure of xylem.
3-Define the anatomical features of phloem.
4-What is periderm? How does periderm formation take place in the dicot stems?
5-What are collateral and bicollateral vascular bundles?

3.11.3 Answer the following questions in about 200 words.

1-What are vascular bundles? Define different types of vascular bundles.

2-Define vascular cambium and its function.

3-What are the differences between xylem and phloem?

UNIT-4 NORMAL AND ANOMALOUS GROWTH

4.1 OBJECTIVES

After reading this unit, students will be able to understand-

- What is Secondary growth?
- About two types of tissue used during secondary growth: vascular cambium and cork cambium.
- What vascular cambium produces and what cork cambium produces?
- How secondary growth does occurs.
- How many types of wood are found in trees?
- How do we determine the age of the tree?
- What is the abnormal behaviour of cambium.

4.2 INTRODUCTION

Remember that all plant stem growth occurs at the meristems of the shoot system because this is where cell division occurs. There are two types of meristem in the plant stem: apical and lateral. As we just reviewed, primary growth occurs at the apical meristem and increases plant stem length. We have previously looked at the basic structures of the shoot system as well as primary growth of the stem. We will now look at another form of growth known as secondary growth of the stem. Before we do, let us review a few key components of the shoot system, which is the above ground structures of plants, including the leaves, buds, stems, flowers and fruits.

Primary growth occurs at the apical meristem and allows the plant stem to increase in length. However, some plants need more than just growth in the length of the stem. We will now look at this type of growth. Remember that all plant stem growth occurs at the meristems of the shoot system because this is where cell division occurs. There are two types of meristem in the plant stem: apical and lateral. As we just reviewed, primary growth occurs at the apical meristem and increases plant stem length. Primary growth occurs when plants grow toward the sunlight necessary for photosynthesis and also sink roots deep into the soil to anchor them and enable them to absorb water and nutrients. This 'up and down' growth is possible due to apical meristem, stem cell like tissue that, upon division, creates an undifferentiated cell that will become either a new root or shoot tip.

Secondary growth happens when stems or branches grow outward (get thicker) This type of growth is possible because some plants (like trees and shrubs) have lateral meristem, another stem cell like tissue. Instead of causing the plant to grow up or down, lateral meristematic tissue causes the plant to increase in girth by adding rings of growth. Now we know how a plant gets taller and its roots get longer. But what about being wider? Even a big tree with an enormous

trunk starts out as a puny seedling. So when the width of a plant or its girth increases is called secondary growth and it arises from the lateral meristems in stems and roots.

As with apical meristems, lateral meristems are regions of high cell division activity. However, the cells they make grow outward rather than upward or downward. Dicots use lateral meristems to add to their width; monocots, however, do not experience secondary growth. We will come back to them later. The lateral meristems that produce secondary growth are called cambia, which just mean a tissue layer that adds to plant growth. The two important ones for secondary growth are the vascular cambium and the cork cambium**.** The vascular cambium produces more vascular tissue (xylem and phloem), which provide support for the shoot system in addition to transporting water and nutrients. Because the xylem and phloem that come from the vascular cambium replace the original (primary) xylem and phloem, and add to the width of the plant, they are called secondary xylem and secondary phloem**.**

Secondary growth is growth at the lateral meristem and increases the girth of the stem. This type of growth is only found in dicots and is not found in monocots. In order to understand why it does not occur in monocots, let us review the structure of vascular tissue in both types of flowering plants. There are two types of vascular tissue: xylem, which moves water and dissolved minerals, and phloem, which moves food in the plant stem. In monocots and dicots, these structures are organized a bit differently.

In monocots, the xylem and phloem are found in paired bundles and are scattered throughout the stem. Remember that monocots are simple flowering plants such as grasses. However, in dicots - which are more advanced flowering plants such as roses and apple trees - the xylem and phloem are found in rings with the xylem on the inside and the phloem on the outside. This organization allows for secondary growth of plant stems.

Cambium

Meristematic tissue responsible for lateral (outward) growth in plants is known as cambium. There are two kinds of cambium in woody plant stems, both of which increase the diameter of stems. First type of cambium is vascular cambium found in the center of the stem; its division produces the plant's secondary vascular tissue (xylem and phloem cells.) The outer ring or near the epidermis the bark of a woody plant also contains a cambium called secondary cambium or cork cambium, which creates cork cells of the outer layer and responsible to give rise the bark.

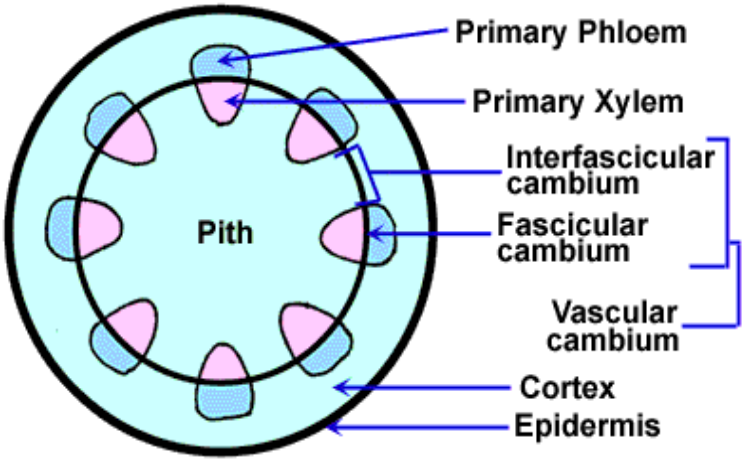


Fig. 4.1: Structure of cambium

The cambium layer consists of a single layer of cell and these cells divide in a direction parallel with epidermis. Each time it divides into two cells and one of the two new cells one remains meristematic and the other differentiates into permanent tissue. If the newly formed cell is near the xylem it will form secondary xylem and if newly formed cell is towards phloem it will develop in secondary phloem. The activity of cambium thus increases and the enlargement of stem takes place and the activity of cambium remains for a considerable long period of time (Fig. 4.1).

4.3 NORMAL BEHAVIOUR OF CAMBIUM

Cells of apical meristems divide, differentiate and develop to form primary tissues. As a result the plant grows in length this is called primary growth. While by the activity of secondary lateral meristems, increase in the circumference/girth of the plant organs due to the formation of secondary tissues in stelar and extra stelar regions, is called as secondary growth. Normally secondary growth takes place in roots and stem of dicots & Gymnosperms. Due to lack of cambium in monocots, secondary growth is absent. But exceptionally secondary, growth takes place in some monocots such as Palm, *Yucca*, *Dracaena*, *Smilax*, *Agave*, Coconut etc.

Secondary growth in dicot stem

Secondary growth in stelar region begins earlier than the extra stelar region. It starts from the development of cambial ring and the detailed activities occur during this is given below (Fig. 4.2, 4.3):

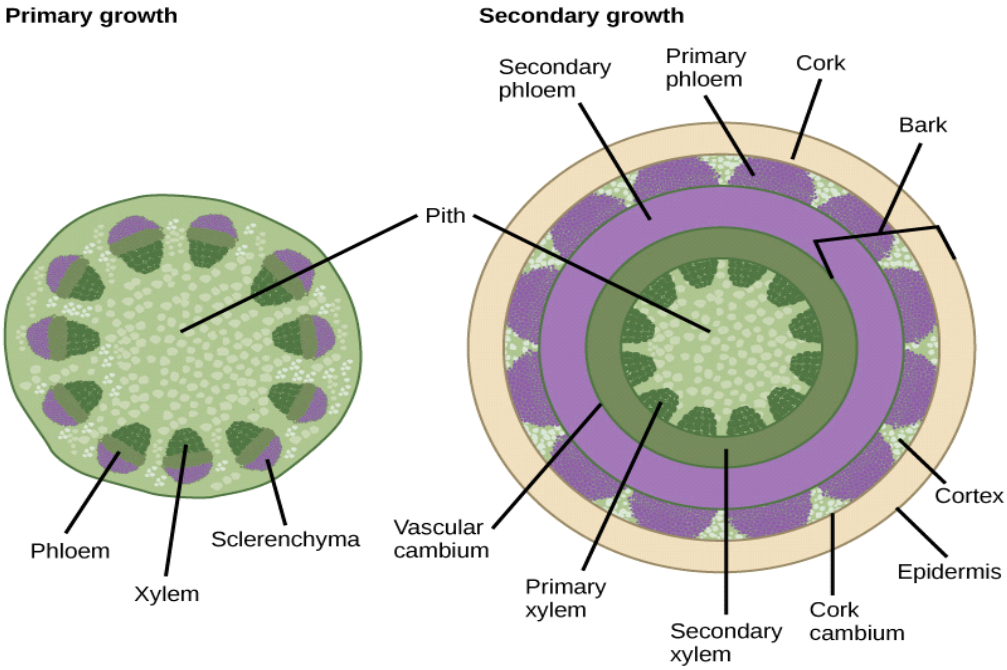


Fig. 4.2 Showing primary growth and secondary growth

Formation of ring of vascular cambium

Vascular bundle comprises xylem and phloem in a bundle and in case of dicot stem these are conjoint, collateral and open type i.e. cambium cells are present in between xylem and phloem cells. A cambium which is present inside the vascular bundle is called intrafascicular cambium. This is a type of primary meristem. When plants become mature then the secondary growth starts and the first step of secondary growth is the formation of cambial ring. For this first of all the cells of medullary rays present in between the vascular bundles become meristematic to form interfascicular cambium this is secondary lateral meristem. Interfascicular cambium is the meristematic cells present outside the vascular bundle and these cells are developed from the medullary cells. Intrafascicular and interfascicular cambia are collectively known as vascular cambial ring. Vascular cambium is formed in the form of a complete ring which is made up of single layer of cells. In dicot stem some part of vascular cambium is primary and some part is secondary. Two types of cells are found in the ring of this vascular cambium.

(i) Fusiform initials

(ii) Ray initials

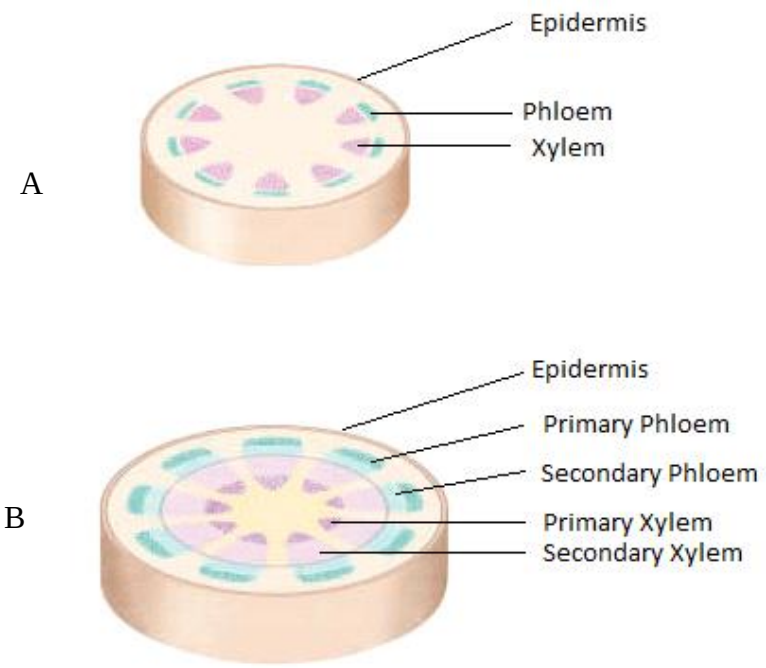


Fig. 4.3: A. T.S. of stem before secondary growth; B. T.S. of stem after secondary growth

Fusiform initials are long with pointed ends, while ray initials are spherical (oval). Amount of fusiform initials is more in vascular cambium. Continuous periclinal divisions or tangential division takes place in fusiform initials. The plane of division in periclinal divisions is parallel to longitudinal axis of a cell. Through this type of activity few cells are formed towards the radius (periphery) and these cells differentiate into secondary phloem or bast and some of the cells are formed towards the central axis and these cells are differentiated into secondary xylem or wood.

Now the complete cambial ring starts producing cells towards inside and outside by division. Normally more secondary xylem is formed as compared to secondary phloem due to unequal distribution of hormones. (secondary xylem is formed 8-10 times more as compared to secondary phloem). By the pressure of secondary phloem; primary phloem is pushed towards the outside and gets crushed. By the pressure of secondary xylem, all the primary tissues such as primary xylem, pith and old secondary xylem degenerates in the centre of the stem. Due to this central part of the stern becomes woody. These activities keep going on continuously in plants throughout.

Before secondary growth the sequences of cells from center towards outside remains pith, primary xylem, cambium, primary phloem, pericycle and endodermis. But due to secondary growth the sequence of the vascular bundle from center changes to primary xylem, secondary xylem, cambium, secondary phloem, primary phloem and then endodermis. Pith crushes due to

the pressure created by the newly formed secondary xylem. Secondary xylem forms in the plant regularly and primary tissues degenerate continuously. This new secondary xylem also degenerate the old secondary xylem.

Waste materials are formed in the stem such as lignin, suberin, tannin, resin-gums etc. due to degeneration of the cells. All these waste materials are filled in the lumen (cavity) of tracheids and vessels of secondary xylem. Because of this, wood in the central region of the stem becomes dark colored (Black brown). It is called heart wood or *Duramen*. The peripheral or outer wood which looks light in color is known as Sap wood or *Alburnum*. As a result of growing of secondary xylem, the diameter of heart wood increases. Physiologically active wood is sapwood and the main function of sap wood is water conduction. Heart wood provides maximum mechanical strength to stem.

Conduction of water is not carried by heart wood because:

- Cavities of tracheids and vessels are progressively filled by waste materials.
- The bladder like in growth of parenchyma cells which enter the lumen of vessels (mainly) and tracheids through the pits in their wall. Such bladder like in growth is called tyloses. Tyloses block the conduction of water.
- In Gymnosperms tylosoids are formed in place of tyloses.
- If the heart wood is destroyed in any stem, then there will be no effect on plants (any vital function is not effected), but if the sap wood is destroyed, then the plant will die because conduction of water will be blocked. Heart wood provides stiffness to the stem. The waste materials of heart wood are antiseptic in nature. Heart wood is resistant to bacteria and fungus. Heart wood has a power of repelling insects- so it is resistant to the termites and in rainy season it does not imbibe water. Thus it is the best quality of wood.
- Study of wood is known as xylotomy.
- If a wood is exposed freely in air then decomposition of sap wood takes place rapidly.
- Position of youngest layer of secondary phloem is just outside the vascular cambium.
- Position of oldest layer of secondary phloem is just inside the primary phloem.
- Position of youngest layer of secondary xylem is just inside the vascular cambium.
- Position of oldest layer of secondary xylem is just outside the primary xylem.
- If xylem is blocked then shoot will die first.

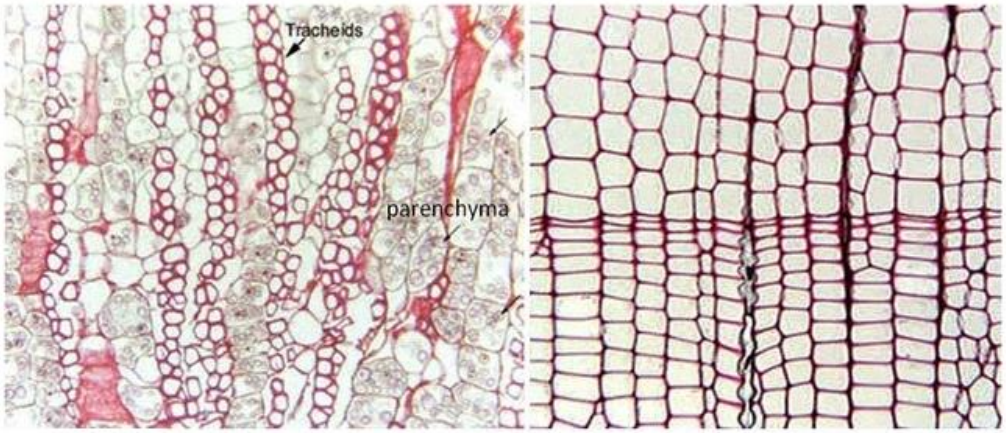
Classification of Wood

(A) On the basis of amount of parenchyma wood is classified into two groups:

1. **Manoxylic wood:** Such type of wood contains more living parenchyma. It is soft and loose wood e.g. *Cycas*. In this secondary vascular tissues with large amounts of softer storage cells (*i.e.* parenchyma) mixed with the wood or xylem cells (e.g. tracheids). The stems of these plants are softer than the wood of trees we use for lumber. Examples of plants with

manoxylic wood are sago palms or cycads, the spurs or short shoots of *Ginkgo* trees, as well as many extinct seed fern groups.

2. **Pycnoxylic wood:** Such wood contains less amount of living parenchyma. It is hard wood. Such types of wood are found in most of the plants and in these secondary vascular tissues with copious amount of xylem cells (e.g. tracheids) and little parenchyma. This wood is much stronger and durable. Examples of plants with pycnoxylic wood are conifers or cone-bearing trees, the long shoots of *Ginkgo*, and Angiosperms (Fig. 4.4).



A **B**

Fig. 4.4: A. Manoxylic wood; B. Pycnoxylic wood

These terms should not be confused with the terms "hard" versus "soft" woods. These terms are used by agro-foresters to make a distinction between conifer trees (soft wood) and angiosperm trees (hard wood). Conifers (mostly evergreen cone-bearing trees such as pines) have wood that is light-weight, light in color, and strong in tension, but weak in shear (along the grains). Therefore this is called "soft wood", which is usually cheaper and used for building inexpensive furniture or used for paper pulp. Flowering plants (mostly deciduous trees such as oak/maple) have wood that is darker in color, heavier in weight, and strong in compression, tension, and shear. Therefore this is called "hard wood", which is used to make durable furniture, flooring, and building structures

(B) On the basis of distribution of parenchyma wood is classified into three groups:

1. Apotracheal: In this type of wood parenchyma is in scattered form e.g. Gymnosperms

2. Paratracheal wood: In this wood parenchyma is arranged or distributed in the form of masses or groups e.g. Dicot plants.

3. Syntracheal wood: In this wood parenchyma is collected around the vessels e.g. *Terminalia arjuna*.

C) Classification based on vessels:

On the 'basis of presence or absence of vessels, wood is classified in two categories

1. Non-porous soft wood: Vessels are absent in such type of wood e.g. Gymnosperms

2. Porous wood: Vessels are present in such type of wood. On the basis of arrangement of vessels porous wood is divided into two groups.

(a) Ring porous wood: Vessels are arranged in the form of a ring in this type of wood. Such wood conducts water more efficiently e.g. in temperate region as in *Dalbergia*

(b) Diffused porous wood: Asystematical distribution of vessels is found in this type of wood in tropical region as in *Azadirachta.*

Formation of Annual rings:

Fig. 4.5: Annual rings

Annual rings are formed due to unequal activity of vascular cambium. The activity of cambium does not remain same; it is changeable in the whole year. Activity of vascular cambium is affected by physiological and environmental factors. In winter or autumn season the activity of the cambium is less and the secondary xylem or wood formed is not extensive through the vascular cambium. Cells formed during this period are small thick walled and have narrow lumens. This is called autumn wood or late wood. The vascular cambium is highly active in spring or summer season and secondary xylem formed during this period is extensive and cells of secondary xylem are larger, thin walled and have wider lumen. This wood is known as spring wood or early wood. The spring wood is lighter in color and exhibits low density where as the autumn (or winter) wood is darker and has higher density (Fig. 4.5).

The autumn and spring wood is formed in the form of rings. The ring of any type of wood is called growth ring. Thus two growth rings are formed in one year. A ring of autumn wood and a ring of spring wood are collectively/known as annual ring. Thus are annual ring consists of two growth rings. The number of annual rings, formed in a tree gives the idea of the age of the tree. The study of determination of age of the plant by these techniques is called **Dendrochronology**. The annual rings are counted from the base of the stem because basal part has maximum annual rings and upper part has less. Therefore, counting from the basal region can give the correct

idea. A piece is taken from the stem up to central region from the base of stem with the help of increment borer instrument. The annual rings are counted from that piece and again inserted (fitted) into the same stem at the same place (Fig.4.6).

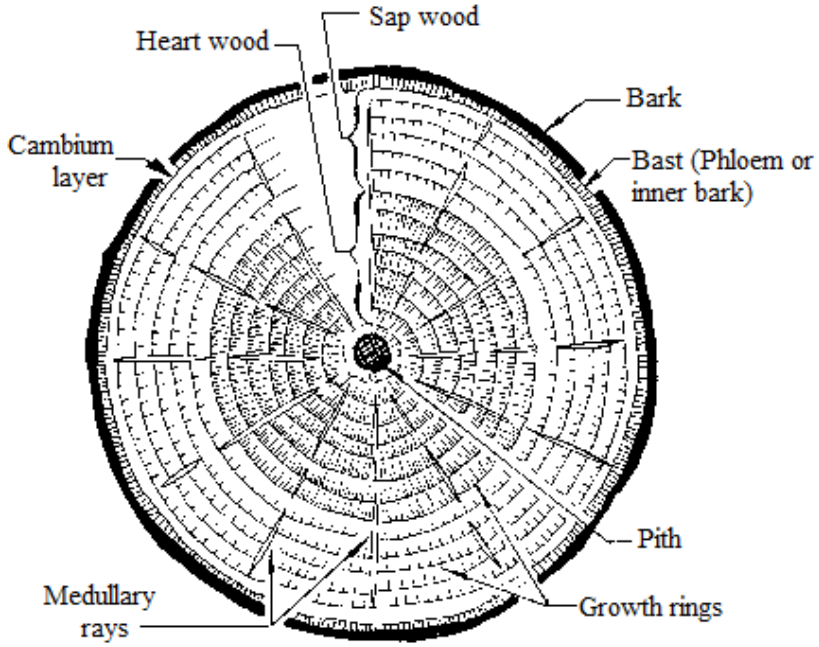


Fig. 4.6: A portion of stem showing annual rings

More distinct annual rings are formed in those regions where climatic variations are sharp, as in temperate plants. Distinct annual rings are not formed in tropical plants. Distinct annual rings are not formed in India except Himalayan regions. Least distinct annual rings are formed in seashore regions because the climate remains same throughout the year. One more thing more clear annual rings are formed in deciduous plants as compared to evergreen plants. Similarly in deserts annual rings are less distinct. Annual rings are bands of secondary xylem and xylem rays. Sometimes drought conditions prevail during the middle of a growing season resulting in formation of more than one annual ring these are called pseudo annual rings.

Secondary Growth in Dicot Root

In dicot roots the vascular bundles are of radial type and in this condition the xylem and the phloem are present in different radii. So there are no cambial cells in between xylem and phloem as in the case of stem. So for the secondary growth in roots first of all, conjunctive tissue becomes meristematic during the secondary growth in a dicot root and form separate curved strips of vascular cambium below phloem bundles. Then after, the cells of pericycle lying opposite to protoxylem also become meristematic to form additional strips of cambium. So the cells present at the base of phloem and the top of xylem first of all become meristematic. Very soon the cells present near to these cells also attain the meristematic behaviour and in this way a

complete ring of vascular cambium is formed. The portion of vascular cambium formed by pericycle is less. The main portion of vascular cambium is formed by conjunctive tissue.

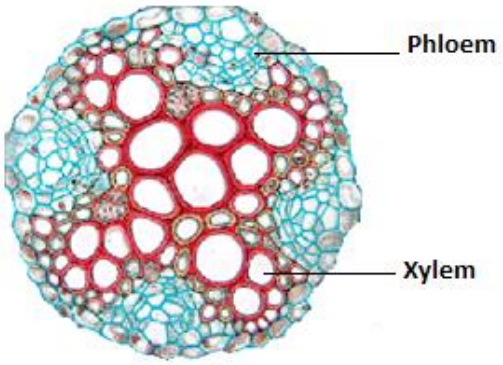


Fig. 4.7: Xylem and Phloem in root

The shape of ring of vascular cambium is wavy in the beginning, but later on it becomes circular due to the pressure of secondary xylem. The portion of vascular cambium formed by conjunctive tissue becomes meristematic first and forms the secondary xylem towards the center. Ultimately the ring becomes circular by the pressure of secondary xylem (pushing outwards). The activity of vascular cambium of root is the same as the activity of vascular cambium of stem. Secondary xylem is formed towards the inner side and secondary phloem is formed towards the outer side by vascular cambium. The portion of vascular cambium which is formed by pericycle is responsible for the formation of pith rays. These are made up of parenchyma. These pith rays are known as primary medullary rays (multiseriate). A few medullary or pith rays are also formed from remaining vascular cambium. These are called secondary medullary rays (uniseriate). Thus two types of medullary rays are found in the secondary structure of roots. The presence of two types of medullary rays is basic characteristic feature of roots. Only secondary medullary rays are found in stem after the secondary growth. Both of them conduct water and food in radial direction (Fig.4.7).

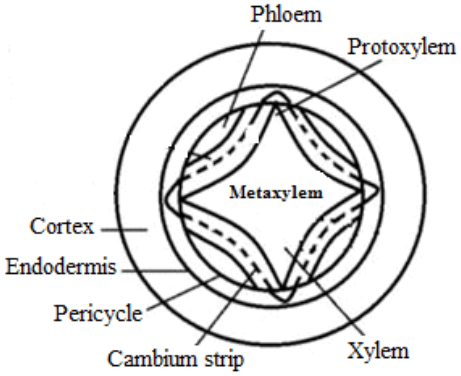


Fig. 4.8 Dicot root

Cork cambium is developed from the pericycle in roots. Cork is formed towards the outside and secondary cortex is formed towards the inner side by the cork cambium. Lenticels are also found in roots but less in number as compared to stem. Cortex completely degenerates in roots after the secondary growth of one or two years. This falls down due to the pressure of cork, whereas in stem, it degenerates after the long duration. Secondary growth is essential in roots to provide strength to the growing aerial parts of the plants and fulfill the requirement of water and minerals. Annual rings are not formed in roots because these are not affected by the changes of environment. Secondary growth is not found in monocot roots (Fig. 4.8).

Functions of Secondary Meristem

1. Healing of wounds

When any plant part gets injured wound is formed there. Boundary of the wound is raised outside and composed of similar type of living cells (parenchyma) called callus. Living cells of wound are responsible to form a cambium. This is called wound cambium. It is also called inducible cambium. This newly formed cambium forms cork towards the outside. This cork covers the wound entirely. Wound cambium is a secondary lateral meristem.

2. Abscission

Falling of any plant organ is called as abscission. Abscission takes place due to formation of abscission layer at the base of plant organ and it is composed of parenchyma. Middle lamella is dissolved in abscission layer during abscission and primary walls also dissolve partially or completely. Sign of leaf fall on stem is called leaf scar and it is a type of wound. The living cells of leaf scar are responsible to form cork cambium, which produce cork. Cork covers the wound. At the site of abscission protective layer is found which is suberized.

3. Knots

Knot is formed when branches are embedded inside the main stem. In most cases knots are caused by the natural growth of the tree, though the specific circumstances under which they form determines how they will appear. As a tree grows and increases the circumference of its trunk, the growing trunk begins to overtake the branches that grow out from it. Knots form around these branches, building up trunk material as the tree continues to expand. The wood of the knot is typically tougher than the surrounding wood and may form a bulge around the branch emerging from its center and known as tight knot. If a branch becomes injured or otherwise dies while still attached to the tree, a loose knot forms as the trunk grows larger. Loose knots are similar to tight knots, but instead of having living wood in the center of the knot there is only a dark plug of dead or decaying material.

4.4 ABNORMAL BEHAVIOUR OF CAMBIUM

The word anomalous means deviating from the general or common order or type. Thus, the term, anomalous growth reflects a **growth condition** which is not commonly seen and which is present in a limited number of families or genera. Plants showing anomalous secondary growth can be studied in two main groups.

(1) Those in which cambium of normal type is present and persists but by peculiarity or irregularity in its activity develop vascular tissues of unusual arrangement.
(2) Those in which the normal cambium either does not develop or is soon replaced by another cambium. This abnormal cambium may either develop from cortex or pericycle and shows abnormal activity.

Several dicots show secondary growth that deviates considerably from the normal secondary growth. The deviating methods of secondary thickenings are called *abnormal* or *anomalous*, although the normal and abnormal forms of growth are not sharply separated from one another. These anomalies may be enlisted as follows:

(a) Anomalous secondary growth in Dicots may be due to following reasons:
  (i) Anomalous position of cambium
  (ii) Abnormal behaviour of normal cambium
  (iii)Accessory cambium formation and its activity
  (iv) Extrastelar cambium
  (v) Interxylary phloem
(b) Absence of vessels in the xylem
(c) Scattered vascular bundles in dicots
(d) Presence of exclusive phloem and xylem bundles
(e) Presence of medullary bundles
(f) Presence of cortical bundles
(g) Intraxylary phloem
(h) Vascular bundles arranged in a ring in monocots

(a) (i) Anomalous/abnormal position of vascular cambium: Normally vascular cambium is circular, but it is folded in stem of some plants. Later on these folds break and separate from each other. Each fold is responsible to form a complete vascular bundle. Many vascular bundles are formed in stem e.g. *Thinouia, Serjania, Bauhinia*.

(ii) Abnormal Activity of vascular cambium: Generally xylem and phloem is formed from the maximum part of the vascular cambium and medullary rays are formed from the few parts of vascular cambium. But in some plants parenchyma (Medullary rays) is formed from the maximum part of the vascular cambium and rarely in some places xylem and phloem are formed e.g. *Aristolochia, Vitis vinifera* (Grape).

(iii) Sequential or successive ring of vascular cambium: In some of the plants, a new ring of vascular cambium is formed each year. This is formed outside the previous ring e.g. *Mirabilis, Boerhaavia, Bougainvillea* etc.

(iv) Formation of vascular cambium from pericycle: Vascular cambium is formed from the pericycle in plants of Amaranthaceae and Chenopodiaceae families. A complete ring of vascular cambium is formed from the pericycle.

(v) Interxylary phloem: This is also called internal phloem. It occurs usually in the form of strands or as a continuous band around the pith. The origin of intraxylary phloem in most plants is primary. The internal phloem develops after the development of external primary phloem. The bundles are treated as bicollateral because of presence of the internal phloem. This type of development is found in Solanaceae, Apocynaceae, Lathyraceae etc.

(b) Absence of vessels in the xylem: Vessels are mainly conducting channels for water but in some species like *Zygozynum, Belliolum, Drimys* and in some aquatic plants like *Elodea, Utricularia, Ceratophyllum* and *Hydrilla* etc. vessels are absent. In these taxa trachieds are the main conducting channels like Gymnosperms.

(c) Scattered vascular bundles in dicotyledons: Vascular bundles are normally arranged in a ring in dicots but in some taxa such as *Thalictrum, Piper, Peperomia, Podophyllum, Papaver, Nymphaea* etc. vascular bundles are scattered. Scattered vascular bundles in dicots recall the arrangement of vascular bundles in monocots.

(d) Presence of exclusive phloem and xylem bundles: Sometimes vascular bundles are incomplete i.e. a bundle is represented either exclusively by xylem or phloem strand. In *Paeonia*, in addition to normal vascular bundle incomplete bundles are also present which are exclusively represented by xylem. Similarly *in Cuscuta, Boerhaavia diffusa, Ricinus communis,* and *Antigonon leptopus* only phloem bundles are present.

(e) Presence of medullary bundles: In some dicots vascular bundles are present in pith and then they are known as medullary bundles. These bundles show a limited amount of secondary growth. These are found in Ranunculaceae, Amaranthaceae, Acanthaceae, Cactaceae and Chenopodiaceae. Their number varies from one to many but they do not supply to the lateral organs like leaf and branch. Their presence is only because of increased needs of translocation and also to the mechanical role in lianas.

(f) Presence of cortical bundles: In some dicots in addition to the normal ring of stelar bundles some vascular bundles are also present in the cortex known as cortical bundles. Morphologically these bundles are leaf traces which traverse through the cortical region of the stem before entering into the petiole. *Casuarina* has a ring of normally oriented cortical bundles below the ridges whereas in *Limonium vulgare* there are numerous irregularly scattered vascular bundles.

(g) Intraxylary phloem: Phloem situated in the inner side of the vascular bundles is known as intraxylary phloem. As it is located in the periphery of the pith it is also known as medullary phloem. It occurs either in the form of a ring (*Asclepias, Convolvulus, Eucalyptus* etc.) or in isolated patches (*Solanum, Capsicum, Calotropis* etc). The internal phloem develops from the provascular tissue. It resembles the external phloem except that fibres are less conspicuous or lacking and sieve tubes and companion cells are in small groups surrounded by parenchyma.

(h) Vascular bundles arranged in a ring in monocots: In monocots the vascular bundles are scattered in the ground tissue. But in some cases as in *Tamus communis* the vascular bundles are arranged in two rings around the pith; the outer ring has only two small bundles which are embedded in the sclerenchymatous pericycle and the inner ring has several large vascular inside the pericycle. In the tubular stems of some of the grasses as *Triticum, Hordeum, Oryza* etc the vascular bundles are arranged in two or more definite rings.

4.4.1 *Bougainvillea* stem

Bougainvillea is a member of the Nyctaginaceae and is an example of a dicotyledonous stem which displays anomalous secondary growth. In the T.S. of *Bougainvillea,* near the centre of the stem, you will see some primary vascular bundles embedded in lignified pith parenchyma. Move the slide towards the outer regions, and you will notice that there has been fairly extensive production of secondary vascular tissue. Secondary phloem and secondary xylem lie on either side of it. The secondary xylem is composed of tracheids, fibers and narrow-diameter vessels. Interspersed with the secondary xylem you will be able to see small pockets of phloem and look like large-diameter metaxylem vessels. These are reminiscent of the primary bundles towards the centre of the stem. These are in fact primary vascular bundles embedded within the secondary xylem, hence the use of the term, anomalous growth in this instance.

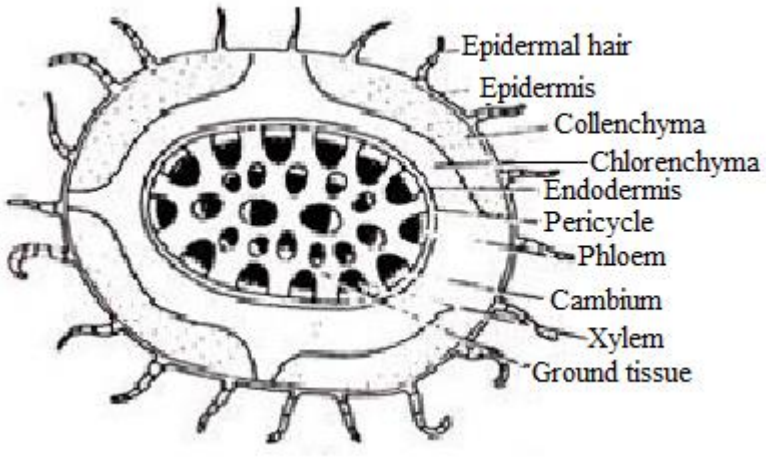


Fig. 4.9: T.S. of *Bougainvillea* stem

The phloem is described as being included phloem, which by definition is phloem tissue which lies between regions of secondary xylem. Whilst the physiological advantage of the formation of included phloem has not yet been studied, one could speculate that in this instance, the included phloem would be well-protected from predators and pests and, of course, be well-supplied with water and nutrient. The anomalous growth results as a result of differential cambial activity. Newly-produced vascular cambia result in the outer lateral meristem becoming quiescent and this cambium returns to activity only when the internal vascular cambium (which produces the individual embedded bundles) becomes less active. Vascular cambia are said to not produce rays in Nyctaginaceae (lateral meristems do), but do produce vessels and associated, axial parenchyma and sometimes fibers to the inside and variable secondary phloem to the outside (Fig. 4.9).

4.4.2 *Nyctanthes* stem

In *Nyctanthes arbor-tristis* stem which is a dicot plant, apart from normal vascular bundles which occur in a ring in the central region, there are four inversely oriented vascular bundles at the four ridges of stem. These cortical bundles are collateral and open. So in addition to the normal ring of stelar bundle some vascular bundles are also present in the cortex, they are known as cortical bundles. Morphologically these are the leaf traces which traverse through the cortical region of the stem before entering into the petiole. These types of vascular bundles are also found in family Crassulaceae, Casuarinaceae and Oleaceae. These cortical bundles are equally active producing cells and helping in secondary growth of the plant (Fig. 4.10).

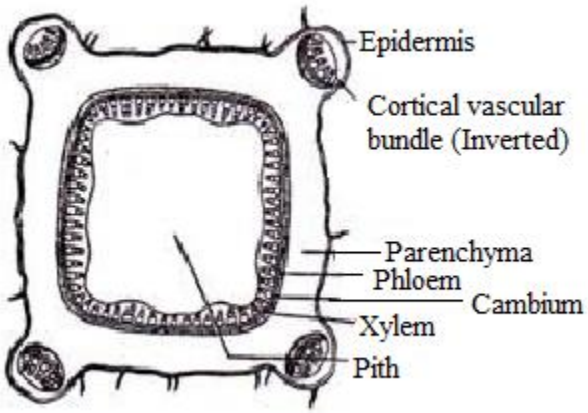


Fig. 4.10: T.S. of *Nyctanthes* stem

4.4.3 *Dracaena* stem

Palm trees are monocots that grow quite tall and thick, yet they lack "normal" secondary growth. *Dracaena* is a monocot but not a true palm, as palms lack the peripheral secondary thickening meristem such as is found in *Dracaena* and *Cordyline*. *Dracaena* is an unusual plant,

in that the vascular bundles are surrounded by very prominent fiber bundles. In this sense, *Dracaena* is not anomalous. The stems undergo a specialized secondary growth, which manifests itself in the production of additional parenchymatous elements. Their later growth pattern is termed diffuse secondary growth, and consists mostly of a proliferation of ground parenchyma cells and additional vascular bundles near the periphery.

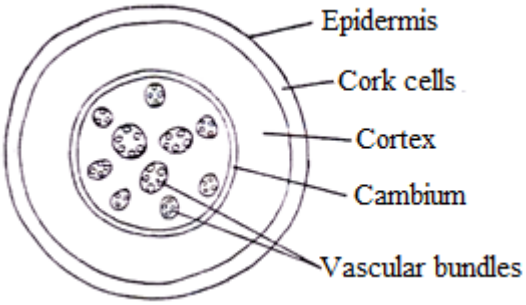


Fig. 4.10: T.S. of *Dracaena* stem

The young *Dracaena* stem has typical structure i.e. epidermis is followed by sclerenchymatous hypodermis. A large number of closed collateral bundles are scattered in ground tissue. One of the outer layers of cells from the ground tissue becomes meristematic and functions as cambium. The cambium formed in the region which has ceased elongating. The activity of this cambium is more on the inner side and very little on the outside where it forms only parenchyma. On the inner side it forms xylem and parenchyma in alternate patches. The inner parenchymatous cells are called conjunctive tissue. After a short while the activity of cambium on inner side changes and above the xylem it starts forming phloem and then again xylem. Thus phloem becomes encircled by xylem and ring of leptocentric (amphivasal) vascular bundle is formed. The xylem formed earlier has bigger vessels and around each vascular bundle is developed a sclerenchymatous sheath. The cambium after sometime alter its activity and forms xylem on the inner side, at those places where it was previously forming the parenchyma and parenchyma in place of xylem. Similar to earlier case again by change in activity it forms a ring of vascular bundles. Activity of cambium goes on changing regularly and more rings of vascular bundles are formed. The last one or two rings of vascular bundles lie in conjunctive tissue. Cork cambium is formed below hypodermis and forms cork and cork cambium in normal fashion (Fig. 4.11)..

4.4.4 *Ficus* root

Ficus is a pantropical genus of trees, shrubs and vines occupying a wide variety of ecological niches; most are evergreen, but some deciduous species are endemic to areas outside of the tropics and to higher elevations. Fig species are characterized by their unique inflorescence and distinctive pollination syndrome, which utilizes wasp species belonging to the Agaonidae family for pollination.

In this furrows of secondary phloem are present in the cylinder of secondary xylem. A peculiar secondary growth takes place due to development of unidirectional and bidirectional arcs of cambium. Unidirectional arc of cambium is that portion of the cambium which produces little or no xylem but extensive amount of phloem, bidirectional arc if cambium produces as much or more xylem then phloem. In the initial stages cambial cylinder produces secondary vascular tissues that have a cylindrical configuration. But subsequently four grooves or furrows of phloem are formed. Other portion of the cambial cylinder except for these four arcs show bicambial activity i.e. they produce as much or more xylem then the phloem. As secondary growth continues furrows of phloem become truncated and the unidirectional and bidirectional arcs of cambium become separated. Initially four furrows of phloem are formed but in the older stems additional furrows may be formed (Fig. 4.12).

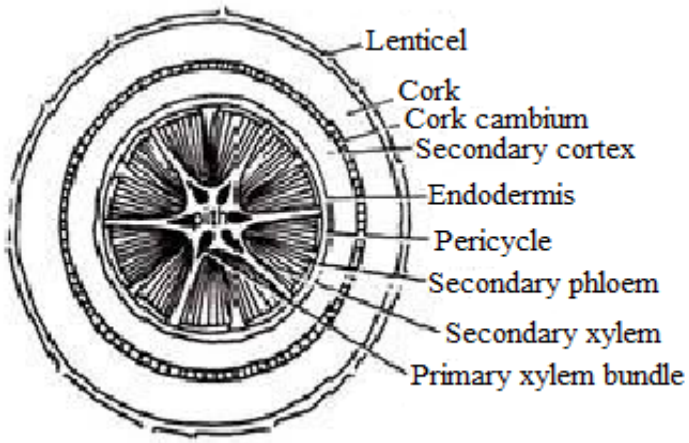


Fig. 4.11: T.S. of *Ficus* root

4.4.5 *Tinospora* stem

Tinospora cordifolia commonly known as Guduchi is an Indian medicinal plant and has been used in Ayurvedic preparations for the treatment of various ailments throughout the centuries. It is a glabrous, succulent, woody climbing shrub native to India. It thrives well in the tropical region, often attains a great height, and climbs up the trunks of large trees. The stem is gray or creamy - white, deeply cleft spirally and longitudinally with the space between spotted with large rosette - like lenticels. The wood is white, soft, and porous, and the freshly cut surface quickly assumes a yellow tint when exposed to air.

Vascular zone is composed of discrete vascular strands with 10 to 12 or more wedge shaped strips of xylem, externally surrounded by semi circular strips of phloem alternating with wide medullary rays; phloem parenchyma contain calcium oxalate crystals; cambium is of 1-2 layers; xylem consists of vessel elements, tracheids, parenchyma and fibres. Vessel elements cylindrical

in shape bearing bordered pits. Medullary rays 15 to 20 cells wide. Pith mostly made up of large thin walled cells containing starch grains.

The presence of discrete vascular strands in the mature stem of *Tinospora cordifolia* is one of the anomalous secondary structures found in Menispermaceae. The cambium forms secondary vascular tissue only in the fascicular region, whereas in the interfascicular areas parenchyma is produced. Thus in the old stem the xylem becomes fissured due to the development broad parenchymatous rays. In such stem parenchyma acts like a shock absorber. It also enables the stem to resist the pulling and compression due to the pressure of high winds. This anomaly is thus an adaptation to the climbing habit of the plant (Fig. 4.13).

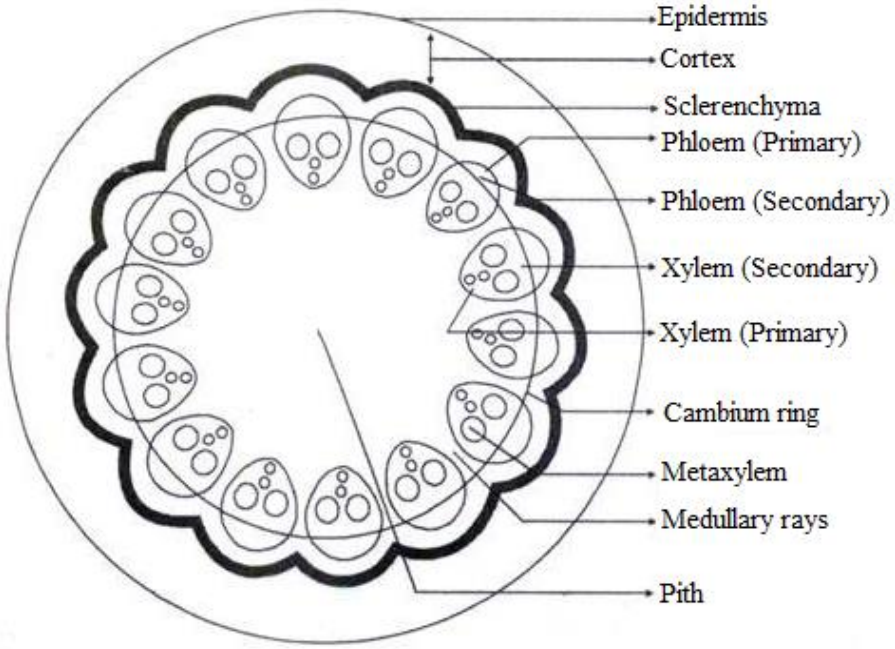


Fig. 4.12: T.S. of *Tinospora* stem

4.5 ACTIVITY OF CORK CAMBIUM

Primary growth in plants yields an outer layer known as the epidermis. In plants that do not have lateral growth, this layer is enough to help protect the inner tissues. When a stem gets thicker, however, this epidermis splits and falls off. The plant would be susceptible to disease and water loss if it weren't for cork cambium. Secondary growth takes place in extra stelar region due to the activity of cork cambium. Cork cambium is also known as phellogen or extra stelar cambium. (The cells of the cork cambium are rectangular). In contrast to vascular cambium the phellogen is relatively simple in structure and composed of one type of cell. Cork cambium arises from the hypodermis or from the outer layer of cortex because they become meristematic. At the time of

their development the first phellogen arises in the subepidermal region. Cork cambium is also formed in the form of a single layered ring.

Cork cambium is a tissue found in many vascular plants as part of the periderm. The cork cambium is a lateral meristem and is responsible for secondary growth that replaces the epidermis in roots and stems. It is found in woody and many herbaceous dicots, Gymnosperms and some monocots, which usually lack secondary growth. Cork cambium is one of the meristems - the series of tissues consisting of embryonic (incompletely differentiated) cells from which the plant grows. It is one of the many layers of bark between the cork and primary phloem. The function of cork cambium is to produce the cork (a tough protective material).

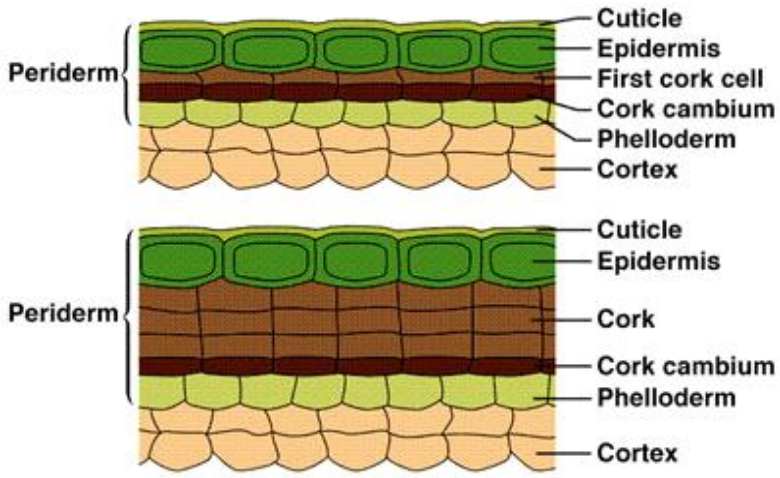


Fig. 4.13: Activity of cork cambium

Phellogen or cork cambium after repeated division gives rise to new cells similar to that of cambial ring. Cork cambium also divides periclinally and it forms some cells towards the outside (epidermis) and some cells towards the inside (cortex). Those cells formed towards outside, their middle lamella is suberized so that these cells become dead and known as Cork or Phellem. Those cell formed towards the inside are differentiated into parenchyma and may contain chloroplasts and these are called secondary cortex or Phelloderm (Fig. 4.13).

Phellogen, phellem and phelloderm are collectively known as periderm. It means the entire secondary tissue in the cortex is known as periderm

Phellogen + Phellem (cork) + Phelloderm = Periderm

Phellem or cork is formed in high quantity and they are like phellogen cells. They are polygonal and uniform in shape with no intercellular spaces. The phelloderm or secondary cortex is in less quantity because activity of cork, cambium is more towards outside. These cells are living with

cellulose walls. Their shape is similar to that of phellogen cell as well. They may be distinguished from cortical cells by their arrangement in radial series.

The periderm thus consists of three different layers:

- phelloderm - inside of cork cambium; composed of living parenchyma cells
- phellogen (cork cambium) - meristem that gives rise to periderm
- phellem (cork) - dead at maturity; air-filled protective tissue on the outside

Growth and development of cork cambium is very variable between different species, and is also highly dependent on age, growth conditions, etc. as can be observed from the different surfaces of bark like smooth, fissured, scaly, flaking off etc.

The **periderm** is the secondary protective (dermal) tissue that replaces the epidermis during growth in thickness of stems and roots of Gymnosperms and dicots (i.e. secondary growth). Unlike typical epidermis, the periderm is a multilayered tissue system, the bulk of which usually constitutes the cork, or **phellem**. There are, however, some exceptions to this in as much as some other structures (*e.g.* potato skin and apple peel) are also periderm. Phellem (the cork) consists of cells that are dead at maturity, and their primary walls become covered from the inside by the secondary wall which consists of parallel suberin lamellae alternating with wax layers. The lateral meristem, (cork cambium or **phellogen**), is one cell layer thick and encircles the stem. It produces periderm centrifugally. The layer of cork cells formed is impermeable for water and gases, but is interrupted at certain points by **lenticels** which function to some extent similar to stomata in the epidermis, and permit gas diffusion.

In some cases parenchyma cells are produced centripetally (i.e. to the inside of the stem or root) by the phellogen as a part of the periderm. These persistent living cells are called **phelloderm** and structurally appear similar to cells of the cortex. The number of layers of cork and phelloderm varies greatly among different species; some plants produce no phelloderm. The most important function of the periderm is to reduce the loss of water and solutes from interior tissues and to protect a plant from unfavorable environmental conditions.

Cork cambium in most stems arises from an outer (subepidermal) layer of the cortex, but in some plants it originates by the periclinal division of the epidermal cells or, alternatively, fairly deeply inside the stem, such as in the primary phloem. In roots, it arises in the pericycle. In woody plants, with the growth of the main stem in thickness, new layers of cork cambium, and accordingly sequential periderm formed in the secondary phloem, cutting off old non-functional phloem tissues. As a result, **rhytidome** or outer bark, is formed consisting entirely of dead cells.

It should be emphasized that bark is not wood. Wood refers only to the secondary xylem. Bark is generally accepted to include all tissues of a plant exterior to the vascular cambium. It can be divided into **inner bark** and **outer bark**. The inner bark includes the region from the vascular cambium to the innermost cork cambium (or phellogen), that is going from one secondary

meristem to a second secondary meristem. The outer bark is composed of all tissues outside of the cork cambium (which are dead) and includes phellem, old secondary phloem, crushed primary phloem, crushed cortex, crushed epidermis and any prior periderms.

It is worth considering the differences between the phellogen (cork cambium) and the vascular cambium. It can be said that the phellogen:

- is not derived from any previous meristem, such as procambium.
- is initially derived from permanent tissues, mostly from the subepidermal layer of the cortex.
- may repeatedly originate progressively in the deeper layers of the stem.
- typically owes its origin to dedifferentiation and redifferentiation into meristematic cells.
- produces its derivatives mainly (or only) to the outside (generally only cork cells are produced).
- lacks intercellular spaces except where lenticels are present.
- produces a single kind of initial that is rectangular in cross-section, and wider tangentially than radially. The cork cells produced have the same shape as the phellogen cells.

As indicated above, phellem cells are dead at maturity, and appear in brick-like rows having their primary walls impregnated with suberin. The plasmodesmata are blocked and usually the contents are filled with tannins or resins, or possibly just air filled as in the case of bottle cork. Some cells, called **phelloids**, have non-cork construction - not suberized but being thin-walled, sclerified or lignified. The highest activity of cork cambium is in winter (autumn) season. Ring of cork cambium remains living only for one year. Each year, a new cambium is formed below the previous cambium. This new cambium is derived from the secondary cortex or phelloderm.

Lenticels: Most of the cells of phellem are dead but at some places living cells are also found. Suberin is not deposited in these places and these loosely arranged cells are called lenticels. Lenticels appear on the outer surface of the plant either in small points or in the form of areas of protuberance. Lenticels are made up of scattered group of living cells. Usually they are formed below the stomata. These cells are known as complementary cells/complementary tissue. The main function of lenticels is exchange of gases between plant and atmosphere. Rows (vertical or longitudinal) of lenticels may occur opposite the medullary rays, facilitating the free exchange of gases. Transpiration also takes place through the lenticels known as lenticular transpiration. Adventitious roots on cutting originate from the living cells of lenticels in vegetative reproduction.

Lenticels are found in most of the woody trees but absent in woody climbers. Lenticels are mainly found on wood stems and they are never found on leaves. Lenticels are present all over the plant body and they are also present on fruits. If lenticels are blocked then root will die first due to lack of food. All the tissues which occur outside the cork cambium are collectively termed

as rhytidome. Rhytidome includes cork and tissues which become dead due to the pressure of cork.

Kinds of bark

1. **Ring Bark:** Continuous bark of equal thickening is called ring bark. It is formed around the stem in the form of a complete ring. In ring bark cork cambium is continuous. A complete distinct ring bark is formed in this plant. Its bark was used as a writing material as a paper in ancient period. Example: Bhojpatra (*Betula utilis*) and it is also formed in *Eucalyptus*.
2. **Scaly Bark:** Discontinuous bark of unequal thickening is called scaly bark. This bark is formed around the stem in the form of pieces or fragments. In scaly bark the ring of cork cambium is not continuous. Highly obvious scaly bark is formed in *Psidium guajava*. Besides this scaly bark is also formed in Neem (*Azadirachta indica*), Mango (*Mangifera indica*) and Imli (*Tamarindus indica*) etc.

4.6 SUMMARY

In plant science, secondary growth refers to the growth that results from cell division in the cambia or lateral meristems and that causes the stems and roots to thicken, while primary growth is growth that occurs as a result of cell division at the tips of stems and roots, causing them to elongate, and gives rise to primary tissue. Secondary growth occurs in most seed plants, but monocots usually lack secondary growth. If they do have secondary growth, it differs from the typical pattern of other seed plants.

In many vascular plants, secondary growth is the result of the activity of the two lateral meristems, the cork cambium and vascular cambium. Arising from *lateral* meristems, secondary growth increases the girth of the plant root or stem, rather than its length. As long as the lateral meristems continue to produce new cells, the stem or root will continue to grow in diameter. In woody plants, this process produces wood, and shapes the plant into a tree with a thickened trunk.

Because this growth usually ruptures the epidermis of the stems or roots, plants with secondary growth usually also develop a cork cambium. The cork cambium gives rise to thickened cork cells to protect the surface of the plant and reduce water loss. If this is kept up over many years, this process may produce a layer of cork. In the case of the cork oak it will yield harvestable cork. Secondary growth also occurs in many non-woody plants e.g. tomato, potato tuber, carrot taproot and sweet potato tuberous root. A few long-lived leaves also have secondary growth.

Abnormal secondary growth does not follow the pattern of a single vascular cambium producing xylem to the inside and phloem to the outside. Some dicots have anomalous secondary growth e.g. in *Bougainvillea* a series of cambia arises outside the oldest phloem.

Most monocots either have no secondary growth or else anomalous secondary growth of some type. For example, palm trees increase their trunk diameter due to division and enlargement of parenchyma cells, which is termed diffuse secondary growth. In some other monocot stems with anomalous secondary growth, a cambium forms, but it produces vascular bundles and parenchyma internally and just parenchyma externally. Some monocot stems increase in diameter due to the activity of a primary thickening meristem, which is derived from the apical meristem.

4.7 GLOSSARY

Apical meristem: Embryonic, totipotent tissue in the tips of the roots and shoots of plants

Cambium: A lateral meristem that produces secondary growth

Collenchyma: Tissue composed of unevenly thickened cell walls; collenchyma cells are flexible and support young parts of the plant without hindering growth; collenchyma cells are composed of cellulose

Cork: A plant tissue composed of cells whose walls are impregnated with suberin and are non-living at maturity; cork is produced by the cork cambium

Cork cambium: A narrow cylindrical sheath of meristematic cells that produces cork cells to replace the epidermis during secondary growth (growth in width)

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

Ectophloic: Having phloem only on the outer side of the stele; compare to amphiophloic

Endarch: A type of xylem maturation in which protoxylem is internal to metaxylem and development proceeds centrifugally (from the inside out)

Epidermis: The exterior tissue, usually on cell thick, of leaves and young stems and roots

Exarch: A type of xylem maturation in which protoxylem is external to metaxylem and development proceeds centripetally (from the outside in)

Fiber: A long-walled plant cell which is often dead at maturity; fibers impart elasticity, flexibility and tensile strength to plant structure

Ground tissue: A tissue consisting mostly of parenchyma cells that makes up the bulk of a young plant

Manoxylic wood: Wood type that contains abundant parenchyma; typical of cycads

Mesarch: A type of xylem maturation in which the protoxylem is embedded in the metaxylem and development proceeds both centripetally (from the outside in) and centrifugally (from the inside out); compare to endarch and exarch

Mesophyll: Parenchyma tissue between the upper and lower epidermis of a leaf

Metaxylem: A type of primary xylem that differentiates and matures later than the protoxylem; generally metaxylem tracheids are longer than protoxylem

Periderm: A tissue primarily consisting of cork cells; outer bark

Phloem: Photosynthate conducting tissue of vascular plants

Pith: The central parenchymatous tissue in a vascular plant axis

Polystelic: Having more than one stele

Primary growth: Growth in length, controlled by the apical meristem

Procambium: The primary meristematic tissue that gives rise to primary xylem and primary phloem; procambia are found in apical as well as intercalary meristems

Protoxylem: The first primary xylem to differentiate and mature, usually before and during elongation of the axis; protoxylem cells are generally smaller in diameter than metaxylem

Pycnoxylic wood: Dense wood that contains little parenchyma; typical of *Archeopteris* conifers

Secondary growth: Growth in width initiated and maintained by the vascular cambium and cork cambium

Secondary xylem: Xylem produced by the vascular cambium

Siphonostele: A type of stele that consists of a ring of vascular tissue surrounding pith

Stele: The central vascular cylinder in stems and roots where the vascular tissue is located

Tracheid: A water conducting and supportive cell type of xylem composed of long, thin cells with tapered ends and walls hardened with lignin

Vascular bundle: A strand of tissue composed mostly of xylem and phloem

Vascular cambium: A lateral meristem that produces secondary vascular tissue in stems and roots

4.8 SELF ASSESSMENT QUESTION

4.8.1 Multiple choice questions:

1. Cork is formed from:

(a) Cork cambium (phellogen) (b) Vascular cambium

(c) Phloem (d) Xylem

2. What is true about a monocot leaf:

(a) Reticulate venation

(b) Absence of bulliform cells from epidermis

(c) Mesophyll not differentiated into palisade and spongy tissues

(d) Well differentiated mesophyll

3. Vascular cambium produces:

(a) Primary xylem and primary phloem (b) Secondary xylem and secondary phloem

(c) Primary xylem and secondary phloem (d) Secondary xylem and primary phloem

4. A bicollateral vascular bundle is characterized by:

(a) Phloem being sandwiched between xylem (b) Transverse splitting of vascular bundle

(c) Longitudinal splitting of vascular bundle (d) Xylem being sandwiched between phloem

5**.** Abnormal/anomalous secondary growth occurs in:
(a) *Dracaena* (b) Ginger
(c) Wheat (d) Sunflower

6**.** Which exposed wood will decay faster?
(a) Sap wood (b) Soft wood
(c) Wood with lot of fibers (d) Heart wood.

7. A narrow layer of thin walled cells found between phloem/bark and wood of a dicot is :
(a) Cork cambium (b) Vascular cambium
(c) Endodermis (d) Pericycle.

8. Periderm is produced by:
(a) Vascular cambium (b) Fascicular cambium
(c) Phellogen (d) Intrafascicular cambium.

9. Casparian strip occurs in a:
(a) Endodermis (b) Exodermis
(c) Pericycle (d) Epidermis.

10. Vascular bundles in a dicot stem are :
(a) Open, collateral, exarch (b) Closed, collateral, endarch
(c) Closed, collateral, exarch (d) Open, collateral, endarch

11. Annual rings are distinct in plants growing in :
(a) Temperate regions (b) Tropical regions
(c) Grasslands (d) Arctic region

12. The lateral roots generally originate in :
(a) Cork cambium (b) Cortex
(c) Pericycle cells lying against protoxylem (d) Endodermal cells lying against protoxylem

13. The best method to determine the age of tree is:
(a) To count the number of leaves (b) To count the number of annual rings
(c) To measure it’s diameter (d) To find out the number of branches

14. The bark of a tree comprises:

(a) All the tissues outside the cork cambium (b) All the tissues outside the vascular cambium
(c) Only the cork (d) Just inside the cork cambium

15. Which of the following give rise to the cork tissue?
(a) Phellogen (b) Periblem
(c) Periderm (d) Phelloderm

4.8.1: Answer key: 1. (a), 2. (c), 3. (b), 4. (a), 5. (a), 6.(a), 7.(b), 8. (c), 9.(a), 10.(d), 11.(a), 12.(c), 13.(b), 14.(b), 15.(a)

4.9 REFERENCES

- Cutler, Botha and Stevenson, Plant Anatomy, an Applied Approach and Raven, Evert and Eichhorn, *Biology of Plants* (6th or later edition) i**s** highly recommende**d** for additional background information.
- Esau, K. & Cheadle, V.I. (1969). Secondary growth in *Bougainvillea*. *Annals of Botany* (N. S.) 33: 807-819.
- Esau, K. 1977. *Anatomy of Seed Plants*. John Wiley Sons. Inc., New York.
- Ewers, F.W. 1982. Secondary growth in needle leaves of *Pinus longaeva* (Bristle-cone pine) and other conifers: Quantitative data. *American Journal of Botany* 69: 1552-1559.
- Fahn, A. (1967). *Plant Anatomy*. Pergamon Press, Oxford.
- Foster, A.S. (1951). *Practical Plant Anatomy*. Van Nostrand, Princeton.
- Vasistha, P.C. (1968). *Plant Anatomy*. Pradeep Publication & Co., Chandigarh.
- Singh, V., Pande, P.C. & Jain, D.K. (2012-2013). *Structure Development and Reproduction in Angiosperms.* Rastogi Publications, Meerut.
- Thompson, N.P. & Heimsch, C. 1964. Stem anatomy and aspects of development in tomato. *American Journal of Botany* 51: 7-19.

4.10 SUGGESTED READINGS

- Pandey, S.N. *Plant Anatomy and Embryology.* Vikas Publishing House Pvt Ltd, New Delhi.
- Pandey. B.P. (2012). Plant Anatomy. S. Chand & Company Ltd., New Delhi
- Integrative Plant Anatomy, 26 Apr 2000, by William C. Dickison Academic Press Inc.
- Roy, P. (2010). *Plant Anatomy.* New Central Book Agency, Kolkata.
- Esau's Plant Anatomy: Meristems, Cells, and Tissues of the Plant Body: Their Structure, Function, and Development, 3rd Edition by Ray F. Evert (Author), Susan E. Eichhorn ISBN-13: 978-0471738435
- *Plant Anatomy: Tissue* by Dr. Manisha Majumdar, Book Rix Edition

4.11 TERMINAL QUESTIONS

4.11.1: Answer the following questions in two or three sentences:

1-Name two plants which possess interxylary phloem?
2-What are the changes involved in formation of heart wood?
3-What is ring bark and scale bark? Give one example each.
4-Differentiate between soft wood and hard wood.
5-Name any two monocot plants in which secondary growth is seen?
6-Name two unusual structure found in the stem of *Boerhaavia*?
7-Why annual rings are prominent in the wood of temperate region?
8-Differentiate between growth and annual rings?
9-What cells give rise to the interfascicular cambium?
10-How accessory cambial rings are found?

4.11.2 Answer the following questions in about 100 words.

1-Cork cambium forms tissues that form the cork. Do you agree with this statement?
2-Explain the process of secondary growth in the roots of woody Angiosperms.
3-Define anomalous secondary growth in *Dracaena* stem.
4-What is periderm? How does periderm formation take place in the dicot stems?
5-What are cortical bundles and how do they arise?

4.11.3 Answer the following questions in about 200 words.

1-Explain the process of secondary growth in the stems of woody Angiosperms with the help of schematic diagrams. What is its significance?
2-What are the various regions of anomalous secondary growth in dicot? Describe the secondary growth in *Boerhaavia*.
3-What are the differences in the secondary growth of stem and root?
4- Give an account of origin, structure and function of periderm?

BLOCK-2 EMBRYOLOGY

UNIT-5 MALE GAMETOPHYTE

5.1 OBJECTIVES

After reading this unit students will be able to understand-

- What is male gametophyte?
- Name the first cell of male gametophyte?
- How does anther looks like?
- What do you understand by the term ‘microsporogenesis”?
- Is there any difference between the terms sporogenesis and microsporogenesis?
- What is the process of development of male gametophyte in Angiosperms?

5.2 INTRODUCTION

A living organism cannot survive forever. For existence each species has to continue and for it, each member must reproduce its own kind. Dear students you all know that plants reproduce by asexual, vegetative and sexual means.

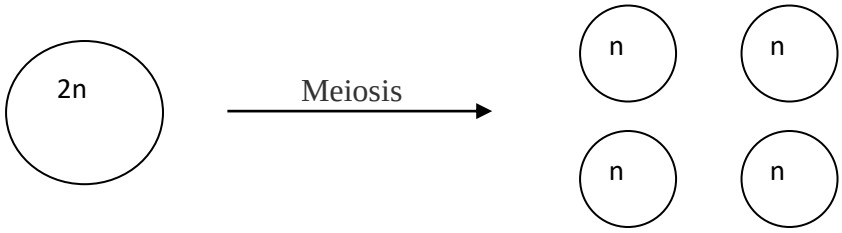
Asexual reproduction is the formation of new individuals from the cell/s of single parent. The offspring will be exact genetic copies of the parent except in specific cases of 'automixis'. Asexual reproduction is of various types (fission, sporulation, budding, fragmentation, parthenogenesis) and vegetative propagation is one of asexual reproduction types. Regeneration of plant from any vegetative part of it i.e. stem cutting, rhizome, tuber, bulb, leaves etc. is known as vegetative reproduction/**vegetative propagation**. Asexual reproduction including vegetative one is an accessory mean of propagation and do not involve genes from different cell lineages whereas in **sexual reproduction**, fusion of two dissimilar gametes from two different parents leads to formation of new combination of genes.

If we are talking about the life cycle of flowering plants, it is characterized by an alternation between a dominant sporophytic generation and a highly reduced gametophytic generation. Dominant **sporophytic generation is diploid** and reduced **gametophytic generation is haploid**.

The normal sexual cycle (amphimixing) involves two important processes:

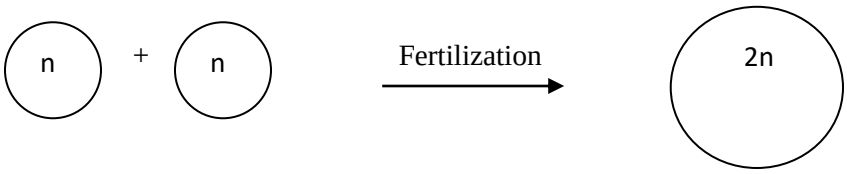
(i) Meiosis and
(ii) Fertilization

In meiosis also known as reduction division, a diploid sporophytic cell (SMC; spore mother cell) gets converted into four haploid gametophytic cells. (‘2n’ number of chromosomes becomes half i.e. ‘n’ number of chromosome)



Diploid sporophytic cell Haploid gametophytic cells

In fertilization, two haploid gametes of opposite sex fuse to form diploid sporophytic generation.



Haploid gametes Diploid sporophytic cell

So we can say, in a sexual cycle a **diploid generation (sporophytic) alternates with a haploid generation (gametophytic)**.

The major function of diploid sporophytic generation is to produce haploid spores, which are the products of meiosis. Spores undergo cell proliferation and differentiation to develop into gametophytes. The major function of gametophytic generation is to produce haploid gametes. The fusion of egg and sperm gives rise to the zygote, which is the beginning of diploid sporophyte generation, thereby completing the life cycle (Gifford and Foster, 1989).

During the Angiosperm life cycle, the sporophyte produces two types of spores, microspores and megaspores. These spores give rise to male gametophytes and female gametophyte, respectively. The Angiosperm gametophyte develops within sporophytic tissues that constitute the sexual organs of the flower.

The male gametophyte, also referred to as the pollen grain or microgametophyte, develops within the stamen's anther and is composed of two sperm cells encased within a vegetative cell (McCormick, 1993, 2004).

The female gametophyte, also referred to as the embryo sac or megagametophyte, develops within the ovule, which is found within the carpel's ovary.

In Angiosperms the gametophytic generation is short and is represented by **embryo sac (on the female side**) and **microspores or pollen grains** (**on the male side).** Remaining part of the life cycle is sporophytic generation.

The sporophyte eventually produces flowers.

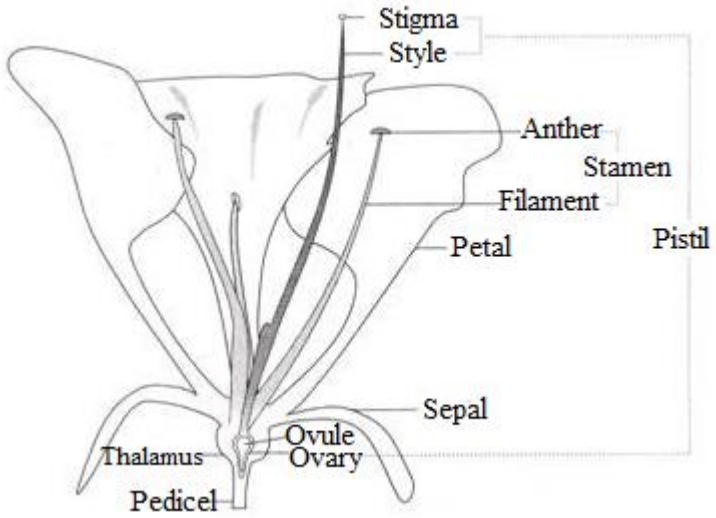


Fig. 5.1: Flower showing reproductive organs

Production of spores and **formation of gametes** are important events in the **sexual reproductive cycle** that take place in the flower.

The floral organ concerned with male sexual reproduction is the **stamen,** and the part of the stamen where events of male sexual reproduction occur is the **anther**. Similarly the floral organ concerned with female sexual reproduction is the **pistil (carpel)**, and the part of the pistil where events of female sexual reproduction occur is the **ovule** inside the **ovary** (Fig. 5.1).

Now it is clear to you that Angiosperm plants are diploid sporophytes i.e. spore bearing plants and haploid spores are developed by meiosis or reduction division. The male spores or **microspores** are developed by meiosis within the **microsporangium (pollen sac)** while the female spores or **megaspores a**re developed by meiosis within the **megasporangium** (ovule). These, in their turns, develop the male and the female gametophytes which are **endosporous** (developing inside the spores).

The process of development of the spore is termed as **sporogenesis**. When it is microspore (pollen), it is termed as **microsporogenesis**. When it is megaspore, it is termed as **megasporogenesis** (Fig. 5.2).

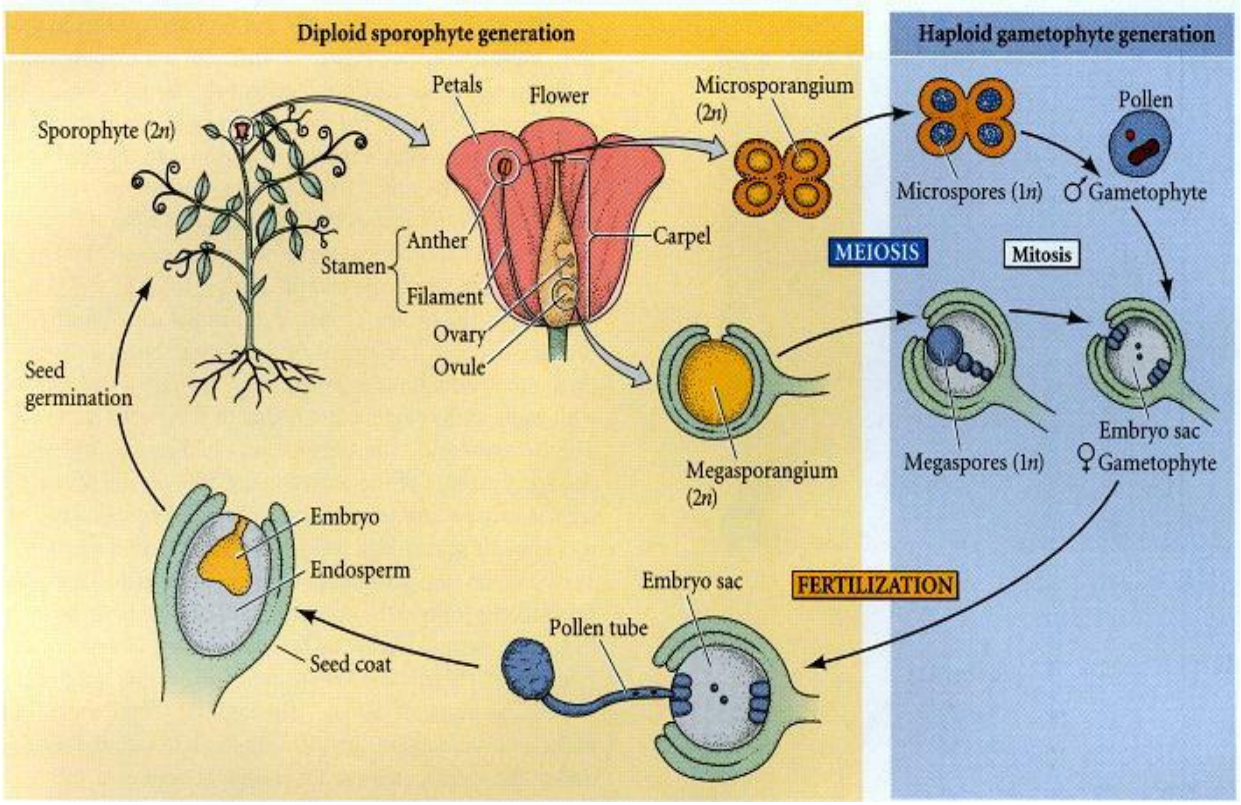


Fig. 5.2: Life cycle of a typical Angiosperm (example - a pea plant). The sporophyte is the dominant generation, but multicellular male and female gametophytes are produced within the flowers of the sporophyte. Cells of the microsporangium within the anther undergo meiosis to produce microspores. Subsequent mitotic divisions are limited, but the end result is a pollen grain. The megasporangium is protected by two layers of integuments and the ovary wall. Within the megasporangium, meiosis yields four megaspores-three small and one large. Only the large megaspore survives to produce the embryo sac. Fertilization occurs when the pollen germinates and the pollen tube grows toward the embryo sac. The sporophytic generation may be maintained in a dormant state, protected by the seed coat

5.3 STRUCTURE OF ANTHER

The fertile portion of stamen is called **anther**. Actually the **stamen** is a slender organ and consists of the **proximal sterile part, the filament (stalk)** bearing at its distal end a **fertile part, the anther**.

A typical anther has two **anther lobes** connected by a **connective** and each anther lobe has two **pollen chambers (microsporangia/pollen sacs). Pollen grains (microspores),** which contribute

the male gametes, are present in each **microsporangium** or we can say are formed within an anther.

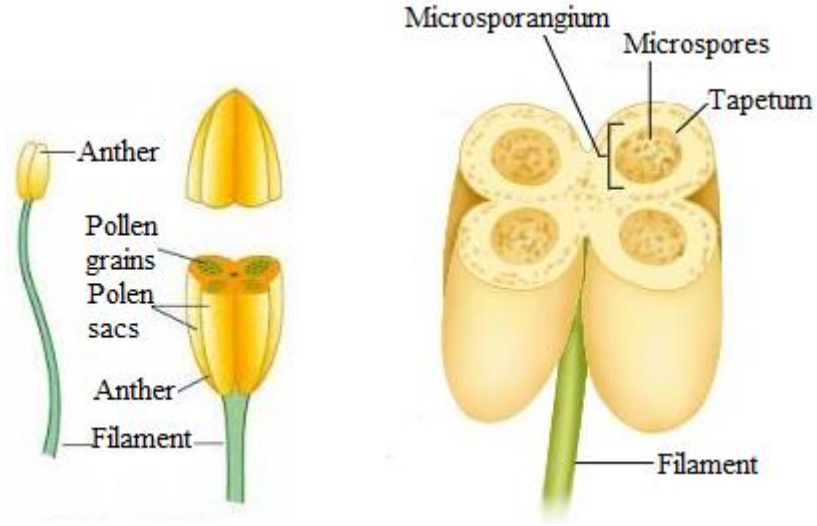


Fig. 5.3: Stamen showing dithecous anther

When the anther has two lobes then it is termed as **dithecous** (two lobed) as in *Citrus* having four microsporangia (Fig. 5.3). Sometimes anther may have single lobe instead of two, at this condition anther is termed as **monothecous** (one lobed) as in *Hibiscus rosa-sinensis* having two microsporangia. A young anther comprises a mass of undifferentiated thin-walled cells bounded by epidermis.

Flowers are structures of sexual reproduction. The essential organs of flower are stamens (microsporophylls) - which make the androecium, and carpels (megasporophylls) - which together form the gynoecium

or

The androecium represents the male reproductive elements of the flower and gynoecium, the female reproductive elements of the flower.

Few terms to remember:

Microsporophyll = Stamen
Megasporophyll = Carpel = Pistil
Microsporangium = Pollen sac = Pollen chamber
Male gametophyte = Germinating pollen grain = Pollens = Microgametophyte
Male gametes = Sperms
Megasporangium = Ovule
Megaspore mother cell = Megasporocyte
Female gametophyte = Embryo sac = Megagametophyte
Female gamete = Egg

Microsporangium (pollen sac/pollen chamber)

The microsporangium is a structure in an anther which produces the microspores and eventually the male gametophyte. A **microsporangium** or future pollen sac is a cylindrical sac which appears circular in transverse section. **It consists of two parts, outer wall** and **central homogeneous mass of sporogenous tissue**. Microsporangial wall has four types of layers:

1. Epidermis (common anther covering)
2. Endothecium
3. 2-3 middle layers and
4. Tapetum

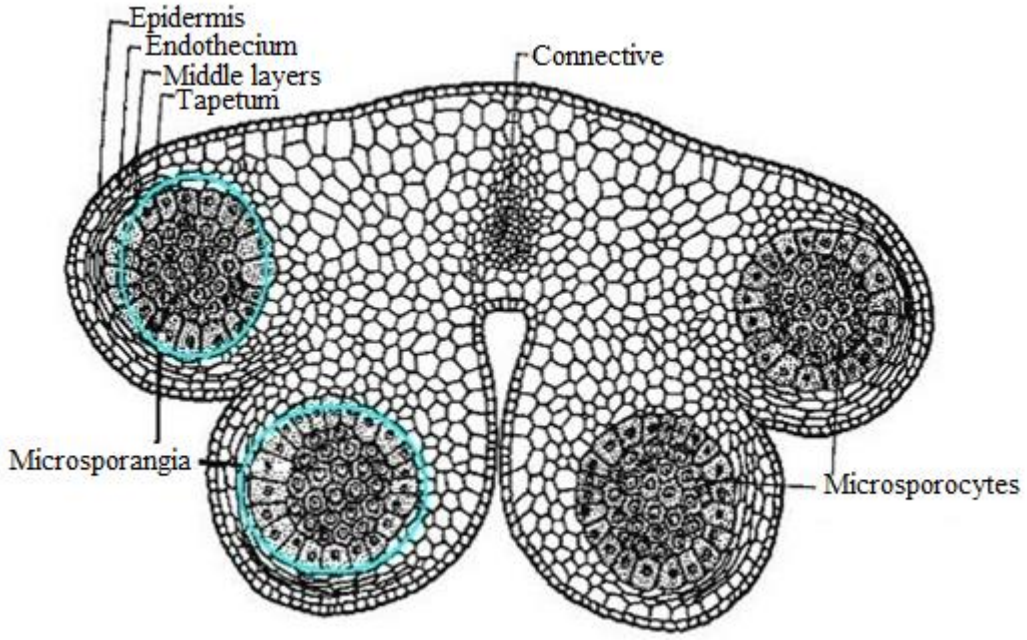


Fig. 5.4 Transverse section of a tetrasporangiate anther showing various tissues

Development of Microsporangium

During the development of microsporangium, the anther is seen initially as a **homogeneous mass of meristematic cells,** oblong in cross section and surrounded by a well defined **epidermis** (Fig. 5.5 A). It then becomes more or less four lobed and in each lobe some **hypodermal cells** become more prominent than the others because of their larger size, more deeply staining cytoplasm and conspicuous nuclei. These cells constitute the **archesporial initials** (archesporium) (Fig.5.5 B). There may be only one archesporial cell in each of the four lobes as in *Boerhaavia.*

The **archesporial cells** divide by periclinal division (in a plane parallel to the outer wall of the anther lobe), cutting off **primary parietal cells** toward the epidermis and **primary sporogenous cells** toward the interior of the anther. Then the cells of parietal layer forms 2-5 layers of anther wall by undergoing a series of divisions, both periclinal as well as anticlinal. The

primary sporogenous cells either directly or after few mitotic divisions functions as **microspore mother cells or pollen mother cells** (MMCs or PMCs).

Anther wall

The mature anther wall as described above, comprises an **epidermis** followed by a layer of **endothecium**, 2 or 3 **middle layers** and a single-layered **tapetum.**

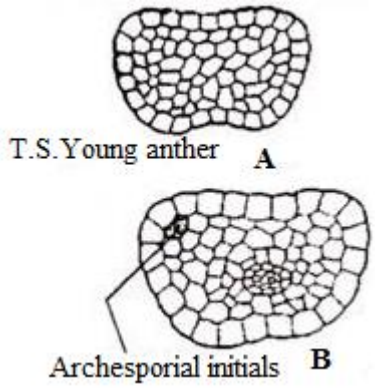


Fig. 5.5: In cross section anther is showing - A: Homogenous mass of meristematic cells surrounded by epidermis; B: Archesporial initials

The outermost layer, **epidermis** undergoes only anticlinal divisions. Its cells are flattened and become greatly stretched so that they keep pace with the enlargement of the anther. The epidermis performs its usual protective function.

Beneath the epidermis **endothecium or fibrous layer** is present. The cells of endothecium are radially elongated which later loses the cell contents, usually becomes fibrous and forms the dry coat of the mature anther. This layer attains its maximum development when anther is ready to dehisce for the discharge of pollen. The presence of fibrous bands, differential expansion of the outer and inner tangential walls and hygroscopic nature of the **endothecial cells help in dehiscence of anthers** at maturity. The cells of endothecium are thin walled along the line of dehiscence of each anther lobe. The **opening** through which the pollen grains are discharged from the pollen sac is called **stomium.** On maturity of the anther, a strain is exerted on the stomium due to the loss of water by the cells of endothecium, with the result the stomium ruptures and the anther dehisces. Mature anther, generally dehisces by means of slit or apical pores. Next to endothecium, **2 or 3 middle layers** are present. The middle layers are usually crushed by the time actual meiosis occurs in the sporogenous cells. In many species the cells of the middle layers are storage centers for starch and other reserves which get mobilized during later development of pollen.

Tapetum is the innermost layer of the anther wall characterized by the presence of dense cytoplasm and prominent nuclei. Tapetum is of considerable physiological significance. It attains

its maximum development at the tetrad stage of microsporogenesis. It is a **nutritive tissue nourishing the developing microspores,** all the food material to the sporogenous tissue must pass through it (Fig.5.6).

Sporogenous tissue

The sporogenous cells may directly functions as **microspore mother cells (MMCs) or pollen mother cells** or they may undergo few mitotic divisions to add up to their number before entering meiosis. Although all the sporogenous cells in the anther are potentially capable of giving rise to microspores, some of them frequently degenerate and absorbed by other cells.

From the above discussion it is clear that the process of development of anther till the MMCs formation is as follows-

Archesporial cell ⟶ Primary parietal cells and Primary sporogenous cells
Primary parietal cells ⟶ Parietal cells and Endothecium
Parietal cells ⟶ Tapetum and Middle cell layer
Primary sporogenous cells ⟶ Pollen mother cells/Microspore mother cells

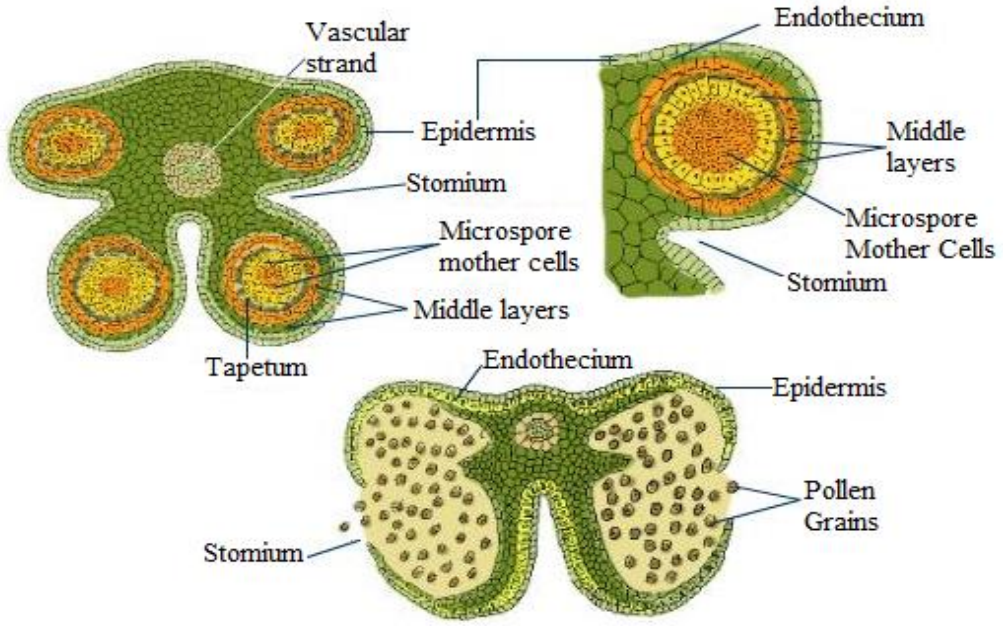


Fig. 5.6: A.T.S. of tetrasporangiate anther; B. Enlarged view of one microsporangium showing four wall layers; C.T.S. of a mature dehisced anther

5.4 MICROSPOROGENESIS

"Microsporogenesis means development of microspores/pollen grains".

The process of development of the microspore (pollen) is termed as **microsporogenesis**. During microsporogenesis the nucleus of each **microspore mother cell** or pollen mother cell (2n) undergo **meiosis** or reduction division, giving rise to four haploid (possessing 'n' number of chromosomes) **microspores**. At the end of meiosis four haploid microspores are enclosed in a common callose wall. The individual spore lacks a wall of its own and it is a callose partition which separates spores from each other. Aggregates of four microspores are called as microspore **tetrads**. Later on each spore forms its own wall (Fig. 5.7).

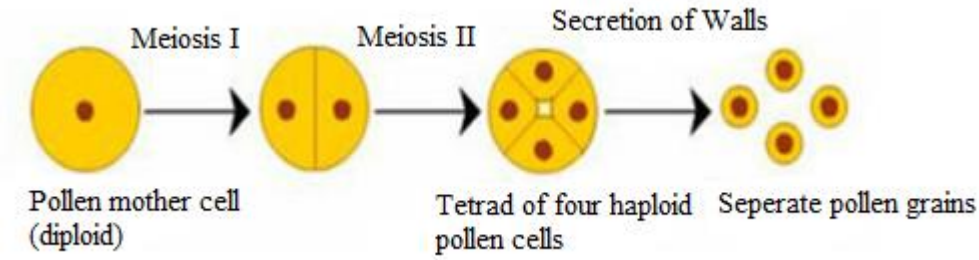


Fig. 5.7: Microsporogenesis

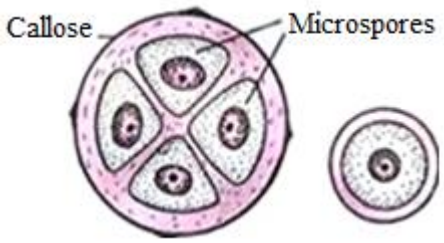


Fig. 5.8: Microspores showing callose wall

Microspore tetrad

All the four spores within a tetrad are completely isolated from one another and from the spores in other tetrads of the locule by means of callose wall (Fig. 5.8).

On the basis of arrangement of spores, tetrads are classified into various types. Usually the arrangement of microspores in a tetrad is tetrahedral or isobilateral. However, other arrangements i.e. decussate, linear and T-shaped tetrads are also found.

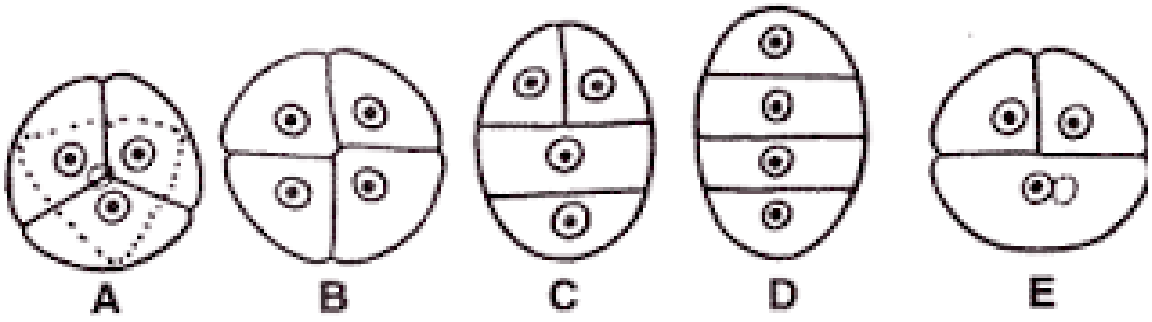


Fig.5.9: Arrangement of microspores/pollen grains, A. Tetrahedral; B. Isobilateral; C. T-shaped; D. Linear; E. Decussate

Tetrahedral: This type of tetrad is very common among dicots. The four microspores are arranged like a quadrant of a sphere so when see from an angle, only three microspores are visible and fourth lies at the back.

Isobilateral: This type of tetrad is very common among monocots. The four microspores are arranged at four corners of a square in one plane.

T-shaped: Out of the four microspores, two lies perpendicular to the others, so that the tetrad has the shape of "T". Example: as in *Aristolochia* (Samuels,1914) and *Butomopsis* (Johri,1936).

Linear: The four microspores aligned linearly as a result of transverse division in mother cell. Example: In some genera of the Asclepiadaceae (Gager, 1902) and in the genus *Halophila* of the Hydrocharitaceae (Kausik and Rao, 1942).

Decussate: A decussate arrangement of the cells has been recorded in *Magnolia* (Farr, 1918), *Atriplex* (Billing, 1934) and many other plants.

In ***Aristolochia elegans*** **all the five types of tetrad have been reported**. The microspores of a tetrad usually separate from each other shortly after meiosis as the anther matures. Now they are called pollens. Pollen grains are uninucleate when they separate from the tetrad and they have their own wall. However, in some plants it has been reported that the microspores of a tetrad tend to remain together in a tetrad and develop into compound structures i.e. Compound pollen grains and Pollinium etc.

Compound pollen grains

Sometimes microspores do not separate from each other and remain stuck together in groups. Such groups are called **compound pollen grains.** Example- *Drosera, Annona, Elodea, Typha* (several members of the Ericaceae, Apocynaceae, Asclepiadaceae, Juncaceae, and Orchidaceae).

Pollinium

All microspores (pollens) in a pollen sac remain together to form a single mass called **pollinium**. Example - *Calotropis*, orchids (members of the family Asclepiadaceae and Orchidaceae respectively). Each pollinium is provided with a stalk called **caudicle** and a sticky base called **disc or corpusculum** (Fig. 5.10).

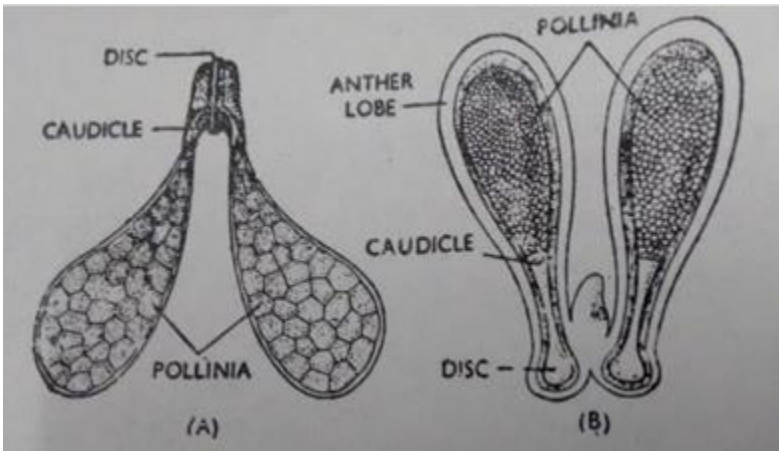


Fig. 5.10 : Pollinia (A). of *Calotropis*; (B). of orchid

Polyspory

Occurrence of more than four spores in a tetrad is called **polyspory**. The phenomenon of polyspory has been reported in a number of plants as an abnormality. Polyspory has been noted in *Hyphaene* by Mahabale and Chennaveeraiah (1957). *Cuscuta reflexa* is a plant where tetrad with as many as eleven microspores have been observed.

Microspores soon dry up and become powdery while the tapetum becomes absorbed. The anther now becomes a dry structure, the partition walls between the sporangia (i.e. loculi) are usually destroyed and the microspores are soon liberated by dehiscence of the anther.

Haploid microspores or pollens are the first cell of male gametophyte.

5.5 DEVELOPMENT OF MALE GAMETOPHYTE IN ANGIOSPERM

Gametogenesis

Process of development of male gametophyte is known as **gametogenesis** and haploid microspores or pollens formed from diploid Microspore Mother Cell (MMC) as a result of meiosis are the first cell (mother cell) of male gametophyte. Therefore **MMC could be called as**

last cell of sporophytic generation or sporophyte. Soon microspores released from the tetrad and are referred to as pollen grains.

Microspore/Pollen grain:
Pollen grains are contained in the microsporangia (pollen chamber). They are very minute in size (approximately 0.025 to 0.125 mm) and are like particles of dust. A freshly formed pollen grain is richly cytoplasmic with a prominent, centrally located nucleus. The wall of the mature pollen grain is stratified. It comprises of two layers. The outer layer is called **exine** and inner layer is termed as **intine**. The term exine and intine were proposed by Fritsch (1837).

Exine: Thick, tough cutinized layer which is often provided with spinous outgrowths or sometimes smooth. The exine is composed of a complex substance, called **sporopollenin**.

Intine: It is thin, smooth, delicate pecto-cellulosic layer lying internal to the exine.

Waxy coating (cutinization of exine) makes the pollen more or less water proof. Sporopollenin is resistant to physical and biological decomposition and due to this property the pollen grain walls are often preserved for long periods in fossil deposits. The pollen wall also protects pollen during its journey from anther to the stigma. At one or more places exine is lacking. These areas are known as **germ pores/slits**. If they are round in appearance then we call them germ pores, if they seems elongated then termed as germinal furrows. Pollen grains are generally **tricolpate** (with three germ pores) in dicots and **monocolpate** (with single germinal furrow) in monocots. Germ pore facilitate the emergence of pollen tube through it at the time of male gametophyte development (Fig. 5.11).

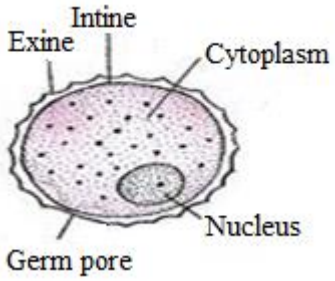


Fig. 5.11: Uninucleate pollen grain

Pollenkitt

It is an oily layer forming a thick viscous coating over the pollen grain surface of many insect pollinated species. The stickiness, odour and colour of the grains are because of the pollenkitt. It comprises mainly of carotenoid or flavonoid pigments which impart the characteristic yellow or orange colour to the pollen. The pollenkitt or the surface pollen cement also contains

glycoproteins, lipids, glycolipids and monosaccharides which are responsible for sticky nature of the pollen). Pollenkitt is believed that it may be contributing in the following ways:

- Acting as insect-attractant.
- Protecting the pollen against the damaging effect of ultraviolet radiation.
- Acting as an adherent to the insect body because of the sticky nature.
- Being hydrophobic, it might even be associated with the dispersal of pollen.
- Functining as the pollen-borne substances involved in sporophytic incompatibility.

The development of the male gametophyte is remarkably uniform in Angiosperms. Microspore is the first cell of a male gametophyte. **This cell undergoes only two divisions.** The second division is concerned with generative cell only and this division may take place either in the pollen grain or in the pollen tube. The life of the male gametophyte is very short as compared to that of the sporophyte.

Pre-pollination development

Pollen grains begin to germinate before pollination.

A pollen grain divides mitotically **(first division)** to form two unequal cells, a **smaller generative cell and a larger vegetative cell (also known as tube cell)** (Fig. 5.12).

Pollen grain

1^{st} mitotic division

Larger vegetative cell (tube cell) Smaller generative cell

The nuclei of both the cells differ in size and structure. The vegetative cell has a prominent nucleus, cytoplasm is rich in RNA whereas generative cell nucleus is small, cytoplasm is almost without RNA.

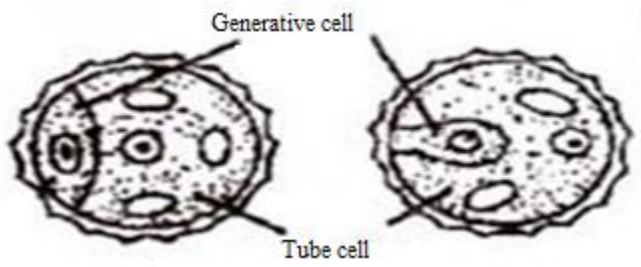


Fig. 5.12: Pollen grain at 2-celled stage having generative cell and tube cell

Larger vegetative cell is central and the generative cell is peripheral in position, initially attached to the intine but later gets detached from it and lies free in the cytoplasm of the vegetative cell (tube cell). At this stage the pollen grain become two celled.

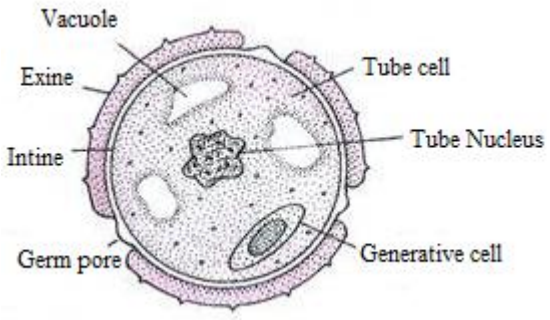


Fig. 5.13: Mature 2-celled pollen grain showing generative cell lies free in the cytoplasm of the vegetative cell (tube cell)

In over 60% of flowering plants, mostly dicots, pollen grains are shed from the microsporangium at 2-celled stage (tube cell + generative cell) for pollination. Generative cell divides after pollination and in the remaining species, pollen grains are shed at 3-celled stage (tube cell + two male gametes). Generative cell divides before pollination (Fig. 5.13).

- When the sperm (gamete) formation takes place after the release of pollen from the anther, it is called 'the pollens are shed at the 2-celled stage' and
- When the sperm (gamete) formation takes place while the pollens are still confined to the anther, it is called 'the pollens are shed at the 3-celled stage'.

Post-Pollination development

Once the pollen grain reaches the receptive stigma by means of pollination, it germinates.
On the stigma the compatible pollen grain absorbs water and nutrients from the stigmatic secretion through its germ pores. The **tube or vegetative cell** enlarges and forms a long slender **pollen tube** by coming out of the pollen grain through one of the germ pores. It secretes pectinase and other hydrolytic enzymes to create a passage for it in the style if the latter is solid. The pollen tube absorbs nourishment from the cells of the style for its growth.

As the pollen germinates and the pollen tube comes out through the germ pore, the generative cell soon divides mitotically for the second time to form two non-motile sperms (**male gametes**). This act is known as **spermatogenesis**. The generative cell or its products, i.e. two male gametes and the tube nucleus migrate into the pollen tube which now represents the mature male gametophyte (Fig. 5.14).

Generative cell 2nd mitotic division ⟶ Two male gametes

Vegetative cell (Tube cell) ⟶ Pollen tube

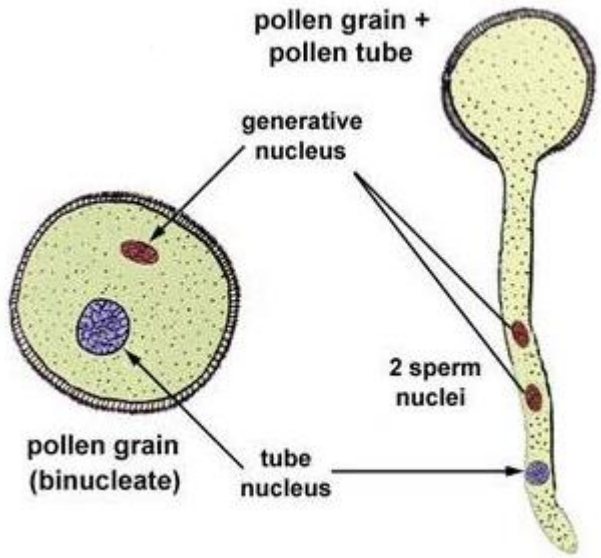


Fig. 5.14: Formation of pollen tube and two male gametes

The generative cell or its products i.e. two male gametes and the tube nucleus migrate into the pollen tube which now represents the **mature male gametophyte**. Each male gamete has a large nucleus which is surrounded by a thin sheath of cytoplasm. The tube nucleus may degenerate completely. Pollen tube not only carries male gametes but also secretes hormones and absorbs food from style (Fig. 5.15).

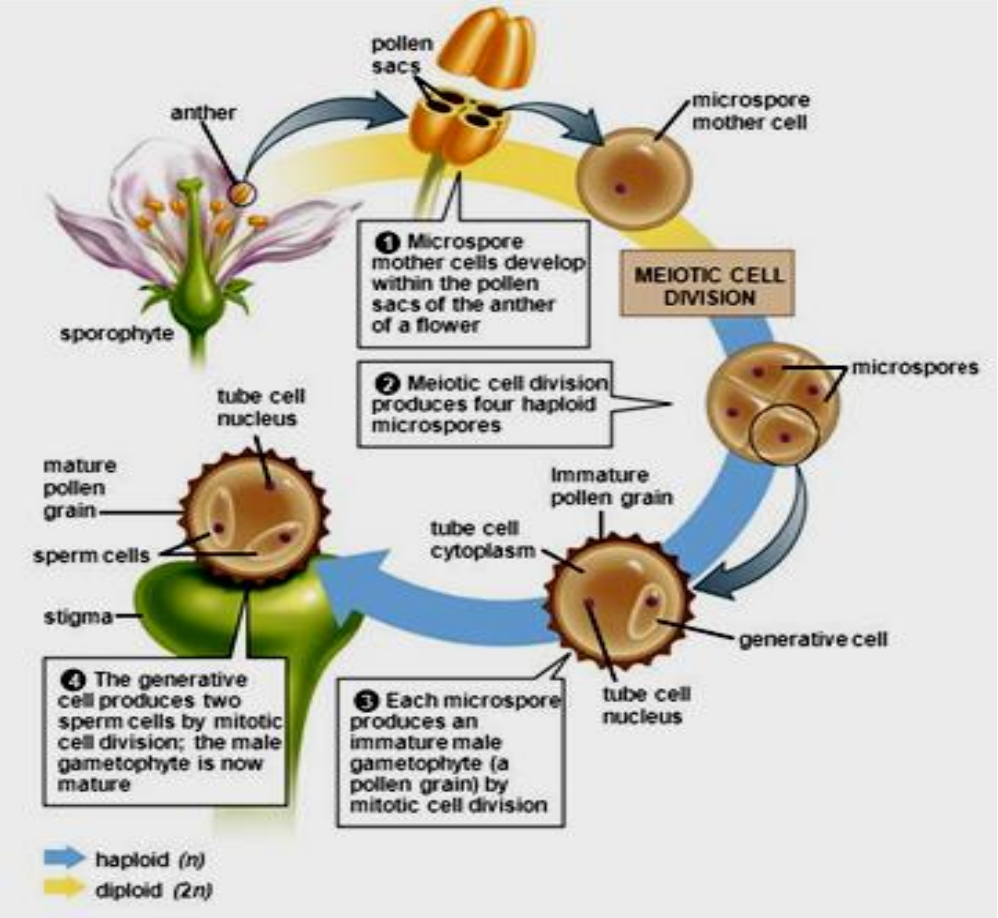


Fig. 5.15: showing male gametophyte development

Palynology

The study of external morphological features of mature pollen grain is known as palynology.

Pollination

It is the process of pollen transfer from anther to stigma of a flower. Therefore for pollination the first and very important requirement is the release of pollen grains from the pollen chamber and for releasing pollens anther must dehisce.

Anther dehiscence

For dehiscence of anther three types of specialized cells are required- stomium, septum and endothecium. The **opening** through which the pollen grains are discharged from the pollen sac is called **stomium. The septum,** that separates the two lobes of an anther, breaks down at a later stage. The cells of **endothecium** are thin walled along the line of dehiscence of each anther lobe. On maturity of the anther, a strain is exerted on the stomium due to the loss of water by the cells

of endothecium, with the result the stomium ruptures and the anther dehisces. Mature anther, generally dehisces by means of slit or apical pores.

5.6 SUMMARY

Alternation of generation is a remarkable aspect of the life cycle of all higher plants. Angiosperm plants are diploid sporophytes i.e. spore bearing plants and the haploid spores are developed by meiosis. In this unit we have discussed the meaning of various terms i.e. gametophyte, male gametophyte, microsporogenesis, etc. and about the structure of anther. We also learnt about the process of microsporogenesis, and various steps of gametophyte development. Besides this we have discussed about various types of tetrads also. Therefore, let us sum it up in key points:

- Gametophyte is the haploid generation producing gametes in plants.
- When we are talking about male then it is said to be male gametophyte.
- Male and female reproductive organs in plants are stamen and carpel, the necessary parts of flower.
- The male spores or microspores (pollens) are developed by reduction division or meiosis within the microsporangium (pollen sac).
- Numerous microspore mother cells are formed in the anther lobe.
- Each mother cell undergoes meiosis or reduction division to form four microspores (pollen grains).
- Microspores thus formed are held together in tetrad.
- The process of development of the microspore (pollen) is termed microsporogenesis.
- On the basis of arrangement of four microspores in a tetrad, there are mainly five types of tetrad. They are: tetrahedral, isobilateral, T-shaped, linear and decussate.
- Each pollen grain has two layers, outer thick, rough exine and inner thin intine having germ pores.
- The pollen grain or microspore is the first cell (mother cell) of the male gametophyte.
- It divides to form two unequal cells, a smaller generative cell and a larger vegetative cell ((tube cell).
- The generative cell further divides mitotically to form two non-motile male gametes whereas tube cell forms pollen tube.
- The pollen grain having male gametes with pollen tube represents the mature male gametophyte.
- Male gametophyte is endosporous i.e. developing inside the spores.
- In some plants gamete formation takes place after the release of pollen from the anther, it is called ‘the pollens are shed at the 2-celled stage’.
- In some plants gamete formation takes place while the pollen are still confined to the anther, it is called ‘the pollens are shed at the 3-celled stage’.

- At maturity, pollen grains released and through various agents i.e. wind, water, animal etc. reach the compatible stigma (either of the same flower or another). This is known as pollination.

5.7 GLOSSARY

Androecium: A collective term used for all the stamens of flower
Anticlinal division: Perpendicular to the surface (at right angle to the surface of apex)
Asexual reproduction: Formation of new individuals from the cell(s) of single parent
Sexual reproduction: Fusion of two dissimilar gametes from two different parents
Anther: Fertile portion of stamen
Connective: The region between the two lobes of an anther
Diploid: Cell having '2n' number of chromosomes
Dithecous: Anther having two lobes
Endosporous: Developing inside the spores
Endothecium: A wall layer found in anther below the epidermis
Exine: Outer thick, rough layer of mature pollen grain
Fertilization: The union of male and female nuclei
Gametes: Sexual cells
Gametogenesis: Process of development of gametophyte
Gametophyte: An individual of the haploid generation producing gametes in plants.
Gynoecium: Refers to all the carpels of a single flower
Haploid: Cell having 'n' number of chromosomes
Intine: Inner thin layer of mature pollen grain
Megasporangium: Ovule
Megaspore: First cell of female gametophyte
Megasporogenesis: The process of formation of megaspores
Meiosis: Process of reduction division in diploid cell to produce haploid cell
Microsporangium (pollen sac) : Chamber producing microspores
Microspores (pollen grains/pollen): First cell of male gametophyte
Microsporogenesis: The process of formation of microspores
Monothecous: Anther having single lobe
Ovule: The megasporangium of seed plant
Palynology: The study of external morphological features of mature pollen grain.
Periclinal division: Division in a plane parallel to the surface
Pollen: The powder produced by anthers consisting of pollen grains
Pollination: Transference of pollen grains from anther to stigma
Pollinium: All pollens in a pollen sac remain together to form a single mass called pollinium
Pollenkitt: Oily layer forming a thick viscous coating over the pollen grain

Polyspory: Occurrence of more than four spores in a tetrad
Sporogenesis: The process of development of the spore
Sporophyte: The spore producing generation in plants
Stamen: Male reproductive part of a flower consists of the filament and the anther
Stomium: The opening through which the pollen grains are discharged from the pollen sac
Tapetum: Innermost layer of pollen sac wall
Tetrad: Aggregate of four spores

5.8 SELF ASSESSMENT QUESTION

5.8.1 Multiple choice questions:

1. Which one of the following is mismatched?
(a) Gynoecium - female reproductive organ (b) Tapetum - one of the layer of anther wall
(c) Anther- fertile part of stamen (d) Pollen grain-diploid

2. A heterosporous plant:
(a) Produces microspores and megaspores (b) Produces only microspores
(c) Produces only megaspores (d) None

3. Microspore develops into a:
(a) Polar nuclei (b) Female gametophye
(c) Male gametophyte (d) Embryo

4. The site of formation of pollen grains is in the:
(a) Pistil (b) Petal
(c) Stamen (d) Stigma

5. Sporophyte is:
(a) Haploid (b) Diploid
(c) May be haploid (d) May be diploid

6. A microspore mother cell is located in the:
(a) Anther (b) Filament
(c) Style (d) Stigma

7. Pollen grains are tricolpate, if they have:
(a) Three germ pores (b) Two germ pores
(c) One germ pore (d) Many germ pores

8. Pollen grains are shed at 2-celled stage, represented by:
(a) Tube cell + vegetative cell (b) Tube cell + generative cell
(c) Tube cell + male gamete (d) None

9. Last cell of sporophytic generation or sporophyte:
(a) Embryo (b) Zygote
(c) Spore (d) Spore mother cell

10. Division in a plane parallel to the outer wall of the anther lobe is called:
(a) Anticlinal division (b) Periclinal division
(c) May be anticlinal or periclinal (d) None

5.8.1 Answer Key:

1. (d), 2. (a), 3.(c), 4.(c), 5.(b), 6.(a), 7.(a), 8.(b), 9.(d), 10.(b)

5.8.2 Short answer questions with answer:

1. Name the first cell of male gametophyte.
Ans: The first cell of male gametophyte is microspore.

2. Name the last cell of male sporophyte.
Ans: The last cell of male sporophyte is microspore mother cell.

3. Can we call first cell (here) as mother cell.
Ans: Yes

4. What is the chemical nature of two layers of microspore?
Ans: The intine is pecto-cellulosic in nature and the exine is composed of sporopollenin.

5. What is the region behind this statement that "Pollen grain walls are often preserved for long periods in fossil deposits".
Ans: Sporopollenin of exine is resistant to physical and biological decomposition and due to this property the pollen grain walls are often preserved for long periods in fossil deposits.

6. What do you understand by the term Pollinium?
Ans: All microspores in a pollen sac remain together to form a single mass called pollinium.

7. Name different types of tetrad.
Ans: Isobilateral, Tetrahedral, Linear, T-shaped, Deccusate.

8. What is the main function of germ pores in pollen grains.
Ans: Germ pore facilitate the emergence of pollen tube through it at the time of male gametophyte development.

9. What kind of enzyme, pollen tube secretes?
Ans: It secretes pectinase and other hydrolytic enzymes to create a passage for it in the style if the latter is solid.

10. How many types of layers are present in anther wall?
Ans: Four types.

11. What is the role of endothecium?
Ans: Endothecial cells help in dehiscence of anthers at maturity.

12. Why tapetum is so important?
Ans: It is a nutritive tissue nourishing the developing microspores, all the food material to the sporogenous tissue must pass through it.

13. Define gametogenesis.
Ans: Process of development of male gametophyte is known as gametogenesis.

14. What do you mean by microsporogenesis?
Ans: The process of development of the microspores (pollens) is termed as microsporogenesis.

15. What do you mean by pollen grains are shed at 2-celled stage or 3-celled stage?
Ans: When the sperm (gamete) formation takes place after the release of pollen from the anther, it is called 'the pollen are shed at the 2-celled stage' and the when sperm (gamete) formation takes place while the pollens are still confined to the anther, it is called 'the pollens are shed at the 3-celled stage'.

16. Which cell takes part in the formation of male gametes?
Ans: Generative cell divides mitotically for the second time to form two non-motile sperms (male gametes).

17. Define Palynology.
Ans: The study of external morphological features of mature pollen grain.

18. What is the difference between microspore and pollen?

Ans: Microspore when develops a exine around it, is called a pollen grain.

5.9 REFERENCES

- Elaine Lopes Pereira Nunes, Cleusa Bona, Maria Cecı´lia de Chiara Moc¸o and Alessandra Ike Coan. (2009) Release of developmental constraints on tetrad shape is confirmed in inaperturate pollen of *Potamogeton*. *Annals of Botany*. Page 1 of 5 doi:10.1093/aob/mcp160, available online at www.aob.oxfordjournals.org
- Gifford, E.M., and Foster, A.S. (1989). *Morphology and Evolution of Vascular Plants*. W.H. Freeman, New York.
- Jonathan Yam and Whitney Hagins.Seedless fruit and methods of Parthenocarpy. *J Experimental Secondary Science*. pp 1-3
- Raghavan,V. (2000). Microsporogenesis and formation of the Male Gametophyte. In: *Developmental Biology of flowering Plants*. pp 186-215

5.10 SUGGESTED READINGS

- Singh,V., Pande, P.C. and Jain, D.K. (2008). *A Text Book of Botany*. Rastogi Publications, Meerut.
- Maheshwari, P. (1950). *An Introduction to the Embryology of Angiosperms* McGraw-Hill, New York .
- Gangulee, H.C., Das, K.S. and C. Datta, C. (1998). *College Botany,* Vol I. New Central Book Agency, Kolkata.
- *Developmental Biology*. 6th edition. Gilbert S.F.Sunderland (MA): Sinauer Associates; Bookshelf ID: NBK9980 (2000).
- Pandey, S.N. (1997). *Plant Anatomy and Embryology.* Vikas Publishing House Pvt Ltd, New Delhi.
- *Plant Physiology and Development*, 6th edition, Lincoln Taiz, Eduardo Zeiger, Ian Max Moller, Angus Murphy.2015 P 761.ISBN: 978-1-60535-255-8
- Singh, V., Pande, P.C. and Jain, D.K. (2012-13). *Structure, Development and Reproduction in Angiosperms.* Rastogi Publications, Meerut.
- Bhojwani, S.S., Bhatnagar, S.P. and P. K. Dantu, P.K. (2015). *The Embryology of Angiosperms*. Vikas Publishing House Pvt Ltd, New Dlhi.

Important website and links

- http://biology.clc.uc.edu/courses/bio106_(accessed on March, 2016).
- http://www.biologyreference.com/(accessed on March, 2016).
- BiologyDiscussion.com

5.11 TERMINAL QUESTIONS

5.11.1 Long answer type questions:

1. Give the structure of pollen grain with well labeled diagram.
2. What is gametogenesis? Explain development of male gametophyte.
3. What are the pre-pollination and post pollination steps in male gametophyte development.
4. Write explanatory note on development of microsporangium.
5. What do you understand by microsporogenesis. How it occurs?
6. How many types of spore tetrads are possible? Give detail account of it.

UNIT-6 FEMALE GAMETOPHYTE

6.1 OBJECTIVES

After going through this unit students will be able to answer:

- What is ovule?
- How many types of ovule are there and on what basis they are differentiated?
- What is megasporogenesis?
- Are all megaspores responsible for female gametophyte formation?
- Is there any difference between female gametophyte or embryo sac?
- Why embryo sacs are classified as monosporic, bisporic or tetrasporic?
- Explain *Polygonum* type of embryo sac.

6.2 INTRODUCTION

Life cycle of plants alternate between multicellular haploid gametophyte and multicellular diploid sporophyte and both differ morphologically as well as functionally.

According to Gifford and Foster (1989), in a life cycle of a plant:

- Diploid sporophyte produces haploid spores as a result of reduction division (meiosis).
- Spores develop into gametophytes.
- Gametophytes produce haploid gametes.
- The fusion of female gamete (egg) and male gamete gives rise to the zygote.
- Zygote is the beginning of diploid sporophyte.

When we are talking about the life cycle of Angiosperms:

- Diploid sporophyte produces two types of spores- microspores and megaspores.
- Microspore develops into male gametophyte as discussed in previous unit and megaspore produces female gametophyte which we will study in this unit.

In the previous unit you have studied about the structure of anther, microsporogenesis as well as the development of male gametophyte in Angiosperms. By going through that unit it must be clear to you that the pollens or microspores are developed by reduction division or meiosis in microspore mother cell within the microsporangium and they represent the first cell of male gametophyte.

The gametophyte in Angiosperms develops within sporophytic tissue –the sexual organs of the flower. The male gametophyte (developing pollen grain composed of two sperm cells encased within a vegetative cell) develops within the anther, fertile part of the stamen (McCormick, 1993, 2004). The female gametophyte (embryo sac) develops within the ovule which is found within the ovary.

In this unit we describe structure and types of ovule, megasporogenesis, megagametogenesis and different types of female gametophytes.

Gynoecium or pistil represents the female reproductive organ in a flower and carpel is a unit of it. A **carpel** consists of a basal swollen **ovary bearing one or more ovules**, a receptive **stigma**, and often a stalk-like **style** between them (Fig. 6.1).

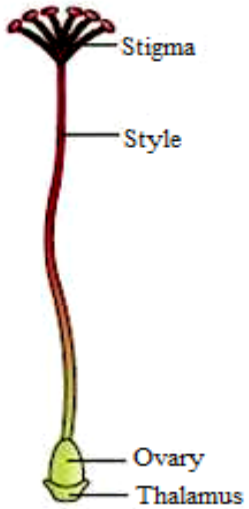


Fig. 6.1 Gynoecium

Ovules as you have read, are enclosed by the ovary wall. The part of the carpellary tissue to which the ovules are attached is called **placenta** and the distribution of ovules in the ovary is described as **placentation.**

Ovule also known as megasporangium is the place of formation of the megaspores and the female gametophyte. The latter, after fertilization produces the embryo and endosperm, while the entire megasporangium with its enclosed structure becomes the seed and the progenitor of the next generation.

6.3 STRUCTURE OF OVULE

The **megasporangium or ovule** consists of **nucellus** and its protective coats, the **integuments**. It is attached to the placenta, on the inner wall of ovary by a stalk called **funiculus (funicle)** and the point of attachment of the body of the ovule to the funiculus is called **hilum.**

A mature ovule, ready for fertilization, consists of nucellus enveloped almost completely by one or two sheaths, known as **integuments**, leaving a small opening at the apical end. This opening is known as **micropyle**. The basal region of the ovule where it is attached to the placenta by funicle, is called **chalaza** and so this side is known as chalazal end. Its opposite end is termed

as micropylar end, the main passage for the entry of the pollen tube into the ovule. In the nucellus, **female gametophyte** is present, also known as **embryo sac.**

Nucellar tissue is parenchymatous and represents the wall of the megasporangium. The nucellus is mostly consumed by the developing embryo sac or endosperm. Each ovule has only one nucellus. However, two nuclei may occur as abnormality within a common fold of integuments as has been observed in *Aegle marmelos* (Fig. 6.2).

The ovule with a **single integument** is called **unitegmic,** with **two integuments** is called **bitegmic** and without integument is called **ategmic.**

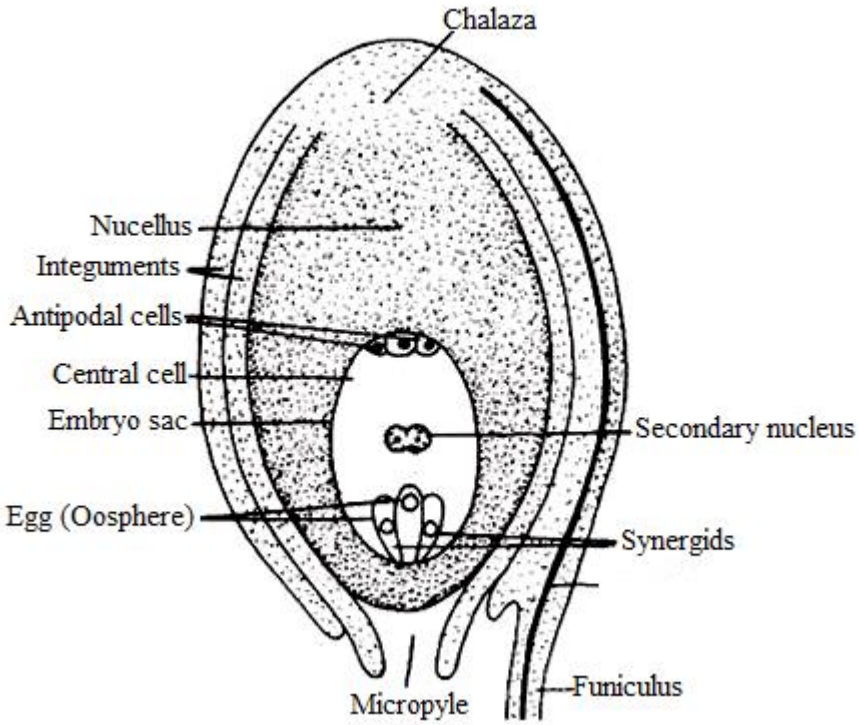


Fig. 6.2: Structure of ovule

Parts of the ovule:

1. **Funiculus** or **Funicle**: A stalk by which ovule is attached to the placenta
2. **Nucellus:** the body of ovule
3. **Integumen**t: the protective covering of nucellus
4. **Micropyle:** small opening formed by two integuments over nucellus
5. **Chalaza:** basal part of the ovule
6. **Hilum:** region where ovule fuses with funiculus
7. **Embryo sac:** female gametophyte located in the nucellus, developed from megaspore

Types of ovule

On the basis of the position of the micropyle with respect to the funiculus, mature ovule can be classified into six main types. These are:

1. Orthotropous
2. Anatropous
3. Campylotropous
4. Amphitropous
5. Hemianatropous
6. Circinotropous

1. **Orthotropous ovule:** Orthotropous ovule is also known as **atropous**. It is upright. In this type the micropyle, chalaza and the funiculus lie in one straight line as in Polygonaceae and Piperaceae (Fig. 6.3).

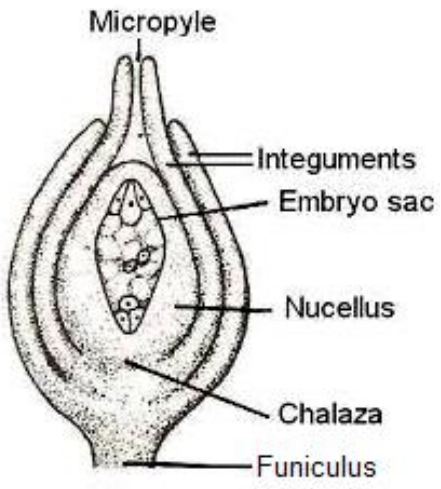


Fig. 6.3: Orthotropous ovule

2. Anatropous ovule: In this type, the funiculus is long; the body of the ovule becomes completely inverted so that micropyle comes to lie close to the base of the funiculus. This happens due to unilateral growth of the ovule. The nucellus remains straight so micropyle and chalaza lie in one line and funiculus lie parallel to it. It is the most common type of ovule in Angiosperms (Fig.6.4).

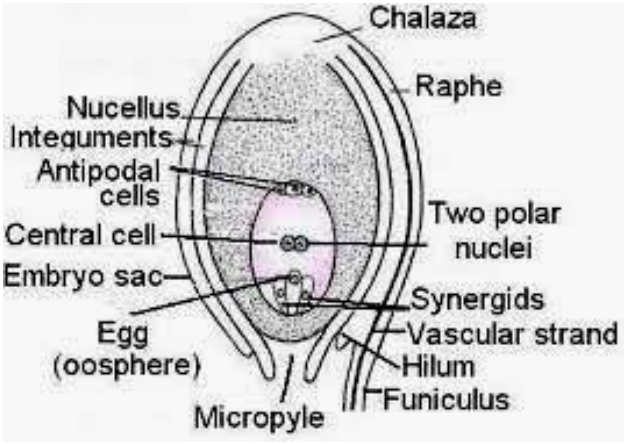


Fig. 6.4: Anatropous ovule

3. Campylotropous ovule: In campylotropous ovules body of the ovule is not completely inverted, the curvature is less than that in anatropous ovules. The micropyle and chalaza do not lie in the straight line and the funiculus lies at right angle to the chalaza as in Chenopodiaceae and Capparaceae (Fig. 6.5).

Fig. 6.5: Campylotropous ovule

4. Amphitropous ovule: It is similar to campylotropous, but in this case the curvature of the ovule also affects the nucellus/embryo-sac so that it bent like 'horse shoe' as in Alismaceae and Butomaceae (Fig. 6.6).

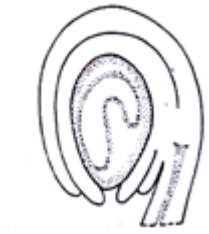


Fig. 6.6: Amphitropous ovule

5. Hemianatropous ovule: Also known as **hemitropous**. In this type of ovule the funiculus is at right angle to the nucellus and the integuments. Micropyle and chalaza, lie in the same plane as in *Ranunculus*, *Nothoscordum*, and *Tulbaghia* (Fig. 6.7).

Fig. 6.7: Hemianatropous ovule

6. Circinotropous ovule: A very peculiar type of ovule is seen in some members of the Plumbaginaceae. Here the nucellar protuberance is at first in the same line as the axis, but the rapid growth on one side causes it to become anatropous. The curvature does not stop but continues until the ovule has turned over completely so that the micropylar end again points upwards. It has been suggested that this kind of ovule, also seen in *Opuntia*, is distinctive enough to merit a separate name, Circinotropous (Archibald, 1939). (Fig. 6.8).

Fig. 6.8: Circinotropous ovule

Depending on the extent of development of the nucellus and on the basis of position of sporogenous cell, ovule can also be categorized as:

1. Tenuinucellate type
2. Crassinucellate type

1. Tenuinucellate type: The archesporial cell directly functions as the megaspore mother cell so that the sporogenous cell is also hypodermal. Such ovules, where the sporogenous cell is hypodermal and the nucellar tissue around it remains single-layered, are called **tenuinucellate**.

2. Crassinucellate type: The hypodermal archesporial cell divides transversely, forming outer parietal cell and an inner sporogenous cell. The parietal cell may either remain undivided or undergo a few divisions (both periclinal as well as anticlinal) so that the sporogenous cell becomes embedded in the massive nucellus. The sporogenous cell may be embedded in the massive nucellus by divisions in the nucellar epidermis. All such ovules where the sporogenous cell becomes subhypodermal, by either above two means, are called **crassinucellate**.

Tenuinucellate ovule: where the sporogenous cell is hypodermal and the nucellar tissue around it remains single layered

Crassinucellate ovule: where the sporogenous cell becomes subhypodermal, either due to formation of parietal cells, or due to divisions in the nucellar epidermis, or both

Development of ovule

The ovule at first arises as a primordium on the placenta in the cavity of the ovary. Later due to meristematic activity of the cells of ovular primordia, the protuberance become prominent and grows into a mass of tissue, **the nucellus**. The initials of two integuments arise at the base of the nucellus. The inner integument which is usually formed first, initiates from the epidermal layer and the outer integument is initiated either dermally or subepidermally. With the differentiation of integuments the ovule begins to curve and by the megaspore tetrad stage it assumes its final shape. Although the integuments initiate later they grow faster than the nucellus. The integuments soon cover the nucellus, leaving a small opening at the tip, the micropyle.

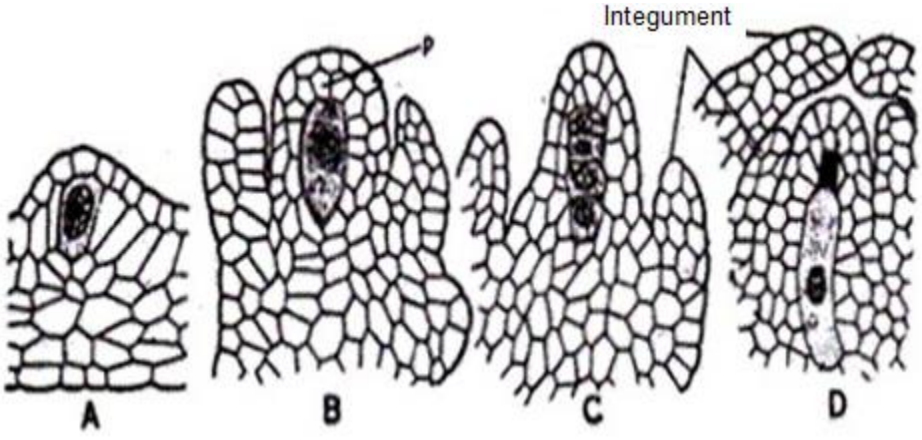


Fig. 6.9: Stages of ovule development and megasporogenesis. A. Protuberance of placental tissue and differentiation of archesporium (shaded); B. Parietal cells and megaspore mother cell (shaded). One integument is developing; C. Linear tetrad of megaspores (shaded); D. Disintegration of the three upper megaspores and enlargement of the functional megaspore. Both integuments have developed.

Female gametophyte development occurs in two phases:
1. Megasporogenesis
2. Megagametogenesis
The developmental pattern exhibited by most species is referred to as the *Polygonum* type because it was first described in *Polygonum divaricatum* (Strasburger, 1879; Maheshwari, 1950).

6.4 MEGASPOROGENESIS

"Development of the megaspore within the ovule (megasporangium) is known as megasporogenesis."

A hypodermal cell in the nucellus (at the micropylar end) differentiates and functions as the **archesporium** (archesporial cell). It is distinguishable from the other cells as it becomes more prominent than its surrounding cells due to its large size, dense cytoplasm and large nucleus.

As it is now clear to you from the previous section that ovule can be categorized into two types (tenuinucellate and crassinucellate), on the basis of position of sporogenous cell. So here in tenuinucellate type of ovule, the archesporial cell directly functions as megaspore mother cell (MMC) and in crassinucellate type of ovule the archesporial cell do not directly behave as MMC and instead of that it divides periclinally into two cells. An outer primary parietal cell (towards epidermis) and an inner primary sporogenous cell. Now this primary sporogenous cell functions as the megaspore mother cell.

Megaspore mother cell is also known as **megasporocyte** having diploid (2n) chromosome number. It undergoes meiosis i.e. reduction division. As a result of this four haploid megaspores are formed. After first meiotic division, the wall is laid down transversely, forming a dyad. The second meiotic division in the two dyad cells is also transverse. In this way a row of four haploid megaspore cells (**linear tetrad)** is formed. Meanwhile, two integuments develop from the base of the ovule. In the linear tetrad **the lowermost megaspore** (the chalazal megaspore) enlarges and becomes **functional.** Rest three megaspores of tetrad do not participate in the formation of female gametophyte and degenerate. (Fig. 6.9, 6.10).

The functional megaspore now forms the female gametophyte (embryo sac). Haploid tetrad of megaspores may be T-shaped, isobilateral or tetrahedral, T-shaped tetrad arises due to vertical division in the micropylar dyad cell and transverse division in the chalazal dyad cell.

Functioning megaspore

During megasporogenesis, the diploid megaspore mother cell undergoes meiosis and gives rise to four haploid nuclei. **Angiosperms exhibit three main patterns of megasporogenesis**, referred to as **monosporic, bisporic, and tetrasporic**. The three types differ mainly in whether

wall (cell plate) formation occurs after these divisions, thus determining the number of meiotic products that contribute to the formation of the mature female gametophyte.

In the monosporic pattern, both meiotic divisions are accompanied by wall formation, resulting in four one-nucleate megaspores (**linear tetrad**). Only one becomes functional and forms the female gametophyte, Subsequently, three megaspores, generally the micropylar-most megaspores degenerates. It is the chalazal megaspore of the tetrad that is functional. Sometimes the female gametophyte (embryo sac) is formed by the micropylar megaspore as observed in Onagraceae.

In the bisporic pattern, wall forms after meiosis I but not after meiosis II. The result is two two-nucleate megaspores (dyad cells). One of the **dyad cells with two haploid megaspore** nuclei contributes towards the formation of female gametophyte (embryo sac) and other dyad cell degenerates.

In the tetrasporic pattern, wall formation fails to form after both meiotic divisions, resulting in one four-nucleate megaspore. **All four megaspore nuclei** participate in the formation of female gametophyte (embryo sac).

Thus, these three patterns give rise to a **single functional megaspore** that contains one (monosporic), two (bisporic), or four (tetrasporic) meiotic nuclei.

If you remember the microsporogenesis where a haploid microspore was said to be the first cell (mother cell) of male gametophyte, similarly here a **haploid megaspore is known as the first cell (mother cell) of female gametophyte**.

In Angiosperms the development of the female gametophyte is completely **endosporous** means within the megaspore.

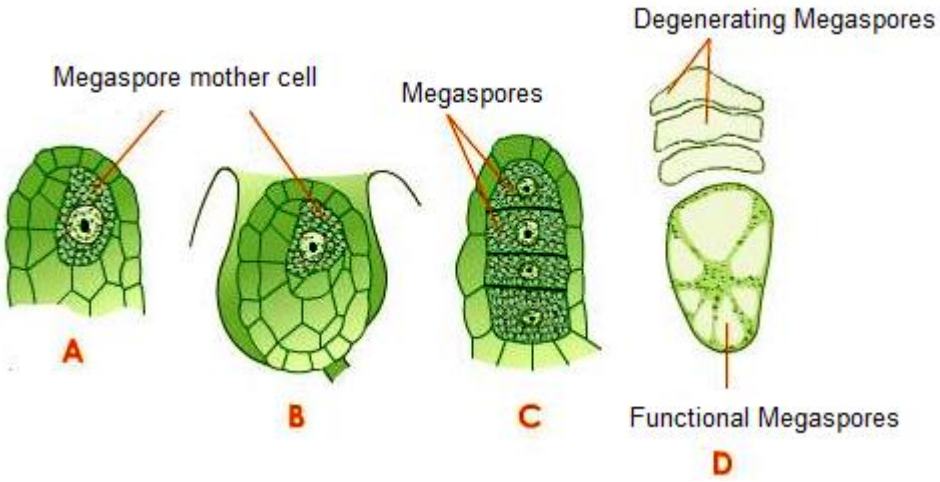


Fig. 6.10: Formation of 4 haploid megaspores from diploid megaspore mother cell

6.5 DEVELOPMENT OF THE FEMALE GAMETOPHYTE OR EMBRYO SAC WITH PARTICULAR REFERENCE TO POLYGONUM TYPE

Megagametogenesis

During megagametogenesis, the functional megaspore gives rise to the mature female gametophyte.

We have already discussed the functional megaspore is the first cell of the female gametophyte or you can also call it as the mother cell of the female gametophyte. It grows in size and forms an embryo sac.

The haploid nucleus of the **megaspore divides mitotically** (non-reductional division) which organize in a definite manner within the embryo sac. Three nuclei at the micropylar end, three at the chalazal end and the remaining two migrate to the centre of the embryo sac. Three nuclei at the micropylar end organize into an egg apparatus. The central large cell of the egg apparatus is called egg cell (female gamete) which is partially surrounded by two lateral synergid cells. Three nuclei of the chalazal end form antipodal cells. The two nuclei which migrates to the centre, called polar nuclei. These polar nuclei later fuse to form a single diploid secondary nucleus (a central cell).

These events result in a mature **seven celled structure called female gametophyte or embryo sac** consisting of three antipodal cells, one central cell having two polar nuclei, two synergid cells, and one egg cell. **Since, this type of embryo sac develops from a single megaspore and has eight nuclei, it is said to be monosporic eight nucleate embryo sac** or ***Polygonum* type** of embryo sac. Throughout the development, the female gametophyte exhibits a polarity along its chalazal-micropylar axis.

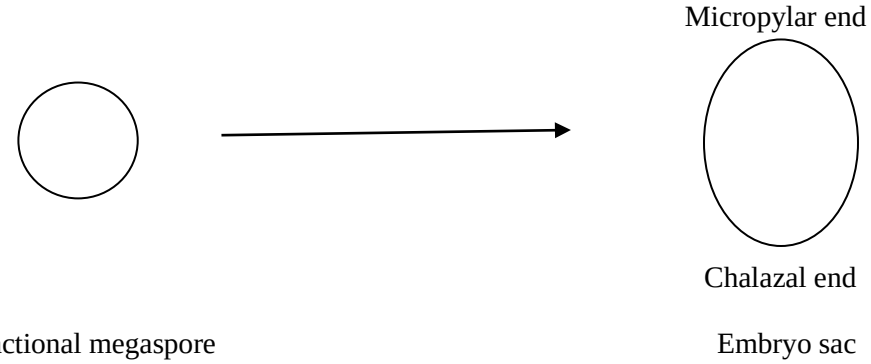
So in a nutshell:

- ***Polygonum* type** is a monosporic eight nucleate, 7-celled embryo sac formed as a result of three divisions in functional megaspore.
- Cells of the egg apparatus and the antipodal cells are uninucleate and haploids whereas the central cell is binucleate or diploid.

It is the most common type of embryo sac and is found in about 81% flowering plants.

Steps of development of the female gametophyte or embryo sac with particular reference to *Polygonum* type:

The development of embryo sac begins as the functional megaspore elongates. You know that in most cases the lowermost megaspore (chalazal) of the linear tetrad becomes functional and rest three degenerates. The elongation is largely along the micropylar-chalazal axis.



The first nuclear division (post- meiotic mitosis) in the megaspore is not followed by wall formation. A large central vacuole appears between the two daughter nuclei. Initially there is no vacuole in the cytoplasm of the megaspore but later small vacuoles appear which may fuse to form large vacuole. As the vacuole expands, the nuclei pushed toward opposite poles of the cell. In this way each pole has one nucleus. Now both the nuclei divide twice, forming four nuclei at each pole. All the divisions are mitotic and without wall formation.

Dear students you can understand that at this stage all the eight nuclei are present in the common cytoplasm because after haploid megaspore formation all the nuclear divisions are not followed by cell wall formation. After the last nuclear division (when there are eight nuclei) the cell undergoes appreciable elongation, so that it looks like sac. The picture must be clear in your mind that out of these eight nuclei, four are at the micropylar end and four at the chalazal end (Fig. 6.11).

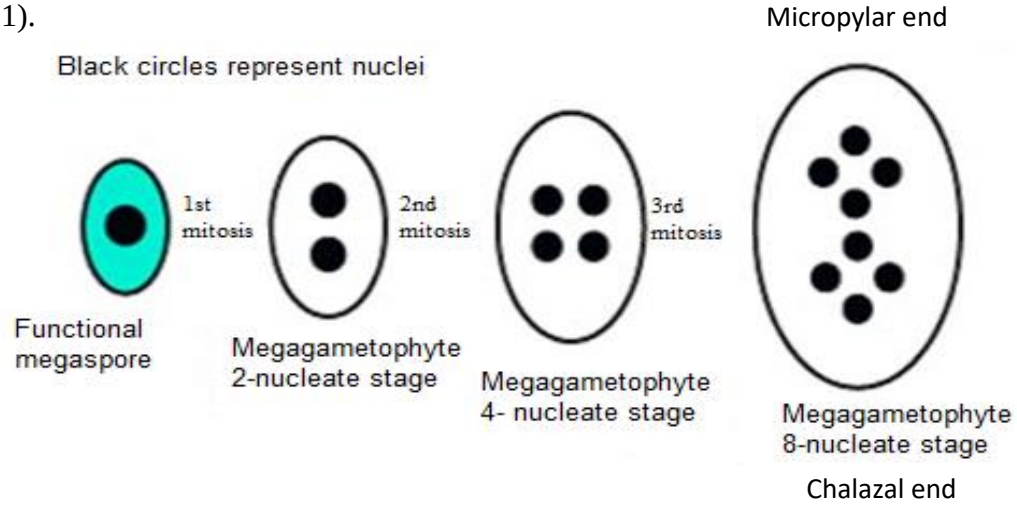


Fig. 6.11: Development of embryo sac up to 8-nucleate stage

Finally the embryo sac becomes organized. Three nuclei at the micropylar end of the embryo sac organize into **egg apparatus** and the fourth one is left free in the cytoplasm of the central cell and moves toward the centre as the upper polar nucleus. The egg apparatus (only upper portion) is attached to the wall of the embryo sac at the micropylar end, its major portion is surrounded by the central cell. In the **egg apparatus** the centre cell is **egg cell (round, also known as ovum or oosphere)** and the rest two side cells are **synergids** which are flask shaped. Only the synergid cells are in direct contact with the wall of the embryo sac. The centre egg cell is situated in such a way so that its upper portion is slightly below the apices of the synergids seems hanging between and below them. All the three cells are of same length therefore the egg cell extends a little more towards the centre cell in comparison to the synergids. Out of the four nuclei at the chalazal end of the embryo sac, three nuclei forms three **antipodal cells** and the fourth one moves toward the centre as the lower polar nucleus.

You have read that one nucleus from each pole moves to the center of the embryo sac, here they may fuse forming the **fusion or secondary nucleus**. The secondary nucleus (if fusion occurred between two polar nuclei) or two polar nuclei (if there is no fusion) remains at the center. (Fig. 6.12, 6.13).

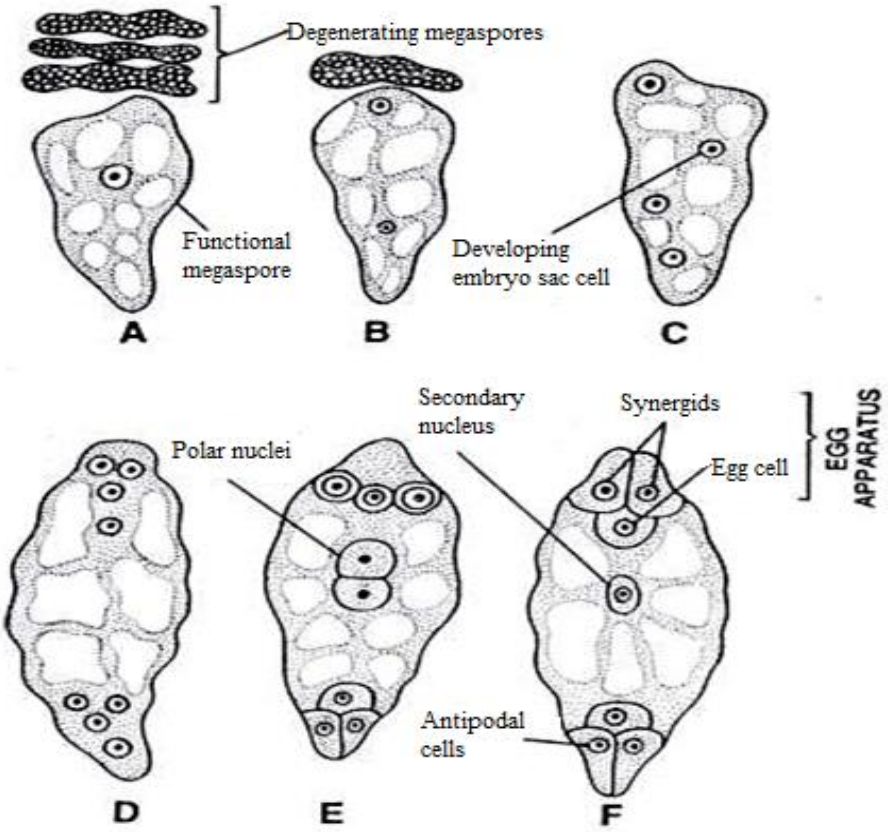


Fig. 6.12: Female gametophyte. A-F: development of the embryo sac (Female gametophyte) of normal type (*Polygonum* type)

- The female gametophyte (embryo sac) is 7-celled (mostly), 8-nucleate structure having **three cells** of egg apparatus (two synergid cells and one egg cell) at the micropylar end, **three cells** (antipodal cells) at the chalazal end and **one cell** (centre cell) in the centre having two polar nuclei.
- This type of embryo sac is designated as the *Polygonum* type.
- This mode of embryo sac development occurs in the majority of flowering plants. According to Davis (1966), about 81 per cent of the families show *Polygonum* type of embryo sac development.
- Two polar nuclei later fuse to form the secondary nucleus so you can also say that the central cell is binucleate or diploid and the antipodal cells and cells of the egg apparatus are uninucleate and haploid.

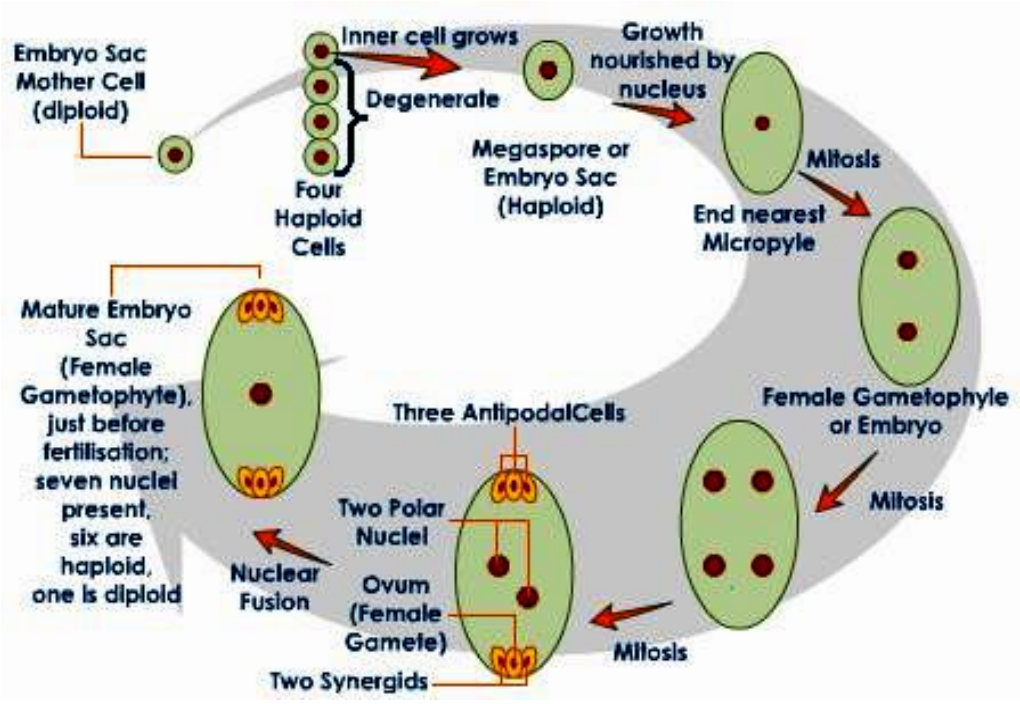


Fig. 6.13: Diagrammatic representation of embryo sac development in Angiosperms

Types of embryo sac

The *Polygonum* type of embryo sac as described above is formed from one of the four haploid megaspore nuclei which in turn formed from diploid megaspore mother cell as a result of meiosis. Although it is the

most common type of mode of embryo sac development in Angiosperms, even there is a substantial number of plants where more than one megaspore nuclei take part in the process.

Therefore **depending on the basis of involvement of number of megaspore nuclei** in its formation, the embryo sac can be of different types:
1. Monosporic
2. Bisporic
3. Tetrasporic

1. Monosporic Embryo sac

Monosporic embryo sac is the one where **only one of the four megaspores** take part in its formation as in the *Polygonum* type. Three of the megaspores, usually those which are at micropylar end, degenerate, leaving only one functional megaspore. In this type of embryo sac all the nuclei are genetically identical because they are formed through mitosis of a single nucleus.

Monosporic embryo sacs are further divided into two types.

1. *Polygonum* type (8 -nucleate)
2. *Oenothera* type (4- nucleate)

1. *Polygonum* type (8-nucleate): As described earlier, it is formed by the chalazal megaspore of the tetrad and is eight nucleate. A mature *Polygonum* type of embryo sac comprises a 3-celled egg apparatus, three antipodal cells and a binucleate central cell (Fig. 6.14).

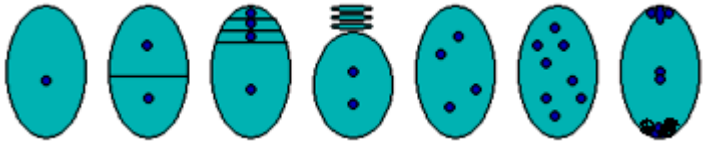


Fig. 6.14: *Polygonum* type embryo sac

This type of embryo sac is the most common and is, therefore, commonly designated as the "Normal type." However, it is also designated as the *Polygonum* type because it was reported for the **first time in *Polygonum divaricatum* by Strasburger (1879)**.

2. *Oenothera* type (4-nucleate): *Oenothera* type of embryo sac is derived from the micropylar megaspore of the tetrad and is four nucleate. The mature embryo sac consists of an egg apparatus and a uninucleate central cell. *Oenothera* type of embryo sac is found in Onagraceae family (Fig. 6.15).

Geert (1908) found that in *Oenothera lamarckiana* the embryo sac is usually formed by the micropylar megaspore of the tetrad, which undergoes only two nuclear divisions instead of the usual three occurring in the *Polygonum* type of embryo sac. In this way, 4 nuclei are produced which organize into the two synergids, the egg and a single polar nucleus. Since the third division is omitted and all the nuclei are situated in the micropylar part of the developing embryo sac, there is neither a lower polar nucleus nor any antipodal cells.

Fig. 6.15: *Oenothera* type

6.6 COMPARISON WITH BISPORIC AND TETRASPORIC TYPES

Bisporic embryo sac

In this type of embryo sac as the name indicates **two megaspore nuclei** participate in its formation. After first meiotic division a dyad is formed by wall formation. Only one of the dyad cells undergoes the second meiotic division and the other one, near to micropyle, degenerates. In the functional dyad cell division is not followed by wall formation and so both the megaspore nuclei participate in the formation of the embryo sac. Each megaspore nucleus undergoes two mitotic divisions forming eight nuclei and mature embryo sac has the same organization like that of the *Polygonum* type.

So you can say the bisporic embryo sacs are 8- nucleate and arise from one of the two dyad cells formed after meiosis I.

Tetrad formed as a result of meiosis in MMC, has four genetically different nuclei. **Being a derivative of two meiotic products, nuclei of a bisporic embryo sac are genetically different** (four nuclei are of one type and other four of a different type).

A bisporic embryo sac was **first described in *Allium fistulosum* (Strasburger, 1879)** and has since been confirmed in several species of this genus.

Bisporic embryo sacs are also of two types:
1. *Allium* type
2. *Endymion* type

1. *Allium* type (8-nucleate): In this type chalazal dyad cell participates in the formation of the embryo sac (Fig. 6.16).

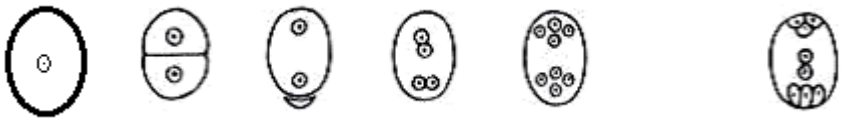


Fig. 6.16: *Allium* type

2. *Endymion* type: In this type micropylar dyad cell participates in the formation of the embryo sac (Fig. 6.17).

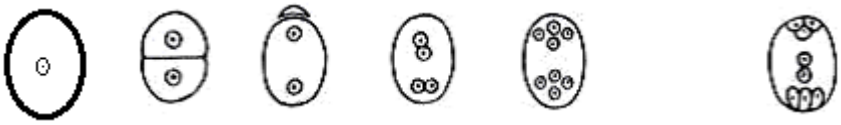


Fig. 6.17: *Endymion* type

Tetrasporic embryo sac

When **four megaspores take part** in the formation of the embryo sac, it is called **tetrasporic embryo sac**. In this type neither of the meiotic division (first as well as second) is accompanied by wall formation so that at the end of meiosis all the four haploid nuclei remain in a common cytoplasm forming a **coenomegaspore (four nuclei inside a cell)** and all the four nuclei of the coenomegaspore take part in the formation of embryo sac. This type of embryo sac is **more heterogenous than a bisporic one because all the four nuclei of the coenomegaspore, products of meiosis are genetically different.**

***Peperomia* type:** Campbell (1899a, b, 1901) and Johnson (1900) reported that in *Peperomia pellucida* each of the 4 megaspore nuclei divides twice, resulting in a total of 16 nuclei which become more or less uniformly distributed in the rather thick layer of cytoplasm lying at the periphery of the embryo sac (Fig. 6.18).

Fig. 6.18: *Peperomia* type

According to P. Maheshwari (1950), they are of 7 types, which are as follows:

1. *Adoxa* type
2. *Penaea* type
3. *Plumbago* type
4. *Peperomia* type

5. *Drusa* type
6. *Fritillaria* type
7. *Plumbagella* type

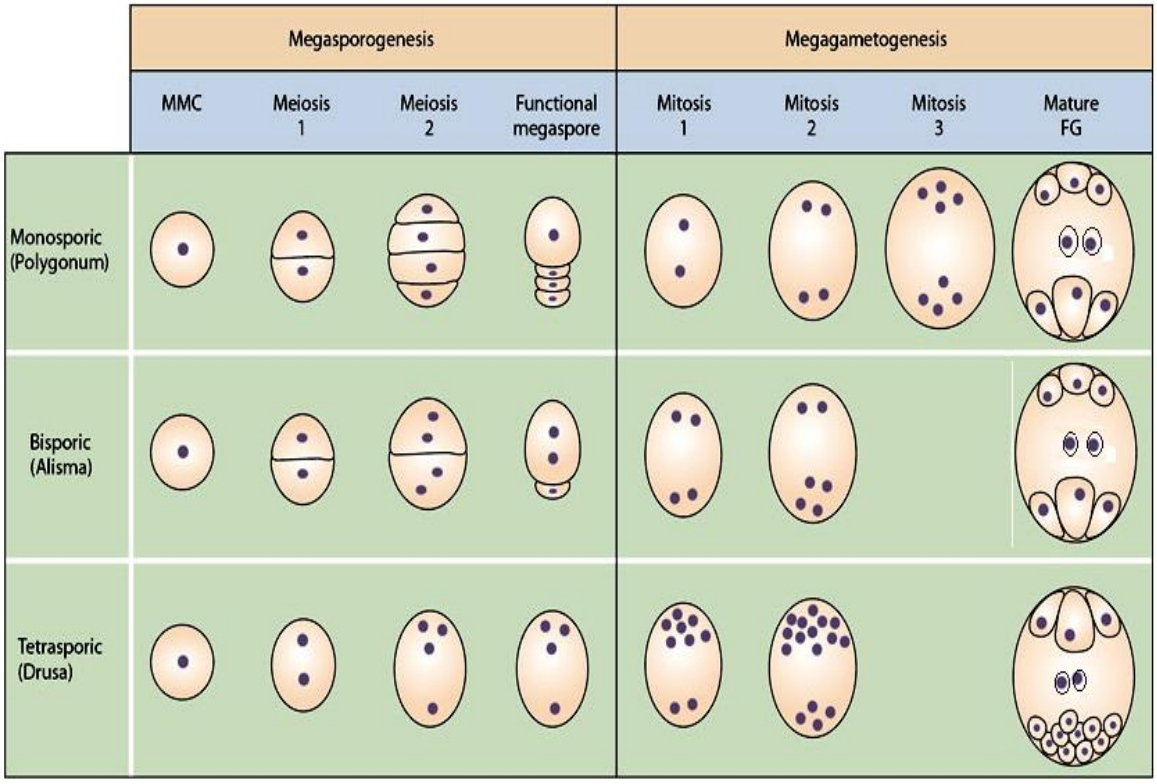


Fig.6.19: Diagrammatic representation of three main types of embryo sac development: monosporic, bisporic, tetrasporic

- Monosporic embryo sac: embryo sac developed from one functional megaspore.
- Bisporic embryo sac: embryo sac developed from two functional megaspore nuclei.
- Tetrasporic embryo sac: MMC by meiosis forms four haploid daughter nuclei. No wall formation is there between these four nuclei and all four nuclei participate in the formation of embryo sac.
- All three patterns give rise to a single functional megaspore that contains either one (monosporic), two (bisporic), or four (tetrasporic) haploid nuclei.
- The nuclei of the bisporic and tetrasporic embryo sacs are not genetically identical as they are in monosporic embryo sacs, because they arise from two or four different meiotic products.

Organisation of mature embryo sac

The egg apparatus: It is composed of an egg and two synergids. Usually the synergids are ephemeral structures which degenerate and disappear soon after fertilization or even before it.
The Antipodals: There are three antipodal cells.

Polar nuclei: The central portion of the embryo sac containing the polar nuclei eventually gives rise to the endosperm and has therefore been called the "endosperm mother cell." The fusion of the polar nuclei may occur either before, or during, or sometimes after, the entry of the pollen tube in the embryo sac. The secondary nucleus formed after fusion usually lies just below the egg and is separated from the antipodal cells by a large vacuole.

Pollination

Dear students after reading male gametophyte unit and this unit about female gametophyte, you should have learnt by now that male gamete is contained within the microspore which develops as male gametophyte while the female gamete (egg) is contained within the female gametophyte which is the embryo sac develops within the megaspore and is located within the megasporangium or ovule. So the next biological phase is **pollination.**

You are also familiar about the term pollination which means **the transfer of the pollen from the anther to the receptive stigma whether of the same flower or of a different flower.**

Based on the destination of pollen grains, two types of pollinations are there:

(1) **Self-pollination:** If the pollen is transferred from anther to the stigma of the same flower, it is called self pollination or **autogamy** as in pea, wheat and rice.

When the pollen of one flower pollinates the stigma of different flower, but on the same plant, it is called **geitonogamy.**

(2) **Cross-pollination:** If the pollen is transferred from anther to the stigma of the another flower, it is called cross pollination or **allogamy** as in hemp and willow.

Cross pollination within a species (may be inter-varietal) is termed as **xenogamy.**

For self pollination flower must be bisexual (hermaphrodite) and only those bisexual flower which achieve anther dehiscence and receptivity of stigma simultaneously means when anther releases pollen grains then stigma should be ready to receive them. For cross pollination flowers are mostly unisexual.

Pollination leads to fertilization and production of seeds and fruits which ensure continuity of plant life.

Agents for pollination

Pollination process can occur by different agencies which can be classified into two categories:

1. Abiotic such as wind (anemophily or anemophilous) and water (hydrophily or hydrophilous) and
2. Biotic such as insects (entomophily or entomophilous), birds (ornithophily or ornithophilous), and bats (cheiropterophily or cheiropterophilous)

Anemophily or anemogamy: Here pollinating agent is wind e.g. in most cereals, poplar, willow, alder, elm, oak, beech, *Urtica*.
Hydrophily or Hydrogamy: Here pollinating agent is water e.g. aquatic plants.
Zoophily or Zoogamy: Again divided into entomophily, ornithophily, and chiropteriphily
Entomophily: Pollinating agents are insects as in *Salvia, Ficus,* orchids etc.
Ornithophily: Pollinating agents are birds as in *Bignonia,* silk cotton etc.
Cheiropterophily: Pollinating agents are bats as in *Bauhinia megalandra, Eperua falcata* etc.
Pollination ends in a copious dusting of the stigma surface with pollen grains.

6.7 SUMMARY

In this unit we have discussed the structure of ovule, types of ovule on the basis of the position of the micropyle with respect to the funiculus, as well as on the basis of dependency on the extent of development of the nucellus and on the basis of position of sporogenous cell. Further development of ovule along with megasporogenesis, female gametophyte development with particular reference to *Polygonum* type was described. In addition to this monosporic embryo sac we have also discussed about bisporic and tetrasporic embryo sacs. Pollination and its agents were also discussed in short. Therefore, the whole unit is summarized in the following key points:

- Gametophyte is the haploid generation producing gametes in plants.
- When we are talking about female then it is said to be female gametophyte.
- The female gametophyte (embryo sac) develops within the ovule which is found within the ovary.
- A carpel consisting of a basal swollen ovary bearing one or more ovules, a receptive stigma, and often a stalk-like style between them.
- Ovule consists of nucellus surrounded by integuments.
- Ovule, on the basis of the position of the micropyle with respect to the funiculus, is of 5 types - Orthotropous, Anatropous, Campylotropous, Amphitropous, Hemianatropous.
- Ovule depending on the extent of development of the nucellus and on the basis of position of sporogenous cell, is of 2 types - tenuinucellate and crassinucellate.
- Female gametophyte (embryo sac) located in the nucellus, developed from megaspore.

- Female gametophyte development occurs in two phases - megasporogenesis and megagametogenesis
- The process of development of the megaspores is termed megasporogenesis.
- The female spores or megaspores are developed from MMC by reduction division or meiosis within the megasporangium (ovule).
- Each mother cell undergoes meiosis or reduction division to form four megaspores.
- Out of four megaspores, only one becomes functional.
- The functional megaspore now forms the female gametophyte (embryo sac).
- So megaspore is the first cell (mother cell) of the female gametophyte.
- On the basis of wall formation after meiotic divisions, Angiosperms exhibit three main patterns of megasporogenesis, referred to as monosporic, bisporic, and tetrasporic.
- Development of the female gametophyte is completely endosporous means within the megaspore.
- During megagametogenesis, the functional megaspore gives rise to the mature female gametophyte.
- Female gametophyte or embryo sac develops from a single megaspore and has eight nuclei, it is said to be monosporic-8-nucleate embryo sac or *Polygonum* type of embryo sac.
- Normal type (*Polygonum* type) of mature embryo sac has 3 antipodal cells at chalazal end, an egg apparatus (one egg cell and two synergids) at micropylar end and two polar nuclei in the centre (total 8 nuclei).
- Two polar nuclei later fuse to form secondary nucleus.
- The central cell is binucleate or diploid and the antipodal cells and cells of the egg apparatus are uninucleate and haploid.
- Depending on the basis of involvement of number of megaspore nuclei in its formation, the embryo sac can be of 3 types- monosporic, bisporic and tetrasporic.
- The nuclei of the bisporic and tetrasporic embryo sacs are not genetically identical as they are in monosporic embryo sacs, because they arise from two or four different meiotic products.
- After development of male and female gametophyte, the next biological phase is pollination, which is must for fertilization.
- Pollination means the transfer of the pollen from the anther to the receptive stigma whether of the same flower (self-pollination) or of a different flower (cross pollination).
- Abiotic (wind and water) and biotic (insects, birds and bats) agents are responsible for pollination.
- On the basis of these agents pollination may be anemophilous (by wind), hydrophilous (by water), entomophilous (by insects), ornithophilous (by birds) and chiropteriphilous (by bats).
- Pollination ends in a copious dusting of the stigma surface with pollen grains.

6.8 GLOSSARY

Bitegmic ovule: Ovule with two integuments
Chalaza: Basal part of the ovule where integument and nucellus connect to the funiculus
Crassinucellate ovule: Where the sporogenous cell becomes subhypodermal, either due to formation of parietal cells, or due to divisions in the nucellar epidermis, or both
Embryo sac: Female gametophyte located in the nucellus, developed from megaspore
Funiculus (Funicle): A stalk of the ovule by which it remains attached to the placenta
Hilum: Region where ovule fuses with funiculus
Integument: The protective covering of nucellus
Megagametogenesis : Development of the mature female gametophyte from functional megaspore
Megasporogenesis: Development of the megaspore within the ovule
Micropyle: Small opening formed by two integuments over nucellus
Nucellus: The body of ovule
Placenta: The part of the carpellary tissue to which the ovules are attached
Placentation: The distribution of ovules in the ovary
Synergids : The cells present on either side of egg cell in mature embryo
Tenuinucellate ovule: Where the sporogenous cell is hypodermal and the nucellar tissue around it remains single layered
Unitegmic ovule: Ovule with a single integument

6.9 SELF ASSESSMENT QUESTIONS

6.9.1 Multiple choice questions:

1. Ovule is also known as:
(a) Megasporangium (b) Microsporangium
(c) Embryo sac (d) Endosperm

2. Stalk by which ovule is attached to the placenta is:
(a) Hilum (b) Funiculus
(c) Style (d) None

3. Another name for female gametophyte is :
(a) Megasporangium (b) Embryo sac
(c) Endosperm (d) Nucellus

4. Orthotropous ovule is also known as :

(a) Hemitropous (b) Anatropous
(c) Orthotropous (d) Atropous

5. Tenuinucellate and crassinucellate are the types of:
(a) Spores (b) Tetrad
(c) Ovule (d) Endosperm

6. Linear tetrad is formed by two meiotic divisions in MMC. Both the divisions are:
(a) First division is transverse, second is vertical
(b) First division is vertical, second is transverse
(c) First division is transverse, second is also transverse
(d) May be any type.

7. Which cell in the embryo sac represent the female gamete:
(a) Egg cell (b) Egg apparatus
(c) Synergids (d) Polar nuclei

8. Female gametophyte is 7-celled in 8 nucleate structure in:
(a) *Peperomia* type (b) *Polygonum* type
(c) *Oenothera* type (d) None of these

9. Secondary nucleus in the embryo sac is:
(a) Triploid (b) Haploid
(c) Diploid (d) Absent

10. The product of fusion of two polar nuclei is:
(a) Zygote (b) Oosphere
(c) Embryo sac (d) Secondary nucleus

11. Which one of the following is the example of monosporic type of embryo sac:
(a) *Polygonum* type (b) *Allium* type
(c) *Endymion* type (d) *Peperomia* type

12. Which one of the following is the example of bisporic type of embryo sac
(a) *Polygonum* type (b) *Allium* type
(c) *Oenothera* type (d) *Peperomia* type

13. Which one of the following is the example of tetrasporic type of embryo sac:
(a) *Polygonum* type (b) *Allium* type

(c) *Endymion* type (d) *Peperomia* type

6.9.1: Answer key:

1. (a), 2.(b), 3.(b), 4.(d), 5.(c), 6.(c), 7.(a), 8.(b), 9.(c), 10.(d), 11.(a),12.(b), 13.(d)

6.9.2: Short answer type questions with answer:

1. Name the first cell of female gametophyte.
Ans: A haploid megaspore is known as the first cell (mother cell) of female gametophyte.

2. Name the last cell of female sporophyte.
Ans: Diploid megaspore mother cell

3. What type of female gametophyte development is reported in Angiosperms?
Ans: In Angiosperms the development of the female gametophyte is completely **endosporous** means within the megaspore.

4. What do you understand by tenuinucellate type and crassinucellate type of ovule?
Ans: In tenuinucellate type of ovule, the archesporial cell directly functions as megaspore mother cell (MMC) and in crassinucellate type of ovule the archesporial cell do not directly behave as MMC and instead of that it divides periclinally into two cells. An outer primary parietal cell (towards epidermis) and an inner primary sporogenous cell. Now this primary sporogenous cell functions as the megaspore mother cell.

5. What is megasporocyte?
Ans: Megaspore mother cell is also known as megasporocyte having diploid (2n) chromosome number

6. What is megasporogenesis?
Ans: Development of the megaspore within the ovule (megasporangium) is known as megasporogenesis.

7. How many types of ovule are there? On what basis they are classified?
Ans: Mature ovule can be classified into five main types. The classification is based on the position of the micropyle with respect to the funiculus.

8. What is the main criteria for classifying embryo sac?
Ans: Depending on the basis of involvement of number of megaspore nuclei in its formation, the embryo sac can be of different types:

9. Why the 7-celled embryo sac is called *Polygonum* type?
Ans: Normal type of embryo sac is also designated as the *Polygonum* type because it was reported for the first time in *Polygonum divaricatum* by Strasburger (1879).

10. Name the patterns of megasporogenesis in Angiosperms.
Ans. Angiosperms exhibit three main patterns of megasporogenesis, referred to as monosporic, bisporic, and tetrasporic.

6.10 REFERENCES

- Gifford, E.M., and Foster, A.S. (1989). *Morphology and Evolution of Vascular Plants*. W.H. Freeman, New York.
- McCormick, S. (1993). Male gametophyte development. *Plant Cell* 5: 1265–1275.
- McCormick, S. (2004). Control of male gametophyte development. *Plant Cell* 16.
- Maheshwari, P. (1950). *An Introduction to the Embryology of Angiosperms.* McGraw-Hill. New York.
- Strasburger, E. (1879). Die Angiospermen und die Gymnospermen. (Jena, Germany: Fischer).
- Yadegari R and Drews G. N. (2004). Female gametophyte development. *Plant Cell*. DOI 10.1105/tpc.018192

6.11 SUGGESTED READINGS

- Singh, V., Pande, P.C. and Jain, D.K (2008). *A Text Book of Botany.* Rastogi Publications, Meerut.
- Maheshwari, P. (1950). *An Introduction to the Embryology of Angiosperms.* McGraw- Hill, New York.
- Gangulee, H.C., Das, K.S. and Datta, C. (1998). *College Botany*. Vol. I. New Central Book Agency, Kolkata.
- *Developmental Biology.* 6th edition. Gilbert S.F.Sunderland (MA): Sinauer Associates; Bookshelf ID: NBK9980 (2000).
- Pandey, S.N. (1997). *Plant Anatomy and Embryology.* Vikas Publishing House Pvt Ltd, New Delhi.
- *Plant Physiology and Development,* 6th edition, Lincoln Taiz, Eduardo Zeiger, Ian Max Moller, Angus Murphy. 2015 P 761.ISBN: 978-1-60535-255-8
- Singh, V., Pande, P.C. and Jain, D.K (2012-13). *Structure Development and Reproduction in Angiosperm.* Rastogi Publications, Meerut.

- Bhojwani, S.S., Bhatnagar, S.P. and Dantu, P. K. (2015). *The Embryology of Angiosperms.* Vikas Publishing House Pvt Ltd, New Delhi.

6.12 TERMINAL QUESTIONS

1. Describe the different types of ovule.
2. Explain the patterns of megasporogenesis and megagametogenesis in Angiosperms.
3. Compare monosporic embryo sac with bisporic and tetrasporic embryo sac.
4. "Agents of pollination", throw light on this sentence.

UNIT-7 FERTILIZATION AND POST FERTILIZATION

7.1 OBJECTIVES

After reading this unit, students will be able to understand:

- What is fertilization?
- About the different ways of entry of pollen tube into the ovule.
- What is syngamy?
- What do you understand by triple fusion?
- In Angiosperms double fertilization is a unique phenomenon. Students will clearly understand about double fertilization.
- What comes in post fertilization developments?
- What is endosperm? How it forms and on what basis endosperm categorized into different types?
- How endospermic seeds differ from non-endospermic seeds?
- Define embryo?
- While going through the chapter, students will encounter the development of dicotyledonous and monocotyledonous embryo.
- Also become familiar with terms like apomixis, apogamy, apospory, parthenogenesis etc.
- What is polyembryony and on what basis it can be classified into different types?
- Define parthenocarpy.

7.2 INTRODUCTION

By going through the male gametophyte and female gametophyte units, it is now clear that gametophytic generation is haploid. The first male gametophytic cell is microspore or pollen grain. The first female gametophytic cell is functional megaspore. The pollen grains are liberated at the 2-celled or 3-celled stage. Female gametophyte is also known as embryo sac and in most of the species it is of *Polygonum* type. After development of male and female gametophyte, the next biological phase is pollination, which is must for fertilization. Pollination ends in a copious dusting of the stigma surface with pollen grains (Fig.7.1).

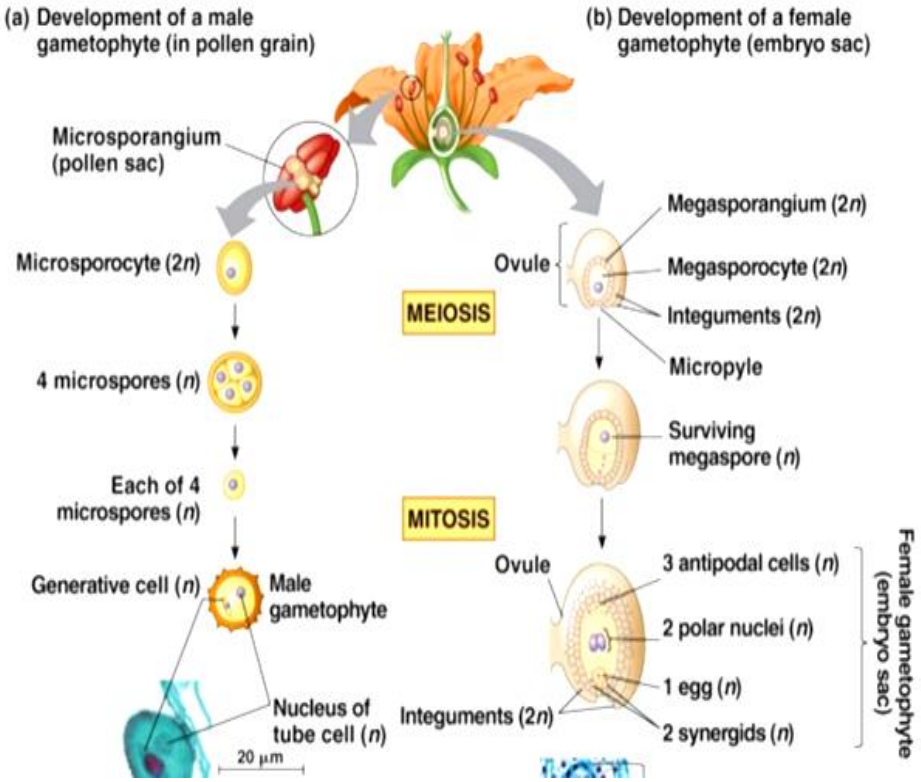


Fig. 7.1: Showing development of male and female gametophyte

In this unit we will discuss about fertilization and post fertilization developments along with some very important phenomena occurring in the life cycle of Angiosperm plants i.e. apomixis, adventive embryony, polyembryony and parthenocarpy.

The capacity to reproduce is one of the most important characteristics of life and is aimed to sustain the individual species. Reproduction methods are mainly of two types- asexual and sexual. In flowering plants sexual method of reproduction requires fusion of two gametes, one from male organ and other from female organ of the plant. The product of the fusion of two different gametes is zygote and this fusion process is known as fertilization.

In Angiosperms fertilization initiates with the compatible type pollen (male gametophyte) reaching the stigma and ends with the fusion of male and female gametes in the embryo sac (female gametophyte). The pollens received by the female reproductive organ i.e. gynoecium are held at the stigma.

There is no such way by which the pollen (male gamete) can reach to the egg (female gamete) in the embryo sac. So to overcome this difficulty pollen germinate on the stigma and forms pollen tube which penetrate the stigmatic tissue, grows down the style, enters the ovary and finally finds

its way into the embryo sac (female gametophyte) through ovule. Here it releases two sperms (male gametes) in the vicinity of the female gametes. Out of the two sperms, one fuses with the egg (syngamy) and forms zygote. The other one fuses with the polar nuclei or the secondary nucleus (triple fusion) and forms primary endosperm nucleus. This phenomenon is known as double fertilization and is a characteristic unique feature of the Angiosperms.

After series of divisions primary endosperm nucleus forms endosperm. Endosperm is very nutritive tissue that nourishes the developing embryo. Zygote or oospore forms embryo, either dicotyledonous or monocotyledonous embryo, as the case may be.

Terms to remember:
Apical cell = terminal cell = (also known as embryo cell)
Basal cell = also known as suspensor cell
Meiosis = reduction division
Syngamy = fertilization
Sexual cycle = amphimixis
Asexual cycle = apomixis
Sperms = male gamete
Egg = female gamete
Vegetative cell = tube cell
Embryo sac = female gametophyte
Zygote = oospore = fertilized egg
Gynoecium = pistil

7.3 FERTILIZATION

"Fertilization is the process of fusion of two dissimilar reproductive units, called gametes."

In flowering plants, the process of fertilization was first discovered by Strasburger in 1884. As described in Unit 6, the female gametophyte (embryo sac) of Angiosperms is situated in the ovule, at a distance from the stigma. There is no such device developed in the gynoecium (pistil) which facilitates transfer of pollen from stigma to embryo sac. Therefore the pollen after reaching to the stigma produces a pollen tube which facilitates transport of male gametes deep into the embryo sac from stigma.

In Angiosperms, the fertilization is being completed as follows:

Germination of pollen grains and growth of pollen tube

When the pollen is shed from anther it has usually two cells:

1. A generative cell
2. A vegetative cell (tube cell)

The generative cell forms two male gametes. Once the pollen has landed on compatible receptive stigma as a result of pollination, its germination starts. On the surface of stigma the pollen hydrates. This means pollen absorbs water from the surrounding and swells. After that the vegetative cell forms a pollen tube. The stigmatic fluid secreted by the stigma contains sugars, lipids and resins, etc. which provides suitable medium for the germination of pollen grains. Pollen grains as well as pollen tube contain an enzyme cutinase which helps in the penetration of pollen tube into the stigmatic tissue. Cutinase as the name indicates degrades the cutin of the stigma at the point of contact with the pollen tube. The entire content of the pollen including two male gametes of generative cell move into the pollen tube (Fig. 7.2).

The growing pollen tube penetrates the stigmatic tissue and pushes its way through the style and then down the wall of the ovary. The style may be hollow or solid. If it is hollow, then the pollen tube grows along the epidermal surface but in case of solid style, the pollen tube travels through intercellular spaces between the cells which lie in its path.

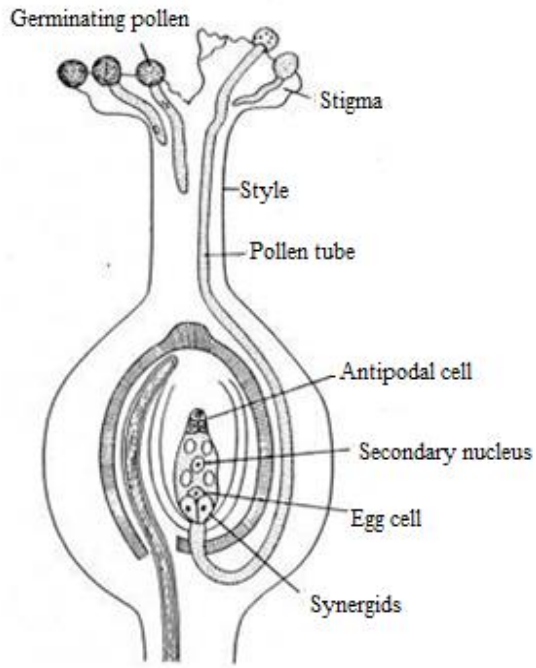


Fig. 7.2: Longitudinal section of a flower showing growth of pollen tube

Entry of pollen tube into ovule

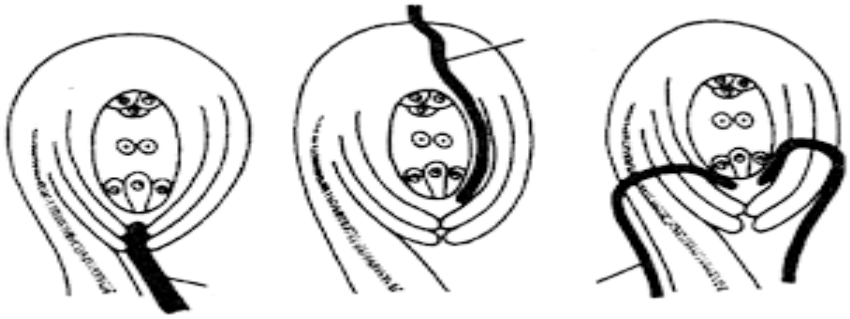
After arriving in the ovary, the pollen tube finds its way into the ovule. The pollen tube may enter into the ovule via three routes.

1. through the micropyle
2. through the chalazal end
3. through the integument

On that basis of modes of entry of pollen tube into the ovule, three terms are given as follows:
1. Porogamy: When the pollen tube enters the ovule through the micropyle, the condition is known as **porogamy**. This is the most common mode of pollen tube entry into the ovule and so the most common type of fertilization.

2. Chalazogamy: When the pollen tube enters the ovule through the chalazal end, the condition is known as **chalazogamy**. This type of pollen tube entry into the ovule and so the type of fertilization is observed in *Casuarina, Betula* and *Juglans regia*. The chalagogamy was first reported by Treub (1891) in *Casuarina*.

3. Mesogamy: When the pollen tube enters the ovule through the integument or through the funiculus, the condition is known as **mesogamy**. This type of pollen tube entry into the ovule and so the type of fertilization is observed in *Cucurbita* (through the integument), and *Pistacia* (through the funiculus).



Porogamy Chalazogamy Mesogamy

Fig. 7.3: Modes of entry of pollen tube into the ovule

Therefore depending on the place of pollen tube entry into the ovule, fertilization may also be called of three types:
1. Porogamous
2. Chalazogamous
3. Mesogamous

Entry of pollen tube into the embryo sac

It does not matter through which way pollen tube enters into the ovule; it always enters in the embryo sac from the micropylar end means **entry of pollen tube in the embryo sac is irrespective of pollen tube entry into the ovule** (Fig. 7.3).

Again the entry of pollen tube into the embryo sac after passing micropyle may be via different passages. It may be:
(i) between the egg cell and one of the synergids e.g. *Fagopyrum*
(ii) between the wall of the embryo sac and one or other synergids.er. *Cardiospermum*
(iii) directly penetrates one of the synergids e.g. *Oxalis*

So we can say that synergids not only play an important role in determining the entry of pollen tube in the embryo sac but they also affect dissemination of male gametes in the embryo sac.

Discharge of male gametes from pollen tube

After reaching the embryo sac the pollen tube burst at its tip and deliver the (two) male gametes. Just prior to bursting of pollen tube the tube nucleus disorganizes. Immediately after releasing, the male gametes show amoeboid movement and one male gamete moves toward the egg and other one move to the polar nuclei (Fig. 7.4).

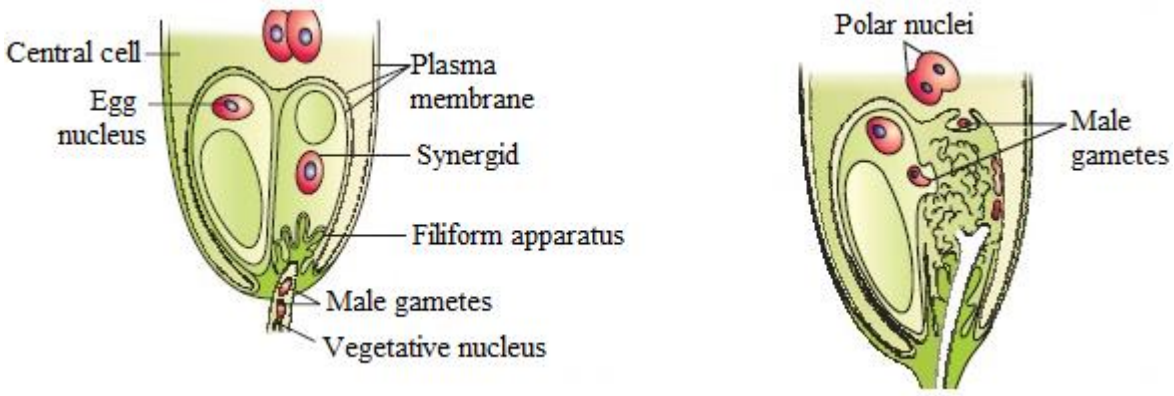


Fig.7.4: A: Enlarge view of an egg apparatus showing path of pollen tube growth; B: discharge of male gametes into a synergid and the movement of the male gametes, one into the egg and the other into the central cell

Syngamy- fusion of gametes

As the one of the male gametes reached the egg, it fuses with it. As a result of this fusion diploid zygote/oospore (2n) forms (because you know the egg and the male gamete, both are haploid). The fusion of male and female gametes is known as **fertilization**. This is also known as **syngamy**.

One of the most significant discoveries was made by **Strasburger in 1884,** as mentioned above. He observed the actual fusion of the male gamete with the female gamete (egg) in *Monotropa.*

Since two male gametes are released by the pollen tube, what happened to the second male gamete? The answer was provided by **S. G.Nawaschin (1898).** He showed that the one male

gamete fused with the egg (syngamy) and the other male gamete with the polar nuclei (triple fusion) while working with *Fritillaria* and *Lilium.* So this was the discovery of double fertilization.

Triple fusion

The other male gamete fuses with the two polar nuclei (or secondary nucleus, if the two have already fused) and so forms triple fusion nucleus (3n), called **primary endosperm nucleus** (Fig. 7.5).

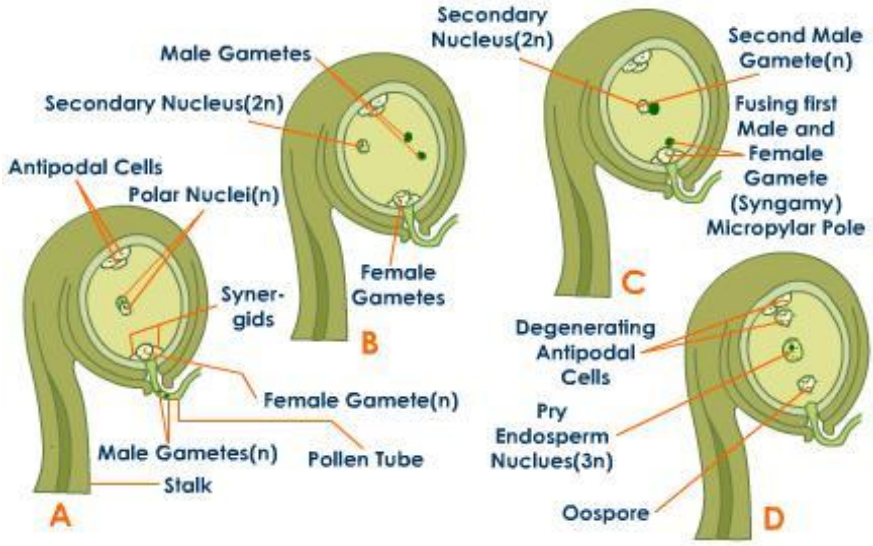


Fig.7.5: Showing syngamy and triple fusion

Double fertilization

Thus in an embryo sac two sexual fusions occur; one is **syngamy** (i.e. fusion of one male gamete with the egg) and another is **triple fusion** (i.e. fusion of other male gamete with the polar nuclei or secondary nucleus), and therefore, the phenomenon is known as **double fertilization** (Fig. 7.6).

As a result of first fertilization the zygote or **oospore** cell is formed which is the **mother cell of the embryo** and is a diploid cell containing 2n complement of the chromosomes. The nucleus of the triple fusion product (**primary endosperm nucleus**) is triploid or 3n. This is the first nucleus of the endosperm.

Double fertilization is a very unique phenomenon in Angiosperms and **discovered for the first time by S.G. Nawaschin (1898) in *Lilium* and *Fritillaria* species** as described above.

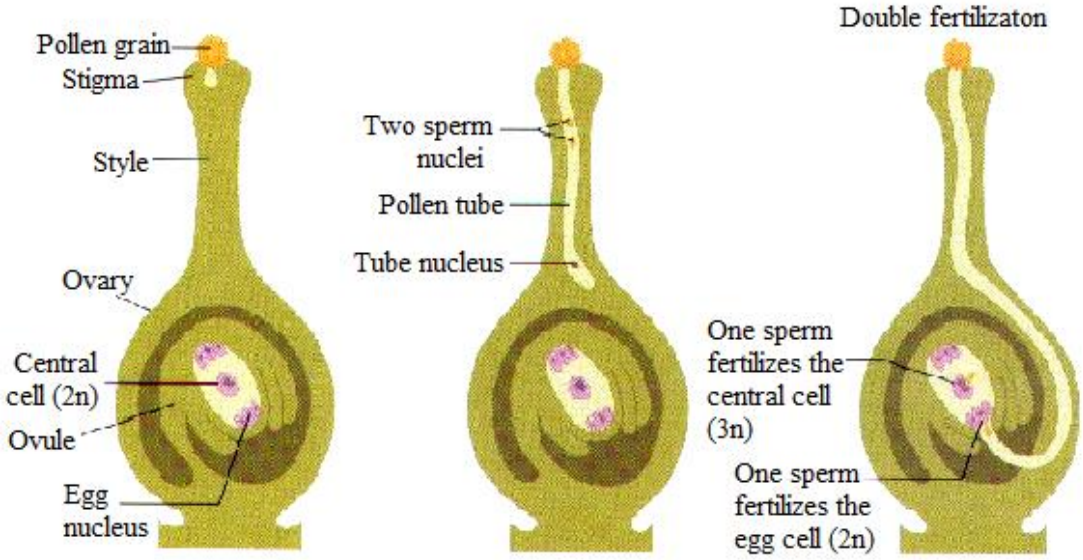


Fig.7.6: Process of double fertilization

7.4 POST FERTILIZATION DEVELOPMENTS

After fertilization, development of the **embryo** and the **endosperm** within the ovule goes side by side. The **oospore (zygote),** formed as a result of fusion of one male gamete with the egg, **develops into the embryo** while the **primary endosperm nucleus-** product of triple fusion, **develops the endosperm**. The other nuclei or cells within the embryo sac (synergids, antipodal cells) disorganize sooner or later.

Development of the Endosperm

The primary endosperm nucleus is a product of triple fusion. This undergoes a series of divisions and ultimately forms **endosperm.** The Angiospermic endosperm is a triploid (3n) tissue as it is a **product of triple fusion.** It is formed either by the fusion of one haploid male gamete and one diploid secondary nucleus (fusion product of two haploid polar nuclei) or by the fusion of three haploid nuclei (one male gamete belongs to male gametophyte and two polar nuclei belongs to the female gametophyte).

It is therefore **distinct from the endosperm of heterosporous Pteridophytes and Gymnosperms** where the endosperm is a simple haploid (n) tissue of the gametophyte not involving any triple fusion like in Angiosperms. **Endosperm is a highly nutritive tissue which provides nourishment to the developing embryo.**

In Orchidaceae and Podostemonaceae, the product of double fertilization soon disintegrates and endosperm development is completely suppressed.

Depending upon mode of development three types of endosperm has been recognized:

1. Nuclear endosperm
2. Cellular endosperm
3. Helobial endosperm

Of these nuclear endosperm is the most common type which occurs in about 56% families of Angiosperms. It is followed by cellular endosperm (reported in 25% families of Angiosperms) and then by helobial endosperm (reported in 19% families of Angiosperms).

1. Nuclear endosperm

In this type of endosperm the division of primary endosperm nucleus and number of subsequent nuclear divisions are not accompanied by wall formation and the nuclei thus produced remain free in the cytoplasm of the embryo sac. They remain in the peripheral layer of the cytoplasm surrounding a large central vacuole. Wall formation occurs at later stage around nuclei. The wall formation is mostly centripetal i.e. from the periphery towards the centre and usually begins from the basal periphery e.g. *Arachis hypogea.*

In some cases the central vacuole may not be filled up even in the mature seed. This is seen in the palms. *Cocus nucifera* is the classical example of this type of nuclear endosperm. Development of endosperm in it deserves special mention. The primary endosperm nucleus undergoes a number of free nuclear divisions. Then the embryo sac gets filled with a clear fluid (watery liquid endosperm) in which numerous nuclei float. It is known as **liquid syncytium.** Gradually nuclei start settling at the periphery with the beginning of peripheral cell wall formation. This forms the coconut meat. In mature coconuts the liquid endosperm becomes milky. The watery endosperm of coconut contains growth promoting 'coconut milk factor' and that is why it is used as a nutrient medium in plant tissue culture experiments. Nuclear endosperm is commonly found in polypetalous dicotyledons (Fig. 7.7).

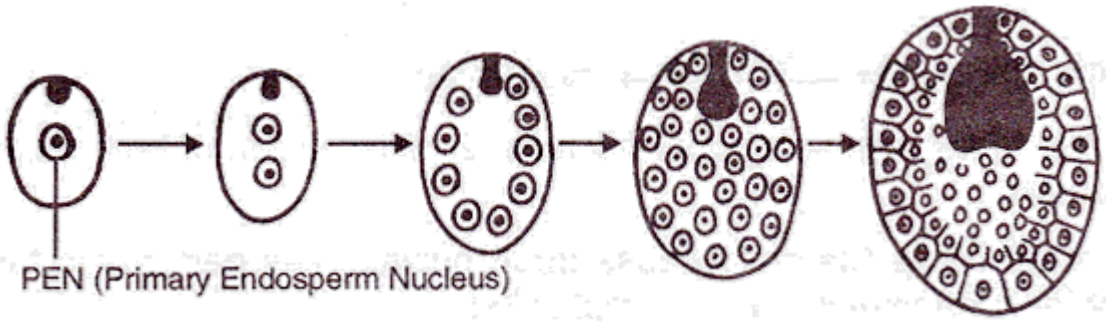


Fig.7.7: Nuclear endosperm formation

2. Cellular endosperm

In this type of endosperm, division of the primary endosperm nucleus is immediately followed by wall formation so that the endosperm is cellular from the beginning. The first wall is laid

down transversely but the subsequent divisions are irregular. *Adoxa, Peperomia, Villarsia* etc. are some common examples. (Fig.7.8).

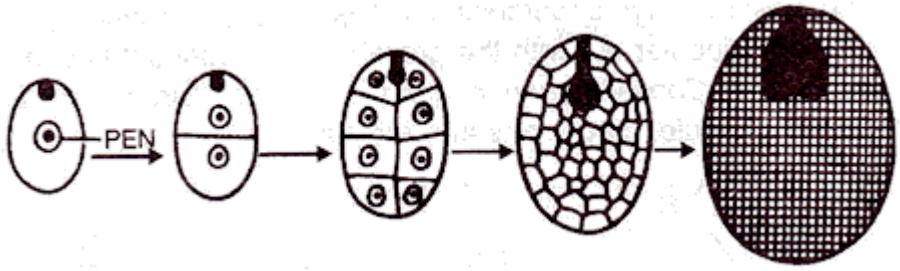


Fig.7.8: Cellular endosperm formation

3. Helobial endosperm

Among members of Helobiales (e.g. *Vallisneria*, *Eremurus*, *Limnophyton* etc.) there is type of endosperm the development which is intermediate between the nuclear and the cellular type. Here the first division of the primary endosperm nucleus is accompanied by the formation of transverse wall. This divides the embryo sac unequally into two compartments - a small chalazal chamber and a large micropylar chamber.

This step is followed by free nuclear division in both the chambers but there are relatively more free nuclear divisions in micropylar chamber in comparison to chalzal one. The chalazal chamber often degenerates. The free nuclear divisions in the micropylar chamber are followed by wall formation and thus a cellular endosperm tissue is formed and usually found in the members of the order Helobiales (Fig.7.9).

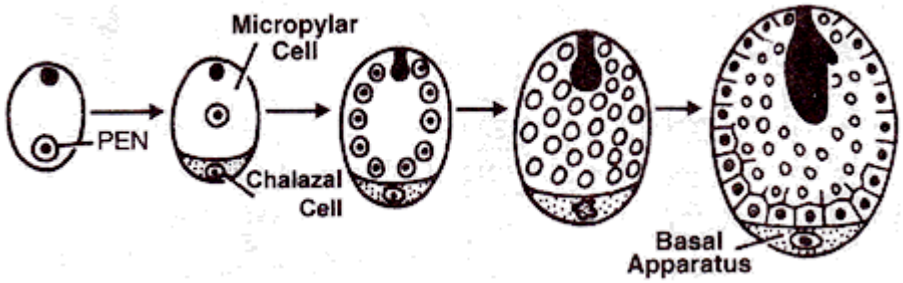


Fig.7.9: Helobial endosperm formation

It is the endosperm, on the basis of which seeds can also be categorized into two categories.

1. Non-endospermic seeds
2. Endospermic seeds

1. Non- endospermic seeds (ex-albuminous seeds)

In plants where the entire endosperm consumed or utilized in the nutrition of the developing embryo, the mature seeds thus formed are without endosperm. Such seeds are termed as non-

endospermic seeds. Example are seeds of beans, peas etc. The non-endospermic seeds store their food material in cotyledons.

2. Endospermic seeds (albuminous seeds)

In plants where the seeds retain endosperm even at maturity and do not consumed or utilized the endosperm completely in the nutrition of the developing embryo. Such seeds are said to be endospermic seeds. Example are seeds of coconut, castor etc. The endosperm present in the seed is utilized after germination in the establishment of young seedlings.

Development of the embryo

After fertilization, a series of changes occurs in the ovule and finally seed is formed. Side by side with the development of the endosperm, the oospore (zygote, the fertilized egg) is developing the embryo after a period of rest.

The process of development of **mature embryo from diploid oospore** is called **embryogenesis.**

Both dicotyledons and monocotyledons begin embryo development in the same way but there is considerable difference in later differentiation. Before proceeding let us discuss about the dicotyledonous and monocotyledonous embryo.

The **dicotyledonous embryo** as the name reflects, has two cotyledons attached laterally to an embryonical axis, whereas in the **monocotyledonous** embryo, the embryonical axis has a single cotyledon at its apex. Due to this organographic difference, it is very easy to distinguish the two types of embryo but there is no fundamental difference in their early stage of development. The development is very similar till the globular stage.

In all Angiosperms the embryogenesis starts with the division in oospore and it divides to develop a two-celled **proembryo** by forming a transverse wall. The cell near the micropyle is termed the **basal cell** and the cell facing towards the centre of the embryo sac is called the **terminal cell**. The basal cell forms the suspensor and may or may not contribute in rest activities so sometimes called as **suspensor cell**, whereas terminal cell is responsible for further development of embryo so called **embryo cell**.

Types of embryo development

On the basis of plane of division of the terminal cell (also known as apical or embryo cell) in the 2-celled proembryo and the contribution of the basal cell and terminal cells in the formation of embryo proper, **six types of embryogeny** (embryo development) have been reported by Johansen (1950) among the Angiosperms.

1. **Onagrad or Crucifer type** (e.g. Annonaceae, Brassicaceae, Onagraceae, Pedaliaceae, Ranunculaceae, Scrophulariaceae).

2. **Asterad type** (e.g. Asteraceae, Balsamianceae, Violaceae, Vitaceae).
3. **Solanad type** (e.g. Campanulaceae, Linaceae, Solanaceae, Theaceae).
4. **Caryophyllad type** (e.g. Caryophyllaceae, Crassulaceae,Haloragaceae).
5. **Chenopodiad type** (e.g. Boraginaceae, Chenopodiaceae).
6. **Piperad type** (e.g. Loranthaceae, Piperaceae).

A. The **terminal cell** of 2-celled proembryo **divides longitudinally:**
(1). The basal cell plays only a minor role or none in the subsequent development of the embryo proper……**Onagrad or Crucifer type**

(2). The basal cell and terminal cell both contribute to the development of embryo proper…… …….. **Asterad type**

B. The **terminal cell** of 2-celled proembryo **divides transversely**
I. The basal cell plays only a minor role or none in the subsequent development of the embryo proper

(3) The basal cell usually forms suspensor……**Solanad type**

(4) The basal cell undergoes no further division and the suspensor, if present, is always derived from the terminal cell…… ….**Caryophyllad type**

II. (5) The basal cell and terminal cell both contribute to the development of embryo proper……..**Chenopodiad type**

These five types of embryogeny are reported in those plants where first division of the oospore (zygote) is transverse forming terminal and basal cell.

Johansen (1950) has also reported a sixth type of embryogeny called **Piperad type**. In this type the first division of the oospore (zygote) is vertical.

- The process of development of **mature embryo from diploid oospore** is called **embryogenesis.**
- In majority of Angiosperm plants, the first division of the zygote is transverse. Rarely it is vertical.
- Six types of embryogeny was reported among Angiosperms by Johansen (1950) - Onagrad or Crucifer type, Asterad type, Solanad type, Caryophyllad type, Chenopodiad type and Piperad type.

Development of dicotyledonous embryo

The classical example is *Capsella bursa-pastoris* (shepherd's purse) of Brassicaceae. The ovule is campylotropous so that the embryo sac and the later developed endosperm as well as embryo are horseshoe-shaped. Here the development of embryo is **Onagrad or Crucifer type.**

1. Z**ygote (oospore) divides transversely**. As a result of this a **two-celled proembryo** is formed.

2. The larger basal cell at the micropylar end is called suspensor cell. The smaller one, away from it termed as terminal cell or embryo cell.

3. The suspensor cell divides transversely a few times to produce a filamentous suspensor of 6-10 cells. The suspensor helps in pushing the embryo in the endosperm.

4. The first cell of the suspensor (towards micropyle) becomes swollen and called **haustorium or vesicular cell**.

5. The last cell of suspensor (towards embryo cell) is known as **hypophysis**. It forms radicle and root cap.

6. The embryo cell undergoes two vertical divisions and one transverse division to form **quadrant** and then **octant stage**. In octant, eight cells arranged in two tiers- epibasal (terminal) and hypobasal (near the suspensor).

The **epibasal cells** eventually form the **two cotyledons and the plumule**. The **hypobasal cells** produce **the hypocotyl**. For this the octant embryo undergoes periclinal divisions producing outer layer of protoderm, procambium and ground meristem.

Protoderm forms epidermis, procambium gives rise to steal or vascular strand and ground meristem produces cortex and pith. It is initially **globular** but with the growth of cotyledons it becomes **heart-shaped** and then assumes the typical shape, e.g., *Capsella bursa-pastoris*.

Structure of Dicot Embryo

The mature embryo consists of an **embryonal axis** having **two cotyledons** (Fig 2.30 H). Embryonal axis above the level of cotyledons forms the plumule (epicotyl) and below the cotyledons, the radical (hypocotyl). Upon germination the plumule forms the shoot and the radical gives rise the root system. The reserve food material in the cotyledons is used in the establishment of young seedlings (Fig. 7.10).

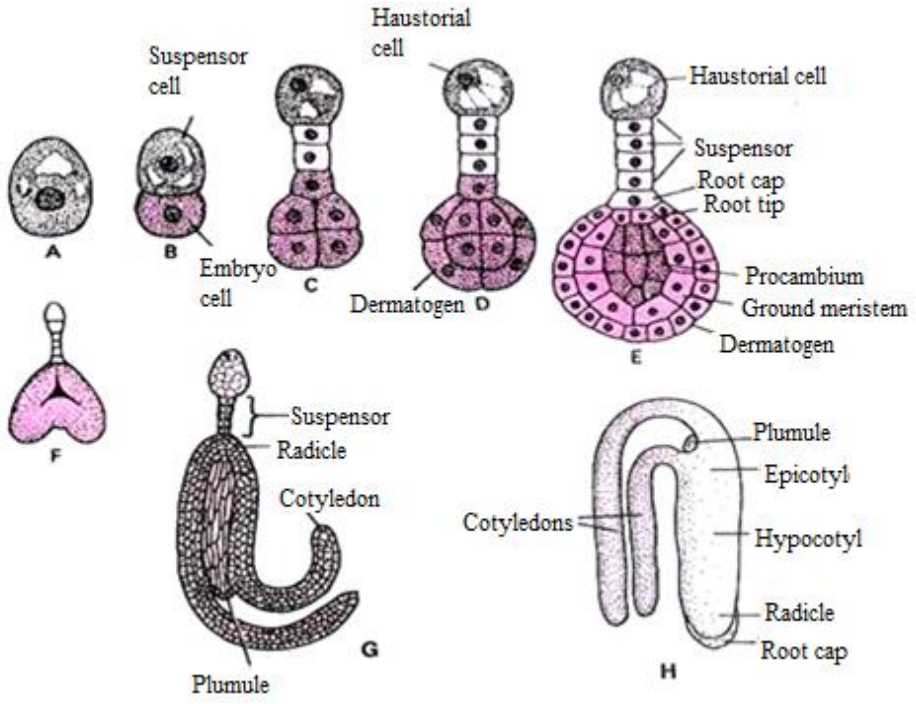


Fig.7.10: Stages in the development of a dicot embryo; A. Zygote or oospore; B. Division of zygote into suspensor and embryo cells; C. Formation of suspensor and embryo octant; D. Pericinal divisions of embryo octants to form outer dermatogens; E. Globular embryo showing regions of radical, procambium, ground meristem and dermatogens; F. Heart-shaped embryo; G. Mature dicot embryo; H. A typical dicot embryo

Dicot embryo

1. A typical dicotyledonous embryo consists of an embryonal axis and two cotyledons.
2. Above cotyledons level, embryonal axis is called epicotyl which forms shoot.
3. Below cotyledons level, embryonal axis is called hypocotyl which forms radicle.
4. The root tip is covered with a root cap (calyptra).

Development of monocotyledonous embryo

There is no essential difference between the embryogeny of monocotyledons and that of dicotyledons but as a single cotyledon develops instead of two from the embryo in monocotyledons, there is some difference in later stages.

We are taking an example of *Luzula forsteri* of Juncaceae for describing the development of monocotyledonous embryo. Here the development of embryo is also **Onagrad or Crucifer type**.

The early development of dicot and monocot embryos are similar upto **octant stage**. Later on differentiation starts.

1. The zygote or oospore elongates and then divides transversely to form basal and terminal cells.
2. The basal cell (towards micropylar end) produces a large swollen, vesicular suspensor cell. It may function as haustorium.
3. The terminal cell divides by another transverse wall to form two cells.
4. The top cell after a series of divisions forms plumule and a single cotyledon.
5. Cotyledon called scutellum, grows rapidly and pushes the terminal plumule to one side. The plumule comes to lie in a depression.
6. The middle cell, after many divisions forms hypocotyl and radicle. It also adds a few cells to the suspensor.
7. In some cereals both plumule and radicle get covered by sheaths developed from scutellum called coleoptile and coleorhiza respectively.

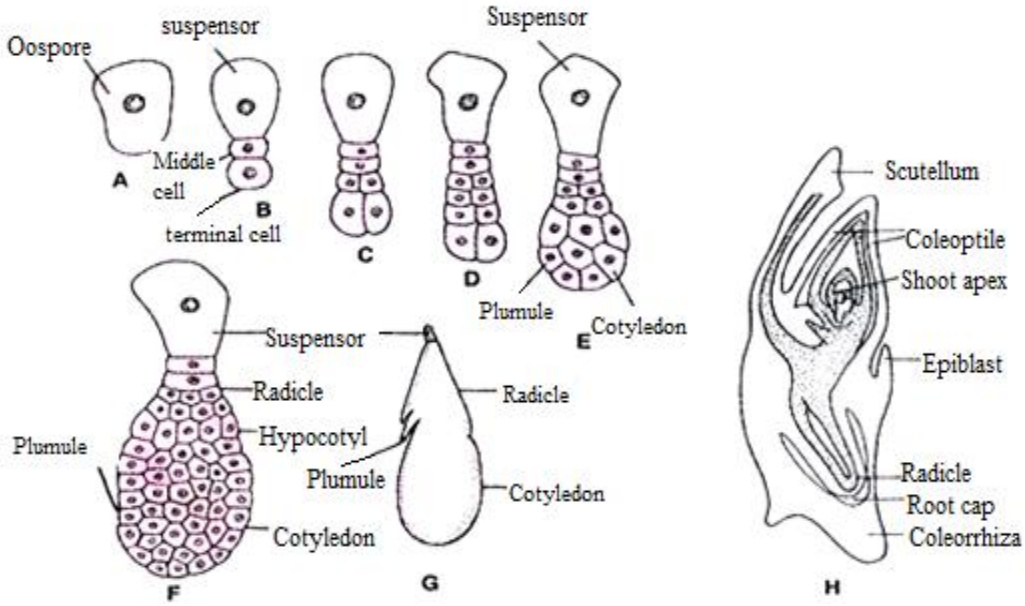


Fig.7.11A-G: Stages in development of a monocot embryo; H.A monocot embryo of a grass

Structure of Monocot Embryo

The embryos of monocotyledons have only **one cotyledon**. In grass family (Poaceae), this cotyledon is called **scutellum**. It is situated towards lateral side of embryonal axis. This axis at its lower end **has radicle** and root cap enclosed in a sheath called coleorhiza (Fig. 7.11).

The part of axis above the level of attachment of scutellum is called **epicotyl.** It has shoot apex and few leaf primordia enclosed in a hollow foliar structure called coleoptile. Epiblast represents rudiments of second cotyledon.

Monocot embryo

1. A typical monocot embryo consists of an embryonal axis and one cotyledon.
2. In grasses, this cotyledon is called scutellum.
3. Embryonal axis at its upper end is called epicotyl which forms shoot.
4. Embryonal axis at its lower end is called hypocotyl which forms radicle.
5. The root tip is covered with a sheath (coleorhiza).

7.5 APOMIXIS

"Reproduction without fertilization"

Apomixis in **flowering plants** is defined as the asexual formation of a seed from the maternal tissues of the ovule, avoiding the processes of meiosis and fertilization, leading to embryo development.

The term Apomixis was first coined by Hacke in 1893. Apomixis, derived from two Greek word "Apo" (away from) and "mixis" (act of mixing or mingling). Winkler (1908) explained the term apomixis as the **substitution of sexual reproduction (amphimixis) by any such method which does not involve meiosis and syngamy**.

or we can say that Winkler used the term apomixis to signify any asexual method of propagation not involving the normal production of embryo by fertilization. It includes even propagation by bulbils.

The first discovery of this phenomenon is credited to **Leeuwenhoek** as early as 1719 in *Citrus* seeds.

When we are talking about asexual formation of seed, in this sense apomixis is synonymous with **agamospermy: seed formation without fertilization of the egg cell.** In some plants **meiosis** [which converts a diploid sporophytic cell into four haploid gametophytic cells] and **fertilization** [where two haploid gametes of opposite sex fuse to re-establish the diploid sporophytic generation], the two very important necessary processes of sexual cycle (amphimixis) are interrupted. Even then a viable embryo if formed resulting into asexual seeds. When these asexual seeds produce plants identical to the female parent are called **apomictic seeds** and the phenomenon is known as **apomixis**.

The plants showing apomixis are known as apomictic plants. It is most common in Poaceae, Asteraceae, Rosaceae and Rutaceae.

When apomixis is the only method of reproduction in a plant species, it is known as **obligate apomixis**. When gametic and apomictic reproduction occurs in the same plant, it is known as **facultative apomixis**.

Apomixis is of the following types as suggested by Maheshwari (1954):
(i) Non-recurrent apomixis
(ii) Recurrent apomixis
(iii) Adventive apomixis
(iv) Vegetative apomixis

(i) Non-recurrent apomixes: Non-recurrent means which cannot be repeated. In this type of apomixis, the megaspore mother cell undergoes normal meiotic division and one of the four megaspores thus formed develops into haploid female gametophyte (i.e. embryo sac). However, **there is no fertilization and the embryo arises directly from normal egg-cell (n).** Since an egg cell is haploid, the resulting embryo will also be haploid and so **sterile**, therefore the process cannot be repeated in the next generation.

Haploid parthenogenesis (the embryo develops from the unfertilized egg) and **haploid apogamy** (the embryo develops from some other cell of the embryo sac like antipodal cell or synergid cell) are non- recurrent apomixis. Such types of apomixis are of rare occurrence.

(ii) Recurrent apomixes: Recurrent means which can be repeated. In recurrent apomixis, the nuclei of the embryo sac are usually diploid. Such embryo sac may arise either from a cell of the archesporium due to disturbance in meiosis (**generative apospory**) or from some other cell of the nucellus due to disintegration of megaspore mother cell (**somatic apospory**).

The embryo subsequently develops directly from the diploid egg-cell without fertilization. **Somatic apospory, diploid parthenogenesis and diploid apogamy** are recurrent apomixis. However, diploid parthenogenesis/apogamy occurs only in aposporic (somatic) embryo-sacs. Therefore, it is the somatic or diploid apospory that constitutes the recurrent apomixis. Such apomixis occurs in some species of *Crepis, Taraxacum, Paa* (blue grass), and *Allium* (onion) without the stimulus of pollination.

(iii) Adventive apomixes: In it, the development of embryo takes place from any diploid cell of the ovule lying outside the embryo sac. Since it takes place outside the embryo sac, it is not grouped with recurrent apomixis, though this is regenerated with the accuracy. In addition to such embryos, regular embryo within the embryo sac may also develop simultaneously, thus giving rise to polyembryony condition as in *Citrus, Opuntia.*

iv) Vegetative apomixes: In some cases like *Poa bulbosa* and some *Allium, Agave* and grass species, vegetative buds or bulbils, instead of flowers are produced in the inflorescence. They

can also be reproduced without difficulty. However, Russian workers do not group this type of vegetative reproduction with apomixis.

Apomixis does not involve meiosis, so there is no segregation and recombination of chromosomes. Therefore it could be useful in preserving desirable characters for indefinite period.

Parthenogenesis

Parthenogenesis means development of an embryo directly from an egg cell or a male gamete or it may be defined as - the development of female gamete into a new individual without fertilization. Parthenogenesis may be haploid or diploid as the case may be.

(i) Haploid parthenogenesis: Generally, normal haploid egg develops into an embryo, so the embryo and resultant plant are haploid. This type of parthenogenesis is termed as haploid parthenogenesis e.g. *Oenothera, Datura*. Plants thus produces are sterile.

(ii) Diploid parthenogenesis: When the cells of embryo sac including egg cell are already diploid as a result of apospory. This diploid egg when develops parthenogenetically into diploid embryo, termed as diploid parthenogenesis e.g. *Taraxacum.*

Apogamy

Apogamy is the development of a sporophyte (i.e. embryo) out of any gametophytic cell without fertilization i.e. the union of gametes. Plants formed in this way are sterile because they are haploid. Example - *Lilium, Nicotiana.* One of the two synergids develops into embryo in *Lilium* while male gamete forms embryo in *Nicotiana* by apogamy (Fig.7.12).

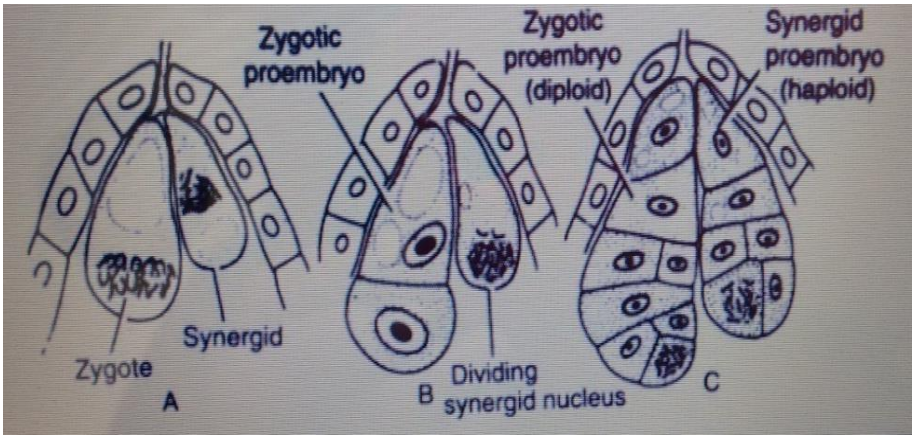


Fig.7.12: Haploid apogamy in *Lilium* A-B: dividing zygote and synergid; C: haploid (synergid proembryo) and diploid (zygotic proembryo) proembryos

Apospory

Apospory was discovered by Rosenberg (1907) in *Hieracium* species. In this type, megaspores are formed by usual process but all the four megaspores degenerates gradually. At the same time, **somatic cells,** usually nucellar cells enlarge and functions as initials of embryo sac. These initials enlarge, undergo mitotic divisions and develop embryo sacs. This type of apospory is also called as **somatic apospory. Aposporic embryo sacs are diploid.** It means the formation of gametophyte (i.e. embryo sac or pollen) on a sporophyte without any reduction division.

- **Apomixis** : reproduction without fertilization
- **Haploid parthenogenesis** : the embryo develops from the unfertilized egg
- **Diploid parthenogenesis** : the embryo develops from diploid egg formed as a result of aposopry
- **Haploid apogamy** : the embryo develops from some other cell of the embryo sac like antipodal cell or synergid cell
- **Apospory:** embryo sac arises from somatic cells like nucellus. Product is diploid.

Therefore **apogamy** is the development of a sporophyte (i.e. embryo) out of any gametophytic cell without fertilization i.e. the union of gametes while **apospory** is formation of gametophyte (i.e. embryo sac) out of sporophytic cell i.e. nucellar cell without reduction division.

Diagrammatic representation of embryo formation in different ways is given (Fig. 7.13).

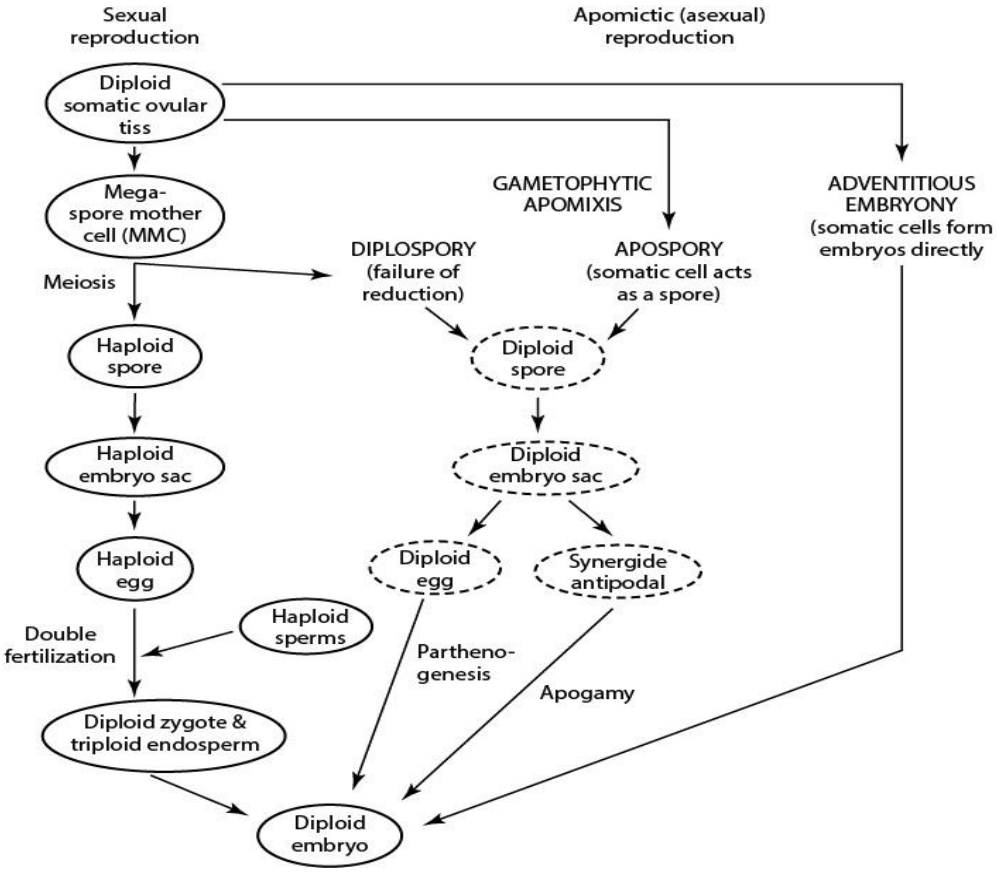


Fig.7.13: Diagrammatic representation of embryo formation through different ways

7.6 ADVENTIVE EMBRYONY

Adventive embryony is an embryony where an embryo develops directly from any diploid sporophytic cell for example- cells of nucellus, integument etc., without formation of gametophyte. This is also known as **adventitious embryony or nucellar embryony** (Fig 2.33). This may be considered as vegetative growth of the category of bulbils. Sometimes this is called **sporophytic budding**.

Or in simple way we can describe it as - cells outside the embryo sac also develop into embryos. These embryos are known as **adventives embryos** and the process as **adventives embryony** (Fig.7.14).

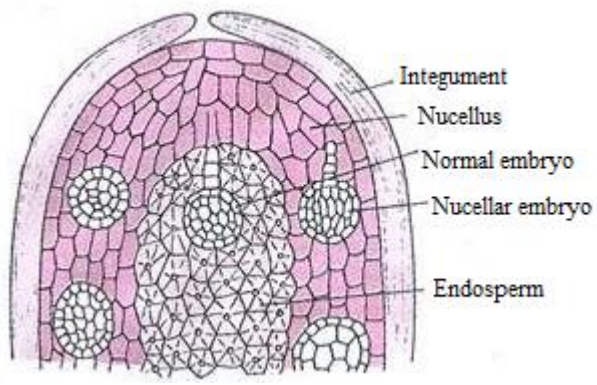


Fig.7.14: *Citrus* ovule (Young seed) in section showing normal and nucellar (adventive) embryos

Adventive embryony has great significance in horticulture and plant breeding. It provides uniform seedlings of the parental line as obtained through vegetative propagation by cuttings.

- When diploid embryo develops from diploid cells of the archesporium, this phenomenon is known as **generative apospory** e.g. *Parthenium argentatum.*
- When diploid embryo develops from any diploid cell of the ovule lying outside the embryo sac, this phenomenon is known as **adventive embryony**. It is also known as **sporophytic budding**.
- When diploid embryo sac develops from the cells of nucellus or integument, it is known as **somatic apospory** e.g. *Hieracium.*

7.7 POLYEMBRYONY AND PARTHENOCARPY

After fertilization, ovules mature into seeds. In normal case, a single embryo is present in each seed but sometimes more than one embryo may present in a seed. When a seed contain more than one embryo, this condition is termed as **polyembryony**. Therefore, polyembryony has been defined by many workers as the occurrence of more than one embryo in a seed or "The development of several embryos within the same ovule."

Polyembryony is very common among Gymnosperms but when we are talking about Angiosperms, it is very rare. You can find it in *Citrus* species like lemons, oranges (Fig.7.15) and also in few *Quercus* species. Additional embryos do not always mature. They may degenerate during the course of development. The mature seed thus has only one embryo. **The first case of polyembryony was reported by Antoni van Leeuwenhoek in 1719 in certain orange seeds.** Since then it has been observed in large number of plants.

Fig.7.15: Multiple seedlings grow from a single mandarin orange seed as the result of polyembryony

Classification of Polyembryony

In broad sense it is of two types:

1. Spontaneous- includes instances of naturally occurring polyembryony.
2. Induced- includes instances of experimentally induced polyembryony.

Ernst (1901, 1910) divides spontaneous polyembrony into two categories:
1. True polyembryony- development of two or more embryo in same embryo sac
2. False polyembryony - development of embryos in more than one embryo sac within the same ovule

Yakovlev (1967) divides spontaneous polyembrony into two categories on genetic basis-
1. Gametophytic: arising from any gametic cell of the embryo sac after or without fertilization.
2. Sporophytic: arising from the zygote, proembryo or the initial sporophytic cells of the ovule (nucellus, integuments).

There are number of factors responsible for polyembryony in Angiosperms and they are:

(1) Cleavage of proembryo
(2) Formation of embryos by cells of the embryo sac other than the egg
(3) Development of more than one embryo sac within the same ovule
(4) Activation of some sporophytic cells of the ovule

On the basis of these above factors the following four types of polyembryony have been recognized in Angiosperms (Braun, 1859).

1. Cleavage polyembryony resulted due to cleavage or splitting of the proembryo.
2. Embryos from cells of the embryo sac other than the egg.
3. More than one embryo sac in the same ovule.
4. Activation of some sporophytic cells of the ovule.

1. Cleavage Polyembryony

Splitting of zygote or proembryo occurs and each split part develops into an individual embryo. This kind of polyembryony is called as **cleavage polyembryony**. It is very common phenomenon among Gymnosperms and also reported in Angiosperm plants e.g. *Erythronium,* (Fig 7.16), Nicotiana *rustica.*

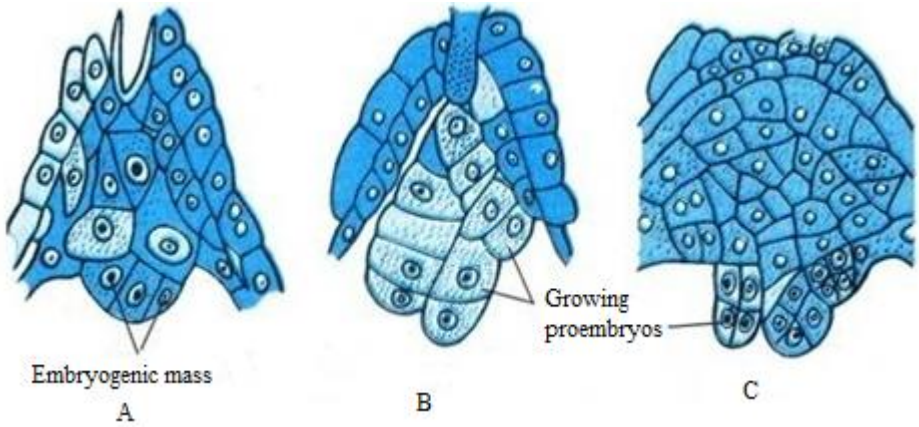


Fig.7.16: A-C showing cleavage polyembryony: A. Embryonic mass formed by the basal cell of the zygote in *Erythronium americanum*; B-C. Differentiation of embryos from the cells of the embryonic mass.

2. Embryos from cells of the embryo sac other than the egg

In this, the additional embryo forms mostly from synergids e.g. *Argemone mexicana, Phaseolus vulgaris.* Synergid embryo thus formed is haploid which can easily be distinguished from diploid zygotic embryo. Embryos can also be formed from antipodal cells e.g. *Ulmus* species (Fig. 7.17).

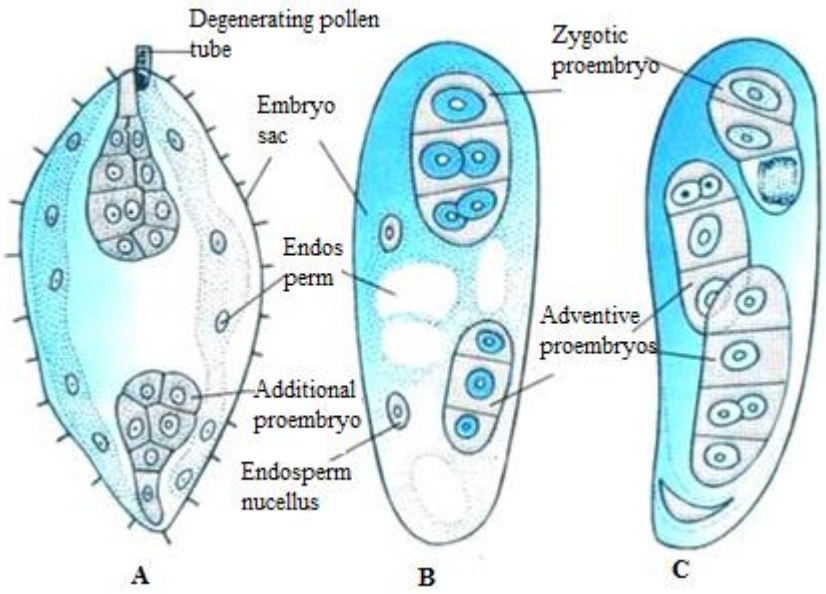


Fig.7.17: Polyembryony: A. Development of embryo from antipodal cells; B-C. Adventive proembryos developed from the cells of nucellus (they grow along with the zygotic embryos)

3. More than one embryo sac in the same ovule

Occurrence of multiple embryo sacs in an ovule may be from derivatives of the same megaspore mother cell or from derivatives of two or more megaspore mother cells or from nucellar cells e.g. twin embryo sacs within an ovule in *Casuarina, Citrus*.

4. Activation of some sporophytic cells of the ovule

We know that the embryos arising from the maternal sporophytic tissues (outside the embryo sac for example- nucellus and integuments) are called **adventive embryos.** Polyembryony because of adventives embryos is the most common type of polyembryony and known as **adventive polyembryony or nucellar polyembryony** e.g. *Citrus, Mangifera, Opuntia.*

Parthenocarpy

The term was introduced by Noll (1902). According to him, parthenocarpy means the development of fruits without pollination or any other stimulus.

According to present concept- **"Parthenocarpy is the formation of fruits without fertilization"** (Nitsch, 1965).

Therefore the fruits which develop without fertilization are called parthenocarpic fruits and the phenomenon is described as Parthenocarpy.

On the basis of requirement of pollination stimulus, it can be categorized into two categories:
(i) Stimulative parthenocarpy: In this type the parthenocarpic development of fruit may require the pollination stimulus.
(ii) Vegetative parthenocarpy: In this type the parthenocarpic development of fruit may occur in unpollinated flowers.

Nitsch (1963) had recognized three types of parthenocarpy:
1. Genetic
2. Environmental
3. Chemically induced

1-Genetic parthenocarpy

When many of the plants cultivated for their fruits show seeded as well as parthenocarpic varities. This type of parthenocarpy is known to arise due to either mutations or hybridization. Example- Seedless navel oranges, *Citrus, Cucurbita, Musa, Punica* and *Vitis.*

2-Environmental parthenocarpy

Variations in environmental conditions such as frost, fog, temperature interfare with the normal functioning of sexual organs and causes parthenocarpy. Example- Seedless olives due to heavy fog (Campbell, 1912), pears due to freezing temperature for 3-19 hours (Lewis, 1942).

3-Chemically induced parthenocarpy

Plant growth regulators like auxins and gibberellins have been successfully use to induce parthenocarpy in a number of plants which normally bear seeded fruits e.g. parthenocarpic tomato, blackberry, strawberry, figs, *Citrus* etc.

7.8 SUMMARY

In this unit we have discussed about fertilization, pathway of pollens to their destination for fertilization. After that we have also learnt post fertilization development process. Along with these topics light have been thrown on apomixis, adventives embryony, polyembryony as well as on parthenocarpy. Therefore, summary of all the topics covered in this unit is given in the following key points:

- Once the pollen grain reaches the receptive stigma, as a result of pollination, it germinates producing a long slender pollen tube. Two male gametes and the tube nucleus migrate into the pollen tube which now represents the mature male gametophyte.
- Pollen tube penetrates the stigmatic tissue and pushes its way through the style and then down the wall of the ovary.

- After arriving in the ovary, the pollen tube may enter into the ovule through micropyle (porogamy), through chalazal end (chalazogamy) or thrugh integuments (mesogamy).
- Irrespective of the route of the entry of the pollen tube into the ovule, it always enters the embryo sac from the micropylar end.
- After entering into the embryo sac, the tip of the pollen tube bursts and the two male gametes are released.
- Out of the two male gametes one male gamete fuses with the egg nucleus and result in the formation of zygote or oospore (2n). Second male gamete fuses with two polar nuclei, resulting into primary endosperm nucleus (3n). This phenomenon is termed as double fertilization.
- After series of divisions primary endosperm nucleus forms endosperm. Endosperm is very nutritive tissue that nourishes the developing embryo.
- Zygote or oospore forms embryo.
- On the basis of plane of division of the terminal cell in the 2-celled proembryo and the contribution of the basal cell and terminal cells in the formation of embryo proper, six types of embryogeny (embryo development) have been reported by Johansen (1950) among the Angiosperms i.e. Onagrad or Crucifer, Asterad, Solanad, Caryophyllad, Chenopodiad and peperad types.
- A typical dicotyledonous embryo consists of an embryonal axis and two cotyledons.
- A typical monocotyledonous embryo consists of an embryonal axis and one cotyledon.
- Reproduction without fertilization is referred as apomixis.
- Apogamy is the development of a sporophyte (i.e. embryo) out of any gametophytic cell without fertilization i.e. the union of gametes.
- Apospory is the formation of gametophyte (i.e. embryo sac) out of sporophytic cell i.e. nucellar cell without reduction division.
- When diploid embryo develops from any diploid cell of the ovule lying outside the embryo sac, this phenomenon is known as adventive embryony.
- Development of several embryos within the same ovule is known as polyembryony.
- Parthenocarpy is the formation of fruits without fertilization.

7.9 GLOSSARY

Adventive apomixes: When the development of embryo takes place from any diploid cell of the ovule lying outside the embryo sac

Adventive embryony: An embryo develops directly from any diploid sporophytic cell without formation of gametophyte

Adventive embryos: The embryos arising from the maternal sporophytic tissues (outside the embryo sac e.g. Nucellus and integuments)

Adventive polyembryony: Polyembryony because of adventives embryos and also known as nucellar polyembryony
Agamospermy: Seed formation without fertilization of the egg cell
Albuminous seeds: Mature seeds with endosperm
Apogamy: Development of sporophyte from a gametophyte without fertilization
Apomixis: Method involving the production of seeds without fertilization; phenomenon of substitution of sexual reproduction by asexual methods
Apospory (somatic): Development of diploid embryo sac from the cells of nucellus or integument
Chalazogamy: Entry of pollen tube through the chalazal end
Cleavage polyembryony: Polyembryony due to splitiing of zygote or proembryo and development of each split part into an individual embryo
Coleoptile: A plumule sheath found in monocot seeds
Coleorrhiza: A sheath surrounding radicle found in monocot seeds
Cotyledon: The first leaf of the embryo
Dicot embryo: Embryonical axis having two cotyledons
Embryo: A young sporophytic plant while still retain in the gametophyte.
Embryogenesis: The process of development of mature embryo from diploid oospore
Endosperm: A nourishing tissue in seed bearing plants that is formed within the embryo sac
Epiblast: A small outgrowth found opposite to scutellum in the embryo of some members of Poaceae
Epicotyl: The stem of an embryo (or seedling) above the cotyledons
Ex-albuminous seeds: Mature seeds without endosperm
Fertilization: Process of fusion of two dissimilar reproductive units, called gametes
Hypophysis: One of the cells of an embryo found in its early stage of development
Integument: An envelope surrounding the nucellus (in ovule)
Liquid syncytium: Clear fluid (here watery liquid endosperm) in which numerous nuclei float
Megasporogenesis: Development of megaspore within ovule
Mesogamy: Entry of pollen tube through the integument or funiculus
Monocot embryo: Embryonical axis having one cotyledon
Monocotyledon: A flowering plant with one cotyledon
Parthenocarpy: Formation of fruits without the act of fertilization.
Parthenogenesis: A special type of sexual reproduction in which egg develops without entrance of a sperm
Polyembryony: A condition when more than one embryo is present in a seed
Porogamy: Entry of pollen tube through the micropyle
Scutellum: Specialized monocot cotyledon
Suspensor: The part of the embryo which connect the main part of the embryo to the basal cell

Triple fusion: A unique feature of flowering plants wherein fusion of one male gamete with the two polar nuclei to form primary endosperm nucleus that give rise to the endosperm
Zygote: The fusion product of an egg and a male gamete, i.e. a fertilized egg

7.10 SELF ASSESSMENT QUESTION

7.10.1 Multiple choice questions:

1. Entry of pollen tube into the ovule through micropyle is called:
(a) Chalazogamy (b) Porogamy
(c) Mesogamy (d) None

2. Entry of pollen tube into the ovule through the chalazal end is called:
(a) Chalazogamy (b) Porogamy
(c) Mesogamy (d) None

3. Entry of pollen tube into the ovule through the funiculus or integuments is called :
(a) Chalazogamy (b) Porogamy
(c) Mesogamy (d) None

4. Product of syngamy is:
(a) Zygote (b) Oosphere
(c) Primary endosperm nucleus (d) Embryo

5. Product of triple fusion is:
(a) Zygote (b) Oosphere
(c) Primary endosperm nucleus (d) Embryo

6. Zygote develops into:
(a) Endosoerm (b) Polar nuclei
(c) Embryo (d) Egg

7. Endosperm is formed from:
(a) Primary endosperm nucleus (b) Secondary nucleus
(c) Egg (d) Embryo

8. Two- celled proembryo has:
(a) Apical cell and terminal cell (b) Suspensor cells only
(c) Spore cells (d) Basal cell and terminal cell

9. In dicots embryo development, the hypophysis is formed from:
(a) Terminal cell (b) Embryo cell
(c) Suspensor cell (d) None

10. The development of several embryos within the same ovule is known as:
(a) Embryony (b) Polyembryony
(c) Both (d) None

11. Double fertilization in flowering plants produces :
(a) Diploid oosphere and diploid endosperm (b) Diploid zygote and triploid endosperm
(c) Diploid zygote and triploid oosphere (d) Diploid endosperm and triploid zygote

12. Endosperm:
(a) Provide nutrient to the embryo (b) First cell of male gametophyte
(c) Produced by syngamy (d) Product of meiosis in microspore mother cell

7.10.1: Answer key: 1. (b), 2. (a), 3.(c), 4.(a), 5.(c), 6.(c), 7.(a), 8.(d), 9.(c), 10.(b), 11. (b), 12. (a)

7.10.2 Short answer questions:

1. On the surface of stigma the pollen hydrates. What does this reflect?
Ans: On the surface of stigma the pollen hydrates. This means pollen absorbs water from the surrounding and swells. Then pollens start germinating.

2. What is the role of enzyme cutinase in pollen tube?
Ans: Pollen grains as well as pollen tube contain an enzyme cutinase which helps in the penetration of pollen tube into the stigmatic tissue. Cutinase as the name indicates degrades the cutin of the stigma at the point of contact with the pollen tube.

3. Who discovered chalazogamy?
Ans: The chalazogamy was first reported by Treub (1891) in *Casuarina*.

4. Is the way of entry of pollen tube to ovule and embryo sac same?
Ans: It does not matter through which way pollen tube enters into the ovule, it always enters in the embryo sac from the micropylar end means entry of pollen tube in the embryo sac is irrespective of pollen tube entry into the ovule.

5. What is the difference between syngamy and triple fusion?

Ans: (i). Syngamy - fusion of one male gamete and one female gamete i.e. egg whereas, triple fusion is fusion of male gamete with secondary nucleus. (ii). Result of syngamy is diploid zygote and result of triple fusion is triploid primary endosperm nucleus

6. Define endosperm.
Ans: The primary endosperm nucleus is a product of triple fusion. This undergoes a series of divisions and ultimately forms highly nutritive endosperm. The Angiospermic endosperm is a triploid (3n) tissue as it is a product of triple fusion

7. What is the main role of endosperm?
Ans: Endosperm is a highly nutritive tissue which provides nourishment to the developing embryo.

8. What is double fertilization?
Ans: In an embryo sac two sexual fusions occur; one in syngamy (i.e. fusion of one male gamete with the egg) and another in triple fusion (i.e. fusion of other male gamete with the polar nuclei or secondary nucleus) , and therefore, the phenomenon is known as double fertilization.

9. Who discovered the process of fertilization in flowering plants?
Ans: In flowering plants, the process of fertilization was first discovered by Strasburger in 1884.

10. Who discovered the phenomenon of double fertilization?
Ans: Double fertilization is a very unique phenomenon in Angiosperms and discovered for the first time by S.G. Nawaschin (1898) in *Lilium* and *Fritillaria* species.

11. Are Angiospermic and Gymnospermic endosperms same?
Ans: Endosperm of Angiosperms is distinct from the endosperm of heterosporous Pteridophytes and Gymnosperms where the endosperm is a simple haploid (n) tissue of the gametophyte not involving any triple fusion like in Angiosperms where it is triploid (3n).

12. Name types of endosperms.
Ans: Depending upon mode of development there are three types of endosperm.
1. nuclear endosperm
2. cellular endosperm
3. helobial endosperm

13. Which type of endosperm is common in Angiosperms?
Ans: Nuclear endosperm is the most common type which occurs in about 56% families of Angiosperms

14. What is liquid syncytium?
Ans: Watery liquid endosperm in which numerous nuclei float.

15. What is embryogenesis?
Ans: The process of development of mature embryo from diploid oospore is called embryogenesis.

16. Name the different types of embryo developments.
Ans: 1. Onagrad or Crucifer type; 2. Asterad type; 3. Solanad type; 4. Caryophyllad type; 5.Chenopodiad type; 6. Piperad type.

17. Differentiate dicot and monocot embryo.
Ans: The dicotyledonous embryo as the name reflects, has two cotyledons attached laterally to an embryonical axis, whereas in the monocotyledonous embryo, the embryonical axis has a single cotyledon at its apex.

18. Who coined the term apomixis?
Ans: The term apomixis was first coined by Hacke in 1893

19. Who discovered the phenomenon of apomixis?
Ans: The first discovery of this phenomenon is credited to Leeuwenhoek as early as 1719 in *Citrus* seeds.

20. Define agamospermy?
Ans: Agamospermy is defined as seed formation without fertilization of the egg cell.

21. What do you understand by apomictic seeds?
Ans: A viable embryo if formed without meiosis and fertilization, and resulting into asexual seeds. When these asexual seeds produce plants identical to the female parent are called apomictic seeds and the phenomenon is known as apomixis.

22. What are obligate and facultative apomixis?
Ans: When apomixis is the only method of reproduction in a plant species, it is known as obligate apomixis and when gametic and apomictic reproduction occurs in the same plant, it is known as facultative apomixis.

23. Why apomixis is important?

Ans: Apomixis does not involve meiosis, so there is no segregation and recombination of chromosomes. Therefore it could be useful in preserving desirable characters for indefinite period.

24. What do you understand by parthenogenesis?
Ans: Parthenogenesis means development of an embryo directly from an egg cell or a male gamete.

25. Define apogamy and apospory.
Ans: Apogamy is the development of a sporophyte (i.e. embryo) out of any gametophytic cell without fertilization i.e. the union of gametes while apospory is formation of gametophyte (i.e. embryo sac) out of sporophytic cell i.e. nucellar cell without reduction division.

26. What is adventive embryony? Is it also known as nucellar embryony or not?
Ans: Adventive embryony is an embryony where an embryo develops directly from any diploid sporophytic cell for example- cells of nucellus, integument etc., without formation of gametophyte. This is also known as adventitious embryony or nucellar embryony.

27. What does the polyembryony means?
Ans: The development of several embryos within the same ovule is polyembryony.

28. Define parthenocarpy.
Ans: Parthenocarpy is the formation of fruits without fertilization.

7.11 REFERENCES

- Jonathan Yam and Whitney Hagins Seedless fruit and methods of Parthenocarpy. *J. Experimental Secondary Science* P1-3
- Photo courtesy of: http://jerry-coleby-williams.net/2012/08/25/natural-cloning-mandarin-style/polyembryony-triple-mandarin-embryo-citrus-reticulata-parramatta-sweets-2-2/

7.12 SUGGESTED READINGS

- Singh,V. Pande, P.C. and Jain, D.K. (2008). *A Text Book of Botany.* Rastogi Punlications, Meerut.
- Maheshwari, P. (1950). *An Introduction to the Embryology of Angiosperms.* Mc Graw -Hill, New York.

- Gangulee, H.C., Das, K.S. and Datta, C. (1998). *College Botany*. Vol. I. New Central Book Agency, Kolkata.
- *Developmental Biology.* 6th edition, by Gilbert S.F.Sunderland (MA): Sinauer Associates; Bookshelf ID: NBK9980 (2000).
- Pandey, S.N. (1997). *Plant Anatomy and Embryology*. Vikas Publishing House Pvt Ltd., New Delhi.
- *Plant Physiology and Development*. 6th edition, Lincoln Taiz, Eduardo Zeiger, Ian Max Moller, Angus Murphy.2015 P 761.ISBN: 978-1-60535-255-8
- Singh,V., Pande, P.C. and Jain, D.K. (2012-13). Structure, Development and Reproduction in Angiosperms. Rastogi Publications, Meerut.
- Pullaiah, T.(2001). *Textbook of embryology of Angiosperms.* Daya Books, New Delhi.
- S S Bhojwani, S.S., Bhatnagar, S.P. and P. K. Dantu,P.K. (2015). *The Embryology of Angiosperms.* Vikas Publication House Pvt Ltd, New Delhi.

7.13 TERMINAL QUESTIONS

7.13.1 Long answer type questions:

1. Define briefly the endosperm development in *Cocos nucifera.*
2. Describe in detail the post fertilization developments.
3. Explain the development of different types of endosperms in Angiosperms.
4. Types of embryo development.
5. Define different types of apomixis.
6. Classify polyembryony.

BLOCK-3 MORPHOGENESIS

UNIT-8 PLANT MORPHOGENESIS AND MORPHOGENETIC FACTORS

8.1 OBJECTIVES

After reading this unit, student will be able:

- To understand concept of morphogenesis and how process of differentiation plays a role in morphogenesis
- To know about how is polarity developed and how it affects morphogenesis
- To understand role of totipotency in growth of plants and in plant tissue culture
- To study about different morphogenetic factors and how they affect morphogenesis of plants

8.2 INTRODUCTION

Morphogenesis is defined as a process concerned with formation and development of whole plant, a part of plant or a specific structure. During very early developmental stages polarity is established at the zygote stage due to which a polar difference is developed at both the ends of zygote. Cytological differences at the two ends determines the position of first cell division and also the fate of the structure which will be produced by the two cells (cells formed by division of zygote). Polarity is not limited to initial developmental stages but polarity is maintained throughout the growth. Plant axis (shoot and root tips) also exhibits polarity. If a portion of shoot is excised (cut) and allowed to regenerate the end toward shoot tips will always form shoot whereas end towards root will regenerate roots. As in stem polarity is also exhibited in other organs like upper and lower surface of leaf, petals, sepals etc. Different parts of plant have different type of morphology. This diversity in different parts of plant is produced due to variation in growth rate of different parts and also because different parts show growth in different dimension. Rate of cell division, cell elongation along with orientation of plane of division and axis of cell elongation altogether establish the form of structure of plant.

Different factors (called as morphogenetic factors) affect growth and development of plants. These factors can be environmental such as light, temperature, water or nutritional factor, physical factors such as gravity, pressure and genetic factor. Genes are considered to be the ultimate factor which growth but they do not regulate growth independently. Instead genes interact with existing environmental conditions to control plant development.

During their growth and development plant, cells exhibits a specific phenomenon called as totipotency. It is the ability of a cell to give rise to different types of cells and eventually lead to regeneration of a complete plant. Meristematic cells get differentiated to attain specific functions once the cell gets differentiated. They lose their ability to divide. However, differentiated plant cells can undergo a process of dedifferentiation (specially during plant tissue culture) and can

again become meristematic. Now, such dedifferentiated cells can again redifferentiate (by a process known as redifferentiation) to form cells and tissues with specific structure and function.

8.3 BASIC IDEA OF MORPHOGENESIS AND CONCEPT OF DIFFERENTIATION

The word morphogenesis comes from Greek words *morphe* (which means shape) and *genesis* (which means creation) to indicate a process of formation of a particular structure with a specific shape and size. Morphogenesis is considered to be a biological process which causes an organism to develop its shape. Morphogenesis is concerned with development of particular part or structure. Plants possess a longer period of morphogenesis. During development plants (unlike animals) do not exhibit a distinct body plan. Plants may grow and develop on and on till they die. This is because plants have meristematic tissue composed of actively dividing cells which result in formation of more and more new tissues, organs and structures throughout the life of plant.

The term differentiation was first of all used by Karl Willhelm. Differentiation refers to a process in which distinct (different) types of cells are formed from a precursor cell. Differentiation is a permanent localized qualitative change in size, biochemistry, structure and function of cells, tissues or organs. A cell which has ability to get differentiated into different cell types of an adult organism is called pluripotent. In plants such cells are also called as meristematic cells.

Different type of structural changes occurs inside a cell during the process of differentiation. These changes may occur in cell wall, protoplasm or both. For example when a cell gets differentiated into tracheary elements it loses its cytoplasm and the cells develop an elastic, strong, lingo cellulosic secondary cell wall to carry out transport of water.

Hence, meristematic cells are group of unspecialized (undifferentiated) cells which are capable of dividing throughout the life of plant and can get differentiated into different types of cells. When a cell gets differentiated it acquires specific morphological, physiological as well as biochemical properties. During growth and development of plant meristematic tissue give rise differentiated tissue where each cell has specified structure and function. Differentiation cells do not have ability to divide. In an another process known as dedifferentiation, differentiated tissue loses its differentiated state and becomes undifferentiated.

Such undifferentiated tissue can again undergo the process of differentiation known as redifferentiation and again become differentiated with specific structure and function. A dedifferentiated cell can divide and produce new cells (Fig.8.1). Dedifferentiation is a commonly observed phenomenon during secondary growth in plants and also during the process of healing of wounds.

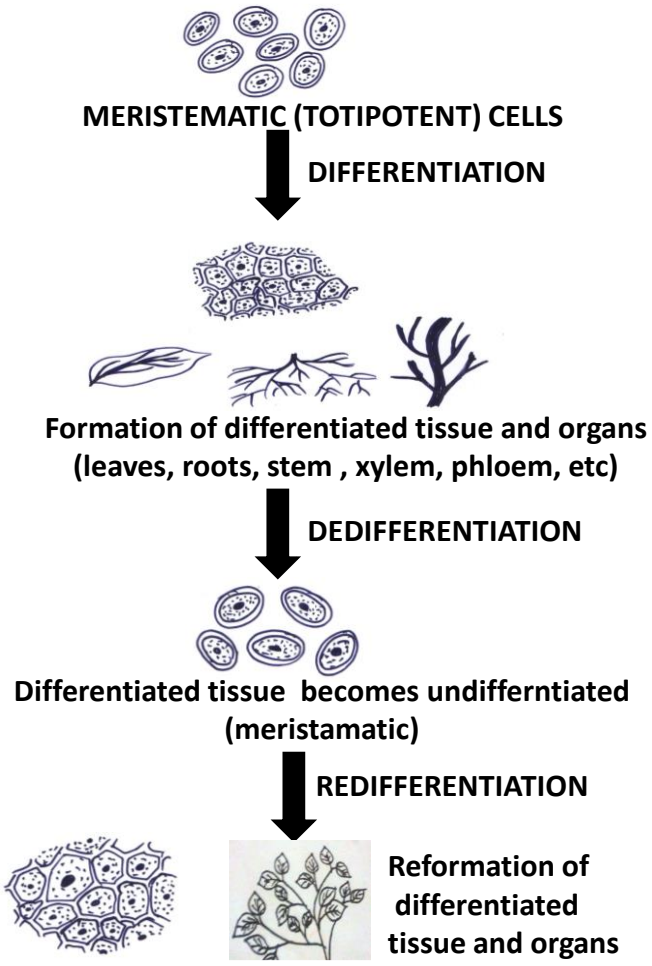


Fig. 8.1: Diagrammatic representation of differentiation, dedifferentiation and redifferentiation

Plant cells are totipotent and possesses an inherent ability to undergo process of differentiation to give rise to different types of cells, which ultimately form different organs in plant system.

8.4 POLARITY

The term polarity means specific orientation of plant activity and morphogenesis in space. Plants are multicellular organisms made up of cells, tissue and organs. As we already know that in a multicellular organism cells, tissue and organs are integrated with one another to bring about overall functioning of an organism. There are many factors which regulate and control this integrated functioning. Among different factors polarity is one the most important factor of plant integrity. In plants axial polarity, dorsiventral polarity and radial polarity are known. However, When we talk of polarity in plants we generally refers to axial polarity. Axial polarity means presence of a well defined longitudinal axis which bears lateral organs such as lateral branches, roots, leaves and flowers. The radial axis is most clearly evident in dicotyledonous species as the

concentric rings of cell layers stem hypocotyl and root with an increase in size across this axis can arise from the generation of new cell layers following divisions in the vascular cambium in the older plant.

There are several factors which influence polarity in plants. Physical factors like light, gravity, electric and magnetic field, chemical agents such as plant growth regulators and ions influence polarization in plants. Polarization is related to axial gradient of bioelectric potential (BEP) which develop from gradient of Ca^{2+}, K^{+}, H^{+} etc. Changes in membrane permeability to these ion generates a dielectric potential. Results obtained from studies conducted on plants such as *Arabidopsis, Capsella bursa-pastoris* have made it clear that apical-basal polarity is determined even before the first zygotic division in the egg.

Early events of zygote polarization have been very well studied in *Fucus* (brown alga). In *Fucus* polarization of zygote is initiated and influenced by various types of stimuli such as unidirectional light, temperature, electric field or chemical gradient. Axis formulation is associated with redistribution of plasma membrane components. Ca^{++} is the most important component which gets accumulated toward basal end during axial axis function.

In *Arabidopsis* during axial polarization, zygote divides by an asymmetric transverse division resulting in formation of two daughter cells of unequal size. One is the basal cell which is derived from vacuolar region and is larger in size and another cell is smaller upper cell which is derived from cytoplasmic region. Upper cell divides to form suspensor (containing six to nine cells). Only the upper most cell of suspensor called hypophysis is actually the part of embryo proper.

Although suspensor cells are known to have different functions such as they physically project the embryo into endosperm, avail a source of hormone and nutrient to the developing embryo, the suspensor cells undergo programmed cell death when embryo reaches its torpedo stage of development.

In *Fucus* the larger upper cell is known to form thallus cell from which develops the thallus structure of mature alga. On the other hand the small basal cell forms rhizoid which undergoes polarized growth. In ferns and mosses polarity can be induced by membrane bound biliprotein phytochrome.

There are two system under which induction of polarity in plants have been studied.

(A)The first system of polarity in plants is **ROOT- RHIZOID POLARITY**, this type of system studied in phaeophycean zygotes and in pteridophytic spores.

Development of polarity occurs parallel to ionic gradient of calcium, potassium and sodium. During polarization an increase influx of calcium ions occurs into the cell present in the future rhizoid pole. On the contrary a decreases influx of calcium ions occur in the opposite pole.

(B) Another system of polarity is **SHOOT-ROOT POLARITY** found in higher plants: (Development / induction of polarity in multicellular plants)

The earliest work related to shoot-root polarity was done by Marquis Duhamel du monceau in eighteenth century. In his work existence of two morphogenetic factors was proposed, one was a heavy root sap and another a light shoot sap. Both morphogenetic factors (shoot sap and root sap) were directed by gravity to their respective poles, where they got accumulated and shoot sap initiated formation of shoots and root sap gave rise to roots.

Zygote displays a specific cell polarity with a vacuolar pole present at micropylar site (which later develops into suspensor or root pole) and an opposed cytoplasmic pole (embryo pole). Establishing polarity is an important event for morphogenesis and development of plant. Particularly in plants polar differences can be identified at very early stage of development after the formulation of zygote. During the process of development of plant, polarity can also be seen in plant axis i.e. in shoot and root tips. This means that once a polarity is established it does not gets altered naturally. So if a part of shoot or root is existed and allowed to regenerate the end toward shoot tips always regenerates into shoot and the opposite end will develop roots. However, during the process of development either removal of one part of plant or changes in a part of plant significantly affect morphogenesis of one or more other parts of plant. This process is called as correlation and is generally mediated through nutrient and plant growth regulators.

8.5 TOTIPOTENCY

8.5.1 Basic concept of totipotency

Totipotency refers to inherent genetic potential of a plant cell to regenerate into complete plant. Plant cells can follow a developmental pathway similar to that of a zygote resulting in formation of new plant. The concept of regeneration the entire plant from a single cell or tissue was conceptualized by G. Haberlandt in 1902, who is known as father of plant tissue culture.

F. C. Steward along with his colleagues developed a method for growing carrot tissue by taking small part, from the secondary phloem region of carrot root. This part was utilized as explants in the experiment. The explants were cultured by placing it onto a liquid medium under aseptic conditions. During the culture process the phloem tissue began to grow. Initially some single cells and some groups of cells became loosened from the surface of growing tissue and started growing separately. Some single cells developed somatic embryos or embryoids by a process

now known as somatic embryogenesis. The embryo ultimately gave rise to shoot and root and the complete plant was regenerated (Fig.8.2).

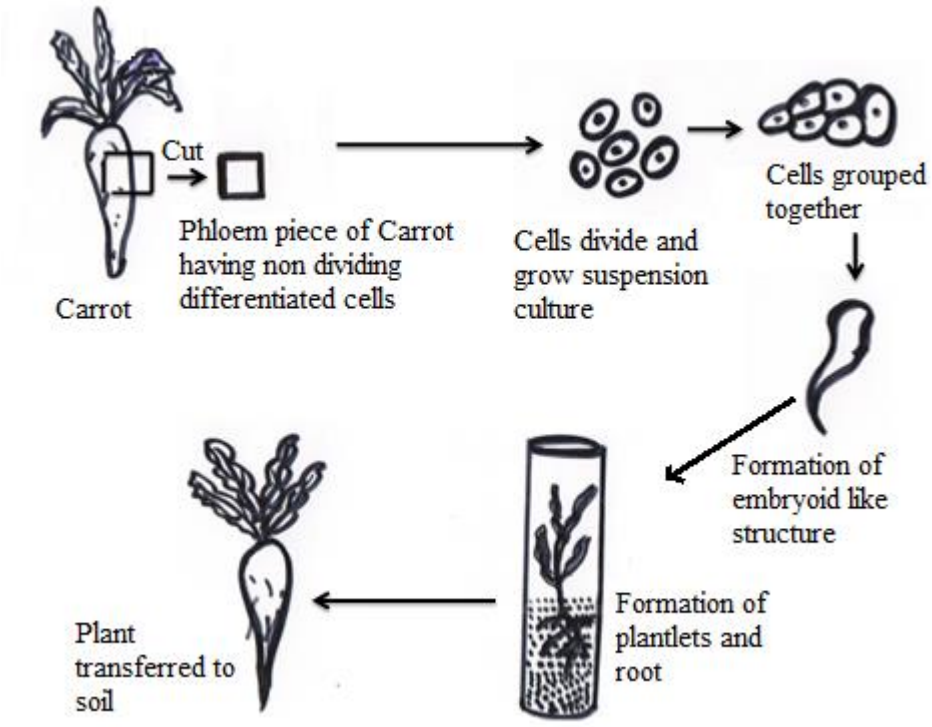


Fig. 8.2: Regeneration of complete plant (carrot) from single cell

8.5.2 Importance or significance of Totipotency

- The most important aspect/application of totipotency is reconstruction or regeneration of complete plant from any tissue or organ.
- Regeneration of plants from somatic cells through their ability totipotency has been utilized for vegetative propagation of many medicinal, aromatic and ornamental plants with economic importance.
- With the development of plant tissue culture technology large number of plants can be produced in short time interval. Totipotency is the underlying principle of regeneration of plants through plant tissue culture. Hence, endangered, rare and scarce plants can be mass propagated through the technique.
- Advancements made in plant science have resulted in development of genetically modified plants. Production of homozygous plants, haploid plants, somatic embryogenesis, somatic hybridization, protoplast (fusion and) culture etc. Totipotency is the basic of all the above mentioned developments made in plant science.
- *In vitro* regenerated cells, tissue, callus with totipotency potential can be preserved for long periods under liquid nitrogen. The process is known as cryopreservation. Whenever required

these cells can be retrieved thawed and can be utilized for regeneration (since they are totipotent)

8.5.3 Totipotency and plant tissue culture

Plant tissue culture also known as *in vitro* micropropagation is a technique utilized for regeneration of plants under controlled conditions. The technique has been successfully utilized for regeneration, conversation of large number of medicinal, aromatic, ornamental and other plants on a large scale.

The entire success of plant tissue culture technology is based upon the totipotency of plant cells.

Normally, we grow plants mainly through seeds or by methods of vegetative propagation including cutting, grafting, layering etc. But through the technique of tissue culture plants can be regenerated by culturing any part of the plant. The part of plant (cell, tissue, organ) excised to culture is called as explant. Explants are transferred to a culture medium aseptically. The cultures are then incubated under suitable temperature with proper light.

Now, during the process of incubation the explants which are differentiated tissue undergo the process of dedifferentiation and become undifferentiated and totipotent. Explants now undergo the process of redifferentiation and start growing to regenerate a new plant (Fig. 8.4).

8.5.4 Different response of totipotent cells during *in vitro* culture process

When totipotent cells undergoes the process of differentiation to form different types of cells and organ (Fig. 8.3, 8.4) there are basically three types of pathways which can be followed:

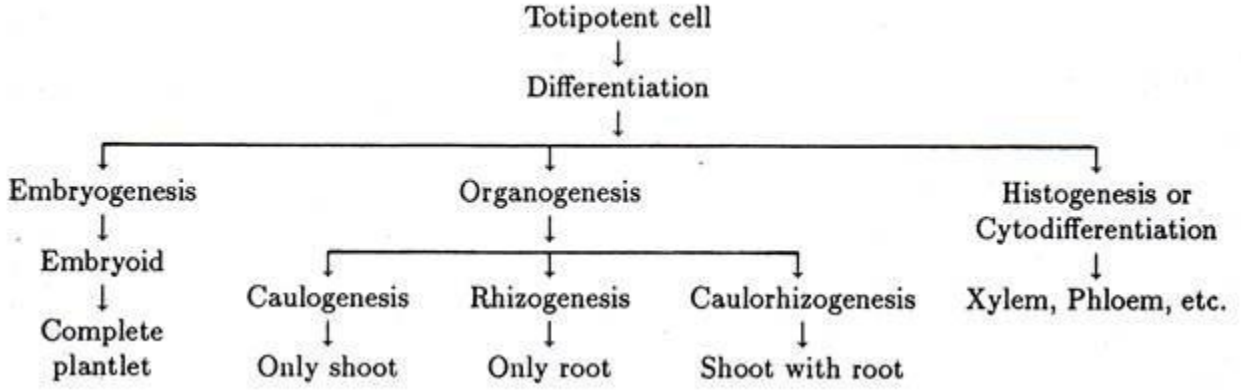


Fig. 8.3: Possible growth fates of totipotent cells during *in vitro* culture

(a) Embryogenesis: Totipotent cells can divide and differentiate to give rise to embryoid structure. Formation of embryo is a bipolar structure and same structure give rise to root as well as shoot i.e. complete plant.

(b) Organogenesis: division and differentiation of totipotent cells may also result in formation of organs. If totipotent cell give rise to only shoot it is known as caulogenesis. However, rhizogenesis is process of formation of roots. In another process called as Caulorizogenesis formation of shoot as well as root occurs simultaneously.

(c) Histogenesis / Cyto differentiation: Totipotent cells may divide and differentiate and give rise to tissues like xylem, phloem etc.

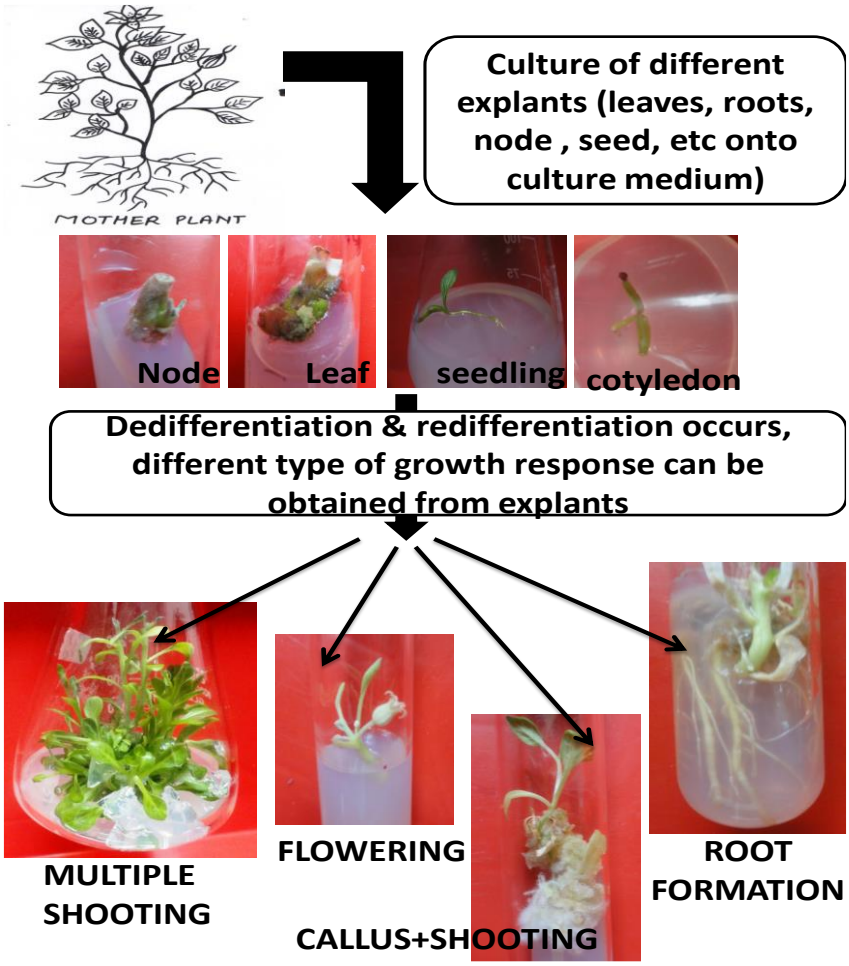


Fig. 8.4: Depicting regeneration of different organs from different explant

In tissue culture process growth can be of two types direct and indirect. In direct growth formation of organs (shoots, roots or embryo) occurs directly from explant whereas in indirect growth first a callus (undifferentiated mass of cell) is formed. By further sub culturing this callus regeneration of shoot and roots can be obtained. The plant regenerated in laboratory conditions are then transferred to soil (natural conditions) through a process known as hardening or acclimatization.

Hence, totipotency forms the basis of plant tissue culture through which large number of plants can be regenerated in comparatively shorter duration of time.

There are several advantages of plant tissue culture.

1. Production of large number of plants.
2. Conservation of endangered species.
3. Production of hybrid plants.
4. Synthesis of secondary metabolites.
5. Production of virus resistant plants through meristem culture.

8.6 MORPHOGENETIC FACTORS

8.6.1 Basic concept and effect of morphogenetic factors

Morphogenetic factors are physiological factors which induce regulate and coordinate morphogenetic events in plants. These factors can be a part of inner or outer environment of the plants.

Morphogenetic factor can be divided into two groups:

(a) Environment factors

(b) Genetic factors

Plants are multicellular organisms which survive in an environment which is complex and keeps on changing. Genetic makeup (genome / total genes present in nucleus) remains unchanged except for rarely occurring somaclonal variation. Now, even since there is no change in genetic constitution of plant but plants do exhibit phenotypic changes i.e. their appearance changes or gets modified with changes in their environment. Such phenotypic changes which occur in plants are considered to have occurred due to environmental factors. However it is quite difficult to judge whether the morphogenetic change occurring in plant is due to a genetic factor or an environmental factor since both environmental as well as genetic factors are operating simultaneously.

Responses such as flowering, thickness of cuticle, height of plant are greatly influenced by environment and gets altered according to the changing environment (change in temperature, pH, light, moisture etc).

Whereas characters such as formation of pits on side walls of vessels, arrangement of leaves etc do not change with the change in environmental conditions. The degree of lobbing in leaves is greatly influenced by changes in temperature.

Another class of factors which influence plant growth or morphogenesis are nutrients. They act as chemical factors, come into plant body from outside and participate in biochemical process

occurring inside the plant. There are several growth substances which significantly influence morphogenesis in plants.

There are three possible attributes of action of morphogenesis factors.

(1) It is not necessary that a morphogenetic factor may directly result in a response but it may act as a stimuli to trigger other biochemical reaction in an organism.

(2) One morphogenetic factor can significantly influence or modify the action of another factor. No factor can act independently; response mediated by each factor is dependent upon the environment as well as on the status of plant.

(3) A plant is not a constant system i.e. character of plant changes from one phase of life cycle to another and also from one part of plant to other part of the plant. Hence, plants may exhibit different response to same morphogenetic factor in different phases of life cycle. And also different part of plant may respond differently to same morphogenetic factor.

8.6.2: Morphogenetic effect of light

As we all know that light is one of the most crucial factors for growth, development and survival of plants. Light is required by the plants for vital processes such as photosynthesis, photo morphogenesis etc. Beside these processes, light also influences several other physiological processes. One of the most prominent effect of light as morphogenesis factor is that any plant reaches its maximum height with optimum growth only when the plant is exposed to sufficient amount of light. If insufficient light is provided the plants exhibited retarded growth even if supplied with sufficient water, nutrients and temperature.

There are three aspects of light which influence growth of plants:

(a) Intensity: It is the measure of brightness of light or other illumination i.e. actual energy of the radiation.

(b) Quality: It refers to wavelength of the light perceived by plants.

(c) Duration: By duration it means the length of lightness (day) and darkness (night) to which a plant is exposed.

The effect of light can have different effect on different parts of plant. Some of the effect of light on plants are:

- Rate of photosynthesis generally increases with increase in intensity of light to a certain extent.
- Intensity of light also affects qualitative traits such as strength of stem, development of xylem and phloem etc.
- Plants grown in shade have comparatively small root system.
- Light intensity is directly proportional to width of stem.
- Some herbaceous plants show zig-zag growth pattern in light but grow straight if same plants are grown in darkness.

- Whenever we think of light and plants. We get an image that light is required for photosynthesis by plants. But light is also needed by plants which lack chlorophyll.
- Etiolation is an important effect of light intensity. Plant grown in darkness are somewhat with pale leaves, weak roots and poorly developed xylem and phloem.
- Longer wavelength of light (red light) enhance elongation cells and tissues whereas blue light tends to present elongation.
- Quality of light also effects flowering in plants.
- Beside quality duration of light (photoperiodism) also effects flowering in plants.
- The length of photoperiod may also effect differentiation of sex e.g. in *Cannabis sativa*, when 16 hour photoperiod is given flowering occurs within 4-6 week. About half plants are male and half females. However, same plants when provided with 8 hours photoperiod, enhanced and fast development occurs with flowering occurring within 3-4 weeks an about half the plants are hermaphrodites and half females.

8.6.3 Morphogenetic effect of water

Water is another important morphogenetic factor which influences growth, development and morphogenesis in plants. Water is one of the key requires for photosynthesis to occur. Deficiency of water results in phenomenon known as xeromorphy. On the contrary presence of excess amount of water results in small roots. Poor development of mechanical and vascular tissue, leaves become then stomata are reduced or absent. These traits are generally regarded as adaptation to survive in aquatic environment.

It has been found through several studies conducted by different scientist that there exists a definite correlation between the amount of water passing through the vascular tissue and the amount of vascular tissue developed.

Water also exerts other morphogenetic effects. Development of positive hydrostatic pressure generally occurs at early and rapid leaf growth and leads to formation of larger leaves. When the hydrostatic pressure is low at later stage smaller leaves are developed.

8.6.4 Morphogenetic effect of temperature

For all living organisms including plants temperature is a crucial factor which influences morphogenesis as well as metabolic processes occurring inside the organism. A peculiar feature about temperature is that most of the response mediated by temperature are equally affected by light.

The most important effect of temperature is on the growth of plant. Like any other living organisms plants also need an optimum temperature for growth and development. However, the optimum temperature may vary from one plant species to another, same plant may require different temperature during different phase of life cycle and moreover optimum temperature may be different for different region of plant.

As we study the concept of photoperiodism similarly there exists thermoperiodism. It refers to daily rhythm in reaction to temperature. If plant is provided with constant temperature throughout 24 hours, many plant show slow growth as compared to the growth obtained when the same plants are grown in comparatively warmer days and cooler nights.

A plant usually contains many buds out of which several buds do not develop. Significant amount of study has been conducted to find out which factors or growth substances decide that which bud will develop and which will not. Temperature is one the crucial factors which influences breaking of bud dormancy. Low temperature is considered to be an effective treatment for breaking dormancy.

Another morphogenetic effect of temperature is observed in form of vernalization. Vernalization is a process of providing low temperature for induction or acceleration of flowering. For some plants vernalization is a must for flower to occur. In Horticulture practice, seeds and seedlings are intentionally given treatment of low temperature to induce early flowering. In a study conducted by Burstrom (1956) it was found that exposure to high temperature results in reduction in length of root cells. This is due to shorter period of cell elongation.

8.6.5 Morphogenetic effect of mechanical factors

Physical factors such as compression, tension, bending and swaying, gravity also effect growth and development of plants. These factors are also referred to as mechanical factors. These factors may be called as mechanical but they are quite simple in character as compared to temperature and light. Mechanical factors influence morphogenesis indirectly by affecting the physiological process occurring in plants.

There are plants which display thigmotropism (response to contact). This type of response also involves morphogenetic changes. For example, when a tip of a tendril is touched by another branch or wire any other material tendril tends to coil around the wire or branch to provide support to the plant. This response involves enhanced growth of tendril in the direction of the support. When the stem of herbaceous plant bents, smaller cells are formed on convex side where as thick walled cells are formed on concave side. This difference is due to mechanical strain. Cells on convex side are under tension and cells on the concave side are under compression.

Gravity is another factor which influences growth of plants. Unlike other morphogenetic factors gravity is continuous, unchanging in intensity and also constant in direction. Downward growth of primary root, upward growth of main stem, etc are considered to be manifestations of geotropic growth reaction. Effect of gravity and light appears to be indistinguishable from one another. A change in relation to one generally produces a change in relation to the other. However unlike light (which directly affects morphogenesis) gravity exerts an indirect effect (by

influencing other factors) on plant. Gravity is also known to play an important role in distribution of growth substances.

8.6.6 Morphogenetic effect of chemical factors

Chemical factors also affect morphogenesis in plants. Normally chemical factor are known to execute their effect on physiological processes occurring inside an organism but beside this they do affect form and structure of plant. Till now we have studied about factors such as light, temperature, water, gravity which execute their effect on plant through external environment. But chemical factors influence morphogenesis through external as well as internal environment.
There is another peculiar feature of chemical factors that their effect can be localized to a particular part of plant instead of affecting the whole plant. Effect of chemical substance varies from time to time and from one phase of plant life cycle to another. Different elements are required by living organisms for several physiological functions. Elements such as O, N, K, Mg, C, Ca are considered to be macro elements. Since they are needed in larger amounts on the other hand elements such as B, Cu, Zn, Co, Mn are known as microelements (trace elements) as they are required in micro quantities by living organisms.

Nitrogen is essential constituent of all the proteins. Nitrogen is also reported to enhance growth of plants. In a study conducted by Burkholder and Mc Veigh in maize plant displayed better meristematic growth and enhanced length and diameter of stem when cultivated in presence sufficient quality of nitrogen. Nitrogen is also known to enhance differentiation in phloem with increased growth of sieve tube and vessels.

The ratio of C/N is also known to affect morphogenesis. Nitrogen is known to support vegetative growth hence plants having low C/N ratio tends to possess few flowers or fruits. Whereas when the ratio of C/N is high abundant flowering and fruiting occurs. Studies conducted have also related C/N ratio to the ratio of shoot length and root length.

Phosphorus is another element which is a prime constituent of nucleic acid (DNA and RNA). Besides being an important part of DNA and RNA, phosphorus also promotes cell division in roots but has little effect on elongation of stem. If we compare effect of phosphorus to that of nitrogen, elongation of stem is promoted by nitrogen but nitrogen does not directly affect cell division.

Calcium is known to support formation of cell wall. However, calcium is not directly a part of composition of cell wall but it produces its effect by bringing changes in cytoplasm. Zn is a trace element but is known to have an indirect effect on maintaining auxin in its active state. Boron is also required for cell wall formation. Deficiency of Boron causes hypertrophy and hyperplasia of tissue.

Plant hormones better known as plant growth regulators also control and coordinate morphogenesis in plants. Auxin and cytokinin remain to be the most significant plant hormones, along with them ethylene is crucial for fruit ripening, Gibberellic acid for germination. Almost all the morphogenesis response or growth shown by plants is mediated by one or the other hormone.

8.6.7 Morphogenetic effect of genetic factor

Genes are known to have specific response to a specific environment. We are very well familiar with George Mendel's law of genetics. In his first law called as Law of dominance he described how inheritance of genes governs formation of tall or short plants in *Pisum sativum*. Both types of plant (tall and short) can be easily differentiated from one another based upon their genetic composition. Transcription and translation of genes leads to synthesis of enzymes which directly regulate or control growth and morphogenesis in plants. Generally any morphogenetic trait is not entirely controlled or affected by a single gene but many genes or polygenes collectively affect morphogenesis. One of the key effects of genes on morphology is seen in extent of growth as well as on distribution of growth. Several examples are available where shape of leaves, flowers, fruits is inherited and controlled by gene expression.

Lamprent in his study found that in pea plant there is a long distance between first and second flower as compared to the total length of inflorescence. This is believed to be controlled by three genes. In corn grass due to a single gene dominant mutation results in formation of narrow leaves, many tillers and less number of male flower as compared to normal plant. In another plant *Aquilegia canadensis* a dwarf race with bushy and compact growth differs from normal plant by a single gene. There is another example of *Acetabularia* (marine alga) in which control of gene over form and morphology was determined. This alga has a branching, rhizoid base from this base rises a stalk which has a hat (umbrella-like structure) A single large nucleus is found to be located in the basal rhizoids.

There are two species of *Acetabularia* one is longer (*A. mediterranea*) and another shorter *(A. crenulata*). Both the species also differ in form of hat. In Hammering's grafting experiment stalk was excised (cut) from longer species and grafted onto the basal portion of shorter species, now a new hat formation will begin from the stalk, at initial stages the newly formed hat may look like the hat to the species to which stalk belongs but finally the hat formed was similar to the hat of species which contributed rhizoid (containing nucleus). Hence, it was clear that formation of hat in *Acetabularia* is controlled by gene present in nucleus (Fig. 8.5).

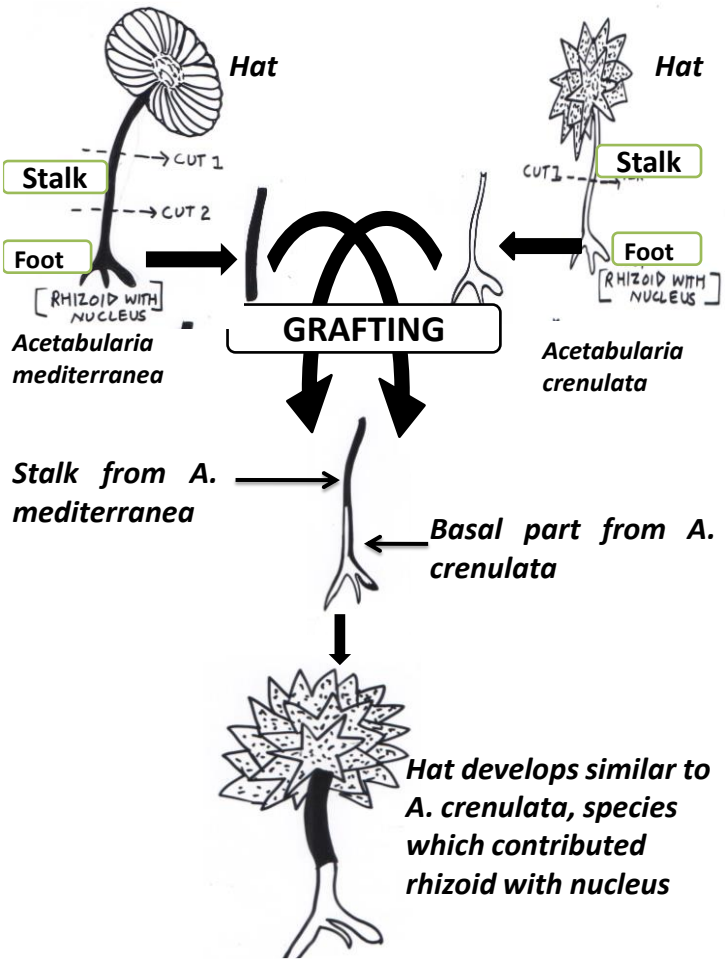


Fig. 8.5: Demonstration of development of Hat in *Acetabularia* to be under genetic control

Some plants develop perfect or complete flower having both male (staminate) and female (pistillate) flowers. Such flowers are also known as hermaphrodite or bisexual and when male and female flowers develop on the same plant i.e. some flowers will be male and some flowers will be female such plant is called monoecious and the condition is known as monoecism whereas when male and female flowers develop on separate plant as seen in case of animals and the plant is called dioecious and the condition is known as dioecism In this case a male plant will develop only male flowers and female plant will develop only female flowers. Some common example of dioecious is strawberry (*Fragaria*). These types of sexual development in flowers are controlled by specific gene. However, environmental factors equally contribute to development of sex of flowers.

Genes also play a crucial role in production and distribution of growth substances which in turn affects morphology of plant. Genes also control photoperiodic effect which regulates flowering in plants. As a result of gene mutation, the flower time and season may get altered.

Most plant are haploid i.e. two sets of chromosome in each nucleus. In some plants number of sets of chromosome is multiplied. Such plants are called polyploids (triploid with three set of genes, tetraploid with four set of genes and so on). Polyploids plants are believed to exhibits better growth in terms of leaves size, enhanced number and size of fruits and flower etc. But this increase is restricted to certain level only. Plants with ploidy level higher than tetra or pentaploid show negative growth in terms of number and size of leaves, flower, fruit and other growth parameters.

8.7 SUMMARY

1. Morphogenesis is defined as a process concerned with formation and development of whole plant, a part of plant or a specific structure.
2. During very early developmental stages polarity is established at the zygote stage.
3. Due to this polarity difference at both the ends of zygote is established according to which different structures are developed at different poles.
4. Axial polarity is most significant polarity pattern in plants which is represented by longitudinal axis which bears lateral organs such as lateral branches, roots, leaves and flowers.
5. Different parts of plant have different type of morphology.
6. This difference develops due to difference in growth pattern, growth rate and different dimension of growth.
7. These factors can be environmental such as light, temperature, water etc
8. Physical factors and mechanical factors also affect morphogenesis in plants.
9. Beside environmental factor, genetic factor (genes) also control and regulate morphogenesis.
10. Genes are considered to be the ultimate control agent of growth and development.
11. However, genes alone do not control growth instead they interact with prevailing environmental conditions to regulate growth.
12. Totipotency is the ability of a cell to give rise to different types of cells and eventually lead to regeneration of a complete plant
13. Totipotency is reconstruction or regeneration of complete plant from any tissue or organ.
14. Totipotency forms the basis of plant tissue culture through which large number of plants can be regenerated in comparatively shorter duration of time.
15. Meristematic cells divide and differentiate to form different types of cells with specific structure and function.
16. Differentiated cells loose their ability to divide.
17. Structural changes occurs inside a cell during the process of differentiation.
18. These changes may occur in cell wall, protoplasm or both.
19. Differentiated plant cells can undergo a process of dedifferentiation and can again become meristematic

20. Such dedifferentiated cells can again differentiate by a process known as redifferentiation.
21. There are two system under which induction of polarity in plants have been studied, they are root- rhizoid polarity and shoot-root polarity.
22. Root- rhizoid type of polarity is studied in Phaeophyceae and Pteridophytes.
23. Shoot-root polarity type of polarity is studied in higher plants.
24. Gradient of calcium, potassium and sodium across the cell membrane play an important role in development of polarity.
25. Once a polarity is established, it does not get altered naturally.
26. Changes in a part of plant significantly affect morphogenesis of one or more other parts of plant.
27. This process is called as correlation.
28. Different factors called as morphogenetic factors affect growth and development of plants.
29. Light is one of the most crucial factors for growth, development and survival of plants.
30. Light influences flowering, height, strength of stem, development of xylem and phloem etc.
31. Plants also need an optimum temperature for growth and development. However, the optimum temperature may vary from one plant species to another.
32. Physical factors such as compression, tension, bending and swaying, gravity also effect growth and development of plants.
33. Chemical factors also affect morphogenesis in plants. Several elements are required by plants for normal formation and growth of tissues and organs.
34. Nitrogen enhances differentiation in phloem and increases growth of sieve tube and vessels.
35. The ratio of C/N is also known to affect morphogenesis.
36. Phosphorus also promotes cell division in roots.
37. Boron is also required for cell wall formation.
38. Genes remain to be the most crucial factor governing growth and development as they control and regulate complete morphogenesis of plant stating from establishment of polarity, vegetative growth and reproductive growth.

8.8 GLOSSARY

Acclimatization: Acclimatization is the process in which an individual organism or plant adjusts to a gradual change in its environment (such as a change in temperature, humidity, photoperiod, or pH), allowing it to maintain performance across a range of environmental conditions

Aromatic plants: Aromatic plants are those which produce and exude aromatic substances (largely ether oils), which are used in making perfumes, in cooking, and in the food, pharmaceutical, and liquor industries

Callus: Callus is defined as an unorganized tissue mass growing on solid substrate

Cryopreservation or cryoconservation: Is a process where organelles, cells, tissues, extracellular matrix, organs or any other biological constructs susceptible to damage caused by unregulated chemical kinetics are preserved by cooling to very low temperatures (typically -80°C using solid carbon dioxide or -196°C

Endangered plant: An endangered plant is a species which has been categorized by the International Union for Conservation of Nature and Natural Products (IUCN) in Red List as likely to become extinct

Etiolation: Etiolation is a process in flowering plants grown in partial or complete absence of light. It is characterized by long, weak stems; smaller leaves due to longer internodes, and a pale yellow color

Genome: The haploid set of chromosomes in a gamete or microorganism, or in each cell of a multicellular organism. It is basically the complete set of genes or genetic material present in a cell or organism

Horticulture: Horticulture is the branch of agriculture that deals with the art, science, technology, and business of growing plants

Hybrid Plants: A hybrid plant is produced when plant breeders intentionally cross-pollinate two different varieties of a plant, aiming to produce an offspring, or hybrid, that contains the best traits of each of the parents

Hydrostatic pressure: Hydrostatic pressure, termed turgor pressure, is generated in plant cells because water moves by osmosis across semipermeable membranes

Hyperplasia: The enlargement of an organ or tissue caused by an increase in the reproduction rate of its cells

Hypertrophy: The enlargement of an organ or tissue from the increase in size of its cells

Micropyle: A small opening in the surface of an ovule, through which the pollen tube penetrates, often visible as a small pore in the ripe seed

Photoperiodism: Photoperiodism is the physiological reaction of organisms to the length of day or night. It occurs in plants and animals. Photoperiodism can also be defined as the developmental responses of plants to the relative lengths of light and dark periods

Programmed cell death: Programmed cell death (PCD) is the death of a cell in any form, mediated by an intracellular program. PCD is carried out in a regulated process, which usually confers advantage during an organism's life-cycle

Rare species: A rare species is a group of organisms that are very uncommon, scarce, or infrequently encountered

Secondary metabolites: Secondary metabolites are compounds produced in other metabolic pathways that are not directly involved in the normal growth, development, or reproduction of an organism

Somaclonal variation: Somaclonal variation is the variation seen in plants that have been produced by plant tissue culture. Chromosomal rearrangements are an important source of this variation

Stimulus: A thing or event that evokes a specific functional reaction in an organ or tissue

Thallus: Thallus is a plant body that is not differentiated into stem and leaves and lacks true roots and a vascular system

8.9 SELF ASSESSMENT QUESTION

8.9.1 Choose the correct option:

1. Axial polarity represents:
   a) Longitudinal axis with lateral branches and roots
   b) Horizontal axis with lateral branches and roots
   c) Both longitudinal and horizontal axis with branches roots and flower.
   d) None of the above

2. Which of the following is not true?
   a) Polarization is related to polarity
   b) BEP develops from gradient of ions.
   c) Ca^{++} ions is most crucial ion for development of polarity
   d) All of the above

3. Rate of photosynthesis:
   a) Infinitely increase with increase in intensity of light
   b) Decrease with increase in intensity of light
   c) Increase with increase in intensity of light to a certain extent
   d) There is no effect of intensity of light on photosynthesis

4. Phosphorus:
   a) Promotes cell division in roots and enhance stem elongation
   b) Inhibits cell division in roots and enhance stem elongation
   c) Promotes cell division in roots and has little effect on stem elongation
   d) Inhibits cell division roots & has little effect on stem elongation

5. Which of the following aspect of light influences morphogenesis:
   a) Duration
   b) Quality
   c) Intensity
   d) All of the above

6. Which of the following statement is false:
a) Temperature significantly affects bud breaking
b) Related growth occurs when insufficient light is supplied
c) All the morphogenetic factor acts independently
d) Blue light prevents cell elongation in plants

7. Choose the correct statement smaller:
a) Under low hydrostatic pressure smaller size leaves develop
b) Under high hydrostatic pressure larger size leaves develop row
c) Hydrostatic pressure has no role in controlling size of leaves
d) Both a and b are correct

8. Study the following statement and choose the correct one:
1. Different parts of same plant may respond differently to same temperature.
2. Thermoperiodism affects flowering but has no effect on fruit setting.
3. Shorter wavelength of light enhances elongation of cells
4. Exposure of high temperature results in reduction in length of root cells
a) Only 2 is correct
b) 1 and 2 are correct
c) 1, 2 and 4 are correct
d) 1, 3 and 4 are correct

9. Which of the following can be used as explant in plant tissue culture:
a) Leaves and nodes
b) Ovule and anther
c) Cotyledons
d) All of the above

10. Totipotency is:
a) Regeneration of complete plant from meristematic cells only
b) Regeneration of complete plant from any tissue or organ
c) Regeneration of complete plant from somatic cells only
d) Regeneration of complete plant from pluripotent cells

11. Callus is:
a) Undifferentiated mass of cells
b) Differentiated mass of cells
c) Redifferentiated mass of cells
d) None of the above

12. Which of the following is not true?

a) Calcium may not be a constituent of cell wall but still it influence cell wall formation
b) Differentiation of phloem and growth of sieve tube and vessels increase in presence of high level of nitrogen
c) Zn is a macronutrient which maintains auxin in its active state
d) Phosphorus promotes cell division in roots but has no effect on elongation of cells of stem.

13. Ratio of C/N significantly affects morphogenesis in plants. Study the following statement and choose the correct option:

1. When ratio of C/N is lower flowering and fruiting both are accelerated
2. When ratio of C/N is high abundant flowering and fruiting occurs
3. C/N ratio also affects length of root and shoot
4. C/N has no effect on length of root and shoot

(a) 1 and 3 are correct
(b) 1 and 4 are correct
(c) 2 and 3 are correct
(d) 2 and 4 are correct

14. A differentiated cell:

a) Is pluripotent
b) Can divide and produce new cells
c) Cannot divide and produce new cells
d) Can give rise to different type of cell

15. Choose the correct sequence:

a) Differentiation → Undifferentiated state → Dedifferentiation → Redifferentiation
b) Dedifferentiation → Undifferentiated state → Redifferentiation → Differentiation
c) Dedifferentiation → Differentiation → Redifferentiation → Undifferentiated state
d) Differentiation → Dedifferentiation → Undifferentiated state → Redifferentiation

16. Dedifferentiation:

a) Is found to occur during primary and secondary growth of plants and during the process of healing of wounds?
b) Is found to occur during secondary growth of plants, during the process of healing of wounds?
c) Is found to occur during primary growth of plants and during the process of healing of wound?
d) Is found to occur during primary and secondary growth of plants?

17. Which of the following is advantage of plant tissue culture?
a) Regeneration of large number of plants
b) Conservation of endangered species
c) Production of virus, disease resistant plants
d) All the above

18. Differentiated cells:
a) Does not possess the ability to divide but can undergo the process of dedifferentiation.
b) Possess ability to divide and can undergo process of dedifferentiation
c) Do not divide but can undergo the process of redifferentiation
d) Posses ability to divide and undergo the process of dedifferentiation

8.9.2. State whether following statements are true or false

1. Polarity is unidirectional.
2. During polarization zygotic division in *Arabidopsis* is asymmetric.
3. Root-rhizoid system is found in higher plants.
4. Once established polarity can be easily altered.
5. Vernalization means treatment of low temperature to induce / enhance flowering.
6. One morphogenetic factor significantly influences other morphogenetic factor.
7. Plants growing in shade comparatively develop smaller root system.
8. Plants which lack chlorophyll do not need light.
9. Photoperiodism has no effect on flowering in plants.
10. Thermoperiodism significantly influences fruit setting.
11. Treatment of high temperature is a good method of breaking bud dormancy.
12. Plants with low C/N ratio exhibit extensive flowering.
13. Differentiated cells lose the ability to divide.
14. Caulogenesis is process of regeneration of shoots.
15. In indirect regeneration no intermediate callus is formed.

8.9.3: Fill up the blanks

1. _____is a phytochrome which induces polarity in moss and ferns.
2._____ and _____ are mechanical factors influencing morphogenesis.
3. _____is a mass of undifferentiated cells.
4. Preservation of cells and tissues under low temperature is called_____
5. Upper part of suspensor cells is called as _____
6. Longer wavelength of light _____elongation of stems.
7. _____elements are required in very less quantity for growth and development of plants.
8. Nitrogen is essential component of _____

9. In plants _____cells are also called as meristematic cells.
10. The inherent property of plant cell to give rise to a complete plant is called _____
11. Phenomenon of mature cells reverting to meristematic state is called as _____
12. Formation of shoot from explants or callus is called as _____
13. _____ is known as Father of plant tissue culture.

8.9.4: Very Short answer type question

1. What do you understand by the term polarity?
2. What is bioelectric potential?
3. What is the cause of xeromorphy?
4. Mention about significance of vernalization in horticulture?
5. On what parameter effect of gravity differs from effect of other morphogenetic factors?
6. How does embryogenesis differ from rhizogenesis?
7. Define caulogenesis?
8. Define the term correlation with reference to morphogenesis in plants?
9. How does hydrostatic pressure affect growth of leaves?

8.9.1 Answers key: 1-(a), 2-(d), 3-(c), 4-(c), 5-(d), 6-(c), 7-(d), 8-(c), 9-(d), 10-(b), 11-(a), 12-(c), 13-(c), 14-(c), 15-(d), 16-(b),17-(d), 18-(a)

8.9.2 Answers key: 1-(T), 2-(T), 3-(F), 4-(F), 5-(T), 6-(T), 7-(T), 8-(F), 9-(F), 10-(T), 11-(F),12-(F), 13-(T), 14-(T), 15-(T)

8.9.3 Answers key: 1- (Biliportein), 2-(Gravity and Pressure), 3-(Callus), 4-(Cryopreservation), 5- (Hypophysis), 6- (Enhances), 7- (Trace), 8- (Protein), 9- (Pluripotent), 10-(Totipotency), 11- (Dedifferentiation), 12 - (Caulogenesis), 13- (G.Haberlandt)

8.10 REFERENCES

- Souter M and Lindsey K (2000). Polarity and signaling in plant embryogenesis. *Journal of experimental Botany* 51: 971-983.
- Nick, P. and Furuya, M. (1992). Induction and fixation of polarity- early stages in plant morphogenesis. *Development, growth and differentiation* 34:115-125.
- Medvedev, S.S. (2011). Mechanisms and physiological role of polarity in plants. *Russian Journal of Plant Physiology* 59:502-514.
- Ramage, M.C. and Williams, R.R. (2002). Mineral nutrition and plant morphogenesis. *In vitro Cell developmental Biology* 38:116-124.

8.11 SUGGESTED READINGS

- *Plant physiology*. Frank B. Salisbury and Cleon W. Ross. Thomson Worsworth.
- *Plant physiology*. Lincoln Taiz and Eduardo Zeiger. Sinauer Associates, Inc. Publishers.
- *Textbook of Plant Biotechnology*. H.S Chawla.

8.12 TERMINAL QUESTIONS

8.12.1. Short answer type question

1. Differentiate between differentiation, dedifferentiation and redifferentiation?
2. Briefly describe about zygotic polarization in *Fucus*?
3. What is thermoperiodism ? How does it differ from photoperiodism?
4. Mention about mechanical plant response thigmotropism?
5. Describe about the effect of temperature as morphogenetic factor on growth and development of plant?
6. Enlist few applications of plant tissue culture?
7. Define totipotency? Mention the significance & importance of totipotency?
8. Mention about different aspects of light which influence growth of plants?
9. Length of photoperiod can also affect sex of plant. Cite a suitable example in support of the statement?
10. How does monoecious flowers differ from dioecious flowers?

8.12.2. Long answer type question

1. Define polarity? Citing suitable examples mention about shoot root and root- rhizoid?
2. Explain the meaning of morphogenetic factors. Mention about different types and function of morphogenetic factors?
3. Define totipotency? Elaborate upon the role of totipotency in plant tissue culture?

UNIT-9 PHYSIOLOGY OF FLOWERING

9.1 OBJECTIVES

After reading this unit students will be able to understand-

- Physiological mechanism for flowering in plant
- Effect of photoperiod on flowering in plants
- Effect of quality of light and phytochrome on flowering
- Role of temperature in regulating flowering
- Practical utilization / application of photoperiodism and vernalization

9.2 INTRODUCTION

After attaining certain growth the plants begin to flower. Flower is reproductive organ of plants and most of the plants utilize the process of flowering as a mode of reproduction. Flowering is followed by pollination, fertilization which ultimately leads to formation of fruits/seeds. The time taken by a plant to flower varies from species to species. For example so many fruiting trees which you commonly see around such as mango tree, guava tree etc take many years before they begin to flower and fruit. Many herbs begin to flower in few months. Such plants have very short vegetative phase and the reproductive phase (flowering) begins early. Different plant species may exhibit different pattern of growth before they begin to flower, for example corn plants does not begin to flower until they have produced certain number of leaves. Plant of bamboo takes several years (more than 30 or 50 years depending upon species) to flower. Flowering in plants crucially depends upon season. Each plant displays a strict and definite pattern of their vegetative and reproductive growth depending upon season. It means that every plant require specific seasonal/ environmental condition before they begin to flower. In this chapter you will come to know how seasons, length of day and night control flowering in plants.

9.3 BASIC CONCEPT OF FLOWERING

After attaining certain vegetative growth, plants undergo structural and functional changes and reproductive growth begins leading to flowering. Flowering in plants is influenced by various experimental factors. Plant will respond to environmental factors (for flowering) only when the plant has reached certain stage of maturity. It means that if a plant is provided with all the favourable conditions required for flowering but if the plant is not mature enough it will not flower. Flowering crucially depends upon developmental status of plants among different environmental factors, length of days, quality and intensity of light and temperature are among the most important factors which control flowering in plants.

After sufficient amount of vegetative growth, if plant is provided with suitable environmental conditions, the development of plant shifts towards reproductive growth. Several changes occur

at metabolic level including changes in kind and amount of hormones produces, production of metabolites required for reproductive growth, etc. Stimulus for flowering is perceived by leaves (discussed in detail later in the chapter), the flowering stimulus from leaves in form of hormones is transferred from leaves to shoot tips / nodes. Formation of floral buds occurs at shot tips which ultimately results in flowering (Fig.9.1).

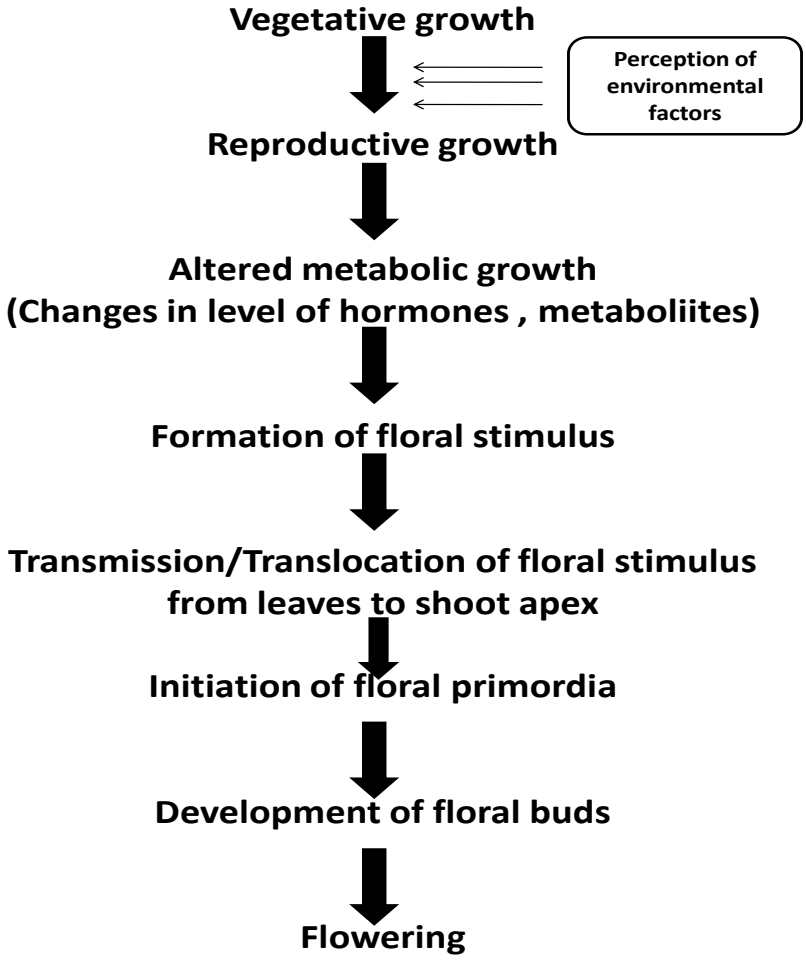


Fig. 9.1: Outline of basic process leading to flowering in plants

9.4 PHOTOPERIODISM

The term photoperiodism was suggested by Garner and Allard (1920) and the term photoperiodism refers to effect of length of day and night on growth and development of plants. Photoperiod is the favourable day length required by plants mainly for flowering to occur.

Garner and Allard (1920) first of all reported the phenomena of photoperiodism. They observed that mutant tobacco plant (Maryland mammoth) and soyabean (*Glycine max*) follow seasonal

dependent pattern of flowering. Soyabean plant flower only in late summer irrespective of the time when the seeds were sown. Effect of various environmental factors such as nutrition, soil moisture on flowering has been analyzed and it was found that none of these factors played a key role in regulating flowering. When plants were placed in dark and provided with shorter light period, flowering was obtained in plants. After this, similar experiments were conducted on different plant species under different photoperiods (short - day, long - day) and found that it was length of day which control flowering in plant (Fig.9.2).

Depending upon the photoperiod requirement plants can be classified into these groups.

I. Short- day plant, II . Long-day plant, III , Day-neutral plants

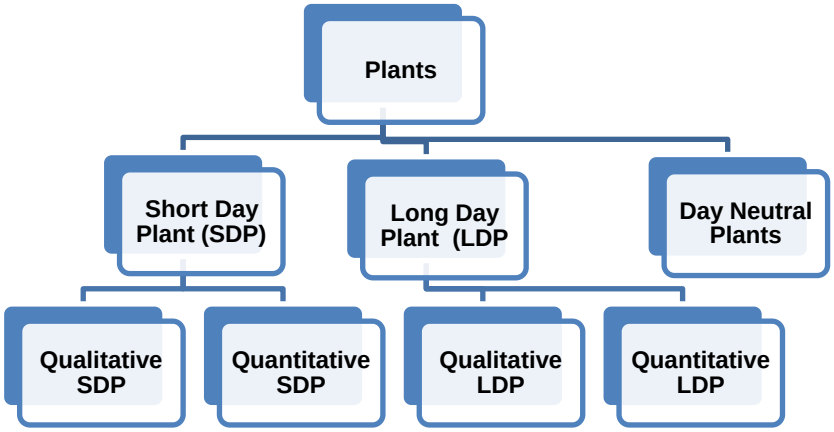


Fig. 9.2: Classification of plants based upon photoperiod requirement for flowering

1. Short - day flowering plants

These are those plants which flower when length of day is shorter than a critical period. These plants need a day length shorter than a critical period to flower. If day length exceeds a critical value then short-day flowering plants fail to flower. For example in soyabean day length of more than 12 hours effectively reduced number of flowers. These plants are also called as long night plants.

Characteristic features of short-day flowering plants

(1) Short - day flowering plants need continuous /uninterrupted long period of darkness to flower. Hence, you can say that in SDP length of day is not as important as period of darkness.

(2) SDP will fail to flower if the continuous period of darkness is interrupted by weak intensity of light given for some time. The plant will also not flower even if a flash of light is given during period of darkness. Moreover, even if weak intensity (dim light) is given to plant for sometimes during the period of darkness flowering is inhibited (Fig. 9.3).

(3) These plants can be made to flower in long day conditions as well by transferring to plants to darkness for sufficient duration.

(4) It is obvious that length of night is more crucial for flowering in SDP than day length. If plants are kept in complete darkness and provided with sucrose externally. They exhibit normal pattern of flowering indicating that the photoperiod (day length) is required only for the process of photosynthesis (Hillman, 1959), (Fig.9.3).

(5) SDP do not flower under alternating cycles of lightness and darkness. The period of darkness which is needed by SDP for flowering showed be continuous. Suppose if a plant requires 16 hrs of darkness for flowering and the plant is given 16 hrs of darkness but not continuously instead in four instalments of 4 hrs each. Now the total period of darkness is 16 hrs but the plant fails to flower because period of darkness is not give continuously (Fig.9.3). Some examples of SDP are: tobacco (*Nicotiana tabacum*), soyabean (*Glycine max*), strawberry (*Fragaria*), coffee (*Coffea arabica*), rice (*Oryza sativa*), *Bryophyllum*, maize (*Zea mays*).

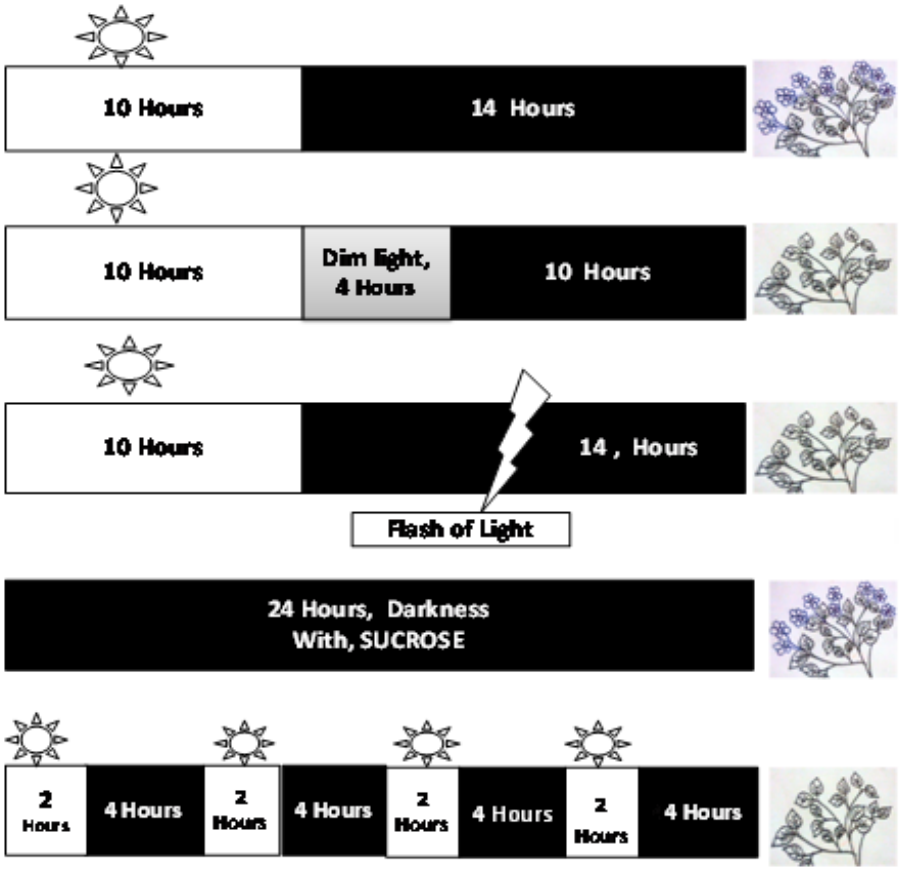


Fig. 9.3: Effect of different photoperiod conditions on flowering in SDP

Types of SDP

Qualitative short - day plants: Also called as absolute or obligatory short-day plants. These plants will flower only under short- day conditions and will never flower under unsuitable (other then absolute short- day) photoperiod e.g. strawberry (*Fragaria*), coffee (*Coffea arabica*), maize (*Zea mays*).

Quantitative short-day plant: Also called as facultative short- day plant. These plants best flower under short-day conditions. However, they may also flower under long- day conditions but with delayed flowering e.g. cotton (*Gossypium hirsutum*).

Short long-day plants: Plant which flower when placed under short -day conditions followed by long days e.g. white clover (*Trifolium repens*).

2. Long-day Flowering plants

These are those plants which flower when provided with longer photoperiods. They need day length longer than a critical period to flower. More than, the requirement of longer photoperiod these plants require short period of darkness for flowering because larger period of darkness inhibits flowering in these plant. Hence, long-day plants are also called as short night plants.

Characteristic features of long-day flowering plants:

(1) Long- day flowering plants flower best in continuous light. They either need little or no darkness for flowering.
(2) Long period of darkness exhibits an inhibitory effect on flowering in long- day plants.
(3) Long day- plants can flower in short -day (days shorter and nights longer) conditions if the period of darkness is interrupted by flash of light.
(4) Unlike short-day plants long- day plants flower normally if light and dark period are provided alternately. Flowering occurs because dark period is not maintained for longer duration and hence cannot exhibit its inhibitory effect on flowering e.g. pea (*Pisum sativum*), peppermint (*Mentha piperita*), barley *(Hordeum vulgare*), rye Grass (*Lolium* spp.), wheat (*Triticum aestivum*), radish (*Raphanus sativus*).

Long- days plants flower when provided with a photoperiod of more then critical length. Period of darkness is believed to have somewhat inhibitory effect on flowering, hence if period of darkness extends beyond a limit flowering is inhibited. However, if a flash of light is given during the period of darkness, the inhibitory effect of darkness is compensated and their plant exhibits normal pattern of flowering. LDP also exhibits flowering kept under continuous light (without any period of darkness). LDP will also flower if exposed to alternate period of light and darkness (Fig.9.4).

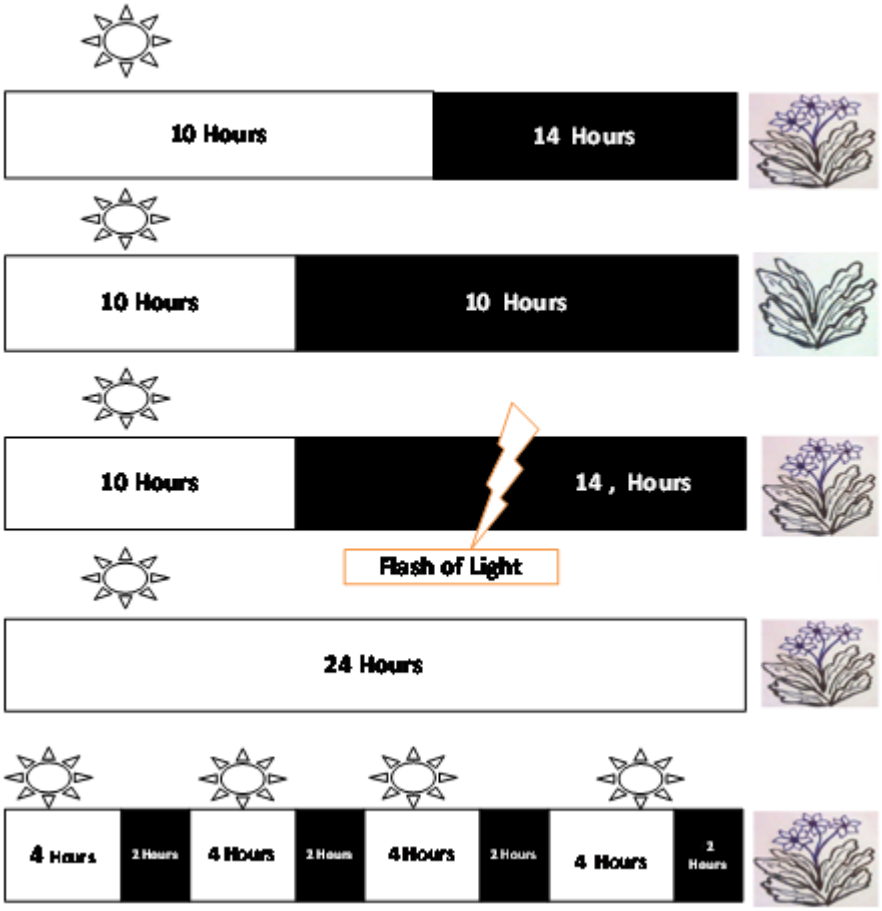


Fig. 9.4: Effect of different photoperiod conditions on flowering in LDP

Types of LDP

Qualitative long-day plants: These plants are also called as absolute long-day plants or obligatory long day plants. These are those plants which flower under only under long day conditions and will never flower under unsuitable / improper) photoperiod e.g. oat (*Avena sativa*), radish (*Raphanus sativus*)

Quantitative long-day plants: Also called as facultative long day plant. These are basically long day flowering plant and flower best under long day conditions. However they may also exhibit flowering under short day delayed conditions e.g. turnip (*Brassica rapa*), garden pea (*Pisum sativum*), spring wheat (*Triticum aestivum*).

Long short-day plant: plants which exhibited flowering when provided with long days followed by short day treatment / exposure e.g. Aloe (*Aloe bulbillifera*), Kalanchoe (*Kalanchoe laxiflora*).

3. Day- neutral Plants

These are those plants which can flower in all photoperiods. There is no seasonal preference for flowering in these plants e.g. tomato (*Lycopersicon esculentum*), bean (*Phaseolus* spp.), cucumber *(Cucumis sativus*).

Normally short-day plants flower when day length is shorter than 11 hours and for long-day plants day length period of 14-16 hours is sufficient for flowering (Warner, 2006). However, day length may vary from species to species.

4. Ambiphotoperiodic plants: These are those plants which flower when photoperiods are shorter than certain length or longer than certain period. *Madia elegans* flowers when photoperiod is either shorter than 14 hours or longer than 18 hours.

5. Intermediate-day plant: These are those plants which flower when exposed to dark period of certain length. For example *Salsola komarovii* flower when provided with darkness period of 12 hours. The plant fails to flower of under short as well longer night length. You can consider these plants as modification of short-day plants.

Quality of light

Green color of visible spectrum have been reported to be ineffective in inducing flower and orange red color (wavelength 660 nm) is by for the most effective wavelength to inhibit flowering. Blue light is known to induce poor flowering. Red portion of the visible spectrum with wavelength 580nm-680nm have been found to be the best portion of spectrum to initiate flowering in short day as well as long-day plants.

Site of photoperiodic induction

M.K. Chailakhyan demonstrated that stimulus for flowering was perceived by leaves. He utilized *Chrysanthemum* plant for the purpose. Leaves were removed (defoliated) from above portion of the plant. After defoliation (from upper part) plants were divided in four groups A,B,C,D (Fig.9.5).

Group A plants were exposed to long days. Upper defoliated portion of group B plants was exposed to long days where lower leafy region was given short day treatment. Contrary to this, upper defoliated portion was provided with long days. Group D plants were provided with short day treatment. Plants of Group B and D exhibited development of flower since leaves in both the group were exposed to short day photoperiod. Leaves perceived the stimulus for flowering, synthesized flowering hormone, which was transported to defoliated upper part and as a result flowering occurred (Fig.9.5).

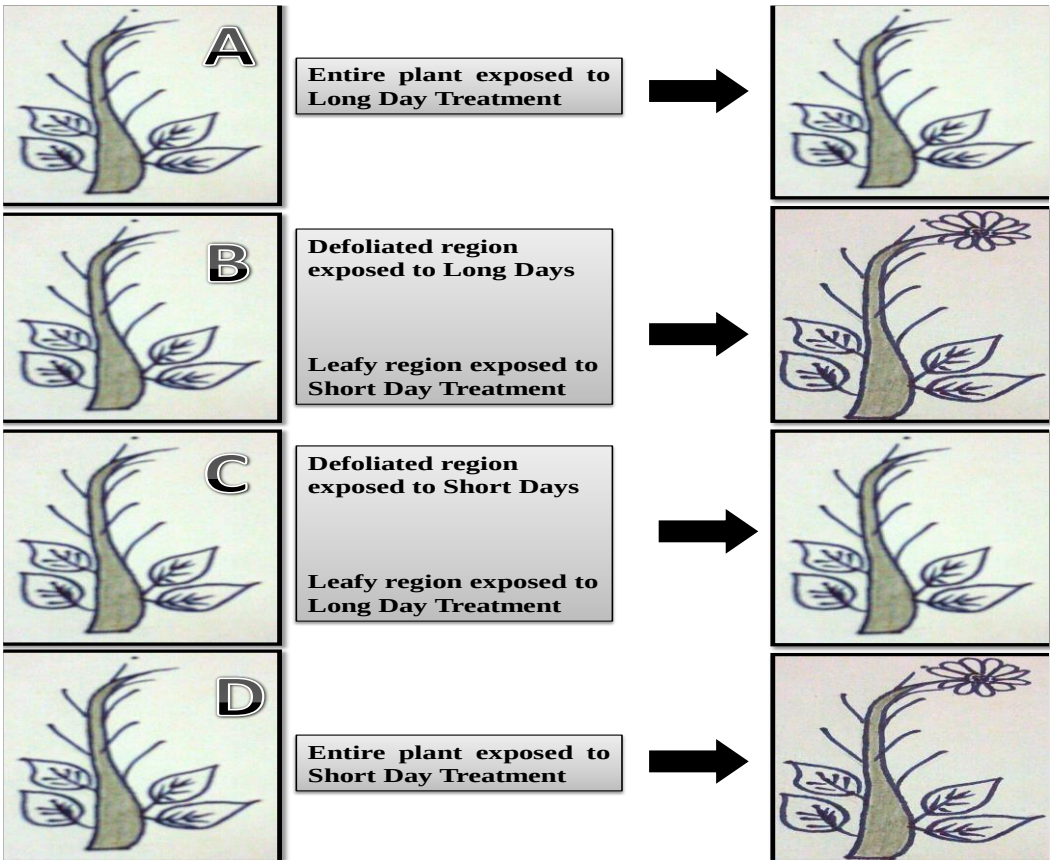


Fig. 9.5: Experiment to demonstrate that flowering stimulus is perceived by leaves

In an another experiment three short day plant (same species) were taken. In the first plant no changes were done, whereas in second plant the plant was completely defoliated and in third plant only one leaf was left intact rest all leaves were removed. All the three plants were exposed to appropriate conditions required by short-day plants to flower. As you might expect in normal plants (in which no changes were done) exhibited flowering and the plant which was completely defoliated failed to develop flowers. Interestingly, the third plant in which only one leaf was left also exhibited normal flowering. Observations made from the above experiment confirmed that flowering stimulus is perceived by leaves and also even a single leaf is sufficient to perceive the flowering stimulus which will make the whole plant flower (Fig. 9.6).

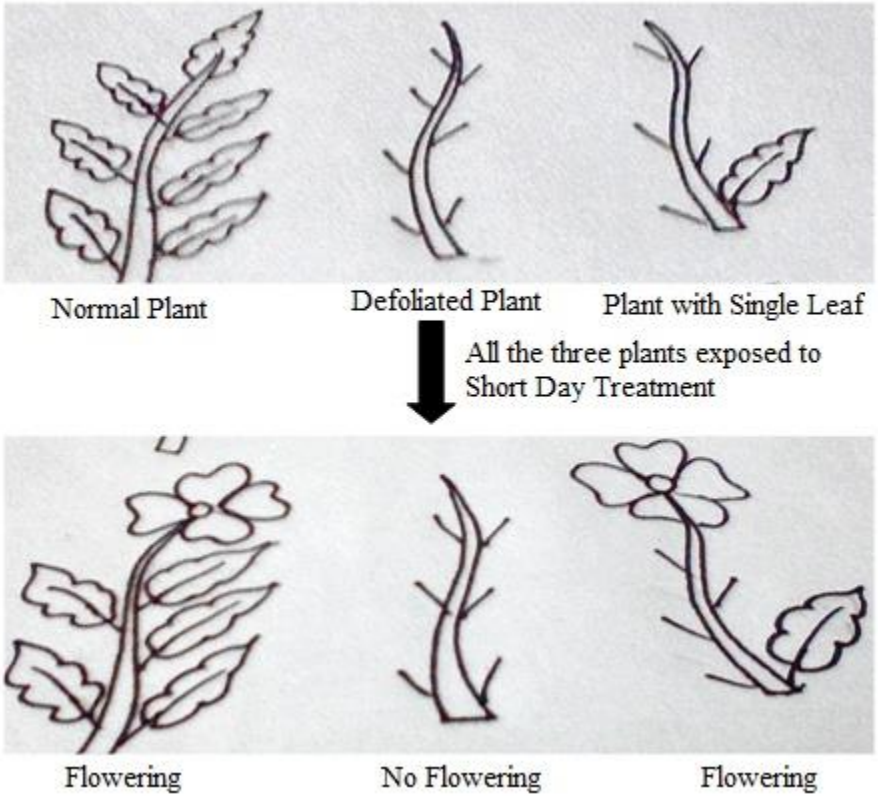


Fig. 9.6: Experiment to demonstrate that flowering stimulus is perceived by leaves

Flowering Hormone

There has been an ever existing evidence of presence of a flowering hormone in plants. The presence of flowering hormone and its translocation has been demonstrated through grafting experiment.

Experiment I: In a grafting experiment one short day plant was provided with short day treatment and other with long day treatment. Obviously plant exposed to short day treatment exhibited flowering and plant exposed to long day failed to flower.

SDP which was given long day treatment which did not flower could be made to flower if grafted with a SDP exposed to proper photoperiod. From the result obtained you can clearly predict that the plant exposed to proper photoperiod synthesized a flowering hormone which was transferred to the grafted plant (exposed to long day) which resulted in flowering response (Fig. 9.7)

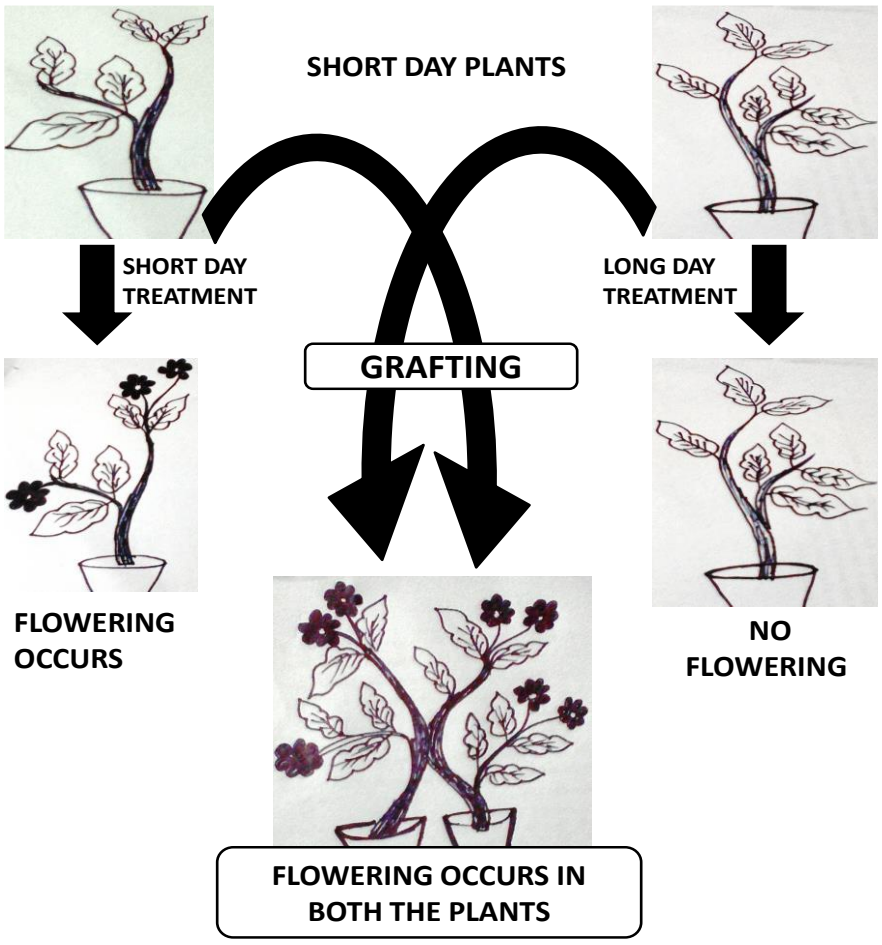


Fig. 9.7: Grafting experiment to show translocation of flowering stimulus

Experiment II: In an another experiment several short-day plants were grafted with one another (as shown in Fig. 9.8). All the plants were given long day treatment except one leaf of one plant (which was given short day treatment). Flowering was observed in all the plants. The results obtained shows that if single leaf receives proper stimulus, the flowering hormone will be synthesized and was transferred from one plant to another. Hence, even if one leaf of the plant receives correct day length, the plant exhibits flowering regardless of the conditions surrounding rest of plant.

Experiments showed that flowering hormone exists in plants and also stimulus for synthesis of flowering hormone is perceived by leaves which is transmitted to buds for flowering to occur. Chailakhyan (1936) has named the flowering hormone as florigen. He gave the name florigen to unknown chemical stimulus which acts as floral inducer.

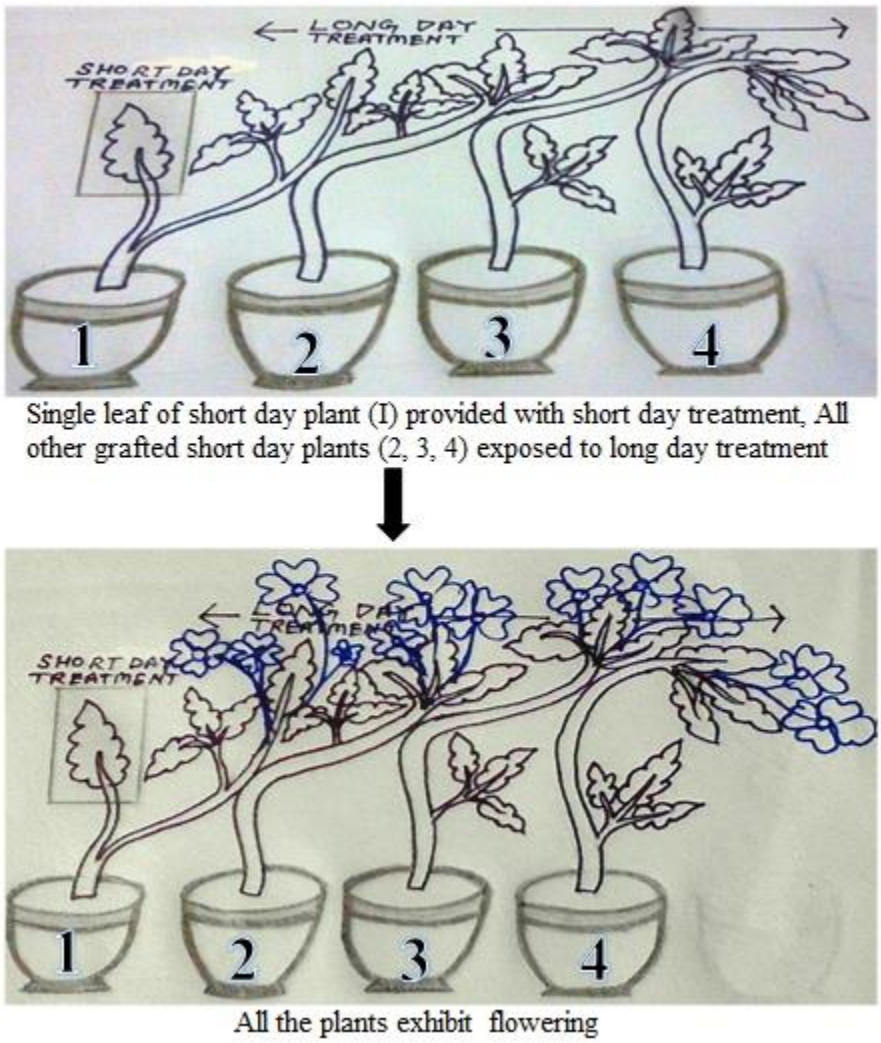


Fig. 9.8: Grafting experiment to show translocation of flowering stimulus

Significance of Photoperiodism

(1) Photoperiodism determines the flowering season of a plant.

(2) Knowledge of photoperiodism can be utilized in keeping plant in vegetative phase to obtain high yield of tubers rhizomes etc. or the plants may be maintained in reproductive stage to yield more flower and fruits.

(3) Annuals can be grown more than once in a year by regulating photoperiod.

(4) By increasing light hours winter dormancy and autumnal leaf fall can be prevented.

(5) By providing requirement photoperiod plants can be made to flower through out the year under green house.

(6) Knowledge of photoperiodism is also useful in setting up of gardens, orchards etc.

The difference between photoperiodism and vernalization is given in Table 9.1 and difference between short-day plants and long-day plants has been given in Table 9.2.

Table 9.1: Differences between Photoperiodism and Vernalization

S.No.	Photoperiodism	Vernalization
1.	It is response of length of light and dark on growth and development of plants	It is acceleration of flowering in plants by providing chilling treatment
2.	Stimulus for photoperiodism is perceived by leaves	Stimulus is perceived by actively dividing cells (mostly meristems)
3.	It prepares the plant for flowering and also initiates Flowering	It only prepares the plant for flowering
4.	It is mediated via florigen	It is mediated via vernalin
5.	Effect of photoperiod once perceived cannot be reserved	Effect of vernalization can be reserved by providing high temperature to plants
6.	Photoperiodic stimulus can be transferred from plant to another by grafting	It cannot be passed through grafting except in *Henbane*

Table 9.2: Differences between short-day plants and long-day plants

S.No.	Short -Day Plant	Long- Day plant
1.	These plant exhibit flowering when exposed to day length shorter than a critical period	These plants flower when exposed to day length longer than a critical period
2.	Generally flower in early spring or autumn	Generally flower in spring or early summar
3.	Plants fail to flower if period of darkness is interrupted by flash of light	Flowering is stimulated if period of darkness is interrupted by flash of light
4.	Gibberellic acid has no effect on flowering in SDP	Gibberellic acid exerts an inductive effect on flowering

Phytochrome

Phytochrome is a pigment found in plants which is known to control development of plants. Phytochrome is a protein with chromatophore. The pigment exists in two interconvertable forms P_R and P_{FR}. The type of phytochrome which absorbs red light is called as P_R and the type of

phytochrome which absorbs far-red light if called $P_{FR.}$ P_R is red light sensitive and P_{FR} is far red light sensitive. Plant utilizes phytochrome to sense the seasonal changes in night length or photoperiod (Fig.9.9).

Phytochrome is mainly produced during darkness and firstly exists as P_R (P600). When exposed to light of wavelength 660nm (red) it is converted into P_{FR} (P730). P_{FR} can be reconverted to P_R if exposed to wavelength of 730nm. Among both the forms of phytochrome, P_R is biologically inactive whereas P_{FR} is biologically active. Many of the physiological changes occurring in plants such as pigmentation, hypocotyls - hook opening, unfolding of leaves (in seedlings), photomorphogenesis, photoperiodism and many others are influenced by phytochrome.

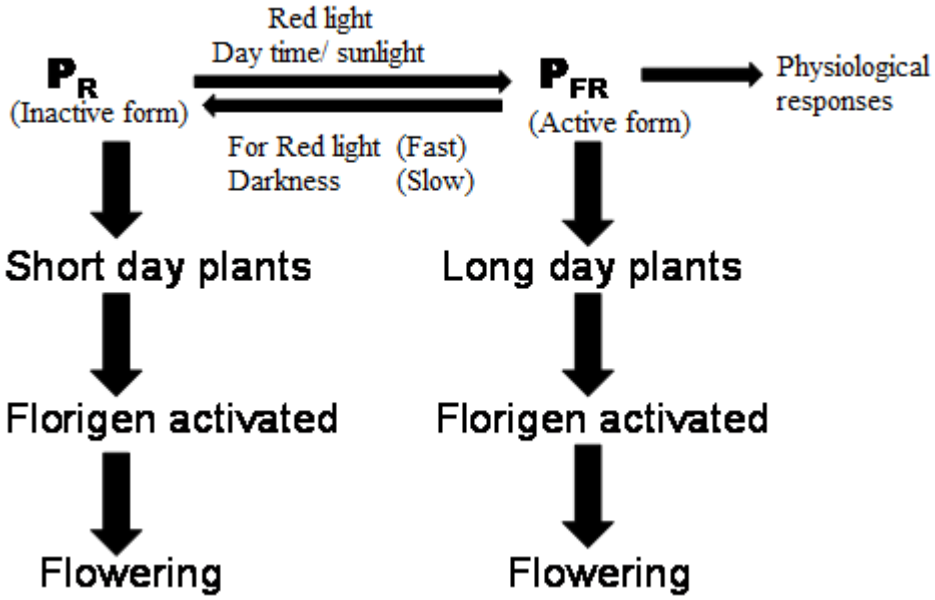


Fig. 9.9: Interconvertable forms of phytochrome

Effect of phytochrome on flowering in SDP

As you have already seen that short-day plants are those plants which show normal flowering when exposed to shorter photoperiod and longer period of darkness. It has already been discussed that longer period of darkness is crucial for flowering to occur in SDP. If the longer period of darkness is interrupted by red light, SDP fail to flower. However, if red light treatment is followed by far red, the inhibitory effect of red right is compensated and plants show normal flowering. If SDP are given alternate treatment of far and far red light then the treatment given in the last will show its effect (Fig.9.10).

Effect of phytochrome on flowering in LDP

We have already discussed that long-day plants are those which flower when provided with longer period of light and shorter period of darkness. You have also seen that longer period of

darkness has an inhibitory effect on flowering in LDP. If LDP is exposed to longer period of darkness it will not flower. However, this longer period of darkness is interrupted by red light, plants show normal flowering as because the period of continuous darkness is not maintained. Red light breaks the longer period of darkness into two shorter periods and hence period of darkness loses its inhibitory effect and flowering occurs. But if red light is followed by far red, the effect of red light is counter acted by far red and red light does not show its effect. As a result, the longer period of darkness can maintain its inhibitory effect and plant fails to flower. As seen in the case of SDP, if Red and far red light are given alternately than the last treatment provided to the plant will exert its effect (Fig.9.10)

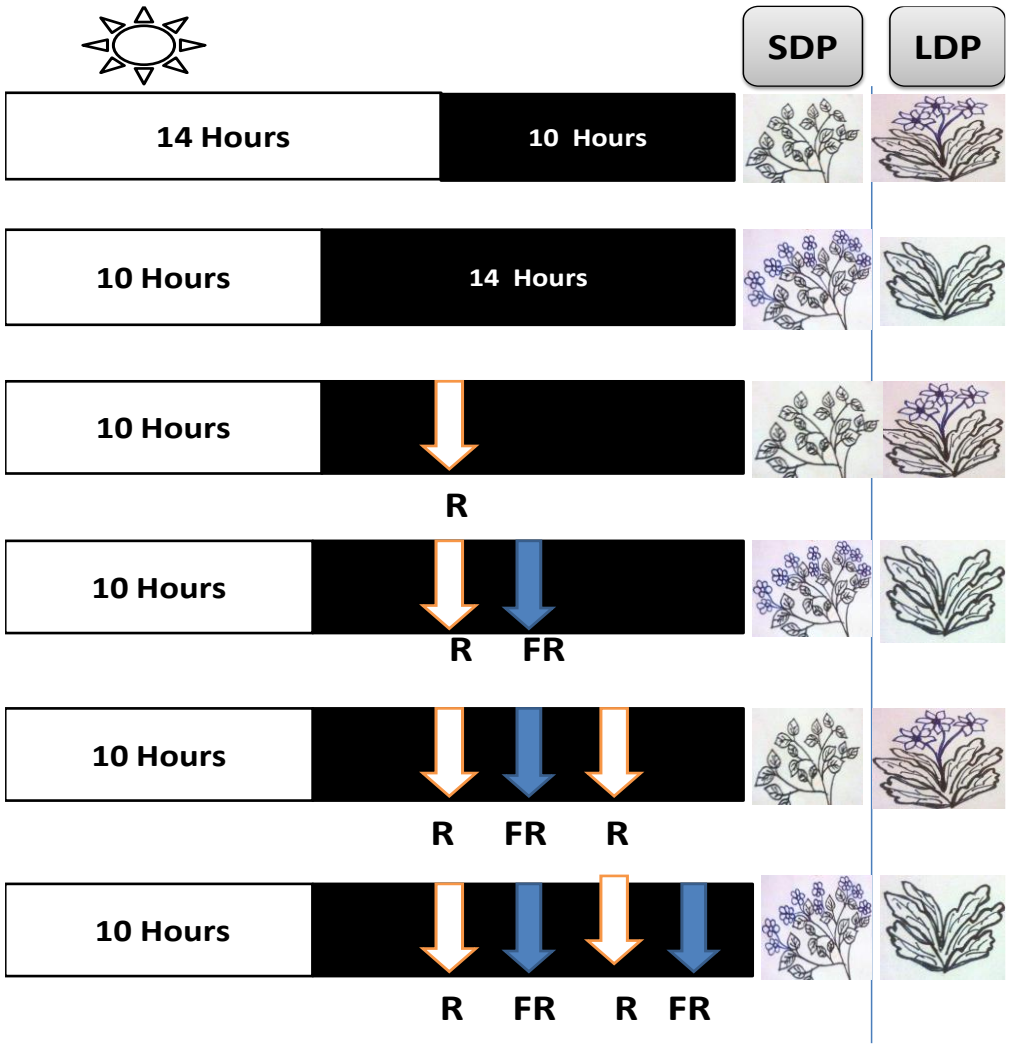


Fig.9.10: Effect of P_R & P_{FR} on flowering in SDP and LDP

Other Factors Influencing Flowering in Plants

(a) Effect of plant growth regulators

Gibberellic acid has been reported to have a positive influence on flowering in long-day plants. There are several long-day plants which under unfavourable conditions (short day condition) provided will gibberellic acid exhibit normal flowering.

Many scientists namely Brian (1959), Chailakhyan (1958), Naylor (1961) have supported association of gibberellin with flowering hormone. It is believed that CO_2 leads to formation of a precursor which is then converted into a hormone (Gibberellin - like). This gibberellin - like hormone as finally converted into florigen (hormone responsible for flowering). Flowering inducing effect of gibberellin have been observed only in long-day plants and not in short-day plants. However application of GA in SDP will cause stem elongation.

In the presence of red light the precursor is converted into GA like hormone and far red light inhibits the action of Gibberellic acid as in the presence of far red light gibberellin like hormone is converted back to the precursor. *Arabidopsis thaliana, Hyoscyamus niger, Lactuca sativa* are some of long-day plants which can be made to flower under short day condition by exogenous application of gibberellic acid. Plants belonging to family Cupressaceae, Taxodiaceae and Pinaceae also show flowering induced by GA.

Although, it is clear that GA influenced flowering specially in long-day plants, there are other hormones also which have been found to effect flowering in plants. Auxin and Cytokinin are two type of plant hormones which are known to control growth and development of plants. These hormones have also been found to have effect on flowering. **Auxins** have been reported to induce flowering in pine apple, *Hysocyamus niger*, wintex barley. Flower inducing effects of auxin on pine apple was discovered in 1942 and since then auxin have been commercially utilized to induce flowering in pine apple varieties. However, auxin has been found to have different response in different plant species. In some plants auxin helps in inducing flowering whereas in other plant it inhibits flowering or have no effect on flowering. It is expected that the plants in which auxin inhibits flowering is via. ethylene production as it is well known that application of auxin leads to production of ethylene.

Similarly**, Cytokinin** have been reported to induce flowering in plant varieties such as *Lemna paucicostata, Perilla, Wolffia, Chrysanthemum* etc. flowering in these plant have been achieved even under non inductive photoperiods by utilization of cytokinin. However, as you have seen in case of auxin that in some plants it induces flowering and in some plants it inhibits flowering. Similarly, cytokinin may also inhibit flowering in some plants. One such plant is *Chenopodium* in which flowering is inhibited in presence of cytokinin.

Abscissic acid cannot induce flowering if the required photoperiod is not provided, however application of abscissic acid under favourable photoperiod enhances reproductive development. *Chenopodium* and *Pharbitis nil* are two such plant species in which flowering in enhanced in favourable season by application of abscissic acid.

Salicylic acid has been known to act as plant growth regulator. Most of the plants do not have a requirement of salicylic acid for flowering however it has been reported to enhance flowering in plants such as *Lemna*. **Ascorbic acid** have also been reported to induce in flowering in plants like *Brassica* and *Lemna*.

(b) Increased Carbon: Nitrogen Ratio

Many scientists including Kraus and Bill have proposed that C/N ratio is also significant in determination of flowering in plants. They conducted their study on tomato plants and have proposed following effects of C/N ratio on plants (Table 9.3).

Table 9.3: Effect of carbon nitrogen ratio on flowering in plants

S. No.	C / N Ratio	Effect
1	**Very high** (High carbohydrate, low nitrogen)	Weak vegetative growth Non fruiting plants
2	**High** (High carbohydrate, moderate nitrogen)	Comparatively less vegetative growth Abundant flowering and fruiting
3	**Low** (High carbohydrate, high nitrogen)	Enormous vegetative growth No flowering
4	**Very low** (Low carbohydrate, high nitrogen)	Decreased vegetative growth Decreased flowering

Plants such as *Pharbitis nil* and *Lemna paucicostata* show increased flowering when C/N ratio is high, as you have already seen that short-day plants require shorter photoperiod (period of light) for flowering, however short day plant *Lemna* was induced to flower even in presence of continuous light when cultured onto nitrogen free medium. When plants are supplied with sucrose, ratio of C/N increases which result in increased flowering in plants such as *Pharbitis nil*, *Anagallis arvensis.*

9.5 VERNALIZATION

Vernalization can be defined as a process or method of inducing early flowering in plants. It is achieved mainly by treatment of seeds at very low temperature.

Generally, you may consider growth and development to be more or less similar; however both (growth and development) are different processes. Growth generally refers to increase in size and weight whereas development includes processes such as differentiation in flowering, pollination and fertilization which ultimately leads to reproduction. Lysenko in 1920-30 postulated the main principle of vernalization. The basic concept remains that by providing specific treatment either

to germinating seed or to the plant one of the two phase of life cycle of plant (i.e. growth and development) can be favored. For example winter wheat is normally grown in winter season but if the seeds of the plant are allowed to germinate in ice box with appropriate suitable light moisture and air, they can be grown in summer as well along with normal flowering.

Flowering is one of the most important process in life cycle of plant since it is the key event for reproductive succession in plants. Most of the plants flower only when they are exposed to proper period of light. Long-day flowering plants need short period of darkness to flower whereas short-day plants require longer period of darkness (continuous) to exhibit flowering. However, day neutral plants are independent of photoperiod and flower irrespective of day night length. There is no doubt about the photoperiod (duration of light to which plant is exposed) remains to the most crucial important factor for following to occur. Beside this, temperature is another factor which also has significant effect on flowering.

If you consider flowering in annuals and biennials, for annuals photoperiod is most crucial for flowering, followed by temperature. However in the case of biennials as you know that biennials are those plants which show vegetative growth in first season and when they have gone through prolonged exposure to low temperature during winter season, they exhibit flowering in next season. If due to any reason these plants do not get exposure to low temperature they fail to flower and will continue to grow vegetatively. However they can be made to flower of the plants that are exhibited to cold treatment following suitable photoperiod. Many biennials such as carrot, cabbage, beet, glove needs cold treatment for flowering to occur (Table 9.4).

Table 9.4: Effect of Vernalization on different categories of plant

S. No.	Plant type	Effect
1	Spring / Summer type annuals	Vernalization has no effect
2	Winter type annuals	Vernalization decreases time to flowering
3	Biennials	Require vernalization to flower

If young plants or moistened seeds of biennial plants are provided with chilling treatment, they can be made to flower in single growing season. Winter annuals respond to low temperature early in their life and can be vernalized before germination (imbibed seeds). However, most biennials grow during first season and flower in summer. Hence they must reach a minimum size before they become responsive towards vernalization treatment

Examples:

- In case of winter rye, if the seeds are treated with the temperature of 1°C for about 4 weeks the plants exhibit flowering in after 11 weeks of plantation. However if the seeds are treated

and germinated at temperature about 18°C no flowering can be obtained after same period of growth (11 weeks) after which vernalized plants flowered.

- *Hyoscyamus niger* (Henbane) is a biennial plant and requires exposure to cold treatment for flowering, besides that there is another interesting fact about flowering in *Henbane.* Plants of *Henbane* will show flowering only if when vernalization is followed by a long day treatment. However if vernalized plants are exposed to short day treatments, plant fail to flower. There is another variety of *Henbane* which is annual and does not require cold treatment for flowering.

Process of Vernalization

Vernalization can be achieved by a very simple process. Seeds to be vernalized should be soaked in water properly, vernalization can never be achieved in dry seeds, it has been reported that seed should contain about 90% water of their dry weight. Seeds then allowed to germinate, followed by treatment of low temperature for suitable period of time. Treated seedlings are slightly dried and then sown for further growth (Fig. 9.11).

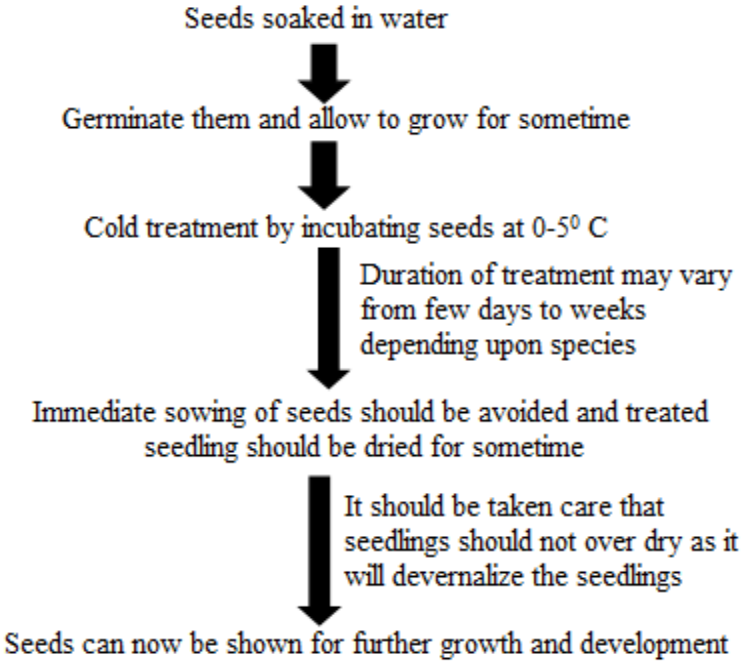


Fig.9.11: Outline of basic process to achieve vernalization

Requirement of vernalization

i) Low temperature: Normally vernalization is achieved in temperature range of zero degree to 10°C, when the temperature is decreased below 0°C the effectiveness of vernalization generally decreases and at about -6°C there is no effect and vernalization cannot be achieved.

ii) Duration of treatment: The time period required for vernalization may vary from species to species and can range from few days to few weeks. Normally the time period of treatment is long since vernalization is considered to be a slow process.

iii) Actively dividing cells: Since vernalization cannot occur in dry seeds due to absence of active embryo, after germination embryo becomes active and can perceive vernalization. In whole plant vernalization signal is perceived by meristematic cells (shoot tips, root apex, developing leaves) shoot apical meristem of *Chrysanthemum* have been demonstrated to perceive vernalization.

iv) Water: Water is an essential requirement for germination and that seeds provided with vernalization treatment also requires water for germination and growth.

Devernalization and Revernalization

Devernalization can be defined as a process in which vernalized seeds / seedlings loses their vernalized states and becomes devernalized. Over drying of vernalized seed / seedling, heat treatment of vernalized seed/ seedling may result in devernalization. An atmosphere of nitrogen in presence of high concentration of CO_2 may also result in devernalization. However, devernalized seeds can be easily revernalized by subjecting them to low temperature. Vernalization response critically depends upon the temperature to which seeds are exposed and also on the duration for which the treatment is maintained. If either of them (temperature or duration) falls short of the requirements then either vernalization may not occur properly or the vernalized seed may be easily devernalized.

Advantages of Vernalization

i) Vernalization shortens the Juvenile or vegetative phase and induces early flowering.
ii) Vernalization can be applied to temperate as well as tropical plants.
iii) Vernalization is expected to increase resistance to cold and diseases.
iv) Biennials can be made to behave as an annual that is flower in one growing season.
v) Early flowering and fruit setting can be achieved in biennials.
vi) Plants can also be made to grow in regions where they normally do not grow.
vii) It can be used in Horticulture. Flowering can be induced into non vernalization plant by grafting a vernalized shoot open.

When proper cell treatment is provided a stimulus is perceived by the dividing cell. Researchers have named the stimulus as vernalin. Formation of vernalin alone is not enough for flowering to occur. After vernalization plants appropriate photoperiod is also required (it should be noted here that proper vernalization only prepares the plant to flower however, photoperiodism provides stimulus for flowering and also induces the flowering). Following proper photoperiod either

vernalin is converted into florigen or vernalin regulates florigen synthesis. Florigen once produced directs reproductive development and flowering is initiated in the plants.

9.6 SUMMARY

1. When plant has completed certain vegetative growth, it makes a transition from juvenile stage of maturity.
2. Most of the plants require favourable environmental to occur. Photoperiod and temperature are the most crucial environmental factors for flowering.
3. The response of plants to day length is known as photoperiodism.
4. Depending upon requirement of photoperiod plants can be short -day plants, long-day plants and day neutral plants.
5. Short - day plants flower when a critical period of darkness is exceeded. Long-day plants exhibit flowering when period of darkness is less than a critical period.
6. When exposed to suitable photoperiod, the photoperiodic stimulus is perceived by leaves and a flowering hormone (florigen) is synthesized.
7. Flowering hormone is translocated to shoot apex where bud formation and flowering takes place. The presence of a hormone which can be transmitted from leaves to short apex have been demonstrated by grafting experiments.
8. Phytochrome is a protein with chromatophore found in plants in two interconvertable forms: P_R and P_{FR}.
9. P_R is red light sensitive and P_{FR} is far red light sensitive. Among the two forms of phytochrome P_{FR} is biologically active form.
10. Some plants require exposure to low temperature (vernalization) for flowering to occur.
11. The effect of temperature on flowering is more profound in biennials as compared to annuals.
12. Temperature in range of 0-5ºC is considered to be most effective to achieve vernalization.
13. Effect of vernalization can be reversed (devernalization) by exposing vernalized seed or plant to high temperature.

9.7 GLOSSARY

Annual: Describes a plant that germinates, grows, flowers and produces seeds all in one growing season

Biennial: Describes a plant that grows for two years, it germinates and develops foliage the first season, and produces flowers and seeds the next season

Chromatophores: are pigment-containing and light-reflecting cells, or groups of cells

Day-neutral plant: Describes plants for which flower initiation is not dependent upon day length

Defoliation: Widespread loss of leaves by using chemicals or manual removal of leaves from plants

Florigen: A hormone believed to be formed in plants responsible for flowering

Grafting: Propagation method in which two pieces of live plant tissue are united by placing their meristems in contact

Long-day plant: Plants which flower when length of day is longer than the period of darkness (night)

Perennial: Describes a plant that lives for more than two years, commonly used to describe herbaceous (non-woody) plants

Photoperiodism: The initiation of flowering based on the relative amounts of darkness and light in a 24-hour period

Phytochrome: A light-sensitive protein pigment involved in the photoperiodic response

Short-day plant: Plants which flower when length of day is shorter than the period of darkness (night) is longer

Vernalization: The promotion of flowering due to exposure to low temperatures, or chilling

9.8 SELF ASSESSMENT QUESTIONS

9.8.1 Objective type Questions:

1-Which is following is not a short day plant:

(a) *Zea mays* (b) *Fragaria*

(c) *Bryophyllum* (d) *Pisum sativum*

2-Vernalization results in:

(a) Early flowering (b) Delayed flowering

(c) No effect on flowering (d) None of the above

3-Vernalization can be applied to:

(a) Tropical plants only (b) Temperate plants

(c) Both tropical and temperate plants (d) None of the above

4-Which the following is not true about vernalization:

(a) It shortens juvenile phase

(b) Helps in crop improvement

(c) It needs chilling treatment followed by high temperature to induce flowering

(d) Biennial can be made to flower early

5-Effect of vernalization can be transferred from one plant to another by grafting in :

(a) *Henbane* (b) Sunflower
(c) *Withania somnifera* (d) *Mucuna pruriens*

6-Which of the following is potent agent of devernalization:
(a) High temperature only
(b) Chilling temperature
(c) High temperature, atmosphere of N_2 and CO_2
(d) Chilling temperature, atmosphere of N_2 and CO_2

7-Vernalization can be best achieved in a temperature range of:
(a) Below - 6ºC (b) 0ºC - 10ºC
(c) 30ºC - 40ºC (d) Above 40ºC

8-Pigment involved in photoperception in flowering is:
(a) Cytochrome (b) Phytochrome
(c) Phycocyanin (d) Pheophytin

9-Pigment that absorbs far and far red light in plant is:
(a) Cytochrome (b) Cholorophyll
(c) Phytochrome (d) Carotene

10-Which of the following statement is correct:
(a) Effect of photoperiod once perceived can be easily reversed
(b) Vernalization prepares the plant for flowering
(c) Vernalization can be passed through grafting
(d) Gibberellic acid induces flowering in SDP

11-Which the following statement is incorrect:
(a) SDP generally flower in early spring or autumn
(b) Stimulus for photoperiodism is perceived by stem.
(c) LDP generally flower in spring or early summer
(d) Photoperiodism is mediated through florigen

12-By regulating and maintaining suitable photoperiod:
(a) Annual can be grown more than once in a year
(b) Annual can be grown once in a year
(c) Annuals can be grown every month
(d) There is no effect of photoperiod on growth of annuals

13-Which of the following is a day-neutral plant:
(a) *Gossypium hirsutum* (b) Spinach
(c) Rice (d) *Glycine max*

14- Which of the following is a long- day plant:
(a) Tomato (b) Coffee
(c) Soyabean (d) Pea

15-By increasing light hours:
(a) Winter dormancy can be prevented (b) Plants can flower throughout the year
(c) Both a and b (d) None of the above

16-Devernalized seeds can be revernalized by subjecting them to:
(a) Chilling temperature (b) High temperature
(c) Gibberellic acid (d) Atmosphere of N_2 and CO_2.

17-Long short-day plants flower when exposed to:
(a) Long-day or short-day (b) Long- day followed by short-day
(c) Short-day followed by long-day (d) Long-day only

18-Which of the following is ambiphotoperiodic plant:
(a) *Madia elegans* (b) *Pisum sativum*
(c) *Aloe bulbillifera* (d) *Avena sativa*

19-Phytochrome is mainly produced during:
(a) Darkness (b) Light
(c) Both a and b (d) None of the above

20-Find out the correct statement:
(a) $P_{FR.}$ is converted to P_R in presence of light
(b) $P_{FR.}$ is converted to P_R in darkness and 670nm red light
(c) $P_{FR.}$ is converted to P_R in darkness and 730nm red light
(d) $P_{FR.}$ is converted to P_R in light and 670 nm for red light

21-P_R and $P_{FR.}$ are:
(a) Sensitive to red light (b) Two interconvertable forms of chlorophyll
(c) Sensitive to far red light (d) Two interconvertable forms of phytochrome

22- $P_{FR.}$ acts as inhibitor of flowering in :

(a) SDP (b) LDP
(c) $P_{FR.}$ never inhibits (d) Both a and b

9.8.2: Fill in the blanks

(a) Both P_R and $P_{FR.}$ can absorb ..... light.
(b) Shorter or longer nights have no effect on flowering in ..... plants.
(c) P_F.....flowering in SDP.
(d) Stimulus for vernalization is mainly perceived by.....
(e) Wavelength .....to..... in red portion of spectrum are most effective to induce flowering.
(f) Biennials require..... treatment for flowering to occur.
(g) Plants which remain vegetative in intermediate day length and flower only in longer or shorter day length are called.....
(h) ……determines flowering season of plants.
(i) ……is a plant hormone which have positive influence on flowering in LDP.

9.8.3: True / false

(1) $P_{FR.}$ stimulates flowering in LDP.
(2) Wavelength 660μm in orange red color is most effective for inhibiting flowering .
(3) Flowering stimulus can be transferred through grafting.
(4) Green light of visible spectrum is most effective in inducing flowering .
(5) Temperature below-6ºC is not suitable for vernalization.
(6) Short long-day plants flower when short days are followed by long-day treatment.
(7) Raddish is an example of short-day plant.
(8) Effect of photoperiod once perceived can be easily reversed.
(9) Long period of darkness exerts inhibitory effect on flowering in long-day plants.
(10) Vernalization increases the time of flowering in winter type annuals.

9.8.1: Answer keys: 1-d, 2-a, 3- , 4-c, 5-a, 6-c, 7-b, 8-b, 9-c, 10-b, 11- b, 12-a, 13-a, 14-d, 15-d, 16-a, 17-b, 18-a, 19-a, 20-c, 21-d, 22-b

9.8.2 : Answer key: a - Blue, b- day neutral, c - induces, d - leaves, e - 580nm, 680nm, f- vernalization, g - ambiphotoperiod, h - photoperiodism, i- gibberellic acid.

9.8.3: Answer key: 1-False, 2- True, 3-True, 4-False, 5-True, 6-True, 7-False, 8-False, 9-True, 10-True

9.9 REFERENCES

- *Plant Phyogy by* Lincoln Taiz and Ederordo Zeiger Sinauev Associates, Inc. publishers.
- *Plant Physiology* by V. Verma, Ane Books India
- Pandey, S.N. and Sinha, B.K. *Plant Physiology,* Vikas publishing House Pvt Ltd,
- Malik, C.P. and Srivastava, S.K. *Text book of Plant Physiology.* Kalyani Publications, New Delhi

9.10 SUGGESTED READING

- Malik, C.P. and Srivastava, S.K. *Text book of Plant Physiology* Kalyani Publications, New Delhi.
- Srivastava, H.S. *Plant Physiology.* Rastogi Publications, Meerut.
- *Plant Physiology* by G. Ray Noggle and George J Fritz Practice Hall of India, Pvt. Ltd., New Delhi.
- *Plant Physiology* by Frank B Salisbury and Deonw Ross Wadsworth Biology Series.
- *Plant Physiology* by Lincoln Taiz and Ederordo Zeiger Sinauev Associates, Inc. publishers.
- Verma, V. *Plant Physiology.* Ane Books India.
- Pandey, S.N. and Sinha, B.K. *Plant Physiology.* Vikas publishing House Pvt Ltd, New Delhi.

9.11 TERMINAL QUESTION

9.11.1: Long answer type

(1) Define Phytochrome and discuss the effect of different types of phytochrome on flowering in plants?

(2) Describe underlying process and mechanism of vernalization. List some practical uses of this phenomenon?

(3) What is photoperiodism? Classify plants based upon their photoperiodic requirement for flowering?

(4) With the help of suitable experiment show that stimulus for photoperiodism is perceived by leaves?

9.11.2: Short answer type question

(1) Differentiate between:

(a) Long-day Plant and short-day plants

(b) Photoperiodism and vernalization

(c) Obligate SDP and Facultative SDP

(d) Qualitative LDP and Quantitative LDP

(2) Explain 'Critical night Length' with respect to flowering in SDP?

(3) How intermediate day length plants differ from ambiphotoperiodic plants?

(4) Define devernalization. How it can be achieved?

(5) Briefly describe about specific photoperiod required for flowering in *Henbane*?

(6) Mention suitable conditions requirement for vernalization to occur?

(7) Experimentally show that stimulus for flowering can be transferred through grafting?

(8) Describe the effect of different plant growth regulator on flowering in plants?

(9) How does C /N ratio influence flowering in plants?

9.11.3 Very short answer type Question:

(1) How can $P_{FR.}$ can be converted in P_R?

(2) What are Day- neutral plants? Give example?

(3) What are long short- day plants? Give examples?

(4) Define Florigen.

(5) Name the biologically active form of phytochrome?

LABORATORY COURSE

UNIT-1L STUDY OF POLLEN GRAINS, PLACENTATIONS, AND OVULES USING TEMPORARY AND PERMANENT PREPARATIONS

1.1 OBJECTIVES

After reading this unit students will be able:

- To describe the temporary and permanent preparations of Pollen grains.
- To know the temporary and permanent preparations of Placentations.
- To understand the temporary and permanent preparations of various types of Ovules.
- To know the development of female gametophyte.

1.2 INTRODUCTION

When using a microscope, slides that are permanent can be examined and stored for a long time, while temporary slides are used for short time observations. Permanent slides must be properly made for successful long-term storage. Specimens must be finely sectioned and properly preserved for preparing a permanent slide.

Most permanent slides use the semi-solid form of mounting medium, which is the most stable. Liquid mounting mediums can also be used on permanent slides. This form suspends the specimen in liquid and uses nail polish to fix the cover slip to the slide. Nail polish makes the slide semi-permanent. It is permanent if left intact and temporary if the cover slip is removed, washed and dried for reuse. Slides using the liquid mounting method must be stored horizontally.

Preparation of a Temporary Slide

A temporary laboratory slide is in laboratories to view mounts under the microscope.

Apparatus: Material to be mounted, slide, watch glass, coverslip, petridish, filter paper, brush, microscope, stain as per the plant material.

Procedure:

1. Take a clean slide and put a drop of glycerine (10%) on the center of the slide. Excess amount of glycerine should be removed from the slide, if present.
2. Transfer material to be mounted on the drop of glycerine with the help of a small brush. Do this step very carefully. If the mount is not correctly placed observation under microscope becomes difficult.
3. Apply a thin coat of glycerine on the lower surface of cover slip to remove the air of this surface and now carefully place the coverslip over the glass slide covering the mount. Take extra care not to crush the mount much.
4. Remove extra fluid present on the slide with the help of filter paper. This is to obtain a clearer view on the microscope. Don't crush the coverslip, because it breaks easily.

5. Observe your mount under a microscope.

Once your slide is ready to be observed under a microscope place it in the viewing area of microscope and view it. Adjust light to obtaina clear view.

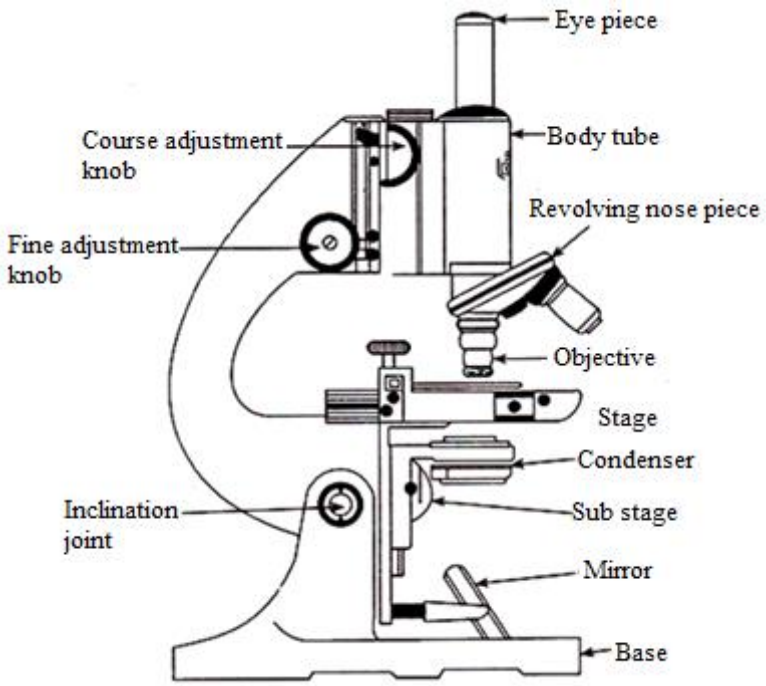


Fig.1.1 The compound microscope showing its various parts

Staining: Usually, material to be mounted is stained. Staining is generally done to get clear image under the microscope. For staining, different stains are commonly used. Iodine solution is commonly used for pollen studies. Take a little Stain in watch glass. Transfer material to be stained onto the watch glass. Wait for a few minutes. Now remove the material from the strain with the help of a brush and place it in watch glass containing clean water. Transfer stained material in drop of glycerine on glass slide. Put coverslip as described above and examine under microscope. When material to be stained is very small, place material in a drop off water. Add a drop off stain to it. After few seconds add drop of glycerine and put coverslip. Remove oozing fluid using filter paper.

Precautions:

1. Don't use excess amount of water.
2. Hold coverslip gently.

3. Use proper staining technique.
4. Don't crush the mount too much.
5. Use brush to transfer mount to slide from watch glass.

Prepare A Permanent Mount:

In certain casespreparations need to be stored permanently for future use. The method of preparation followed is described below.

1. The section is first stained with principal stain(aqueous hematoxylin, safranin or crystal violet).
2. The section is then washed with water till no more stain dissolves and water remains colourless.
3. Section is passed through a graded series of alcohol for dehydration. Different cavity blocks or petri dishes or watch glasses are filled with requisite amount of alcohol, (beginning with 30% alcohol) and the section is transferred to it. These cavity blocks or petri dishes or watch glasses should always be covered. The section is transferred to next higher series of alcohol after every 30 minutes which helps in gradual dehydration. In case if you don't want to disturb the section, used alcohol is removed by glass dropper. All the 30% alcohol is replaced with 50% alcohol. This procedure is repeated till 70% of alcohol grade is reached.
4. At this stage, counterstained is employed (e.g. safranin, fast green or erythrosine prepared in 80% or 90% alcohol).
5. This stain acts quickly and as such section is washed immediately after the requisite time is over.
6. De-staining is done by washing sections with90% or 100% alcohol.
7. The section is now transferred to absolute alcohol for complete dehydration.
8. Clearing now begins with 25% of xylol (25 cc of xylol and 75 cc of absolute alcohol). The sections are gradually passed through xylol series of 25%, 50%, 70%, 90% and finally transferred to pure xylol. If dehydration is not complete, pure xylol turns white or turbid. At this stage section should be passed through reverse series.
9. Pure xylol is the last stage of clearing. Section is now ready for mounting.
10. Mounting is done in Canada balsam or DPX mountant.

Mounting an Object

Mounting is necessary to properly position an object for clear view. Lactophenol, glycerine and glycerine jelly are used for temporary mounting while Canada balsam and DPX is used for permanent mounting.

(a) **Canada balsam:** It is a resin obtained from a conifer-*Abies balsamea*, most suitable for permanent slide preparation. The material to be mounted should come through alcohol (dehydration) and xylol (clearing) series.

(b) **Lactophenol:** It is a mixture of equal parts of phenol crystals, lactic acid, glycerine (sometimes two parts) and distilled water. Stains may be mixed with this medium (e.g. cotton blue in Lactophenol used to stain fungi) or copper acetate is added to preserve green colour of the pigment.

(c) **Glycerine:** Pure glycerine diluted to 10-25%is widely used. Semi-permanent and temporary preparations are mounted in glycerine.

(d) **Glycerine jelly:** Jelly is also used for mounting. It is made of gelatin 1: glycerine 7 water 6.Warm the gelatin for two hours by adding water. Phenol (1 %) is added later. Add crystals of safranin if desired. Allow the solution to cool and settle into jelly. Many other mounting media like cedar oil, dammar, balsam, venetian turpentine and synthetic resins are also used.

1.3 STUDY OF TEMPORARY AND PERMANENT SLIDE PREPARATIONS OF VARIOUS TYPES OF POLLEN GRAINS, PLACENTATION AND OVULE

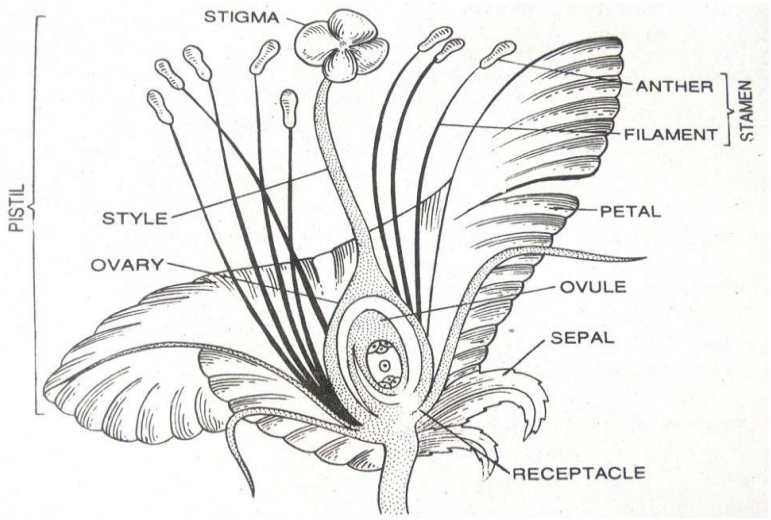


Fig.1.3. General Plan of a typical flower, Flower in section view (L.S.)

1.3.1- Pollen Grains

Pollen is a fine to coarse powdery substance comprising pollen grains which contain partial or fully developed male gametophytes Pollen grains have a hard coat made of sporopollenin that protects the gametophytes during the process of their movement from the stamens to

the pistil of flowering plants. The study of pollen is called **palynology** and is highly useful in paleoecology, paleontology, archaeology, and forensics.

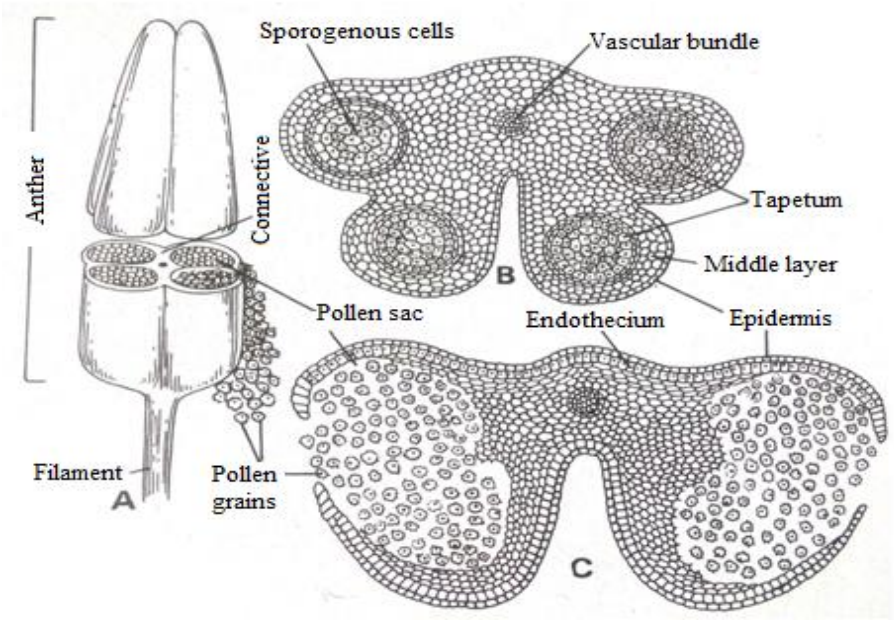


Fig. 1.4 Stamen. A- Pollen showing filament and anther lobe. The anther lobe have been cut transversely to show microsporangia and microspores (pollen grains). B- T.S. of young anther lobe showing four microsporangia .C- T.S. of anold anther.

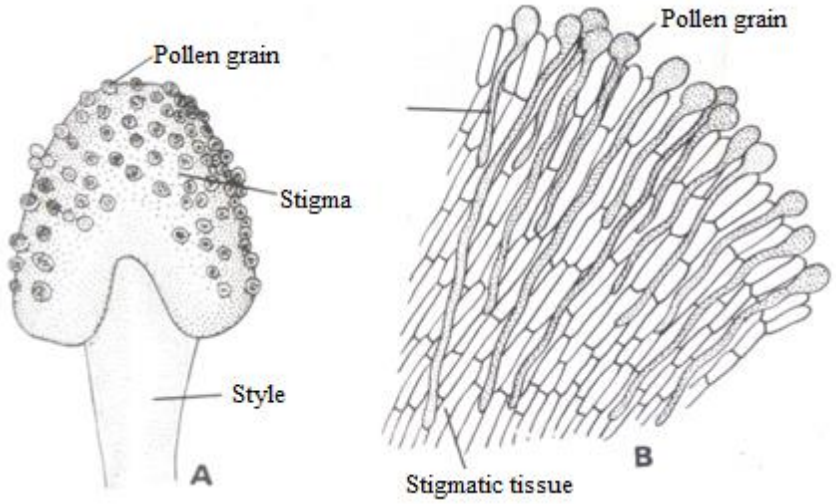
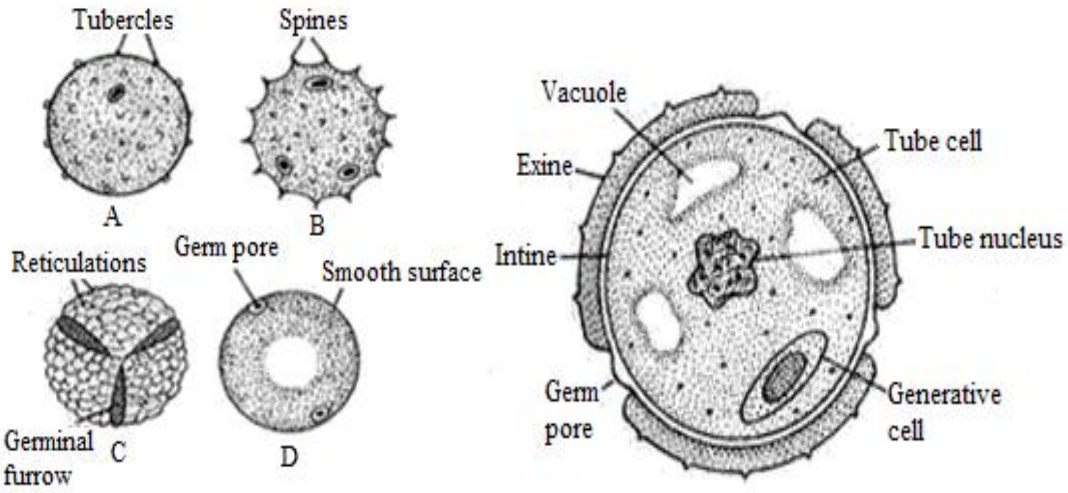


Fig.1.5. A- Pollen grains deposited on stigma; B- Pollen grains germinating through stigmatic tissue forming pollen tubes

The pollen grain of flowering plants is a haploid, uninucleate cell with double layered wall, an inner layer, the **intine**, and an outer layer, the **exine**. The intine is thin and

consists of cellulose, while the outer layer, the exine is thick, sometimes spiny, very resistant to disintegration, and composed of sporopollenin.



(A) **(B)**

Fig. 1.6(A) Common pollen grain sculpturing (B) section of a mature 2 celled pollen grain of an angiosperm.

Characters of Pollen Grains for Study

1. Polarity: The pollen grains are often formed in tetrads. While in tetrad, one end of the individual grain is noted. Accordingly following are the axis: **Polar axis** (Hypothetical line connecting the two poles) and **Equatorial axis** (Hypothetical line that lies perpendicular to the polar axis).

(a) **Proximal pole**: The end of the pollen grain is directed towards the center of the tetrad.

(b) **Distal pole**: The end of the pollen grain is directed away from the center of the tetrad.

2. Symmetry: Pollen grains may be symmetrical (bilateral or radial) or asymmetrical (without any symmetry).

3. Apertures: The exine of pollen grains are often provided with apertures which are thin, more or less distinctly delimited areas formed only of a hyaline membrane.

4. Shape of pollen grain: It is determined by Px100/E formula, where P is the polar diameter and E the equatorial diameter.

5. **Exine stratification.** The wall is made of intine and exine. The intine is colourless and disappears during the slide preparation. Exine consists of two layers, the inner homogenous layer, the **endine** and the outer heterogeneous layer, the **ectine**. The ectine is composed of radial

rods, the columellae, which are either free at their tips or are united to form a layer called **tegillum**.

6. **Exine ornamentation.** The following are some of the patterns.
(1) The columellae forming the ectine produce **Pilate** pattern with bright and dark areas.
(2) In some other cases columellae are arranged regularly and are fused to produce areas or **lumina**, the intervening areas between lumina being called **muri**.
(3) When a network is produced the pattern is **reticulate** which may be **retipilate** with incomplete fusion of columellae, **foveolate** with circular closely placed lumina, **scrobiculate** with circular but distantly placed lumina, and **fossulate** with elongated lumina.
(4) When lumina are parallel the pattern is called **striate** and when reticulate it is **rugulate**.
(5) A network with raised areas is called **areolate**.
(6) In some cases excrescences such as minute granules are present on the tegillum, the pattern is **granulose**, as **spinulose** (pointed or blunt ends), **gemmate (**rounded warts**)**, **verrucate** (base of the warts is not constricted), **tuberculate** (tubercles present), **spinose** (pointed), **baculate** (rod shaped) and **clavate** (club shaped).

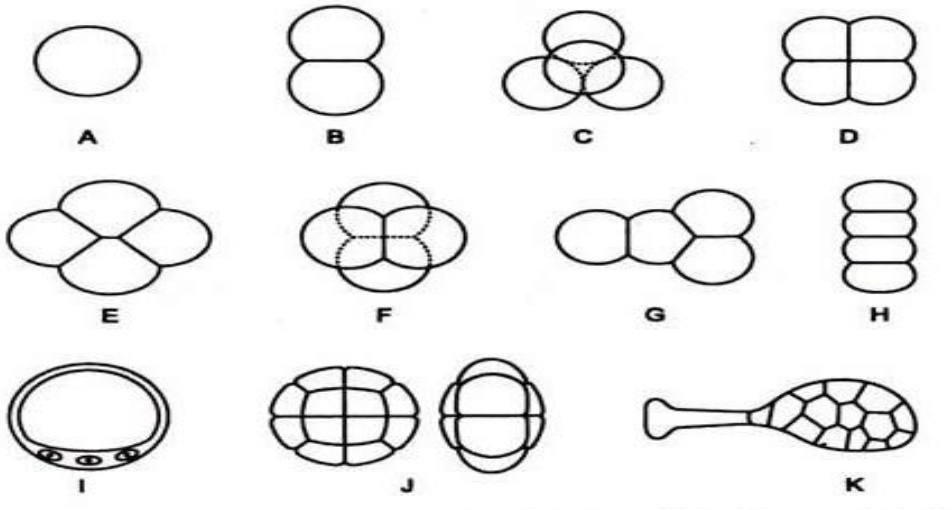


Fig. 1.7- Pollen units (A= Monad, B= Dyads, C= Tetrahedral tetrad, D= Tetragonal tetrad, E= Rhomboidal tetrad, F= Decussate tetrad, G= T-Shaped tetrad, H= Linear tetrad, I=Cryptotetrad, J = Polyads, K= Pollinia.

The pollen grains do not remain united at maturity, and are dissociated into single pollen grain called **monad**. Sometimes rarer types like dyads (two pollen grains), Octads (eight pollen grains) and Polyads (many pollen grains) are also observed.

Dyads: Pollen grains which are united in pairs and shed from the anthers as doubles are called dyads. The dyads are formed due to the incomplete break up of individual grain or monad.

Tetrads: Four pollen grains are united to form tetrad. Tetrads are the unseparated product of meiosis. Tetrads maybe categorized into different types based on their arrangement.

Tetrahedral tetrad: Pollen grains are arranged in two different planes. Three grains are in one plane and one lies centrally over the other three. In some cases, the pollen grains are released from the anther in the tetrad condition. These types of tetrads are called obligate or permanent tetrads, viz., *Rhododendron* (Ericaceae).

Tetragonal tetrad: All the four pollen grains are arranged in one plane e.g., *Typha latifolia* (Typhaceae).

Rhomboidal tetrad: All pollen grains are arranged in one plane forming rhomboidal shape e.g., *Annona muricata* (Annonaceae).

Decussate tetrad: Pair-wise the pollen grains are at right angle to each other, e.g., *Magnolia grandiflora* (Magnoliaceae).

T-Shaped tetrad: The first division of pollen mother cell is transverse to form a dyad. The upper or lower cell of dyad undergoes a vertical or longitudinal division instead of transverse, yielding either straight or inverted T-shaped configuration, e.g., *Polyanthes*.

Linear tetrad: The first division of pollen mother cell is transverse and a dyad is formed. Each cell of the dyad again divides transversely to form a linear tetrad, e.g., *Mimosa pudica.*

Cryptotetrad: Here tetrads are formed without partition walls between the four compartments. One out of the four nuclei develops normally and the rest three obliterate. Thus an apparent monad but homologous to the tetrad is formed also called **Pseudomonad**, e.g., Cyperaceae.

Polyads: In most of the Mimosaceae members each of the tetrad cells divides once or twice or more, yielding a group of 8 to 64 cells which remain together after maturity. These compound grains are usually held together in small units and are called Polyadse g., *Acacia auriculiformis*.

Preparation of Pollen Grains for Study:

The following are the steps in the preparation of slides for pollen study.

(A) Collection of the material

1. The polliniferous material (anthers) is freshly collected. If possible use only freshly opened flowers so that contamination from other pollens is avoided.
2. The anthers are picked by a clean forceps.

(B) Preparation of the material

1. The anthers are tapped by needles or glass rod on a clean slide to obtain a mass of pollen grains.
2. This mass of pollen grains is picked up by the flat end of the forceps and transferred to the centre of another clean microscopic slide.

(C) Pre-treatment

1. Few drops of alcohol is added to the pollen grains to remove waxy surface from the pollen grain and to separate them from one another.
2. The ring developed by alcohol is wiped clean with cotton moistened with alcohol.

(D) Mounting

1. A small pellet of glycerin jelly pre-stained with methyl green is taken. It is placed over the mass of pollen grains. Coverslip is also placed over the pellet.
2. A small piece of paraffin wax (melting point 60-70°C) is placed close to the coverslip.
3. Both, jelly and wax are warmed over the flame of the spirit lamp in such a way that while the jelly spreads a little, the remaining vacant space below the coverslip is occupied by wax.

Germination of Pollen Grains

Requirements:

Anthers of *Antirrhinum* (snap dragon), *Catharanthus roseus* (Periwinkle; Sadabahar), *Papaver somniferum* (Poppy; Afim) or any other easily available plant, sugar, boron, cavity slides, coverslips, microscope, water, etc.

Procedure

1. Prepare 15% sugar solution by dissolving 1.5 gm sugar in 100 ml of distilled water.
2. Add a pinch of boron to sugar solution.
3. Clean the cavity slide and place a drop of this solution in the cavity.
4. Remove mature anthers from freshly opened flowers. Crush them on a slide. Collect the pollen grains with a brush from the crushed anthers. Dust the brush free of anthers in the cavity filled with solution.
5. Place a cover slip over the cavity.
6. Allow the slide to remain as such for a few hours or overnight.
7. Remove the coverslip slowly. Mount the coverslip on a fresh and clean slide in a drop of safranin. The lower side of the coverslip with germinated pollen grains should be in contact with safranin.
8. Observe the slide.

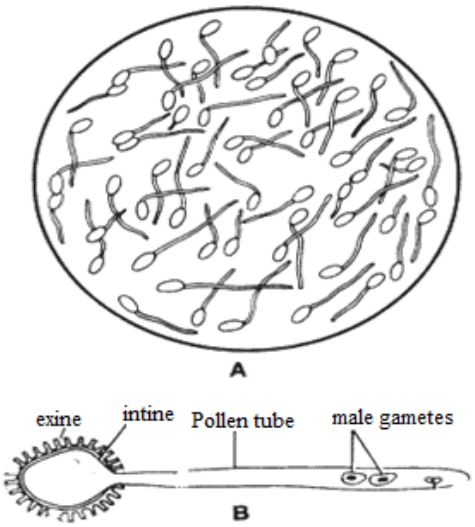


Fig. 1.8- Pollen grains: Germination of pollen grains A. seen under the light microscope, B. Details of the structure.

Observations

The following characters are observed.

1. Numerous germinated pollen grains are seen.
2. A pollen grain has a distinct ornamented exine with germ pores.
3. Intine lies internal to exine. It is thin and uniform.
4. Intine forms a pollen tube that comes out through one of the germ pores.
5. Pollen tube shows a vegetative nucleus and two small male gametes.

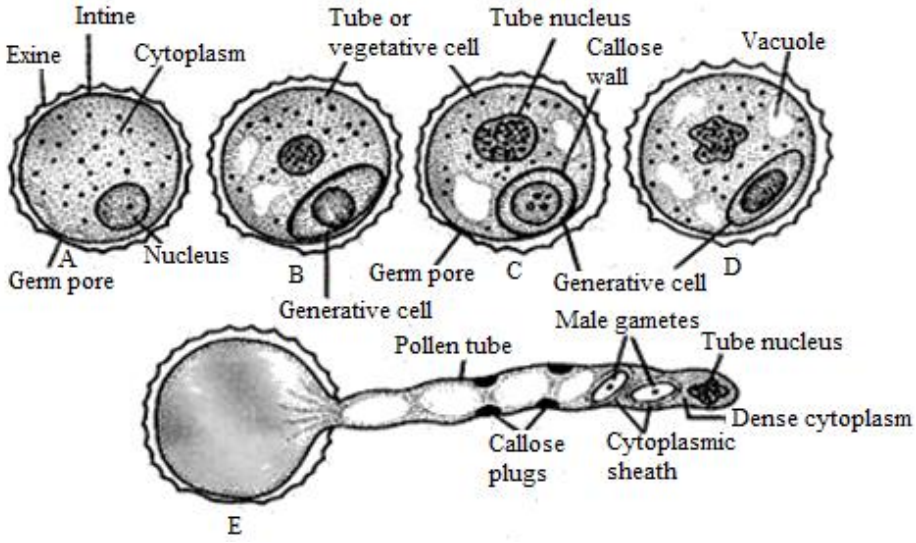


Fig.1.9. Germination of pollen grain and formation of male gametophyte in an angiosperm

Preparing a Permanent Mount for Pollen Grains

Permanent slides of pollen grains can be used as a reference for identifying unknown pollen samples. It is therefore important, that the pollen grains remain in an authentic, natural shape. The preparation and mounting of the pollen can introduce artifacts: the pollen may lose some of its pigment, start to shrink and shrivel or absorb water and swell. A careful preparation is therefore necessary. There are several methods of preparing pollen grains, each one offers advantages and disadvantages.

Mounting Techniques

(i) Glycerol wet mount: Place a small drop of glycerol on a clean slide and tap the anthers of the plant so that the pollen falls into the glycerol. If necessary, carefully separate large chunks of pollen grains by stirring. Place a cover slip on top and seal the sides of the cover slip with nail polish. Use a very small amount of glycerol to make sure that the nail polish has enough area to stick the coverslip to the slide. Glycerol wet mounted slides can be stored for months if properly sealed with nail polish. The glycerol will withdraw water from the pollen. If the pollen is not dry, then there is a possibility of the pollen to shrink.

(ii) Air mounts (dry mounts): In this case, no liquid mounting medium is used. A cover slip is placed on top of the pollen grains and sealed on the side, either with nail polish or with tape. Nail polish may flow very quickly between cover slip and slide, so it may be best to use a nail polish of high viscosity (by letting some solvent evaporate first).

(iii) Glycerol jelly: This is a very popular mounting medium for pollen. It is phenol-free (antiseptic additive) and therefore non-hazardous. It contains 10g of gelatin, 35ml distilled water and 30ml of glycerol (glycerin). After mounting, the sides of the cover slip need to be sealed. Due to the lack of an antiseptic, it is also necessary to work in a sterile manner, otherwise there is the risk of fungal growth in the medium. It is a good to treat the pollen grains first in alcohol to reduce the chance of fungal contamination by spores.

(iv) Non-water-based mounting media: Euparal is a mounting medium which is not water based. Specimens which are present in alcohol can be directly transferred to Euparal. Place a pollen suspension on the slide and let the alcohol evaporate. Before mounting pollen in Euparalthe pollen are first washed in alcohol and then compared to the original shape. Washing in alcohol may result in an unacceptable shrinking of the pollen or unacceptable loss of pigments, if not, then mounting the pollen in Euparal may be an alternative.

1.3.2- Placentations

In biology, placentation refers to the formation, type and structure, arrangement or position of the placenta. The function of placenta is to transfer nutrients, respiratory gases, and water from maternal tissue to a growing embryo, and in some instances to remove waste from the embryo.

In botany, the term placentation most commonly refers to the arrangement of placenta inside the ovary. In flowering plants, placentation occurs where the ovules are attached inside the ovary. The ovule inside an ovary is attached via funicle. The part of the ovary where the funicle is attached is referred to as the placenta.

Types of Placentations:

1. Marginal: The ovary in which the placenta forms a ridge along the ventral suture of the ovary and the ovules develop on two separate rows is known to have marginal placentation. The ovules are borne along the junction of the two margins of the carpel. It occurs in monocarpellary and unilocular ovary, e.g., Leguminosae (pea, beans).

2. Parietal: The placenta is formed by the swelling up of cohering margins, where later on develop the ovules in rows. Here ovary is one-chambered but it becomes two- chambered due to the formation of the false septum. The ovules develop on the inner wall of the ovary or on peripheral part. It occurs in bicarpellary or multicarpellary but unilocular ovary, e.g., mustard, cucurbita and Argemone.

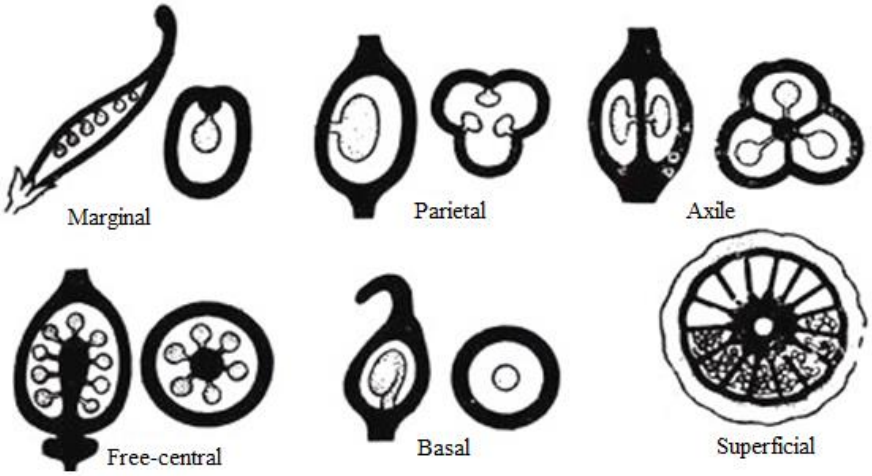


Fig. 1.10- Types of Placentations

3. Axile: The placentae develop from the central axis which corresponds to the confluent margins of carpels. The ovary is sectioned by radial spokes with placentas in separate locules. It occurs in bi-to multilocular ovary, e.g., Solanaceae (apple, hibiscus) and Malvaceae.

4. Free-central: The placenta develops in the centre of the ovary as a prolongation of floral axis and the ovules are attached on this axis. The ovules are borne on central axis and septa are absent. It occurs in multicarpellary but unilocular ovary, e.g., Dianthus, Primula and Sandalwood

5. Superficial or Laminar: The ovules develop over the entire inner surface of the carpels. It occurs in multicarpellary ovary. Ovary is multilocular and syncarpous e.g., Nymphaea.

6. Basal: The placenta is at the base (bottom) of the ovary. The placenta develops directly on the thalamus and bears a single ovule at the base of the unilocular ovary, e.g., Compositae (sunflower).

7. Apical: where one or few ovules develop at the top of a simple or compound ovary.

Preparation of Placentation for Study

Requirement: Commonly available flowers, forceps, razor/scalpel blade, brush, slides, cover slip, filter paper, dissecting microscope, compound microscope, etc.

Preparation of material: Dissect the ovary of freshly collected flower. Cut the T.S. of ovary, mount it on a slide and observe the type of placentation.

Procedure:

1. Take a clean slide and put a drop of water on the middle of the slide. The drop of water is where the mount is to be transferred to.
2. Transfer material to be mounted on the drop of water with the help of a small brush. Do this step very carefully. If the mount is not correctly placed observation under microscope becomes difficult.
3. Add glycerine to the mount. Now carefully place the coverslip over the glass slide covering the mount.
4. Remove extra fluid present on the slide with the help of filter paper.
5. Observe your mount under the microscope.

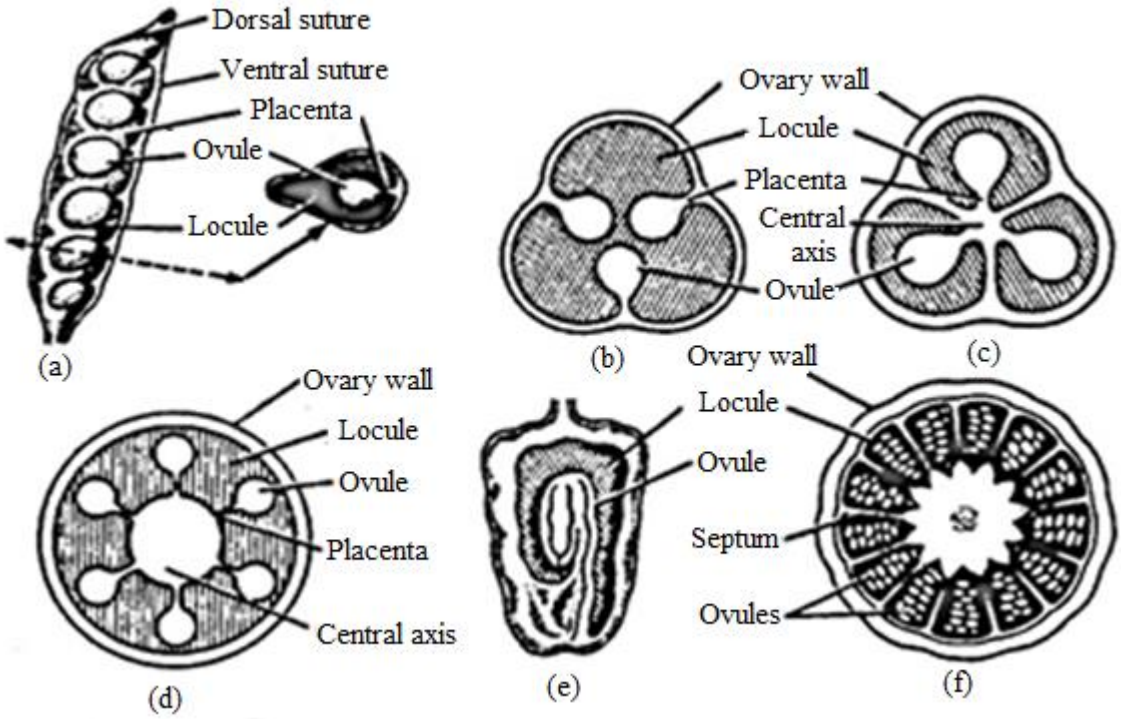


Fig.1.11 Detailed Diagram showing placentations: (a) Marginal, (b) Parietal, (c)Axile, (d) Free-central, (e) Basal, (f) Superficial.

Observations study:

1. The Ovary consists of the ovary wall, the locule or locules, and in a multilocular ovary, the partitions.
2. The ovules are found to be situated on the inner or adaxial (ventral) side of the ovary wall.
3. The ovule-bearing region forms the placenta.
4. In a carpel the placenta occurs close to the margin (Marginal).
5. The ovules develop on the inner wall of the ovary or on peripheral part (Parietal).
6. The ovary is sectioned by radial spokes with placentas in separate locules (Axile).
7. The ovules are borne on central axis and septa are absent (Free-central).
8. The ovules develop over the entire inner surface of the carpels (Superficial).

Placentation can also be seen (generally more easily) from the observation of older ovaries, and fruits (while stamens have to be observed on fresh or even non-open flower). But sometimes, septa tend to secondarily disappear in ovaries with axile placentation. Thus when a cross-section of ovary or a fruit show only one locule, it is necessary to observe a longitudinal section of the ovary to say whether the placentation is free-central or axile.

1.3.3- Ovule

Components of ovule:

In seed plants, the ovule ("small egg") is the structure that gives rise to and contains the female reproductive cells. It consists of three parts: The **integument** forming its outer layer, the **nucellus**, and **embryo sac** or **female gametophyte** formed from haploid megaspore at its center.

The Nucellus

The nucellus is the largest part of the ovule. It houses the embryo sac as well as nutritive tissue and actually remains present in some flowering plants after fertilization as a source of nutrients for the embryo.

The Integuments

The integument is the tough outer protective layer of the ovule. In the diagrams below we can see that gymnosperms, such as pine trees and spruce trees, usually have one integument in an ovule, so we call them unitegmic. On the other hand, angiosperms, like maples and daisies, typically have two integuments, and we call them bitegmic. The integument encloses the nucellus except for a small gap, which is called the *micropyle*.

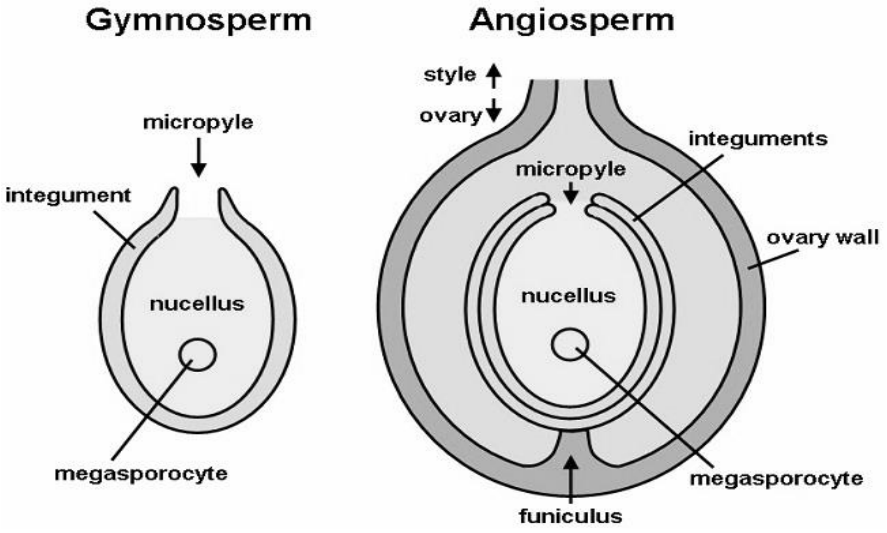


Fig.1.12 Ovules showing integument of gymnosperms and angiosperms

Types of Ovule:

1. Orthotropous (Atropous)-This is where the body of ovule is straight, so that the micropyle lie on the same vertical axis with the funicle and chalaza, e.g. **Polygonium.**

2. Anatropous-In this type the body of the ovule becomes completely inverted, so that the micropyle and hilum come to lie very close to each other. The hilum is a scar that marks the point where the ovule remains attached to the funicle.The micropyle and chalaza lie on the same vertical axis but not funicle, **e.g., Helianthus, Castor.**

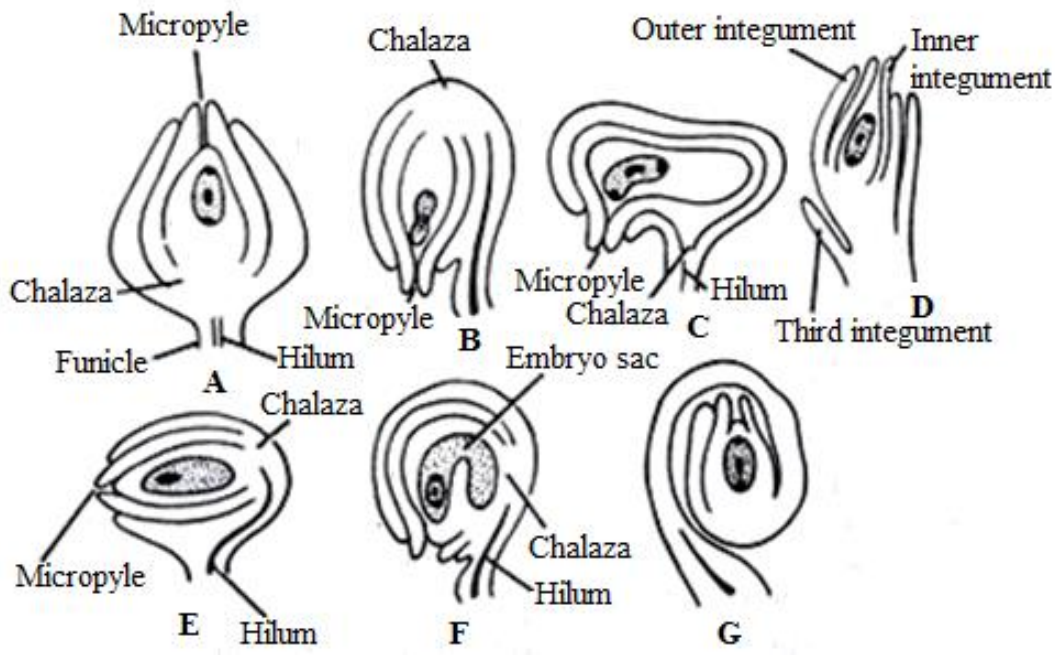


Fig.1.13 Types of ovules: (A) Orthotropous, (B) Anatropous, (C) Campylotropous, (D) Ovule with three integuments, (E) Hemi-anatropous, (F) Amphitropous, (G) Circinotropous

3. Campylotropous-When the micropylar end of the ovule is bend downwards hence the micropyle and chalaza do not lie on the same straight line, it is called campylotropous, **e.g. Pea, Mustard.**

4. Hemi-anatropous-In this type, the nucellus and integuments lie more or less at right angles to the funicle. The micropyle and chalaza lie in one straight line e.g**. Ranunculus**.

5. Amphitropous-When the curvature of the ovule is so much pronounced that the embryo sac bends like a horse-shoe, the ovule is called amphitropous, **e.g. Poppy.**

6. Circinotropous-In this type, the nucellar protuberance is at first in the same line as the axis, but the rapid growth on one side makes it anatropous. The curvature continues till the ovule has turned over completely with the micropylar end again pointing upward, **e.g. Opuntia.**

Study the Slide Showing L.S. Of Anatropous Ovule

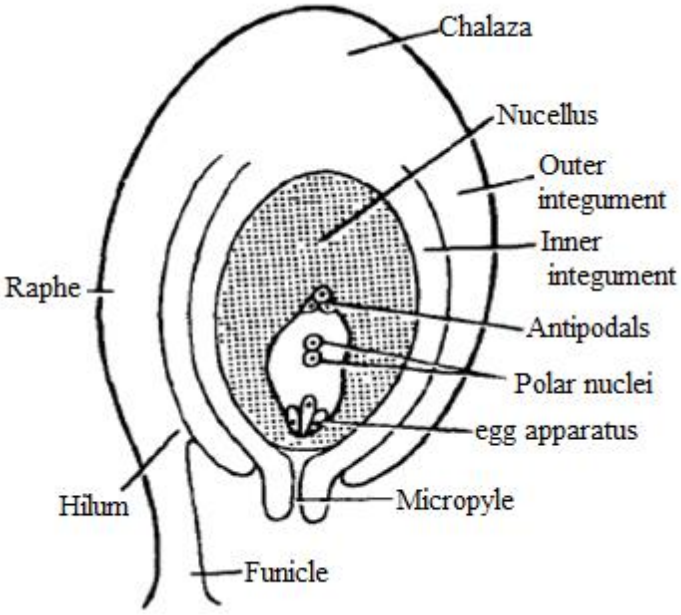


Fig.1.14 The Ovule, L.S. of anatropous ovule

Observations:

The following characters are observed.

1. Anatropous ovule is most common among angiosperms.
2. The ovule is a rounded structure attached to the placenta by a stalk, the funicle. The place of attachment of funicle to the body of the ovule is known as hilum.
3. The basal region of the ovule, where from integuments arise, is known as chalaza.
4. In anatropous ovules, the funicle extends above, along the body of the ovule to form a ridge, known as raphe.
5. The ovule consists of integuments, nucellus and embryo sac.
6. Integuments which may be one (unitegmic) or two (bitegmic) surround the nucellus. These extend well beyond the nucellus to form a narrow opening called micropyle.
7. Nucellus lies below the integuments. If it is massive, ovules are called crassinucellate and if scanty, these are called tenuinucellate. Unitegmic ovules are crassinucellate and bitegmic ovules are tenuinucellate.
8. Enveloped by nucellus is the female gametophyte or embryo sac. A typical embryo sac shows an egg apparatus consisting of an egg and two synergids towards micropyle. In the centre are 2 polar nuclei and 3 antipodal are present at the chalazal end.

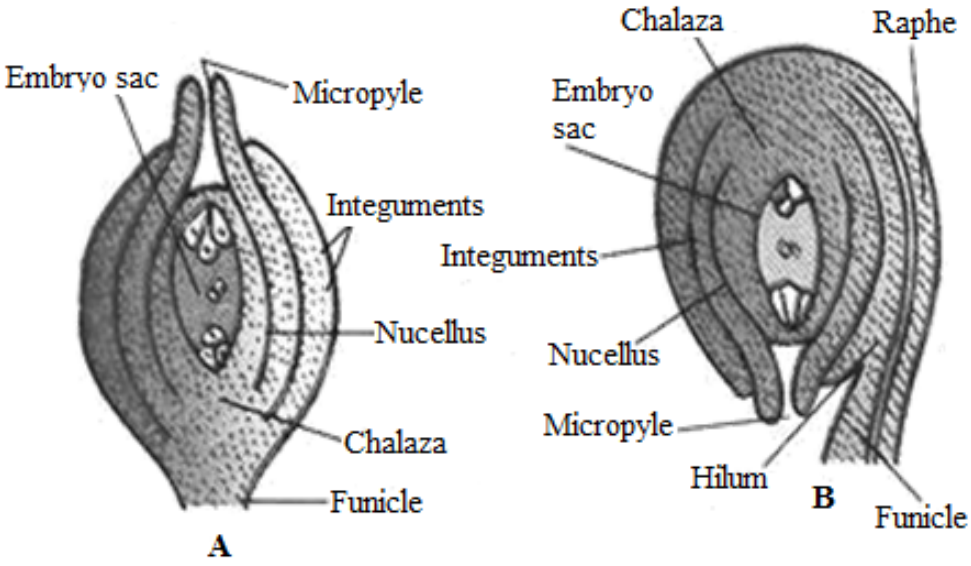


Fig.1.15 Comparing ovules, (A) L.S. of Orthotropous, straight ovule, (B) L.S. of curved anatropous ovule

The Female Gametophyte

The female gametophyte also called the **embryo sac** in angiosperms develops from free nuclear divisions in megaspore nucleus.

Embryo sac: The embryo sac has the egg-apparatus towards the micropylar end. The egg-apparatus has one egg cell (female gamete) and two **synergids.** The egg cell, which is enlarged lies below the synergids. At the chalazal end of the embryo sac there are three **antipodal** cells. These antipodal cells have no definite function and soon gets disorganized. At the center of the embryo sac there are two polar nuclei or their fusion product known as **secondary nucleus.**

Development of female gametophyte:

1. The functional megaspore forms female gametophyte or embryo sac.
2. The nucleus of megaspore divides into two, four and finally eight daughter nuclei. Four of which are located at each pole.
3. One nucleus from each pole migrates to the center to form two polar nuclei which later on fuse to form a diploid fusion or secondary nucleus.
4. Three nuclei at the base of embryo sac form antipodal cells. The remaining three nuclei at the micropylar end get surrounded by cytoplasm to form pyriform cells.
5. These three cells together constitute egg apparatus, which consists of an egg and two other cells known as synergids. The synergids bear special cellular thickenings at the micropylar tip called filiform apparatus, which play an important role in guiding pollen tubes into synergids.

6. The egg cell fuses with one male gamete during fertilization and gives rise to zygote which ultimately develops into embryo, while synergids get disorganized soon after fertilization. The antipodal cells sooner or later also get disorganized.
7. Another male gamete fuses with two polar nuclei or secondary nucleus to form a triploid (3X) primary endosperm nucleus which later on develops into endosperm.

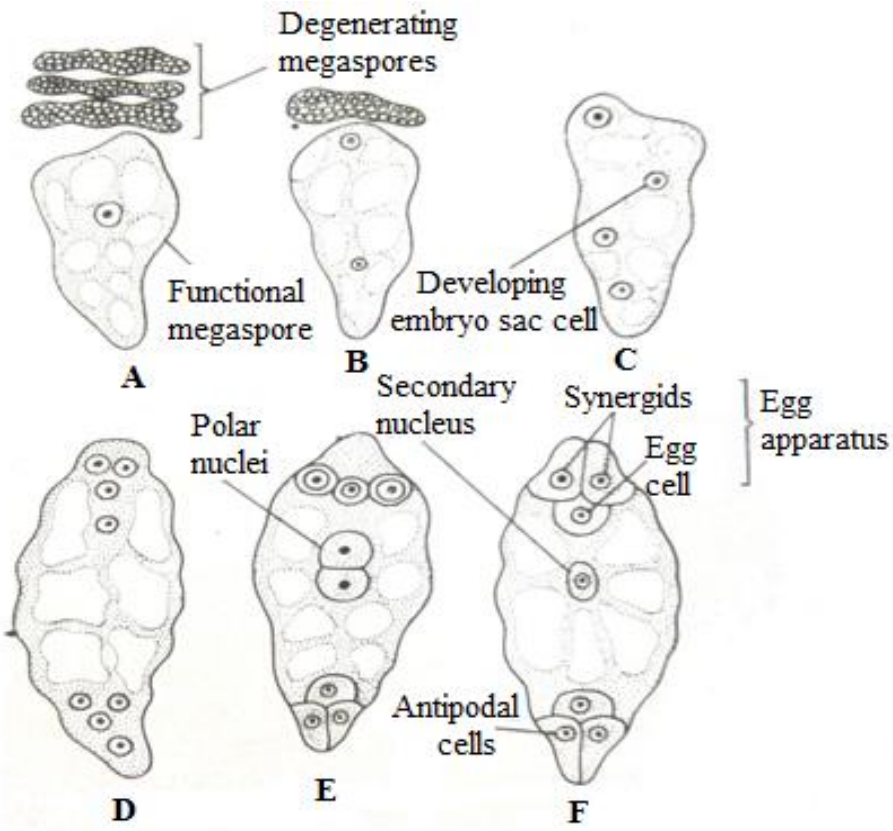


Fig. 1.17 Female gametophyte. A-F, Development of embryo sac of normal type (*Polygonium* type)

To Dissect Out Heart-Shaped Embryo:

Take a small seed of mustard. Locate the micropyle under the dissecting microscope. Remove the seed coat starting from this point. A small white to yellowish embryo can be seen under the microscope.

Observations:

It shows following characters.
1. Heart-shaped embryo consists of a suspensor and a heart -shaped mass of cells.
2. The suspensor is a row of cells arranged in a single line.
3. The uppermost cell of suspensor lies closer to micropyle. It is swollen and is known as vesicular cell.

4. The lowermost cell of suspensor lies close to the embryo proper. It is known as hypophysis.
5. Heart-shaped embryo is formed as a result of cell divisions in globular embryo at places where cotyledons develop.
6. Heart-shaped embryo is differentiated into outer dermatogen, middle periblem and innermost plerome.

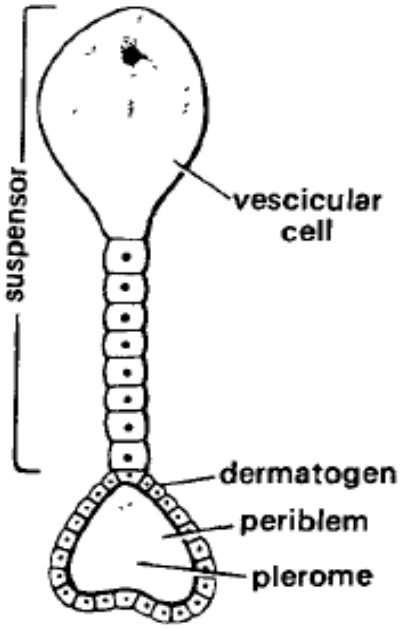


Fig.1.18-Embryo, a heart shaped embryo

1.4 SUMMARY

The mounting of specimens on microscope slides is often critical for successful viewing. The problem has been given much attention in the last two centuries and is a well-developed area with many specialized and sometimes quite sophisticated techniques. In a dry mount, the simplest kind of mounting, the object is merely placed on the slide. A cover slip may be placed on top to protect the specimen and the microscope's objective and to keep the specimen still and pressed flat. This mounting can be successfully used for viewing specimens like pollen. In a wet mount, the specimen is placed in a drop of water or other liquid held between the slide and the cover slip by surface tension. This method is commonly used, for example, to view microscopic organisms that grow in pond water or other liquid media, especially when studying their movement and behavior. The mounting medium is the solution in which the specimen is embedded, generally under a cover glass. Simple liquids like water or glycerol can be considered

mounting media, though the term generally refers to compounds that harden into a permanent mount.

The pollen grain of flowering plants is a haploid, uninucleate cell having two layers, the outer layer **exine** and inner layer **exine**. Permanent slides of pollen grains can be used as a reference for identifying unknown pollen samples. It is therefore important, that the pollen grains remain in an authentic, natural shape. The pollen grains do not remain united at maturity, and are dissociated into single pollen grain called **monad**. Sometimes rarer types like dyads, Octads and Polyads also occur. The pollen grain is uninucleate in the beginning. At the time of liberation, it becomes 2 celled, with a small generative cell and a vegetative cell. In the nutrient medium, the pollen grain germinates. The tube cell enlarges and comes out of the pollen grain through one of the germ pores to form a pollen tube. The tube nucleus descends to the tip of the pollen tube. The generative cell also passes into it. It soon divides into two male gametes.

In botany, the term placentation most commonly refers to the arrangement of placentae inside an ovary. The part of the ovary where the funicle attaches is referred to as the placenta. The types of placenta are, Marginal, Axile, Superficial, Basal, Parietal, Free-central and Apical.

Mature ovule contains an embryo sac also known as female gametophyte. Female gamete (egg) fuses with male gamete to form zygote which later on develops into embryo.

1.5 GLOSSARY

Abiotic: of or characterized by the absence of life or living organisms.
Adaxial: situated on the side toward the axis or stem.
Amphitropous: (of an ovule) inverted so that the funicle is in the middle of one side.
Antipodal: Cells present towards the chalazal end
Aperture: an opening, as a hole, slit, crack, gap, etc.
Authentic: having an origin supported by unquestionable evidence.
Bicarpellary: (of an ovary) having two carpels.
Biotic: pertaining to life.
Campylotropous:(of an ovule) curved so that the micropyle and funiculus almost touch.
Chalaza: the basal part of an ovule, where the integuments and nucellus are joined.
Clavate: shaped like a club with the thicker end uppermost.
Cohering: to stick or hold together in a mass that resists separation.
Columellae: any of various small, column-like structures of animals or plants; rod or axis.
Compound ovary: an ovary composed of more than one carpel.
Confluent: flowing or running together; blending into one.
Congruent: If one thing is congruent with another thing, they are similar or fittogether well.

constituents.

Cross-pollination: the transfer of pollen from the flower of one plant to the flower of a plant having a different genetic constitution

Curvature: the act of curving or the state of being curved.

Dermatogen: a thin outer layer of the meristem in embryos and growing points of roots and stems, which gives rise to the epidermis.

Distal: situated away from the point of origin or attachment.

Dyad: a secondary morphological unit, consisting of two monads.

Embryo sac: the structure within a plant ovule that contains the egg cell; develops from the megaspore

Embryo: the rudimentary plant usually contained in the seed**.**

Equatorial: of, like, or existing at or near the equator.

Exine: the outermost coat of a pollen grain or a spore.

Fertilization: the union of male and female gametes, during sexual reproduction, to form a zygote

Filiform: having the form of a thread, filamentous.

Fossulate: hollowed; grooved.

Foveolate: having foveolae, or very small pits.

Funicle: the stalk of an ovule or seed.

Gemmate: having buds; increasing by budding.

Gynoecium: the pistil or pistils of a flower; the female parts.

Haploid: pertaining to a single set of chromosomes.

Heterogeneous: composed of parts of different kinds; having widely dissimilarelements or

Hilum: the mark or scar on a seed produced by separation from its funicleor placenta.

Homogeneous: composed of parts or elements that are all of the same kind.

HyalineMembrane: The thin, clear basement membrane between the inner fibrous layer of ahair follicle and its outer root sheath.

Integument: the protective layer around an ovule that becomes the seed coat.

Intine: the inner coat of a spore, especially a pollen grain.

Locule: a small compartment or chamber, as the pollen-containing cavitywithin an anther.

Lumina: (of a cell) the cavity that the cell walls enclose.

Megasporangium:a sporangium containing megaspores.

Micropyle: the minute orifice or opening in the integuments of an ovule.

Monad: any simple, single-celled organism.

Multicarpellary: (of a plant gynoecium) having or consisting of many carpels.

Multilocular: having or comprising several small cavities or compartments.

Nucellus: the central part of a plant ovule containing the embryo sac.

Oblate: having an equatorial diameter of greater length than the polar diameter.

Obliterate: to remove or destroy all traces of; do away with; destroy completely.

or spores, sometimes in masses.

Ornamented: if something is ornamented with attractive objects or patterns, is decorated with them.

Orthotropous: (of an ovule) straight and symmetrical, with the chalaza at the evident base and

Paleoecology: the branch of ecology dealing with the relations and interactions between ancient life forms and their environment.

Paleontology: the science of the forms of life existing in former geologic periods, as represented by their fossils.

Palynology: the study of live and fossil spores, pollen grains, and similar plantstructures.

Peripheral: pertaining to, situated in, or constituting the periphery.

Placenta: the part of the ovary of flowering plants that bears the ovules.

Placentation: the disposition or arrangement of a placenta or placentas.

Pollen grain: a single granule of pollen.

Pollen: the fertilizing element of flowering plants, consisting of fine, powdery, yellowish grains

Pollentube:the protoplasmic tube that is extruded from a germinating pollengrain and grows.

Pollination: the transfer of pollen from the anther to the stigma.

Polliniferous: producing or bearing pollen.

Prolongation: the state of being prolonged.

Proximal: situated close to the centre, median line, or point of attachment or origin.

Raphe:(in certain ovules) a ridge connecting the hilum with the chalaza.

Ridge: a ridge is a raised line on a flat surface.

Scar: a mark indicating a former point of attachment, as where aleaf has fallen from a stem.

Scrobiculate: furrowed or pitted.

Self pollination: the transfer of pollen from the anther to the stigma of the sameflower, another

Shrivel: to contract and wrinkle, as from great heat, cold, or dryness.

Spinose: full of spines; spiniferous; spinous.

Suture: a line of junction between two parts.

Syncarpous: of the nature of or pertaining to a syncarp.

Synergids: one of two small cells that lie inside the embryo sac of a flowering plant and nourish

Tetrad: a group of four cells formed by meiosis from one diploid cell.

Tuberculate: having tubercles.

Unilocular: having or consisting of only one loculus, chamber, or cell.

Ventral: of or designating the lower or inner surface of a structure.

Vesicular: characterized by or consisting of vesicles.

1.6 SELF ASSESSMENT QUESTIONS

1.6.1 One wordAnswers type questions:

1. The arrangement of ovules within the ovary wall known as?
2. Fertilization in which pollen tube enter the ovule through micropyle is called?
3. An ovule which becomes curved so that the nucellus and embryo sac lie at right angles to the funicle is?
4. The deposition of pollen on stigma of another flower of the same plant is known as?
5. The ovary in which the placenta form a ridge along the ventral suture of the ovary and ovules develop on two separate rows is known as?
6. The placenta develops from the central axis which correspond to the confluent margins of carpels, known as?
7. The ovule is located inside the portion of the flower, which is called?
8. The integument encloses the nucellus except for a small gap, which is called?
9. Ovules are attached to the placenta in the ovary through a stalk-like structure known as?
10. When ovule is curved in such a way, so that the micropyle and chalaza do not lie on the same straight line, called?

1.6.2 Fill in the blanks:

1. Study of pollen grains is known as_____
2. A stamen consists of_____ and _____
3. The anther that consists of only one anther lobe is called_____
4. Phenomenon of the formation of more than one embryo per ovule is called_____
5. A small pore in the ovule through which the pollen tube enters is called_____
6. Inside ovary, ovule develop from a special tissue called_____
7. For staining material_____ solution is commonly used.
8. Canada balsam is a resin obtained from a conifer_____
9. Semi-permanent and temporary preparations are mounted in _____
10. Pollen grains have hard coat made of _____
11. The outer and inner layer of pollen are_____ and_____
12. Intine of pollen grains is composed of_____ and_____

1.6.3 Multiple choice questions:

1. When pollen from one flower are deposited on the stigma of another flower borne on the same plant the pollination is known as

(a) Self pollination (autogamy) (b)Self pollination (geitonogamy)
(c) Cross pollination (allogamy) (d) Anemophily

2. Marginal placentation is found in
(a) Poppy (b) Mustard
(c) Sunflower (d) Pea

3. Double fertilization is a characteristic of
(a) Gymnosperm (b) Angiosperm
(c) Pteridophytes (d) Bryophytes

4. How many male cells are there in the pollen tube of angiosperm
(a) One (b) Two
(c) Three (d) Four

5. Type of placenta in which ovary is syncarpous unilocular and ovules on sutures is called
(a) Apical placentation (b) Parietal placentation
(c) Marginal placentation (d) Superficial placentation

6. Abiotic means of pollination is carried by
(a) Animals (b) Insects
(c) Water (d) Birds

7. After fertilization the seed coat develops from
(a) Chalaza (b) Ovule
(c) Embryo sac (d) Integuments

8. Which of the following statements is true for the pollen tube
(a) It shows only tip growth
(b) It is composed of three non cellular zones
(c) It shows chemostatic movements
(d) It shows radial cytoplasmic streaming

9. Pollen grains are shed at which stage
(a) Two celled (b) Three celled
(c) Single celled (d) Usually at two celled, but sometimes three celled

10. Filiform apparatus is characteristic of
(a) Egg (b) Synergids
(c) Antipodal cells (d) Anther wall

11. Pollen grains of flower pollinated by insects or wind are not

(a) Large and showy (b) Rough and sticky
(c) Smooth and dry (d) Rough and dry

12. Pollinia are sac like structures
(a) In which anther lobes are present
(b) Which are present in megasporangia
(c) In which pollen grains are present in mass
(d) Which secret yellow substance called pollenkit material

13. The ovule develop over the entire inner surface of the carpels
(a) Basal (b) Free-central
(c) Marginal (d) Superficial

1.6.1 Answer key: 1-Placentation, 2- Porogamy, 3- Hemi-anatropous, 4-Geitonogamy, 5-Marginal Placentation, 6-Axile placentation, 7- Gynoecium, 8-Micropyle, 9- Funicle, 10-Anatropous ovule.

1.6.2 Answers Key: (1) Palynology, **(2**) Filament and Anther, **(3)** Monothecous, **(4)** Polyembryony,**(5)** Micropyle, **(6)** Placenta, **(7)** Iodine, **(8)** *Abies balsamea*, **(9)** Glycerine, **(10)** Sporopollenin, **(11)** Exine and Intine, **(12)** Lipid and Protein

1.6.3 Answers key:1-(a), **2-**(d), **3-**(b), **4-**(b), **5-**(b), **6-**(c), **7-**(d), **8-**(a), **9-**(d), **10-**(b), **11-**(a), **12-**(c), **13-**(d)

1.7 REFERENCES

- Bhojwani, S, S, and Bhatnagar, S. P. 2008. The Embryology of Angiosperms.
- Bendre, A.M. & Ashok Kumar 2012. *A Text Book of Practical Botany II*
- Pandey, B.P. 2009. *College Botany vol. II.*
- http://www.biologydiscussion.com
- http://www.microbehunter.com
- https://biologydictionary.net
- http://www.readorrefer.in

1.8 SUGGESTED READINGS

- Bhojwani, S, S, and Bhatnagar, S. P. 2008. The Embryology of Angiosperms. Vikas Publishing House, New Delhi.

- Maheshwari, P, 1950. An Introduction to the Embryology of Angiosperms. MacGraw Hill, New York.
- Endress,P. K. 2011.Angiosperm ovule: diversity, development, evolution. Ann. Bot. 107(9):1465-1489.
- Rodkiewicz, B.1970. Callose in cell wall during megasporogenesis in angiosperms. Planta 93; 37-47.
- Tilton, V. R. 1980. Hypostase development in Ornithogalum caudatum (Liliaceae) and notes on other types of modifications in the chalaza of Angiospermous ovules. Can. J. Bot. 58: 2059- 2066.
- Wallwork, M. A.B. and Sedgley, M. 2000. Early Events in the Penetration of the Embryo Sac in *Toreniafournieri* (Lind.) Annals of Botany 85: 447-454.

1.9 TERMINAL QUESTIONS

1. Describe the procedure of temporary mount preparation. How it is different from permanent mount?
2. What are the common media used for mounting an object. Describe any four of them.
3. Write a short essay note on pollen grains. What are the various characteristics of pollen grains for study?
4. Illustrate the entire procedure for the germination of pollen grains. Also draw the schematic diagram showing germination of pollen grains.
5. Discuss the different mounting techniques to prepare a permanent mount for pollen grains.
6. What is placentation? Write about the different types of Placentations with example.
7. Write a short essay note on ovule. Explain different components and functions of ovule.
8. Draw a labelled diagram of the longitudinal section of an anatropous ovule. Also write the observation characteristics.
9. Write about the different stages of ovule development and female gametophyte.
10. Draw a detailed diagram showing longitudinal section of ovule of a typical angiosperm.

UNIT-2L DEMONSTRATION OF USUAL TECHNIQUES OF PLANT ANATOMY, SECTION CUTTING, T.S., L.S. OF LEAF, STEM AND ROOT

2.1 OBJECTIVES

This unit is written to explain the following points:

- The student will be familiar with the general features of microscopy and different parts of compound microscopes.
- Students would be able to learn different techniques of anatomy like sectioning and staining along with mounting media and mounting techniques.
- What are the common stains for plant cells.
- Section cutting techniques for leaf, stem and roots
- Anatomical features of root, stem and leaves

2.2 INTRODUCTION

The practical knowledge develops the scientific outlook of the subject and while working in the laboratory a rational approach develops in the students. Here a clear cut differentiation develops in the mind of the student regarding the theory of the subject. As in all experimental sciences, research in plant anatomy depends on the laboratory methods that can be used to study cell structure and function. Many important advances in understanding cells have directly followed the development of new methods that have opened novel avenues of investigation. The basic method used in plant anatomy, or the study of internal plant structure, is the preparation of thin slices which are studied microscopically. From this the science "derives its name (in Greek, *anatome* means "dissection"). The emergence of the field of plant anatomy is closely related to the invention and perfection of the microscope. The English physicist R. Hooke observed in 1665 the cellular structure of thin slices of cork, elder pith, and wood from various plants, using a microscope of his own improved design.

The objectives of undertaking practical studies in botany are to:

- develop practical skill for better understanding through firsthand experience;
- demonstrate the principles covered in the theory;
- develop observational skill in the form of identifying and locating desired parts in specimen;
- develop manipulative skills in arranging and handling the apparatus and instruments
- collect material and to mount it and to develop skill in preserving biological material and specimens;
- draw, label and record experimental results and interpret them.

Through practical work, not only the theoretical concepts are tested but also it trains you in the scientific method.

Some Common Instruments for Anatomy

There are some instruments, which you will use frequently while working in the laboratory. One of these is the compound microscope.

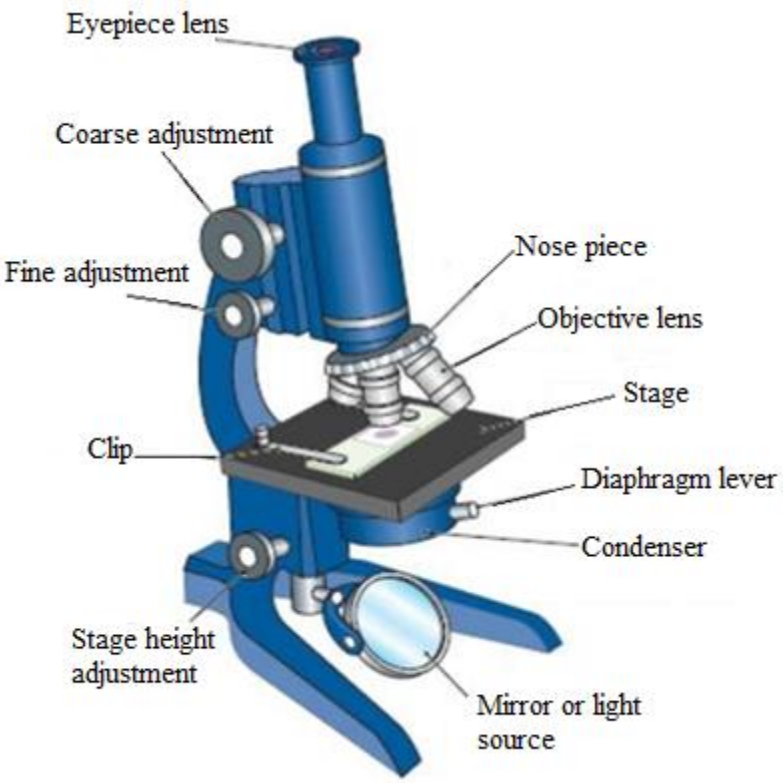


Fig.2.1: Compound Microscope

(1) Compound Microscope: It is an indispensable instrument in a Biology laboratory. Study the diagram of the microscope and compare it with an actual one in the laboratory.

Eye-Piece: Contains lenses to increase magnification.

Body Tube: Holds lenses of eyepiece and objectives at proper working distance from each other.

Arm: Supports body tube and coarse adjustment.

Nose Piece: Permits interchange of low and high powered objectives.

Coarse Adjustment: Moves body tube up and down to the correct distance from the specimen for focusing the object.

Objective lense: Lenses of different magnification as 10X, 40X etc.

Stage: Supports slide over hole that permits light from mirror below.

Diaphragm: Regulates amount of light passing through the specimen.

Stage Clips: Hold slide firmly in place.

Base: Firm support bearing weight of microscope.

Mirror: Reflects light upward through diaphragm and hole in stage.

Fine Adjustment: Meant for the exact focusing by moving stage or body tube up or down very slightly.
Inclination Joint: Permits tilting to adjust the eye level.

Using the Microscope:

- Always use both hands when carrying the microscope, one hand beneath the base and the other holding the arm of the microscope in an upright position.
- Walk, holding the microscope close to your body.
- Set the microscope at least 5 inches from the edge of the table to avoid its knocking off accidently.
- Always clean the lenses and mirror of the microscope with the lens paper/ cloth. Otherwise there might be scratches on them.
- Adjust the mirror by slightly tilting it and by seeing through the eye piece so that sufficient light enters the microscope when you view under low magnification objectives.
- Place the prepared slide directly over the hole in the stage.
- Secure the slide on the stage with the stage clips to prevent accidental movement of the slide.
- Look through the eye piece and slowly bring the low magnification objective towards the material by using the coarse adjustment until the specimen comes into view.
- To change to high power, rotate the nose-piece to bring the high power objective in position (taking precaution that the body tube does not move up or down).
- Look through the eye piece, if the light is insufficient, open out the diaphragm slightly.
- Gently raise the objective by using fine adjustment. If the image worsens without improving, start lowering the objective by the same fine adjustment.
- Do not use coarse adjustment while viewing under high power. By gently moving up and down you will be able to get a clear focus.
- While removing the slide from the stage release the spring clips. Do not allow the stage clips to extend out of the stage.
- When work gets over, rotate nose piece such that the objective lens is not over the hole in the stage.
- When not in use keep it covered by a polythene cover and/or lock it in its box.

(2) A simple hand lens: Contains a single double convex lens mounted on a handle. Can magnify things four to five times and used for smaller magnification.

(3) Scalpel: Works like a knife, used to cut out thin slices and peel.

(4) Fine scissors: Used for cutting.

(5) A pair of forceps: Used for picking up very thin slices or material.

(6) Fine needles: Used for (i) adjusting sample/teasing any biological material on a glass slide without touching it, placing the cover slip on the slide.

(7) Fine hair brush: Mainly used for transferring material for mounting on the slides.

(8) Spatula: Used to pick up solid chemicals.

(9) Glassware:
(i) **A dropper:** Used for putting a drop of liquid on the slide.
(ii)**Plain glass slides:** Used for preparing temporary or permanent mounts.
(iii) **Cover slips (Very thin glass cover):** Used for covering the material placed on glass slide to be observed under the microscope. This protects the objective lens.
(iv) **Petridish:** Is a shallow dish often with a cover. Used for soaking specimen for the purpose of preservation, staining etc. Also used to keep a medium on which bacteria or small organisms may be cultured.
(v) **Beaker:** Available in various sizes from 100 ml to 1000ml. Used for preparing and storing chemicals and performing experiments.
(vi) **Flask:** A bottle with a narrow neck used in the laboratory for performing experiments (keeping solution, for heating solution etc).
(vii) **Funnel:** Available in various sizes i.e. in different diameter of the mouth of the funnel. Used during filtration of solutions.
(viii) **Pipette**: A slender graduated glass tube for measuring and transferring known volume of liquid.
(ix) **Spirit lamp or Bunsen burner**: Used for heating. It should be extinguished immediately after use.

2.3 DEMONSTRATION OF USUAL TECHNIQUES

2.3.1-Plant anatomy

Plant anatomy is a basic core subject in the study of Botany. The cells of plants are quite minute and microscopic in size, so cannot be observed by naked eyes. Such objects are visible only under microscopes. Our eye has limited magnification or resolution power so unable to distinguish the objects smaller than 0.1 mm. Moreover the living cells are transparent in ordinary light and cannot be distinguished among various cellular components. The microscopes are the most important tools in the plant anatomy and their magnification power is achieved by lenses of various types.

Solid material should be sectioned in several planes in order to discover the distribution of the various tissues within it. The complete investigation of axial structures, such as stem or root, normally requires a transverse (cross) section at one or more levels and radial longitudinal, and tangential longitudinal sections at different depths from the surface to the center. Foliar structures generally require transverse and vertical longitudinal sections may occasionally be necessary. Hand sections of plant organs can be obtained readily and this, together with the use of simple staining schedules, allows the visualization of the structure using a light microscope. The exercise can be performed by students at all levels after having demonstrated the techniques to them. Students will need help initially in identifying cell and tissue types. Color photographs will be useful to serve as a guide for identification purposes. Some of the techniques are given below.

2.3.2-Section cutting

Free Hand Sectioning Methods

Most plant parts are too thick to be mounted intact and viewed with a microscope. In order to study the structural organization of the plant body, sections have to be made so that enough light can be transmitted through the specimen to resolve cell structures under the microscope. A free hand section is the simplest method of preparing specimens for microscopic viewing. This method allows one to examine the specimen in a few minutes. It is also suitable for a variety of plant materials, such as soft herbaceous stems and small woody twigs. The fixation of materials is generally not required for temporary preparations.

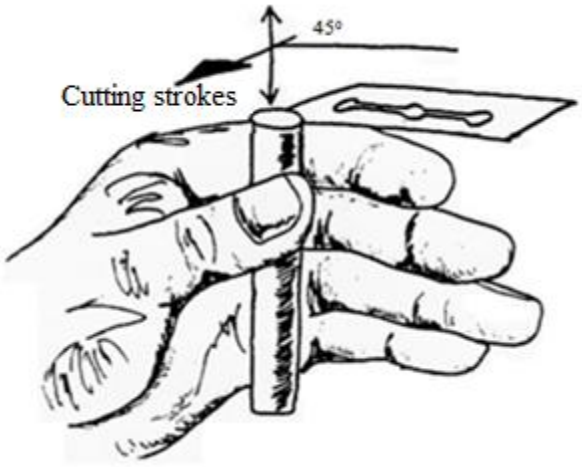


Fig.2.2: One method of holding a specimen for free hand sectioning

In order to reveal the cellular organisation, the plant material is usually cut into following types of sections:

1. **Cross Section**: Here the section is cut at the right angle to the plant material and it is of two type:

a) **Transverse section**: Section at right angle to vertical axis of the material such as in root and stem as in figure given below.

Fig.2.3: T.S. of Tomato and Banana

b) **Vertical Section**: Section at right angle to the transverse axis such as in leaves and thallus.

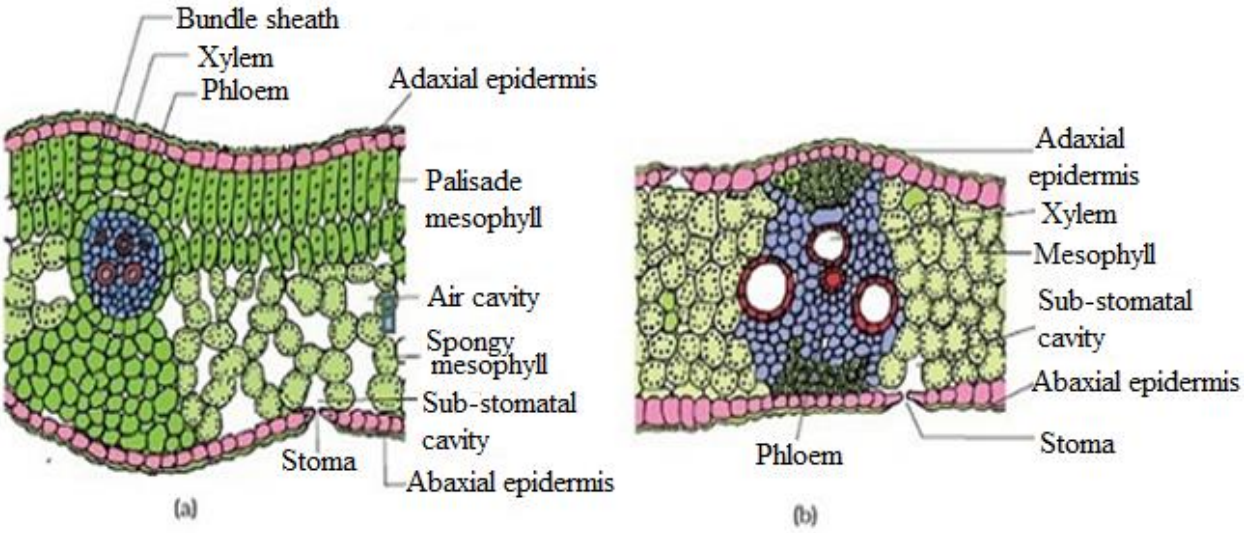


Fig.2.4: V.S. of Dicot and Monocot Leaf

2. Longitudinal Section: The section is cut at right angle to the transverse axis and is also of two types:

a) **Radial Longitudinal Section (RLS):** In this the section is cut through the radius

b) **Tangential Longitudinal Section (TLS):** In this the section is cut through the tangent and do not pass through the central part.

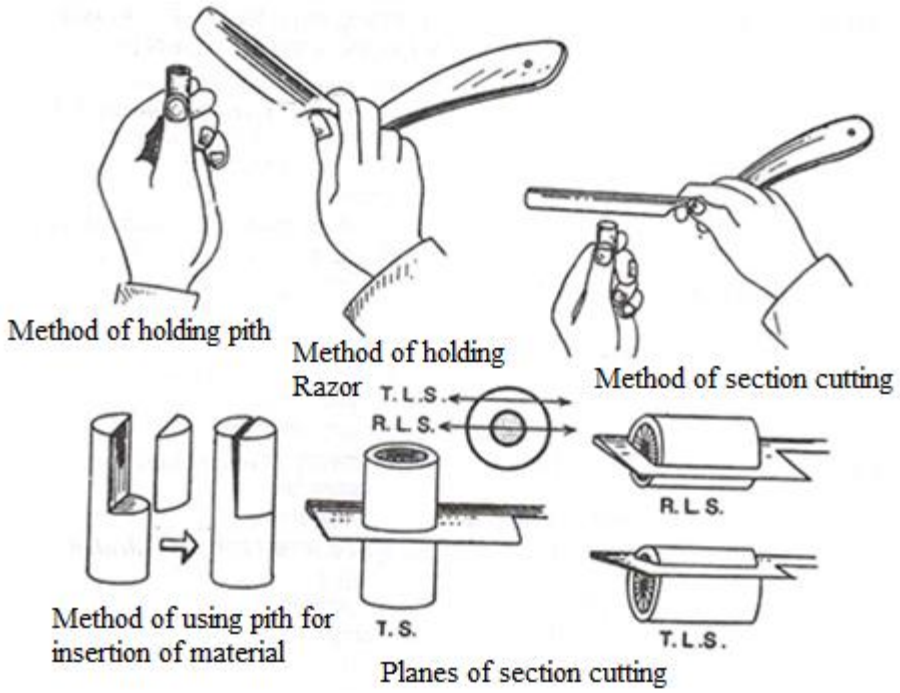


Fig.2.5: Sectioning procedure for TLS and RLS

Procedures of Section cutting:

1. Obtain a new double edge razor blade. To minimize the risk of cutting oneself, cover one edge of the razor blade with masking tape. Rinse the blade with warm tap water to remove traces of grease from the surface of the blade if necessary.
2. Hold the plant material firmly. The material should be held against the side of the first finger of the left hand (or right hand) by means of the thumb. The first finger should be kept as straight as possible, while the thumb is kept well below the surface of the material out of the way of the razor edge.
3. Flood the razor with water. This will reduce the friction during cutting as sections can float onto the surface of the blade. Take the razor blade in the right hand (or left hand) and place it on the first finger of the left hand (or right hand), more or less at a right angle to the specimen.
4. Draw the razor across the top of the material in such a way as to give the material a drawing cut. This results in less friction as the razor blade passes through the specimen. Cut several sections at a time. Sections will certainly vary in thickness. However, there will be usable ones among the "thick" sections!
5. Transfer sections to water, always using a brush, not a forceps or needle.
6. Select and transfer the thinnest sections (the more transparent ones) onto a glass slide and stain (see next section).

Note: For cross sections, special care should be taken during sectioning to see that the material is not cut obliquely. In our experience, as long as the sections are not obliquely sectioned, even "thick" sections are usable. During sectioning, a number of sections should be cut at the same time and one should not worry about the section thickness at this time. By slightly and progressively increasing the pressure with the razor blade on the first finger, and simultaneously exerting increasing pressure onto the specimen by the thumb, a number of sections can be cut without moving the material or the thumb.

For delicate and hard to hold specimens such as thin leaves and tiny roots, additional support can be used to facilitate hand sectioning. The following methods will allow for the sectioning of thin leaves and small, soft specimens such as roots. Tissue pieces can be inserted into a small piece of pith such as a carrot root. Once the tissue is firmly in place, the hand sectioning technique can be applied.Longitudinal sections are also difficult to obtain by hand without supporting material as small stem and root pieces are difficult to hold with one's finger. However, by cutting a v-shaped notch into the pith support, it is possible to hold the tissue firmly for free hand sections.

Preparation Techniques: Dry Mounts, Wet Mount, Squash, Staining

Permanent Preparation: There are several ways to prepare slides for histological analysis. To notice or detect physiological changes, it is needed to embed the samples in resin or paraffin. First of all, the section fixing should be as soon as possible and we can fix it in several reagents. We can use FAA (Formaldehyde, acetic acid and Alcohol 70% or 50%. After that dehydration in an ethanol series (10, 30, 50, 70, 90, and 100%) for 20-30 minutes in each ethanol grade is done. After finishing the dehydration you need to make a transition of ethanol to resin (3:1, 1:1, 1:3,) the time for each step is variable depending upon the size of your sample. After this we can mount the section in Canada balsam or DPX and cover it with cover slip.

Mounting Media and Mounting

For temporary preparations: Glycerine medium with 10 to 20% pure glycerine + 90 to 80ml water or lacto phenol medium is used for temporary preparation. It is prepared by adding equal amount of phenol, lactic acid, glycerine and distilled water.

For permanent preparations: Canada balsam and DPX mountant are used for permanent slide as mounting media.

Mounting is done at the center of the slide. For this put a drop of mounting media at the center of the slide and the material is transferred in the drop of medium with help of a brush. With the help of a needle we put cover slip over it gently in such a way that air bubble should not be there. Extra amount of fluid can be removed by blotting paper.

Maceration Technique

A maceration method has been very useful in studying the features of intact cells. In this maceration procedure, the middle lamella, which normally cements adjacent cells together, is dissolved by acid which allows the cells to separate from one another.

Maceration fluid preparation: The maceration fluid is prepared by combining 1 part of a 30% solution of hydrogen peroxide, 4 parts of distilled water, and 5 parts of glacial acetic acid. Be sure to use a clean bottle and prepare this solution in the fume hood. Avoid contact with the solution, wear gloves if necessary.

Procedures: Temporary preparations

1. A variety of plant tissues such as soft pith tissues and woody xylem samples can be studied using this technique. Cut plant tissues into small pieces and place these into a vial containing the maceration fluid. The volume of fluid required is approximately 10X the volume of the tissue.
2. Cap tightly. Place the vials in an oven at about 56^0C for 1-4 days. The duration of maceration depends on the nature of the material. For soft tissues, such as the sunflower stem, 12-24 hours is sufficient.
3. If the maceration has been completed, the fluid will be clear and the tissues appear whitish to translucent. Often, the tissue remains intact after this treatment. If the material is not as described, add fresh maceration fluid and leave it for an additional one to two days.
4. When the maceration is complete, gently rinse tissue in three changes of water (several hours between each change) and leave the tissue in water overnight. Give the material a final rinse in water and store in water or 30% glycerol solution.
5. If necessary, transfer a small mass of cells into a vial containing water; otherwise simply process the tissue using the original vial. Be sure to cap the vial tightly, and shake vigorously until the water becomes clouded with cells.
6. Apply a small drop of the mixture to a glass slide, cover it with a cover glass and examine.

Staining - Application of stain to a sample to color cells, tissues, components, or metabolic processes. This process may involve immersing the sample in a dye solution and then rinsing and observing the sample under a microscope. Some dyes require the use of a mordant, which is a chemical compound that reacts with the stain to form an insoluble, colored precipitate.

Botany specimens from differing divisions (based on their taxonomy) respond to stains in a unique way. Stains that Bryophytes require might not be the same for Algae or Fungi, or even Pteridophytes. The most commons stains used in laboratory work are Aniline blue, Fast green, Safranin, Cotton blue, Methylene blue or Crystal violet. Media used for mounting may vary between Glycerine 10%, Glycerine jelly, Lactophenol, Erythrosine or Canada balsam (or D.P.X. Mountant) depending on whether they are for temporary or permanent preparations.

Algae: Temporary preparations

- Single staining: Iodine solution, Aniline blue - 0.1% aqueous, Fast green - 0.5% aqueous.
- Mounting media: Glycerine 10 % or glycerine jelly

Fungi: Temporary preparations

- Single staining: Cotton blue, Aniline blue
- Mounting media: Lactophenol or glycerine 10%

Bryophytes: Temporary preparations

- Single staining: Safranin or Fast green
- Mounting media: glycerine 10% or glycerine jelly

Pteridophytes: Temporary and permanent preparations

Double staining

- Primary stains: Safranin and Crystal violet
- Secondary stains: Fast green, Aniline blue and Erythrosine
- Mounting media: Glycerine 10% for temporary preparations and Canada balsam or D.P.X. Mountant for permanent preparations.

Gymnosperms: Temporary and permanent preparations

Double staining

- Primary stains: Safranin and Crystal violet
- Secondary stains: Fast green , Aniline blue and Erythrosine

Mounting media: Glycerine 10% for temporary preparations and Canada balsam or D.P.X. Mountant for permanent preparations

Mixtures of Some Common Stains

Crystal violet:

- Crystal violet: 3 g
- Distilled water: 80 ml
- Ethyl alcohol (95%): 20 ml, dissolved and mixed with 0.8 g of ammonium oxalate.
  It is a violet dye and is used to stain the lignified tissues.

The color of stain by gentian violet depends on the acidity. At pH 1.0, the dye is green, but in an alkaline solution it is colorless.

Methylene blue:

- Methylene blue: 0.3 g
- (0.01%) distilled water - 100 ml
- Ethyl alcohol (95%): 30 ml, dissolved and mixed with potassium hydroxide
  It is used to stain cellulose walls.

Safranin:

- Safranin: 0.25 g
- Alcohol (95%): 10 ml
- Distilled water: 100 ml
  This is mainly used to stain lignified tissues.

Fast Green:

- Fast Green: 0.5 g
- Alcohol (95%): 100c.c.
  Fast green is the green dye used to stain thin walled tissue.

Common Biological Stains: Different stains react or concentrate in different parts of a cell or tissue, and these properties are used to advantage to reveal specific parts or areas. Some of the most common biological stains are listed below. Unless otherwise marked, all of these dyes may be used with fixed cells and tissues; vital dyes (suitable for use with living organisms) are noted.

Carmine: Carmine is an intensely red dye used to stain glycogen, while Carmine alum is a nuclear stain. Carmine stains require the use of a mordant, usually aluminum.

Crystal violet: Crystal violet, when combined with a suitable mordant, stains cell walls purple. Crystal violet is the stain used in Gram staining. Crystal violet stains the acidic components of the neuronal cytoplasm a violet colour, specifically nissl bodies.

Eosin: Eosin is most often used as a counter stain to haematoxylene, imparting a pink or red colour to cytoplasmic material, cell membranes, and some extracellular structures. It also imparts a strong red colour to red blood cells. Eosin is a red dye that stains cytoplasm. It is water-soluble and thus can be used to follow water movement through plants.

Acid fuchsine: Acid fuchsine may be used to stain collagen, smooth muscle, or mitochondria. Acid fuchsine is used as the nuclear and cytoplasmic stain. Acid fuchsine is also a traditional stain for mitochondria. The dye fuchsine is a biological stain that is produced by oxidation of a mixture of aniline and toluidine, producing a brilliant bluish red.

Haematoxylene: Haematoxylene is a nuclear stain. Used with a mordant, haematoxylene stains nuclei blue-violet or brown. It is most often used with eosin in H&E (haematoxylene and eosin) staining—one of the most common procedures in histology.

Iodine: Iodine is used in chemistry as an indicator for starch. When starch is mixed with iodine in solution, an intensely dark blue colour develops, representing a starch/iodine complex. Starch is a substance common to most plant cells and so a weak iodine solution will stain starch present in the cells. Iodine is one component in the staining technique known as Gram staining, used in microbiology.

Methyl green: Methyl green is used commonly with bright-field microscopes to dye the chromatin of cells so that they are more easily viewed.

Methylene blue: Methylene blue is used to stain animal cells, such as human cheek cells, to make their nuclei more observable. Also used to stain the blood film and used in cytology.
Safranin: Safranin (or Safranin O) is a nuclear stain. It produces red nuclei, and is used primarily as a counter stain. Safranin may also be used to give a yellow colour to collagen.

2.3.3- T.S.of leaf, stem, root

Leaf, stem and root of plants are made up of different types of tissues. These tissues form different layers in the composition of leaves, stems and roots. To study the structural details of the leaf, stem or root of a monocot or dicot plant, it is essential to be familiarized with the sectioning and staining techniques used with plant materials. It is also necessary to take the sections with uniform thickness so that the light passes through them equally and the different tissues found in the material are clearly visible under the microscope. To examine the tissues clearly, it is desirable to stain the section with suitable stains, as different stains colour the tissues differently.So till now we have studied all these aspects of practical. Now we are going to read the anatomy of leaf, stem and root.

Leaf Anatomy

T.S. Leaf: Leaf comprises following cells or tissue:

- **Epidermal layer-**barrel shaped cells on the dorsal and ventral surfaces.
- **Palisade mesophyll** – tightly packed cells that absorb light
- **Spongy mesophyll** – loosely packed cells with air spaces
- **Stomata** – pore-like openings for taking in CO_2 and releasing O_2
- **Guard cells** – cells that open and close the stomata

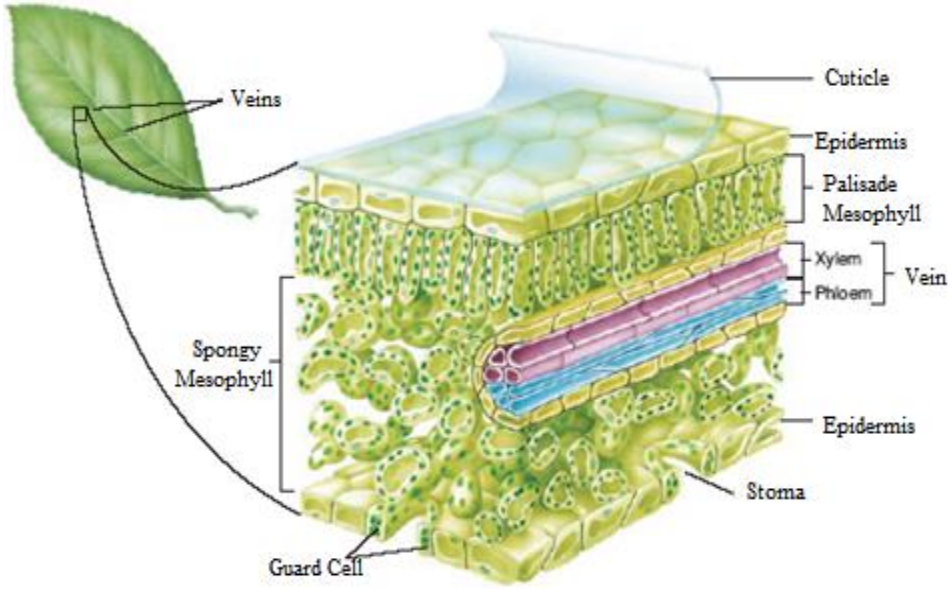


Fig.2.6: Leaf Anatomy

Leaves are of two types; dorsiventral common in Dicots and isobilateral common in Monocots. These are clearly distinguished on the basis of their venation, mesophyll cells, and distribution of stomata and even on the basis of their colour.

Leaf of Banyan (Dicot):

A transverse section through the leaf of Banyan (*Ficus benghalensis* of family Moraceae) would reveal the anatomical characters more or less similar to the previous one.

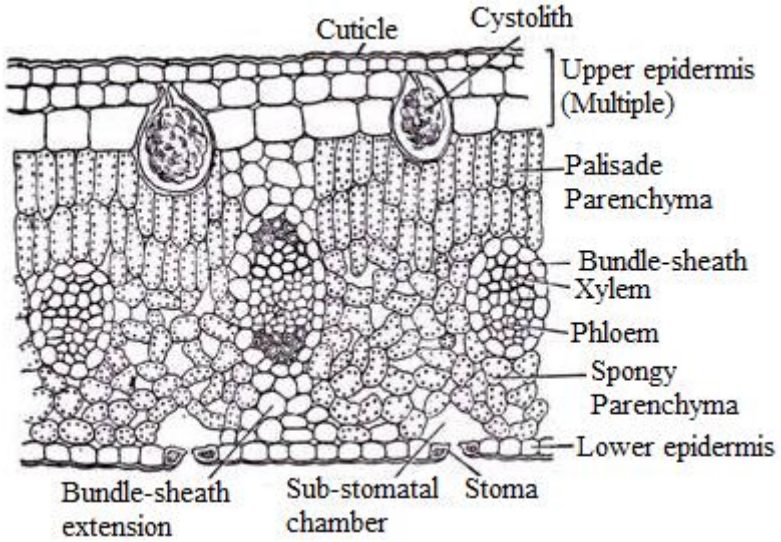


Fig.2.7: T.S. *Ficus benghalensis* leaf

I. Epidermis:

The upper epidermis is multiseriate, being made of a few layers of cells. Lithocysts are frequently present and well-developed calcium carbonate crystals, the cystoliths, occur here and there. The lower epidermis is uniseriate. The outer layer of upper epidermis and the lower epidermis as a whole are made of compactly-arranged tubular cells with cutinised outer walls having cuticle. The degree of cutinisation is more pronounced on the upper side. Stomata occur on the lower epidermis.

II. Mesophyll:

It is differentiated into palisade and spongy cells. Two or three layers of columnar cells with abundant chloroplasts remain arranged more or less at right angles to the upper epidermis. These are palisade cells. This is the principal photosynthetic tissue. The spongy cells occurring towards lower epidermis are isodiametric, and often irregular in shape, and have profuse intercellular spaces. The number of chloroplasts is naturally much smaller here in comparison to palisade cells.

III. Vascular bundles:

The bundles are as usual collateral and closed ones, with xylem lying on the upper and phloem on the lower sides. They remain surrounded by parenchymatous bundle sheath. In case of bigger bundles bundle sheath extensions are present.

2. Leaf of Maize (Monocot):

A section through a leaf of maize (*Zea mays* of family Graminaceae) shows the following structure:

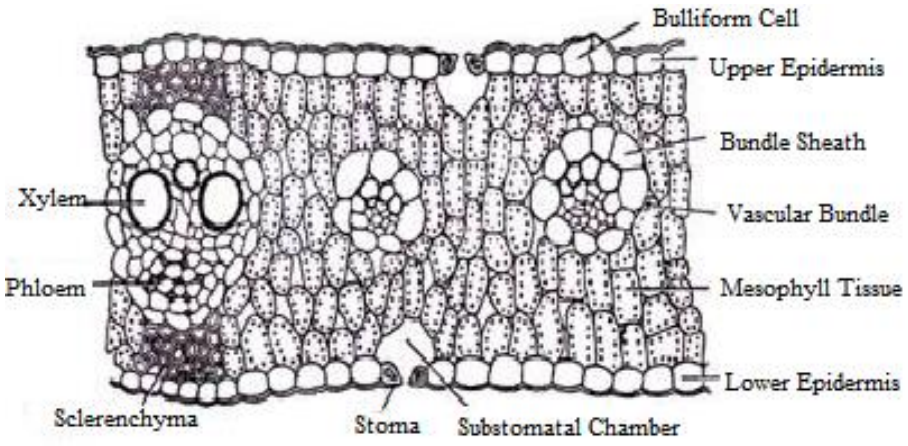


Fig.2.9: *Zeamays* leaf T.S.

I. Epidermis:

Both upper and lower epidermal layers are uniseriate and composed of more or less oval cells with cuticularised outer walls. Upper epidermis may be easily identified due to presence of large and empty bulliform cells. Stomata occur on both the epidermal layers.

II. Mesophyll:

The mesophyll does not show differentiation into palisade and spongy cells, but is made of rather compactly-arranged isodiametric cells.

III. Vascular bundles:

The bundles are collateral and closed ones which remain arranged in parallel series. Majority of the bundles are small, but fairly large bundles occur at regular intervals. Small bundles have xylem on the upper and phloem on the lower sides surrounded by large parenchyma cells forming the bundle sheath.The cells of the sheath contain plastids, often with starch grains. It is assumed that this layer serves as a temporary storage tissue, apart fromconducting the products of photosynthesis to the phloem. Xylem, as usual, consists of tracheary elements, and phloem of sieve tubes and companion cells.

Floating Leaf of Water-lily:

A section through the leaf of water lily (*Nymphaea stellata* of family Nyphaeaceae) would reveal the following anatomical structure. As an aquatic plant it has extremely reduced vascular and supporting tissues and well-formed air chambers.

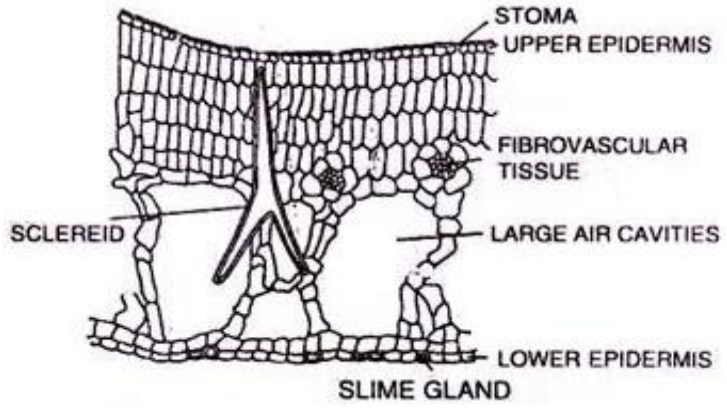


Fig. 2.10: T.S. Nymphaea Leaf

I. Epidermis:

Epidermal layers are uniseriate both on the adaxial and abaxial sides. They are composed of closely-set cells. Stomata occur on the upper side. Moreover, there is deposition of waxy matters which prevents wetting and clogging of the stomata.

II. Mesophyll:

It is differentiated into palisade and spongy cells. A few layers of columnar cells occur towards the adaxial side forming the palisade. The spongy cells present towards lower epidermis and irregular in outline. Large air chambers are present in the mesophyll. Elongated sclerotic cells—the trichosclereids commonly called 'internal hairs', often with branched ends are frequently present.

III. Vascular Bundles:

These are very much reduced. As usual they are composed of xylem and phloem, and remain surrounded by parenchymatous bundle sheath.

Stem Anatomy

The stem and other plant organs are primarily made from three simple cell types: parenchyma, collenchyma, and sclerenchyma cells. Parenchyma cells are the most common plant cells. They are found in the stem, the root, the inside of the leaf, and the pulp of the fruit. Parenchyma cells are responsible for metabolic functions, such as photosynthesis. They also help repair and heal wounds. In addition, some parenchyma cells store starch. Collenchyma cells are elongated cells with unevenly-thickened walls. They provide structural support, mainly to the stem and leaves. These cells are alive at maturity and are usually found below the epidermis.

Dicot stems

Dicot stems with primary growth have pith in the center.

Vascular bundles forming a distinct ring visible when the stem is viewed in cross section.

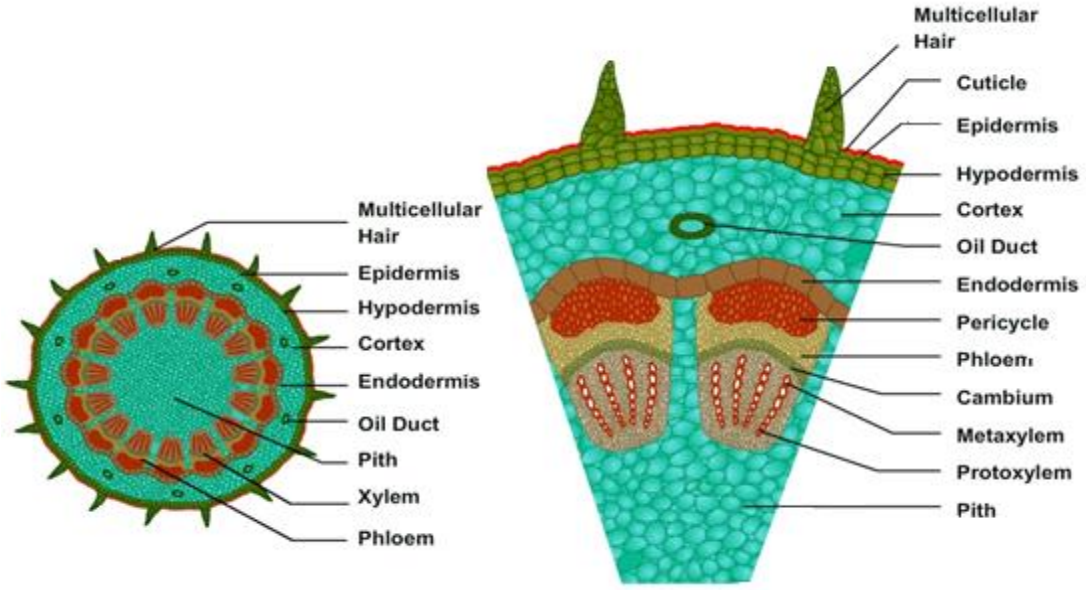


Fig. 2.11 Detailed Structure of Dicot Stem T.S.

- The outside of the stem is covered with an epidermis, which is covered by a waterproof cuticle.
- The epidermis may also contain stomata for gas exchange and multicellular stem hairs called trichomes.
- Cortex comprises the cells of collenchyma, and parenchyma.
- A cortex is flanked by hypodermis (collenchyma cells) outside and endodermis (starch containing cells) inside.
- Collenchyma cells lie under the epidermis and constitute three to four layers of cells with cell walls thickened at the corners. The collenchyma cells contain chloroplasts.
- The parenchyma cells make up the bulk of the cortex. They synthesized organic food (mainly starch) is stored here. The intercellular air spaces are responsible for gaseous exchange.
- Endodermis is starch sheath which forms the innermost layer of the cortex.
- This is a single layer of tightly-packed rectangular cells bordering the stele of the stem.
- The cells of this tissue store starch. It allows solutions to pass from the vascular bundles to the cortex.
- The vascular cambium cells divide to produce secondary xylem to the inside and secondary phloem to the outside.
- As the stem increases in diameter due to production of secondary xylem and secondary phloem, the cortex and epidermis are eventually destroyed.

- Before the cortex is destroyed, a cork cambium develops there. The cork cambium divides to produce waterproof cork cells externally and phelloderm cells internally.
- These three tissues form the periderm, which replaces the epidermis in function. Areas of loosely packed cells in the periderm that function in gas exchange are called lenticels.

Monocot stems

- Vascular bundles are present throughout the monocot stem, although concentrated towards the outside and not in a ring as found in dicots.
- The shoot apex in monocot stems is more elongated.
- Monocots rarely produce secondary growth and are therefore seldom woody, with Palms and Bamboo being notable exceptions.
- However, many monocot stems increase in diameter via anomalous secondary growth occur.
- Monocot stems, such as corn, palms and bamboos, do not have a vascular cambium and do not exhibit secondary growth by the production of concentric annual rings.
- They have scattered vascular bundles composed of xylem and phloem tissue.
- Each bundle is surrounded by a ring of cells called a bundle sheath.
- The structural strength and hardness of woody monocots is due to clusters of heavily lignified tracheids and fibers associated with the vascular bundles.
- The following illustrations and photos show scattered vascular bundles in the stem cross sections of corn (**Zea mays**):

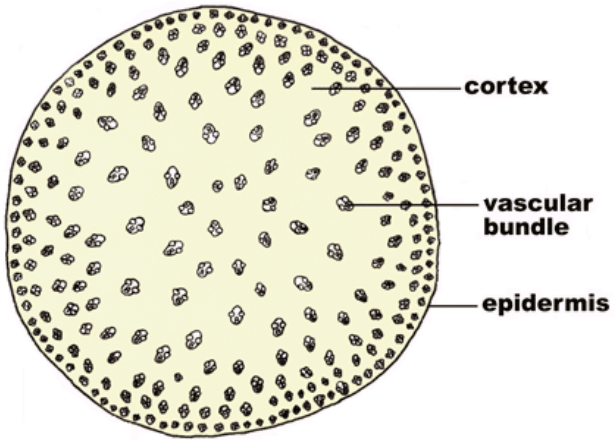


Fig. 2.12. Zea mays Stem T. S.

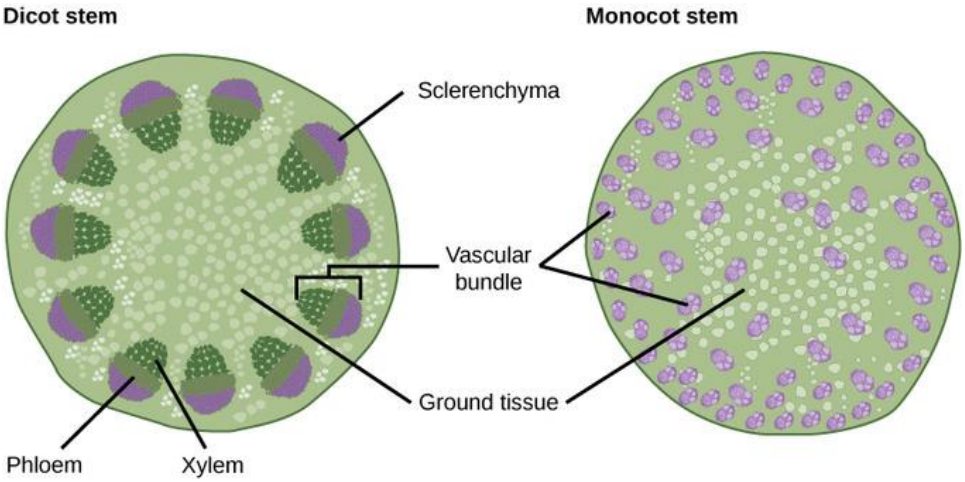


Fig.2.13: Comparative Anatomy of Dicot and Monocot Stem

Root Anatomy

In vascular plants, the **root** is the organ of a plant that typically lies below the surface of the soil. Roots can also be aerial or aerating, that is growing up above the ground or especially above water. The root is best defined as the non-leaf, non-node bearing parts of the plant's body. However, important internal structural differences between stems and roots exist.

When dissected, the arrangement of the cells in a root is root hair, epidermis (epiblema), cortex, endodermis, pericycle and, lastly, the vascular tissue in the centre of a root to transport the water absorbed by the root to other places of the plant.

Monocot Root: The typical monocot roots show following features:

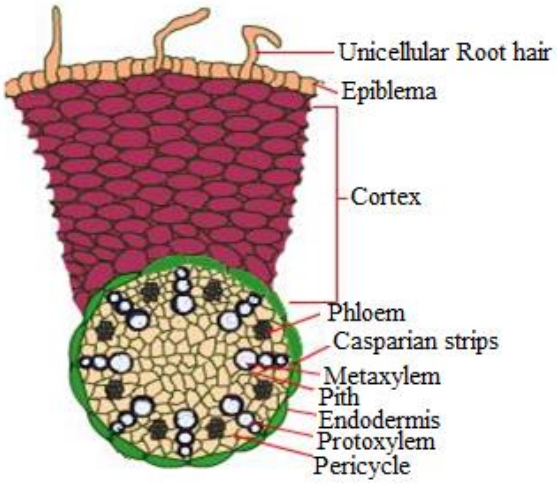


Fig. 2.14: Monocot Root T.S. (*Zeamays*)

1. **Epiblema** is the outermost single layer made from compactly arranged parenchymatous cells without intercellular space. Usually Epiblema has no stomata but bears unicellular epidermal root hairs and less amount of cutin. It contains more cuticle than dicot roots. The root hairs and thin walled epidermal cells take part in the absorption of water and minerals from the soil. The epiphytes have several layered hygroscopic epidermis, called **velamen tissues**. It is made from spongy dead cells which help in absorption of water from atmosphere.
2. **Cortex** is a multi-layered well developed and made from oval parenchymatous cells with intercellular spaces. The intercellular spaces usually help in gaseous exchanges, storage of starch, etc. Cortex helps in mechanical support to the roots (like hypodermis to stem).
3. **Endodermis** is innermost layer of cortex made from barrel shaped parenchyma. It forms a definite ring around the stele. These cells are characterized by the presence of casparian strips. It is deposition of suberin and lignin, on their radial and tangential walls. Usually passage cells are absent in monocot roots. Due to presence of **casparian strips**, endodermis forms water tight jacket around the vascular tissues, hence it is also called biological barrier.
4. **Pericycle** is uniseriate (multiseriate in Smilax) and made from thin walled parenchymatous cells. It is outermost layer of stellar system. Usually it is made from parenchymatous cells but it may become sclerenchymatous in older roots. Several lateral roots arise from this layer.
5. **Vascular bundle** is radial, arranged in a ring (except mangrove, which also contains lenticels), **polyarch** (presence of many alternating xylem and phloem bundles). Xylem and phloem are found at different radii alternating with each other (radial). The number of xylem and phloem vary from, 8 to 46 (100 in *pandanus*). The xylem is exarch, i.e. the protoxylem lies towards periphery and metaxylem toward center.

The phloem is also exarch (protophloem towards the periphery and metaphloem towards the center). Secondary growth is absent in monocot roots due to lack of vascular and cork cambium. **Conjunctive tissue** is parenchymatous tissues which separates xylem and phloem bundles.

6. **Pith** is large, well developed portion of monocot root. It occupies the central portion and made from thin walled parenchymatous tissue with intercellular spaces. It contains abundant amount of starch grains.

Primary Structure of Dicot root

The transverse section of the dicot root shows the following plan of arrangement of tissues from the periphery to the centre.

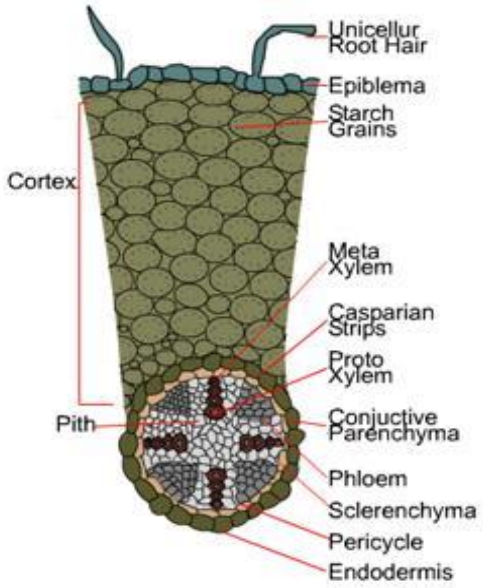


Fig. 2.15- T.S. of Dicot Root of Gram

1. Epiblema or Epidermis - It is the outermost unilayered with several unicellular root hairs. It consists of thin walled, compactly arranged living parenchymatous cells. Usually epiblema is characterised by absence of stomata and cuticle.

2. Cortex - It is thin walled, multi-layered region made from circular or polygonal parenchymatous cells usually with intercellular spaces. The cortical cells have no chloroplast but may contain leucoplast for storage of starch grains. The cortex is responsible for transportation of *water and salts* from the root hairs to the center of the root.

3. Endodermis - It is the innermost layer of cortex and covers the stele. It consists of compactly arranged barrel shaped parenchyma without intercellular spaces. Most of the cells are characterised by the presence of special thickening of suberin and lignin on their radial and tangential walls called **casparian strips**. Some endodermal cell near protoxylem has no casparian strips and called **passage cells** or transfusion cells. These cells allow radial diffusion of water and minerals through the endodermis.

4. Pericycle - It is the outermost layer of stele and composed of uniseriate layer of parenchymatous cells without intercellular spaces. Some dicots and hydrophytes do not bear pericycle. Several lateral roots and lateral meristem arise from pericycle region (hence lateral roots are endogenous in origin). At the time of secondary growth, it produces secondary cambium or phellogens.

5. Vascular bundles - They are 2-8 in number, radial and arranged in ring. Xylem and phloem bundles are separated from each other by parenchymatous cells called conjuctive or **complementary tissue**.

- **Xylem** is exarch (i.e. protoxylem towards the periphery and metaxylem towards the centre) and consists of tracheids, vessels, xylem parenchyma and xylem fibres.

- The **phloem** forms oval masses beneath the pericycle, alternating with xylem bundles. Phloem consists of sieve tubes, companion cells and phloem parenchyma. Usually phloem fibers are absent or reduced.

6. Pith - it is feebly developed and centrally located. It consists of thin walled, polygonal parenchyma cells with intercellular spaces. In dicots roots, it may be reduced or absent. It helps in storage of food materials.

2.3.4-L.S. of leaf, stem and root

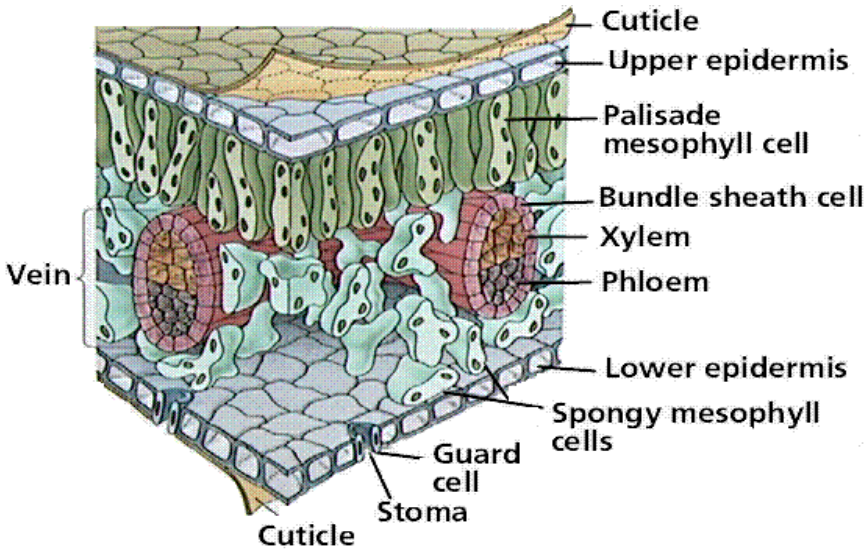


Fig. 2.16: L.S. of Leaf

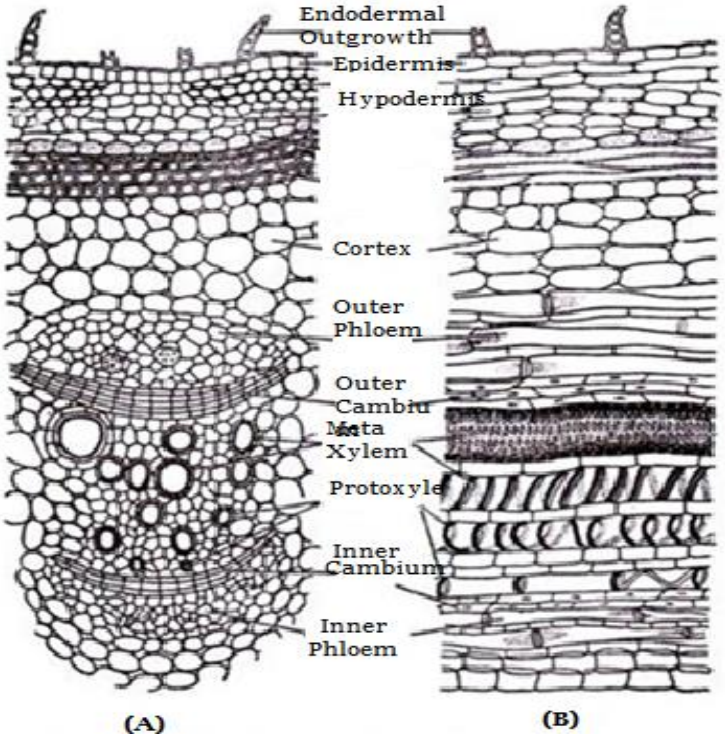


Fig. 2.17. *Cucurbita* Stem (A) T.S. and (B) L.S

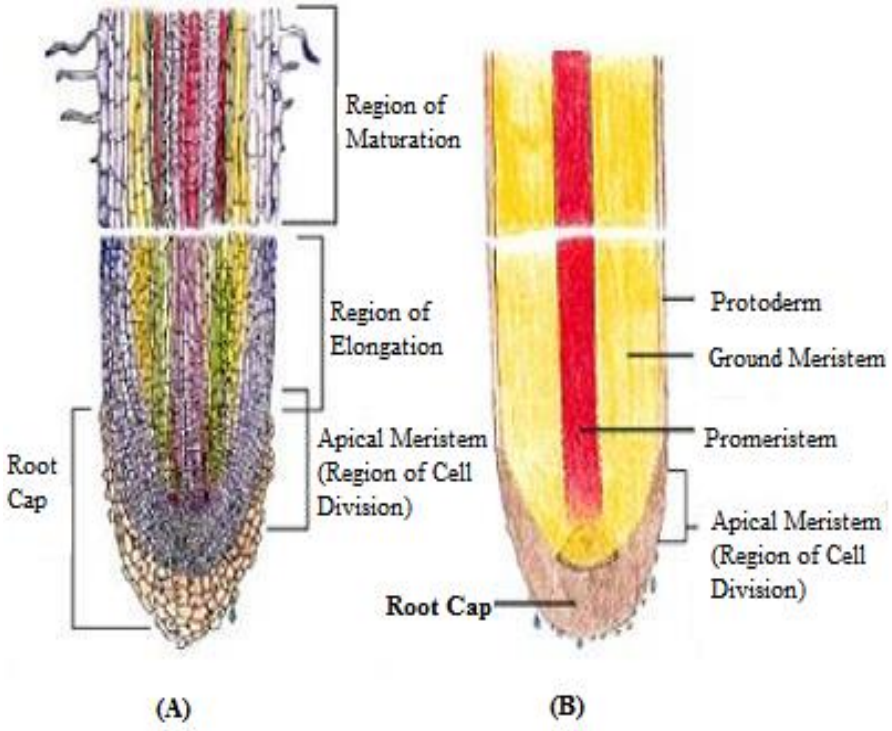


Fig.2.18. Dicot Root Tip L.S.

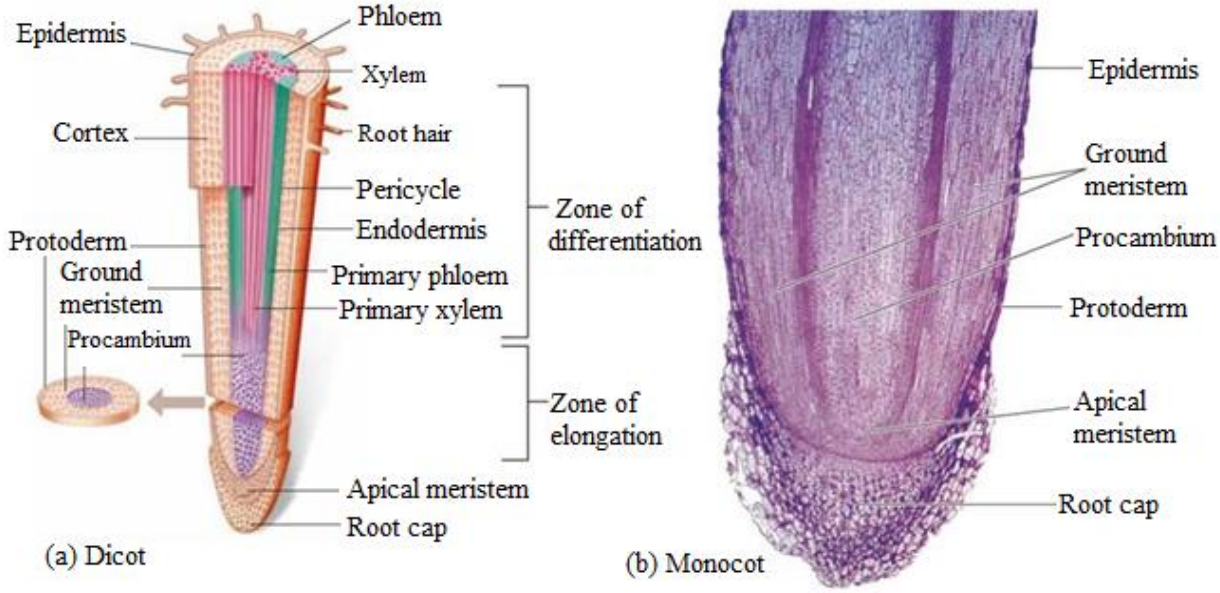


Fig. 2.19. Dicot and Monocot Root Tip L.S.

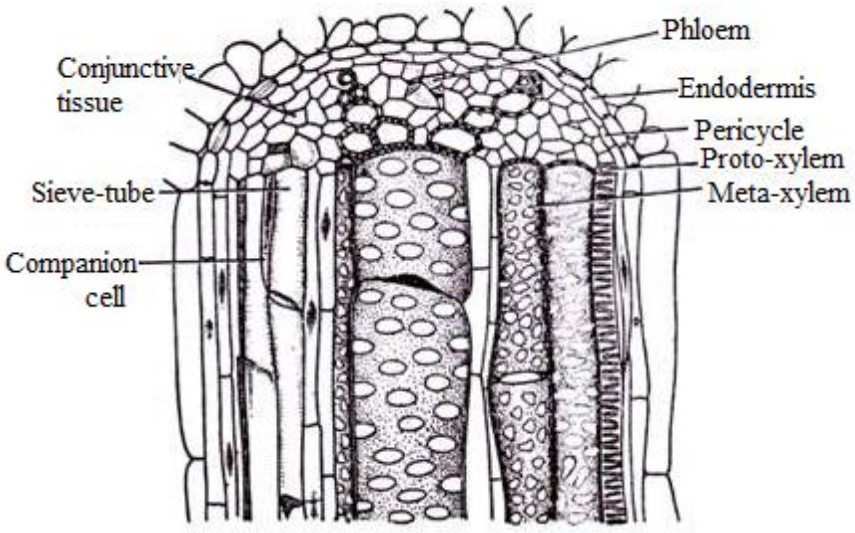


Fig. 2.20 Longitudinal Section of a Dicot Root

2.4 SUMMARY

The unit comprising different practical tools, sectioning procedure, process of staining the section. Microscopes are important tools for observation due to immense resolution power and the magnification of the microscope is determined by multiplying the magnification of the eyepiece by the magnification of the objective lens. In order to reveal the cellular structure, plant materials are being cut in various planes. Normally cross and longitudinal sections are taken for the study. These sections are stained through chemical stains and then after mounting we put them under microscope for the study. Unit has detailed idea regarding all the glasswares and the sectioning procedure along with staining. Root, stem and leaves anatomical features have been described in detail with the differentiating features between dicot and monocot plants.

2.5 GLOSSARY

Microscopy: Technique to see or visualize the microscopic objects though lenses.
Cross Section: Here the section passes at the right angle to the material.
Cyanin: Blue pigment.
Double Staining: Use of two dyes for coloring the different tissues of plants.
Longitudinal Section: Section is cut at the right angle to the transverse axis.
Maceration: Process of separation of cells from the surrounding cells.
Mounting: Keeping a section on the slide.
Sectioning: Process of cutting plant material into thin slices.
Staining: Technique to colour the tissues by different chemicals.
Squash: Technique for studying cell by crushing them over a slide.
Cuticle - The waxy, water-repelling layer on the outer surface of a leaf that protects it from dying out.
Epidermis - The protective, outer layer of cells on the surface of a leaf. The guard cells (and stoma) are part of the epidermis.
Guard Cell - One of a pair of sausage-shaped cells that surround a stoma (a pore in a leaf).
Lamina - The blade of a leaf.
Mesophyll - The chlorophyll-containing leaf tissue located between the upper and lower epidermis. These cells convert sunlight into usable chemical energy for the plant.
Palisade Mesophyll - A layer of elongated cells located under the upper epidermis. These cells contain most of the leaf's chlorophyll, converting sunlight into usable chemical energy for the plant.

Sclerenchyma- Tissue composed of thick-walled cells containing lignin for strength and support.

Spongy mesophyll - The layer below the palisade mesophyll; it has irregularly-shaped cells with many air spaces between the cells. These cells contain some chlorophyll. The spongy mesophyll cells communicate with the guard cells (stomata), causing them to open or close, depending on the concentration of gases.

Stoma - (plural stomata) a pore (or opening) in a plant's leaves where water vapor and other gases leave and enter the plant. Stomata are formed by two guard cells that regulate the opening and closing of the pore. Generally, many more stomata are on the bottom of a leaf than on the top.

2.6 SELF ASSESSMENT QUESTION

2.6.1 Objective type questions:

(i) Casparian strips have compound:

(a) Cellulose (b) Lignin

(c) Suberin (d) Cutin

(ii) A parenchymatous sheet of tissues separates the phloem strands from xylem and it becomes:

(a) Pericycle (b) Endodermis

(c) Stele (d) Cambium

(iii) Which is incorrect for monocot root?

(a) The pericycle gives rise to lateral roots (b) Cambium is absent

(c) The pith is present (d) Secondary growth does not occur

(iv) Pith is composed of:

(a) Collenchyma (b) Parenchyma

(c) Sclerenchyma (d) None

(v) Which is correct for dicot root?

(a) Vascular bundles are scattered irregularly in ground tissues

(b) The vascular bundles are open

(c) Cambium is absent

(d) There is no hard bast

(vi) Which meristem helps in increasing girth?

(a) lateral meristem (b) intercalary meristem

(c) primary meristem (d) apical meristem

(vii) Pith and cortex do not differentiate in
(a) monocot stem (b) dicot stem
(c) monocot root (d) dicot root

(viii) Where do the casparian bands occur
(a) epidermis (b) endodermis
(c) pericycle (d) phloem

(ix) Bordered pits are found in
(a) sieve cells (b) vessel wall
(c) companion cells (d) sieve tube wall

(x) Vertical section such as in leaves and thallus.
(a) Section at right angle to the transverse axis (b) Section at 180^0
(c) Section at 90^0 (d) Section 120^0

2.6.2 Short answer question.

i. What is sectioning?
ii. What is staining?
iii. What is a transverse section?
iv. Define single staining in plants.
vi. What is mounting?
vii. What is a chlorenchyma?

2.6.3Answer the following questions in about 100 words.

i) What are the different parts of compound microscope?
ii) Name different types of stains used for algae, fungi, bryophytes, pteridophytes and gymnosperms.
iii) Define sectioning technique.
iv) What is double staining?
v) Name the glassware used during anatomical study.

2.6.1 Answer Key:

i. (b) ii. (d) iii. (a) iv. (b) v. (b), vi. (b), vii. (a), viii.(b), ix. (d), x. (b)

2.7 REFERENCES

- Carlquist, S, Schneider EL. Origins and nature of vessels in Monocotyledons. I. Acorus. International Journal of Plant Sciences. 1997; 158:52–56.
- Craig, Richard and Vassilyev, Andrey. "Plant Anatomy". McGraw-Hill. Archived from the original on 24 July 2010.
- Esau, K. 1977, Plant anatomy. New York: John Wiley & Son.
- Esau's Plant Anatomy, Meristems, Cells, and Tissues of the Plant Body: their Structure, Function, and Development. 3rd edn.". *Annals of Botany*, 99 (4): 785–786.
- Fahn, A. 1990, Plant Anatomy, Pergamon Press, Oxford 4th Edn.
- Pandey,B. P. 2001, Plant Anatomy, Published by S. Chand Publisher.
- Pandey B.P, 2012, Modern Practical Botany, Vol II, S.Chand& Co., New Delhi.
- Pandey, S.N. 1997, Plant Anatomy and Embryology, Vikas Publication House Pvt Ltd.
- Singh, V., 2010, Plant Anatomy and Embryology of Angiosperms, Global Media Publications.
- Sharma A.K. & Sharma R. 2010, Structure, Development and Reproduction in Flowering Plants, Jagdamba Publishing Co, New Delhi.

2.8 SUGGESTED READINGS

- Esau K. 1977, Plant anatomy. New York: John Wiley & Son.
- Fahn, A. 1990, Plant Anatomy, Pergamon Press, Oxford 4th Edn.
- Pandey,B. P., 2001, Plant Anatomy, Published by S. Chand Publisher.
- Pandey, S.N., 1997, Plant Anatomy and Embryology, Pub. Vikas Publication House Pvt Ltd.
- Singh, V., 2010, Plant Anatomy and Embryology of Angiosperms, Global Media Publications.
- Vasishta, P.C. 1968, Plant Anatomy, Pradeep Publication & Co Chandigarh.

2.9 TERMINAL QUESTIONS

2.9.1. Answer the following questions in about 100 words.

i) Bring out the characters of transverse section of a dicot leaf.
ii) Explain the anatomical feature of monocot leaf.
iii) Draw a labeled diagram of dicot stem T.S.
iv) Compare the anatomical features of dicot and monocot root.

v) Cut a transverse section of young stem of a plant from your garden and observe it under the microscope. How would you ascertain whether it is a monocot stem or a dicot stem? Give reasons.

2.9.2 Answer the following questions in about 200 words.

i. Write an essay on the structure and function of microscope.
ii. Describe in detail with diagram about the anatomy of monocot stem.
iii. Define the anatomy of dicot root in detail.
iv. Define the transverse section of dicot stem.

UNIT-3L NORMAL AND ABNORMAL SECONDARY GROWTH IN *BOERHAVIA, BOUGAINVELLIA, NYCTANTHES, SALVADORA, DRACAENA* AND *TINOSPORA*

3.1 OBJECTIVES

In this section student will be able to understand:

- What is normal secondary growth and how does it take place?
- What is anomalous secondary growth?
- What are the reasons of anomalous secondary growth?
- Explanation of different types of anomalous secondary growth in different species?
- Significance of anomalous secondary growth?

3.2 INTRODUCTION

In botany, **secondary growth** is the growth that results from cell division in the cambia or lateral meristems and that causes the stems and roots to thicken, while **primary growth** is growth that occurs as a result of cell division at the tips of stems and roots, causing them to elongate, and gives rise to primary tissue. Secondary growth occurs in most seed plants, but monocots usually lack secondary growth. If they do have secondary growth, it differs from the typical pattern of other seed plants.

In many vascular plants, secondary growth is the result of the activity of the two lateral meristems, the cork cambium and vascular cambium. Arising from *lateral* meristems, secondary growth increases the girth of the plant root or stem, rather than its length. As long as the lateral meristems continue to produce new cells, the stem or root will continue to grow in diameter. In woody plants, this process produces wood, and shapes the plant into a tree with a thickened trunk.

Because this growth usually ruptures the epidermis of the stem or roots, plants with secondary growth usually also develop a cork cambium. The cork cambium gives rise to thickened cork cells to protect the surface of the plant and reduce water loss. If this continues for many years, this may produce a layer of cork. In case of oak it will yield harvestable cork.

Anomalous secondary growth refers to the deviation of the secondary growth from the normal type of growth. It is also known as abnormal or more appropriately unusual secondary growth, as the term encompasses some less common type of secondary growth patterns. Though secondary growth is an exclusive feature of dicotyledonous plants, but there are some monocotyledons that also show secondary growth.

3.3 NORMAL AND ABNORMAL SECONDARY GROWTH IN PLANTS

Normal Secondary Growth

It is the result of the activity of the vascular cambium, which occurs in between xylem, and phloem of each vascular bundle. Hence, it is known as intra-fascicular cambium or fascicular cambium. In addition, towards the beginning of secondary growth there is a process of dedifferentiation in some of the parenchyma cells of the medullary rays which are at the level of cambium cells and adjoining the vascular cambium. As a result, these cells now become meristematic and represent the inter-fascicular cambium. The meristematic cells of the intra-fascicular cambium and inter-fascicular cambium join and result in the formation of a continuous strip of meristem called cambium ring. The cambium ring at this stage has primary xylem on its inner surface and primary phloem on its outer surface.

The cambium ring exhibits mitotic activity on both the sides. The mitotic activity on the inner surface results in the formation of cells, which differentiate into xylem. It represents the secondary xylem. Similarly, the mitotic activity on the outer surface results in the formation of cells, which differentiate into phloem. It represents the secondary phloem. Due to the formation of secondary xylem, the primary xylem becomes pushed more towards the pith and the pith gets slightly reduced. However, the secondary phloem grows and completely masks the primary phloem. Hence, it is not visible.

The mitotic activity of the cambial ring is purely seasonal. It occurs only twice during every year, once in the spring and once in the autumn season. Thus, every year two sets of secondary xylem and two sets of secondary phloem are formed. Each year, the mitotic division of the cambial ring usually begins in the spring season. The secondary xylem that is formed in the spring season is therefore known as springwood or early wood, while the secondary xylem formed in the autumn is known as autumn wood or late wood. The springwood is generally characterized by the presence of xylem vessels having wider lumen. This is because, spring is the ideal season for growth and the water requirement of the plant is more in the spring. The autumn wood has xylem vessels with narrow lumen, since water requirement in the winter is less.

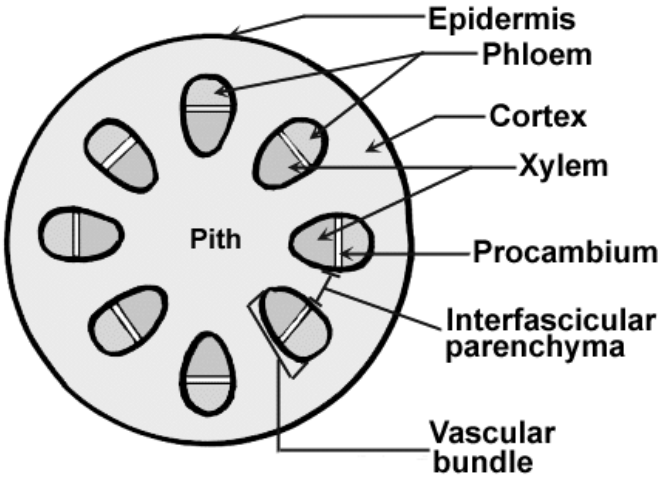


Fig.3.1. T.S. Dicot Stem before Secondary Growth

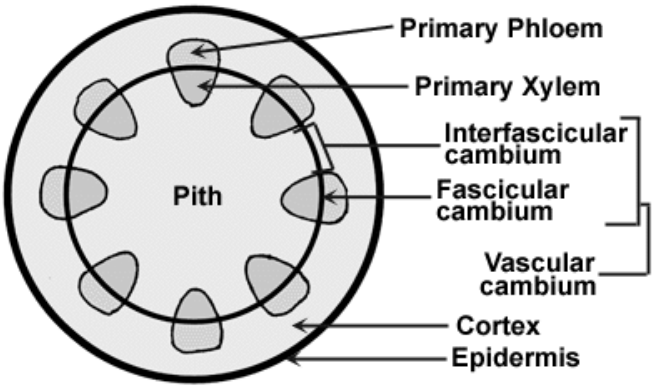


Fig.3.2. T.S. Dicot Stem after Initiation of Secondary Growth

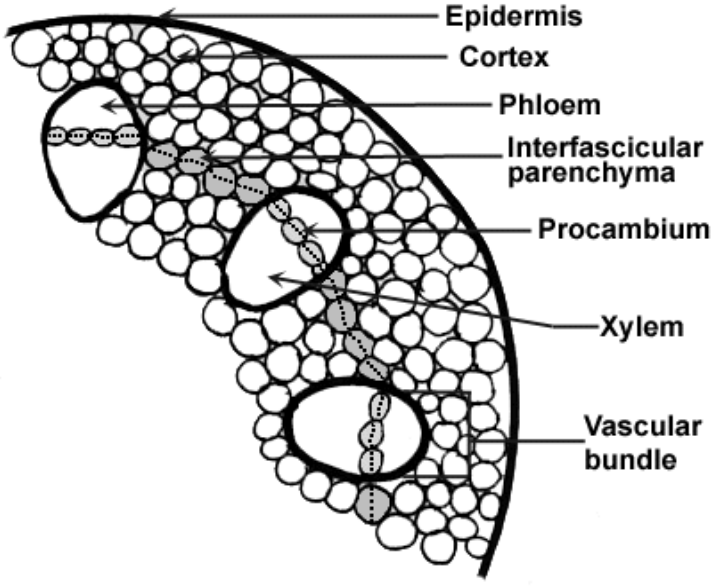


Fig.3.3. T.S. Dicot Stem Showing Formation of Cambium Ring

Activity of Cork Cambium

In dicot plants due to secondary growth a pressure is put on the epidermal layer and then the parenchymatous cells of the cortex become meristematic behaving like cambium and known as cork cambium or phellogen. It produces cork cells or phellem (bark) towards outside containing a waxy substance known as suberin. The bark protects the plant against physical damage, insects, termites, microbes and helps reduce water loss. The cork cambium also produces a layer of cells known as phelloderm, which grows inwards. The cork cambium, cork cells, and phelloderm are collectively termed as periderm. The periderm substitutes for the epidermis in mature plants. In some plants, the periderm has many openings, known as lenticels, which allow the interior cells to exchange gases with the outside atmosphere. This supplies oxygen to the living- and metabolically-active cells of the cortex, xylem, and phloem.

Mechanism of Secondary Growth

1. The mature dicot stem shows secondary growth and increases in girth.
2. This results by the activity of two lateral meristems--- vascular cambium and cork cambium or phellogen.
3. Secondary growth occurs in two parts—
   A) In stelar region by the vascular cambium
   B) In extrastelar region by the phellogen (cork cambium)

A) Sec. Growth in Stelar Region by Vascular Cambium

1. Interfascicular cambium develops outside the vascular bundles joining intrafascicular cambium and forming complete ring.

2. Now this complete ring produces cells towards inside as secondary xylem and towards outside as secondary phloem. Thus new cells produced and the girth increases slowly.

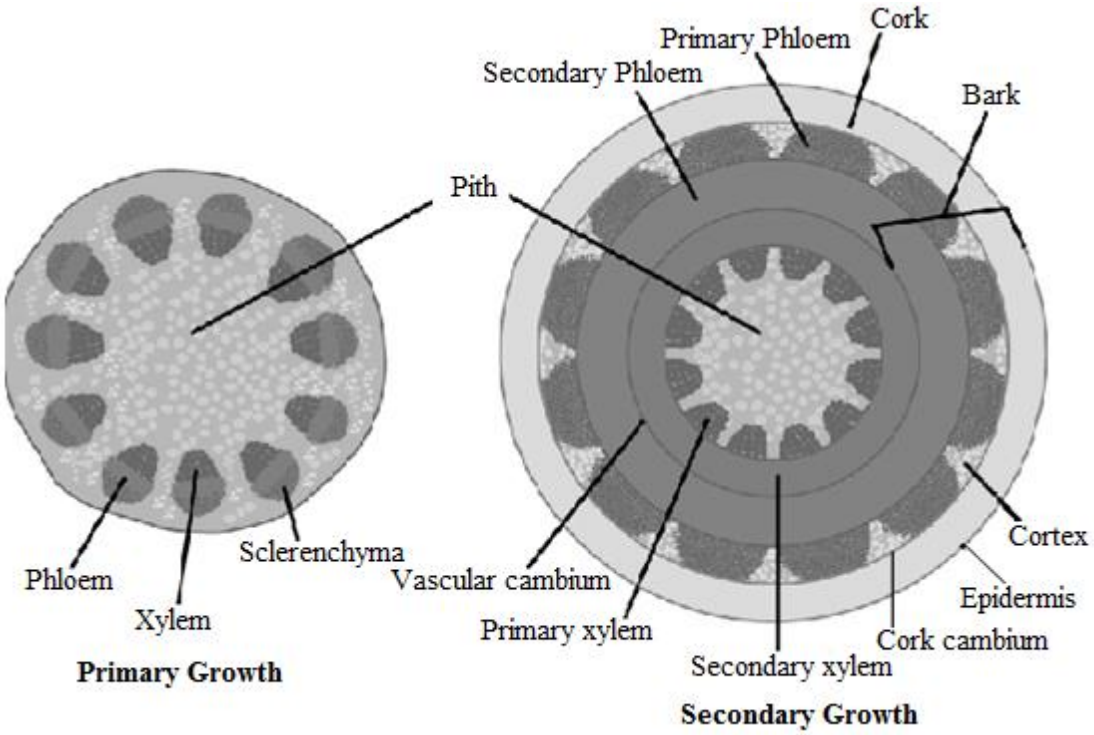


Fig.3.4. Primary and Secondary Growth in Cross Section

Formation of Annual Rings

- The vascular cambium remains active throughout life.
- Activity of vascular cambium is affected by seasonal variations.
- Variation in activity is seen during different seasons, resulting in shape and structure of wood. Because the activity of the cambium varies so the production of new cell also varies resulting in a clear cut colour variation in different seasons.
- This results in the formation of annual rings, which is used to determine the age of the tree.

B) Sec. Growth in Extra Stelar Region by Cork Cambium

- As long as the lateral meristems continue to produce new cells, the stem or root will continue to grow in diameter.
- In woody plants, this process produces wood, and shapes the plant into a tree with a thickened trunk.
- This growth usually ruptures the epidermis of the stem or roots, plants with secondary growth usually also develop a cork cambium.
- For this any outer layer of cortex becomes meristematic and begins to divide. This is known as Phellogen or cork cambium.

- The cork cambium gives rise to thickened cork cells to protect the surface of the plant and reduce water loss.
- Phellogen or cork cambium divides to produce outer cork (Phellem) and inner secondary cortex (phelloderm).
- All the three tissues phellem, phellogen and phelloderm are together known as periderm.

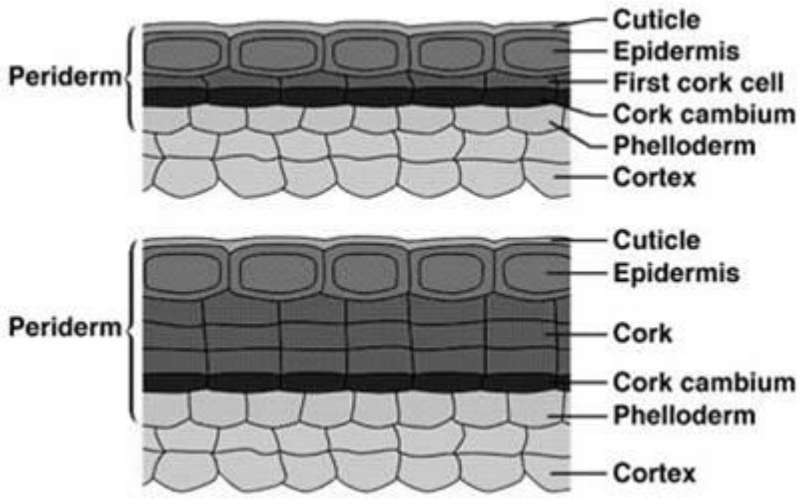


Fig.3.5. Activity of Cork Cambium

Anomalous Secondary Growth in Dicot Stem

Anomalous or unusual secondary growth may occur due to:

1. Unusual position of the vascular cambium
2. Unusual activity of the vascular cambium
3. No development of usual cambium or if so happens, its replacement by other accessory cambium formation and its activity
4. Formation of included or interxylary phloem
5. Development of interxylary cork

For better understanding, the anomalous secondary growth may be studied under the above stated categories in some representative plant species:

Unusual Position of the Vascular Cambium

The vascular cambium normally lies in between primary xylem and primary phloem. But sometimes, in plants such as *Thinouia sp., Serjania sp., Paullinia sp., Bauhinia langsdorffiana*, etc. cambium may be present elsewhere and its location may not be well differentiated. Cambium in such unusual position shows unusual activity resulting in anomalous secondary structure.

Unusual Activity of the Vascular Cambium

The cambium is normal in position but it shows an abnormal activity leading to irregular arrangement of secondary tissues. Such as formation of unusually large amount of secondary vascular tissue and formation of increased size of vascular bundles. This may happen when only intrafascicular cambium is active and it forms secondary vascular tissues in the region of vascular bundles only, e.g. stem of *Cucurbita* sp.

One more thing can happen i.e. formation of wide medullary rays. A normal cambium ring is formed by the union of intra- and interfascicular cambium, but it shows unusual activity where interfascicular cambium forms parenchyma only resulting in the formation of wide medullary rays.

Cambium in the Form of Folds or Ridges

In the young stem of climbers e.g. *Thinouia scandens*, the cambium is thrown into folds or ridges. At the time of secondary growth, the cambium separates at the folds and gives rise to separate groups of vascular tissues, resulting in a lobed stem

Cambium in the form of Separate Strips

This is commonly found in climbers, *Serjania ichthyoctona* and *Paullinia* of family Sapindaceae. The cambium here originally appears in many separate strips, each of which surrounds small parts or may be some strands of primary xylem and phloem. As the secondary growth starts, each cambial strip forms a separate entire ring of its own secondary tissue and behaves normally by cutting secondary xylem inside and secondary phloem outside. The mature stem thus has many distinct vascular bundles. Such a stem seems to be made up of many fused stems. In older stems, the discrete vascular bundles develop their own periderm and may progressively get separated from each other. The stem thus, seems to be made up of a number of strands of smaller stems closely suppressed to each other, resembling strands in a rope.

Accessory Cambium formation and its Activity

In many genera, a new cambium ring or accessory cambial rings originate(s) in the cortex or pericycle where either the normal cambium ring is altogether absent, e.g. *Amaranthus*, or there is cessation of its activity, e.g. *Boerhaavia*. The unusually positioned cambium (referred to as extrastelar in origin and accessory cambium) behaves unusually resulting in the formation of successive rings of vascular bundles embedded in parenchyma or conjunctive tissue. Here, the first ring of cambium arises in the pericycle region and it shows unusual activity by cutting off secondary xylem in patches alternating with parenchyma cells (or conjunctive tissue) on the inner side whereas externally, initially forming parenchymatous layers and afterwards forming secondary phloem. It forms a complete ring of vascular bundles and then it stops functioning. A

new ring of cambium called accessory cambium is formed from the parenchyma cut off externally by the earlier cambium. This newly formed cambium also behaves unusually in a similar manner forming another ring of vascular bundles embedded in parenchyma and then becomes inactive. Likewise, more accessory cambia are formed giving rise to successive rings of vascular bundles.

Formation of Included or Interxylary Phloem

The groups of secondary phloem cells embedded in the secondary xylem is referred to as included or interxylary phloem. By definition, it is 'the phloem that develops within secondary xylem'. These are formed due to unusual activity of the cambium.

Sometimes small segments of the cambium at different intervals starts cutting secondary phloem towards inside instead of secondary xylem (abnormal behavior). After sometimes these segments behave normally and as usual cut secondary xylem towards inside. Thus inwardly formed secondary phloem gets embedded in secondary xylem.

Sometimes small segments of cambium cease to function and their cells converted into secondary phloem. New cambial strips develop outside. Later these newly formed cambial strips unite with the edges of general cambium and the normal activity of cambium is resumed and thus the phloem cells get embedded in secondary xylem.

Consequently, included phloem is observed in several dicot families such as Asclepiadaceae, Nyctaginaceae, Onagraceae, Salvadoraceae, and Amaranthaceae. Included phloem is a characteristic feature of some xerophytic plants and has a physiological significance. Being embedded in the xylem tissue, they are retained and they continue to function even in the unfavourable conditions. They serve to assimilate food for the developing buds on the restoration of favorable conditions.

3.3.1-*Boerhaavia* (Family: Nyctaginaceae)

Boerhaavia shows anomalous secondary growth due to anomaly in its primary structure as well as accessory cambium formation and its activity at the time of secondary growth. The young stem is typically dicotyledonous in structure with a few unusual features:

Epidermis

- Single layered epidermis consists of small, radially elongated cells.
- Multicellular epidermal hairs arise from some cells.
- A thick cuticle is present on the epidermis.
- Some stomata are also present.

Cortex

- It is well differentiated and consists of few layered collenchymatous hypodermis followed by chlorenchyma.
- Collenchyma is 3 to 4 cells deep, but generally near stomata it is only one layered.
- Chlorenchyma is present inner to collenchyma in the form of 3 to 7 layers.
- Chlorenchymatous cells are thin walled, oval, full of chloroplasts and enclose many intercellular spaces.
- Endodermis is clearly developed and made up of many, tubular, thick-walled cells.

Pericycle

- Inner to the endodermis is present parenchymatous pericycle but at some places it is represented by isolated patches of sclerenchyma.

Vascular System

- Vascular bundles are present in three rings. In the innermost ring are present two large bundles; in the middle ring the number ranges from 6 to 14 while the outermost ring consists of 15 to 20 vascular bundles.

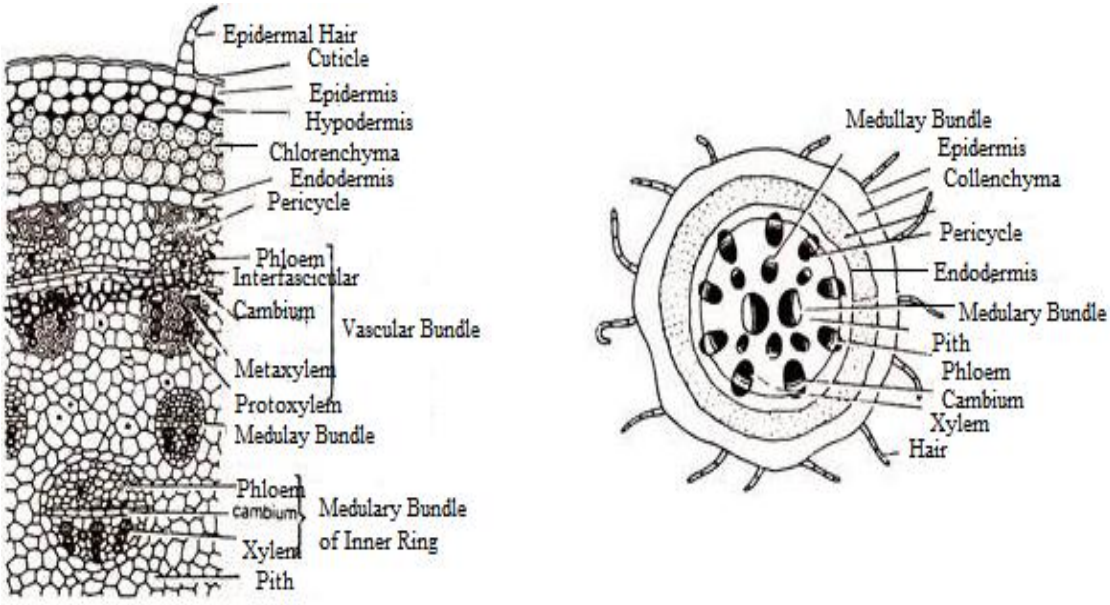


Fig.3.6. Anomalous Secondary Growth in *Boerhaavia*

- Vascular bundles are conjoint, collateral, open and endarch.
- There are three rings of vascular bundles which are primary in origin – the innermost two large medullary bundles, middle ring composed of 6-14 loosely arranged bundles and the outermost ring of 15-20 small bundles.

- Two vascular bundles of the innermost ring are large, oval and lie opposite to each other with their xylem facing towards center and phloem outwards.
- Middle ring consists of 6-14 small vascular bundles.
- Vascular bundles of inner and middle rings may show a little, restricted secondary growth with only small increase in size, and the intrafascicular cambium in these bundles behave normally.
- It forms secondary xylem towards inside and secondary phloem towards outside with primary phloem pushed to lie only as a cap like structure towards outside.
- The cambium of the outermost ring of the vascular bundles forms a complete ring at the time of secondary growth by the union of inter- and intrafascicular cambium.
- The intrafascicular cambium forms secondary xylem on the inside and secondary phloem on the outside, whereas the interfascicular cambium forms conjunctive tissue on the inside and parenchymatous tissue on the outside.
- The interfascicular cambium functions for some time, and then it ceases its activity.
- Soon after, a new accessory cambium ring arises by the union of the secondary parenchyma cells lying above and the cells of pericycle positioned outside the phloem.
- This first accessory cambium ring behaves in a similar manner as of the vascular cambium, forming secondary xylem alternating with conjunctive tissue on the inner side and secondary phloem above parenchyma tissue on the outside.
- As a result, another ring of vascular bundles is formed which are of secondary origin. This process may be repeated to form four or more successive rings of vascular bundle.

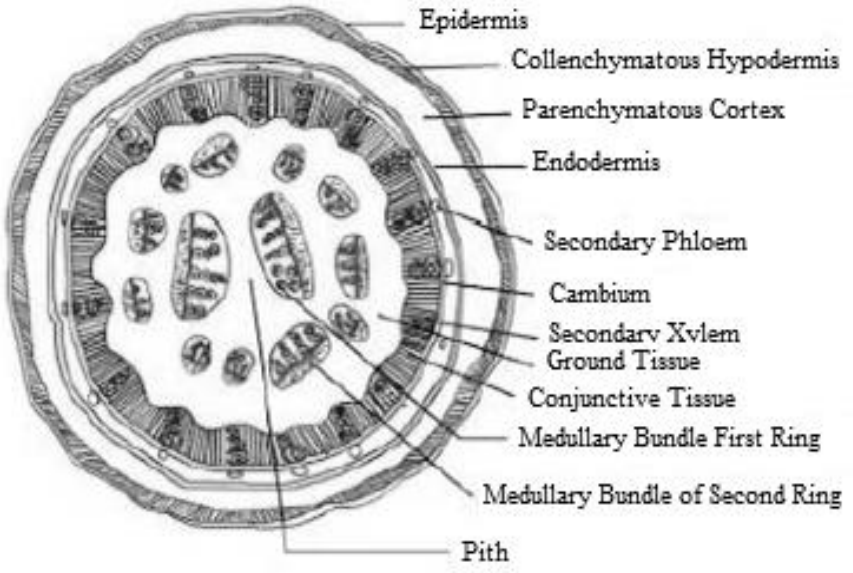
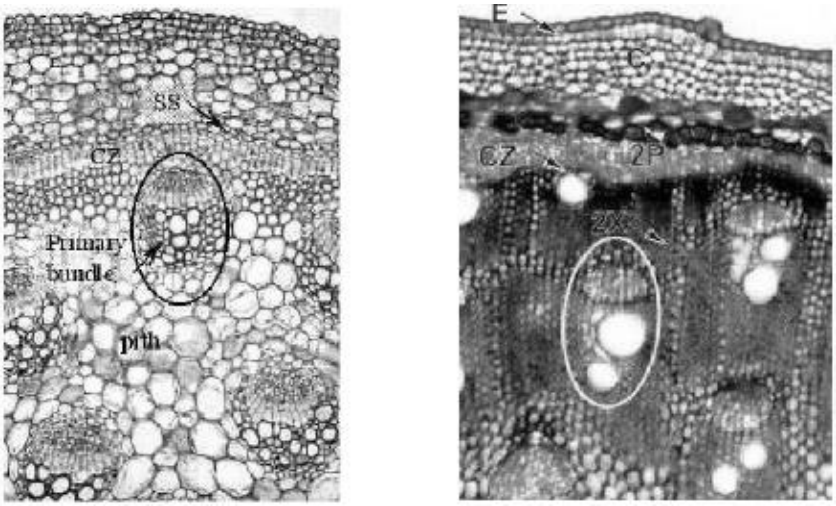


Fig.3.7. T.S. *Boerhaavia* Stem

- The stem in *Boerhaavia* contains well-defined anomalous secondary growth, which is characterized by the presence of successive rings of xylem and phloem.
- Alternate bands of lignified and parenchymatous bands are distinct in the stem.

3.3.2-*Bougainvellia* (Family: Nyctaginaceae)

Bougainvillea is a member of the Nyctaginaceae and is an example of a dicotyledonous stem which displays **anomalous secondary growth**.



(A) *Bougainvillea* Young Stem (B) *Bougainvillea* Old Stem

Fig. 3. 8. T. S. *Bougainvillea* stem

- The stem is circular in outline when young.
- It has a uniseriate epidermis covered by a thick cuticle, collenchymatous hypodermis and a well-developed parenchymatous cortex.
- The ill-defined endodermis is followed by a pericycle made of parenchyma with intermittent sclerenchymatous patches.
- In the TS, near the center of the stem, we can see some **primary vascular bundles** embedded in lignified pith parenchyma.
- The primary vascular bundles are seemingly scattered in the ground tissue and are not arranged in a ring.
- The first ring of cambium arises from the pericycle thus is extrastelar in origin. This is followed by formation of successive rings of cambia, though it was also believed that all the secondary tissue derivatives arise from a single cambium.
- Each cambial ring cuts off xylem alternating with parenchyma internally and, phloem and alternating patches of parenchyma externally.
- The parenchyma so formed usually gets lignified, which is then referred to as conjunctive tissue.

- Thus, concentric rings of vascular bundles are formed embedded in conjunctive tissue.
- In some species, the conjunctive tissue is sclerenchymatous only and is hardly distinguishable from the tracheary elements of the embedded vascular bundles.
- The phloem appears as an isolated patch actually surrounded by the conjunctive tissue, which is often mistaken to be included phloem.
- Secondary phloem and secondary xylem lie on either side of it. The secondary xylem is composed of **tracheids, fibres** and **narrow-diameter vessels**.
- Interspersed with the secondary xylem you will be able to see small pockets of phloem and what vessels look like large-diameter metaxylem.
- These are reminiscent of the primary bundles towards the center of the stem. These are in fact primary vascular bundles embedded within the secondary xylem, hence the use of the term, **anomalous growth** in this instance.
- The phloem is described as being **included phloem**, which by definition is phloem tissue which lies between regions of secondary xylem.
- The **anomalous growth** results as a result of differential cambial activity. Newly-produced vascular cambia result in the outer lateral meristem becoming quiescent and this cambium returns to activity only when the internal vascular cambium becomes less active.
- Vascular cambia do not produce rays in Nyctaginaceae but do produce vessels, axial parenchyma and sometimes fibers to the inside and variable secondary phloem to the outside.

3.3.3-*Nyctanthes* (Family–Oleaceae)

The outline of T.S. appears quadrangular and reveals the following tissues from outside with-in:

Epidermis

- Single-layered epidermis consists of rectangular cells.
- A thick uninterrupted cuticle is present on the epidermis.
- Many multicellular hairs are present.

Cortex

- It is differentiated into collenchyma and parenchyma.
- Collenchyma is several cells deep below the four protruded comers while only few layers deep at the other places just beneath the epidermis.
- Parenchyma is present below the collenchyma. Many intercellular spaces are present. The region extends up to the vascular tissue.

Cortical bundles

- Four vascular bundles are present in the cortex, situated one in each protruded bulge.

- Each conical bundle faces its pointed xylem end towards outer side, i.e., epidermis, and is conjoint, collateral, open and exarch.
- These bundles may show secondary growth at maturity.

Endodermis

- Not well-developed.

Pericycle

- It is in the form of sclerenchymatous patches.

Vascular System

- It consists of primary phloem, secondary phloem, cambium, secondary xylem and primary xylem.
- Primary phloem is crushed and irregularly present in patches below pericycle.
- Secondary phloem is present in the form of a continuous ring and consists of sieve tubes, companion cells and phloem parenchyma
- Cambium is one to three cells thick continuous layer present in between phloem and xylem.
- Secondary xylem is present just inner to the cambial ring and consists mainly of thick walled wood parenchyma and fibres. Tracheids and vessels are also present
- Primary xylem is situated just near the pith facing its protoxylem towards the centre.

Pith

- It is thin walled and parenchymatous.

Abnormality

- Abnormality in Nyctanthes is the presence of cortical bundles, which are inversely oriented, 4 in number and never directly connected with the main axial ring of the vascular cylinder. These are leaf trace bundles.
- Cortical bundles have also been reported in some other families such as Casuarinaceae (Casuarina), Umbelliferae (Eryngium), Papilionaccae (*Latkyrusmarytimus*). Mclastomaccac, Rutaccae, etc.

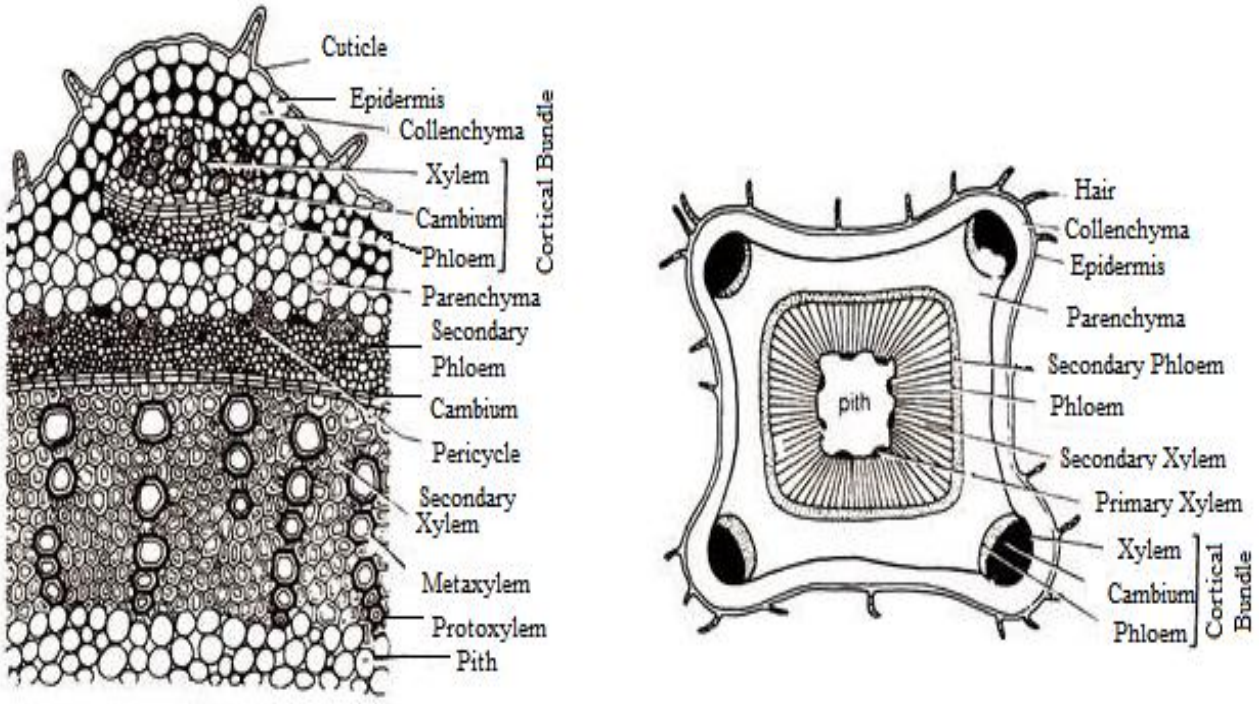


Fig. 3.9. *Nyctanthes* T.S.

3.3.4-*Salvadora* (Family: Salvadoraceae)

T.S. reveals the following tissues from outside within:

- Epidermis isthe outermost layer with barrel shaped cells. Cells are covered by a thick cuticle.
- Cortex consists of parenchymatous hypodermis, few layers of chlorenchyma and an innermost layer of endodermis.
- Hypodermis is generally thin walled, parenchymatous, but sometimes 2-3 layers of collenchyma are seen.
- Chlorenchyma is present inner to the hypodermis. It is 3-5 cells deep and cells are filled with chloroplasts.
- Few layers of parenchyma are also present below the chlorenchyma. The cells contain intercellular spaces.
- Endodermis is the innermost layer of cortex made up of barrel shaped cells which contain starch grains.
- Pericycle is a discontinuous layer present in the form of patches consisting of many widely spaced strands of thick walled fibres.
- Vascular bundles are conjoint, collateral, open and endarch.
- Vascular system composed of primary phloem, secondary phloem, cambium, secondary xylem, primary xylem and included phloem.

- Primary phloem is crushed and found in the form of patches.
- Secondary phloem is present just outside the cambium in the form of a ring.
- Cambium strip consists of rectangular cells arranged in radial rows.
- Secondary xylem forms a complete cylinder. It is represented by wide vessels and xylem parenchyma.
- Many medullary rays traverse the secondary xylem.
- Wide vessels of metaxylem and narrow protoxylem vessels can be observed in the primary xylem present near the pith.
- Secondary xylem is interrupted by many groups of thin walled phloem representing included or interxylary phloem or phloem islands.

Pith

It is well developed, thin-walled, parenchymatous and present at the center.

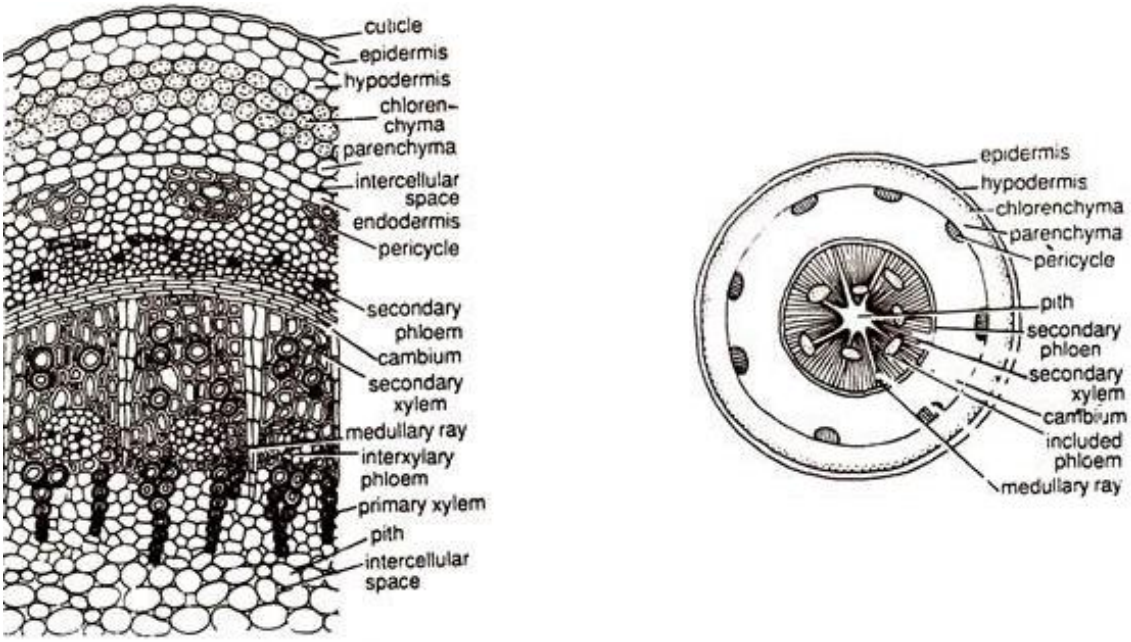


Fig. 3.10.T.S. Salvadora Stem

3.3.5-*Dracaena* (Family: Asparagaceae)

Normally the vascular bundles of the monocotyledonous stems are closed ones. Thus due to absence of the cambium" they lack secondary growth in thickness and the vascular system is wholly composed of primary tissues.The bundles remain irregularly scattered in the ground tissues, where the limits of cortex and other ground tissues can be hardly seen.Some monocotyledons belonging to the family Liliaceae, mainly the arborescent ones like *Dracaena,*

Yucca, Cordyline, Agave, Aloe and others exhibit a peculiar type of secondary increase in thickness, an account of which is given here.

Dracaena (the Dragon's blood tree) is the only monocot which have secondary growth in roots. *Dracaena* is a monocot. The stems undergo a specialized secondary growth, which manifests itself in the production of additional parenchymatous elements. Their later growth pattern is termed diffuse secondary growth, and consists mostly of a proliferation of ground parenchyma cells and additional vascular bundles near the periphery.

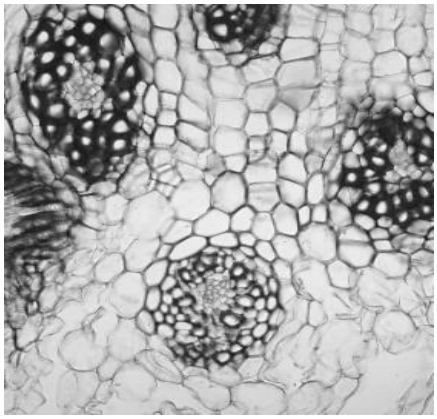


Fig. 3.11 T.S. Dracaena Stem

Young stem has a typical monocot structure having single epidermis, sclerenchymatous hypodermis and numerous closed, collateral vascular bundles scattered in the parenchymatous ground tissue.

In Dracaena secondary growth is due to:

A) Extrastelar cambium ring in monocot stem at the cortex
B) Abnormal activity of the cambium

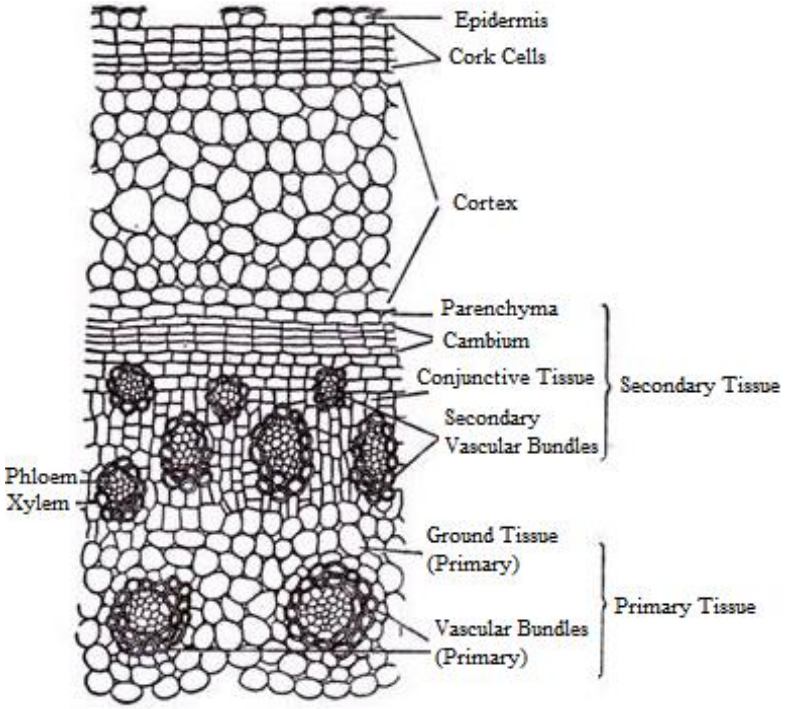


Fig. 3.12 T.S. *Dracaena* Stem Showing Special Type of Secondary Growth

When secondary growth starts:

- Formation of secondary meristem or secondary cambium occurs in the inner region of parenchymatous cortex.
- The activity of cambium is abnormal.
- It produces secondary vascular bundles on its inner side only and parenchymatous cells on the outer side.
- Secondary vascular bundles are amphivasal where phloem is surrounded by xylem.
- The secondary ring of vascular bundles are alternating in position with the first ring.
- The vascular bundle in the last inner ring is embedded in a mass of lignified conjunctive tissue.
- Cork cambium activity is normal and produces cork and secondary cortex in the outer region.

3.3.6-*Amaranthus* Stem (Family: Amaranthaceae)

The primary structure of the stem shows a number of shallow ridges and furrows with a thickly cuticularised single layer of epidermis.

Epidermis

- It consists of single layer of barrel shaped cells covered externally by thick cuticle.
- Lateral and inner walls are thin.

Cortex

- It is well differentiated into collenchyma and chlorenchyma.
- Collenchyma is present just below the epidermis. It is more prominent below ridges. Comers of the cells are thick and the cells are-oval or polygonal in shape.
- Chlorenchyma is present inner to collenchyma. Thin walled cells are spherical to oval in shape, filled with chloroplasts and contain many intercellular spaces.
- Endodermis is poorly developed and sometimes absent. The cells are elongated and lack casparian strips.

Pericycle

- It consists of few layers of thin walled, compactly arranged cells. It becomes sclerenchymatous in older stems.

Vascular System

- The normal ring of vascular bundles is absent. Instead there are two rings of medullary bundles formed by the activity of accessory cambia.
- The first accessory cambium differentiates in the pericycle. It behaves unusually by first forming small amount of parenchyma on the outside, and then cutting xylem alternating with parenchyma on the inner side and consequently forming phloem alternating with parenchyma on the outside.
- As a result, a ring of conjoint, collateral, endarch and open type of vascular bundles is formed which gets embedded in parenchymatous tissue.
- After sometime, this cambium ceases to function and becomes passive. A second accessory cambium arises from the parenchyma cut off by the previous one on the outside.
- It also behaves in a similar fashion producing a second ring of vascular bundles again included in the parenchyma, but alternating to the first one.
- Similarly, numerous accessory cambia develop consecutively producing consecutive rings of vascular bundles, giving a scattered appearance in the ground tissue of the stem.
- The final accessory cambium ring forms sclerenchyma alternating with xylem internally thus the last ring of vascular bundles seems to be embedded in sclerenchyma.
- On maturity, sometimes the medullary bundles along with some adjoining parenchyma may degenerate creating cavity.
- Primary phloem is crushed and present in patches.
- Secondary phloem is present in the form of a complete ring which consists of sieve tubes, companion cells and phloem parenchyma.

- Cambium is distinct and present in one to many layers located in between phloem and xylem.
- Secondary xylem remains embedded in conjunctive tissue and consists of proto-and metaxylem vessels and abundant parenchyma.
- Conjunctive tissue is present in abundance and consists of thick walled and lignified cells.
- Primary xylem is present near the pith facing its protoxylem towards centre.
- Though the normal vascular cambium is not formed but the cork cambium is formed and functions normally.

Medullary Bundles

Many scattered medullary bundles are present in the pith.

Each medullary bundle is conjoint, collateral and endarch, with the cambium either feebly developed or functionless or absent.

Pith

It is parenchymatous and cells show some intercellular spaces.

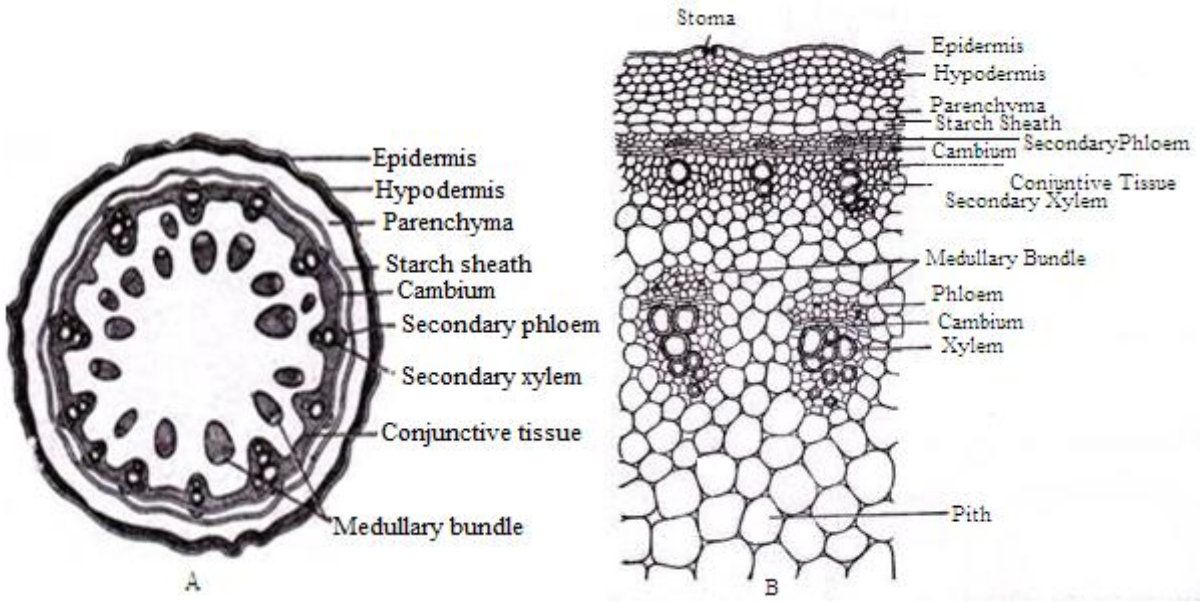


Fig. 3. 13. Stem of *Amaranthus* Showing Secondary Growth (A) T.S. Stem (Diagrammatic) (B) Magnified View of Stem T.S.

Secondary Growth in *Amaranthus* Stem

The vascular bundles are medullary ones. They are large in number and remain scattered in the pith. The bundles are collateral and open. Cambial activity is confined to the individual bundles,

and it ceases soon. Secondary growth occurs due to development of a new meristem— the secondary cambium outside the stele. The cambium cuts off similar bundles with xylem on the inner side and phloem on the outer side. The secondary bundles remain embedded in thin-walled conjunctive tissue, which is wavy in outline on the inner side.

In the pericycle region the outer primary bundles become meristematic and develop few layered cambium. This cambium cuts collateral vascular bundles towards inner side consisting of secondary phloem and secondary xylem. Cambium also cuts many layered parenchymatous conjunctive tissue which becomes lignified and thick walled. The vascular bundle lies completely embedded in conjunctive tissue.

Significance of Anomalous Secondary Growth

It is believed that plants show anomalous secondary growth primarily because of two reasons:

i) As an adaptation to the environment – Some anomalies in the plant structure arise in response to the environment to cope with it. Such forms are termed as adaptive type. This includes the plants with:

a) Climbing habits – This is found in climbers and lianas. The climbers should have soft tissue like parenchyma or secondary phloem in abundance to promote their flexibility and twining or twisting habit. These tissues may also split the solid woody cylinder into strands helping the plant to climb, e.g. *Aristolochia* and *Tinospora* show fluted vascular bundles; Bignonia show phloem wedges; *Leptadenia*and *Thunbergia* show presence of interxylary phloem.

A flattened stem is sometimes encountered in climbers which helps the plant to hold onto the support while climbing, e.g. as seen in Bauhinia.

In some other climbers, such as *Serjania, Thnouia,Ichthyoctona*and*Paullinia,* cambium develops in the form of separate strips and the mature stem has many distinct vascular bundles which develop their own periderm and may progressively get separated from each other. The stem thus, seems to be made up of a number of strands of smaller stems closely oppressed to each other, resembling strands in a rope. This provides strength to the stem against extension and breakage facilitating twisting and twining.

b) Storage roots - Many plants have storage roots where the reserve food material is stored in the parenchymatous tissue. A considerable amount of storage parenchymatous tissue is formed as a result of anomalous secondary growth in them which is considered to be an adaptation to their storage function, e.g. *Beta vulgaris, Raphanus sativus, Ipomoea batatas*and*Daucuscarota*.

c) Floating habits – The parenchymatous tissue when encloses a lot of air space (referred as aerenchyma) can provide buoyancy to the aquatic plant, e.g. in *Jussiaea*, cork cambium produced at the time of secondary growth gives rise to parenchyma only that help in buoyancy.

ii) Variation in the cambial activity – In nature there is variation in the position, development, behaviour and/or nature of cambium found in some plants leading to varied structural organizations. Such forms, with structural anomalies which are not because of the environment, are referred to as non-adaptive type. This is found in many plants such as, *Boerhaavia, Mirabilis, Amaranthus, Chenopodium, Bougainvillea, Dracaena*etc.

3.4 SUMMARY

Most monocots either have no secondary growth or else anomalous secondary growth of some type. For example, palm trees increase their trunk diameter due to division and enlargement of parenchyma cells, which is termed *diffuse secondary growth.* In some other monocot stems with anomalous secondary growth, a cambium forms, but it produces vascular bundles and parenchyma internally and just parenchyma externally.The word anomalous means deviating from the general or common order or type. Thus, the term, anomalous growth reflects a growth condition which is not commonly seen and which is present in a limited number of families or genera. This exercise explores a few examples of anomalous growth; bear in mind, there are many to choose from! The examples here illustrate aspects that are common - and include multiple cambia, included vascular bundles, and multiple vascular cylinders. Whereas the development, arrangement, activity of the vascular cambium in most woody dicotyledonous and Gymnospermous plants tends to be very similar, there are some alternatives which produce new secondary tissues that do not follow a normal pattern. As a result, the secondary plant structures that are formed are termed anomalous. Most anomalous growth is associated with the formation of multiple cambia.

3.5 GLOSSARY

Cell membrane: A slim layer of fat and protein that surrounds a cell though still located inside the cell wall. It is semi-permeable, which means it allows for some substances to pass through it while keeping others out.

Cell wall: A tough, rigid layer that surrounds a plant cell. The cell wall is located outside of the cell membrane and acts to support, filter incoming substances, and protect the cell from over-expansion due to water intake. Cell walls can also attach to other cell walls to help form the structure of a plant.

Chlorophyll: A green molecule vital for photosynthesis. Chlorophyll captures light energy from the sun in order to convert carbon dioxide and water into oxygen and sugar for plant consumption.

Chloroplast: Disc-shaped organelle containing chlorophyll and the location where photosynthesis occurs.

Collenchyma: Tissue composed of cells with unevenly thickened walls.
Cotyledon: One of the first leaves of the embryo of a seed plant; seed leaf.
Cristae: The folded membranes inside the mitochondria. The walls of the cristae contain proteins and are the site where cell energy production occurs (ATP is produced).
Cytoplasm: A gooey substance that contains all the cell's organelles outside of the nucleus. Most cellular activity occurs within the cytoplasm.
Dicotyledon: Flowering plants that have two seed leaves that emerge after germination.
Monocotyledon: Flowering plants that have one seed leaf that emerges after germination.
Parenchyma: Thin-walled cells, varying in shape, size, and function.
Plasmodesmata: The site where communication and transport of materials between plant cells occurs.
Phloem: The food-conducting tissue of a vascular plant.
Schlerenchyma: Tissue composed of thick-walled cells containing lignin for strength and support.
Sieve element: Cell in the phloem tissue concerned with longitudinal conduction of food materials. In flowering plants, it is called a sieve-tube element.
Sieve tube: A series of sieve-tube elements arranged end to end and interconnected through sieve plates.
Vacuole: Membrane-lined area within a plant cell that is filled with water. This organelle takes up much of the space inside a cell and help maintains its shape and size.
Vessel: A tube-like series of vessel elements with open ends. The walls that join the members have perforations or holes in them to allow water to pass through freely.
Vessel element: Individual cells that make up vessels.

3.6 SELF ASSESSMENT QUESTION

3.6.1 Multiple choice questions:

i) Casparian strips are present in
(a) Cortex (b) Epidermis
(c) Stele (d) Endodermis

ii) The outer most part of the stele consists of one or more layers of parenchymatous cells. The outer layer of this parenchyma is called:
(a) Cortex (b) Epidermis
(c) Stele (d) pericycle

iii) The type of arrangement in which protoxylem lies towards the outside and metaxylem lies towards the inside is called:

(a) Mesarch (b) Endarch
(c) Exarch (d) None

iv) The case in which xylem is present towards the inner side and phloem is present towards the outer side of vascular bundle is:
(a) Collateral (b) Bicollateral
(c) Concentric (d) Bilateral

v) Cambium is absent in:
(a) Monocot (b) Dicot
(c) Gymnosperm (d) None

vi) Exarch and polyarch vascular bundles occur in
(a) Monocot stem (b) Monocot root
(c) Dicot stem (d) Dicot root

vii) The endodermis in dicot stem is also called
(a) Starch sheath (b) Mesophyll
(c) Pili (d) Bundle sheath
viii) Polyarch condition is seen in
(a) Monocot stem (b) Monocot root
(c) Dicot root (d) Dicot stem

ix) Which of the following is seen in a monocot root?
(a) Large pith (b) Vascular cambium
(c) Endarch xylem (d) Medullary ray

x) Well developed pith is found in
(a) Monocot stem and dicot root (b) Monocot and dicot stems
(c) Dicot stem and dicot root (d) Dicot stem and monocot root

3.6.2 Fill in the blanks:

i) The meristems present at the tips of roots and shoot are called …… meristems.
ii) The meristem situated at the bases of internodes is called …… meristem.
iii) One or More layers of cortex below the epidermis become thick wall to form ……
iv) The inner layer of endodermis is called ……
v) Cells of root caps in many parts form a constant structure called……..
vi) Secondary growth includes the formation of secondary vasculartissues and ……
vii) The walls of epidermal cells of leaves of the xerophytic plantsundergo……

3.6.1 Answer Key:

i. (d), ii. (d), iii. (c), iv. (a), v. (a), vi. (b), vii. (a), viii (b), ix. (b), x (a)

3.6.2 Answer Key:

i) apical, **ii)** Intercalary, **iii)** Endodermis, **iv)** pericycle, **v)** columella, **vi)** Periderm **vii)** lignification

3.7 REFERENCES

- Carlquist, S. 2004: Bot. J. Linn. Soc.146:, (2) 129-143
- Carlquist, S, Schneider EL. Origins and nature of vessels in Monocotyledons. I. Acorus. International Journal of Plant Sciences.1997; 158:52–56.
- Craig, Richard and Vassilyev, Andrey. "Plant Anatomy".McGraw-Hill.Archived from the original on 24 July 2010.
- Eames, Arthur Johnson and MacDaniels, Laurence H. (1947).*An Introduction to Plant Anatomy* 2nd ed. McGraw-Hill, New York, (1st ed., 1925.
- Eames, Arthur Johnson and MacDaniels, Laurence H. (1947).*An Introduction to Plant Anatomy* 2nd ed. McGraw-Hill, New York, .(1st ed., 1925.
- Esau, Katherine (1965). *Plant Anatomy* 2nd ed. Wiley, New York.
- Esau's Plant Anatomy, Meristems, Cells, and Tissues of the Plant Body: their Structure, Function, and Development. 3rd edn.". *Annals of Botany,* 99 (4): 785–786.
- Fahn, A. 1990, Plant Anatomy, Pergamon Press, Oxford 4th Edn.
- Meicenheimer, R. *History of Plant Anatomy*. Miami University.
- Pandey,B. P. 2001, Plant Anatomy, Published by S. Chand Publisher.
- Pandey B.P, 2012, Modern Practical Botany, Vol II, S.Chand& Co., New Delhi.
- Pandey, S.N. 1997, Plant Anatomy and Embryology, Vikas Publication House Pvt Ltd.
- Rajput, K. S. &Rao, K. S. 1998: Cambial anatomy and absence of rays in the stem of *Boerhaavia* species (Nyctaginaceae). — *Ann. Bot. Fennici*35: 131–135.
- Singh, V., 2010, Plant Anatomy and Embryology of Angiosperms, Global Media Publications.
- Sharma A.K. & Sharma R. 2010, Structure, Development and Reproduction in Flowering Plants, Jagdamba Publishing Co, New Delhi.

3.8 SUGGESTED READINGS

- Esau, K. 1977, Plant anatomy. New York: John Wiley & Son.

- Esau K. 1977, Plant anatomy. New York: John Wiley & Son.
- Fahn, A. 1990, Plant Anatomy, Pergamon Press, Oxford 4th Edn.
- Pandey,B. P., 2001, Plant Anatomy, Published by S. Chand Publisher.
- Pandey, S.N., 1997, Plant Anatomy and Embryology, Pub.Vikas Publication House Pvt Ltd.
- Singh, V., 2010, Plant Anatomy and Embryology of Angiosperms, Global Media Publications.
- Vasishta, P.C. 1968, Plant Anatomy, Pradeep Publication & Co Chandigarh.

3.9 TERMINAL QUESTIONS

3.9.1 Short Answer type Question:

1. What are the differences between normal and anomalous secondary growth?
2. Define activity of cork cambium.
3. Define the mechanism of secondary growth.
4. What are spring and autumn wood?

3.9.2 Long Answer type Question:

1. How dicot stem increases in its girth define in detail?
2. Define secondary growth in Dracaena?
3. What is accessary cambium and define its function.
4. How secondary growth occurs in Salvadora?
5. Secondary growth in Boerhaavia occurs due to accessory cambium formation, define this.

UNIT-4L STRUCTURE AND ANATOMY OF SOME IMPOPRTANT STEM

4.1 OBJECTIVES

After reading this unit students will be able:

- to explain the stem anatomy of *Hydrilla,* an aquatic plant sps.
- To know morphological and anatomical features of *Ranunculus*sps.
- to describe the stem anatomy of *Euphorbia*sps.
- to understand ecologicaland anatomical differences among *Hydrilla, Ranunculus* and *Euphorbia.*

4.2 INTRODUCTION

The plants which characteristically grow in certain environment often show the structure which is believed to be adapted to that particular environment. In the course of evolution, many species have become adapted in their structural and physiological features to habitats either with an excessive water or deficiency in water.

Plants that live wholly or partly submerged in water or in very wet places are known as hydrophytes while the larger numbers of plants grow under average conditions of moisture and temperature. Plants of habitat that usually show neither an excess nor a deficiency of water are known as mesophytes.Plants that grow in places where the evaporation stress is high and the water supply is low are known as xerophytes.Mesophytes are therefore, intermediate between hydrophytes and xerophytes.

In this section we have chosen one hydrophyte (*Hydrilla*), onemesophyte (*Ranunculus*) and one xerophyte (*Euphorbia*) to study the anatomical detailswith variation in stem anatomy.

Hydrophytes are characterized by large number of air chambers within the tissue of stem and leaves which help them in buoyancy as well as storing oxygen.Root system, vascular tissue, stomata etc are poorly developed. Cuticle layer in leaves is mostly absent.Thick walled tissue is altogether absent.

In mesophytes the root system is well developed with the tap-root system and branched in dicotyledons, while a cluster of fibrous roots in monocotyledons; root hairs are abundantly produced for the absorption of water from the soil.

In mesophytes the stem is solid, erect and normally branched. All the different kinds of tissues, particularly the mechanical and conducting tissues have reached their full development. The aerial parts of plants such as the leaves and the branches are provided with cuticle. In

dorsiventral leaves the lower epidermis is provided with numerous stomata, there are few stomata or none at all on the upper surface.

In erect leaves of most monocotyledons, stomata are more or less equally distributed on both surfaces. The stomata are relatively uniform in structure and the guard cells show a maximum capacity for movement. The anatomy of mesophytic plants is quite normal and no special adaptations are found in them.

Xerophytes growing in very dry places can withstand a prolonged period of drought uninjured, and for this purpose they have certain peculiar adaptations. The xerophytic plants have to guard against excessive evaporation of water, by reducing evaporating surfaces. Plants produce a long tap root which goes deep into the sub-soil in search of moisture. To retain the water absorbed by the roots, the leaves and stems of some plants become very thick and fleshy (e.g., *Aloe, Agave*). Multiple epidermis sometimes develops in the leaf (e.g., *Nerium*). In xerophytes certain structural features are also common. Leaves are thick and leathery, with a well-developed cuticle and abundant hairs. Well differentiated mesophyll and more than one layer of palisade tissue (e.g., *Nerium*). The walls of epidermal and sub-epidermal cells are frequently lignified, and a distinct hypodermis, may be present. They have a well-developed vascular system and often an abundance of sclerenchyma, either in the form of sclereids or fibres. The leaf is sometimes cylindrical or rolled. This organization is to protect the stomata, which may occur in furrows.

4.3 STRUCTURE, ORGANIZATION OF THE SHOOT AND ANATOMY OF-

4.3.1-*Hydrilla verticellata*

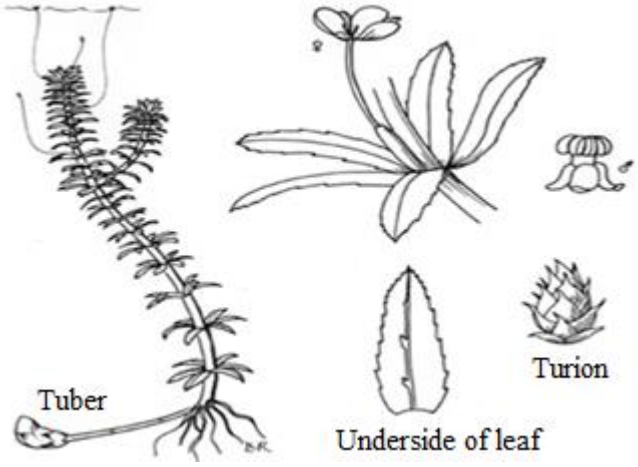


Fig. 4.1 *Hydrilla* Plant Morphology

Hydrilla (Waterweed) is a genus of aquatic plant, usually treated with one species, *Hydrilla verticillata*. The stems grow up to 1–2 m long. The leaves are arranged in whorls of two to eight around the stem, each leaf 5–20 mm long and 0.7–2 mm broad, with serrations or small spines along the leaf margins; the leaf midrib is often reddish when fresh. It is monoecious (sometimes dioecious), with male and female flowers produced separately on a single plant; the flowers are small, with three sepals and three petals, the petals 3–5 mm long, transparent with red streaks. It reproduces primarily vegetatively by fragmentation and by rhizomes, and flowers are rarely seen. They have air spaces to keep them upright.

The inflorescences are unisexual, arising from spathes situated in the leaf axils; each flower has three sepals and three petals. All six perianth parts are clear or translucent green (the sepals usually slightly reddish).The male spathe is about 1.5 mm long, solitary in the leaf axils, somewhat spiny. The female spathe is about 5 mm long, solitary in the leaf axils. There are three petals, three stamens and three styles. The ovary is cylindrical to narrowly conical and is enclosed in the base of a hypanthium; the style is as long as the hypanthium and there are three stigmas.

Chambers and passages filled with gases are commonly found in the leaves and stems of hydrophytes. The air chambers are large, usually regular, intercellular spaces extending through the leaf and often for long distances through the stem (e.g., *Hydrilla, Potamogeton, Pontederia*).

The spaces are usually separated by partitions of photosynthetic tissue only one or two cells thick. The chambers prepare an internal atmosphere for the plant. These air- chambers on the one hand give buoyancy to the plant for floating and on the other they serve to store up air (oxygen and carbon dioxide).

Here, very thin partitions enclose air spaces and the whole structure consists of very feeble tissue. Aerenchyma is phellem formed by a typical phellogen of epidermal or cortical origin. At regular intervals individual cells of each layer of phellem elongate greatly in the radial direction while the other cells of this layer remain small.

However, the term aerenchyma is applied to any tissue with many large intercellular spaces, but such aerenchyma is quite distinct from the typical aerenchyma mentioned above which is of secondary origin.

Stem Apex of *Hydrilla*

Fig. 4.2. Stem Apex of Hydrilla Showing Bud Development

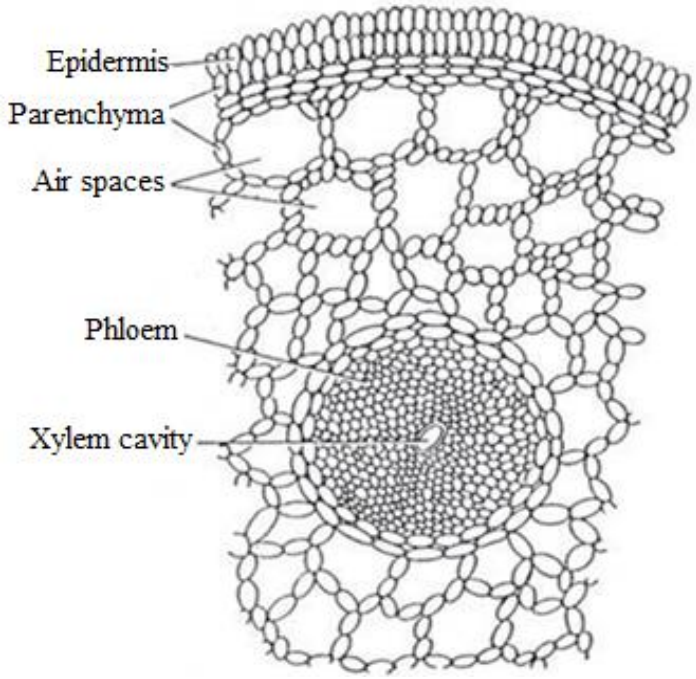


Fig. 4.3.T.S. of *Hydrilla* Root Showing Air Spaces in Cortex and Single Xylem Cavity

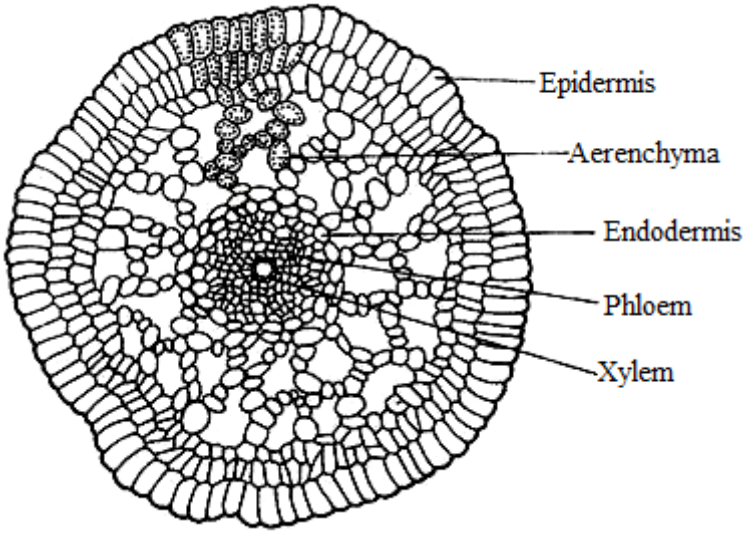


Fig. 4.4.T.S. of *Hydrilla* Stem Showing Aerenchyma

- Stem is usually weak and flexible. Sometimes it is covered by a gelatinous sheath which serves as protection against periodic desiccation.
- The cuticle is either altogether absent or very poorly developed. The epidermis is always single-layered and thin-walled; this character facilitates direct absorption of gases and mineral salts dissolved in water.
- The cortex is very broad and occupies bulk of the stem. The outer layers of the cortex are parenchymatous and usually without inter-cellular air spaces, whereas the inner cortex is aerenchymatous and possesses symmetrically arranged large air spaces. The air filled in these cavities adds to the buoyancy of the plant and secondly facilitate the exchange of gases during respiration and photosynthesis.
- The cells of the cortex contain chloroplasts and assist in carbon assimilation.
- Usually there is no marked distinction of endodermis and pericycle. Sometimes the inner-most layer of the cortex is regarded as endodermis.
- Vascular tissue is poorly developed and does not show marked differentiation of phloem and xylem. An air cavity is mostly present at the center of the vascular strand that adds to the buoyancy of the plant. Sometimes, xylem is represented by a single strand present in the center of the stele (e.g., *Hydrilla, Potamogeton, Elodea* etc.)
- There is no mechanical tissue present in the stem of the submerged plant. Water column itself provides mechanical support to the plant.

4.3.2-*Ranunculus scleretus*

Ranunculus sceleratus is an annual herb growing up to half a meter tall. The leaves are more or less glabrous (hairless) and have small blades each deeply lobed or divided into three leaflets.

They are borne on long petioles. The flowers are 5-10mm across with five or fewer yellow petals a few millimetres long and reflexed sepals. The fruit is an etario ofachenes borne in cluster.

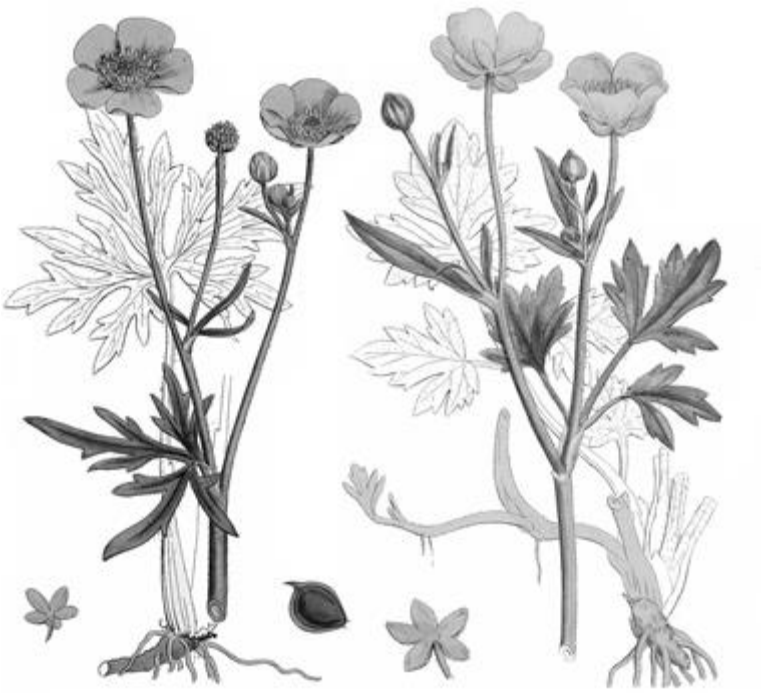


Fig. 4.5 Morphology of *Ranunculusscleretus*

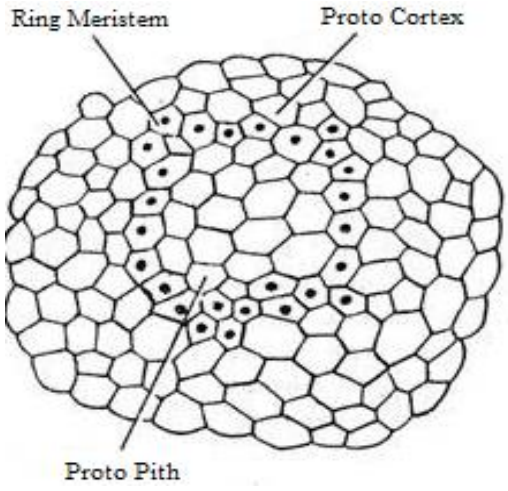


Fig. 4.6 T.S.Through Shoot Apex of *Ranunculus* sp

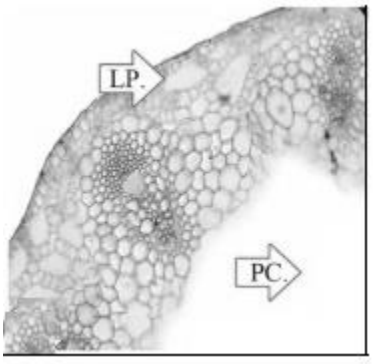
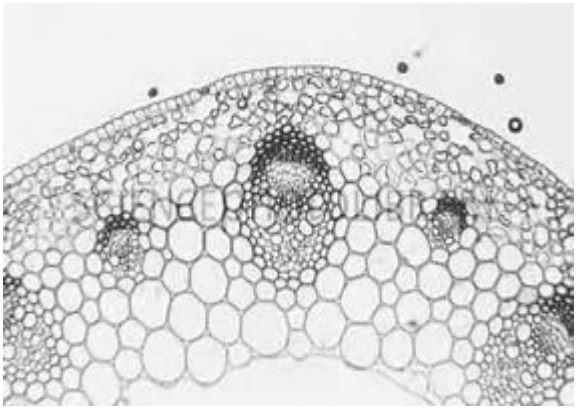
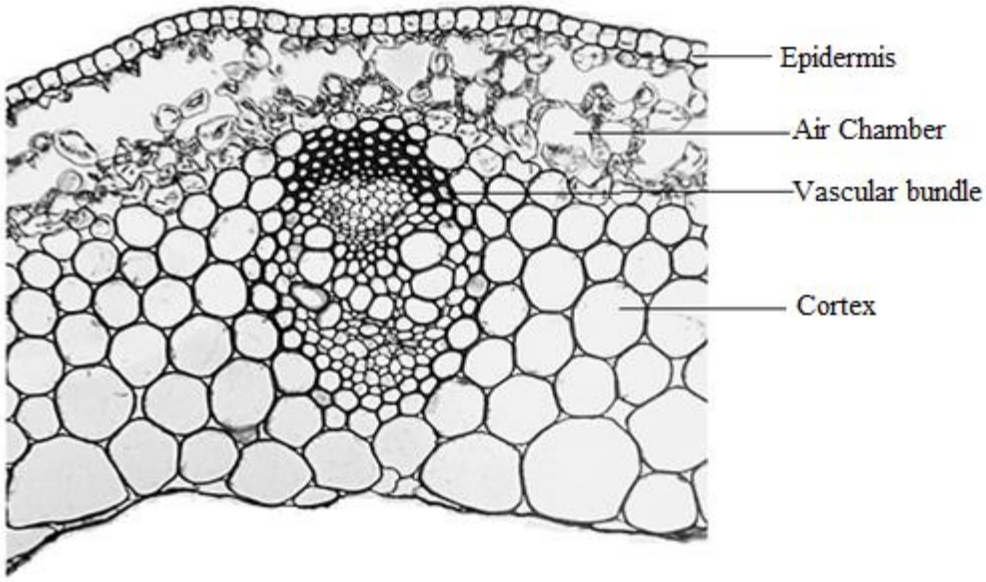


Fig. 4.7 T.S. Through Stem of *Ranunculus*sps. Showing Lacunate Parenchyma (LP) & Pith cavity (PC)



(A)



(B)

Fig. 4.8 Stem T.S. of *Ranunculus scleretus* (A&B)

In this low magnification image of a *Ranunculus* stem, attributes of a dicot stem can be viewed. This dicot stem contains vascular bundles arranged in a concentric ring. This is somewhat similar to the arrangement of vascular tissues in a monocot root.

- The epidermis appears thicker due to a cuticle or waterproofing layer. Trichomes and stomata may be present.
- The cortex is made up of the multiple layers of cells between epidermis and pericycle. There are three sub-zones in the cortex. The outer sub-zone is called hypodermis. The hypodermis is composed of a few layers of collenchyma. The middle layer is composed of thin-walled parenchyma with distinct air spaces called aerenchyma. The innermost layer is called endodermis.
- Endodermal cells are rich in starch grains and hence this layer is also called the starch sheath. Pericycle is present on the inner side of endodermis and above the phloem. The pericycle is in the form of semi-lunar patches of sclerenchyma.
- A large number of vascular bundles are arranged in a ring. It is important to remember that the ring-like arrangement of vascular bundles is the characteristic of dicot stem. Each vascular bundle is conjoint, collateral and open. Protoxylem is endarch. Each vascular bundle is capped by sclerenchymatousfibers.
- Usually pith is composed of rounded parenchymatous cells; with large intercellular spaces but in case of *Ranunculus* it is hollow. The deepest parenchyma cells of this stem are fragmented during growth to produce the hollow pith area.

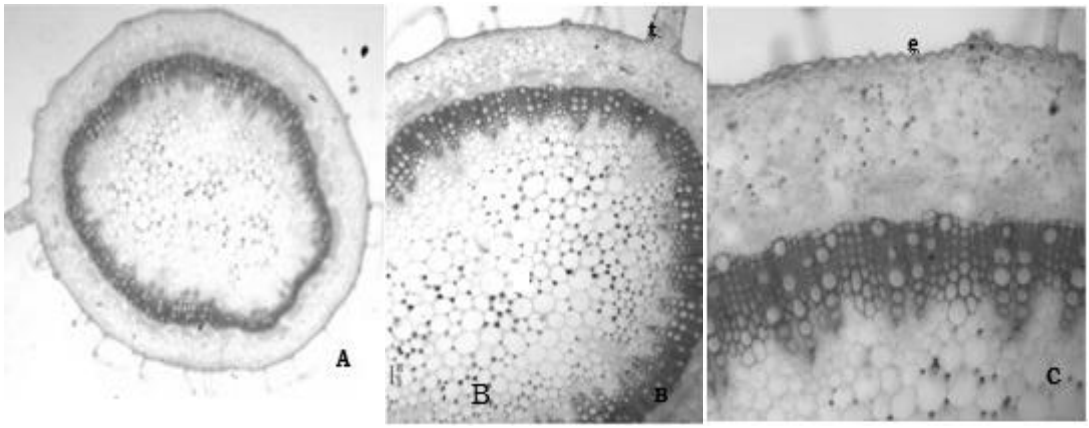
4.3.3-*Euphorbia hirta*

Euphorbia hirta L. belongs to the family Euphorbiaceae, widespread at low altitudes throughout the tropics and subtropics. It prefers sunny to lightly shaded dry conditions. It is an early colonizer of bare ground. *E. hirta* is one kind of weed in cultivated fields of perennial crops, grasslands, roadsides, gardens, lawns, fallow lands, ditch banks and waste places. It is a slender-stemmed when mature, annual hairy plant with many branches from the base to top, spreading upto 40 cm in height, reddish or purplish in colour.*Euphobiahirta* is used in the treatment of gastrointestinal disorders, bronchial and respiratory diseases, and in conjunctivitis. Hypotensive and tonic properties are also reported in *E. hirta*.

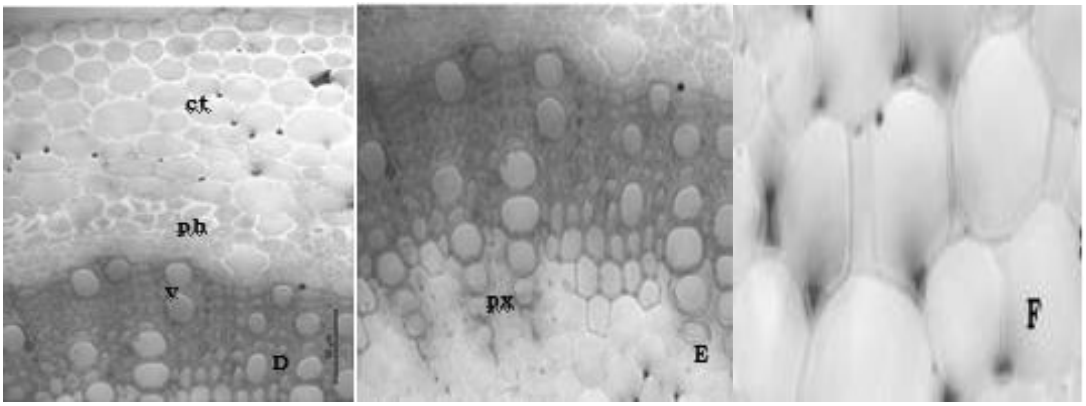
Fig.4.9 Morphology of *Euphorbia hirta* Plant

Macroscopic characters of *E. hirta* leavesshows composition of leaf is simple with dark green color having no odour, about 2-6cm. long in size, shape is ovate, texture is hairy, apex is acute and midrib is distinct on both the sides. T.S. of leaf revealed the presence of stomata on upper and lower epidermis. Powder characteristics revealed the presence of starch granules.

Stem Anatomy:



A. T.S. of Stem B. One Portion Enlarged C. Cortical Region Enlarged



D. Cortical Region E. Vascular Region Showing Protoxylem F. Enlarged View of Pith

Fig. 4.10: Anatomy of *Euphorbia hirta Stem at Different Places*

- Cross section of the stem wasgenerally circular shape.
- Covered with thickcuticle layer, epidermis was uniseriate with epidermal cells elongated, compactly arranged, bearingunicellular and multicellular trichomes (Fig.8.10 B).
- The thickness of the cuticle is greater than normal, like that of plants of semi- xerophytic habitats.
- Cortex was distinctly formed inabout 10-12 rows. Both the parenchymatous and chlorenchymatous cells were present in cortex. The cellswere rich in chloroplasts, therefore it was chlorenchyma.
- Laticifers were also present in the cortex zone.
- The genus *Euphorbia* has got wavy central cylinder usuallybecause of the bundle cap which prolongated and projectedalong the phloem positions. This waving may be initiated bytwo ways:

1) Variations in the vascular bundle positions, there are internal vascular bundles and external ones which alternate with one another.
2) Variations among bundle capdiameters, bundle cap of one isprojected out and the other in.

- In *E. hirta*, the centralcylinder was slightly wavy, wide and with peripheral positionbecause of their similar size and regular positions of the bundlecaps.
- Tracheary elements of wood resembled with vessels and tracheids, which were in radial rows.
- Xylem parenchyma was distinct within thewood. Wood arms (xylary arms) projected clearly toward thepith that will be single, double or triple.
- The phloemwas in external position and usually surrounded by thickfibrous tissue which resemble the bundle caps.
- The phloemwas differentiated by 4-6 narrow parenchymatous layers.
- The sieve elements and other cells of phloem were distributedregularly between the bundle caps and xylem, and there werefew fibers, or these cell elements present as an islands betweenthe bundle cap fibers.
- The pith has adistinct gap or central cavity at maturity.
- At theimmature stage, the pith cells were big, with thin walls anddistinct intercellular spaces. Pith cells were similar in shapeusually spherical or polyhedral.

4.4 SUMMARY

Let's take a look at the anatomy of dicotyledonous and monocotyledonous plants. In this section we have discussed *Hydrilla* a monocot, *Ranunculus* and *Euphorbia* spp. the dicots

Hydrilla stem is usually hollow with no secondary growth. The anatomy of monocot and dicot stem are similar, however some notable differences are as follows:

- The hypodermis of the cortex in monocots is made of sclerenchymatous cells.
- Vascular bundles are numerous but scattered, conjoint and closed, surrounded by the ground tissue.
- Phloem parenchyma is absent.
- Plant shows hydrophytic characters as the vascular tissue is poorly developed and does not show marked differentiation of phloem and xylem. An air cavity is mostly present at the center of the vascular strand in addition to air chambers in the cortical region that adds to the buoyancy of the plant.

Dicotyledonous stem of *Ranunculus* and *Euphorbia* is usually solid. The transverse section of their stems consists of the following parts:

- Epidermisis the outermost protective layer which is covered with a thin layer of cuticle.
- Epidermis possesses trichomes and a few stomata.

- Cortex is multi-layered sandwiched between epidermis and pericycle.
- The outer hypodermis, the middlecortical layers and the inner endodermis together make the three subzones of cortex.
- Next to endodermis is the pericycle which is constituted of semi-lunar patches of sclerenchyma.
- Ring arrangement of vascular bundles is present (only in dicot stem).
- Vascular bundle is conjoint, collateral and open with endarch protoxylem.
- Pith is evident and is made of parenchymatous cells but hollow in *Ranunculus*.

4.5 GLOSSARY

Adaxial Surface-The upper surface of a leaf, harvests light. This side is closer to the meristem in the leaf primordia.
Aerenchyma- Parenchyma with large intercellular air spaces.
Angular -Ridged along its length, these ridges appearing as angles in the cross-section.
Antrorse -Projecting forwards; used for an arrangement of hairs, the anther or less commonly the column wings.
Apex -The tip or end.
Bilocular -With two cavities or locules.
Bisexual -Both male and female sexes present.
Bristly - With stiff hairs or bristles.
Dimorphic -Existing in two different forms.
Dimorphism -The non-flowering plants are strikingly different to the flowering plants.
Dissected -Deeply divided into segments.
Distal - Away from the base towards the apex.
Distichous -In two ranks; usually applied to the arrangement of leaves or flowers.
Dorsal -The upper or outer surface or edge.
Epidermis -The outermost layer of cells covering the leaves.
Mesarch - A type of xylem maturation in which the protoxylem is embedded in the metaxylem and development proceeds both centripetally (from the outside in) and centrifugally (from the inside out); compare to endarch and exarch
Mesophyll -Parenchyma tissue between the upper and lower epidermis of a **leaf**
Metaxylem -Type of primary xylem that differentiates and matures later than the protoxylem; generally metaxylem tracheids are longer than protoxylem
Parenchyma -The most common type of plant cell; thin-walled cells varying in size, shape, and function
Periderm -A tissue primarily consisting of cork cells; outer bark
Phloem -Photosynthate conducting tissue of vascular plants

Pith -The central parenchymatous tissue in a vascular plant axis
Prostrate - Lying flat.
Proximal - Situated near the point of attachment.
Sessile - Without a stalk, pedicel or petiole.
Sheath - The base of a leaf or bract which embraces a bud or axis.
Shoot -A horticultural term used by growers for a new growth.
Terminal - The apex or end.
Vascular -Said of plants which have water-conducting tissue.

4.6 SELF ASSESSMENT QUESTIONS

4.6.1Objective type Questions:

1. A bicollateral vascular bundle is characterized by
(a) phloem being sandwiched between xylem (b) transverse splitting of vascular bundle
(c) longitudinal splitting of vascular bundle (d) xylem sandwiched between phloem.

2. A narrow layer of thin walled cells found between phloem/bark and wood of a dicot is
(a) cork cambium (b) vascular cambium
(c) endodermis (d) pericycle

3. Casparian strip occurs in a
(a) endodermis (b) exodermis
(c) pericycle (d) epidermis

4. Vascular bundles in a dicot stem are
(a) Open, collateral, exarch (b) Closed, collateral, endarch
(c) Closed, collateral, exarch (d) Open, collateral, endarch

5. Annual rings are distinct in plants growing in
(a) Temperate regions ` (b) Tropical regions
(c) Grasslands (d) Arctric region

6. The lateral roots generally originate in
(a) Cork cambium (b) cortex
(c) pericycle cells lying against protoxylem (d) Endodermal cells lying against protoxylem

4.6.2 Fill in the blanks:

1. The inner most layer of the cortex is distinct and well developed in primary roots. It is called_____.

2. A band of suberin develops all around the cell in the middle of the transverse and radial walls. This suberin band is called _____ strip.

3. The outer most part of the stele consists of one or more layers of parenchymatous cells. The outer layer of this parenchyma is called _____

4. In case _____ xylem is present towards the inner side and phloem is present towards the outer side of vascular bundle.

5. In case_____ , phloem is present on both side of xylem.

6. In case of_____ bundles, on type of vascular tissue (xylem or phloem) completely surround the other type of tissue.

7. Vascular bundles having cambium between xylem and phloem are called _____ type.

8. A narrow strip of meristematic cells is present between the xylem and phloem in the vascular bundles of dicots and gymnosperms. This strip of meristematic cells is called vascular……..

9. The phellogen produces a group of loosely placed cells at certain points. These loosely placed cells are called ____

10. The tissues in which the cells are undifferentiated and capable of division are called _____

4.6.1 Answers Key: 1. (d), 2.(c), 3. (a), 4. (d), 5. (a), 6.(c)

4.6.2 Answers Key: 1. Endodermis, **2.** Casparian, **3.** Pericycle,
4. Collateral, **5.** Bicollateral, **6.** Concentric**, 7.** Open, **8.** Cambium, **9.** Lenticels,
10. Meristem

4.7 REFERENCES

- *"The Plant List: Ranunculus sceleratusL.".Royal Botanic Gardens, Kew and Missouri Botanic Garden. 2013*. Retrieved *27 May* 2016.
- Metcalfe CR and Chalk L. 1950. Anatomy of the Dicotyledons: Leaves, Stem and Wood in Relation to Taxonomy with Notes on Economic Uses. Oxford: Oxford Clarendon Press, v. 1. 1500 p.
- Prajapati ND, Purohit SS, Sharma AK and Kumar T. 2003.Handbook of Medicinal Plants. Jodhpur, India: Agarbios.
- Raju VS and Rao PN. 1977. Variation in the structure and development of foliar stomata in the Euphorbiaceae. *BotanicalJournal of the Linnean Society*, 75: 69-97.
- Rosowski JR. 1968.Laticifer morphology in the mature stem and leaf of *Euphorbia supina*.*Botanical Gazette*, 129: 113-120.
- Sehgal L and Paliwal GS. 1974. Studies on the leaf anatomy of *Euphorbia* venation patterns. *Botanical Journal of theLinnean Society*, 68: 173-208.

- Sereena K and Shahida TA. 2015. Comparative anatomical and histochemical studies of *Euphorbia hirta*L. and*Euphorbiathymifolia*L. (stem). *IJPSR*, 6 (2): 772-777.
- Solereder H. 1908. Systematic Anatomy of the Dicotyledons.Oxford, Clarendon Press.p. 643.
- Sultana RS. 2016. Stem and leaf anatomy of *Lantana camara*L. - a Plant of the Verbenaceae Family. *Int. J. Curr. Res.Biosci. Plant Biol.*, 3 (1): 27-31.
- Williamson EM. 2002. Major Herbs of Ayurveda. China: Churchill Livingstone.
- Zahra NB, Ahmad M, Shinwari ZK, Zafar M and Sultana S. 2014.Systematic Significance of anatomical characterizationin some euphorbiaceous species.*Pak. J. Bot*., 46 (5): 1653-1661.

4.8 SUGGESTED READINGS

- Arber, A. (1950): *The natural philosophy of plant form*, Cambridge, UK.
- Esau K. (1977): Anatomy of Seed Plants. John Wiley & Sons, Inc., Delhi
- Hort, A. (1949): *Theophrastus: Enquiry into plants*, with an English Translator, London, UK.
- Pandey, S.N. &Chadha, A. (1997)- Plant Anatomy and EmbryologyPaperback, Vikas Publication House Pvt Ltd; First edition
- Pandey, B.P., 2012, 'Plant Anatomy' by S. Chand, Publication, New Delhi
- Pijush Roy, 2010; Plant Anatomy Paperback – New Central Book Agency.
- Sporne, K. R. (1974): *The Morphology of Angiosperms*. M/s Hutchinson & Co (Pub) Ltd., London, UK.
- Wardlaw, C. W. (1968): *Morphogenesis in Plants*. Methuen & Co. Ltd, London, UK.

4.9 TERMINAL QUESTIONS

4.9.1. Short Answer type Question:

1. Describe the cortex of *Hydrilla*.
2. Explain the vascular bundles in *Hydrilla.*
3. Describe waving in central cylinder of *Euphorbia* stem.
4. Is there any similarity in *Ranunculus* stem and monocot root anatomy?
5. Define the anatomical difference in vascular bundles of *Hydrilla* and *Euphorbia.*

4.9.2 Long Answer type Question:

1. Draw a well labeled diagram of *Hydrilla* stem T.S. and describe in detail.
2. Draw and comment on the anatomical features of *Euphorbia* stem.
3. How *Ranunculus* shows some specific features in its stem anatomy? Describe and draw its anatomical features.

UNIT-5L TO STUDY THE INTERNAL STRUCTURE OF ANTHER THROUGH T.S.

5.1 OBJECTIVES

After reading this unit, students will be able-

- To study the structure of Flower
- To study the male reproductive part of Flower
- To study the T. S. of Anther
- To understand the procedure for preparing the T. S. of Anther

5.2 INTRODUCTION

The **angiosperms** are seed-bearing plants that produce flowers. The seeds, which contain the plant embryo, are produced in the flower. All the parts of a flower are actually modified leaves that are specialized for their roles in the reproductive process. Let us try to understand a typical flower with the help of Fig.3.1.

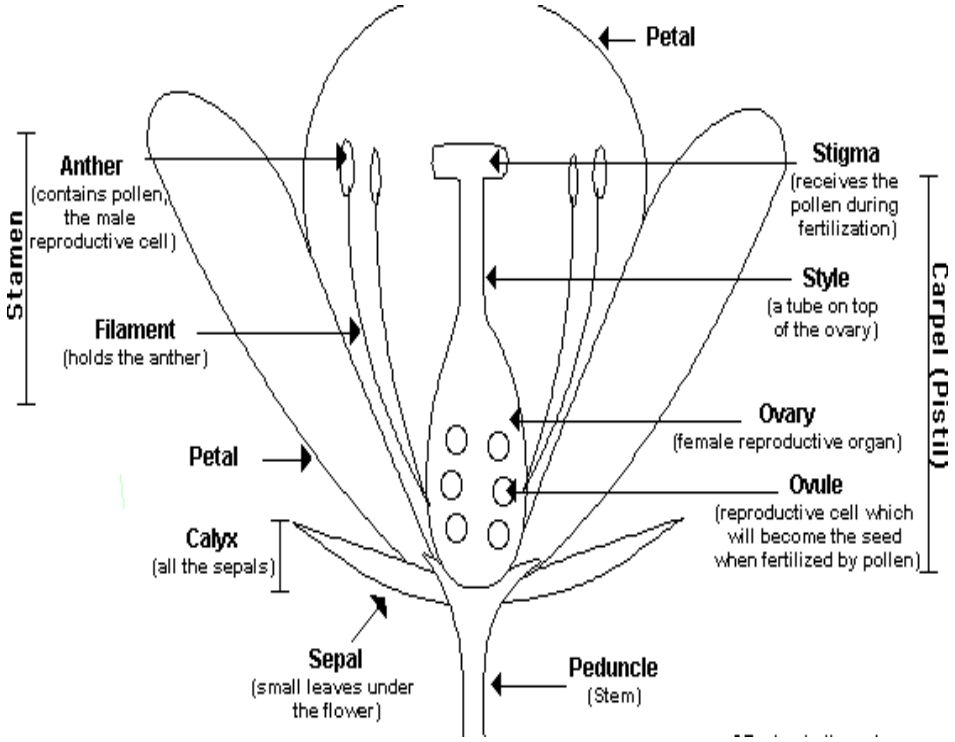


Fig. 5.1 Structure of flower

Flower parts are arranged in circles called **whorls**. They are attached at the enlarged base of the flower, the **receptacle**. As we know that flowers are made up of both sterile and fertile parts which are arranged in four whorls. These include the calyx, the corolla, the androecium and

the gynoecium. Both the calyx (collective term of sepals) and corolla (collective term of petals) are the sterile parts, while the androecium and gynoecium are the fertile structures.

Basically, the androecium is referred to be the third set of floral organs composed of stamens or microsporophylls. Ordinarily, each stamen is composed of a slender stalk-like filament supporting a knob-like spore case or the anther. Each anther consists of two lobes (anther lobes) connected by a tissue (connective) which can be in some cases clearly seen on the dorsal side as an extension of the filament. Each anther lobe, again, has two pollen sacs or pollen chambers (microsporangia) placed longitudinally. Special cells within the pollen sacs undergo meiosis to form **pollen grains**. Each pollen grain contains two male gametes. When the pollen grains mature, the pollen sacs split open to release the dust-like **pollen**.

The **pistil** (carpel) is the female reproductive organ and consists of three parts: the stigma, style, and ovary. The **stigma** is an enlarged receptive part at the top of the pistil that becomes moist and sticky when mature. The **style** is the middle thin portion of the pistil. It can be long and slender, short, or even absent, depending upon the species. The **ovary** is the enlarged structure at the bottom of the pistil. The ovary contains one or more hollow compartments called **locules**. Each locule contains one or more **ovules**. Embryo sac (female gametophyte) is present in each ovule which has one **egg.**

Pollination occurs when pollen grains land on the sticky surface of the stigma and are trapped there. The pollen grain germinates and a **pollen tube** emerges due to the release of enzymes by stigmatic surface that digest the cell wall of pollen grain. The pollen tube grows down through the style to the ovary and enters the ovule, making a continuous passage for the two male gametes to enter the embryo sac inside the ovule. **Fertilization** occurs by fusion of male gamete with egg.

The fertilized egg ultimately develops into an **embryo**. The wall of the ovule thickens and forms a **seed**, thus enclosing and protecting the embryo. The ovary develops into a **fruit**.

After getting a concise knowledge about a flower, we will try to study the morphological features of the flower. For this purpose we will obtain a single flower and observe its parts carefully.

The **sepals** form the outermost whorl of the flower. The sepals are leaf-like structures that are usually green in color. Sometimes, the sepals are the same color as the petals, or appear to be another set of petals of a different color. The function of the sepals is to protect the inner part of the flower before it blossoms. Gently remove the sepals, tape them into position onto the paper, and label them. On the chart, observations should be recorded.

The **petals** are found directly under the sepals. The color and odor of the petals help to attract birds and insects to the flower for pollination. Gently remove the petals, tape them into position onto the paper, and label them. On the chart, observations are recorded.

The stalk-like structures inside the petals are the **stamens**, the male reproductive part. Depending on the species, the stamens may be attached to the receptacle, to the petals, or to the pistil. The enlarged portion at the top of the stamen is the **anther**. Inside the anther are **pollen sacs**, which produce pollen grains. When the **pollen grains** mature, the pollen sacs split open, releasing the dust like pollen grains. The filament is the thin structure that supports the anther.

After examining flower morphologically let us try to know more about the stamen-

Stamens (Fig.3.2) are the male reproductive organs of a flower. Each stamen consists of an anther borne on a stalk-like filament. The anther dehisces at maturity in most of the angiosperms by a longitudinal slit to release the pollen grains. The pollen grains contain the highly reduced male gametophyte. These microgametophytes carry of male gametes that play a key role in plant reproduction during the process of double fertilization.

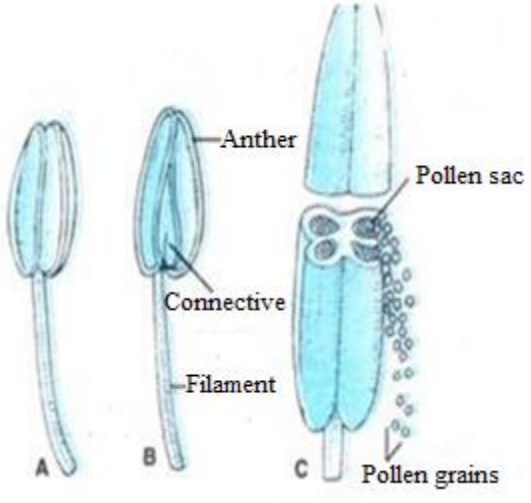


Fig.5.2 Stamen: A ventral view; B. Dorsal view; c. Three dimensional cut section of anther

5.2.1 Structure of Anther

As we can see in the Fig.3.2, a typical anther is a bilobed, dithecous structure with two microsporangia in each lobe. Therefore, an anther is a tetrasporangiate structure with four microsporangia. The non-sporangial tissue that joins the two anther lobes is known as the connective. A single vascular strand is embedded in the connective. In each lobe the two microsporangia are separated by a strip of sterile tissue. In a mature anther, the two sporangia in an anther lobe become confluent due to the enzymatic lysis of the sterile tissue to form a single

locule. In some plants such as *Hibiscus rosa-sinensis*, the anther is one lobed consisting of two microsporangia which are fused at maturity to form a single locule (monothecous).

5.3 T. S. OF ANTHER

The transverse section of Anther (Fig.3.3) reveals that the mature anther wall is made up of the following four layers:-

Epidermis
Endothecium
Middle layers
Tapetum

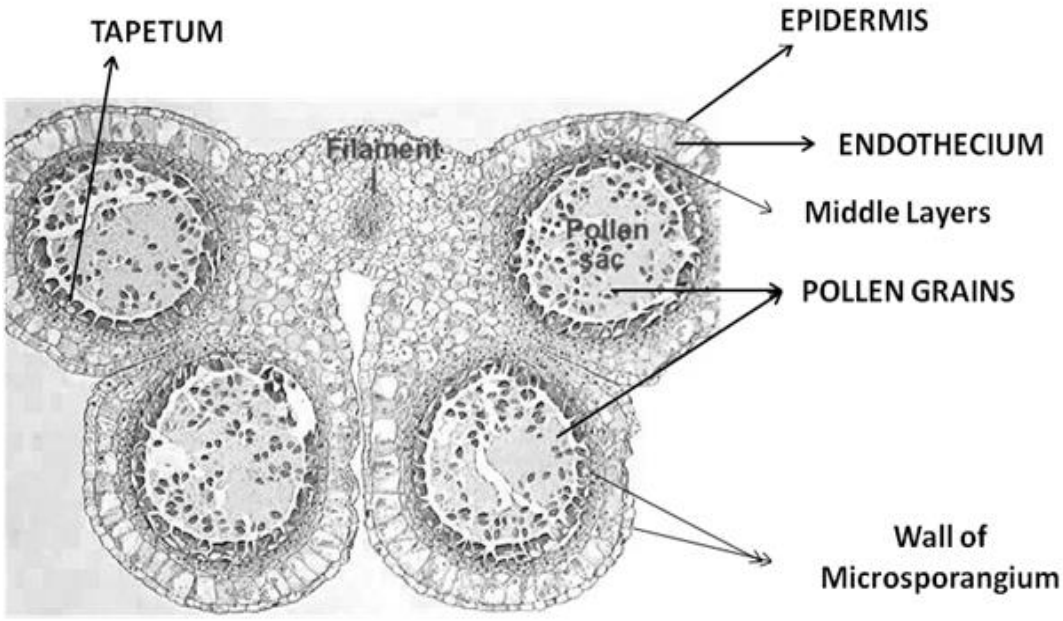


Fig.5.3 T.S. of Anther

5.3.1 Epidermis

The epidermis is the outermost layer of the anther and made up of tangentially stretched and flattened cells. Epidermis has a protective function. The epidermis prevents water loss from the anther, together with the endothecium provides structural support to the anther and plays a role in the anther dehiscence (Goldberg *et al*. 1993). In a mature anther, the epidermal cells are greatly stretched and flattened. In xerophytic plants, the epidermal cells are stretched to such an extent that these cell loose contact among themselves and appear as withering remains in a mature anther. The epidermal cells in the stomium region differentiate into small, specialized cells that split at maturity to facilitate dehiscence and release of pollen grains (Fig.3.4).

5.3.2 Endothecium

Endothecium also known as the subepidermal layer is the hypodermal layer that persists in the mature anther. Endothecium is usually single layered having radially elongated cells which attain maximum development when the anther is ready to dehisce for the discharge of mature pollens. The radial and inner tangential wall of endothecium cells are characterized by deposition of fibrous thickening bands. The outer tangential walls remain thin.

The endothecial cells at the junction of two pollen sacs (stomium) of anther lobe lack thickening in case of longitudinally dehiscing anthers. Thus the presence of fibrous bands, differential expansion of tangential wall layers and hygroscopic nature of endothecial cells play an important role in the dehiscence of anthers.

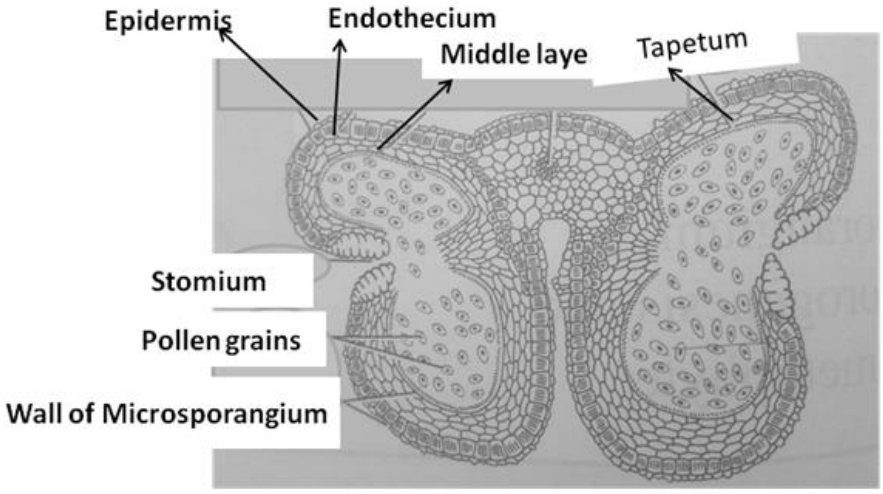


Fig. 5.4 T.S. of Anther showing the stomium region

5.3.3 Middle Layers

In general the cells of middle layers are ephemeral and, as a rule, become flattened and crushed during meiosis in the microspore mother cells. However, in some plants one or more middle layers may persist in anthers such as *Lilium*while in others, such as *Wolffia*and *Vallisneria*, middle layers are absent. The cells are flattened, thin-walled, uninucleate and vacuolated. In most angiospermic plants, the middle layers are referred to be the storage centers of reserve food material such as starch and other reserves which gets mobilized during the later development of pollen.

5.3.4 Tapetum

Tapetum is the innermost layer of the anther wall and is present in the form of a homogenous layer that completely surrounds the sporogenous tissue. It is usually single layered and has several nutritive and secretory functions related to pollen development and pollen germination. In many angiosperms, the tapetum is of dual origin. The outer portion of the tapetum (Fig.3.5), is contributed by the parietal layer (P-Tepetum) while the inner portion is derived from the connective tissue (C-Tapetum). The tapetum cells contain prominent nuclei and dense cytoplasm with an abundance of organelles such as mitochondria, plastids, endoplasmic reticulum, dictyosomes, vesicles and ribosomes.

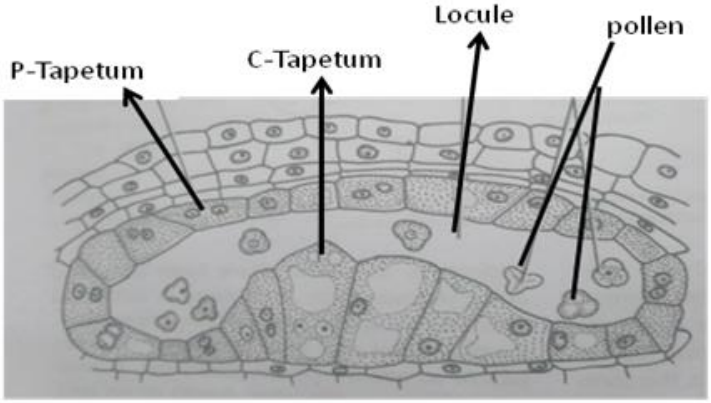


Fig.5.5 Tapetum showing dimorphic nature

The tapetum attains its maximum development at the tetrad stage of microsporogenesis. Later on it starts degenerating and is completely degenerated by the time the anther is ready to dehisce.

5.3.4.1 Function of Tapetum

The tapetum appears to play a significant role in the development of pollen. Its precocious degeneration during premeiotic and meiotic stages or its cellular persistence for unusually long period results in pollen sterility. Tapetum also helps in the transport of foodmaterial to the inside of the anther. The food material stored in tapetal cells is not utilised in the early stages of anther development. But after pollen mother cells have undergone meiosis the protoplast of tapetal cells enters into the pollen chamber to form periplasmodium which provides nutrition to the developing pollen grains and also helps in the exine formation.

5.4 PROCEDURE

For making neat and clean slide of Anther (T. S. of Anther), we should take few precautionary steps. These steps involve fine handling of plant material and a complex multi-stage process of staining to prepare microscope slides. This will depend on the amount and type of anther material available. Following are the major requirements that should be available with us-

- High power microscopes
- Suitable plant material as a source of anthers
- Plain slides
- Cover slips
- Staining solutions as safranin or fast green

5.4.1 Using the Microscope

- Lighting the slide is crucial, and students will need to use the condenser correctly, in order to achieve good contrast. Use of fine focus (and an oil immersion lens if available) will allow better viewing.
- It is important for us to obtain very young anthers to study, in which the pollen mother cells on the inner wall of the anther are still active. Look for white, translucent anthers.
- Some anthers do not take up the stain very readily, so it is wise to use a number of different sources of material. Possible sources of material are floral buds (in late summer or early autumn – before sprouting) of *Allium*, *Lilium*, or young buds of *Pelargonium* sp., *Tradescantia* sp. etc.

5.4.2 Location of Anthers in an Inflorescence

- Select the very smallest single bud on the inflorescence, which will also be the youngest.
- Carefully hold the base or stalk of the bud with forceps and pick the green sepals off with a mounted needle.
- Remove the small white petals that are around the outside of the flower and expose the ring of stamens in the middle of the flower. In some flowers, the stamens may themselves be attached to very small petals, do not discard these by mistake.

5.4.3 Preparation of Anthers for Further Investigation

- Anthers should appear white and translucent. If in doubt, try a smaller bud. Leave them attached to the flower throughout the staining process, as they are very easily lost.
- Stain anthers with safranin and sometimes with acetocarmine for five to six minutes and boil them upto three minutes in acetocarmine solution with the help of sprit lamp to see the cell division.
- Detach two or three anthers and transfer to a clean slide.
- Mature anthers can also be selected from the flower for section cutting.
- Then cut sections of anthers transversely in bulk quantity.
- Select the best sections and cover them with cover slips.

5.5 SUMMARY

The **angiosperms** are seed-bearing plants that produce flowers. The seeds, which contain the plant embryo, are produced in the flower. All the parts of a flower are actually modified leaves that are specialized for their roles in the reproductive process. Flower parts are arranged in circles called **whorls**. They are attached at the enlarged base of the flower, the **receptacle**.

As we know that flowers are made up of both sterile and fertile structures arranged in four whorls. Both the calyx and corolla are the sterile structures of a flower, while the androecium and gynoecium are the fertile structures.

The unitof the androecium is called the **stamen** and is the male structures in the flower. The stamen is made up of the filament and the anther. The **filaments** are the slender stalks and the **anther** is at the top of the stamen that contains pollen. The anthers often appear as yellowish because they contain pollen grains.

The well-differentiated anther wall comprises an epidermis, an endothecium, 1-3 middle layers and the tapetum. The epidermis is protective, The endothecium develops fibrous bands of lignocellulosic secondary thickening that provides the mechanical force for anther dehiscence. The middle layers are short-lived and get crushed during pollen development. The cells store nutrients for the developing pollen. Tapetum is the inner most nutritive layer which plays a crucial role in pollen development.

5.6 GLOSSARY

Angiosperm: A plant of a large group that comprises those that have flowers and produce seeds enclosed within a carpel, including herbaceous plants, shrubs, grasses, and most trees.

Receptacle: The modified or expanded portion of the stem or axis that bears the organs of a single flower or the florets of a flower head.

Filament: Part of a stamen, the male part of a flower

Pollination: A process by which pollen is transferred to the female reproductive organs of a plant, thereby enabling fertilization to take place.

Pollen tube: A hollow tube which develops from a pollen grain when deposited on the stigma of a flower. It penetrates the style and conveys the male gametes to the ovule.

Stamen: The male fertilizing organ of a flower, typically consisting of a pollen-containing anther and a filament.

Double fertilization: This process of forming a zygote and endosperm is called double fertilization, and it is unique to angiosperms.

Dithecous: The anther type, which contains two anther lobes connected to each other by connective.

Anther: An anther is the part of a stamen that produces and releases the pollen grains.

Anther dehiscence: Splitting of the anther at maturity along a built-in line of weakness.
Anther locule: A liquid filled cavity within the anthers in which the pollen grains develop and ripen.
Endothecium: The hypodermal layer of the anther wall characterized by the deposition of fibrous bands of lignocellulosic thickenings that provides the mechanical force for anther dehiscence.
Microgametogenesis: The process of formation of male/micro-gametes from the microspores.
Microsporogenesis: The series of events that lead to the development of haploid, uninucleate microspores from microspore mother cells within the microsporangium.
Pollenkitt: An oily, thick, viscous coating present over the pollen grain surface of many insect pollinated species that helps in adhering pollen grains together, adhering of pollen to insect pollinators and also to the stigma surface.
Tapetum: The innermost layer of the anther wall that plays an important secretory and transport function in pollen development, pollination and pollen germination.

5.7 SELF ASSESSMENT QUESTIONS

5.7.1 One Word Answer Type Questions:

1. Which substance is present in the fibrous thickenings of microsporangium?
2. Which is the innermost layer of microsporangial wall?
3. Which plant produce compound microspores?
4. Name the substance present in the exine of pollen grain.
5. Name the normal type of arrangement of microspore tetrad in angiosperms.
6. The pollen grains are liberated in angiosperm at what stage?
7. Which provides nutrition to the developing pollen grains and also helps in the exine formation?

5.7.2 Fill in the Blanks:

1. The branch of botany, which deals with the study of pollen, is……
2. The layer of cells present below the epidermis of a microsporangium is known as……
3. Each microspore mother cell undergoes **…….** and forms four haploid microspores.
4. The process of formation of microspors from the sporogenous tissue is known as **……**
5. The fertilized egg becomes an **……**
6. Special cells within the pollen sacs undergo meiosis to form **……...**
7. The outer portion of the tapetum is contributed by the parietal layer reffered as **……**
8. ……of endothecial cells play an important role in the dehiscence of anthers.

5.7.1 Answers Key:

1. Cellulose, 2. Tapetum, 3. *Acacia*, 4. Sporopollenin, 5. Tetrahedral type, 6. 2-celled stage, 7. Periplasmodium

5.7.2 Answers Key:

1. Palynology, 2. Endothecium, 3.Meiosis, 4. Microsporogenesis, 5. Embryo, 6. Pollen grains, 7. P- Tapetum, 8. Hygroscopic nature

5.8 REFERENCES

- Bharti Chaudhry and MR Vijayaraghavan (1995) Structure and Development of Anther, Pollen and Exinal connections in Jojoba (*Simmondsiachinensis*) Proceedings of Indian National Science Academy B61 No3 pp199-208.
- Goldberg R, Beals T, Sanders P (1993) Anther development: basic principles and practical applications. The Plant Cell 5, 1217–1229.
- Teagen D. Quilichini, Carl J. Douglas and A. Lacey Samuels (2014) New views of tapetum ultrastructure and pollen exine development in *Arabidopsis thaliana* Annals of Botany 114: 1189–1201.
- R. J. Scott, M. Spielmanand H. G. Dickinson(2004). Stamen: Structure and Function. The Plant Cell, Vol. 16, S46–S60, (Supplement)

5.9 SUGGESTED READINGS

- S. S.Bhojwani, S. P. Bhatnagar and P. K. Dantu (2014). The Embryology of Angiosperms. 6th ed. New Delhi. Vikas publishing house private limited
- Shivanna K. R (2003). Pollen Biology and Biotechnology 1st ed. New Hampshire, USA, Science Publishers Inc. ISBN 1-57808-241-2(PB)
- B. P., Pandey (2012). Practical Botany Vol. II S. Chand and Company, Pvt. Ltd. Ramnagar, New Delhi- 110055.
- O. P., Sharma (2014). Pragati Practical Botany. Vol. II Pragati Prakashan, Meerut.
- V. Singh, P. C. Pande and D. K. Jain (2015).A Text Book of Botany: Structure Development and Reproduction in Angiosperms Published by Rastogi Publications, Shivaji Road, Merrut.

5.10 TERMINAL QUESTIONS

1. Draw a well-labeled sketch of Flower.
2. Draw three dimensional cut section of anther and describe the procedure.

3. Describe the procedure for the preparation of neat and clean slide of Anther.
4. Draw the T. S. of anther and describe it.
5. Draw the structure of a typical tetrasporangiate anther with well-differentiated wall layers and describe it.

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