



“शिक्षा मानव को बन्धनों से मुक्त करती है और आज के युग में तो यह लोकतंत्र की भावना का आधार भी है। जन्म तथा अन्य कारणों से उत्पन्न जाति एवं वर्तमान विषमताओं को दूर करते हुए मनुष्य को इन सबसे ऊपर उठाती है।”

— इन्दिरा गांधी

“Education is a liberating force, and in our age it is also a democratising force, cutting across the barriers of caste and class, smoothing out inequalities imposed by birth and other circumstances.”

— Indira Gandhi

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PLANT ANATOMY AND EMBRYOLOGY

This practical course is designed to give you hands-on experience of Plant Anatomy and Embryology that you have studied in the theory course. In this course you will study the structural details of plants organs, various tissues and cells present in them, along with developmental and reproductive events related to the formation of a new plant. The course is of 2 credits. You will observe the structural details of plant organs, tissues involved in their growth, development and series of reproductive events noted in plants. The course consists of 14 practicals. These practicals need to be conducted in 8 sessions of 4 hours each.

To ensure successful conduct of these practicals within the prescribed time frame, a brief theoretical background, a list of materials used, procedure that needs to be followed and observations that need to be made will be provided to you. After performing these practical exercises you will be able to provide detailed information about various types of cells, tissues present in various plant organs such as root, stem and leaves, and meristematic tissues involved in their growth and development. You will also be able to enlist series of reproductive events involved in the development of a plant. This course is aimed to develop your observational skills as you will note characteristic features of different cells, tissues and various meristematic zones found in different plant organs.

During hands-on laboratory work one needs to be mentally alert for the best outcome. By the end of this course it is expected that you will get trained and develop the ability to work independently and be confident about the observations you made. It is necessary that you cut some sections of the materials given to you, observe them carefully and note the major observations, analyze them and reach a conclusion. The studies should be repeated to get a valid result. The repetition of the study will strengthen your observation skills and confirm your results. You should spend some time discussing your results with the counsellor. This will help you to reach a conclusion. Besides observing the structural features, you are also expected to see and try to find out something new or interesting in your observations.

You should realise that conducting a practical is an expensive exercise. It also requires participation of counselor or technical staff of the laboratory; therefore, the whole practical session should be taken seriously and utilised fruitfully to develop scientific temperament.

Laboratory exercises are interesting and enjoyable. If you come well prepared with some theoretical knowledge pertaining to the practical's to be conducted, you can utilise the time in the best way. Some of the theoretical background required for undertaking the practical exercises has also been provided to you through the laboratory manual.

Objectives

After completing the exercises, you should be able to :

- ❖ identify various cells and tissues based on their characteristic features by preparing temporary slides and seeing the permanent slides;
- ❖ describe the characteristics of plant organs such as root, stem and leaf;
- ❖ identify the growing tips of stem and root (root and shoot apex) based on your observations of various tissues (meristematic) present in them using permanent slides;
- ❖ provide information about the structural details of the flower (main reproductive part of the plant);

- ❖ provide details about the various agents of pollination after studying them by means of pictures and photographs;
- ❖ describe the minute structural details of the male and female gametophyte by observing the permanent slides;
- ❖ provide structural details of various types of embryos after studying them by means of permanent slides;
- ❖ identify structural details of xerophytic leaf and hydrophytic stem; and
- ❖ dissect a seed to study the location and structure of an embryo and endosperm.

Instruments and other requirements

Before you start the laboratory Course, we suggest you prepare a small laboratory kit which should contain the following:

- a dissecting needle,
- a pair of forceps,
- a clean piece of cloth,
- a brush,
- sharpened pencils,
- an eraser, and
- a lab coat
- Petri dishes
- Razor
- Sanitizer

Along with it a small practical notebook needs to be carried before coming to the laboratory.

Instruments such as microscopes, glass slides, coverslips, stains, and blotting paper will be provided to you from the laboratory.

Laboratory Etiquettes

To attain most knowledge during the laboratory exercises, one needs to be keen, hardworking, and sincere with an analytical frame of mind. The present laboratory course will increase your knowledge about the subject and develop your analytical and observational skills. Some points mentioned below can prove useful to you before you come to the laboratory

- read the laboratory exercise along with the theory given in the course book.
- complete the assigned work within the suggested time frame. This will happen once you make proper planning of the experiment.
- optimum and judicious utilization of the facilities provided to you.

- follow the instructions given in the practical manual as well as the counsellor strictly.
- get your observations notebook checked regularly after completing each experiment.
- handle all the laboratory equipment and materials carefully.
- if your observations are different from the expected ones, try to find out the reasons and discuss the doubts with the counselor.

By following these points, the success and satisfaction will be yours. We wish you best of luck.



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EXERCISE 1

TO STUDY PLANT TISSUES

Structure

- | | | | |
|-----|-------------------|-----|---|
| 1.1 | Introduction | 1.5 | Observations on Simple Permanent Tissues |
| | Objectives | | Parenchyma |
| 1.2 | Study Guide | | Collenchyma |
| 1.3 | Material Required | | Sclerenchyma |
| 1.4 | Procedure | 1.6 | Observations on Complex Permanent Tissues |
| | | | Xylem |
| | | | Phloem |

1.1 INTRODUCTION

Plant organs are made of different types of tissues. These tissues perform important functions: i) they provide the mechanical strength to the plants; ii) conduct water, minerals and nutrients to the various parts of the plant body; and iii) help in metabolism, storage and secretion of enzymes and hormones and secondary metabolites. A plant tissue is a group of cells of common origin. These cells may or may not be similar in shape, size and in function. Based on their development plant tissues fall into two categories, the meristematic tissue, and permanent tissues. The former are immature undifferentiated tissues, and its cells are capable of division as well as of self-perpetuation. Permanent tissues are derived from the meristem by gradual differentiation and they are mature. Their cells do not divide.

Permanent tissues fall into two categories: i) simple permanent tissues; and ii) complex permanent tissues. The former type is composed of similar homogenous cells. The latter type is composed of heterogenous cells and is made of different types of cell elements. In this laboratory exercise, you would be observing the different types of tissues under the microscope and sketch the structure of the tissues in your observation notebook and become familiar with their functions as well.

Objectives

After doing this exercise you would be able to:

- ❖ identify and describe the structure of different types of plant tissues and make sketches of them; and
- ❖ describe the functions of simple and complex plant tissues.

1.2 STUDY GUIDE

For doing satisfactory work you must read the following Units before coming to the laboratory.

The UGC (CBCS) Course -Core Botany, Paper III - Plant Anatomy and Embryology (BBYCT-135), Unit-1.

1.3 MATERIALS REQUIRED

1. Permanent slides of plant tissue showing parenchyma, collenchyma, and sclerenchyma.
2. Permanent slides of plant tissue showing elements of xylem and phloem.
3. Compound microscope.

1.4 PROCEDURE

You will be provided permanent slides of simple and complex tissues. You must observe them carefully and draw them into your notebook and write the comments. Do not copy figures from the book and only draw as you see them in the microscope.

1.5 OBSERVATIONS ON SIMPLE PERMANENT TISSUES

Simple permanent tissues are generally of three types: i) Parenchyma; ii) Collenchyma; and iii) Sclerenchyma. You will observe permanent slides showing each of these tissues.

1.5.1 Parenchyma

Place a parenchyma slide preparation under the microscope and focus it. Observe closely the structure of the parenchyma cells and make a neat diagram in your observation notebook. Record the structural details of the tissues.

You will find:

- The cells are of different shapes and sizes but mainly are circular and isodiametric.
- They may have intercellular spaces (Fig. 1.1) or are compactly arranged.

- Most of the lower plants are formed of parenchymatous cells.
- Meristems are also parenchymatous.
- Epidermis, cortex, pith, mesophyll of leaves, pulp of fleshy fruits and embryonic tissues are composed of parenchyma cells

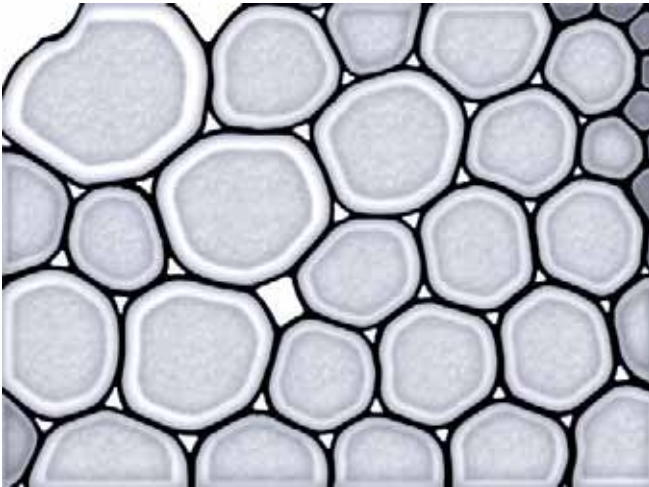


Fig. 1.1: Parenchyma.

- These cells have an active protoplast.
- Parenchyma cells have functions of photosynthesis, storage of food material, secretion, and excretion.
- They occur as part of xylem and phloem and conduct water and nutrients in solution.
- In leaves, parenchyma cells have chloroplasts in them, hence called **chlorenchyma**.
- In aquatic plants, they acquire large air spaces to enable the plants to float in water, hence called **aerenchyma** cells (Fig. 1.2).

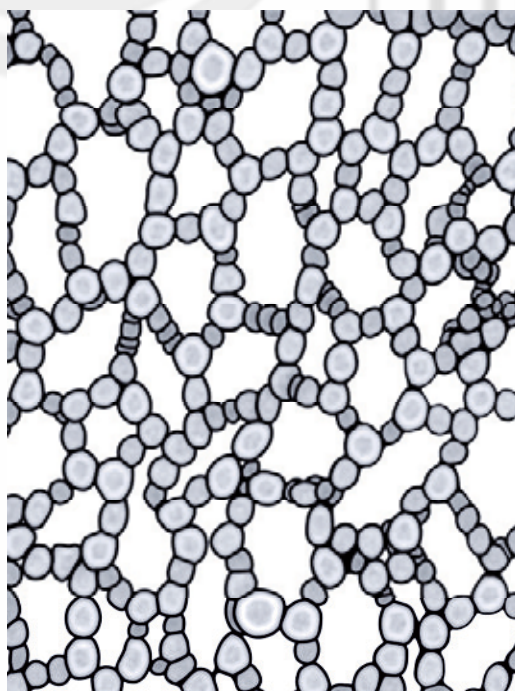


Fig. 1.2: Aerenchyma.

1.5.2 Collenchyma

Observe a permanent preparation of collenchyma tissue under a microscope. Make a neat sketch of the cells in your observation notebook. Record the details of the structure.

You will find:

- Collenchyma cells are circular and isodiametric (in cross section). Some of the cells may also be polygonal in shape.
- The cells wall is thick, more so in corners of the cells with or without intercellular spaces between them (Fig. 1.3).

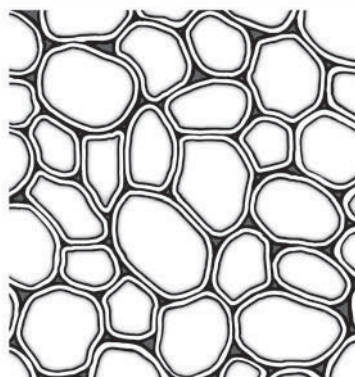


Fig. 1.3: Collenchyma.

- Young stems, petioles of leaves, and stalk of flowers, ribbed stems, and petioles as well as the square stems of certain plants have collenchyma cells.
- Collenchyma is a tissue providing mechanical strength and elasticity to the growing plant organs.

1.5.3 Sclerenchyma

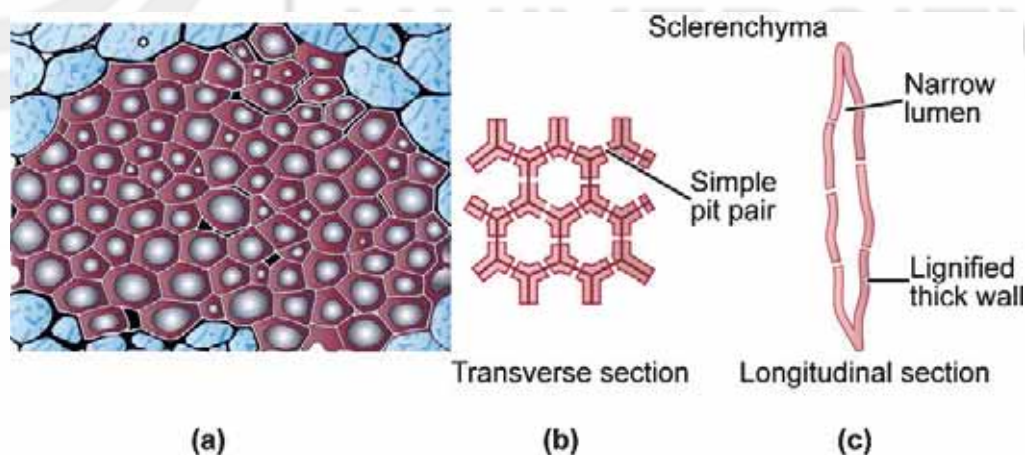


Fig. 1.4: Sclerenchyma fibers a) Seen as a part of vascular bundle; b) T.S. showing pits; and c) L.S. showing narrow lumen.

Observe a preparation of sclerenchyma cells under a compound microscope. Make a neat sketch of the tissue as observed in your record notebook. Write down the structural details.

- These are found in cortex, pericycle, xylem and phloem. They provide mechanical strength to plants.

You will see that the size and shape of sclerenchyma cells is variable and at least two types could be distinguished :

- Elongated cells called **sclerenchyma fibers** (Fig. 1.4 a-c).
- Short cells either isodiametric or irregular in shape with lignified walls called **sclereids** (Fig. 1.5).

Make drawings of both types of cells in your notebook. You will find that sclerenchyma fibers are long cells with pointed needle like ends (Fig. 1.4c).

- They are dead cells with no protoplast in them; and highly thick walled, the thickening is due to a substance called lignin (Fig 1.4 a-c).
- The sclereids are of varied shapes (spherical, oval, or cylindrical). These cells possess lignified secondary cell walls.
- These are also dead cells with no protoplast in them (Fig. 1.5).
- Sclereids are found in cortex, phloem, pith, seed coats and fruit wall.
- They provide mechanical strength to plant parts.

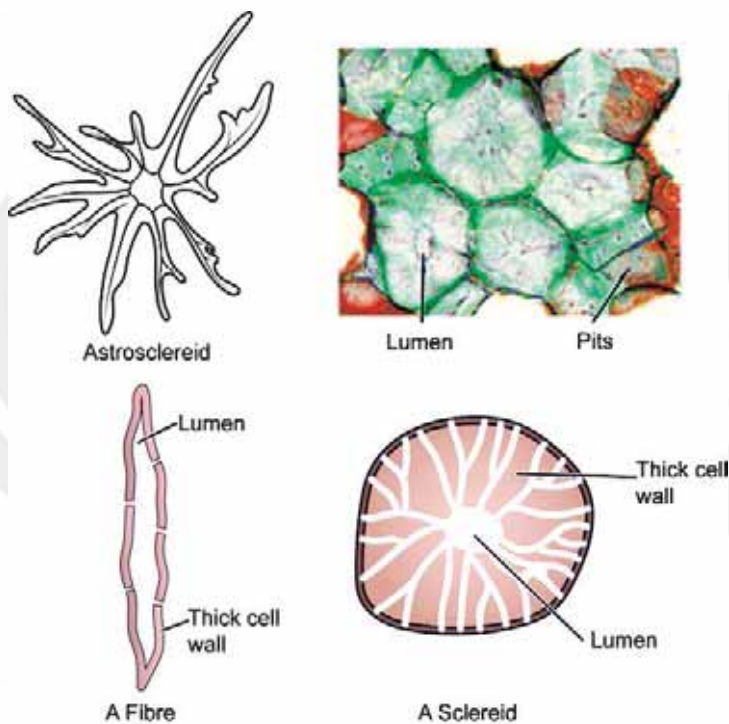


Fig. 1.5: Sclereids.

1.6 OBSERVATIONS ON COMPLEX PERMANENT TISSUES

The cells of complex permanent tissues, though of common origin, are composed of different types of cell elements. These different cellular elements form an integral part of a structure of plant and carry out a specific function. By way of analogy with animal tissues, you may recall that blood is a complex tissue formed of different types of cellular elements. Among plants xylem and phloem are examples of complex tissues. These tissues principally perform

the function of transport of water and nutrients in plants, hence called vascular tissues. The vascular tissues perform a function like the ones performed by the blood vascular system in animals.

1.6.1 Xylem

Xylem is a complex tissue forming a part of vascular system. Its major function is conduction of water and solutes, mainly minerals. It also provides mechanical support to plants. Since it is a complex tissue it consists of different types of cells and elements. The cell elements that form xylem are a) tracheids; b) vessels; c) xylem fibers; and d) xylem parenchyma.

Place a permanent slide preparation of xylem under a microscope and observe the various elements:

a) Tracheids

- A tracheid is an elongated cell (Fig. 1.6).
- The cells are devoid of any protoplast; hence they are dead cells.
- The tracheids have a lumen without any contents in them. The walls of tracheids are thicker, secondary, and lignified.
- Depending on the type of thickenings, tracheary elements are classified into annular (ring like thickenings), spiral, reticulate (the walls present a network like appearance), scalariform (ladder like) and pitted (with holes) (Fig.1.6).

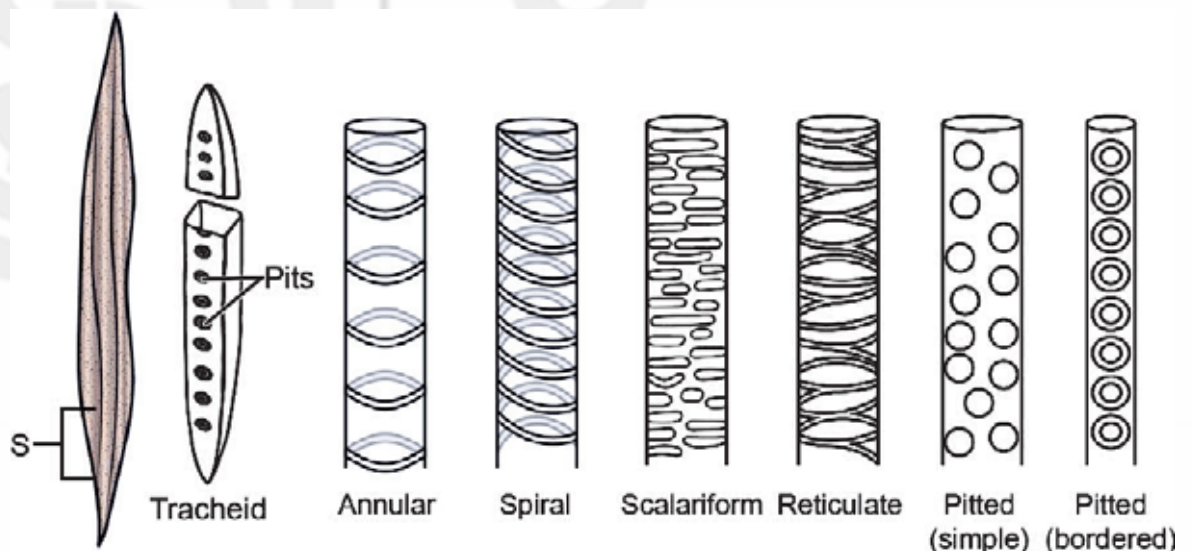


Fig. 1.6: Types of Tracheary elements.

b) Vessels

- These are long tube-like bodies which conduct water and solutes (Fig. 1.7).
- A trachea is a vessel formed from a row of cylindrical cells arranged in longitudinal series.
- The partition walls between the cells are perforated so that the entire structure is a long continuous vessel. These partitions are called perforation plates.

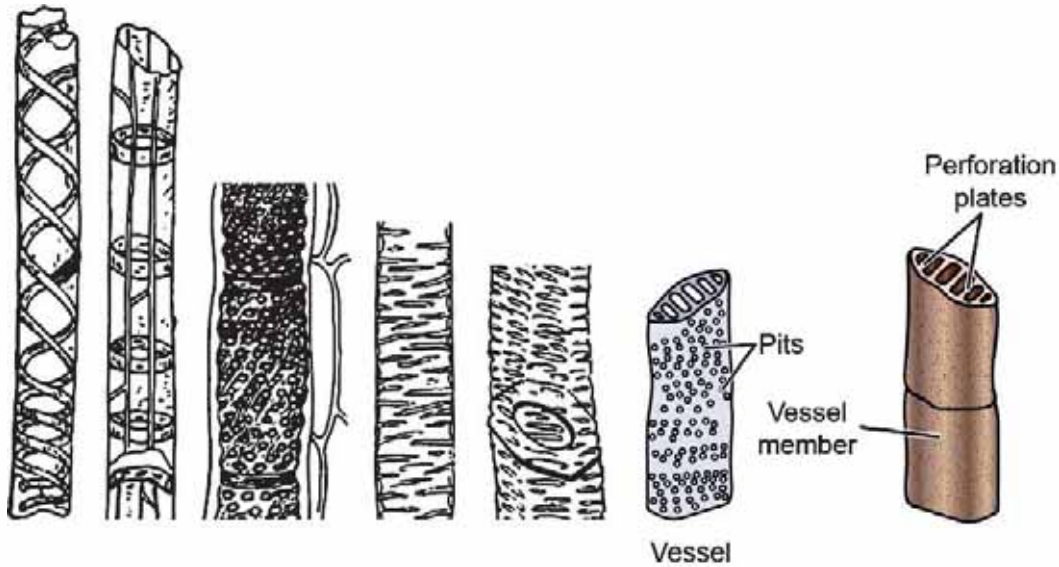


Fig. 1.7: Types of xylem vessels.

c) Xylem Fibers

These are the dead cells which provide mechanical support to the plant. They are long cells with lignified walls tapering at both ends (Fig. 1.8).



Fig. 1.8: A wood fiber (Xylem sclerenchyma).

d) Xylem Parenchyma

- These are the only living component of the xylem of most plants.
- Parenchyma is abundant in the secondary xylem of most plants. The cells may be thin walled or thick walled (Fig. 1.9).
- The cells have a storage function, mostly starch and fatty substances are stored.

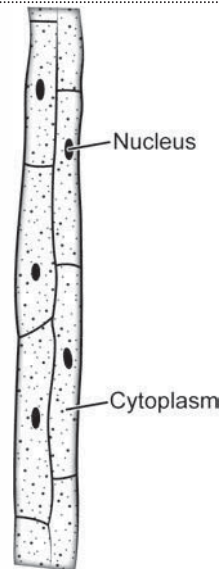


Fig. 1.9: Xylem parenchyma.

1.6.2 Phloem

Observe a permanent slide preparation of phloem under the microscope. You will see that the phloem is a complex tissue composed of four elements: a) sieve elements; b) companion cells; c) Phloem Parenchyma; d) Phloem fibers.

a) Sieve elements

These are the most important elements of phloem. You will be amazed to know that these cells though living are without functional nucleus at maturity. A sieve element consists of sieve tubes. Sieve tubes are cells arranged in a longitudinal series with perforations in the cell wall, called sieve plate (Fig. 1.10). The individual members of a sieve-tube are called sieve-tube members or sieve-tube elements. Therefore, in the sieve plate cytoplasmic connections are established between neighboring cells. The cytoplasmic connections are formed at the middle lamellar region of two adjacent sieve tube elements. These constitute sieve pores. A sieve plate may consist of one or many sieve pores. Sieve tubes have a conducting function.

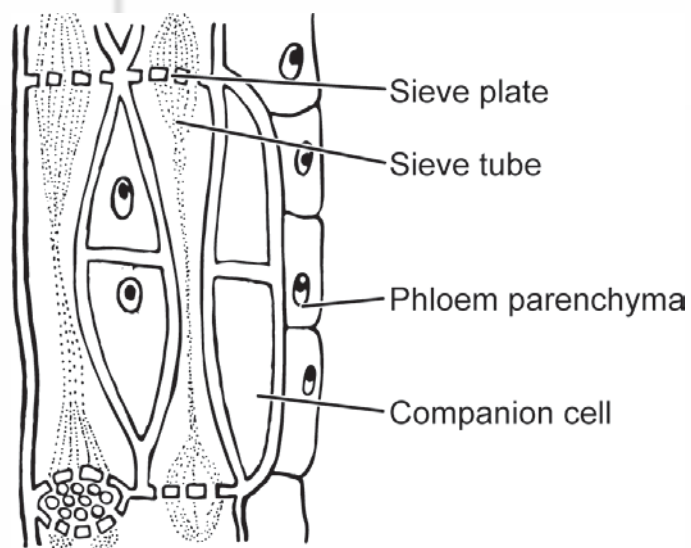


Fig. 1.10: Phloem tissue showing sieve elements, companion cells and phloem parenchyma.

Sieve elements at maturity are conspicuously devoid of a functional nucleus and organized protoplasm.

b) Companion Cells

As their name suggests, the companion cells are closely associated with sieve tubes of flowering plants both during development as well as during functioning. They are small elongate cells with dense cytoplasm and prominent nuclei (Fig. 1.11). They occur on the lateral walls of sieve tubes. Companion cells accompanying a sieve tube may be a single cell of equal length or a mother cell may be divided transversely forming a series of companion cells. Sieve tubes and companion cells originate from the same mother cell. The companion cells function if sieve tubes are functional. The companion cells are firmly attached to sieve tubes.

c) Phloem Parenchyma

These living cells are also associated with sieve elements. They are living cells with protoplasm in them (Fig. 1.11). They are concerned with storage of organic food materials.

d) Phloem Fibers

These are sclerenchymatous cells. These are dead elongated cells with lignified walls having single pits. The fibers are of commercial importance as they are used for the manufacture of ropes and cords.

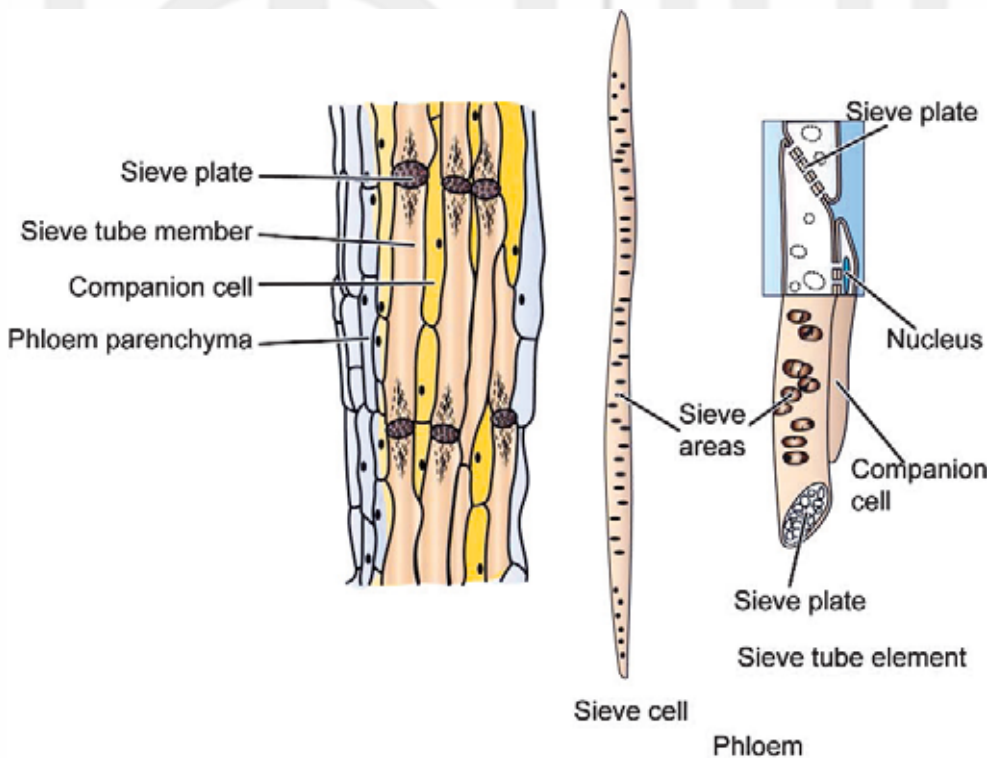


Fig. 1.11: Companion cells and Phloem parenchyma.

EXERCISE 2

TO STUDY PRIMARY MERISTEMS

Structure

2.1	Introduction	2.4	Procedure
	Objectives	2.5	Observations
2.2	Study Guide		L.S. Shoot apex
2.3	Material Required		L.S. Root apex

2.1 INTRODUCTION

In previous exercise you have studied that most of the higher plants possess a complex internal structure which is composed of different types of tissues. Groups or masses of cells which are structurally similar or dissimilar, have a common origin perform or help to perform a common function are called a **tissue**. Each tissue performs a specific function. Different types of tissue function in coordination with one another in a plant body.

Meristematic tissue are composed of immature cells that are either preparing to divide or are in a state of division or retain the power of division. The term meristem (Gr. *Meristos* = divisible) was coined by **C. Nageli** (1858).

Based on ability of cell multiplication, tissues are grouped into *two* principal types:

1. Meristems or Meristematic Tissue, and
2. Permanent Tissue which you have read in previous exercise.

In this exercise, you will study the meristematic tissue with the help of permanent slides.

Objectives

After observing the permanent slides of various meristematic tissues, you would be able to:

- ❖ distinguish between the shoot and the root apex;

- ❖ know the position of various derivatives of the meristematic tissue in the shoot and root apex; and
- ❖ describe the characteristic features of each of the components of the shoot and root apex.

2.2 STUDY GUIDE

For doing satisfactory work you must read the following Units before coming to the laboratory.

The UGC (CBCS) Course-Core Botany, Paper III - Plant Anatomy and Embryology (BBYCT-135), Unit 2.

2.3 MATERIALS REQUIRED

Permanent slides of :

1. L.S. of shoot apex.
2. L.S. of root apex.
3. Photographs of L.S. shoot and root apex.
4. Compound Microscope

2.4 PROCEDURE

You will be provided permanent slides of L.S. of root and shoot apices. You must observe them carefully under the compound microscope, both under low and high power. In addition, you will also be provided with photomicrographs of the same. Make neat, well- labelled diagrams (both outline and a portion enlarged to show the cellular details) in your notebook and write comments. Do not copy figures from the book and only draw as you see them in the microscope or in the photograph.

2.5 OBSERVATIONS

In this exercise in the slides provided to you will see that meristematic cells show the following characteristic features:

- Composed of immature cells present in a state of division and growth.
- Cells are round, oval, polygonal, or rectangular.
- Possess thin cellulosic primary cell walls with numerous plasmodesmata.
- No intercellular spaces are found.
- Presence of abundant cytoplasm with one to many nuclei, and very small vacuoles.

Meristematic tissue are classified based on i) mode of origin and development; ii) Position in the plant body; and iii) Function.

Based on the position in the plant body, the meristems are classified into i) apical meristem; ii) lateral meristem; and iii) intercalary meristem (Fig. 2.1).

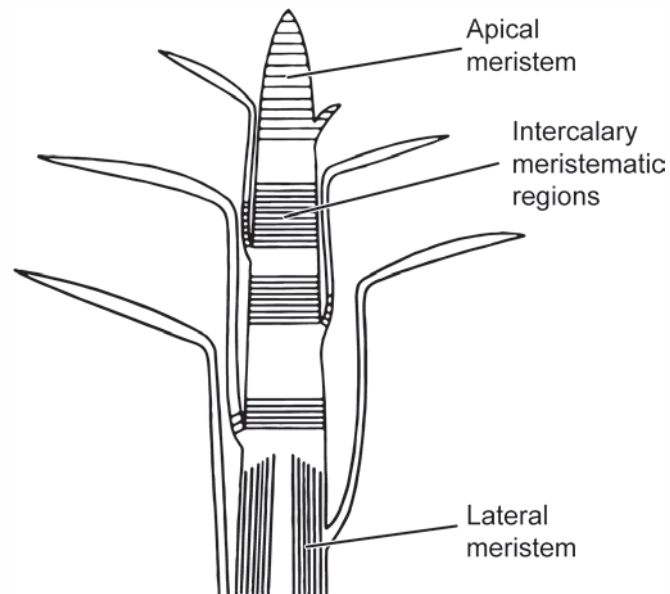


Fig. 2.1: A schematic diagram indicating the position of three types of meristems in a longitudinal section.

You will observe the anatomical features in the slide of L.S. of shoot apex:

2.5.1 LS. Of Shoot Apex

- The shoot apex appears to be hemispherical in a longitudinal section.
- An apical promeristem consists of two zones, **tunica**, and **corpus**.
- Tunica forms the outermost covering, and it is one to two layered.
- Cells of the tunica divide **anticlinally** (perpendicular to the shoot) and increase the surface area of the meristem.
- Immediately below the tunica lies the core region called corpus. This zone has larger cells which divide both anticlinally and periclinally (parallel to the shoot), thus dividing in all the planes and contribute to the depth and mass of the inner tissues (Fig. 2.2).

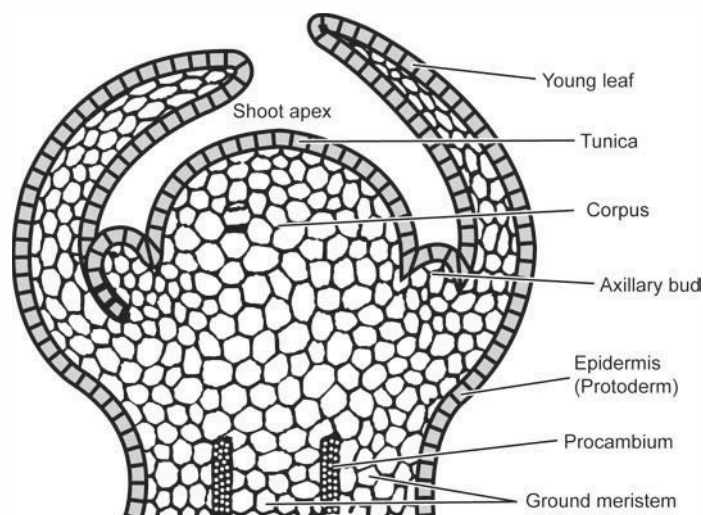


Fig 2.2: L.S. of a vegetative shoot apex.

- Activity in corpus raises the height of the plant.
- Corpus contributes to the formation of internal structures to the stem and leaves.
- **Rib meristem** is present just below the corpus and is in the form of cells arranged in regular files (Fig. 2.3 a, b). Rib meristem gives rise to the pith.
- Cells of the peripheral region outside the rib meristem constitute the **flank meristem** (Fig. 2.3 a, b). These cells are actively dividing and give rise to lateral organs like leaves and branches.

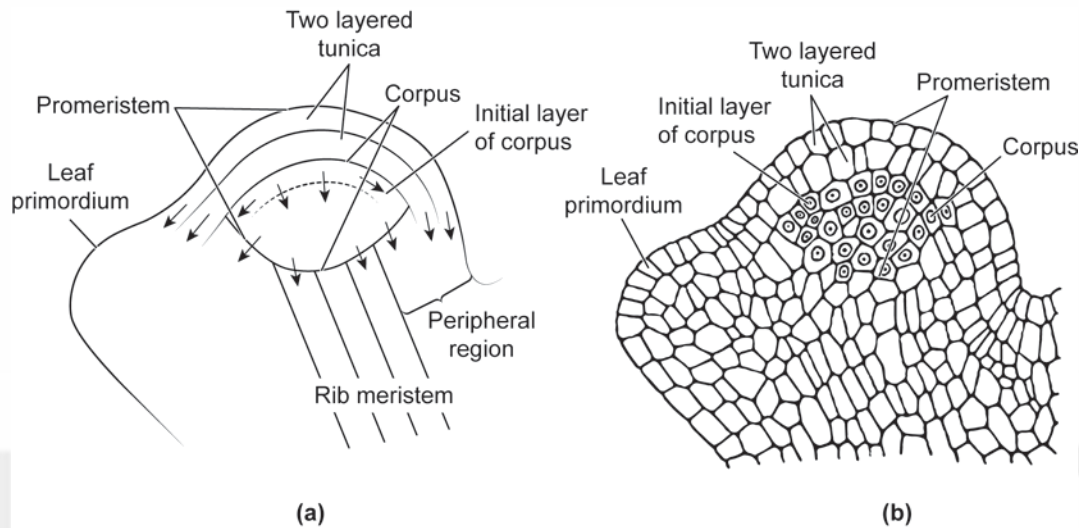


Fig: 2.3: LS shoot apex. a) Outline diagram showing different regions; b) Cellular diagram highlights the tunica and corpus.

2.5.2 L.S. of Root apex

You will observe the following features in the slide on T.S. of root apex (Fig. 2.4 a, b):

- In the slide you will see the L.S of the root seems to be gradually tapering.
- The root apical meristem is less complex than that of the shoot (Fig. 2.4).

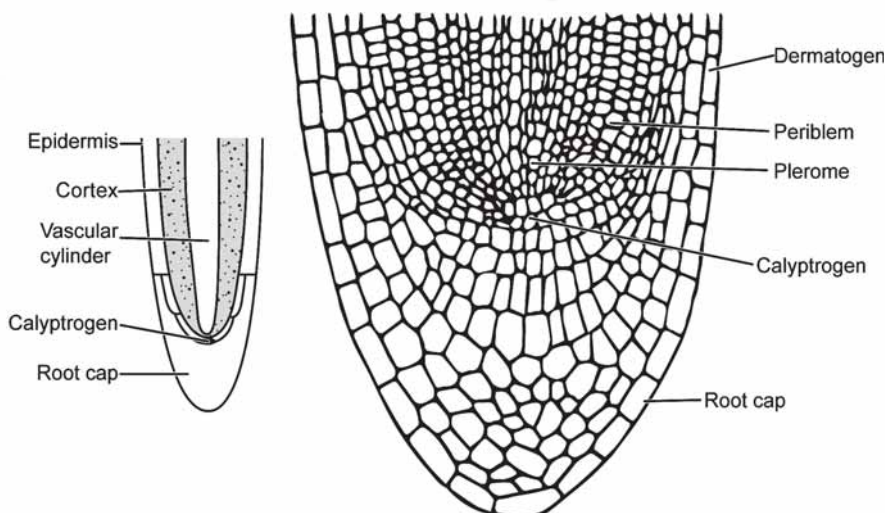


Fig. 2.4: L.S. Root Apex a) An outline diagram highlighting different regions; b) A portion enlarged to show cellular details.

- A root cap protects the root tip and helps in gravitropism of roots. It generally consists of 3-4 layers of cells. The root cap secretes a mucilaginous polysaccharide mucigel.
- The apical initials in the root constituting a central cup like region of passive cells lying between the root cap and active root meristem is called a **quiescent center**, which serves as a reservoir
- Closer to the root apex lies a specialized zone **calyptrogen** which gives rise to the root cap tissue.
- The sub terminal root apex lies inside the calyptrogen. Cells of the root apex form various root tissue.
- Root apex shows three distinct zones-**plerome**, **periblem** and **dermatogen**.
- Dermatogen is single layered and gives rise to the outer layer of epiblema.
- The cortex of the root is contributed by the periblem. Periblem can be many layered.
- Plerome is the innermost zone which differentiates into pericycle, medullary rays, pith, and the vascular bundles.

Intercalary Meristem

As the name suggests, this type of meristem is located at the base of the internode. It is separated from both the apical and the lateral meristems. Intercalary meristem is present in grasses and some families of dicots. Since the derivatives of this meristem add permanent tissue in basipetal succession, its activity results in stem elongation.

EXERCISE 3

TO STUDY THE INTERNAL STRUCTURE OF MONOCOT AND DICOT STEM

Structure

3.1	Introduction	3.5	Observations
	Objectives		Study of T.S. of Monocot Stem- <i>Zea mays</i>
3.2	Study Guide		Study of T.S. of Dicot Stem - <i>Helianthus</i>
3.3	Material Required		
3.4	Procedure		

3.1 INTRODUCTION

You have already studied in Unit-4 that the stem is usually an aerial part of the plant axis which bears leaves and reproductive bodies. Stem is one of the most important organs of the plant. The stem comprises three tissue systems, viz., epidermal, ground and the vascular system. In monocot stem and dicot stem you will observe sharp differences in their internal as well as external structures. Among the differences between in monocot and dicot stem major one is orientation of vascular bundles which you will observe in the TS.

In this exercise, you will study the internal structure of a monocot (*Zea mays*) and a dicot stem (*Helianthus*).

Objectives

After observing the permanent slides of *Zea mays* and *Helianthus*, you would be able to:

- ❖ identify the position of various tissues in a typical monocot and a dicot stem;
- ❖ appreciate the structure of a collateral, conjoint, open as well as closed vascular bundles, and
- ❖ know the typical Y-shaped arrangement of the xylem elements in a monocot stem.

3.2 STUDY GUIDE

For doing satisfactory work you must read the following Units before coming to the laboratory.

The UGC (CBCS) Course -Core Botany-, Paper III - Plant Anatomy and Embryology (BBYCT-135), Unit 4.

1.3 MATERIALS REQUIRED

1. Compound Microscope
2. Permanent slides of:
 - a) T.S. of *Zea mays* stem
 - b) T.S. of *Helianthus* stem (young)

3.4 PROCEDURE

You will be provided permanent slides of T.S. of monocot and dicot stem. You must observe them carefully under the compound microscope, both under low and high power. Make neat, well-labelled diagrams (both outline and a portion enlarged to show the cellular details) in your notebook and write comments. Do not copy figures from the book and only draw as you see them in the microscope.

3.5 OBSERVATIONS

3.5.1 Study of T.S. of Monocot Stem of *Zea mays*

You will observe the following features in the transverse section of *Zea mays* stem (beginning from the periphery):

The transverse section appears almost circular in outline.

Epidermis

- It is made up of single layer of cells with their outer walls covered over with a thick cuticle.
- Few stomata are present but multicellular hairs are absent.

Hypodermis

- There are two to four layers of sclerenchyma below the epidermis.
- Intercellular spaces are absent in this tissue.
- Sclerenchyma provides mechanical support to plant.

Ground tissue

- The entire mass of parenchymatous cells from below the hypodermis to the center forms ground tissue. Many intercellular spaces are present.

- There is no differentiation between cortex, endodermis, pericycle and pith.
- The cells contain reserve food materials.
- Cells of the peripheral ground tissue are smaller, polygonal, and compactly arranged while they become bigger, rounded, and loosely arranged, towards the center.

Vascular Bundles

- Vascular bundles are embedded and scattered within the ground tissue (Fig. 3.1).

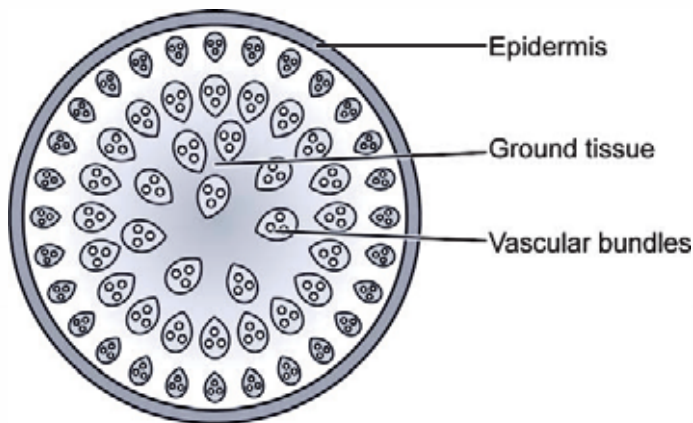


Fig. 3.1: Diagrammatic representation of T.S. of young stem.

- Vascular bundles are oval shaped.
- Vascular bundles are conjoint, collateral, endarch and closed.
- Each vascular bundle is surrounded by few layers of sclerenchymatous cells called **bundle sheath** (Fig. 3.2).
- Bundle sheath is prominent toward the upper and the lower margins of the bundle.

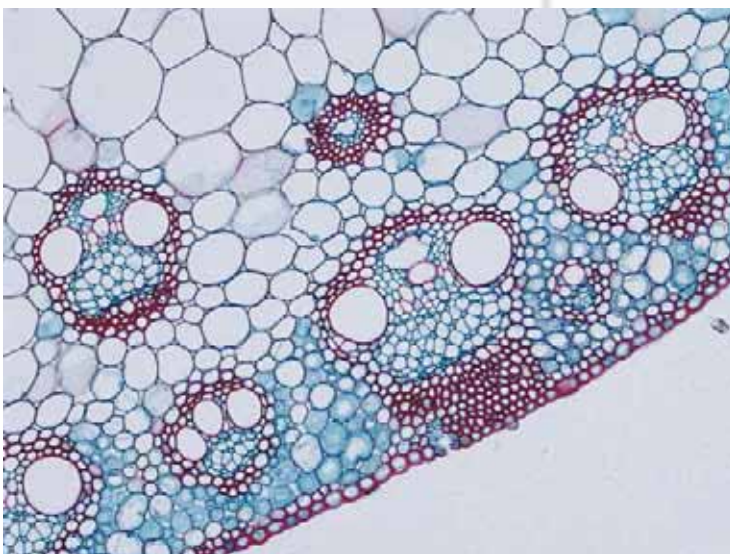


Fig. 3.2: A section enlarged of T.S. *Zea mays*.

a) Xylem

- It consists of vessels, tracheids and xylem parenchyma.
- Metaxylem and protoxylem are present. They are arranged like a **Y**. Two big metaxylem vessels with pitted thickenings represent the divergent ends of the Y.
- The protoxylem is positioned radially towards the center representing the long arm of the Y. The protoxylem consists of two smaller vessels.
- In mature vascular bundle, the lower most protoxylem element disintegrates and form a cavity called **lysigenous cavity** or **protoxylem lacuna**. This cavity is filled with water and is surrounded by parenchymatous cells (Fig. 3.3).

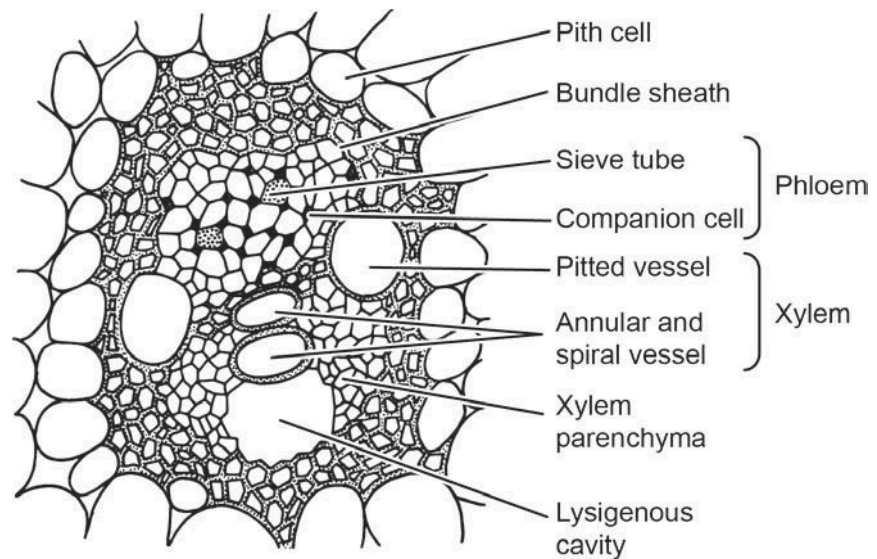


Fig. 3.3: Detailed structure of a vascular bundle of *Zea mays*.

b) Phloem

- Positioned above the xylem.
- Consists of sieve tube elements and companion cells and phloem parenchyma. Phloem fibers are absent.
- A small band of **crushed**, obliterated phloem occurs near the periphery of the bundle which represents **protophloem**.
- **Metaphloem** is the **functional** part and it lie just below protophloem and extends up to Y- shaped xylem, consisting of very prominent sieve tubes and companion cells (Figs. 3.2, 3.3).

Pith

- Pith is not differentiated

Identification

- 1) **Stem:** Vascular bundles are conjoint, collateral and endarch.
- 2) **Monocotyledonous stem.**

- Endodermis and pericycle absent.

- Cortex undifferentiated. Ground tissue present.
- Closed vascular bundles (cambium absent); numerous and scattered.
- A prominent bundle sheath present.

3.5.2 Study of T.S. of Dicot Stem of *Helianthus*

You will observe the following features in the transverse section of a dicot stem - *Helianthus annuus* (beginning from the periphery):

The transverse section appears almost circular in outline.

1) Epidermis

- It is the outermost layer.
- Made up of single layer of parenchyma cells.
- Outer wall is covered with cuticle.
- Epidermis is protective in function.
- Presence of multicellular hair and stomata.

2) Cortex

- Positioned below the epidermis.
- Many layered.
- Can be divided into three regions:

a) Outer Cortex

- Consists of 3 - 5 layers of collenchyma cells. Intercellular spaces present.
- Corners of the cells show thickenings (cellulose impregnated with pectin).
- Hypodermis provides mechanical support to the stem.
- Cells of the outer layers could be chlorenchymatous.

b) Middle cortex

- Made up of few layers of chlorenchymatous cells.
- It is involved in photosynthesis.

c) Inner cortex

- Made up of few layers of parenchymatous cells.
- Intercellular spaces present.
- Helps in gaseous exchange and storage of reserve food materials.

3) Endodermis

- Innermost layer of cortex delimiting it from the stele.
- Consists of a single layer of barrel shaped cells, which store starch grains. So, it is also called **starch sheath**.
- Casparian strips clearly visible.

4) Stele

- Central part of the stem inner to endodermis.
- Consists of pericycle, vascular bundles and pith.

a) Pericycle

- Occurs between vascular bundle and endodermis.
- Multilayered.
- Alternating patches of sclerenchyma and sclerenchyma present. Sclerenchyma positioned between endodermis and phloem of the vascular bundles, parenchyma present above the medullary rays.

b) Vascular Bundles

- Arranged in the form of a ring around the pith (Fig. 3.4 a).
- Conjoint, collateral, endarch and open (fascicular vascular cambium present) (Fig. 3.4 b).
- Cambium in the form of a thin strip of single layer rectangular, thin walled of cells extending between xylem and phloem in between metaxylem and metaphloem.

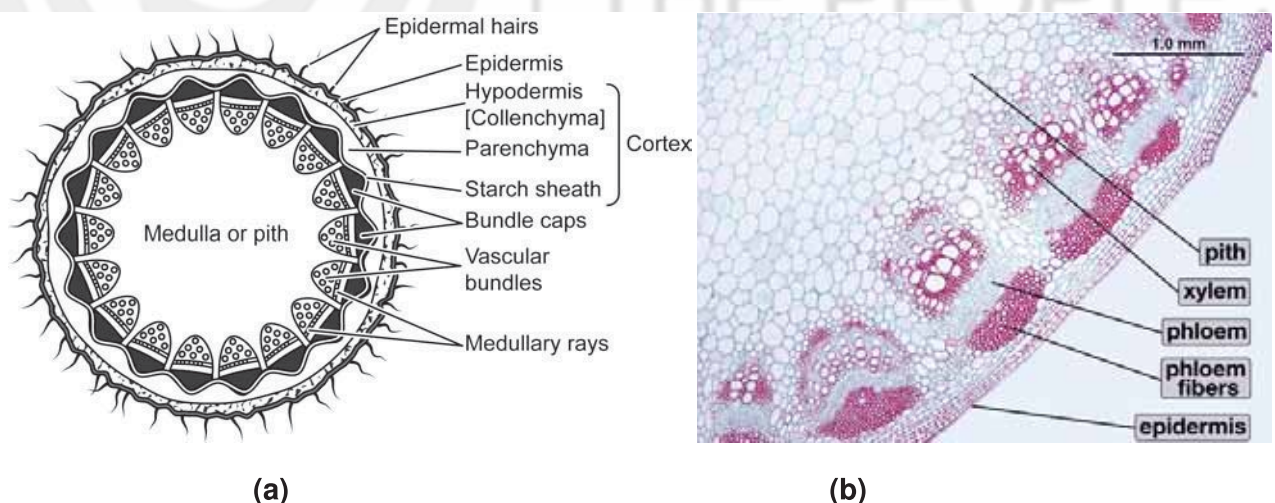


Fig. 3.4: a) Outline diagram of TS of sunflower stem; b) A section enlarged to show cellular details.

- Xylem situated towards the inner side of each vascular bundle. Consists of xylem vessels, tracheids, xylem fibers and xylem parenchyma.
- Phloem present below the sclerenchyma patches of pericycle. Consists of sieve tubes, companion cells and phloem parenchyma

c) Pith

- Large, extensively developed central parenchymatous zone .
- Thin walled cells with intercellular spaces.
- Helps in the storage of food materials.
- Identification

1. Stem.

Vascular bundles conjoint, collateral and endarch.

2. Dicotyledonous stem.

- a. Well differentiated epidermis, cortex, and vascular tissue,
- b. Vascular bundles conjoint, collateral, open; arranged in a ring.
- c. Well developed pith.

Acknowledgements

Fig 3.2 : https://live.staticflickr.com/4066/4426026912_ea91128f35_b.jpg

Fig. 3.4 b : <https://i.pinimg.com/originals/8f/0b/db/8f0bdb60380d51aeb296aba9f825b2cf.jpg>

EXERCISE 4

TO STUDY THE INTERNAL STRUCTURE OF A MONOCOT AND DICOT ROOT

Structure

4.1	Introduction	4.4	Procedure
	Objectives	4.5	Observations
4.2	Study Guide		Monocot Root
4.3	Material Required		Dicot Root

4.1 INTRODUCTION

You have already studied in Unit-3 that the internal structure of root shows many differences from that of the stem. Following are some of the distinguishing features of root anatomy which will help you to identify it in a transverse section: The root is lined by an epidermis from which arises short lived unicellular hair while stem has multicellular hair. Roots do not possess hypodermis whereas it may be collenchymatous or sclerenchymatous in stems. Distinct endodermis and pericycle layers are present in roots. In the other hand they are not very distinct in stems. In roots the vascular bundles are radial, closed, with exarch xylem. The vascular bundles are conjoint, collateral in stems. The protoxylem is exarch in roots while in stems it is endarch.

Monocot and dicot roots can be distinguished by some features .The pericycle in dicot roots will give rise to secondary roots and cork cambium whereas only lateral roots arise from the pericycle in a monocot root. Also, the vascular bundles are diarch to hexarch in dicot roots as against polyarch condition in monocot roots. Another typical difference is that of cambium which is absent in monocot but present in dicot roots.

In this exercise, you will study the internal structure of a monocot (*Zea mays*) and a dicot (*Helianthus*) root.

Objectives

After observing the permanent slides of *Zea mays* and *Helianthus*, you would be able to:

- ❖ identify the position of various tissues in a typical monocot and a dicot root;
- ❖ appreciate the structure of diarch to polyarch, radial, collateral, open as well as closed vascular bundles; and
- ❖ know the formation of cambial ring, the products of vascular and cork cambium in the dicot roots.

4.2 STUDY GUIDE

For doing satisfactory work you must read the following Units before coming to the laboratory.

The UGC (CBCS) Course-Core Botany, Paper III - Plant Anatomy and Embryology (BBYCT-135), Unit-3.

4.3 MATERIALS REQUIRED

You will be provided with the following permanent slides:

1. T.S. of *Zea mays* root.
2. T.S. of *Helianthus* root.
3. T.S. of old *Helianthus* root showing secondary growth; and
4. Compound microscope

4.4 PROCEDURE

- Observe all the given permanent slides carefully under the compound microscope, both under low and high power.
- Make neat, well-labelled diagrams (both outline and a portion enlarged to show the cellular details) and write comments.
- Do not copy the diagrams from the book.

4.5 OBSERVATIONS

4.5.1 Monocot Root

The transverse section appears almost circular in outline. You will observe the following features in the transverse section of *Zea mays* root (Fig. 4.1 a, b) (beginning from the periphery):

Epiblema

- Root epidermis single-layered. Also called piliferous layer.

- Cuticle absent.
- Cells barrel shaped.
- Unicellular hair emerge from few cells.

Cortex

- Occupies a large part of the root.
- Several layers deep.
- Cells thin walled and parenchymatous.
- Many intercellular spaces present in cortex.
- An old root shows suberisation in a few outer layers of cortex resulting in thick collenchymatous zone called exodermis.

Endodermis

- Innermost layer of cortex.
- Separates underlying vascular tissue from the cortex.
- Forms a definite ring around the stele.
- Consists of many compactly arranged, barrel- shaped cells.
- Casparian strips present on the radial and transverse walls.
- Some thin-walled endodermal cells called **passage cells** lay opposite to protoxylem.

Pericycle

- Present inner to the endodermis.
- Cells thin walled and form a complete ring.

Vascular Tissue

- Composed of alternating strands of phloem and xylem located on alternate radii.
- Vascular bundles radial, exarch and polyarch.
- Xylem elements consist of vessels, tracheids and xylem parenchyma.
- Protoxylem located close to the pericycle; cells small in diameter and their walls show annular or spiral thickenings.
- Metaxylem vessels face towards the center; have larger diameter.
- Innermost metaxylem vessel very large and spherical or oval.
- Metaxylem shows reticulate pittings.
- Phloem consists of sieve tubes and companion cells.
- A thick-walled, parenchymatous **conjunctive tissue** present in between the vascular bundles.

Pith

- Occurs in the center.
- Cells parenchymatous with many intercellular spaces.
- Pith cells may sometimes become thick walled and lignified.

Identification

a) Root

- Unicellular hair.
- Vascular bundles radial and exarch.
- Cortex is massive and undifferentiated.

b) Monocotyledonous root

- Polyarch condition in xylem.
- Cortex undifferentiated. Ground tissue present.
- Pith well differentiated.
- Secondary growth is absent.

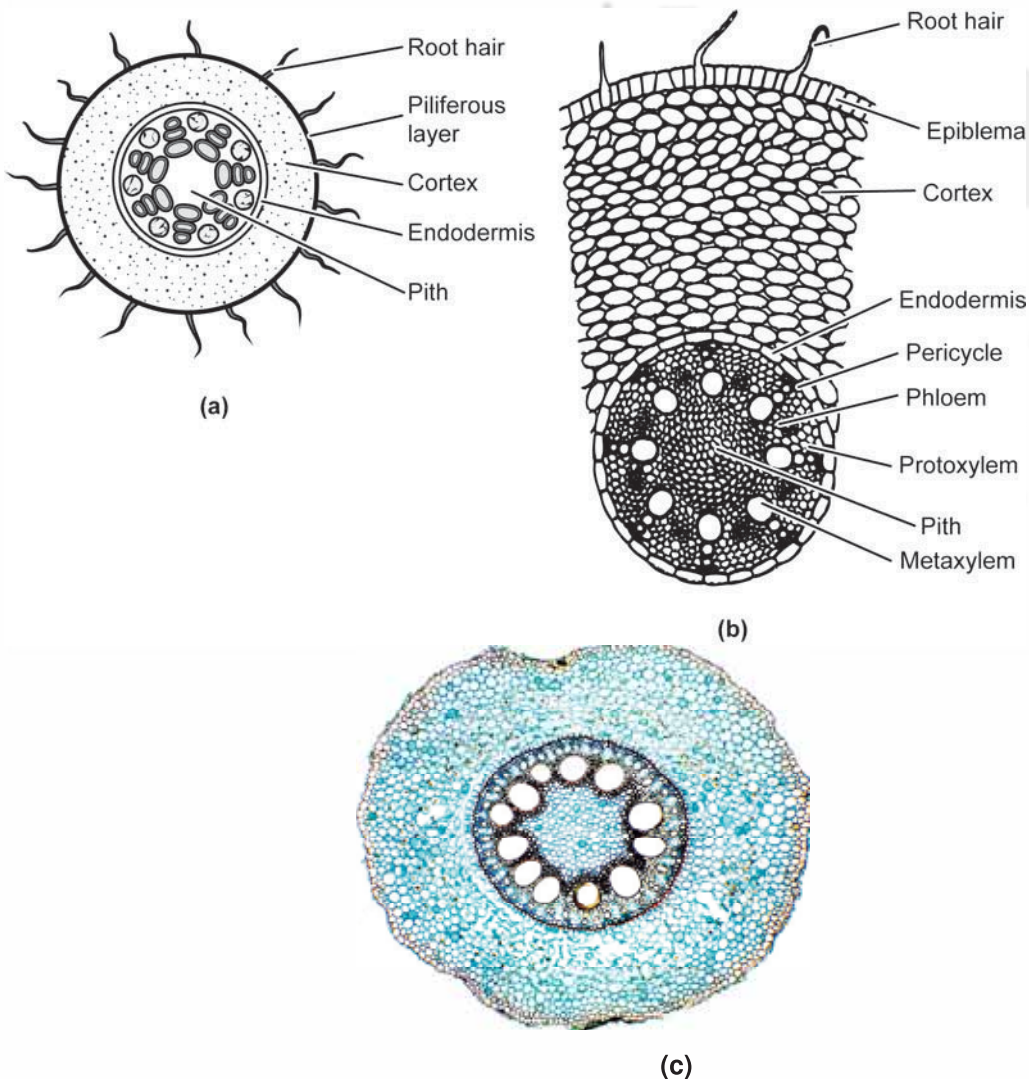


Fig. 4.1: T.S. of monocot root (*Zea mays*). a) An outline diagram; b) an enlarged portion showing the cellular details; c) a stained sectional view.

4.5.2 Dicot Root

The transverse section appears almost circular in outline. You will observe the following features in the transverse section of *Helianthus annuus* root (Figs. 4.2 a, b) (beginning from the periphery):

The T.S shows the following structures:

Epiblema or Epidermis

- Densely arranged cells with thin cell walls forming the outermost single layer.
- Several cells have thin outgrowths known as root hairs.

Cortex

- Present under the epidermis consisting of several layers of thin-walled parenchyma cells.

Endodermis

- They are barrel-shaped cell forming the innermost layer of the cortex. They are densely arranged ,single cell layer.
- A Casparian band is formed due to the radially arranged cell walls that are thickened.

Pericycle

- Under the endodermis is a thin-walled layer of cells known as pericycle.

Vascular bundles

- It consists of radial bundles having alternating 2 to 6 arrangement of bundles of phloem and xylem.
- Bundles of xylem are exarch wherein the metaxylem is present towards the center and the protoxylem at the outer side.
- In outline, xylem vessels are polygonal.

Phloem bundles

- Comprises of companion cells, parenchyma, and sieve tube.

Conjunctive tissue

- They are the parenchyma cells that separate the xylem and phloem bundles from each other.

Pith

- They are significantly absent or occur rarely.

Identification

a) Root

- Unicellular hair.
- Vascular bundles radial and exarch.
- Cortex is massive and undifferentiated.

b) Dicot Root

- The epidermis shows the presence of unicellular hair.
- Absence of hypodermis.
- Radially arranged vascular bundles.
- The number of phloem/xylem bundles is not more than 6.
- The xylem is exarch.
- Absence or rare occurrence of pith.

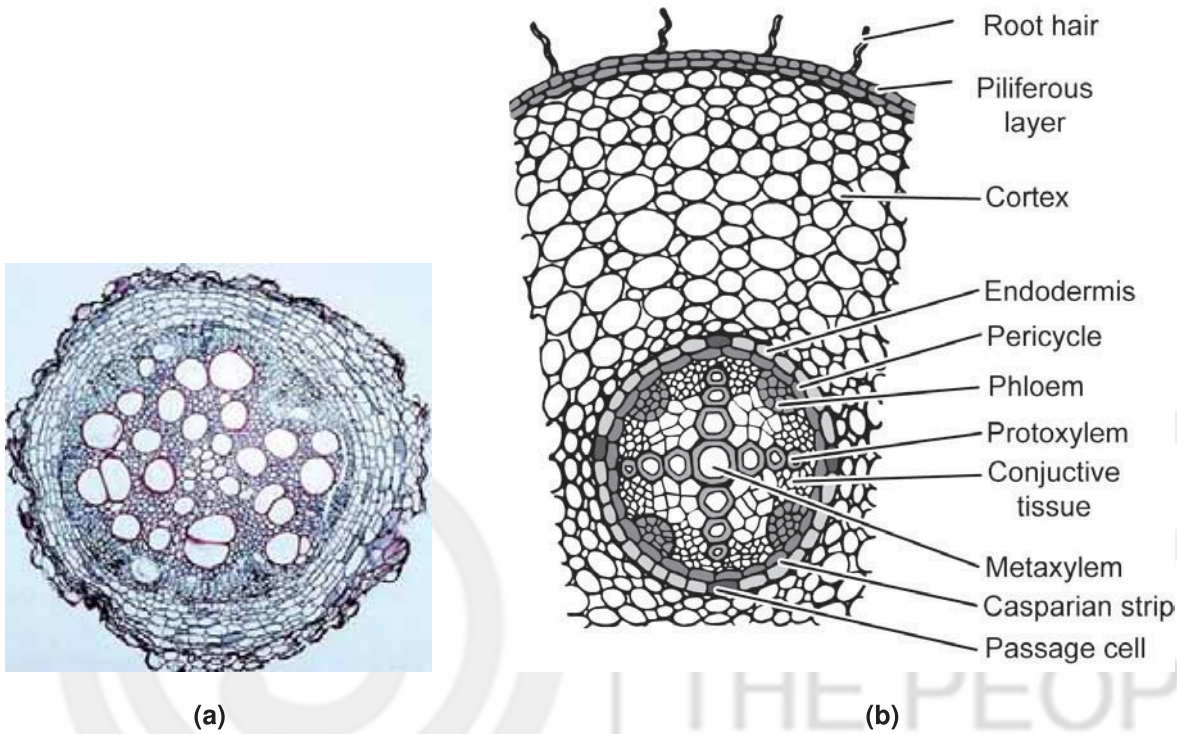


Fig. 4.2: T.S. of a dicot root (*Helianthus*). a) Stained sectional view; b) An enlarged portion showing cellular details of dicot root.

Acknowledgement

Fig. 4.1 c <https://imgc.allpostersimages.com/img/print/u-g Q10H9QA0.jpg?w=550&h=550&p=0>

Fig. 4.2 a : <https://www.google.com/imgres?imgurl=https%3A%2F%2Fi.pinimg.com>

EXERCISE 5

TO STUDY THE INTERNAL STRUCTURE OF MONOCOT AND DICOT LEAF

Structure

5.1	Introduction	5.5	Observations
	Objectives		Study of T.S. of isobilateral leaf of <i>Zea mays</i>
5.2	Study Guide		Study of T.S. of dorsiventral leaf of <i>Helianthus annuus</i>
5.3	Material Required		
5.4	Procedure		

5.1 INTRODUCTION

You have already studied in Unit-5 that the internal structure of leaf shows many differences from that of the stem. Each leaf possesses photosynthetic tissue which is positioned between the upper and the lower epidermis. There is a vascular tissue and the xylem always faces towards the upper epidermis and the phloem towards the lower one. Two types of leaves, viz., dorsiventral (mostly found in dicots) and isobilateral (mostly found in monocots) vary in their internal structure. These leaves are also referred to as unifacial and bifacial leaves, respectively. In the present exercise, you will study the characteristic anatomical features of a monocot isobilateral leaf (*Zea mays*) and a dicot dorsiventral leaf (*Helianthus*) with the help of permanent slides.

Objectives

After observing the permanent slides of T.S. of leaves of *Zea mays* and *Helianthus*, you would be able to:

- ❖ know the position of various tissues in a typical isobilateral and a dorsiventral leaf;
- ❖ appreciate the different types of mesophyll patterns in these two types of leaves; and
- ❖ describe the conjoint, collateral and closed vascular bundles.

5.2 STUDY GUIDE

For doing satisfactory work you must read the following Units before coming to the laboratory.

The UGC (CBCS) Course-Core Botany, Paper III - Plant Anatomy and Embryology (BBYCT-135), Unit-5.

5.3 MATERIALS REQUIRED

1. Compound Microscope.
2. Permanent slides of:
 - a) T.S. of *Zea mays* Leaf
 - b) T.S. of *Helianthus* Leaf

5.4 PROCEDURE

You will be provided permanent slides of T.S. of *Zea mays* leaf and *Helianthus* leaf. You must observe them carefully under the compound microscope, both under low and high power. Make neat, well-labelled diagrams (both outline and a portion enlarged to show the cellular details) in your notebook and write comments. Do not copy figures from the book and only draw as you see them in the microscope.

5.5 OBSERVATIONS

Observe the following anatomical features in the transverse section of monocot leaf of *Zea mays* provided to you (Fig. 5.1).

5.5.1 Study of T.S. of isobilateral leaf of *Zea mays*

Epidermis

- Prominent lower (abaxial) and upper (adaxial) epidermal layers seen.
- Both layers are thickly cuticularized.
- Both layers possess stomata (leaves **amphistomatic**).
- Epidermis is made up of parenchymatous cells.
- Some cells of upper epidermis are large, empty, colorless, and thin walled; called **bulliform cells** or **motor cells**.
- Bulliform cells help in rolling of leaves during drought and water stress (xerophytic adaptation).

Mesophyll

- It is the ground tissue is present between both epidermal layers.
- Mesophyll cells are irregularly arranged, isodiametric with small intercellular spaces; contain chloroplasts.
- Is not differentiated into palisade and spongy parenchyma.

Vascular bundles

- Many large and small sized vascular bundles are present.
- Each vascular bundle is surrounded by parenchymatous **bundle sheath**.
- Cells of the sheath possess plastids and starch grains.
- A patch of sclerenchyma each present above and below the larger vascular bundles ; extends up to the upper and lower epidermal layers, respectively.
- Xylem is present towards upper epidermis (**adaxial** side) and phloem towards lower epidermis (**abaxial** side).
- Vascular bundles are conjoint, collateral and closed.

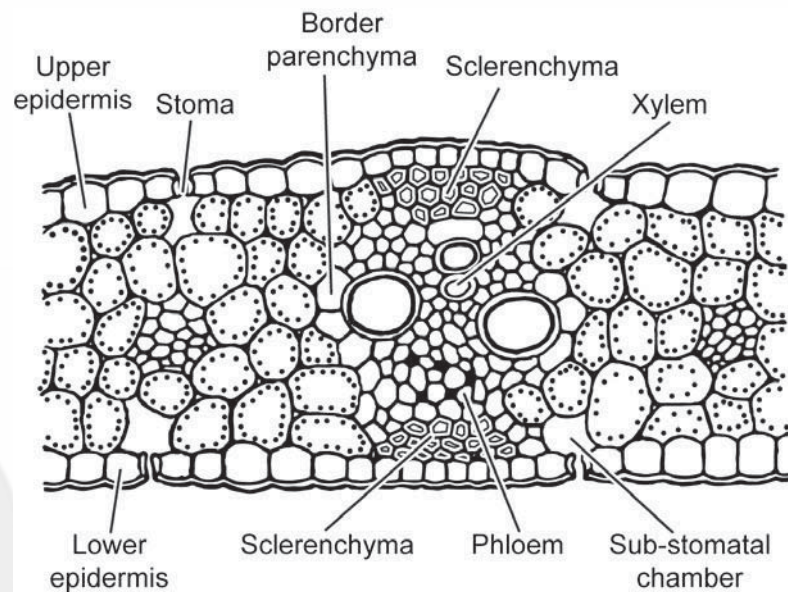


Fig. 5.1: T.S. of a monocot leaf.

Identification

1. Mesophyll is not differentiated into palisade and spongy parenchyma.
2. Stomata are present on both epidermal layers.

5.5.2 Study of T.S. of dorsiventral leaf of *Helianthus annuus*

Now you will observe the salient features in internal structure of dicot or dorsiventral Leaf .You will observe the following anatomical features in the transverse section of *Helianthus annuus* leaf (Fig. 5.2).

Epidermis

- Both surfaces of the leaf covered by a single layered epidermis.
- i) **Upper (Adaxial) Epidermis**
- Made of single layered closely packed parenchymatous cells without intercellular spaces.
 - Thick cuticle.
 - Stomata if present are few.

ii) Lower (Abaxial) Epidermis

- Single layer of parenchymatous cells are with a thin cuticle.
- More stomata present on the lower than at the upper surface.

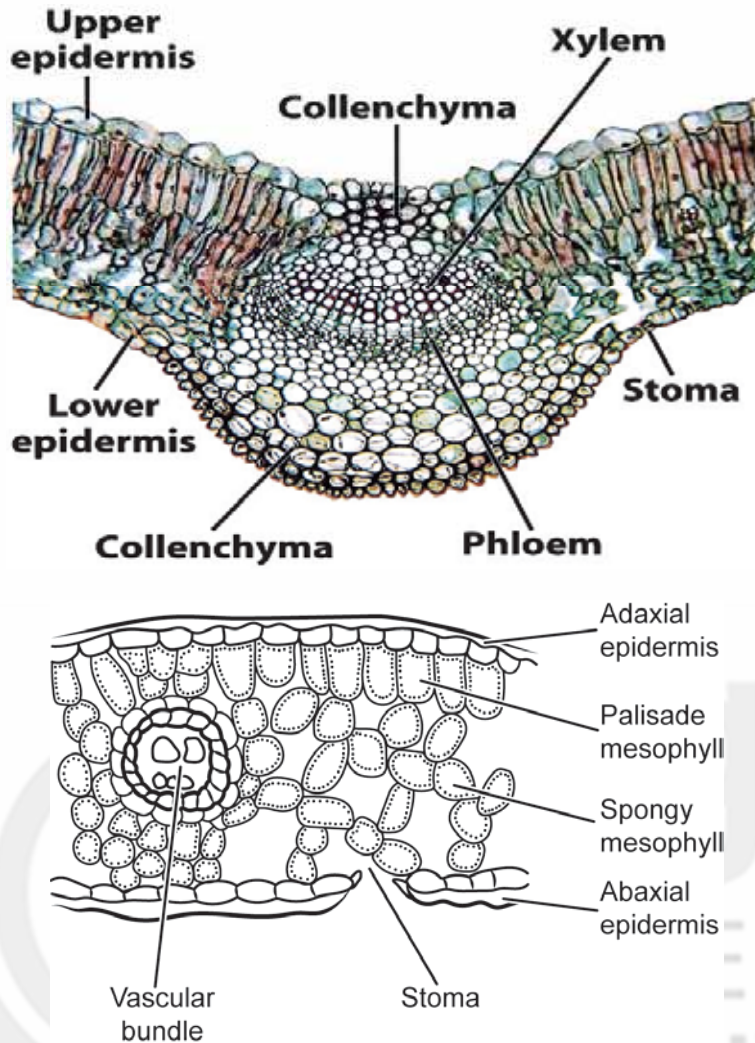


Fig. 5.2: a) Photograph and cellular diagram of T.S. of a dicot leaf.

Mesophyll

- The tissue present between the upper and lower epidermis.
- Differentiated into **Palisade parenchyma** and **Spongy parenchyma**.
- Palisade contains vertically elongated cylindrical cells in one or more layers; compactly arranged cells with no intercellular spaces; chloroplasts abundant.
- Spongy parenchyma is situated below the palisade; Consists of loosely arranged irregular cells with thin walls and large intercellular spaces.

Vascular bundles

- One large vascular bundle in the midrib; many small vascular bundles on either side.
- Vascular bundles are conjoint, collateral and closed.

- Each vascular bundle surrounded by a sheath of parenchymatous cells called **bundle sheath/border parenchyma**.
- Each vascular bundle consists of xylem lying towards the upper epidermis and phloem towards the lower epidermis.
- Metaxylem is situated toward the lower epidermis and protoxylem toward the upper epidermis.

Identification

Dorsiventral leaf:

- Mesophyll differentiated into palisade and spongy parenchyma.

Acknowledgement

Fig.5.2 (a) : (<https://i.pinimg.com/originals/86/1d/16/861d16c697765eb9e3c9a932f8d24b16.jpg>)



EXERCISE 6

TO STUDY SECONDARY GROWTH IN DICOT STEM AND DICOT ROOT

Structure

6.1	Introduction	6.5	Observations
	Objectives		Study of Secondary Growth in a Dicot Stem
6.2	Study Guide		Study of Secondary Growth in a Dicot Root
6.3	Material Required		
6.4	Procedure		

6.1 INTRODUCTION

You have already studied the internal structure of stem and root in Exercises 3 and 4. In long-lived dicotyledonous plants the older regions of the root form secondary vascular tissue. This is because the primary vascular tissues may not be sufficient to sustain the needs of roots. The secondary vascular tissues are formed from two lateral meristems: the vascular cambium and the cork cambium. Activity of the vascular cambium or intrastelar cambium and cork cambium (extrastelar cambium or phellogen) results in an increase in diameter of the axis (shoot and root) and is called secondary growth.

In the present exercise you will study the secondary growth in a dicot stem and dicot root with the help of permanent slides.

Objectives

After going through these permanent slides of secondary growth in a dicot stem and root, you would be able to:

- ❖ study the sequence of differentiation of different cells and tissues at the time of secondary growth; and
- ❖ differentiate between the vascular and cork cambium.

6.2 STUDY GUIDE

For doing satisfactory work you must read the following Units before coming to the laboratory.

The UGC (CBCS) Course-Core Botany, Paper III – Plant Anatomy and Embryology (BBYCT-135), Unit-6.

6.3 MATERIALS REQUIRED

1. Compound Microscope
2. Permanent slides of:
 - a) T.S. of old *Helianthus* stem showing secondary growth, and
 - b) T.S. of old *Helianthus* root showing secondary growth.

6.4 PROCEDURE

You will be provided permanent slides of T.S. of old stem and root of *Helianthus* showing secondary growth. You must observe them carefully under the compound microscope, both under low and high power. Make neat, well- labelled diagrams (both outline and a portion enlarged to show the cellular details) in your notebook and write comments. Do not copy figures from the book and only draw as you see them in the microscope.

6.5 OBSERVATIONS

6.5.1 Study of Secondary Growth in a Dicot Stem

You will observe the following features in the transverse section of an old *Helianthus* stem (Fig. 6.1-beginning from the periphery):

- Since sunflower is an annual herbaceous plant, it does not follow the pattern of secondary growth seen in typical dicots. For example, periderm formation does not take place as there is no initiation of cork cambium.
- Secondary growth is restricted to the stellar region only and there is only one xylem ring.

A T.S. through mature sunflower stem appears roughly circular in outline and reveals the following details:

In intrastelar region

1. When the secondary growth begins **fascicular/ intrafascicular cambium** becomes active.
2. The permanent cells present in between the vascular bundles become meristematic and form cambium strip- **Interfascicular Cambium**.
3. Intrafascicular and interfascicular cambium grow laterally and forms **cambium ring**.

4. Activity of cambium ring begins. The cambium is more active on the inner side than the outer side.
5. Cambium adds **more secondary xylem towards inner side** and relatively **less amount of secondary phloem towards outer side**.
6. The cambium forms radial narrow bands of parenchyma cells running across the secondary xylem and secondary phloem is called as **Secondary medullary rays**.
7. Primary xylem, Primary phloem and pith gets crushed due to formation of more and more secondary tissues.

Vascular system

- In each vascular bundle (Figs. 6.1, 6.2 a) between xylem and phloem a strip of intra-fascicular cambium is present. Between vascular bundles, medullary rays composed of parenchyma are formed.
- A few parenchyma cells of these rays adjacent to intra-fascicular cambium differentiate into meristematic cells and constitute inter-fascicular cambium.
- Both inter and intra-fascicular cambium join to form a complete **ring** of cambium called **vascular cambium**, which initiates intra-stelar secondary growth Figs 6.2 b, c).
- The vascular cambial cells continuously divide and form numerous daughter cells to inner and outer sides. The derivatives produced inward (toward pith) mature into secondary xylem elements, while those produced outward (toward cortex) differentiate into secondary phloem. Secondary xylem tissue produced is much larger than that of secondary phloem tissue as cambium adds more secondary xylem towards inner side and relatively less amount of secondary phloem towards outer side (Fig. 6.2d).
- Due to the formation of secondary xylem and phloem, primary xylem, and phloem separates. The cambium forms radial narrow bands of parenchyma cells running across the secondary xylem and secondary phloem is called as **Secondary medullary rays**.
- The primary phloem is pushed outward is finally crushed due to internal pressure created by the formation of more and more secondary tissues.

Identification

Stem : vascular bundles are conjoint, collateral and endarch.

Dicot stem: Cortex is well differentiated. Endodermis is conspicuous opposite to protoxylem and pericycle surface.

Secondary growth in dicot stem: Secondary xylem (towards inside of the cambial ring) much more massive than the secondary phloem (produce outward of the cambium towards the cortex).

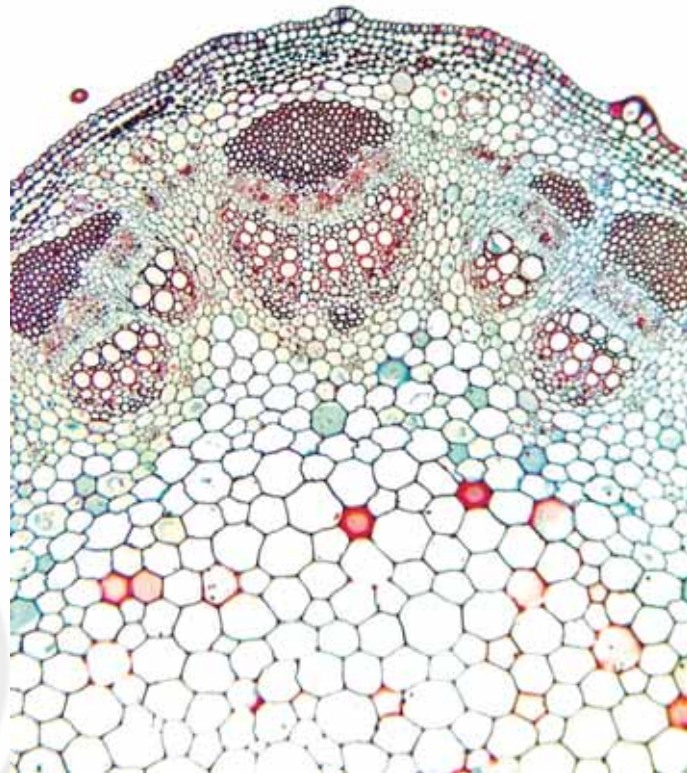
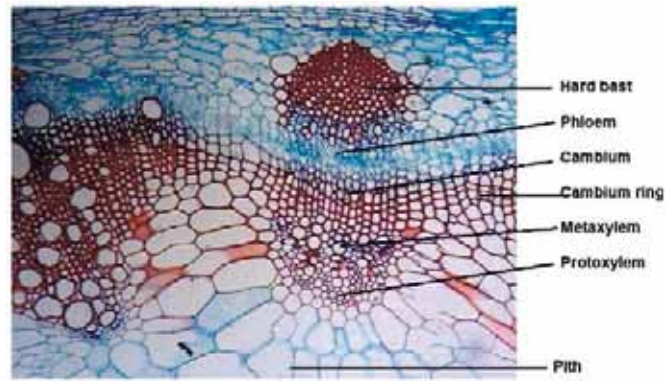


Fig. 6.1: Part of T.S. of old sunflower stems showing secondary growth.

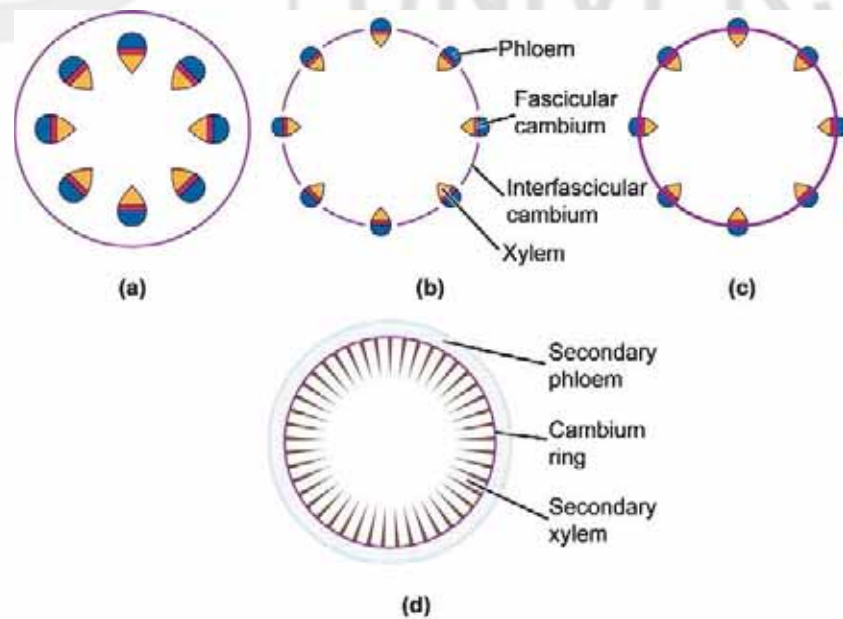


Fig. 6.2: Diagrammatic representation of various stages of secondary growth in the sunflower stem.

6.5.2 Study of Secondary Growth in a Dicot Root

You will observe the following features in the transverse section of an old *Helianthus* root (Fig. 6.3 beginning from the periphery):

The transverse section appears almost circular in outline.

Outline of the transverse section of the dicot root undergoing secondary growth is discontinuous due to sloughing off the epidermis. It shows the following plan of arrangement of tissues from the periphery to center.

- Secondary cortex consists of thin walled cells with numerous intercellular spaces.
- When secondary growth in a root is extensive, the primary phloem, endodermis and the cortex become crushed and eventually get sloughed off.
- At a later stage, cortex and pericycle may get peeled off.

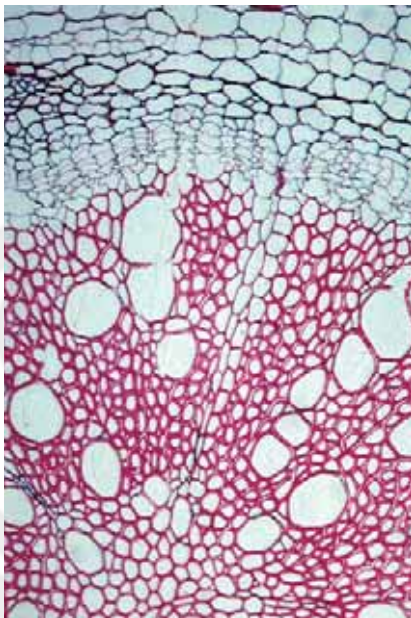


Fig. 6.3: A portion of sunflower root showing secondary growth.

Stele

All the tissues present inside endodermis comprise the stele.

Secondary growth initiates the formation of new cylinder of cork parenchyma inside.

Vascular system

- The undifferentiated procambial cells lying between primary xylem and primary phloem differentiates into cambium. In a tetrarch root, there appear four separate strips of cambium while two separate strips are formed in diarch roots.
- The strips become continuous laterally because of tangential division of pericycle cells external to each protoxylem pole. The cells in the cambium strips which cut off internal to the cambial strips forms secondary xylem and those toward outer surface gives rise to secondary

phloem. As a result, collaterally arranged strands of secondary vascular tissue are built up between the adjacent primary xylem plates (Fig. 6.4).

- Primary xylem arches are persistent and occupies center of the axis.
- Here and there the cambial cells destined to form secondary xylem elements and secondary phloem elements remain undifferentiated and parenchymatous. Such radiating plates of parenchymatous cells are known as **rays** (Fig. 6.5).

Pith is absent.

Identification

1. Root

- Unicellular root hairs.
- Vascular bundle radial.
- Protoxylem exarch.

2. Dicotyledonous root

- Xylem groups are four showing tetrarch condition.
- Cortex uniformly parenchymatous.
- Pith is negligible.

Dicot root with secondary growth

Secondary phloem elements and secondary xylem elements are formed due to extensive activity of vascular cambium. No cork is formed in this herbaceous stem.

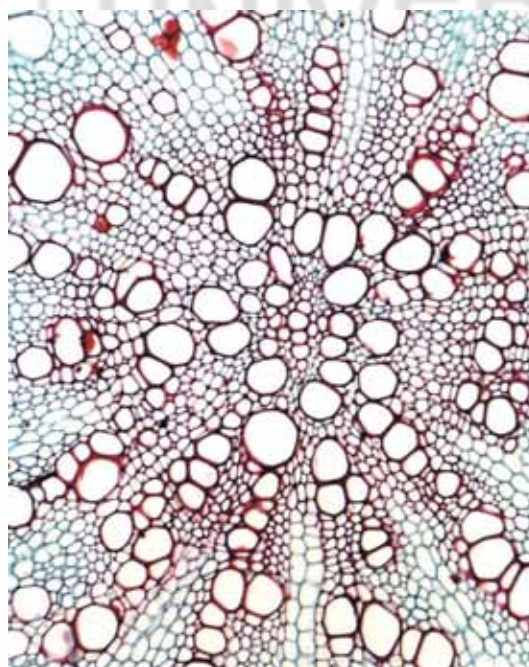


Fig. 6.4: A part of old root of sunflower showing extensive secondary growth occurring during one growing season.



Fig. 6.5: A portion of Fig. 6.4 magnified shows large diameter secondary xylem vessels (V), which are arranged in files/rows. Narrower vessels and tracheids (T) are interspersed. Xylem is separated by parenchyma rays of variable width.

Acknowledgement

- Fig. 6.1 : <https://www.google.com/url?sa=i&url=https%3A%2F%2Fwww.pinterest.com%2Fpin%2F373306256592778641%2F&psig=A0vVaw25mkhMSul9yNjG0k7TU0a3&ustc>
- Fig. 6.3 : https://botweb.uwsp.edu/anatomy/images/rootsecondarygrowth/images_c/anat0894.jpg
- Fig. 6.4 : http://virtualplant.ru.ac.za/Main/ANATOMY/Variations%20in%20roots_files/Helianth-old-root.jpg
- Fig. 6.5 : http://virtualplant.ru.ac.za/Main/ANATOMY/Variations%20in%20roots_files/Helianth-old-rootdet.jpg

EXERCISE 7

TO STUDY A XEROPHYTE LEAF AND A HYDROPHYTE STEM

Structure

7.1	Introduction Objectives	7.5	Observations Adaptive Anatomical Characters of <i>Nerium</i> Leaf
7.2	Study Guide		
7.3	Material Required		Adaptive Anatomical Characters of <i>Hydrilla</i> Stem
7.4	Procedure		

7.1 INTRODUCTION

You have already read in Unit 8 regarding the influence of environment on the external and internal structures of plants. Based on the available water and other factors operative at the habitat, the plants are classified into three main groups: Hydrophytes, mesophytes and xerophytes. These are characterized by characteristic morphological, anatomical, and physiological adaptations.

In this exercise, you will study the internal structure of a xerophyte (*Nerium* leaf) and a submerged hydrophyte (*Hydrilla* stem) with the help of permanent slides.

Objectives

After observing the permanent slides of *Nerium* and *Hydrilla* you would be able to:

- ❖ appreciate the unique anatomical features of xerophytes and hydrophytes; and
- ❖ describe the typical anatomical adaptations shown by these plants in response to the ecological conditions.

7.2 STUDY GUIDE

For doing satisfactory work you must read the following Units before coming to the laboratory.

The UGC (CBCS) Course-Core Botany, Paper III - Plant Anatomy and Embryology (BBYCT-135), Unit-8.

7.3 MATERIALS REQUIRED

You will be provided with the following permanent slides

1. T.S. of *Nerium* leaf.
2. T.S. of *Hydrilla* stem ;and
3. Compound microscope.

7.4 PROCEDURE

You will be provided permanent slides of T.S. of *Nerium* leaf and *Hydrilla* stem. You must observe them carefully under the compound microscope, both under low and high power. Make neat, well- labelled diagrams (both outline and a portion enlarged to show the cellular details) in your notebook and write comments. Do not copy figures from the book and only draw as you see them in the microscope.

7.5 OBSERVATIONS

7.5.1 Adaptive Anatomical Characters of *Nerium* Leaf

You will observe the following adaptive anatomical features in the permanent slide of T.S. of *Nerium* leaf (Fig. 7.1a, b)

- A dorsiventral leaf having distinct midrib with wings on either of its sides when seen in T.S.
- Distinct upper and lower epidermis present.
- Both epidermal layers thickly cuticularized.
- Both epidermises are multiseriate; each epidermis with 3-4 layers of parenchymatous cells.
- Less number of stomata; confined to the folded parts on lower epidermis only; covered with hairs (trichomes).
- Absence of stomata in adaxial epidermis.
- Sunken stomata in stomatal crypts on abaxial surface.
- Mesophyll differentiated into palisade and spongy parenchyma.
- Compactly arranged palisade tissue present both below the epidermis (4-5 layers) as well as just above the lower epidermis (1-2 layers); chlorenchymatous.
- Spongy parenchyma present between the palisade of lower and upper epidermis. Cells loosely arranged and form large air chambers.
- Well-developed vascular bundles: those in the midrib is larger than the ones in the wings.

- Each vascular bundle is conjoint and collateral.
- Vascular bundle is surrounded by a parenchymatous bundle sheath.
- The protoxylem is located towards the upper epidermis and the metaxylem towards the lower epidermis.

Xerophytic Features

- Presence of thick cuticle on upper and lower epidermis.
- Presence of wax on the epidermal cells to reflect light.
- Both epidermal layers multiseriate to check transpiration.
- Highly sunken stomata; on lower surface only; covered with hairs.
- Presence of palisade near both epidermal layers.
- Well-developed vascular system.

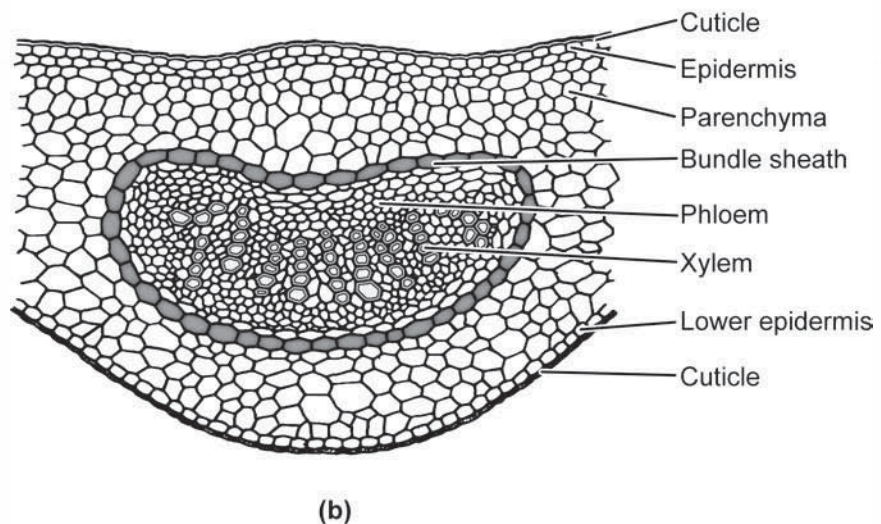
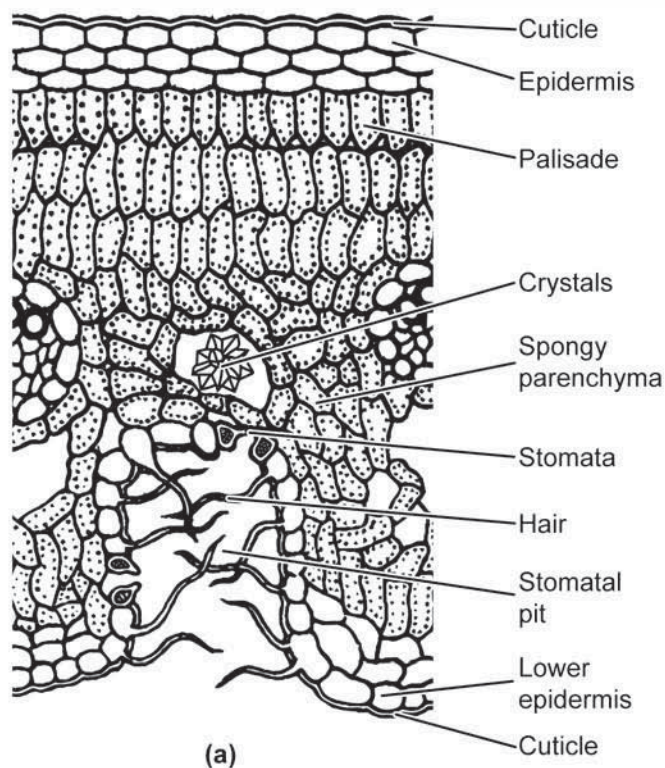


Fig. 7.1: T.S. Nerium leaf: a) Sunken stomata on the lower epidermis; b) Portion of leaf midrib to show the disposition of vascular bundles.

7.5.2 Adaptive Anatomical Characters of *Hydrilla* Stem

Observe the following adaptive anatomical features in the transverse section of stem of *Hydrilla* provided to you (Fig. 7.2).

- Almost circular outline of stem. Thin walled single layer parenchymatous epidermis without cuticle.
- Most of the stem occupied by thin parenchymatous cortex extensively traversed by air cavities (aerenchyma).
- Distinct endodermis and pericycle present that enclose the vascular tissue.
- Extremely reduced vascular tissue with thin walls.
- Stele mainly composed of phloem.
- Xylem represented by only a central cavity.
- Phloem represented by an outer broad zone

Hydrophytic features

- Absence of cuticle; presence of thin walled epidermis.
- Absence of supporting mechanical tissue.
- Presence of extensive aerenchyma for buoyancy and oxygen supply to the underwater organs through air chambers.
- Reduced vascular system.
- Comparatively well-developed phloem.
- Xylem reduced to merely a central cavity.

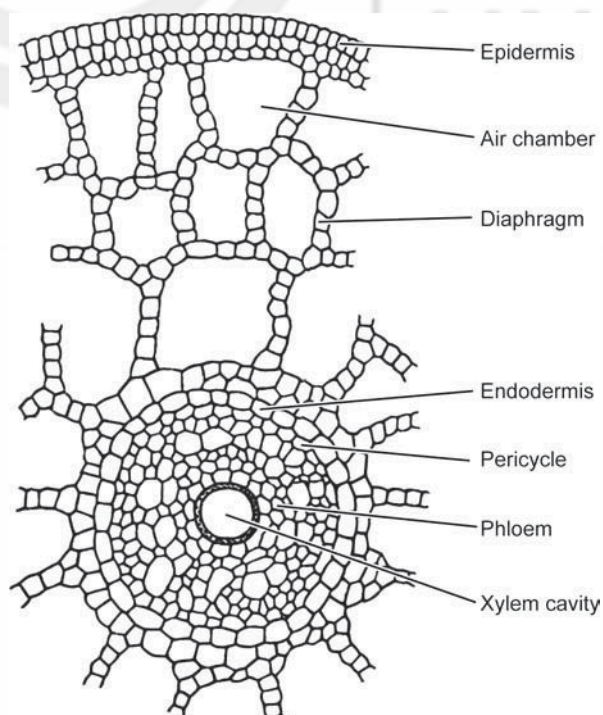


Fig. 7.2 T.S. of *Hydrilla* stem.

TO STUDY THE STRUCTURE OF ANTHER AND TAPETUM

Structure

8.1	Introduction	8.5	Observations
	Objectives		T.S. of a young (developing) Anther
8.2	Study Guide		T.S. of Anther Showing Tetrads
8.3	Material Required		T.S. of Mature Anther showing Pollen Grains
8.4	Procedure		T.S. <i>Tradescantia</i> Anther Showing Amoeboid Tapetum
			T.S. <i>Lilium</i> Anther showing Secretary Tapetum

8.1 INTRODUCTION

You have already studied in Unit - 9 that sexual reproduction in flowering plants requires the coordinated development of two reproductive organs of the flower, the anther, and the pistil. Both anther and pistil show characteristic structures and developmental phases which can be observed through microscopical techniques. The microscopical technique plays a dominant role in the study of sexual reproduction of angiospermic plants. It is very difficult to observe all the structures directly since they are not only microscopic but also because these gametophytic tissues are deeply embedded in the tissues of the sporophyte

In sexual reproduction basic processes are meiosis and fusion of gametes. During meiosis there is rearrangement of genes and then reduction of the number of chromosomes and subsequently fertilization restores original diploid chromosome number.

Thus, prepared slides of male and female reproductive organs can help you understand better the development of these structures as described in Unit 9.

Objectives

After observing the permanent slides, you would be able to:

- ❖ describe the structure of the anther;
- ❖ give structural details in the development of male gametophyte; and
- ❖ differentiate between the amoeboid and secretory tapetum.

8.2 STUDY GUIDE

For doing satisfactory work you must read the following Units before coming to the laboratory.

The UGC (CBCS) Course-Core Botany, Paper III - Plant Anatomy and Embryology (BBYCT-135), Unit-9.

8.3 MATERIALS REQUIRED

1. A compound microscope.
2. The following permanent slides:
 - a) T.S. *Lilium* young (developing) anther;
 - b) T.S. *Lilium* anther showing tetrads and tapetum;
 - c) T.S. mature *Lilium* anther showing pollen grains and degenerating tapetum;
 - d) T.S. *Tradescantia* anther showing amoeboid tapetum ; and
 - e) T.S. *Lilium* anther showing secretory tapetum.

8.4 PROCEDURE

You must observe the slides provided to you carefully under the compound microscope, both under low and high power. Make neat, well- labelled diagrams (both outline and a portion enlarged to show the cellular details) in your notebook and write comments. Do not copy figures from the book and only draw as you see them in the microscope.

8.5 OBSERVATIONS

You have been provided with different permanent slides showing the different developmental stages of an anther. Observe each one of them carefully.

8.5.1 T.S. of a Young (developing) Anther

T.S. of a young anther (Fig. 8.1) shows the following features:

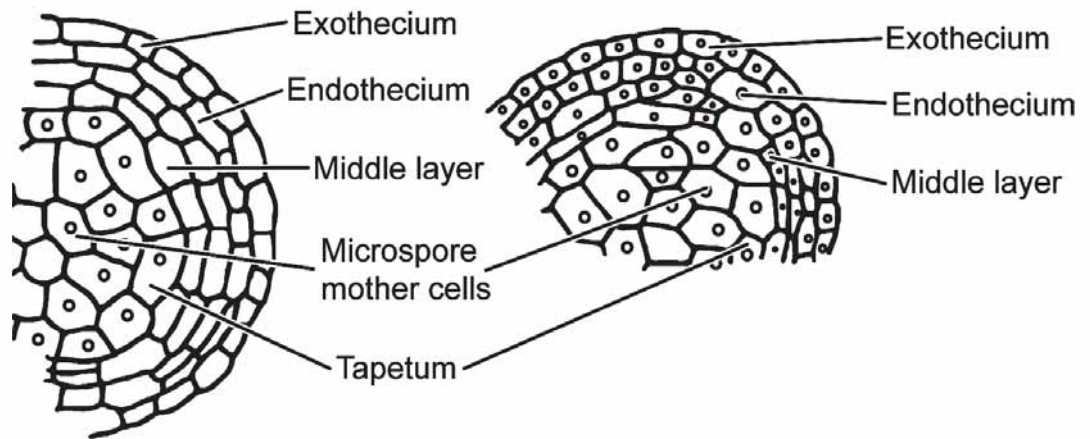


Fig. 8.1: T.S. of young developing anther.

1. It is a multicellular - four cornered structure.
2. It has four microsporangia, one in each corner, and an intervening connective tissue which is linked with the filament.
3. A single layered exothecium (epidermis) forms the outer wall layer. Cuticle is present.
4. At four corners of the anther, below the epidermis, the derivatives of some cells form archesporial cells
5. Archesporial cells divide to give rise to the primary parietal cell towards outside and the primary sporogenous cell towards inside.
6. The primary parietal cells divide periclinally to form 4-5 cell layers, of which outermost differentiates as endothecium, 2-3 form the middle layers and innermost differentiates as tapetum. Tapetum helps in nutrition of developing pollen inside.
7. The primary sporogenous cells divide mitotically to many sporogenous cell which round off to become microspore mother cells (MMCs). MMC divide by meiosis to form tetrads of microspores. Each microspore forms a pollen grain (Fig.8.2).

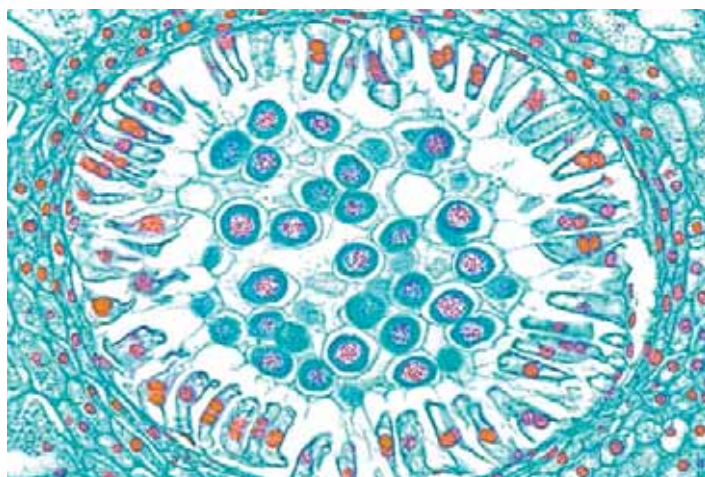


Fig.8.2: A photomicrograph of T.S. young anther.

8.5.2 T.S. of Anther Showing Tetrads

Slide of a slightly more mature anther in T.S. (Fig. 8.3 a, b) shows the following features:

1. During microsporogenesis distinct changes occur in the microsporangia.
2. The middle layer(s) usually get crushed gradually and ultimately degenerate.
3. The cells of the tapetum, in contrast enlarge and develop a complex ultrastructure, which indicates that they have become metabolically very active.
4. The cells of exothecium get stretched and endothecium develops fibrous thickening, the cells can also enlarge and become more vacuolate.
5. After microsporogenesis tetrads are formed. Each microspore mother cell divides meiotically to produce four haploid microspores.
6. The microspores start to differentiate whilst still associated in tetrads and encapsulated by callosic wall.

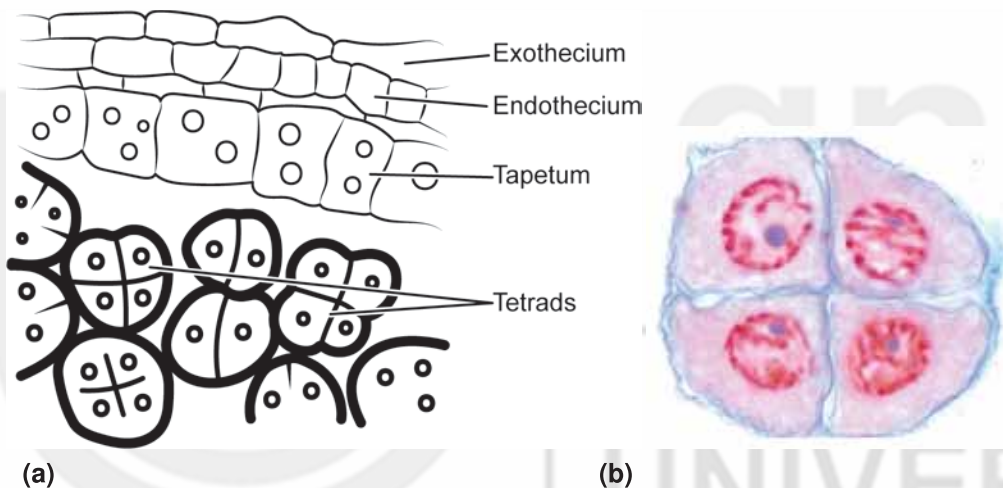


Fig. 8.3: a) T.S. anther showing tetrads; b) A tetrad enlarged.

8.5.3 T.S. of Mature Anther Showing Pollen Grains

A mature anther at the time of dehiscence shows the following structure in a transverse section (Fig. 8.4a, b):

1. A typical anther is tetrasporangiate. It has a column of sterile tissue called the connective on either side of which is an anther lobe.
2. The mature anther wall comprises an exothecium and on inner side a layer of endothecium, 2 or 3 middle layers and a single layer tapetum.
3. Tapetum is the innermost layer of anther wall and attains its maximum development at the tetrad stage of microsporogenesis. Typically, tapetum is composed of single layer of cells characterized by the presence of dense cytoplasm and prominent nuclei.
4. The sporogenous cells can directly function as microspore mother cell or undergoes few mitoses to add up their number and then enter meiosis.

5. Each pollen mother cell by a meiotic division give rise to a group of four haploid microspores.
6. Prior to dehiscence, the tapetum and the middle layers degenerate.
7. The cells of the endothecium are radially elongated and exhibit,characteristic fibrous thickenings.

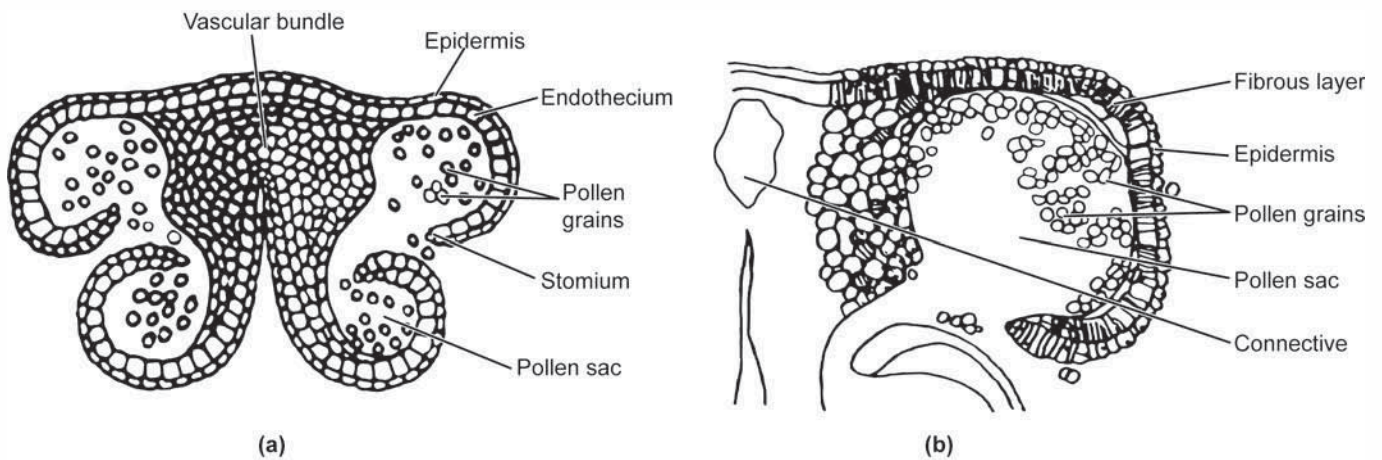


Fig. 8.4: a,b) T.S. mature anther showing dehiscence.

Now you will study the types of tapetum through the permanent slides. Tapetum can be amoeboid or secretory type.

8.5.4 T.S. *Tradescantia* Anther Showing Amoeboid Tapetum

1. It is also called invasive or periplasmodial type of tapetum(Fig 8.5).
2. The tapetal cells break down early in development by their radial and inner walls and release their protoplasmic contents into the sporangial cavity.
3. These protoplasts fuse and form a common mass called tapetal-periplasmodium.
4. The common mass surrounds and nourishes the developing microspores.

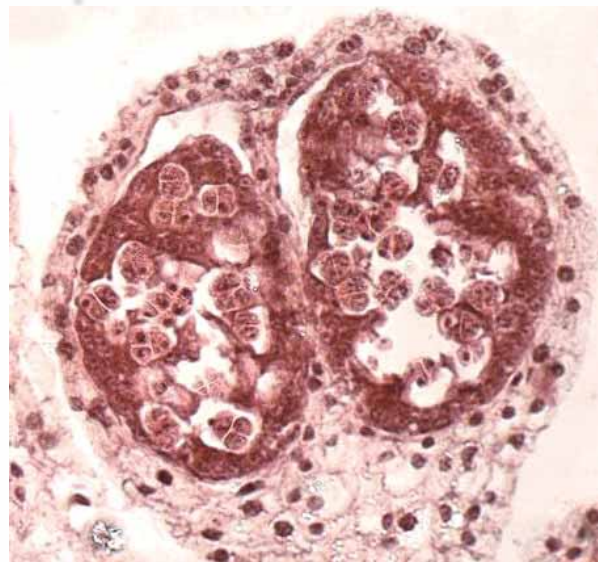


Fig. 8.5: T.S. *Tradescantia* anther showing Amoeboid tapetum.

8.5.5 T.S. *Lilium* Anther Showing Secretory Tapetum

1. It is also called glandular or parietal tapetum.
2. It is the most common type of tapetum seen in majority of angiosperms.
3. Tapetal cells remain intact throughout the microspore development (Fig. 8.6).
4. Tapetal cells secrete nutritive substances through their inner walls.
5. Tapetal cells start degenerating at the time of tetrad formation and anther dehiscence.

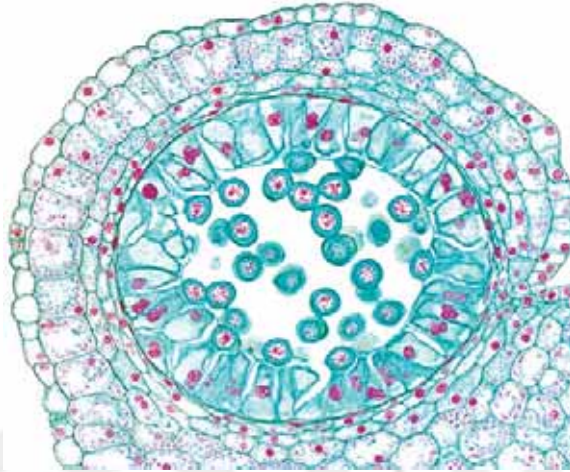


Fig. 8.6: T.S. *Lilium* anther showing Secretory tapetum.

Acknowledgements

- Fig. 8.1b** : <https://encrypted-tbn0.gstatic.com/images?q=tbn%3AANd9GcSKBbD qtAm GOT ySB-BU1fAIAcc9YcaDRfHV5tpaQ03GxCxoCH>
- Fig. 8.2b** : <https://lh3.googleusercontent.com/proxy/gZGidJZMtSRHkaq3HBpEkEIM-KMGzQy7isw2pcig39SNDeshkHH5kxo2EzTXEo1A16LKrVpRxyRLeinaUCJEDerYLsmkaSwW4vgsJlc726F9GXak>
- Fig. 8.4** : <http://docsdrive.com/images/ansinet/ijb/2008/fig1-2k8-241-244.jpg>
- Fig. 8.5** : data: image/jpeg;base64,/9j/4AAQSkZJRgABAQAAQABAAD/

EXERCISE 9

TO STUDY THE TYPES OF OVULES WITH THE HELP OF PERMANENT SLIDES

Structure

9.1	Introduction	9.5	Observations
	Objectives		Atropous/Orthotropous Ovule
9.2	Study Guide		Anatropous Ovule
9.3	Material Required		Campylotropous Ovule
9.4	Procedure		Hemianatropous Ovule
			Amphitropous Ovule
			Circinotropous Ovule

9.1 INTRODUCTION

An ovule is defined as a megasporangium with its protective coats (integumented megasporangium). It is attached to the placenta on the ovary wall. Within an ovule the female gametophyte (the embryo sac) is formed. You have already studied about the structure of the mature ovule in theory Unit 9.

The mature ovules can be classified into different types based on the relative position of the micropyle with respect to the chalaza or funiculus. These include Orthotropous (Atropous), Anatropous, Hemianatropous, Campylotropous, Amphitropous, and Circinotropous. Most common among these are the anatropous types of ovules which occur in majority of the families of dicots and monocots. The other types are restricted to certain families.

In this exercise you will study the different types of ovules with the help of permanent slides.

Objectives

After going through these prepared slides of female gametophyte you would be able to:

- ❖ distinguish between the various types of ovules, and

- ❖ describe the structural uniqueness of each type of ovule with reference to the degree of its curvature.

9.2 STUDY GUIDE

For doing satisfactory work you must read the following Units before coming to the laboratory. The UGC (CBCS) Course-Core Botany, Paper III - Plant Anatomy and Embryology (BBYCT-135), Unit-9.

9.3 MATERIALS REQUIRED

1. A compound microscope and
2. The following permanent slides :
 - a) T.S. *Piper nigrum* / *Polygonum* ovule;
 - b) T.S. *Helianthus* / *Tridax* / *Ricinus* ovule;
 - c) T.S. *Ranunculus* ovule;
 - d) T.S. *Brassica* / *Chenopodium* / *Pisum* ovule;
 - e) T.S. *Botomus* / *Alisma* / *Papaver* ovule; and
 - f) T.S. *Opuntia* / *Plumbago* ovule.

9.4 PROCEDURE

Observe the permanent slides provided carefully under the compound microscope both under low and high power. Note down the structural details of each type of ovule. Make neat, well-labelled diagrams (both outline and a portion enlarged to show the cellular details) in your notebook and write comments. It is advised that figures should not be copied from the book but should be drawn after observing the slides under the microscope.

9.5 OBSERVATIONS

You have been provided with permanent slides of various plants showing different types of ovules. Observe each one of them carefully and note down the characteristic features of each one of them.

9.5.1 Atropous/Orthotropous Ovule

The ovule of this type will show the following structural details (Fig. 9.1a, b) :

- a) The ovule is straight/upright.
- b) The micropyle, chalaza and funicle lie in straight line, i.e. on the same vertical axis,

Example- Polygonaceae, Piperaceae.

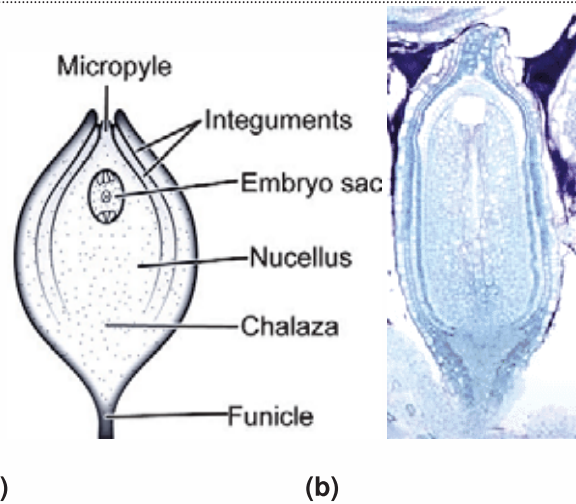


Fig. 9.1: a) Line diagram of orthotropous ovule; b) Microscopic representation of ovule as observed at 40X magnification.

9.5.2 Anatropous Ovule

The ovule of this type shows the following structural details (Fig. 9.2):

- a) The whole body of the ovule is inverted by 180° so that the micropyle comes close to the base of the funicle.
- b) The micropyle and chalaza lie on the same axis while the funicle lies parallel to this axis.

Example: Most of the families of dicots and monocots

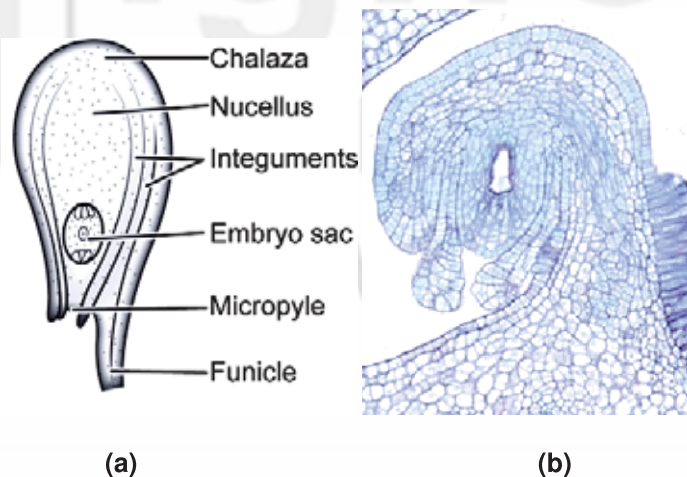


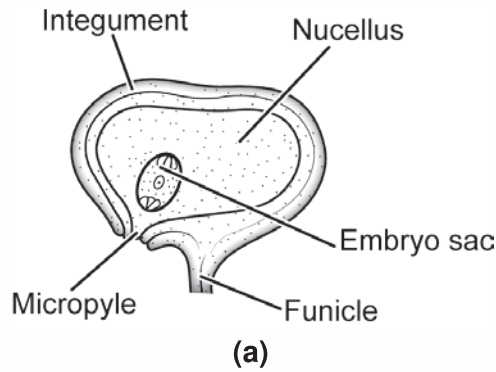
Fig. 9.2: a) Line diagram of anatropous ovule; b) Microscopic representation of ovule as observed at 40X magnification.

9.5.3 Campylotropous Ovule

This type of ovule show the following structural details (Fig.9.3)

- a) Ovule has a curved body, but its curvature is less than that of the anatropous ovule.
- b) The micropyle and chalaza are not in a straight line and the funicle lies at right angles to the chalaza,

Example- Capparidaceae, Chenopodiaceae, Brassicaceae.



(b)

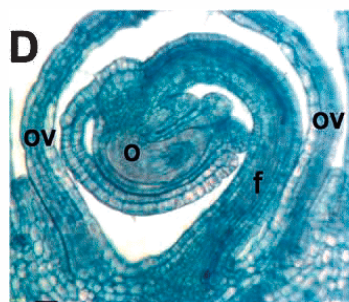
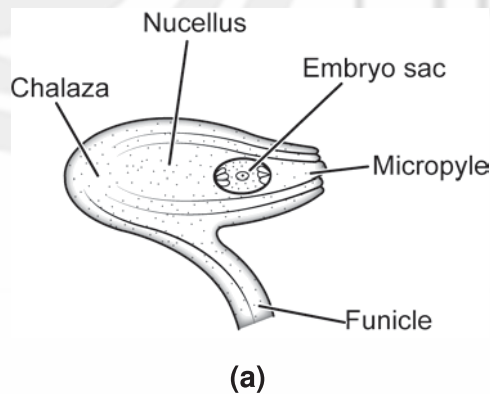
Fig. 9.3: a) Line diagram of Campylotropous ovule; b) Microscopic representation of ovule as observed at 40X magnification.

9.5.4 Hemianatropous Ovule

This type of ovule shows the following structural details (Fig. 9.4):

- a) The body of the ovule is placed transversely or turned through 90° to the funicle.
- b) The micropyle and chalaza are on a horizontal line while the funicle lies at right angles to this line,

Example- Ranunculaceae, Primulaceae.



(b)

Fig.9.4: a) Line diagram of ovule; b) Microscopic image taken at magnification 40X.

9.5.5 Amphitropous Ovule

The ovule of this type shows the following structural details (Fig. 9.5):

- a) Curvature of the body of ovule is pronounced like that of anatropous ovule.
- b) Curvature of the ovule also affects the nucellus and the embryo-sac becomes horseshoe shaped.

Example - Alismaceae, Butomaceae, Loganiaceae, Papaveraceae.

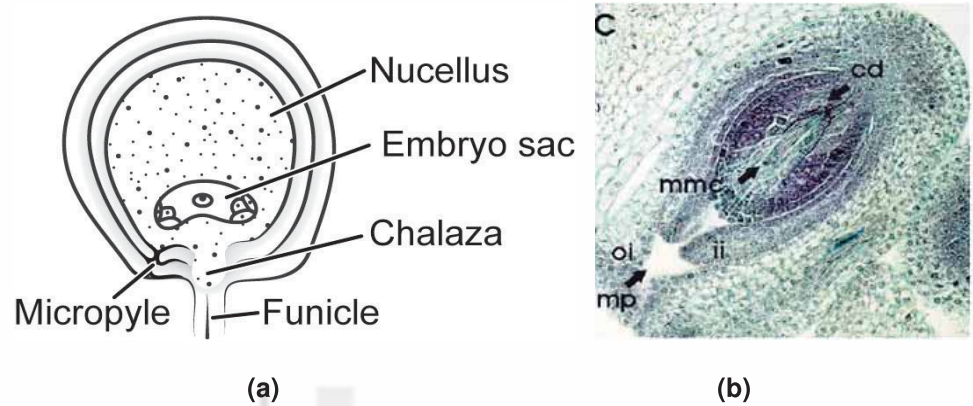


Fig. 9.5: a) Line diagram of ovule; b) Microscopic image of ovule observed at magnification 40X.

9.5.6 Circinotropous Ovule

The ovule of this type shows the following structural details (Fig. 9.6):

- a) The funicle is very long and forms a complete circle around the body of the ovule.
- b) The unilateral growth of the ovule initially becomes anatropous. Thereafter the curvature continues till the micropyle again points upwards,

Example- Cactaceae, Plumbaginaceae.

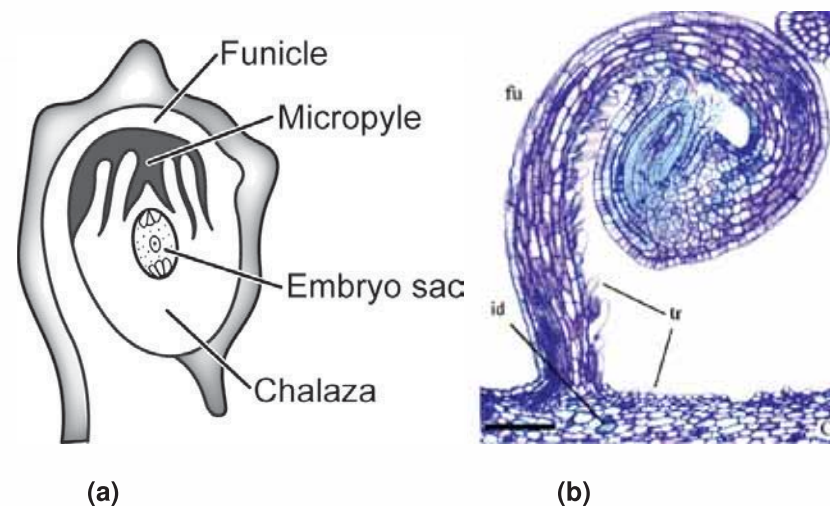


Fig. 9.6: a) Line diagram of ovule; b) Microscopic image taken at 40X magnification.

Acknowledgements

Fig. 9.1b, Fig. 9.2b	:	Endress, Peter. (2011). Angiosperm ovules: Diversity, development, evolution. <i>Annals of Botany</i> . 107. 1465-89).
Fig. 9.3b	:	https://slideplayer.com/slide/4897864/16/images/4/L.S..Campylootropous+Ovulel.jpg
Fig. 9.4b	:	https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fwww.researchgate.net
Fig. 9.5b	:	https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fwww.researchgate.net%2Fprofile%
Fig. 9.6b	:	https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fwww.researchgate.net%2Fprofile%



EXERCISE 10

TO STUDY THE FEMALE GAMETOPHYTE-POLYGONUM TYPE OF EMBRYO SAC

Structure

10.1	Introduction	L.S. Ovule showing Linear Tetrad of Megaspores
	Objectives	
10.2	Study Guide	L.S. Ovule showing Binucleate Embryo Sac
10.3	Material Required	L.S. Ovule showing Four-nucleate Embryo Sac
10.4	Procedure	
10.5	Observations	L.S. Ovule showing Eight-nucleate Mature Embryo Sac
	L.S. Ovule showing an Archesporial Initial	Study of Monosporic Embryo Sac development with the help of Photographs
	L.S. Ovule showing a Megaspore Mother Cell	
	L.S. Ovule showing two celled stage (Dyad) of Megaspore Mother cell	

10.1 INTRODUCTION

Megasporogenesis involves the development of a linear tetrad of megaspores from the megaspore mother cell by meiosis. One, two or all the four megaspores may contribute to the formation of the female gametophyte or the embryo sac. In majority of the angiosperms, the development of the embryo sac begins with usually the megaspore positioned at the chalazal end of the tetrad. This behaves as a functional megaspore and finally develops into a 7-celled, 8 nucleate embryo sac. Such type of embryo sac is called as the Monosporic 8-nucleate or Polygonum type. Interestingly, the haploid megaspore nucleus divides thrice to form eight nuclei which are all genetically identical. This exercise will help you to identify the various stages of megagametogenesis as viewed in longitudinal sections of ovules.

Objectives

After going through the permanent slides and photographs of monosporic embryo sac, you would be able to:

- ❖ describe the various developmental changes occurring in the ovule during megagametogenesis and
- ❖ appreciate the structure of a mature monosporic 7-celled, 8-nucleate embryo sac.

10.2 STUDY GUIDE

For doing satisfactory work you must read the following Units before coming to the laboratory.

The UGC (CBCS) Course - Core Botany-, Paper III – Plant Anatomy and Embryology (BBYCT-135), Unit-9.

10.3 MATERIALS REQUIRED

1. A Compound Microscope
2. Permanent slides
 - a) L.S. Ovule showing an Archesporial Initial ;
 - b) L.S. Ovule showing a Megaspore Mother Cell;
 - c) L.S. Ovule showing two celled stage (Dyad) of Megaspore Mother cell;
 - d) L.S. Ovule showing Linear Tetrad of Megaspores;
 - e) L.S. Ovule showing Binucleate Embryo Sac;
 - f) L.S. Ovule showing Four-nucleate Embryo Sac; and
 - g) L.S. Ovule showing Eight-nucleate Mature Embryo Sac
3. Photograph of:
Monosporic Embryo Sac development

10.4 PROCEDURE

You must observe the slides of monosporic embryo sac provided to you carefully under the compound microscope, both under low and high power. You will also be provided with photomicrographs of the sections of the ovule with the embryo sac.

Make neat, well- labelled diagrams (both outline and a portion enlarged to show the cellular details) in your notebook and write comments. Do not copy figures from the book and only draw as you see them in the microscope.

10.5 OBSERVATIONS

You have been provided with different permanent slides and photomicrographs showing the different stages of monosporic embryo sac development. Observe each one of them carefully.

10.5.1 L.S. Ovule showing an Archesprial Initial

- The ovule is poorly differentiated and shows only integuments and nucellus. The nucellus is covered by one or two integuments. The distal region of nucellus is left uncovered by integuments. This is called Micropyle.
- The parietal cell may or may not divide further. If not, the nucellus remains very insignificant. Such an ovule is called "Tenuinucellate". If primary parietal cell divides further, it forms massive nucellus, such an ovule is called "Crassinucellate". All the cells of nucellus are diploid.
- The hypodermal cell below the epidermis at the distal region of nucellus differentiates into **Archesprium** (Fig. 10.1).

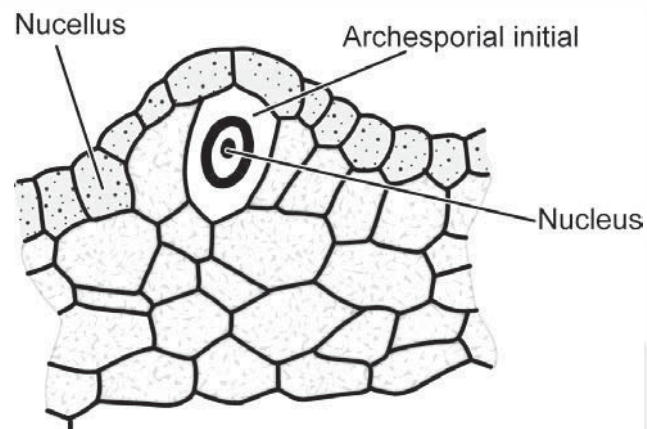


Fig. 10.1: L.S. ovule showing an archesporial initial.

10.5.2 L.S. Ovule showing a Megaspore Mother Cell

- Archesporial cell divides transversely into an outer (distal) primary parietal cell and an inner (abaxial) sporogenous cell. The diploid sporogenous cell functions as **Megaspore mother cell**.
- The megaspore mother cell is conspicuous because of its much larger size, denser cytoplasmic content, and more prominent nucleus (Fig. 10.2).

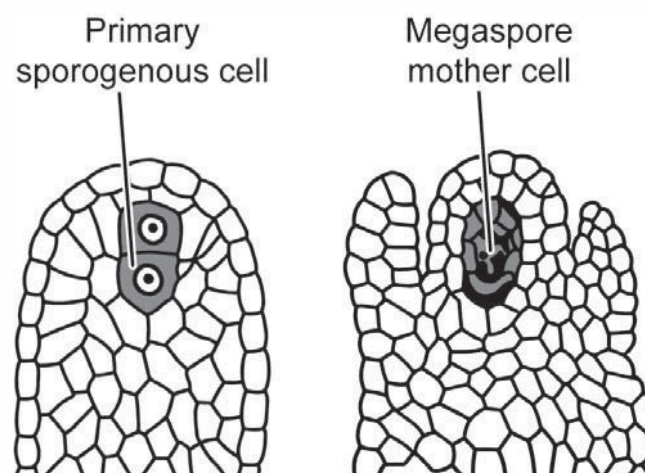


Fig. 10.2: L.S. ovule showing the parietal layers, sporogenous cell and the megaspore mother cell.

10.5.3 L.S. Ovule showing Two celled stage (Dyad) of Megaspore Mother cell

- a) Megaspore mother cell. This cell divides meiotically to produce first a dyad of two haploid megaspores. (all other cells of an ovule are diploid). Since these are formed after reduction division and thus each cell contains haploid set of chromosomes.
- b) Two cells are present one above the other (Fig. 10.3).
- c) From these two cells, a linear tetrad of megaspores will be formed.

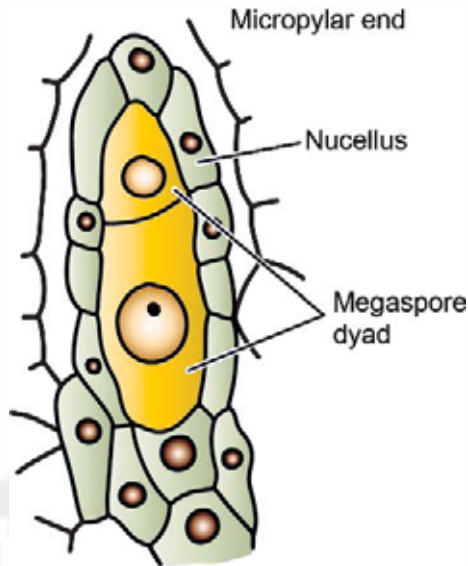


Fig. 10.3: L.S. ovule showing Megaspore mother cell (2-celled stage).

10.5.4 L.S. Ovule showing Linear Tetrad of Megaspores

- a) In the nucellus, a few cells below the nucellar epidermis lies a linear tetrad of megaspores (Fig. 10.4 a).
- b) As a rule, one of the four resulting megaspores (the lowermost or chalazal megaspore) is functional and persists, while the other three **distal ones** are non-functional and degenerate (Fig. 10.4 b).

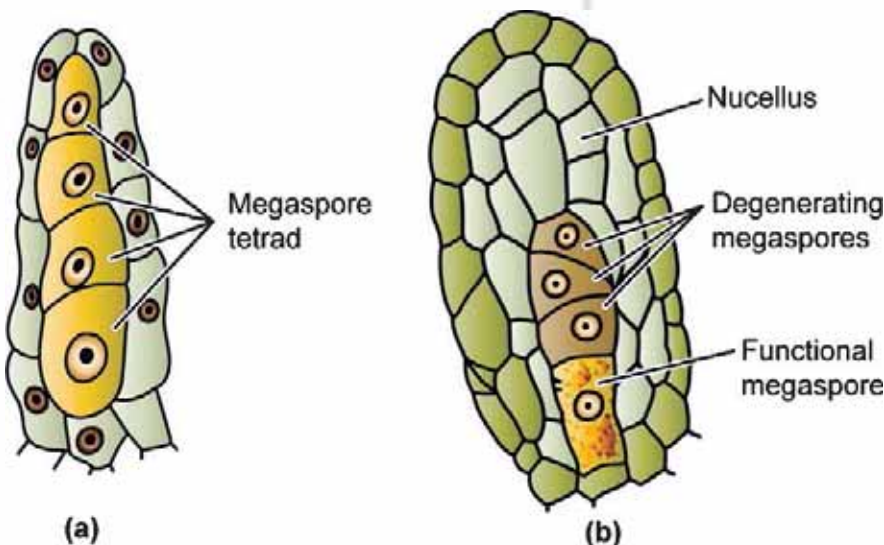


Fig. 10.4: a) L.S. ovule showing linear tetrad of megaspores; b) L.S. ovule showing a functional megaspore and the degenerating ones.

- c) The persisting, functional megaspore is large with dense protoplasm and a prominent nucleus (Fig. 10.4).
- d) This persisting functional megaspore develops into the embryo sac, the female gametophyte.

10.5.5 L.S. Ovule showing Binucleate Embryo Sac

- a) Two nuclei are present in the embryo sac.
- b) These two nuclei are formed by the division of the nucleus of the functional megaspore.
- c) Three degenerating megaspores can still be seen at the top of the sac.
- d) The two nuclei lie at the two poles as they are separated by a large vacuole (Fig. 10.5).

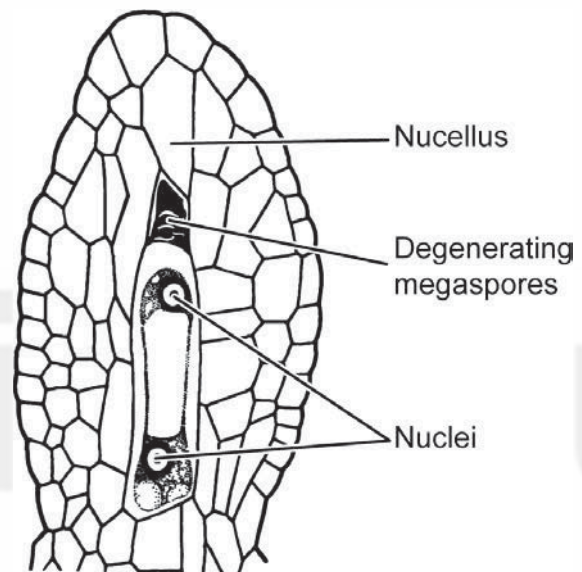


Fig. 10.5: L.S. ovule showing binucleate embryo sac.

10.5.6 L.S. Ovule showing Four-nucleate Embryo Sac

- a) Embryo sac contains four nuclei.
- b) Two nuclei are present near chalazal and other two near the micropylar end (Fig.10.6).
- c) A large central vacuole is present.
- d) At micropylar end traces of degenerated megaspores can be seen.

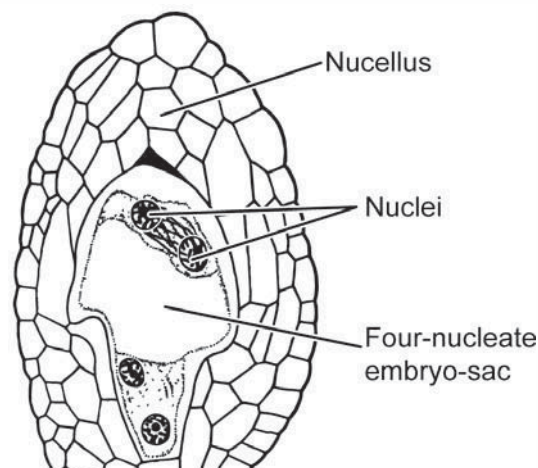


Fig. 10.6: L.S. Ovule showing Four-nucleate embryo sac.

10.5.7 L.S. Ovule showing Eight-nucleate Mature Embryo Sac

- a) The mature embryo sac (Fig.10.7and Fig.10.8) is enclosed usually in a thin layer of nucellus, which is enclosed in the integuments.
- b) The integuments are absent only at the micropylar end where nucellus is exposed.
- c) The 8-nucleate Polygonum-type embryo sac has an egg apparatus, two polar nuclei and three antipodals.
- d) The three nuclei which move towards micropylar end form egg-apparatus (with one egg cell and two synergids).
- e) The three antipodal cells are at located at the chalazal end.
- f) An egg cell has a large vacuole towards its micropylar end while synergids have a small vacuole toward its chalazal end.
- g) Two polar nuclei are in the center of the embryo sac. These later fuses just before fertilization to form the secondary nucleus.
- h) The three antipodal cells located at the chalazal end also degenerate soon, either before or just after fertilization.
- i) Since this embryo sac develops from a single megaspore, it is known as monosporic, 8-nucleate Polygonum-type embryo sac (Fig. 10.8).

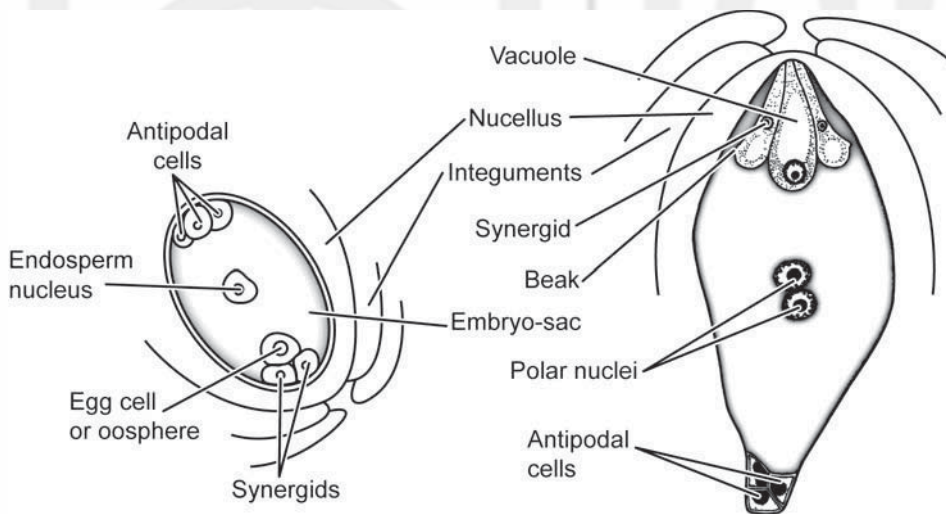


Fig. 10.7: L.S. Ovule showing Eight-nucleate Mature Embryo Sac.

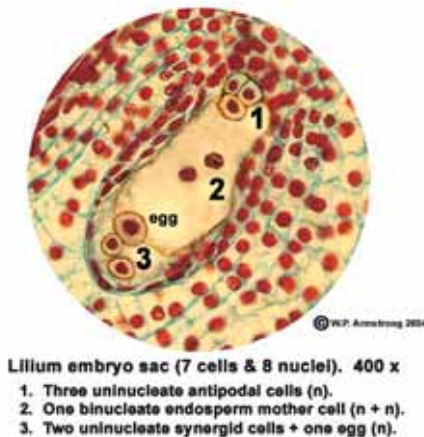


Fig. 10.8: A portion of photograph showing mature embryo-sac of *Lilium*.

10.5.8 Study of Monosporic Embryo Sac development with the help of Photographs

You will now study and sketch the developmental stages of a Polygonum-8 nucleate-Monosporic embryo sac with the help of the following photographs of L.S. of Ovules (Figs 10.9).

A large megaspore mother cell is present below the nucellar epidermis. It has a prominent nucleus and cytoplasm of low density as compared to surrounding nucellar cells.

- The megaspore mother cell has undergone the first reduction division and formed two cells, the micropylar dyad cell and the chalazal dyad cell. Both the cells possess cytoplasm of low density.
- The two dyad cells undergo the second meiotic division to form a 'T'-shaped tetrad of megaspores. The density of cytoplasm is low as compared to the surrounding nucellar cells, in all the four spores.

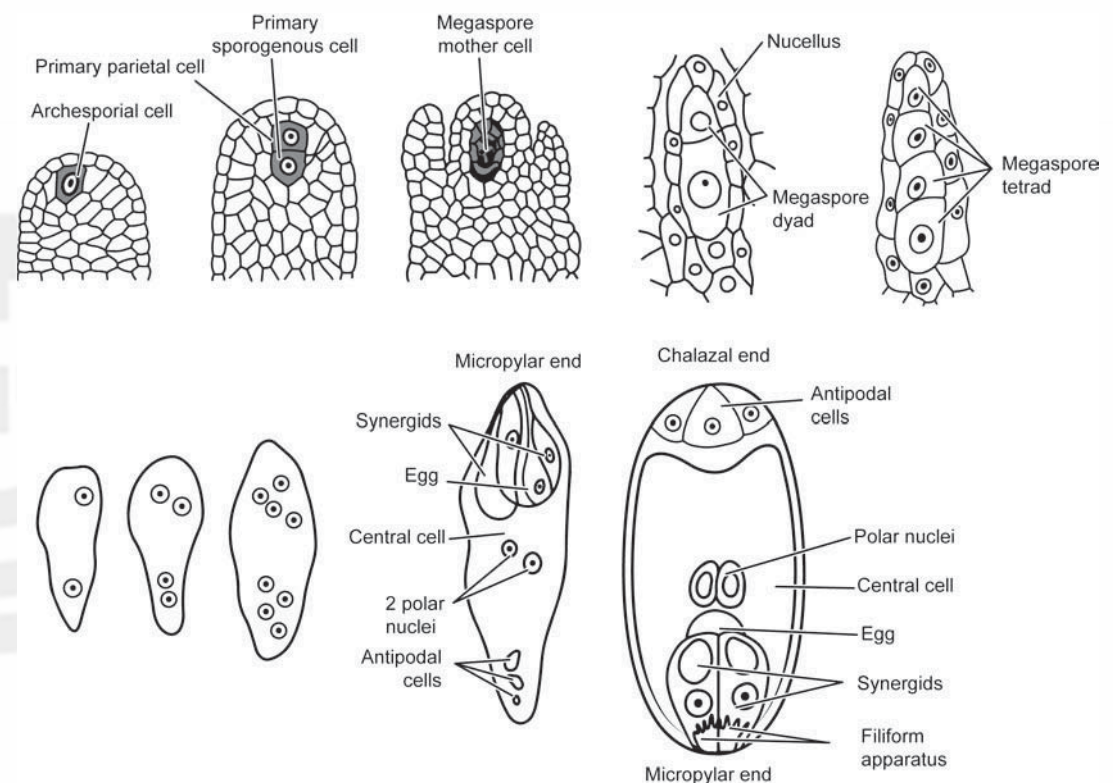


Fig. 10.9: Diagrammatic representation of developmental stages of monosporic Embryo sac.

- The three micropylar megaspores degenerate and the chalazal most megaspore is functional. The chalazal megaspore enlarges and participates in gametogenesis to form the embryo sac. This type of embryo sac development in which a single chalazal megaspore gives rise to the embryo sac is known as the Polygonum (monosporic) type. The megaspore enlarges with the formation of vacuoles. The density of cytoplasm has increased.
- The megaspore nucleus has divided mitotically, and the two nuclei are present near the two ends of the gametophyte separated by a large central vacuole.

- e) The two nuclei after reaching the two ends of the gametophyte undergo the second mitotic division and form four-nucleate gametophyte; the two nuclei at the micropylar end are separated from the two chalazal nuclei by a large central vacuole. The gametophyte has enlarged considerably.
- f) The four nuclei undergo the third mitotic division to form eight nuclei, four at micropylar region and four at chalazal region. Walls form around the nuclei delimiting seven cells, three at the micropylar end, three at the chalazal end and one large cell at the center with two nuclei.
- g) The three cells (two synergid cells and an egg cell) at the micropylar end constitute the egg apparatus. The synergids have wall ingrowths at the micropylar region called the filiform apparatus and a polarized cytoplasm. The nucleus and most of the cytoplasm is concentrated at the micropylar end whereas the chalazal part is vacuolate. The egg cell also shows polarized cytoplasm, with the nucleus and most of the cytoplasm at the chalazal end and a vacuole at the micropylar part. The three cells at the chalazal region are called the antipodals and contain dense cytoplasm and prominent nucleus. The large cell in the center, the central cell, is highly vacuolated and contains the diploid secondary nucleus formed by the fusion of two haploid polar nuclei.
- h) The filiform apparatus of the synergids is prominent.
- i) The antipodals show the presence of wall ingrowths for active absorption of nutrients.

Acknowledgement

Fig. 10.8 : <https://www2.palomar.edu/users/warmstrong/images/embsac4.jpg>

EXERCISE 11

TO STUDY ULTRASTRUCTURE OF MATURE EGG APPARATUS CELLS THROUGH ELECTRON MICROGRAPHS

Structure

11.1	Introduction	11.4	Procedure
	Objectives	11.5	Observations on Ultra structure of Egg Apparatus
11.2	Study Guide		
11.3	Material Required		

11.1 INTRODUCTION

You have already studied in unit 13 of the theory course that the egg apparatus in angiosperms differentiates at the micropylar end of the embryo sac and generally consists of the egg cell and two synergid cells. The egg cell is the female gamete which after syngamy forms the zygote. The synergids are ephemeral cells adjoining the egg cell and help in the process of fertilization. The egg and the two synergids are arranged in a triangular fashion and share common walls with each other and the surrounding central cell. This exercise will help you to appreciate and interpret the electron micrographs and you will be able to describe the ultrastructural features of the egg apparatus.

The walls of the egg and synergids are thicker at the micropylar region and become thinner toward the chalazal region of the cells.

Objectives

After going through these electron micrographs of the egg apparatus you would be able to :

- ❖ comprehend the fine structure of the egg cell and the two synergids; and
- ❖ appreciate the ultrastructure of the synergids in their supportive role with filiform apparatus.

11.2 STUDY GUIDE

For doing satisfactory work you must read the following Units before coming to the laboratory.

The UGC (CBCS) Course-Core Botany, Paper III - Plant Anatomy and Embryology (BBYCT-135), Unit-11, 12 and 13

11.3 MATERIALS REQUIRED

You will be provided with the electron micrographs of the egg apparatus of a monosporic embryo sac.

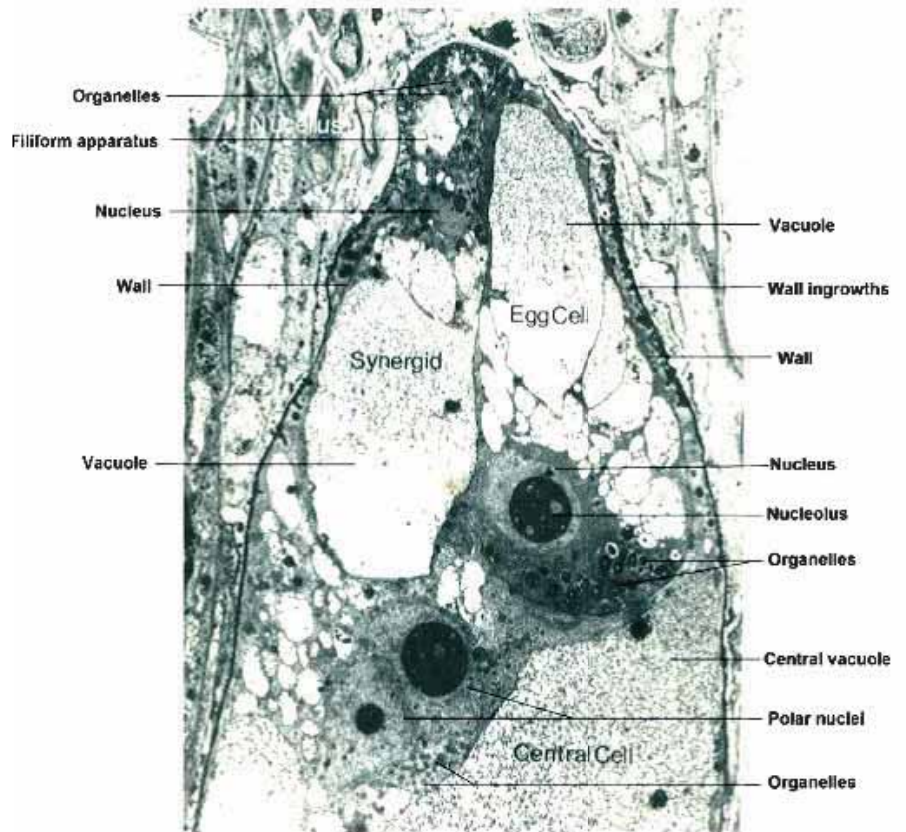
11.4 PROCEDURE

You must observe the electron micrographs provided to you carefully. Identify the various organelles in each cell. Make neat, well-labelled diagrams in your notebook and write comments.

11.5 OBSERVATIONS ON ULTRASTRUCUTRE OF EGG APPARATUS

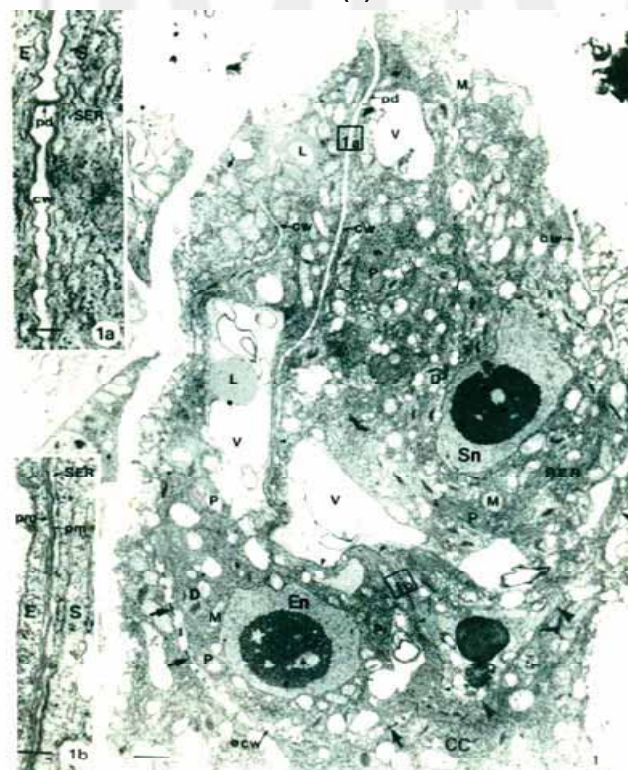
The electron micrographs of the egg apparatus reveal the following ultrastructural features:

- The micrograph (Fig. 11.1a) shows sections of a synergid cell, egg cell and part of central cell.
- The synergid cell which is positioned on the left is covered by a clear wall and contains a large vacuole toward the chalazal end. However, most of the cytoplasm along with the nucleus are localized or concentrated at the micropylar end.
- A portion of wall ingrowths of filiform apparatus (functioning as transfer cells) is also seen at the micropylar end.
- The cell positioned on the right is egg cell. A wall delimits the cell. The cell contains nucleus and large amount of cytoplasm at the chalazal end while the micropylar part is occupied by a large vacuole and less cytoplasm.
- Two polar nuclei of the central cell are also seen positioned adjacent to the egg apparatus. A part of the central vacuole is also observed. Ingrowths of embryo sac wall/central cell wall are seen at the micropylar region around the egg apparatus cells.



Transmission electron micrograph of micropylar region of embryo sac with a synergid and an egg cell

(a)



Transmission electron micrograph of micropylar region of embryo sac with an egg cell and a synergid

(CC- central cell, CW- cell wall, D- dictyosome, E- egg cell, ecw- egg cell wall, En- egg nucleus, L- lipid, M- mitochondrion, P- plastid, pd- plasmodesmata, pm- plasma membrane, RER- rough endoplasmic reticulum, S- synergid cell, SER- smooth endoplasmic reticulum, Sn- synergid nucleus. V- vacuole)

(b)

Exercise 11 To Study Ultrastructure of Mature Egg Apparatus Cells Through Electron Micrographs

- An egg cell is observed on the left side of the electron micrograph (Fig. 11.1 b). Wall of the egg cell is seen to be thicker toward micropylar region.
- The wall gradually thins and becomes discontinuous at the chalazal region (arrows). Plasmodesmata are present in the wall of the micropylar region (inset 1a). There are discontinuities at the chalazal region of the wall which show electron dense deposits (arrows).
- The nucleus is present at the chalazal end of the cell and is surrounded by a large amount of cytoplasm.
- A vacuole is seen towards the micropylar end. The cytoplasm is rich in mitochondria, plastids, endoplasmic reticulum, dictyosomes and ribosomes. Very few vesicles are observed around the dictyosomes and ER indicating their inactive state.
- The synergid is seen on the right. It shows a thicker wall towards micropylar region which becomes thinner towards the chalazal region (arrowheads). The wall appears to be absent at the chalazal-most region (inset 1b).
- Part of chalazal vacuole is also visible. The micropylar part is filled with dense cytoplasm and nucleus is also present.
- The cytoplasm is rich in plastids, mitochondria, ribosomes, endoplasmic reticulum and dictyosomes.
- Numerous vesicles are also seen in the cytoplasm, especially around dictyosomes, indicating that this organelle is in an active state.

Acknowledgements

Fig. 11.1a : Wilms, H.J. 1981. Ultrastructure of the developing embryo sac of spinach. Acta Bot. Neeri. 30: 75-99

Fig. 11.1b : Summer, M.J. and Van Caseele, L. 1989. The ultrastructure and cytochemistry of the egg apparatus of *Brassica campestris*. Can. J. Bot. 67: 177-190

EXERCISE 12

TO STUDY POLLINATION TYPES AND SEED DISPERSAL MECHANISMS WITH THE HELP OF PHOTOGRAPHS AND SPECIMENS

Structure

12.1	Introduction Objectives	12.6	Observations on Seed Dispersal Mechanisms Anemochory (Dispersal by Wind)
12.2	Study Guide		Hydrochory (Dispersal by Water)
12.3	Material Required		Autochory (Dispersal by Mechanical means)
12.4	Procedure		Zoochory (Dispersal by Animals)
12.5	Observations on Pollination Mechanisms Anemophily (Pollination by Wind) Hydrophily (Pollination by Water) Zoophily (Pollination by Animals)		

12.1 INTRODUCTION

You have already read about pollination and the dispersal of seeds in Unit 15. You are also aware that pollination is the act of transferring pollen grains from the male part of the plant i.e., anther of a flower to the female part of the plant i.e., stigma.

Plants can be: Self-pollinating (autogamy) - where the plant can fertilize itself, and Cross-pollinating - the plant needs a vector (a pollinator or wind or water)

to get the pollen to another flower of the same species either on the same plant (Geitonogamy) or on different plants (Xenogamy). In genetic sense, it is Xenogamy which is considered to be true cross pollination within a species.

In the present exercise you will be made familiar with various agencies (both abiotic and biotic) which facilitate the transfer of pollen during cross pollination.

In addition, you will also know about the dispersal mechanisms in angiosperms. Seed dispersal is the movement or transport of seeds away from the parent plant. Plants have very limited mobility and consequently rely upon a variety of dispersal vectors to transport their propagules, including both abiotic and biotic vectors.

The means by which seeds are dispersed depend on a seed's structure, composition, and size. Seeds can be dispersed by wind, water, animals or by mechanical means. Plants show various structural modifications/adaptations which help in the dispersal of their propagules. Modifications like aril, caruncle and elaiosomes also help in seed dispersal.

This exercise will make you familiar with diverse aspects of pollination and seed dispersal mechanisms.

Objectives

After going through the specimens and photographs of various vectors responsible for pollination and means of dispersal of seeds/fruits you would be able to appreciate:

- ❖ various abiotic and biotic agencies operative in pollination and seed and fruit dispersal;
- ❖ structural modifications in the plants to ensure pollination and seed/fruit dispersal; and
- ❖ morphological specializations/modifications seen in the pollinators and fruit dispersing biotic agents themselves illustrating co-evolution.

12.2 STUDY GUIDE

For doing satisfactory work you must read the following Units before coming to the laboratory.

The UGC (CBCS) Course -Core Botany, Paper III - Plant Anatomy and Embryology (BBYCT-135), Unit 10 and Unit-15.

12.3 MATERIALS REQUIRED

You will be provided with various specimens and photographs depicting the various types of pollinating and fruit dispersing mechanisms.

12.4 PROCEDURE

Draw the various figures from the pictures provided to you in your practical records book. Write suitable notes on each pollinating agency (viz.,

Anemophily, Hydrophily, Entomophily, Ornithophily, Malacophily and Chiropterophily) and seed dispersal agency (viz., **Anemochory, Hydrochory, Zoochory and Autochory**) and special modifications like **aril, caruncle** and **elaiosomes**.

12.5 OBSERVATIONS ON POLLINATION MECHANISMS

You will observe and sketch the following Pollination Types:

12.5.1 Anemophily (Pollination by Wind)

Flowers show following modifications :

- i) Anemophilous or wind-pollinated flowers are inconspicuous and not showy.
- ii) They are also devoid of scent or nectar.
- iii) They produce a very large quantity of dusty pollens (Figs. 12.1, 12.2).



Fig.12.1: Windblown pollen from the pine *Pinus contorta* (*Encyclopedia Britannica*).



Fig. 12.2: Pollen being released from *Carex pendula* (Pendulous Sedge grass).

- iv) Stigma is branched and bushy and is capable of catching pollens from air easily e.g., cereals (Fig. 12.3).
- v) Flowers are often unisexual and occur in bunches.

- vi) The anthers are often versatile, swinging freely in air and the pollens are dry, light, and smooth-walled (e.g., Grasses).

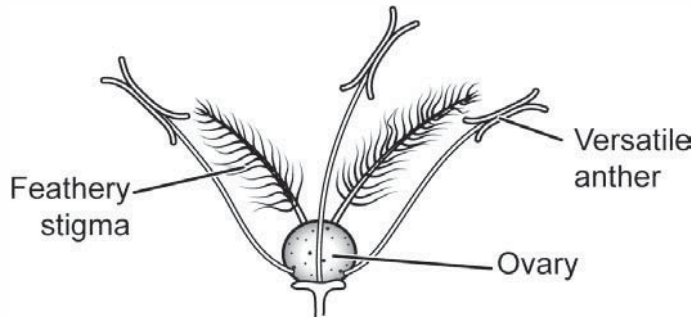


Fig. 12.3: Feathery stigmas and versatile anthers in a flower of grass to facilitate trapping of pollen.

12.5.2 Hydrophily (Pollination by Water)

Flowers show following modifications:

- i) Flowers are small and inconspicuous,
- ii) Perianth and other floral parts are unwettable,
- iii) Nectar and odor are absent,
- iv) Pollen grains are light and unwettable due to presence of mucilage cover,
- v) Stigma is long, sticky but unwettable. Hydrophily is seen in members of the families Ceratophyllaceae, Potamogetonaceae, and Hydrocharitaceae.

Hydrophily occurs only in some 30 genera of mostly monocots e.g., *Vallisneria*, *Zostera*, and *Ceratophyllum*. Interestingly, many aquatic plants with emergent flowers, pollination occurs by wind or insects and not by hydrophily, e.g., Lotus, Water Lily, Water hyacinth.

Hydrophily is of two types - **hypohydrophily** and **epihydrophily**. Hypohydrophily occurs below the surface of water, e.g., *Zostera*, *Ceratophyllum* (Fig. 12.4) and *Najas*.

Epihydrophily takes place over the surface of water, e.g., *Vallisneria*.



Fig. 12.4 : *Ceratophyllum*

- Pollination takes place on the water surface (epihydrogamous) in the common water weeds like *Vallisneria* and *Hydrilla*.
- The dioecious plant *Vallisneria* (Fig.12.5) having strap-shaped leaves grows in the mud at the bottom of stagnant water.

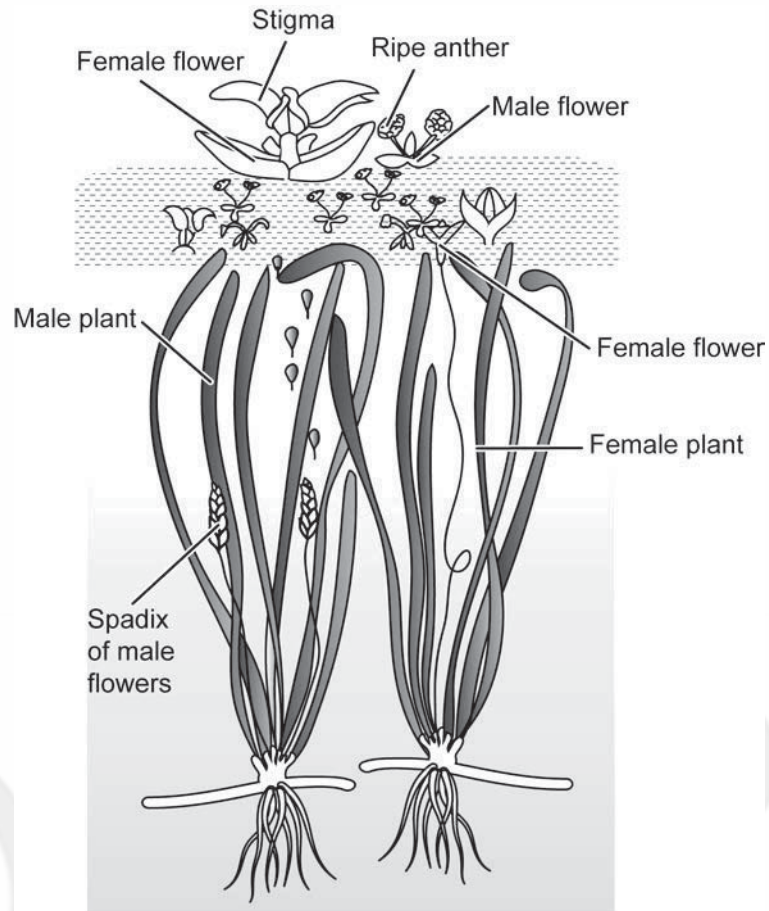


Fig. 12.5: Epiphyllous in *Vallisneria spiralis*.

- The male flowers are borne low down amongst the radical leaves on short-stalked spadix inflorescences out of which the individual flowers get detached and float freely in large numbers on the water surface.
- These flowers open on the surface and the three perianth leaves open widely exposing the two stamens vertically.
- The female flowers are borne singly on long wiry stalks which grow in such a manner that the flowers float on the water surface when mature.
- As the female flower is somewhat waxy, it causes a slight depression in the film of water because of surface tension. This depression, as well as wind, causes the detached male flowers to cluster around the floating female flower and, when the anthers burst, sticky pollens get attached to the stigma.
- Soon after pollination, the long stalk of the female flower begins to coil bringing the female flower again below water level until it reaches almost the tank base where the fruit matures.

12.5.3 Zoophily (Pollination by Animals)

It is pollination through the agency of animals. Insects are the most common type of animal pollinators (**Entomophily**). Other pollinators are birds

(**Ornithophily**), bats (**Chiropterophily**), snails (**Malacophily**), human beings (**Anthophily**). Some primates (e.g., Lemurs), arboreal rodents and reptiles (Gecko Lizard, Garden Lizard) have also been found to accomplish pollination inadvertently.

i) Entomophily

- Insect-pollinated flowers are made attractive to insects in different ways (color, scent, nectar, edible pollen).
- The pollens are sticky with a rough surface so that they may easily stick to insect limbs.
- The stigma also is similarly sticky to be able to receive the pollens more easily. **Moths** and **Butterflies** (Figs 12.6, 12.7) are very efficient insect pollinators (**Lepidopterophily**).



Fig.12.6: Pollination by butterfly in *Trifolium pretense* (Red clover).



Fig.12.7: Pollination by Carolina moth in *Nicotiana tabacum*.

Some flies pollinate flowers of the Arum family (Fig. 12.8)



Fig.12.8: Pollination by fly in skunk cabbage *Symplocarpus foetidus*.

- Bees are the most common among the insect pollinators (**Melittophily**).

- They obtain pollen grains and nectar and collect them in pollen baskets (Fig. 12.9).



Fig.12.9: Pollination by honeybee.

- Although ants with their smooth bodies are not considered to very effective in transferring pollen grains, they are known to pollinate flowers of *Coronopus didymus*, *Medicago sativa* and *Melilotus officinalis*.
- Pollination by ants is called **Myrmecophily**.

Special Adaptations Seen in Entomophilous Flowers :

- Some plants have special adaptations which ensure cross pollination by the insect visitors. For example, a **turn-pipe or lever-mechanism** operates in flowers of *Salvia* to promote cross pollination. *Salvia*, which is pollinated by bees, has protandrous flowers with bilipped corolla (Fig. 12.10). The lower lip functions as a landing platform for the insect. Each stamen possesses a long connective which bears a fertile anther lobe at the upper end and sterile plate-like anther lobe at the lower end. Two sterile anther plates are present side by side and block the path of the insect.

As the insect moves inward a young flower in search of nectar, its head pushes the sterile anther plates and forces the fertile anther lobes to strike against its back. At the same time, the style in older flowers brings the stigma in such a position that it brushes against the back of the insect and collects pollen grains brought by the insect from a young flower.

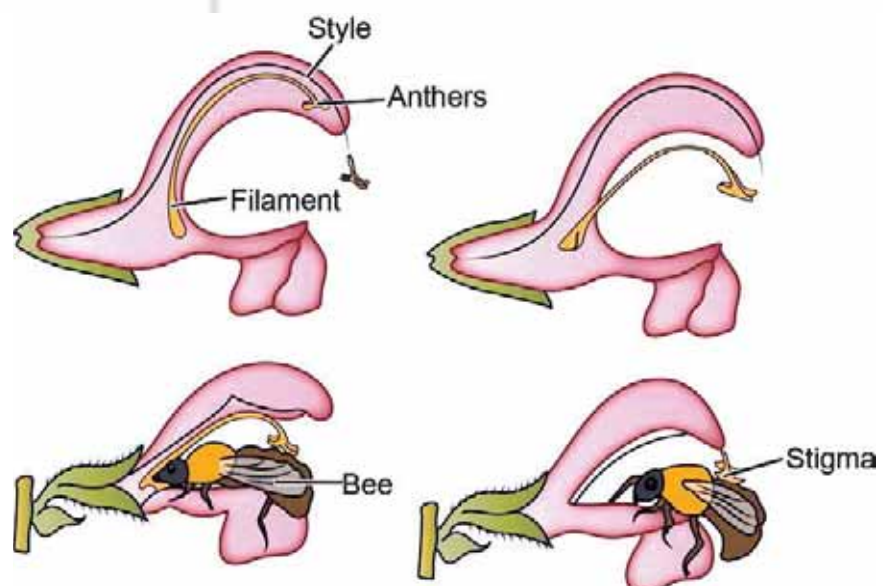


Fig. 12.10: Turn-pipe or lever mechanism in *Salvia*.

- b) Flowers of Fig (*Ficus carica*) and their pollinating agent, the female gall wasp *Blastophaga* show mutual dependence. Hypanthodia of the plant possess gall flowers for feeding the wasp. The wasp spends its early life inside the hypanthodium (Fig. 12.11).



Fig. 12.11: Fig –Moth association.

The young wasp coming out of the hypanthodium having mature male flowers drops the pollen inside another hypanthodium having mature female flowers (trap door mechanism). It deposits its eggs in gall flowers.

- c) Another interesting example of moth –flower association is seen between *Pronuba (Tageticula) yucca sella*, a moth which deposits its eggs in the ovary of *Yucca* flower. Simultaneously, it collects pollen and deposits the same in the hollow of stigma to effect pollination (Fig. 12.12).



Fig. 12.12: *Pronuba* moth on *Yucca* flowers.

- d) Pseudocopulation: In several species of the orchid like *Ophrys speculum* the shape, color, markings, and odor of the flowers is like the female moth *Colpa* (Fig. 12.13). The *Ophrys* employs sexual deceit to look like the female to get pollination done by the moth. The male moth matures earlier than the female. It mistakes the *Ophrys* flower for female *Colpa* moth and tries to copulate (pseudocopulation). In this attempt, it pollinates the flowers.



Fig. 12.13: Pseudocopulation in the orchid *Ophrys*

ii) Ornithophily

- Bird-pollinated flowers are few. These flowers tend to be large, colorful, often tubular and secrete copious nectar. They also tend to be unscented.
- Tiny birds like the hummingbirds and the honey-thrushes feed on the nectar of flowers like *Bignonia capreolata* and *Fuchsia magellanica* (Fig. 12.14) and pollinate them.
- Large flowers of *Strelitzia* (Musaceae) are pollinated by a honey bird called *Nectarina afra*.
- Silk-cotton (*Salmalia* or *Bombax*), *Erythrina* and a few other trees are visited by crows and mynas when in flowering and play part in the pollination.



Fig.12.14: Pollination in *Fuchsia magellanica* flower by hummingbird.

- Some other pollinating birds are Crow, Bulbul, Parrot and Mynah.
- Ornithophilous plants are very few as compared to entomophilous plants.
- Common bird pollinated plants are *Bombax* (Red Silk Cotton), *Erythrina* (Coral Tree), *Callistemon* (Bottle Brush), *Butea monosperma*, *Bignonia*, *Lobelia*, *Agave*, and *Grevillea*.

iii) Chiropterophily

- It is cross pollination performed by bats (Fig. 12.15).
- Bats are nocturnal flying mammals which can transport pollen over long distance, sometimes over 30 km.
- Chiropterophilous flowers are dull-colored and showy with strong fermenting or musty odor, abundant nectar, and pollen grains.
- Flowers secrete even more abundant nectar than the ornithophilous flowers. Flowers open at night.
- They are often large, bell-shaped and have a ball of stamens. Flowers are typically borne away from the trunk or other obstructions to facilitate easy access to bats.
- Common examples of chiropterophilous plants are *Kigelia pinnata* (Sausage Tree), *Adansonia dictate* (Baobab Tree), *Anthocephalus* (Kadam Tree) and *Bauhinia megalandra* and *Eperua falcate*.



Fig. 12.15: Pollination by bats in Saguaro cactus.

iv) Malacophily

- Snails are known to perform pollination in *Arisaema* (Snake or Cobra Plant) and some arum lilies (Fig 12.16).



Fig. 12.16: Pollination by snail.

v) Anthophily (Artificial Pollination)

- Plants are hand pollinated to ensure cross pollination between selected varieties in the artificial breeding programs, e.g., artificial pollination has been an age-old practice in date-palm.

12.6 OBSERVATIONS ON SEED DISPERSAL MECHANISMS

Seeds are dispersed by various agencies. Now you will study the various modifications shown by plants.

12.6.1 Anemochory (Dispersal by Wind)

For easy dispersal by wind seeds must be light so that their buoyancy may enable them to float on air over long distances. Plants that produce wind-blown seeds e.g. *Dandelion* often produce lots of seeds to ensure that some of the seeds are blown to areas where the seeds can germinate.

Seeds specially adapted for wind dispersal are characterized by the following :

Very small, dry, and dusty seeds e.g. Orchid's seeds are carried by wind like pollens. Seeds of *Cinchona* are also extremely small and winged. Certain seeds are provided with appendages which act like parachutes in helping them to float in air (parachute mechanism).

Coma is a tuft of hair developed as a crown on the seeds of *Calotropis*, *Holarrhena*, *Alstonia* (two tufts) and most plants of Apocynaceae and Asclepiadaceae. Hairy outgrowths on the testa completely cover cotton seeds.

Seeds of *Moringa oleifera*, *Oroxylum indicum*, *Lagerstroemia speciosa*, *Swietenia mahogany*, *Cinchona*, etc., are provided with wings developed from the testa (Fig.12.17 a-e).



Fig. 12.17: Wind-dispersed seeds. a) *Dandelion*; b) *Calotropis*; c) *Alstonia*; d) *Lagerstroemia*; e) *Moringa*

12.6.2 Hydrochory (Dispersal by Water)

Seeds dispersed by water are provided with a coat which is waterproof, salt-resistant, and buoyant. Seeds of many aquatic plants like waterlily, *Alisma*, *Sagittaria*, and *Nelumbo* (Fig.12.18a) are very light and waterproof so that they can float easily. Many of these seeds are provided with spongy arils rendering them more buoyant. Seeds dispersed by water are contained in light and buoyant fruit, giving them the ability to float e.g., coconuts (Fig. 12.18 b). Similarly, willow and silver birches produce lightweight fruit that can float on water.



Fig. 12.18.: Seed dispersal by water. a) *Nelumbo*; b) Coconut.

12.6.3 Autochory (Dispersal by Mechanical means)

All dehiscent fruits scatter the seeds when they burst. This dehiscence is accompanied by the expression of great force in many fruits so that seeds are jerked a considerable distance away from the mother plant. Such fruits are called explosive fruits.

Legumes of the mountain climber *Bauhinia vahlii* similarly explode with loud noise scattering the seeds yards away in all directions. The ripe pods peas,

beans etc., suddenly twist on bursting and thus scatter the seeds. Ripe fruits of *Impatiens balsamina* (balsam) explode suddenly when touched. The valves are bent, and the seeds are jerked off forcibly. In certain fruits the seeds are discharged through small openings on the fruits. The outlets are so narrow that only a few seeds can escape at a time. In *Argemone mexicana* and the poppies of Papaveraceae, after the apertures are opened by porous dehiscence, as the capsule swings in air, the minute seeds are dispersed through the pores (Fig.12.19).



Fig. 12.19: Capsule of Poppy.

12.6.4 Zoochory (Dispersal by Animals)

Animals can disperse seeds by excreting or burying them.

Seeds of many plants are provided with hooks, spines, barbs, or stiff hairs so that if an animal grazes or brushes against them, these stick to the animal's body or clothing (Fig.12.20).

Many seeds, as those of *Aegle marmelos*, *Plumbago*, Mistletoe, etc., are sticky and are thereby benefited. Humans also play a role as dispersers by moving fruit to new places and discarding the inedible portions containing the seeds.

In some seeds, post-fertilization developments in seed coats leads to formation of appendages like aril, caruncle, elaiosomes etc. which help in their dispersal.



Fig.12.20: Seed Dispersal with the help of Animals.

i) Aril

An aril, also called an arillus. It is a specialized outgrowth from a seed that partly or completely covers the seed. An aril grows from the attachment point of the seed to the ovary (from the funiculus or hilum). It is regarded as third

integument. Arils are often edible enticements, encouraging transport by animals and thereby assisting in seed dispersal. Examples - Nutmeg, pomegranate, and Litchi (Fig. 12.21).



Fig. 12.21: Arils. a) *Myristica* and b) Litchi.

ii) Caruncle

It is also known as Strophiole. It is a fleshy, whitish beak like structure arises due to proliferation of cells at the tip of outer integuments is called as caruncle e.g. Castor (*Ricinus communis*) (Fig. 12.22). This structure has the ecological function of promoting seed dispersal by ants (myrmecochory).

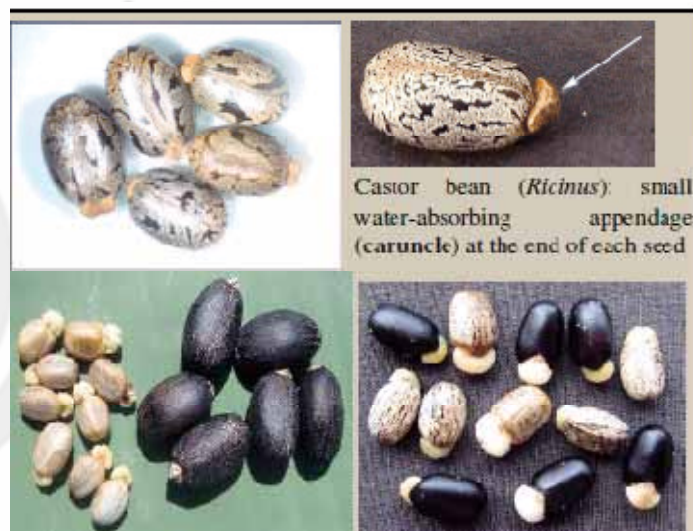


Fig. 12.22: Caruncles in *Ricinus communis*.

iii) Elaiosomes

Elaiosomes ("oil body") are fleshy structures that are attached to the seeds of many plant species. The elaiosome is rich in lipids and proteins and may be variously shaped. Many plants have elaiosomes that attract ants, which take the seed to their nest and feed the elaiosome to their larvae. After the larvae have consumed the elaiosome, the ants take the seed to their waste disposal area, which is rich in nutrients from the ant frass and dead bodies, where the seeds germinate. This type of seed dispersal is termed myrmecochory.

This is mutualistic relationship, as the plant benefits because its seeds are dispersed to favorable germination sites, and because it is planted (carried underground) by the ants. Elaiosomes mostly develop from seed tissues (chalaza, funiculus, hilum, raphe) and serve the main function, i.e., attracting ants (Fig.12.23).



Fig.12.23: Elaiosomes attracting ants.

Acknowledgements

- Fig. 12.1** : <https://www.google.co.in/search?hl=en&tbm=isch&sxsrf=AC YBGNQZQfRZ4DVQKrw-p5uZumcDQfGpYw%3A1580192584857&source=hp&biw=1093&bi>
- Fig. 12.2** : <https://cdn.britannica.com/s:700x500/19/5619-004-06529655/Spikes-sedge-showing-inflorescences-parts-wind-pollination.jpg>
- Fig. 12.3** : https://www.google.co.in/search?hl=en&tbm=isch&sxsrf=AC YBGNS2fvwk-kEoTf_a9x6CeAWFKdZvYA%3A1580193820251&source=hp&biw=1093&bih=486&ei=HNqvXpruDPjYz7sP_LSAgA4&q=Feathery+stigma+for+anemophily&og=Feathery+stigma+for+anemophily&gs_l=img.3...1908.18240..19049...1.0..0.269.475.1.0j26j4.....0....1..gws-wiz-img.....35i39j0i0i131j0i8i30j0i24j0i30.fc2dkgu8V2o&ved=0ahUKEwia7NHa2KXnAhV47HMBHXwaAOAQ4dUDCAY&uact=5#imgrc=ZC9AbHd2xvCUxM:
- Fig. 12.6** : <https://beehealth.bayer.us/-/media/Bayer-CropScience/Marketing/BeeHealth/Who-Can-Help/Gardeners/Butterflies-Bats-Birds/Feed-a-Bee-19.ashx?h=500&w=750&la=en&hash=9FE443DBDA217215D3716EEA9C68CCEFE300D2F5>
- Fig. 12.7** : https://www.chicagobotanic.org/images/plantinfo/smartgardener/sweatbee_alexwild.jpg
- Fig. 12.8** : <https://i.ytimg.com/vi/vltDGUooUvU/hqdefault.jpg>
- Fig. 12.9** : <https://i.pinimg.com/564x/5d/fc/35/5dfc354c0efeb5bf8f0eaae3292c475c.jpg>

- Fig. 12.10** : <http://www.drgpbiology.com/wp-content/uploads/2017/04/Pollination-in-Salvia.png>
- Fig. 12.11** : https://perryponders.com/wp-content/uploads/2018/07/figs-2619978_1920.jpg
https://www.eurekalert.org/multimedia/pub/web/179274_web.jpg
- Fig. 12.12** : <https://19mvmv3yn2qc2bdb912o1t2n-wpengine.netdna-ssl.com/science/files/2013/03/yucca-moth.jpg>
- Fig. 12.13** : <http://www.orchidspecies.com/orphotdir/ophrysspeculum.jpg>
- Fig. 12.15** : <http://b50ym1n8ryw31pmkr4671ui1c64-wpengine.netdna-ssl.com/wp-content/blogs.dir/11/files/2014/06/Mexican-long-tonuged-bat-agave-MERLIN-TUTTLE-183x300.jpg>
- Fig. 12.16** : <https://www.growsmartgrowsafe.org/Images/ContentPages/SlugsAndSnails.jpg>
- Fig. 12.18** : https://abugseyeviewjc.weebly.com/uploads/4/7/6/4/47644513/6518800_orig.jpg
<https://i.pinimg.com/originals/94/0e/99/940e99584cf333afe952a406b87051a8.jpg>



EXERCISE 13

TO DISSECT ENDOSPERM FROM A DEVELOPING SEED

Structure

13.1	Introduction	13.4	Procedure
	Objectives	13.5	Observations
13.2	Study Guide	13.6	Precautions
13.3	Material Required		

13.1 INTRODUCTION

Endosperm is the nutritive tissue for the developing embryos in angiosperms. The endosperm is the product of double fertilization and is usually triploid. Sometimes it is consumed by the developing embryo or it may persist in mature seed to support the growth of embryo during germination. Endosperm of some species develops a special structure called haustorium which modifies variously and extremely to get the metabolites for developing embryo. Haustoria which develop at chalazal end are known as chalazal haustoria and the ones that develop at micropylar end are micropylar haustoria. In some plants haustoria develops at both the ends. In *Cucumis sativus* haustoria develops at the chalazal end. The endosperm is of nuclear type. The chalazal region extends into a long tubular haustorium with flattened spoon shaped tip.

Objectives

After studying this unit, you would be able to:

- ❖ to dissect out the endosperm along with haustorial region from the given seeds of *Cucumis sativus*;
- ❖ recognise endosperm haustoria; and
- ❖ describe its structure and possible functions.

13.2 STUDY GUIDE

For doing satisfactory work you must read the following before coming to the laboratory. The UGC (CBCS) Course of Core Botany (CBCS) - Botany Paper III: Plant anatomy and embryology, BBYCT-135, Unit 12-Endosperm.

13.3 MATERIALS REQUIRED

- Dissecting microscope
- Needles
- *Cucumis sativus* seeds at various stages of development
- 1.5% safranin
- Spirit lamp
- Slides
- Cover slips

13.4 PROCEDURE

You will be provided the seeds of *Cucumis sativus* at various stages of ripening.

1. Split open the seed in two parts with the help of a blade or scalpel and you will be able to see the gelatin-like endosperm haustorium attached at the chalazal end.
2. With the help of forceps and needle gently take out the haustorium and place it on a clean slide. Take out the endospermic haustoria carefully so that globular embryo at the micropylar end remains intact. You can see coenocytic tail like structure at the chalazal end.
3. Add few drops of safranin/acetocarmine and warm it a little. Then place a coverslip carefully and observe the morphology of the structure.

13.5 OBSERVATIONS

You should be able to observe a haustorium with a distinct globular end followed by long tubular region. The tubular region is coenocytic region. This region contains dense cytoplasm and numerous nuclei. The globular end is formed of cells. Draw a labelled diagram of the haustoria and embryo in your notebook as you see in your slide. Fig 13.1 has been provided for your reference.

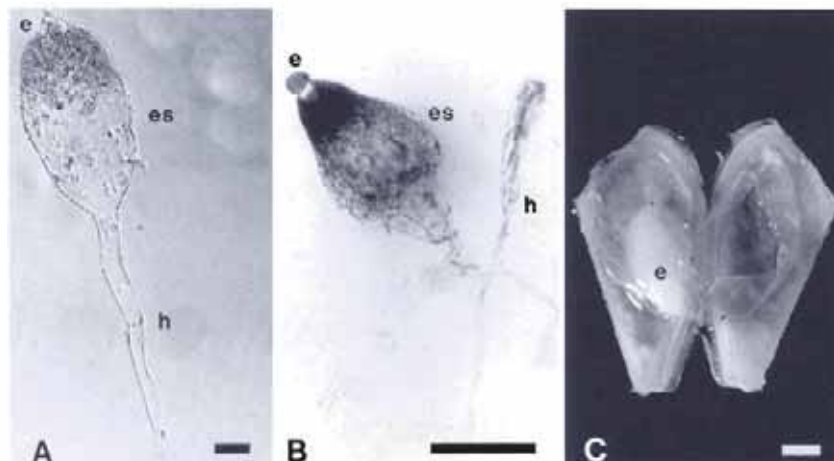


Fig 13.1: *Cucumis sativus*. a) Proembryo with free-nuclear endosperm; b) late globular embryo with cellular endosperm; c) Cotyledon embryo without endosperm, (just after dissection) h-haustorium, es-embryo sac.

13.6 PRECAUTIONS

1. Dissect out endosperm along with the embryo and haustorium carefully so that the entire structure is transferred to the slide.
2. Take care not to overheat the slide.

Acknowledgement

Fig.13.1 : <https://d3i71xaburhd42.cloudfront.net/5ba2b21bdc23676924960c2a94bdbbbac48bdc24/3-Figure2-1.png>



EXERCISE 14

TO CALCULATE PERCENTAGE OF GERMINATED POLLEN IN A GIVEN MEDIUM

Structure

- | | | | |
|------|------------------------------|------|--------------------------|
| 14.1 | Introduction | 14.5 | Observations and Results |
| | Objectives | 14.6 | Precautions |
| 14.2 | Study Guide | | |
| 14.3 | Material Required | | |
| 14.4 | Procedure | | |
| | Study of Pollen grains | | |
| | Preparation of Medium | | |
| | Preparation of Hanging Drop | | |
| | Germination of Pollen Grains | | |

14.1 INTRODUCTION

Flowering plants are diploid and specialised organs for reproduction are present in flower. The stamens are the male organs. In most angiosperms each stamen is composed of an anther and a filament. Anther mostly contains four microsporangia which are joined to the connective. The anther wall consists of four layers: i) The epidermis (exothecium), ii) endothecium, iii) middle layer(s), and iv) tapetum. In each microsporangium the central region contains microspore mother cells, which eventually forms the pollen grains.

Each microspore mother cell undergoes meiosis resulting in the formation of tetrad of cells. Each cell of this tetrad has one haploid nucleus. Each microspore starts to differentiate while still associated in a tetrad and gives rise to pollen grain.

The second phase is microgametogenesis, where a pollen grain undergoes mitotic division and forms a large vegetative cell and a small generative cell.

The pollen grain at this stage is 2-celled. The generative cell in many species divides to form two sperm cells, before the germination of pollen tube. The pollen grains at this stage are termed 3-celled pollen grain. Pollen grains (whether 2-celled or 3-celled) are carried to the stigma by various agents. Pollen grain after landing on a stigma germinates by producing pollen tube through germination aperture (germ pore). Pollen tube travels through stigma and style and reaches the embryo sac and finally enters the ovule where double fertilization occurs.

Objectives

After studying this unit, you would be able to:

- ❖ know the structure of pollen grains;
- ❖ observe the pollen germination through spore aperture or germ pore; and describe the temporal growth of pollen tube; and
- ❖ calculate the germination percentage of pollen given for experiment.

14.2 STUDY GUIDE

For doing satisfactory work you must read the following before coming to the laboratory. The UGC (CBCS) Course of Core Botany (CBCS)- Botany Paper III: Plant anatomy and embryology BBYCT-135 ,Unit 10-Pollination.

14.3 MATERIAL REQUIRED

1. Pollen grains from *Tradescantia*, *Vinca* and *Impatiens*
2. Slides, Cavity slides, Cover slips
3. Brewbaker and Kwack medium
4. Dissecting instruments
5. Microscope

14.4 PROCEDURE

Here for studying the germination of pollen grains you will use hanging drop technique. But first you must learn and practice the technique of making hanging drops as described below and then use the technique for the study of germination of pollens.

a) Study of Pollen grains

It will be beneficial to you if you get acquainted with the pollen grains (Fig. 14.1). First, examine whole mount of the pollen grains under lower power and then under high power of the microscope (14.2). Draw the structure of pollen grains and then observe the difference in size, surface appearance and shape.



Fig. 14.1: Pollen and plant of *Tradescantia*.

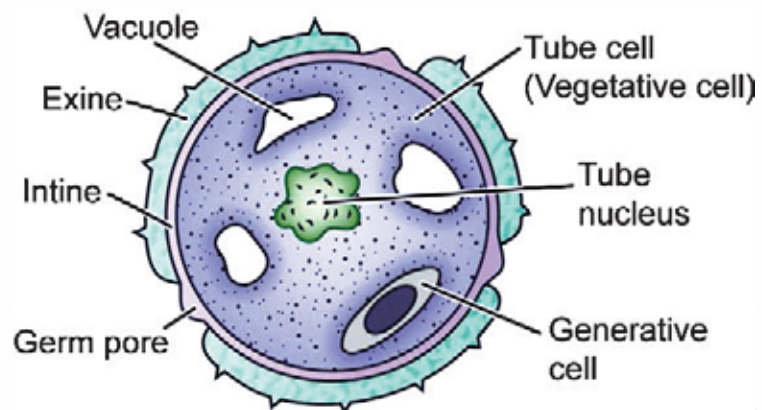


Fig 14.2: Diagrammatic representation of mature pollen grain.

b) Preparation of medium

The composition of some of the successful media used pollen germination are given below :

Composition of Brewbaker and Kwack medium

- 10% sucrose
- 100mg l⁻¹ boric acid
- 300mg l⁻¹ calcium nitrate
- 200mg l⁻¹ magnesium sulfate
- 100mg l⁻¹ potassium nitrate

First, you must prepare Stock solutions of all the constituents, except sucrose and then store it in refrigerator at 4° C. Sucrose should be weighed and added at the time of medium preparation. Freshly prepared Brewbaker & Kwack medium should be used as and when required to test the *in vitro* pollen germination.

Germination of pollen grains will be carried out using hanging drop and suspension culture methods which is described below.

c) Preparation of Hanging drop

- 1) Apply a thin film of petroleum jelly or any sealing substance around the rim of the cavity slide.

- 2) Place carefully 50 μl drop of culture medium (described above) on a clean dry cover glass or coverslip. The volume of the culture medium drop should be such that it does not spread and come in contact with the rim or the bottom of the cavity, or with the sealing substances.
- 3) Add a suitable amount of pollen grains to the medium drop and mix thoroughly with a needle to obtain a homogeneous pollen suspension.
- 4) Carefully invert the cover glass with the pollen suspension over the cavity such that the pollen culture drop is suspended in the center of the cavity. Pollen grains move to the lower meniscus of the hanging drop and are exposed to the atmosphere of the cavity.
- 5) Apply gentle pressure around the edges of the coverslip, to seal the cavity (with the coverslip and the sealing material applied earlier around the rim of the cavity or edge of the coverslip, Fig.14.3).

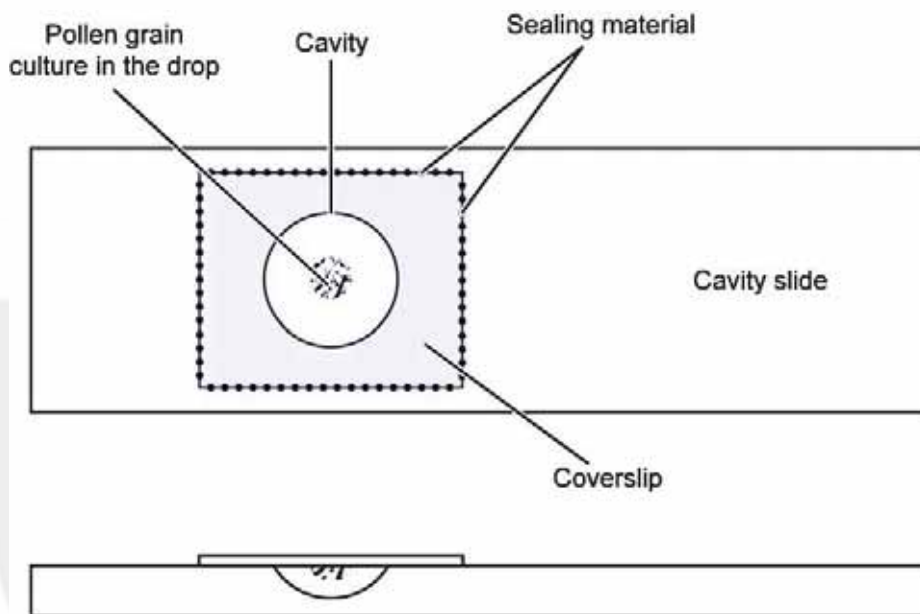


Fig. 14.3: A hanging drop culture from two views, a) Frontal- view of slide; b) Side- view of slide with pollen grains in the culture drop.

- 6) Now label the culture slide. The slide is ready for observation. Record the time taken for germination.

d) Germination of Pollen Grains

At the time of experiment the laboratory temperature preferably should be between 25-30°C. Otherwise if the temperature is too low you must maintain the temperature by incubating the slide in an incubator at 25°C.

14.5 OBSERVATIONS AND RESULTS

Examine after half an hour, and further at half-hourly intervals for the next three hours. Each time count the number of pollen grains germinated. Estimate the rate of germination of pollens over the period and calculate the percentage. If you have time you can repeat using other samples of pollen. When the sample is well germinated you can transfer it to another slide, stain with acetocarmine and examine the tube structure and two male nuclei (Fig. 14.4).

- Several pollen grains germinate and put forth pollen tubes. Count the total number of pollen grains and the number of germinated pollen grains in 3-5 different microscope fields. Tabulate your observations and calculate the percentage of pollen germination.
- Name of the plant used as source of pollen.....
- Number of pollen grains in a field of microscope = N
- Number of germinated pollen grains in a field of microscope = n
- Percent pollen germination = $n / N \times 100$ or $100n / N$
- Percent pollen germination

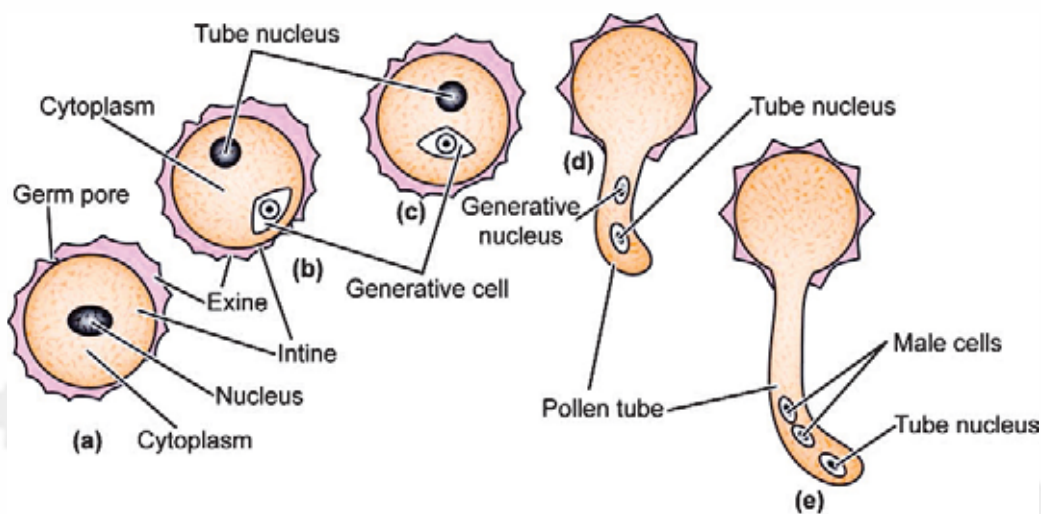


Fig. 14.4: Various stages in the germination of pollen grain.

Table 14.1: Calculation of percentage pollen germination in *in-vitro* pollen culture.

Microscope Field No.	No. of Pollen grains in the field	No. of Pollen grains germinated	Percentage germination
1)			
2)			
3)			
4)			
5)			

$$\text{Percent pollen germination} = \frac{\text{Pollengerminated}}{\text{Total pollen observed}} \times 100$$

Acknowledgement

Fig. 14.1 : <https://encryptedtbn0.gstatic.com/images?q=tbn%3AANd9GcQfD45UxOE4QD-7U6Q8T4SN9ooSAleQzUPRBIXs18-btqPwzbG9>

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