

CELL BIOLOGY AND PLANT STRUCTURE AND DEVELOPMENT

M.Sc. BOTANY
SEMESTER-II, PAPER-VI

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M.Sc. BOTANY: CELL BIOLOGY AND PLANT STRUCTURE AND DEVELOPMENT

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FOREWORD

Since its establishment in 1976, Acharya Nagarjuna University has been forging ahead in the path of progress and dynamism, offering a variety of courses and research contributions. I am extremely happy that by gaining 'A+' grade from the NAAC in the year 2024, Acharya Nagarjuna University is offering educational opportunities at the UG, PG levels apart from research degrees to students from over 221 affiliated colleges spread over the two districts of Guntur and Prakasam.

The University has also started the Centre for Distance Education in 2003-04 with the aim of taking higher education to the door step of all the sectors of the society. The centre will be a great help to those who cannot join in colleges, those who cannot afford the exorbitant fees as regular students, and even to housewives desirous of pursuing higher studies. Acharya Nagarjuna University has started offering B.Sc., B.A., B.B.A., and B.Com. courses at the Degree level and M.A., M.Com., M.Sc., M.B.A., and L.L.M., courses at the PG level from the academic year 2003-2004 onwards.

To facilitate easier understanding by students studying through the distance mode, these self-instruction materials have been prepared by eminent and experienced teachers. The lessons have been drafted with great care and expertise in the stipulated time by these teachers. Constructive ideas and scholarly suggestions are welcome from students and teachers involved respectively. Such ideas will be incorporated for the greater efficacy of this distance mode of education. For clarification of doubts and feedback, weekly classes and contact classes will be arranged at the UG and PG levels respectively.

It is my aim that students getting higher education through the Centre for Distance Education should improve their qualification, have better employment opportunities and in turn be part of country's progress. It is my fond desire that in the years to come, the Centre for Distance Education will go from strength to strength in the form of new courses and by catering to larger number of people. My congratulations to all the Directors, Academic Coordinators, Editors and Lesson-writers of the Centre who have helped in these endeavors.

*Prof. K. Gangadhara Rao
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M.Sc. BOTANY
SEMESTER-II, PAPER-VI
PRACTICAL-II
206BO24-CELL BIOLOGY AND PLANT STRUCTURE AND
DEVELOPMENT
SYLLABUS

Cell Biology:

- 1) Preparation of Slides, Observation and Identification of Various Stages of Mitosis from *Allium Cepa* Roots
- 2) Determination of Mitotic Index (MI)
- 3) Effect of Chemical Mutagens on Dividing Cells (Mitosis)
- 4) Study of Meiotic Stages in Flower Buds of *Allium Cepa* / *Tradescantia Spathacea*
- 5) Study of Photographs of Chromosomes with Different Banding Patterns
- 6) Study of Microscopic Pictures of Polytene Chromosomes, Lampbrush Chromosomes, B-Chromosomes etc.

Plant Structure and Development:

- 1) Observe the Variations in Anatomical Features of Dicots
- 2) Observe the Variations in Anatomical Features of Monocots
- 3) Observation of Anomalous Secondary Growth in Dicots (*Boerhaavia* or *Bignonia*)
- 4) Observation of Anomalous Secondary Growth in Monocots (*Dracaena*)
- 5) Study the Variations in Anatomical Features of Leaves
- 6) Study of Structure and Types of Stomata
- 7) Study of Anatomical Differences Between C4 and CAM Plants

CONTENTS

S.No.	TITLE	PAGE No.
CELL BIOLOGY		
1	OBSERVATION AND IDENTIFICATION OF VARIOUS MITOTIC STAGES FROM <i>ALLIUM CEPA</i> (ONION) ROOTS	1-5
2	DETERMINATION OF MITOTIC INDEX	6-9
3	EFFECT OF CHEMICAL MUTAGENS ON DIVIDING CELLS (MITOSIS)	10-12
4	STUDY OF MEIOTIC STAGES IN FLOWER BUDS OF <i>ALLIUM CEPA</i>	13-16
5	STUDY OF PHOTOGRAPHS OF CHROMOSOMES WITH DIFFERENT BANDING PATTERNS	17-22
6	STUDY OF MICROSCOPIC PICTURES OF POLYTENE CHROMOSOMES, LAMPBRUSH CHROMSOMES AND B-CHROMOSOMES	23-28
PLANT STRUCTURE AND DEVELOPMENT		
1	VARIOUS TYPES OF TISSUES AND TISSUE SYSTEMS	29-38
2	NODAL ANATOMY	39-40
3	ANATOMICAL FEATURES OF DICOT ROOT	41-42
4	ANATOMICAL FEATURES OF MONOCOT ROOT	43-45
5	ANATOMICAL FEATURES OF DICOT STEM	46-47
6	ANATOMICAL FEATURES OF MONOCOT STEM	48-49
7	ANOMALOUS SECONDARY GROWTH IN DICOTS (<i>BOERHAVIA</i>)	50-51
8	ANOMALOUS SECONDARY GROWTH IN MONOCOTS (<i>DRACAENA</i>)	52-53
9	ANATOMICAL FEATURES OF DICOT LEAF (<i>NERIUM</i>)	54-55
10	ANATOMICAL FEATURES OF MONOCOT LEAF (<i>ZEA MAYS</i>)	56-57
11	STUDY OF STRUCTURE AND TYPES OF STOMATA	58-59
12	STUDY OF ANATOMICAL DIFFERENCES BETWEEN C4 AND CAM PLANTS	60-62

CELL BIOLOGY

EXPERIMENT-1

OBSERVATION AND IDENTIFICATION OF VARIOUS MITOTIC STAGES FROM *ALLIUM CEPA* (ONION) ROOTS

Aim: Preparation and study of mitosis in onion root tips

Principle: Somatic growth in plants and animals takes place by the increase in the number of cells. A cell divides mitotically to form two daughter cells wherein the number of chromosomes remains the same (i.e., unchanged) as in the mother cell. In plants, such divisions rapidly take place in meristematic tissues of root and shoot apices, where the stages of mitosis can be easily observed. In animals, mitotically dividing cells can be easily viewed in the bone marrow tissue of a vertebrate, epithelial cells from gills in fishes and the tail of growing tadpole larvae of frog. The root tip meristem is usually a denser white and more rounded than the cut end. Chromosomes in root tip tissue are made visible with the stain. Dividing cells (if present) will show up clearly with chromosomes in different forms according to the stage of mitosis. Individual chromosomes (as tightly-coiled threads) are visible during anaphase. The links between the cellulose walls of plant cells are broken down by the treatment with hydrochloric acid. This ensures that the stain can penetrate the cells and allows the tissue to be squashed out one cell thick.

Materials:

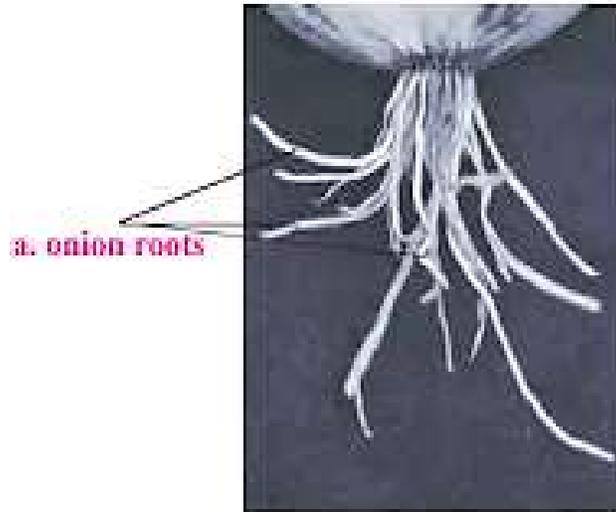
Plant material: Onion root tips

Chemicals: Carnoy's fixative / Acetic alcohol (acetic acid 1 part and ethyl alcohol 3 parts v/v), 2N hydrochloric acid, 70% ethanol, Acetocarmine or acetoorcein (2%), Acetic acid (45%).

Glassware and Equipment: Microscope slides and cover slips, Pasteur pipettes, Teasing needles, Fine forceps and razor blade, Watch glass, Filter paper / blotting paper, Compound microscope, Spirit lamp, Water bath.

Procedure:

- Select a few medium-sized onion bulbs and place the bulbs on glass tubes (of about 3-4 cm. diameter) filled with water and allow for the growth of root tips (Figure).
- Care should be taken so that the stem portion of the bulb (basal part) just touches the water. A few drops of water may be added periodically to compensate evaporation losses. New roots may take 3–6 days to grow.
- Cut 2–3 cm long freshly grown roots and transfer them to freshly prepared fixative, i.e., aceto-alcohol (glacial acetic acid and ethanol in 1:3 ratio).
- Keep the root tips in the fixative for 24 hours and then transfer them to 70% ethanol (for preservation and use in future).



Developed Onion Root Tips

Preparation of Slide:

- Take one or two preserved roots, wash them in water on a clean and grease free slide. Place one drop of N/10 HCl on the root tip followed by 2–3 drops of aceto-carmin or aceto-orcein stain on it.
- Leave the slide for 5–10 minutes on a hot plate (or warm it slightly on spirit lamp). Care should be taken that the stain is not dried up. Carefully blot the excess stain using blotting paper.
- Now cut the comparatively more stained (2–3 mm) tip portion of the root and retain it on the slide and discard the remaining portion. After (10–20 seconds) put one or two drops of water and blot them carefully using blotting paper.
- Again put a drop of water on the root tip and mount a cover slip on it avoiding air bubbles. Place the slide in between the folds of blotting paper using the fingers in such a way that the cover slip mounted on the slide is properly held.
- Now slowly tap the cover slip using the blunt end of a pencil so that the meristematic tissue of the root tip below the cover slip is properly squashed and spread as a thin layer of cells.
- Carefully seal the margins of the cover slip using molten paraffin wax or nail polish. This preparation of onion root tips cells is now ready for the study observation of mitotic cell division stages.

Study of Slide:

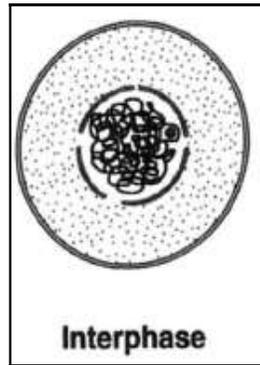
- Place the slide on the stage of a good quality compound microscope. First observe it under the lower magnification (10 X objective) to search for the area having a few dividing cells.
- Examine the dividing cells under higher magnification of the microscope to observe the detailed features of mitosis.

Observation:

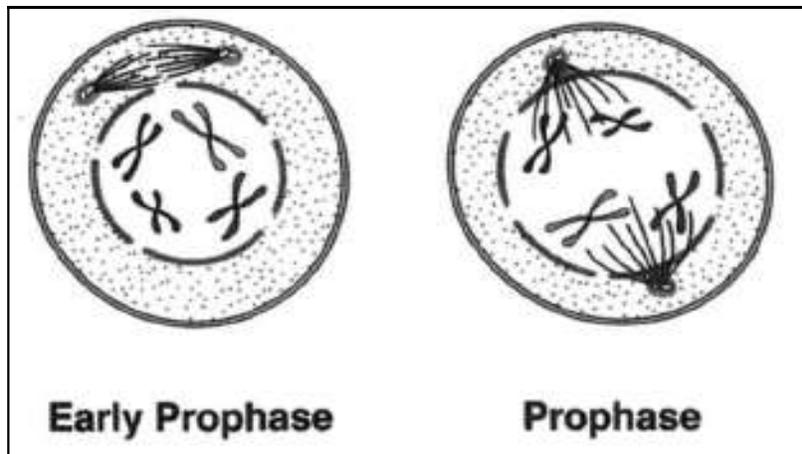
The stages of mitosis can be broadly categorised into two parts: karyokinesis (division of nucleus) followed by cytokinesis (division of cytoplasm, and ultimately of the cell). Those cells, which are not in the phases of cell division, are considered to be in interphase.

Interphase:

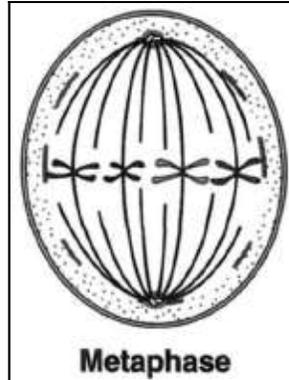
The cells are mostly rectangular, oval or even circular in shape, with almost centrally situated densely stained nucleus. The chromatic (coloured) material of the nucleus is homogeneous and looks granular. The boundary of the nucleus is distinct. One or few nucleoli (sing: nucleolus) can also be observed inside the nucleus.

**Stages of Mitosis:**

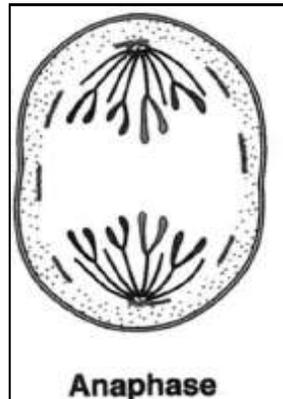
Prophase: Intact nuclear outline is seen. The chromatin (seen as a homogeneous material in the nucleus at interphase) appears as a network of fine threads (chromosomes). Nucleoli may or may not be visible. If the cell under observation is in the early stage of prophase then the chromatin fibres (chromosomes) are very thin. However, in the cells at late prophase, comparatively thicker chromatin fibres would be visible. Besides this, in the late prophase the nuclear membrane may not be noticed.



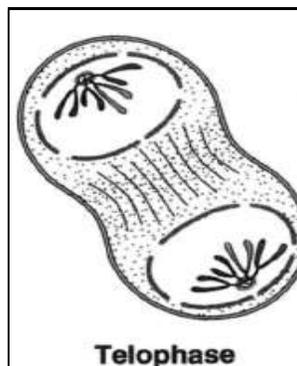
Metaphase: The nuclear membrane disappears. Chromosomes are thick and are seen arranged at the equatorial plane of the cell. Each chromosome at this stage has two chromatids joined together at the centromere, which can be seen by changing the resolution of the microscope. Nucleolus is not observed during metaphase.



Anaphase: This stage shows the separation of the chromatids of each chromosome. The chromatids separate due to the splitting of the centromere. Each chromatid now represents a separate chromosome as it has its own centromere. The chromosomes are found as if they have moved towards the two poles of the cell. The chromosomes at this stage may look like the shape of alphabets 'V', 'J' or 'I' depending upon the position of centromere in them. Different anaphase cells show different stages of movement of chromosomes to opposite poles, and they are designated to represent early, mid and late anaphase.

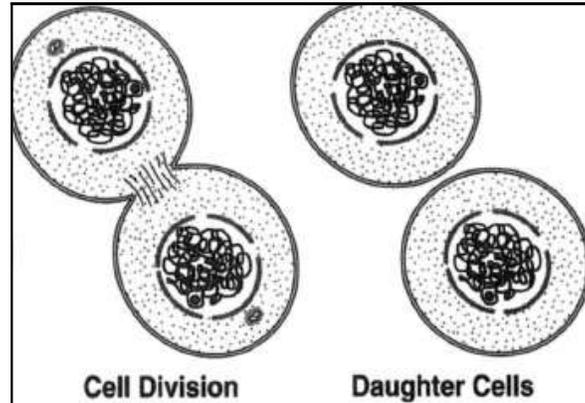


Telophase: Chromosomes reach the opposite poles, lose their individuality, and look like a mass of chromatin. Nuclear membrane appears to form the nuclei of the two future daughter cells.



Cytokinesis:

In plants, a cell plate is formed in the middle after telophase. The plate can be seen to extend outwards to ultimately reach the margin of the cell and divide the cell into two. Such cell plates are characteristic of plant cells. However, in an animal cell, the two sides of the cell show pushings or constrictions formed from the peripheral region in the middle of the cell, which grow inward and meet to divide the cell into two daughter cells.



EXPERIMENT-2

DETERMINATION OF MITOTIC INDEX

Aim: To calculate the mitotic index from onion root tip.

Principle: Mitotic index is defined as a ratio of the total number of dividing cells (n) and the total number of cells (N) in a particular vision field chosen randomly under the microscope. By randomly selecting 5 to 10 such vision fields, you can estimate the Mitotic Index for any given plant material. With the help of Mitotic Index, you can observe that how cells differ in their ability to divide. The mitotic index provides a measure of the capacity of cells to divide and of the rate of cell division. It is used to identify the sites of growth within a tissue and to determine which cell types are dividing.

Materials:

Plant material: Onion Root Tips

Chemicals: Carnoy's fixative / Acetic alcohol (acetic acid 1 part and ethyl alcohol 3 parts v/v), 2N hydrochloric acid, 70% ethanol, Acetocarmine or acetoorcein (2%), Acetic acid (45%).

Glassware and Equipment: Microscope slides and cover slips, Pasteur pipettes, Teasing needles, Fine forceps and razor blade, Watch glass, Filter paper / blotting paper, Compound microscope, Spirit lamp, Water bath.

Procedure:

Preparation of slide:

- Take one or two preserved roots, wash them in water on a clean and grease free slide.
- Place one drop of N/10 HCl on the root tip followed by 2–3 drops of aceto-carmine or aceto-orcein stain on it. Leave the slide for 5–10 minutes on a hot plate (or warm it slightly on spirit lamp). Care should be taken that the stain is not dried up.
- Carefully blot the excess stain using blotting paper. Now cut the comparatively more stained (2–3 mm) tip portion of the root and retain it on the slide and discard the remaining portion.
- After 10–20 seconds, place one or two drops of water and blot them carefully using blotting paper. Again, put a drop of water on the root tip and mount a cover slip on it avoiding air bubbles.
- Place the slide in between the folds of blotting paper using the fingers in such a way that the cover slip mounted on the slide is properly held. Now slowly tap the cover slip using the blunt end of a pencil so that the meristematic tissue of the root tip below the cover slip is properly squashed and spread as a thin layer of cells.

Observation Under the Microscope:

Place the prepared slide under the microscope for observation. Take a thorough survey of the whole slide.

Selection of Vision Field and their Photographs:

Select some (5-10) random vision fields in the slide and take their 5-10 high quality photographs with a digital camera.

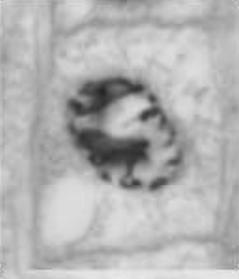
Counting the total number of cells present in the vision field:

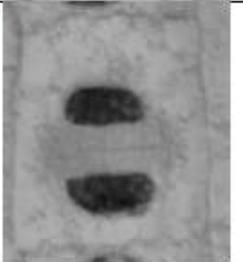
Carefully observe the photographs taken by you and try to count the total number of cells present in the photograph of vision fields. (Figure 8.1 and 8.2; Table 1).

Identification of different stages of mitosis:

Here some key features to identify and differentiate the cells that in which stage of mitosis they are:

Table 1. Identification of the Different Stages of Mitosis Division

S. No.	Stage of Division	Key Features	Photograph
1	Interphase	One intact nuclear region	
2	Prophase	Condensed chromosomes inside a nuclear membrane	
3	Metaphase	Condensed chromosomes present along the equator of the cell	

4	Anaphase	Two separate clusters of chromosomes at two different poles of the cell	
5	Telophase	Two nuclear regions present within a single cell (difficult to see as cytokinesis occurs concurrently)	

Count the number of cells that are actually in dividing state present in the vision field:

- Now count the number of dividing cells that means the cells which are under any stage of mitosis e.g. (Prophase, Metaphase, Anaphase or Telophase) in the vision field.

Calculate the Mitotic index by using the following formula -

$$\text{Mitotic Index (MI)} = \frac{n}{N}$$

Where, n = Number of cells undergoing mitosis

N = Total number of cells in the vision field check for visibility



**A Microphotograph Showing Various Cells
(With and without visible chromosomes) Under Microscope**



A Microphotograph Showing Counts of Cells under Mitosis and Total Number of Cells under Microscope

Observation:

Cells in mitosis = 20

Total number of cells = 75

Mitotic index = $20/75 = 0.267$

EXPERIMENT-3

EFFECT OF CHEMICAL MUTAGENS ON DIVIDING CELLS (MITOSIS)

Aim: To study the effects of physical and chemical mutagens on a biological system and observe mutation frequency.

Introduction: Mutagens are agents that cause mutations in the DNA of organisms. These mutations can be induced by physical or chemical agents.

Principle:

Chemical mutagens including alkylating agents (e.g., EMS), base analogs, and intercalating agents cause DNA damage, base mispairing, cross-linking, or spindle apparatus disruption. These alterations interfere with normal chromosome replication and segregation during mitosis. As a result, treated cells may exhibit changes in the mitotic index and structural chromosomal aberrations such as chromosome stickiness, bridges, laggards, fragments, multipolar spindles, and disturbed metaphase or anaphase stages. By comparing treated cells with untreated controls, the extent of cytotoxic and genotoxic effects of chemical mutagens can be assessed. The frequency of abnormal mitotic stages and reduction in mitotic index serve as indicators of the mutagenic potency of the chemical agent and its impact on cell division. This protocol thus provides a simple and reliable cytogenetic assay to evaluate the effect of chemical mutagens on mitosis.

Materials Required:

Onion root tips and culture media. Ethyl methanesulfonate (EMS), Nitrosoguanidine (NG), Microscope, Protective equipment (gloves, lab coat, goggles)

Procedure:

Treatment with Chemical Mutagens:

- Select healthy, uniformly sized onion bulbs (*Allium cepa*) and allowed to germinate by placing them over water so that young roots develop. When the roots attain a length of about 1–2 cm, select them for treatment.
- The bulbs are then transfer to beakers containing different concentrations (0.05%, 0.1%, 0.2%) of a chemical mutagen prepared in distilled water.
- Expose the roots to the mutagen solution for a specific duration (1–3 hours).
- After treatment, wash the roots thoroughly with distilled water to remove residual mutagen.
- Untreated roots maintained in distilled water serve as the control.

Fixation of Root Tips:

Following treatment, excise the actively growing root tips (1-2 mm) using a sharp blade. Immediately fix these root tips in aceto-alcohol (glacial acetic acid and ethanol in 1:3 ratio) for 12–24 hours at room temperature. Fixation preserves cellular structures and arrests mitosis at various stages by denaturing proteins and stabilizing chromosomes.

Hydrolysis:

Remove the root tips from the fixative and wash with distilled water. Then subject the root tips for acid hydrolysis by treating with 1N hydrochloric acid (HCl) at 60 °C for 5 minutes. Hydrolysis softens the cell walls and enhances stain penetration, facilitating clear visualization of chromosomes.

Staining:

- After hydrolysis, rinse the root tips thoroughly with distilled water and transfer to a clean microscope slide.
- Add one or two drops of acetocarmine or aceto-orcein stain, and gently warm for a few minutes to intensify staining. The stain binds specifically to chromatin, making chromosomes clearly visible under the microscope.

Slide Preparation (Squash Technique):

- Trim the stained root tips to retain only the meristematic region and cover with a clean coverslip.
- Apply gentle pressure using a blunt object or thumb wrapped in blotting paper to squash the tissue evenly, spreading the cells into a single layer without damaging chromosomes.
- Blot the excess stain carefully.

Microscopic Observation:

Observe the prepared slides under a compound microscope, first under low power to locate dividing cells and then under high power to examine different stages of mitosis. Record the frequency of normal and abnormal mitotic figures to assess the effect of chemical mutagens on cell division.

Calculation of Mutation Frequency

$$\text{Mutation Frequency} = \frac{\text{Number of Mutants}}{\text{Total Number of Organisms}} \times 100$$

Precautions:

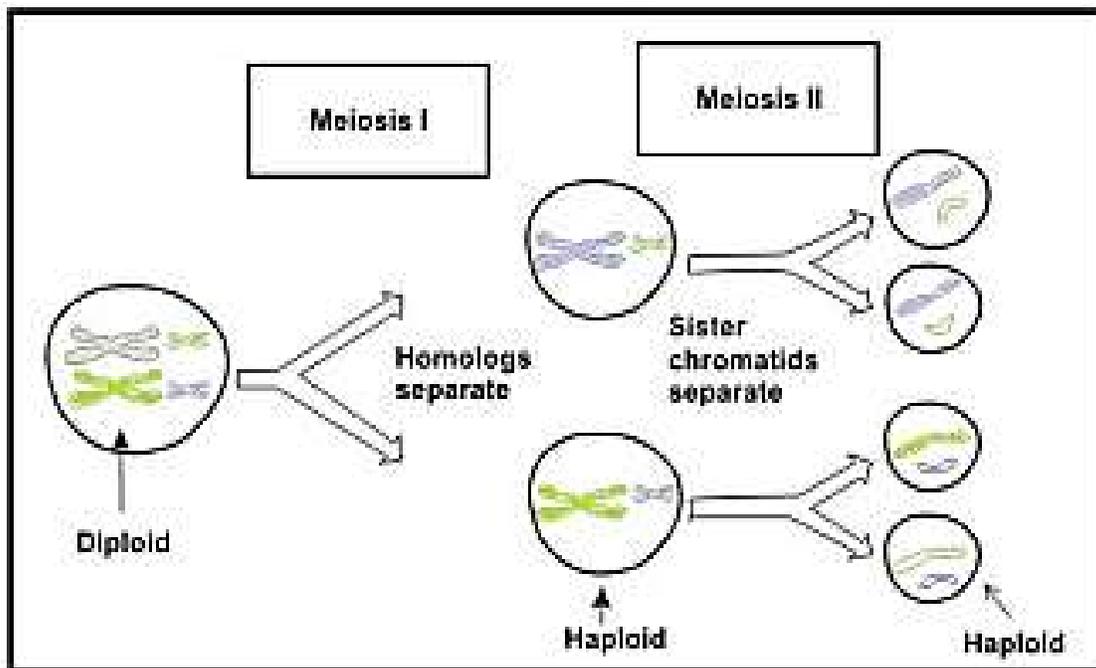
- 1) Handle mutagens with care and use protective equipment.
- 2) Maintain proper controls.
- 3) Ensure accurate timing and dosing.

EXPERIMENT-4

STUDY OF MEIOTIC STAGES IN FLOWER BUDS OF *ALLIUM CEPA*

Aim: To observe the various stages of meiosis occurs in flower buds.

Introduction: The word meiosis is derived from the Greek word meaning ‘diminution’ as the process results in daughter cells with half the number of chromosomes. It is a specific type of cell division that occurs in the reproductive tissues of diploid organisms and results in the formation of haploid gametes. The chromosome number is restored following fertilisation. During meiosis a diploid mother cell gives rise to four haploid daughter cells after two rounds of successive chromosome segregation events that is preceded by only one round of DNA replication in the S phase. These two multistep division stages are called meiosis I (reductional division) and meiosis II (equational division). It is essentially a division cycle that helps in maintaining the chromosome numbers of a species through successive generations.



Meiosis (I and II) Results in the Production of Haploid Cells

Materials Required:

Onion flower buds, Carnoy's fixative / Acetic alcohol (1:3 v/v), 70% Alcohol, Acetic acid (45%), Acetocarmine or acetoorcein (2%), 2N hydrochloric acid, Slides and cover slips, Pasteur pipettes, Teasing needles and fine forceps, Razor blade, Watch glass, Filter paper / blotting paper, Spirit lamp, Compound microscope.

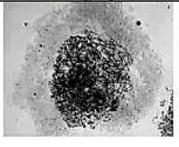
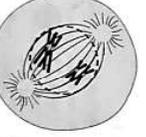
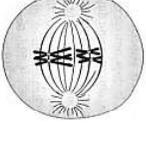
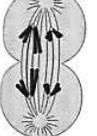
Procedure:**Pre-Lab Preparations:**

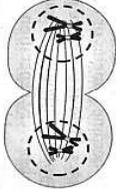
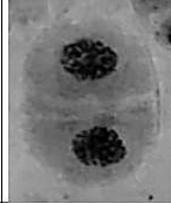
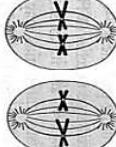
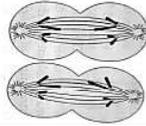
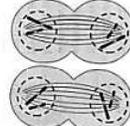
- Pluck the onion buds (preferably in the early morning hours) and transfer them to Carnoy's fixative (acetic -alcohol; 1:3v/v) for 24 hrs.
- At the end of the fixation period, transfer the buds to a storage solution (70% ethanol) and keep these vials at 4-5° C until required for the experiment.

Preparation of Stained Squashes:

- Remove the anthers from the onion buds stored in 70% ethanol.
- Transfer the anthers to a watch glass and wash thoroughly with water (at least 3 times).
- Drain off the water with a Pasteur pipette and add a few drops of 2N HCl. Leave it for 10-15 min at room temperature to allow softening of the tissue. The process can be hastened by hydrolyzing over the flame of a spirit lamp.
- Remove HCl with a Pasteur pipette and wash the anthers again with water.
- Transfer the anthers to 2% acetocarmine or acetoorcein and stain for 10 minutes.
- Destain the tissue by transferring it to a clean slide and add few drops of 45% acetic acid from a dropping bottle.
- Place carefully a cover slip on the stained anthers and transfer the slide between the folds of filter paper to remove excess acetic acid. Apply moderate pressure in a vertical direction with your thumb or with the flat bottom of a pencil. Tapping flattens the cells and spreads the chromosomes. A properly squashed tissue should be about three times of its original size.
- Examine the slides under the compound microscope at 10X and 40X.

Different Stages of Meiosis Including Key Features, Photographs and Diagrams

S. No.	Stages of Division		Key Features	Photographs	Diagrams	
1	MEIOSIS I	PROPHASE I	LEPTOTENE	Chromosomes, each consisting of two sister chromatids, begin to condense.		
2			ZYGOTENE	Homologous chromosomes begin to pair.		
3			PACHYTENE	Homologous chromosomes are fully paired.		
4			DIPLOTENE	Homologous chromosomes separate, except at chiasmata.		
5		DIAKINESIS	Paired chromosomes condense further and become attached to spindle fibers.			
6		METAPHASE I	Paired chromosomes align on the equatorial plane in the cell.			
7		ANAPHASE I	Homologous chromosomes disjoin and move to opposite poles of the cell.			

8		TELOPHASE I	Chromosome movement is completed and new nuclei being to form.		
9	MEIOSIS II	PROPHASE II	Chromosomes, each consisting of two sister chromatids, condense and become attached to spindle fibers.		
10		METAPHASE II	Chromosomes align on the equatorial plane in each cell.		
11		ANAPHASE II	Sister chromatids disjoin and move to opposite poles in each cell.		
12		TELOPHASE II	Chromosomes decondense and new nuclei begin to form.		
13		Cytokinesis	The haploid daughter cells are separated by cytoplasmic membranes.		

EXPERIMENT-5

STUDY OF PHOTOGRAPHS OF CHROMOSOMES WITH DIFFERENT BANDING PATTERNS

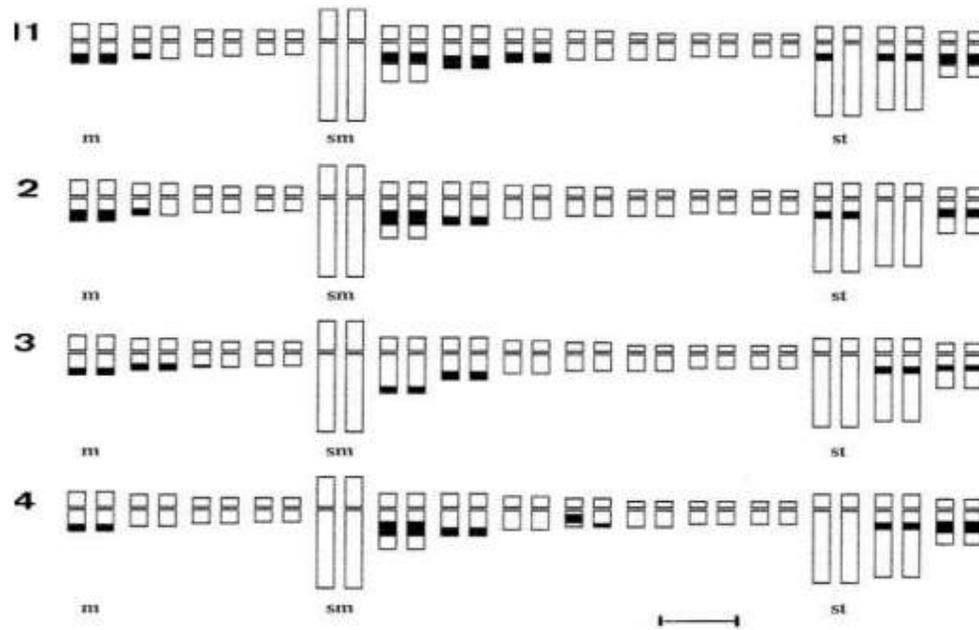
Chromosome banding refers to the pattern observed comprising of light and dark stain on staining. The bands distinguish various locations in the chromosome. The dark and light bands refer to the adjacent segments present in the chromosome which helps in genomic study and evaluation. Chromosome banding pattern is useful to 1. To identify different regions in chromosomes. 2. To differentiate between euchromatin and heterochromatin. 3. To identify abnormal chromosome. 4. To understand various clinical features of chromosomes. 5. To identify different genes and their functions. 6. To understand the molecular basis of any genetic disease. 7. To devise strategy to stabilize the unnatural changes in chromosome.

Types of Banding:

(i) Q Banding:

The fluorescent dyes like quinacrine and quinacrine mustard bind preferentially to certain mitotic chromosomes region.

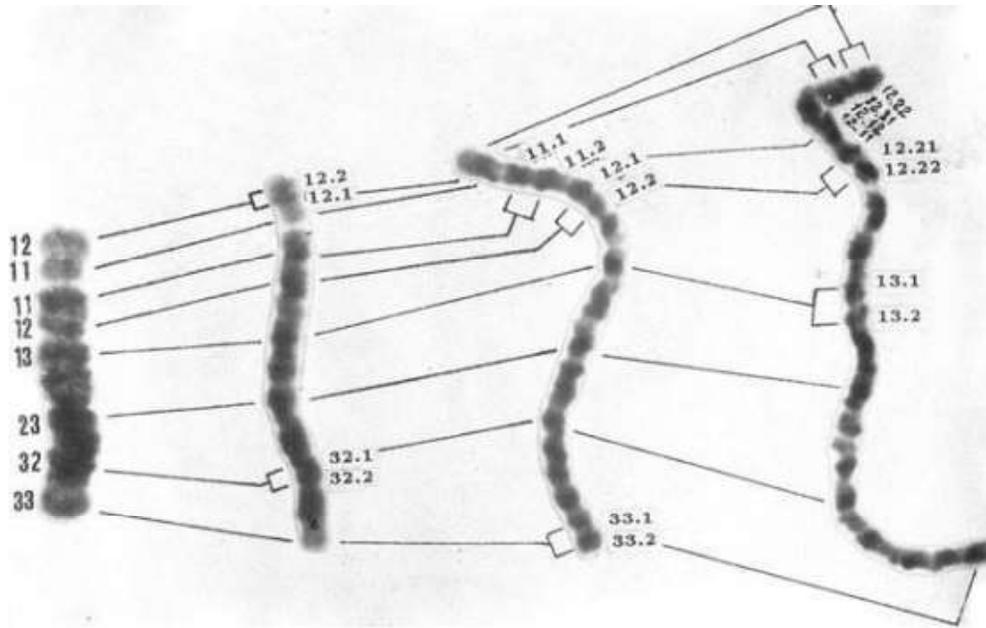
- 1) These fluorescent dyes interact with AT base pairs and hence staining is more in the case of AT rich regions.
- 2) AT rich regions appear as bright bands called the Q bands.
- 3) These bands help in identifying all the chromosomes and their homologues in most of the species.
- 4) It does not require any pre-treatment and is the simplest banding method.
- 5) It reveals the unique patterns of brightly fluorescent (light) regions alternate to non-fluorescent (dark) regions which are produced in each chromosome.
- 6) Although, fluorescent bands are not permanent, and for visualization, ultra violet light is needed.
- 7) This method does stain the chromosome ends and hence its use is considered to limited extent.
- 8) Q banding is commonly used in *Triticum*, *Scilla*, *Allium*, *Crepis*, *Lilium*, *Secale* and *Vicia*.



Q Banding Patterns in Plant Chromosome

(ii) G Banding (Gustav Giemsa):

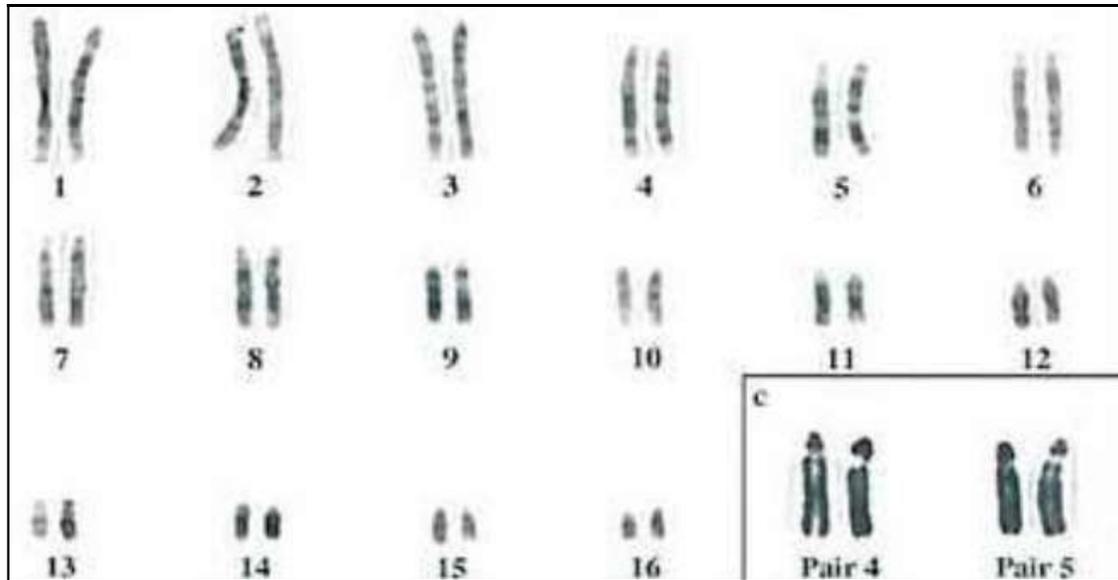
- 1) The Giemsa stain is used along with trypsin, urease as protease for staining and it yields the banding pattern of normal mitotic chromosomes.
- 2) The dark regions formed are similar to Q bands and are called G bands.
- 3) The light regions are similar to the non-fluorescent dark bands which rose by the use of fluorescent dyes.
- 4) A large number of bands of chromosome are produced in prophase and pro-metaphase.
- 5) The prophase and metaphase chromosomes contain a basic chromomeric structure which is enhanced due to which G bands are produced.
- 6) This enhancement is caused by inducing some rearrangement of fibres away from the light bands toward the G bands.
- 7) In few species of plants like *Tulipa gesneriana*, *Pinus resinosa* and *Vicia hajsatana*, the G bands are generated.
- 8) Tribe like *Triticeae* fails to produce G bands in the chromosome due to the increased condensation of the plant chromosomes.
- 9) Improper pre-treatment of plant chromosomes cause alteration in the organization of their chemical constituents which make unresponsive to the procedure of G banding.



G Banding Chromosome Patterns in Plants

(iii) R Banding:

- 1) R stands for Reverse in R banding.
- 2) This technique was developed by Dutrillaux and Lejeune in the year 1971.
- 3) A reverse pattern from the G and Q banding methods is produced by mild denaturation by heat and subsequent staining of chromosomes with Giemsa or a fluorochrome.
- 4) R bands are produced by GC specific fluorochromes.
- 5) Dark R bands are produced, if chromosomes are stained by Giemsa.
- 6) The dark R bands produced are similar to light bands produced by G banding technique.
- 7) When fluorochrome dye like acridine orange or olivomycin are used, bands produced are the reverse of Q banding technique. The R bands are of fluoresce bright green and the faint red colour indicates non-R bands.
- 8) This method is used in detecting structural rearrangements which involve ends of chromosomes or telomeres stained as T bands.
- 9) R bands are produced by some plant species only such as *Scilla siberica*, *Vicia faba*, *Allium* sp.
- 10) The R banding is produced when DNA and proteins in the G and R bands are denatured selectively under different pH conditions, salt concentrations and temperature.



R- Banding Pattern in *Gonatodes Humeralis*

(iv) C Banding:

The chromosomes are stained with Giemsa and Ba(OH)₂ in the constitutive heterochromatin regions.

- 1) These regions are called as C bands which are present proximal to the centromeres of all the chromosomes.
- 2) Constitutive heterochromatin resembles with the satellite DNA, consisting of short, highly repeated base pairs sequences in tandem repeats among one or more regions of nearly all chromosomes of most of the species.
- 3) The location of C bands is at various sites and also next to centromeres of each chromosomes and next to secondary chromosome constrictions
- 4) C banding technique is limited as it does not allow recognition of individual chromosomes with accuracy and precision.
- 5) The C bands are present in many species of *Aegilops*, *Agropyron*, *Elymus*, *Hordeum*, *Secale*, *Triticum*, etc.
- 6) Some plant species like *Allium crinatum*, *Horden* spp. and *Agropyron elonga* do not reveal C bands next to the centromeres of their chromosomes.
- 7) Due to this, individual chromosomes in the somatic cells can be identified by their C banding patterns.
- 8) Through this technique, 21 chromosomes are identified in the genome of Chinese Spring and Norim 61 of *T. aestivum*.
- 9) This method is used in detecting aneuploids translocation and other structural rearrangements and precise physical mapping of genes in the chromosomes.

- 10) The A and B genomes of *T. turgidum* have been identified which are considered as inconsistent.

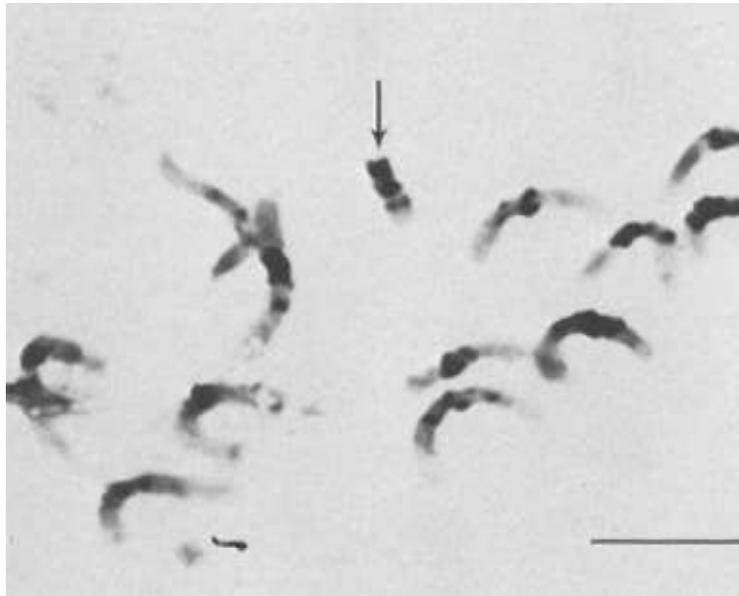


C-Banding Patterns in Plant Chromosome

(v) N Banding:

This technique was developed by Matsui and Sasaki in 1973.

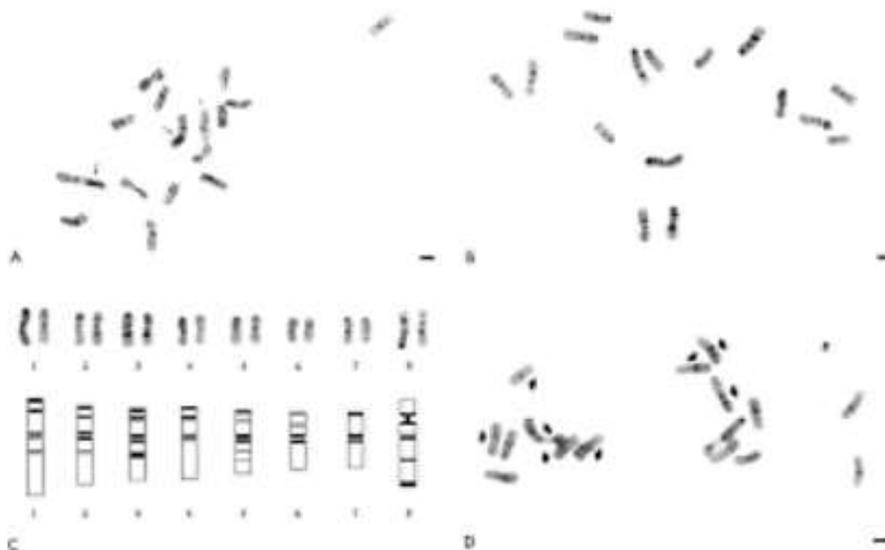
- 1) It stains the NORs in the chromosomes in the mammalian species which are different from the N bands revealed in plant species.
- 2) The presence of N bands in plant species has some examples such as *Triticum* and *Aegilops* species.
- 3) The 21 chromosomes of common wheat and the 14 chromosomes of *Aegilops variabilis* have been identified by N banding patterns.
- 4) The chromosomes of barley, rye, lentils and *Elymus* spp. have also been identified by using this technique.
- 5) This method can identify various types of aneuploids, alien additions and substitution lines and translocation and deletions.
- 6) Some N bands are unknown and hence cannot be detected by this method as in *T. aestivum*.
- 7) Many N bands are known to occupy the same position as that of C bands and hence it can be concluded that 2 classes of heterochromatin occur in wheat, rye and other species.
- 8) Some regions of heterochromatin stains positive for both C and N banding. These are known as C+N bands.
- 9) C+N bands possess multiple copies of the (GAA)_n (GAG)_n sequences in DNA.



N-Banding Patterns in Barley

(vi) Analysis:

- 1) A unique banding pattern of chromosome is revealed by this method.
- 2) It is more reliable staining method for the identification of chromosomes.
- 3) Five major banding techniques naming, Q, G, R, C and N provide precise cytogenetic and phylogenetic analysis.

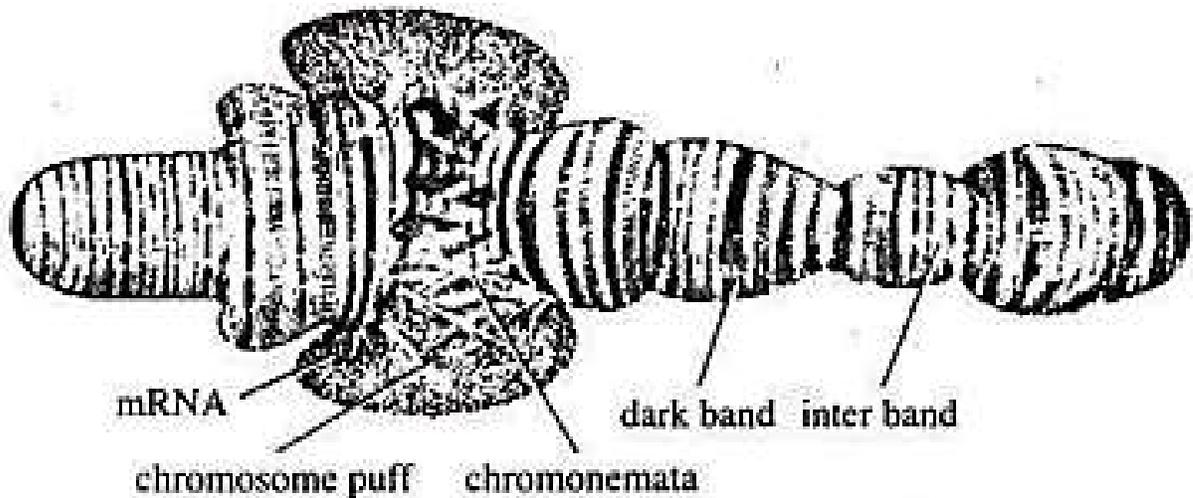


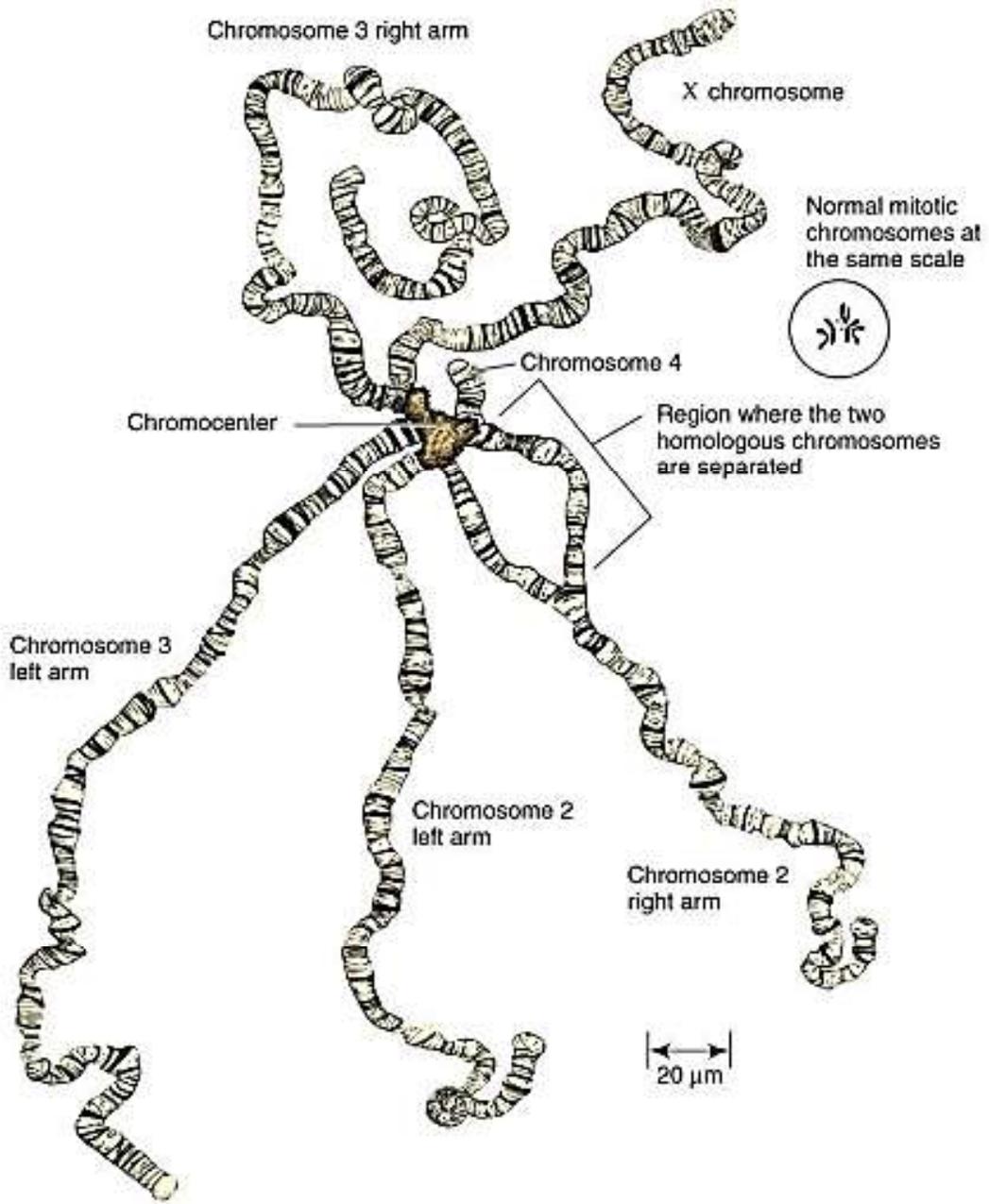
Banded Chromosome Patterns of *M. sativa*

EXPERIMENT-6**STUDY OF MICROSCOPIC PICTURES OF POLYTENE CHROMOSOMES, LAMPBRUSH CHROMSOMES AND B-CHROMOSOMES****1. Polytene Chromosomes:**

- a) Polytene chromosomes are large chromosomes which have thousands of DNA strands.
- b) They provide a high level of function in certain tissues such as salivary glands.
- c) Polytene chromosomes are found in dipteran flies (*Drosophila*, *Chironomus*, *Rhynchosciara* etc.) and group of arthropods of the class Collembola, a protozoan group Ciliophora, mammalian trophoblasts and antipodal, and suspensor cells in plants.
- d) In insects, polytene chromosomes are commonly found in the salivary glands; they are also referred to as "salivary gland chromosomes".
- e) They are produced when repeated rounds of DNA replication without cell division forms a giant chromosome.
- f) Thus polytene chromosomes form when multiple rounds of replication produce many sister chromatids which stay fused together.
- g) The large size of the chromosome is due to the presence of many longitudinal strands called chromonemata, hence the name polytene (many stranded).
- h) The chromosomal strands are formed after repeated division of the chromosome in the absence of cytoplasmic division. This type of division is called endomitosis.
- i) They are about 0.5 mm in length and 20 μm in diameter.
- j) The polytene chromosome contains two types of bands, dark bands and inter bands.
- k) The dark bands are darkly stained and the inter bands are lightly stained with nuclear stains.
- l) The dark bands contain more DNA and less RNA.
- m) The interbands contain more RNA and less DNA.
- n) The amount of DNA in interbands ranges from 0.8 - 25%.
- o) The bands of polytene chromosomes become enlarged at certain times to form swellings called puffs.
- p) The formation of puffs is called puffing. In the regions of puffs, the chromonemata uncoil and open out to form many loops.
- q) The puffing is caused by the uncoiling of individual chromomeres in a band. The puffs indicate the site of active genes.

- r) The chromonemata of puffs give out a series of many loops laterally.
- s) As these loops appear as rings, they are called Balbiani rings after the name of the researcher who discovered them.
- t) They are formed of DNA, RNA and a few proteins.
- u) In addition to increasing the volume of the cells' nuclei and causing cell expansion, polytene cells may also have a metabolic advantage as multiple copies of genes permits a high level of gene expression.
- v) Polytene chromosomes, at interphase, are seen to have distinct thick and thin banding patterns. These patterns were originally used to help map chromosomes, identify small chromosome mutations, and in taxonomic identification. They are now used to study the function of genes in transcription.

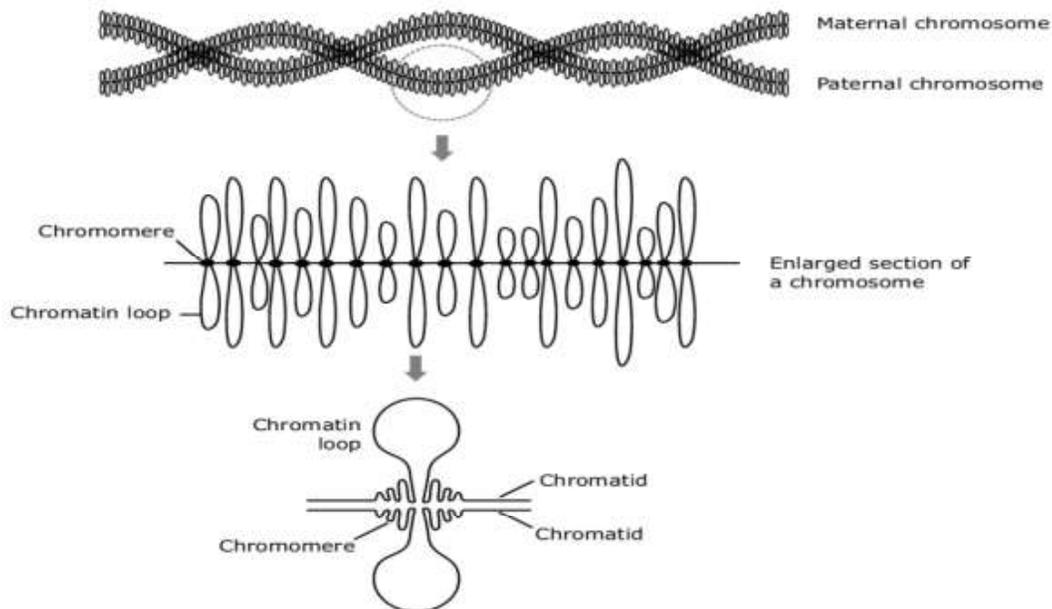


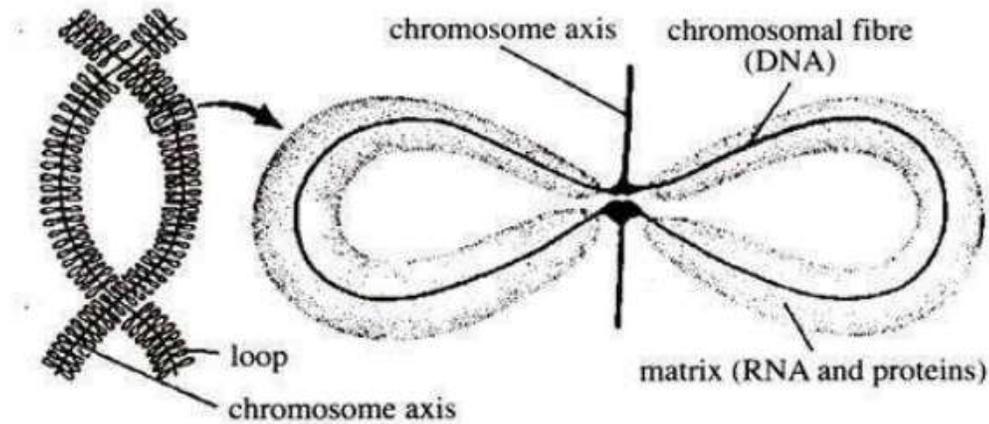


Polytene Chromosome of Insects

2. Lampbrush Chromosomes:

- a) Lampbrush chromosomes are a special form of chromosomes found in the growing oocytes (immature eggs) of most animals, except mammals.
- b) They were first described by Walther Flemming in 1882.
- c) Lampbrush chromosomes of tailed and tailless amphibians, birds and insects are described in best.
- d) Amphibian and avian lampbrush chromosomes can be micro surgically isolated from oocyte nucleus (germinal vesicle) with either forceps or needles.
- e) Chromosomes transform into the lampbrush form during the diplotene stage of meiotic prophase I due to an active transcription of many genes.
- f) They are highly extended meiotic half-bivalents, each consisting of 2 sister chromatids.
- g) Lampbrush chromosomes are clearly visible even in the light microscope, where they are seen to be organized into a series of chromomeres with large chromatin loops extended laterally.
- h) Each lateral loop contains one or several transcription units with polarized RNP-matrix coating the DNA axis of the loop.
- i) Giant chromosomes in the lampbrush form are useful model for studying chromosome organization, genome function and gene expression during meiotic prophase, since they allow the individual transcription units to be visualized.
- j) Lampbrush chromosomes are widely used for high-resolution mapping of DNA sequences and construction of detail cytological maps of individual chromosomes.



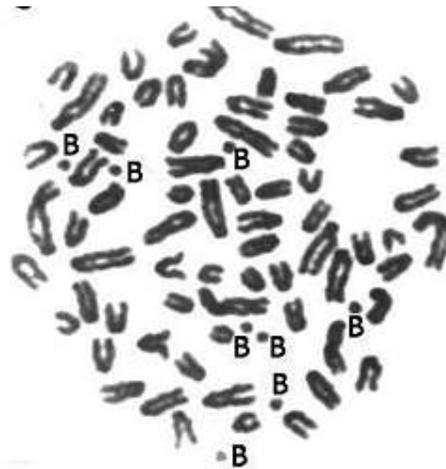


Lampbrush Chromosomes

3. B- Chromosomes:

- a) The B-chromosome is a small chromosome found on the second sex chromosome of all sexually reproducing mammals.
- b) The B-chromosome is responsible for the production of a single protein called beta-globulin.
- c) It is a non-essential part of the karyotype and occurs ubiquitously in all mammalian species.
- d) The B-chromosome is not inherited and is neither expressed nor recombined.
- e) B-chromosomes are also known as supernumerary, lineage-specific or accessory chromosomes.
- f) These chromosomes are not necessary for an individual's life but act as an extra chromosome to the standard complement.
- g) In maize, B chromosomes lead to a reduction in fertility and increase the percentage of abortive pollen.
- h) Most B chromosomes are heterochromatic and are not responsible for coding anything.
- i) In humans, B chromosomes interact with standard chromosomes and play an important role in the evolution of genetic material. Thus, B chromosomes can be useful for studying various evolutionary processes related to molecular biology.
- j) B-chromosomes are a type of chromosome in humans. They occur in females and are the primary cause of Turner syndrome, which affects development in females.
- k) B-chromosomes are found in all vertebrates, from fish to humans, and have been found to have significant roles in several pathways of the cell, but little is known about their regulation.

- l) B-chromosome is one that has a complete set of genes. This is the default state for all cells, as most human cells do not have a B-chromosome.
- m) In certain cases, a cell may have an extra B-chromosome. B chromosomes are extra chromosomes that are different from the 46 standard pairs of chromosomes. They can be added to one or both copies of the 23 pairs in a human cell. They do not contribute to the normal functions of an individual, nor are they present in all organisms.
- n) In some species, B-chromosomes can be quite large, with as many as eight copies of the chromosome.
- o) They contain large numbers of genes, as much as 1/4 of a chromosome's DNA, which can either be expressed or silenced in the cell.
- p) The B-chromosome has the same properties as a normal chromosome in that its sequences can be transcribed and translated to produce proteins.
- q) B-chromosomes are a part of the DNA of the human genome.
- r) They are often used to indicate past and present human migration patterns and admixture.
- s) B-chromosomes are extra chromosomes that occur in about 15% of eukaryotes. When autosomes and sex chromosomes are derived in intra and inter species crosses, there is a maximum chance of generation of B chromosomes.
- t) B chromosomes can be useful for studying various evolutionary processes related to molecular biology.
- u) The B-chromosome is associated with reduced female fertility and also contributes to evolution.
- v) B-chromosome is a special chromosome found in some species of plants, animals, and insects.
- w) It is distinguished by a centromere that is typically located at the centromeric region of the chromosome and contains heterochromatic regions at both ends.



B- Chromosomes

PLANT STRUCTURE AND DEVELOPMENT**EXPERIMENT-1****VARIOUS TYPES OF TISSUES AND TISSUE SYSTEMS**

Fixed or fresh plant parts are collected (locally available plants) and take thin sections (freehand sections). Stain section with 1% safranin (1 g safranin in 100 ml of 70% ethyl alcohol). Destain sections in acid water (3% HCl) if excess stain prevails.

Primary Plant Body:

Entire plant body is derived from the shoot and root apices. Meristematic tissue is located in shoot and root apices. The meristematic cells undergo divisions and derivatives of these cells are differentiated into permanent tissues.

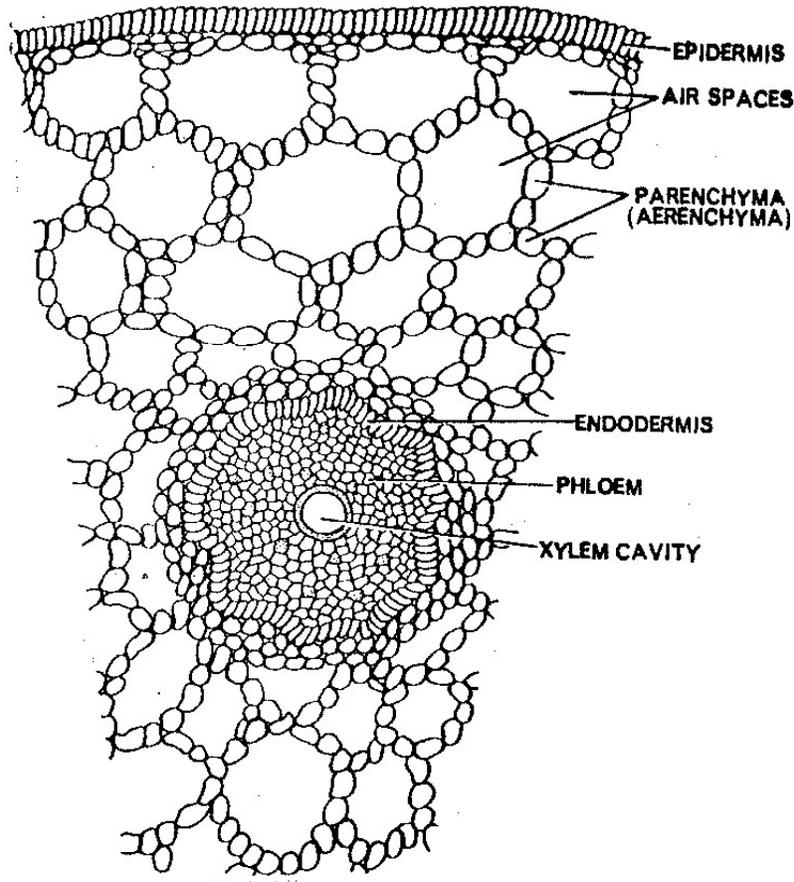
Permanent tissues may be classified into three main groups: (1) Simple (parenchyma, collenchma and sclerenchyma), (2) Complex (xylem and phloem), (3) Secretory tissues.

Tissue systems are of three types found in the primary plant body. These are as follows:

- 1) Epidermal tissue system, i.e., epidermis with stomata, trichomes etc.
- 2) Ground tissue system, i.e. ground tissue with cortex, pericycle, medullary rays, pith etc.
- 3) Vascular tissue system, e.g. vascular bundles with xylem and phloem.

1) Parenchyma:

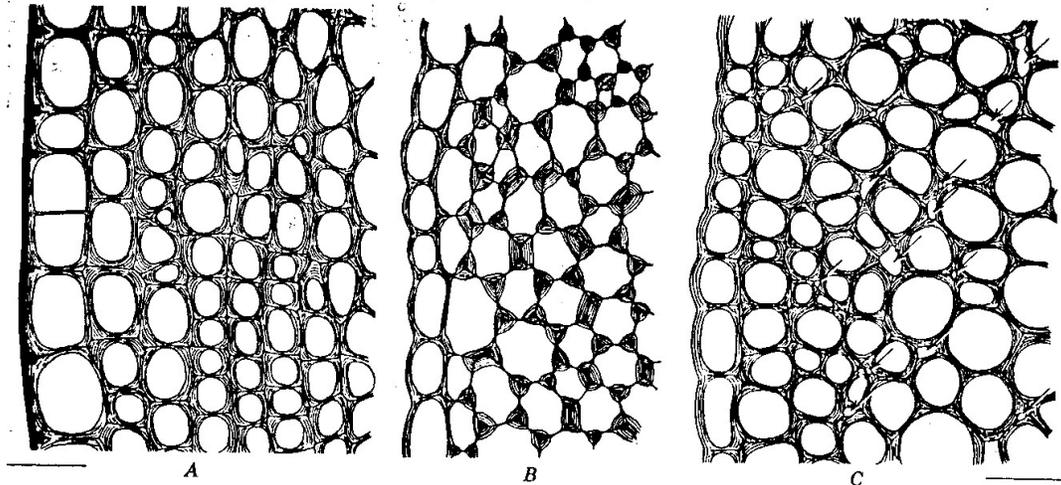
- a) Parenchyma is the most common tissue which is morphologically and physiologically simple and unspecialized.
- b) Parenchyma is found in the cortex, pith, pericycle and mesophyll of leaf.
- c) Parenchymatous cells are thin-walled, compactly arranged (palisade tissue of leaf) or with small intercellular spaces. Numerous, larger intercellular spaces (parenchyma) are found in aquatic plants such as *Eichhornia*, *Hydrilla*, *Potamogeton*, etc. Aerenchyma in aquatic plants provide buoyancy to float easily in or on the water.
- d) Storage parenchyma in potato contain ergastic substances like starch grains and protein granules.
- e) Parenchymatous cells covered with cuticle on leaf epidermis are protective in nature which prevent transpiration and also provide mechanical strength to plant organ.



Transection of *Potamogeton* Stem (Hydrophyte)

2) Collenchyma:

- a) Collenchyma is a living tissue composed of more or less elongated cells with thick primary non-lignified walls. On the basis of thickenings on cell wall and arrangement of cells, the following three types are recognized.
- b) **Angular Collenchyma:** The thickenings are found at the corners or angles of the cells, e.g. stems of *Cucurbita*, *Datura*, *Lycopersicon* etc. This parenchyma is without intercellular spaces.
- c) **Lacunar or Tubular Parenchyma:** This parenchyma is with larger intercellular spaces. The thickenings are restricted to the walls of the regions bordering air spaces, e.g. *Salvia*, *Malva*, aerial roots of *Monstera*, *Lactuca* etc.
- d) **Plate or Lamellar Parenchyma:** The thickenings occur chiefly on the tangential walls and lesser in the radial walls. As a result, cells appear like plates, bands or lamellae, e.g. *Raphanus*, *Sambucus* etc.



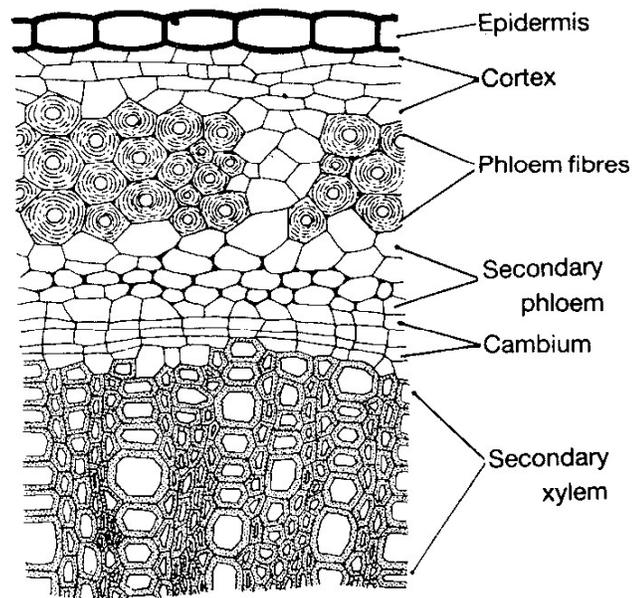
Collenchyma in Stem Transections*.

A. Lamellar Parenchyma in *Sambucus*,

B. Angular Collenchyma in *Cucurbita*, C. Lacunar Parenchyma in *Lactuca*

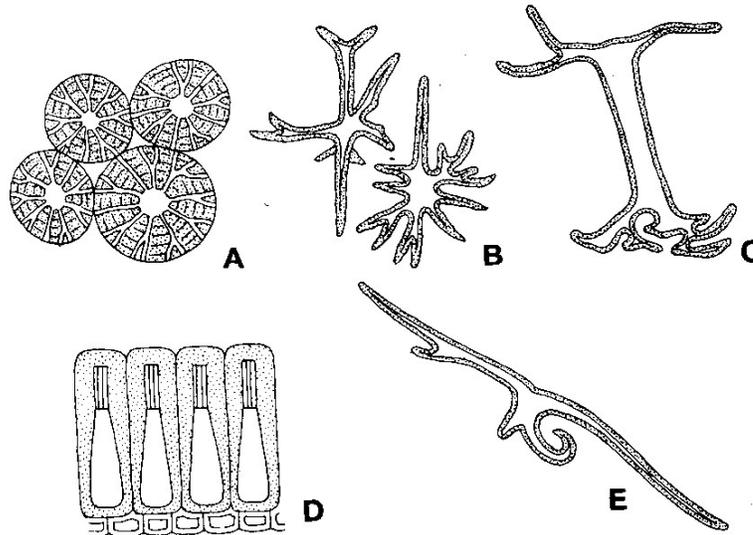
3) Sclerenchyma:

- a) Sclerenchyma is a dead tissue with lignified thickenings on the cell walls. It provides mechanical strength to the plant body. Sclerenchymatous cells show much variation in form, structure, origin and development, and are classed into two groups namely, **fibres** and **sclereids**.
- b) **Fibres:** These are classified into two groups, the xylary fibres (intra-xylary) and extra-xylary fibres.
- c) Xylary fibres are also known as wood fibres. Some of the xylary fibres are **libriform fibres** and **fibre tracheids**. The libriform fibres are characterized by very thick secondary wall and simple pits, while fibre tracheid by relatively thin walls and bordered pits.
- d) Cortical fibres (found in cortex), peri-vascular fibres (periphery of the vascular cylinder) and phloic fibres (phloem fibres) are come under the category of extra-xylary fibres.
- e) **Sclereids (sclerotic cells):** Greater deposition of lignin is found, as a result lumen becomes narrow in these cells. Five categories of sclerieds are given below:



Transection of Stem of *Linum Usitatissimum* (Flax) Showing Phloem Fibres

- Brachysclereids (stone cells) are roughly isodiametric found in cortex, pith and phloem stems and flesh of fruits, e.g. *Pyrus*.
- Macrosclereids are elongated columnar ones, commonly form palisade like epidermal layer, e.g. seed coats of legumes.



Different Types of Sclereids

A. rachysclereids, B. astrosclereids, C. osteosclereid, D. macrosclereids

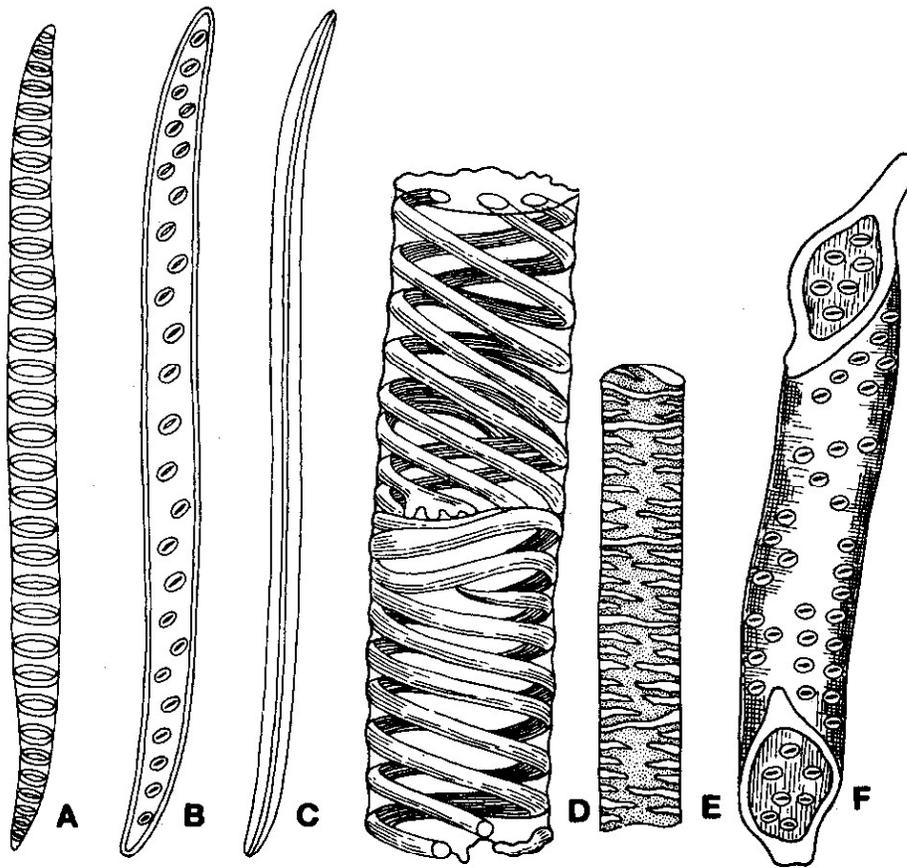
- (i) Osteosclereids are bone or barrel-shaped with dilated ends. Commonly found in leaves and seed coats of monocots.
- (ii) Astrosclereids are star-shaped (stellate type) found in leaves of *Nymphaea* and, stem and leaves of *Trochodendron*.
- (iii) Trichosclereids are hair-like branched and unbranched ones found in hydrophytes and also aerial roots of *Monstera*.

4) Xylem

Xylem is a complex tissue, consisting of tracheids, vessels, xylem fibres and xylem parenchyma. Xylem involves in conduction of water and provides mechanical strength to the plant body.

Tracheids:

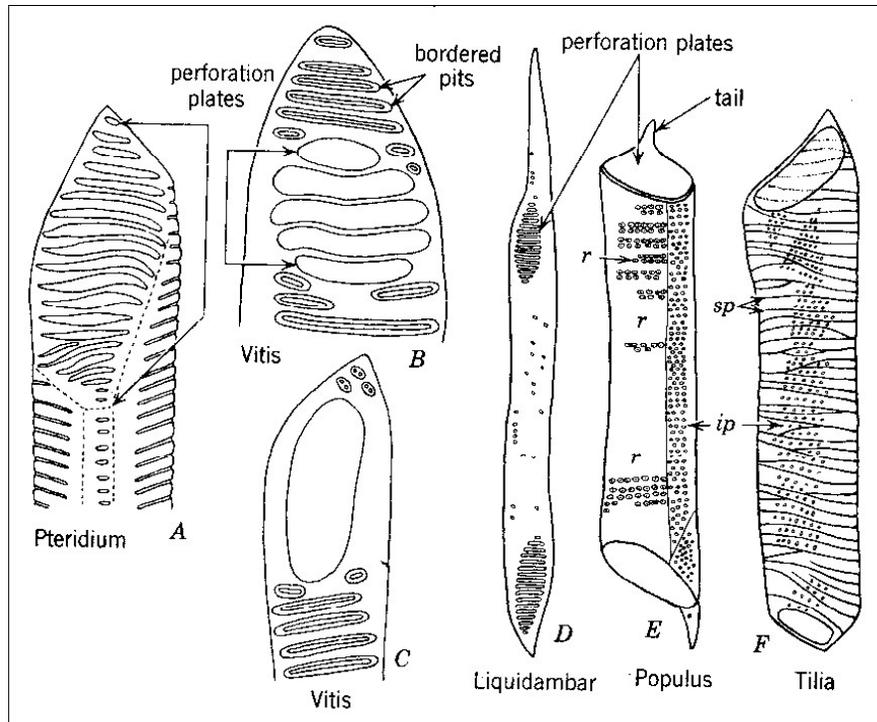
- a) Tracheid is an elongated structure with tapering ends. It has got hard, thick and lignified walls and a large lumen. The tracheids are considered as primitive and found in the ancient vascular plants. These are the only elements found in the fossil seed plants.
- b) A tracheid differs from xylem fibres in having: (i) the cell wall comparatively less lignified, (ii) large number of pits, (iii) bordered pits and (iv) a very large lumen.
- c) Lignified secondary walls are highly characteristic and these are deposited in different pattern such as annular (ring type), spiral, scalariform (ladder type), reticulate and pitted.
- d) Generally, tracheids with annular and spiral thickenings are found in the early formed primary xylem whereas scalariform and pitted tracheids in later formed primary xylem and also in secondary xylem.
- e) Vessels or trachea: Vessel members are joined one end to another end and form the vessel.
- f) It is long, cylindrical, tube-like structure with lignified walls and a wide lumen.



Different Types of Tracheids

A. annular, B and C. tracheidal fibres, D. spiral, E. scalariform and F. pitted.

- (i) The characteristic feature of vessel members is that they have **perforation plates** (end walls).
- (ii) If perforation plate containing one large pore, it is called **simple perforation plate**; if there are several pores, it is known as **compound (multiple) perforation plate**.
- (iii) In compound perforation plates, pores are arranged in several patterns. When the pores are arranged in ladder-like manner, it is called *scalariform*. In some cases, perforations are circular and grouped together, the perforation plate is called *foraminate* type.



End walls of vessel member showing various types of perforation plates.

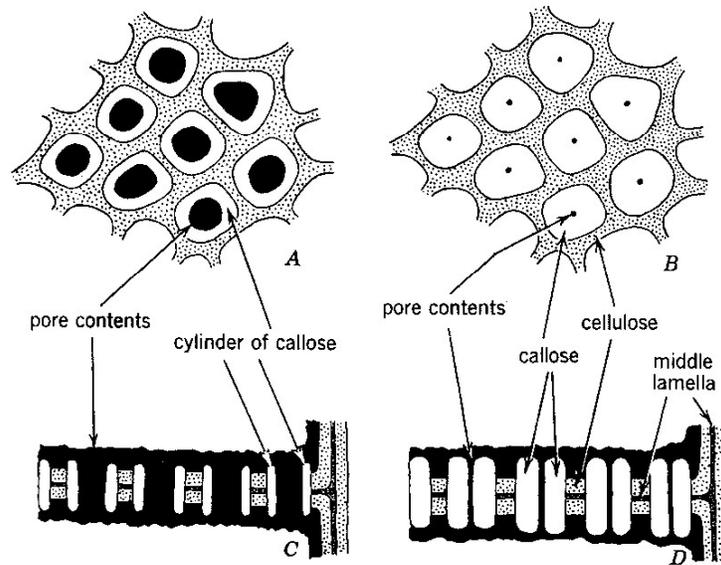
A. and B. scalariform type, C. simple type, D-F. Complete vessel members, D. scalariform perforation plates, E. simple perforation plates, F. simple perforation plates, (*ip*- intervessel pitting; *r*- ray contact areas; *sp*- spiral thickenings).

5) Phloem:

Phloem is a complex tissue, consisting of sieve elements, companion cells, phloem fibres and phloem parenchyma. Phloem can be detected by staining with aniline blue or resorcin blue (Iacmoid).

Characteristic Features:

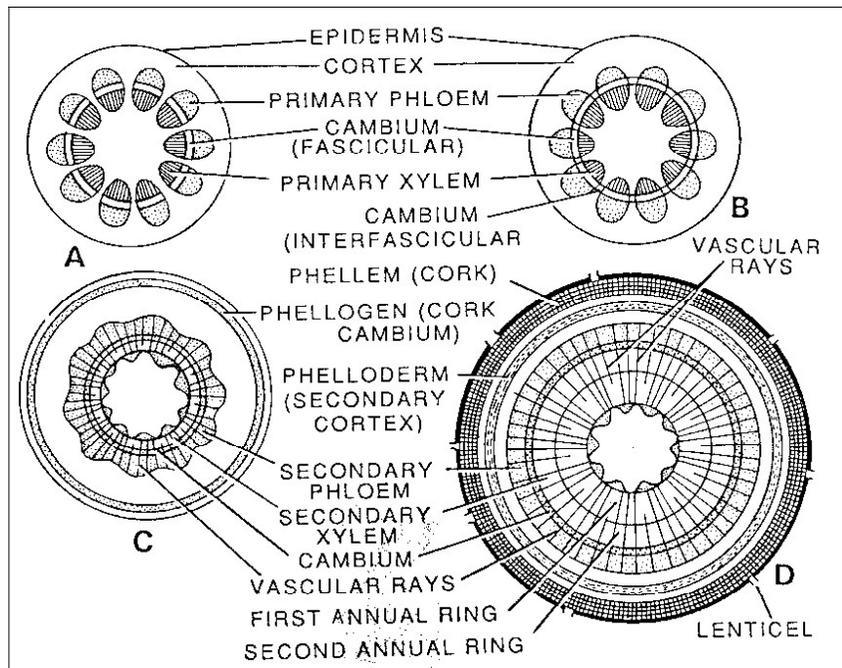
- Sieve elements include two types of cells, the less specialized **sieve cells** and the more specialized **sieve-tube members**.
- The longitudinal series of sieve-tube members constitute the sieve tube.
- The most characteristic features of sieve elements are the sieve areas on their walls and disappearance of nucleus in their protoplasts.
- Sieve-tube members have got highly specialized sieve areas and these are localized in the form of sieve plates.



Sieve Areas in Angiosperm Sieve Tube

A, B. Surface Views, C, D. Sectional Views. A, C. Illustrate Younger Sieve Areas; B, D. Older Sieve Areas

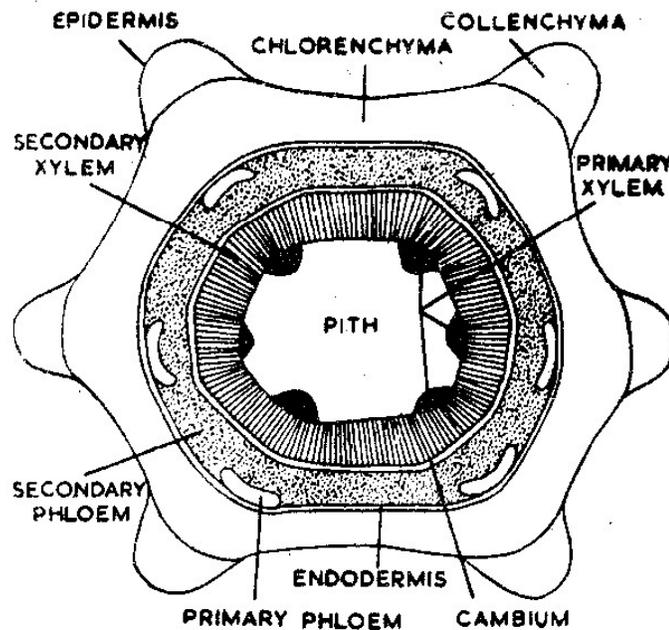
Secondary Plant Body:



Secondary Growth in Dicot System

Locally available dicot stems (thick and mature) are collected and study the secondary structures. Secondary plant body is formed by the activity of lateral meristems such as vascular cambium and phellogen. By the activity of these meristems, the girth of the stem is increasing and giving rise to secondary plant body.

Vascular cambium cuts off secondary phloem towards outer side and secondary xylem towards innerside. Secondary xylem formed during spring and also of autumn constitute together to form annual ring or growth ring. During secondary growth periderm is formed by the activity of phellogen.

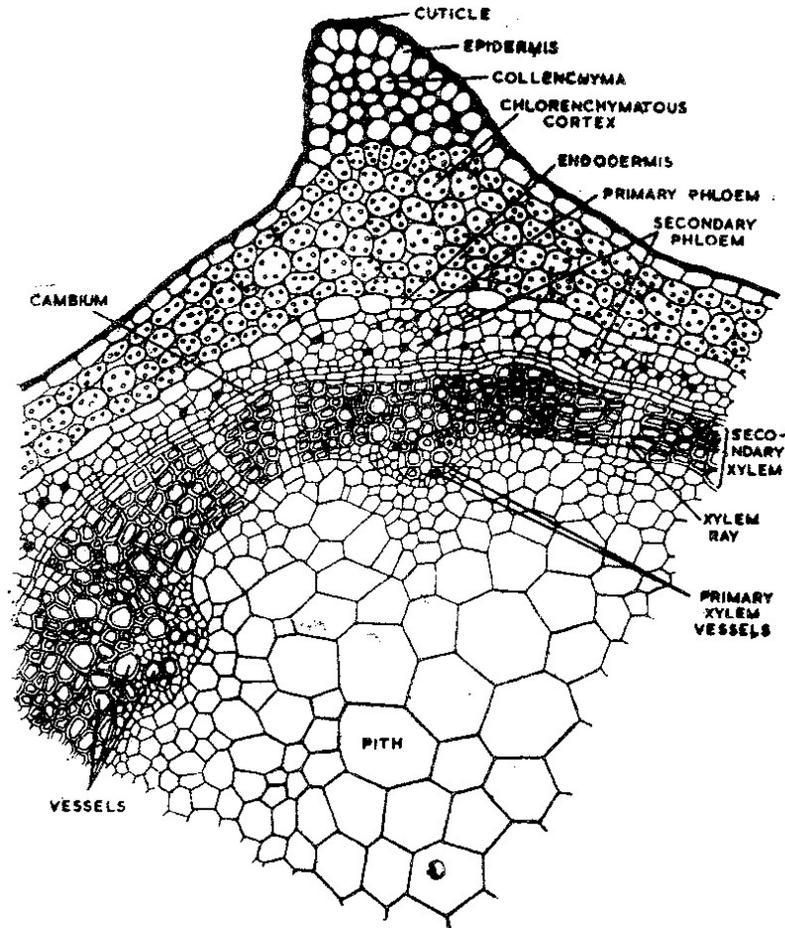


Outline Diagram of Transection of Stem of *Peristrophe Bicalyculata*

Secondary Growth in Stem of *Peristrophe Bicalyculata*

- The stem is angular in outline in transection and shows six distinct ridges and furrows.
- Epidermis is single layered with thin-walled parenchymatous cells.
- Cortex is collenchymatous in ridges, whereas in furrows it is chlorenchymatous.
- Endodermal cells are barrel-shaped, with casparian strips on their radial walls.

- e) In the primary stem, six vascular bundles are present corresponding to ridges; these are conjoint, collateral, open and endarch.



Transection of Stem of *Peristrophe Bicalyculata* – A Cellular Details

- a) During secondary growth, vascular cambium cuts off the secondary phloem towards outside and secondary xylem towards inside. Secondary xylem is interrupted by narrow rays, that may be uniseriate or biseriate.
- b) Pith is made up of thin-walled polygonal cells.

EXPERIMENT-2

NODAL ANATOMY

To study the structure of the node, take series of sections from internode to the above node and stain them with 10% phloroglucinol and 5% conc. nitric acid. Xylem takes dye slowly and stains dark orange red.

In angiosperms, stem possesses nodes and internodes. The place where leaf traces or branch traces arise is referred to as **node**. The structure of vascular cylinder shows variations in the regions of nodes and internodes.

Leaf Traces:

In the nodal region, a part of the vascular cylinder enters in the leaf is known as **leaf trace** (vascular supply to the leaf). A leaf trace may also be defined as a vascular bundle that connects the vascular bundle of the leaf with that of the stem. The vascular supply that goes to branch at the nodal region, is known as **branch trace**.

Leaf Gaps:

In higher plants such as ferns, gymnosperms and angiosperms, there is a discontinuity of vascular cylinder of stem just above the diverging leaf traces. This break up region of vascular cylinder made up of parenchyma is known as **leaf gap** (nodal lacuna). Leaf traces are always accompanied by leaf gaps. However, in Lycopsidea leaf gaps are absent and these nodes are known as **Cladosiphonic nodes**. Branch traces are also accompanied by branch gaps.

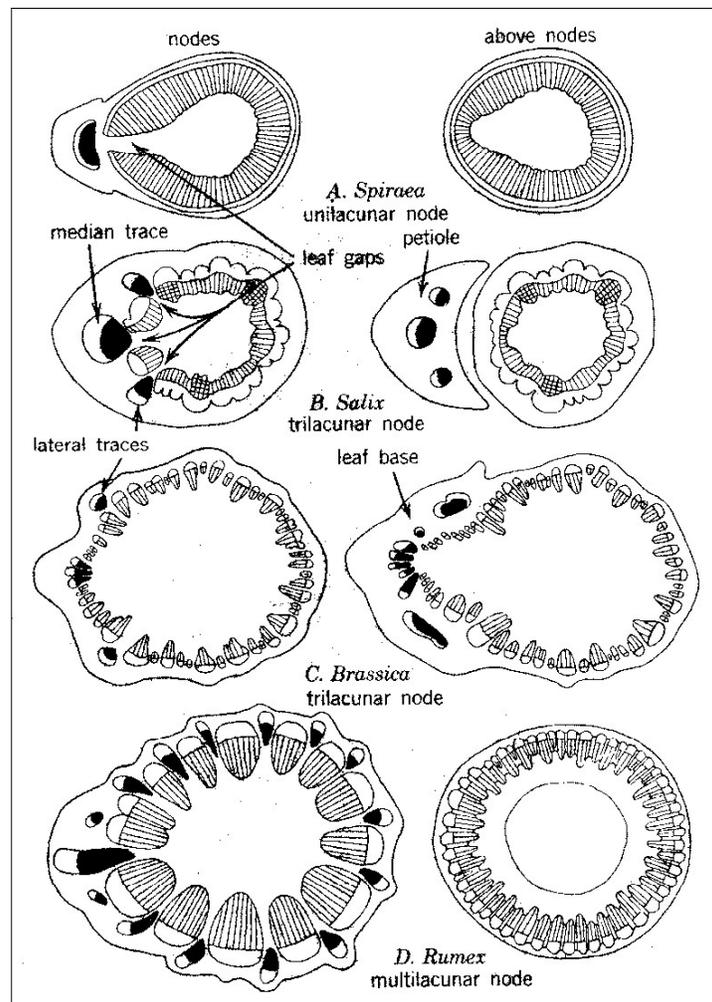
In some ferns, such as *Pteridium* and *Pteris*, the leaves are so crowded that the gaps formed at the successive nodes overlap one another, as a result actual vascular cylinder becomes dissected. Presence of branch gaps further complicates the structure.

In general, at the internodal region solid vascular cylinder is present whereas at the node it becomes dissected due to presence of leaf gaps and branch gaps.

Types of Nodes:

The arrangement of leaf traces and their complexes varies in different groups of plants and is related to phyllotaxis. There are four basic types of nodes found in dicotyledons. These are as follows:

- Unilocunar node** with a single leaf gap and a single leaf trace, e.g. *Spiraea*, and it is found in the opposite and whorled phyllotaxis.
- Unilocunar two trace** is found in opposite leaves, e.g., *Clerodendron*, *Veronica* etc.
- Trilocunar node** with a three leaf gaps and three leaf traces, e.g., *Salix*, *Brassica*, *Leptadenia* etc.
- Multilocunar node** with several leaf gaps and leaf traces associated with a single leaf, e.g. *Rumex*, *Ricinus* etc.



Transection of Stems of Dicotyledons Showing Different Types of Nodes

EXPERIMENT-3

ANATOMICAL FEATURES OF DICOT ROOT

Aim: To identify and make section cutting of monocot root sample.

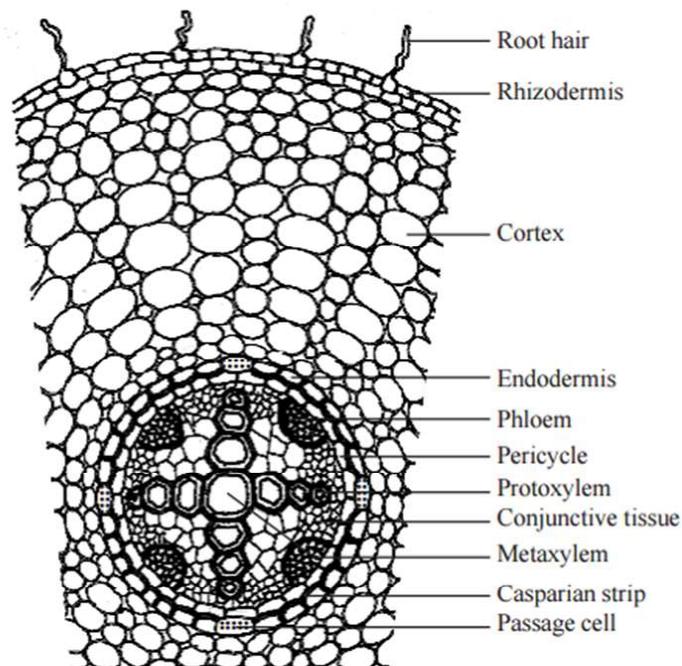
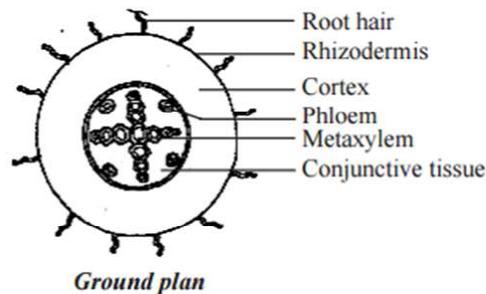
Material: Dicot root (*Cicer arietinum*)

Apparatus: Compound microscope, slides, blades and brush.

Chemicals: Glycerol and Safranin.

Procedure:

Take fresh roots of *Cicer arietinum* and make thin transverse sections of the root. Observe the sections of root under microscope and note the detailed features of dicot root.



Transverse Section of Dicot Root (*Cicer arietinum*)

Transverse Section of *Cicer arietinum* Root Shows Following Anatomical Features-**Epidermis:**

- Consists of closely packed elongated cells with thin walls that usually lack a cuticle and stomata.
- In some dicotyledons, thickened outer walls occur in root parts growing in air.
- The root epidermis (also known as piliferous layer, rhizodermis, or epiblema) is typically uniseriate.
- Most of the epidermal cells extend out in the form of tubular unicellular root hairs.
- Some roots also develop a specialized layer of exodermis beneath the epidermis.
- The exodermis arises from one or several of the sub-epidermal layers of the cortex.
- The cell walls of exodermis become suberized.

Cortex:

- The cortex is massive and thin-walled rounded or polygonal parenchyma cells having sufficiently developed intercellular spaces among them.
- The parenchyma cells of cortex contain abundant starch grains in them.
- The presence of schizogenous spaces are large and form distinct air spaces.
- The cortex of roots is generally devoid of chlorophyll.
- Various secretory structures are found in the root cortex.

Endodermis:

- The endodermis is uniseriate and almost universally present in the roots.
- Cells of endodermis are living.
- The endodermis is characterized by the presence of Casparian strips or Casparian bands on their anticlinal walls.
- The pericycle of relatively young roots consists of thin-walled parenchyma.

The vascular System:

- The xylem forms discrete strands, alternating with phloem.
- The roots typically show exarch xylem.
- The phloem is also centripetally differentiated, i.e., the protophloem occurring closer to the periphery than the metaphloem.

EXPERIMENT-4**ANATOMICAL FEATURES OF MONOCOT ROOT**

Aim: To identify and make section cutting of monocot root sample.

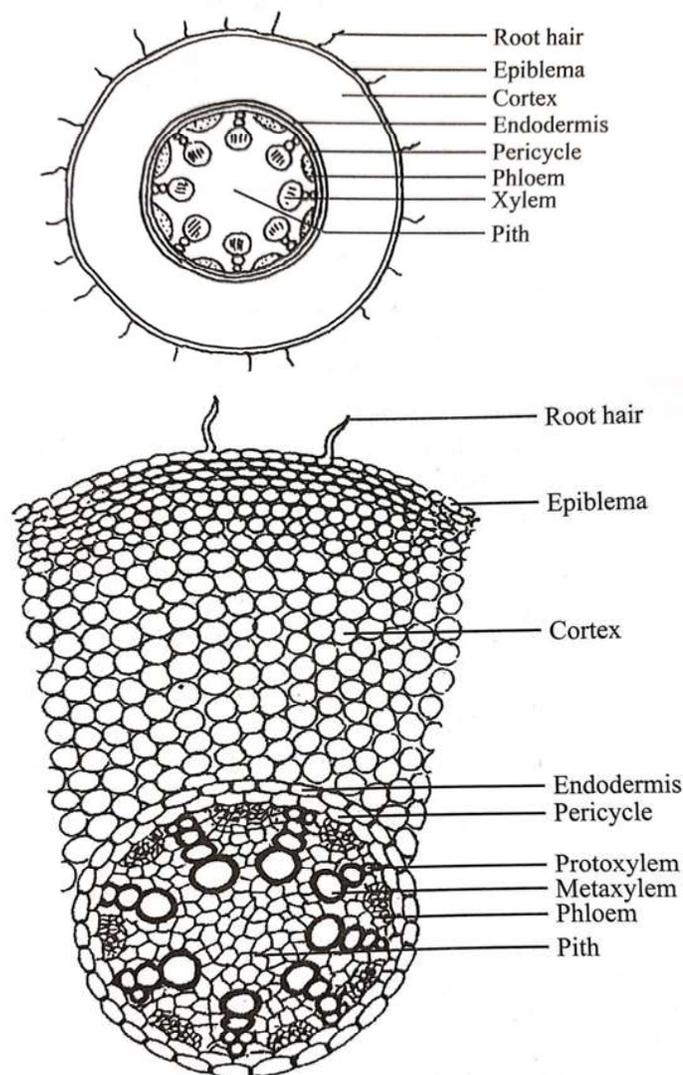
Material: Monocot root (*Canna indica*)

Apparatus: Compound microscope, slides, blades and brush.

Chemicals: Glycerol and Safranin.

Procedure:

Take fresh roots of *Canna indica* and make thin sections of the root. Observe the sections of root under microscope and the detailed features of monocot root.



Transverse Section of Monocot Root *Canna indica*

Transverse Section of *Canna indica* Root Shows the Following Anatomical Features-**Epidermis:**

- The epidermis or outermost layer of the root is commonly known as rhizodermis, epiblema, or piliferous layer.
- It is uniseriate and composed of compact tabular cells having no intercellular spaces and stomata.
- The cell walls develop fibrous thickenings.

Exodermis:

- Generally, beneath the epidermis, there are present one or more layers of exodermis.
- Usually, exodermis consists of a single row of cells with thickened outer and lateral walls except for certain passage cells which remain thin-walled.
- A typical characteristic of the root epidermis is the development of root hair.
- The tubular unicellular root hairs are present on this layer.

Cortex:

- Cortex consists of thin-walled parenchyma cells having sufficiently developed intercellular spaces among them.
- The starch grains are abundantly present in cortical cells.
- The sclerenchyma cells are commonly found in the cortex of monocotyledons.

Endodermis:

- The endodermal cells possess Casparian strips in their anticlinal walls.
- The strip is typically located close to the inner tangential wall.
- Thick-walled passage cells are formed opposite the protoxylem poles.

Pericycle:

- It is usually uniseriate and composed of thin-walled parenchymatous cells.
- In the monocotyledons, the pericycle often undergoes scarification in older roots, partly or entirely.
- The pericycle gives rise to lateral roots only.

Vascular Tissue:

- The phloem of root occurs in the form of strand near the periphery of the vascular cylinder, beneath the pericycle.
- Xylem forms discrete strands, alternating with the phloem strands.
- The xylem is exarch, i.e., the protoxylem lies towards the periphery and the metaxylem towards the centre.
- The phloem is also exarch.

EXPERIMENT-5

ANATOMICAL FEATURES OF DICOT STEM

Aim: To identify and make section cutting of dicot stem sample.

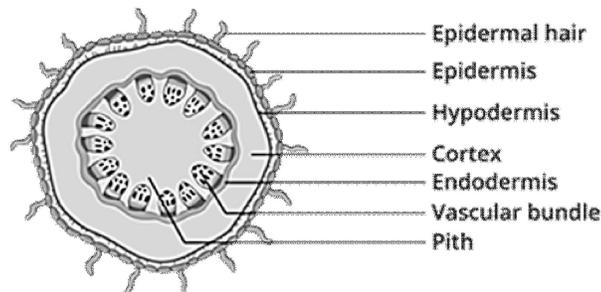
Material: Dicot stem (*Tridax procumbens*)

Apparatus: Compound microscope, slides, blades and brush.

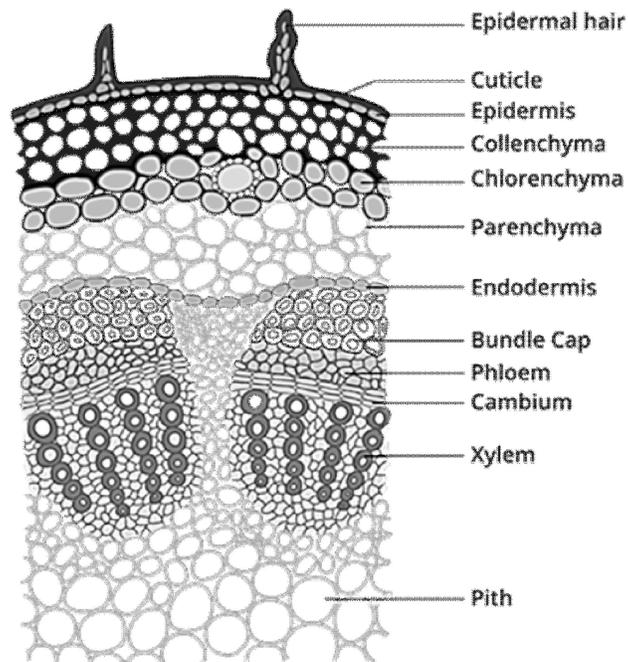
Chemicals: Glycerol and Safranin.

Procedure:

Take fresh stem samples of *Tridax procumbens* and make a thin section of the stem. Observe the sections of stem under microscope. Note the detailed features of dicot stem.



Ground plan



A sector enlarged

Transverse Section of Dicot Stem *Tridax procumbens*

Transverse Section of *Tridax procumbens* Stem Shows Following Anatomical Features**Epidermis:**

- This forms outer most layers and consists of single row of cells and fit along their radial walls with a well-defined cuticle over it.
- It bears some multicellular hairs and few stomata but no chloroplasts except in the guard cells. Cortex It lies in between epidermis and pericycle and consists of hypodermis externally, general cortex centrally and endodermis internally.

Hypodermis (collenchyma):

- This lies immediately below the epidermis and consists of 4 to 4 layers of collenchymatous cells.
- These cells are thick at the corners against the intercellular spaces owing to deposit of cellulose and pectin. The cells are living and contain number of chloroplasts.
- General cortex lies internal to hypodermis and consists of few layers of thin walled large rounded or oval parenchymatous cells. There are clear intercellular spaces in it.

Endodermis:

- This is innermost layer of the cortex consisting of barrel shaped cells and surrounding the stele.
- Endodermis is conspicuous outside the hard bast but often loses its identity on either side. It contains many starch grains and is also known as starch sheath.

Vascular Bundles:

- Protoxylem vessels with smaller cavities and annular or spiral thickenings are always placed towards centre, and metaxylem vessels, with wider cavities and reticulate scalariform or pitted thickening, towards, the epidermis.
- This arrangement, typical of stems, is called endarch.
- Phloem parenchyma lies outside or towards the circumference.
- A strip of lateral meristem, called cambium, is present between xylem and phloem.

EXPERIMENT-6**ANATOMICAL FEATURES OF MONOCOT STEM**

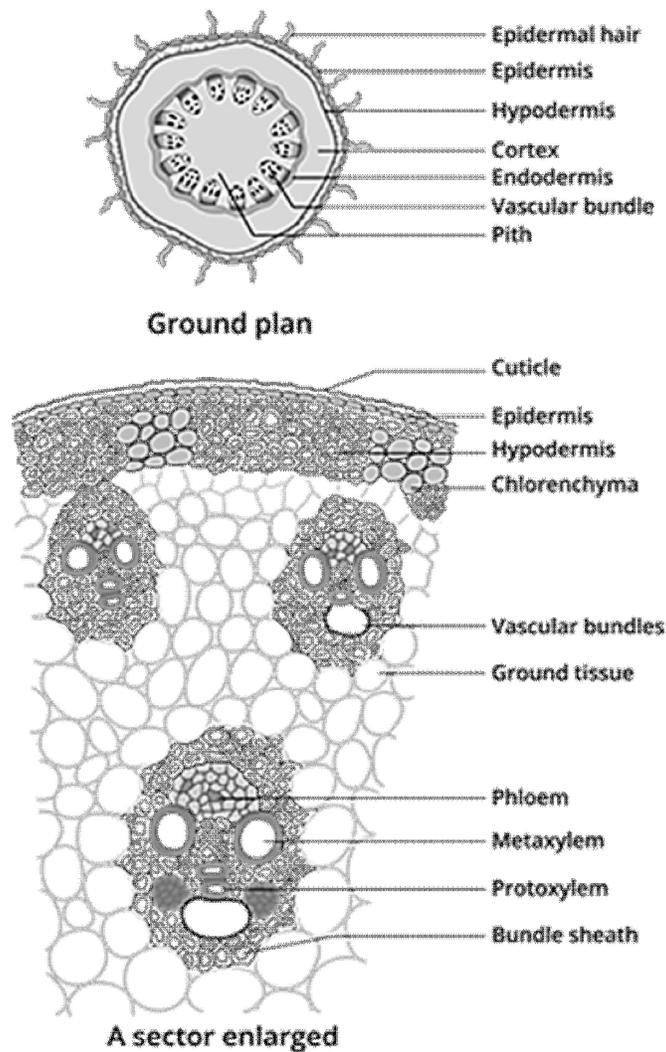
Aim: To identify and make section cutting of monocot stem sample.

Material: Monocot stem (*Canna indica*)

Apparatus: Compound microscope, slides, blades and brush.

Chemicals: Glycerol and Safranin.

Procedure: Take fresh stem samples of *Canna indica* and make a thin section of the stem. Observe the sections of stem under microscope. Note the detailed features of monocot stem.



Transverse Section of Monocot Stem *Canna indica*

Transverse Section of *Canna* Stem Shows the Following Anatomical Features-**Epidermis:**

- Made up of single layer of cells with thick cuticle.
- Stomata are present.
- Multicellular hairs are absent.

Hypodermis:

- Two-Four layer of sclerenchyma present below the epidermis and intercellular spaces are absent.
- Entire mass of parenchymatous cells next to hypodermis from ground tissue.
- No differentiation between cortex, endodermis and pericycle.
- In the periphery ground tissue cells are smaller and compactly arranged and towards the centre the cells are bigger and loosely arranged.
- Vascular Bundles are present in this tissue.

Vascular Bundles:

- Many vascular bundles are scattered in the ground tissue.
- Each vascular bundle is parenchymatous surrounded by a sheath of sclerenchymatous fibre.
- The sheath is extensively developed at the upper and lower faces of vascular bundles.

EXPERIMENT-7

ANOMALOUS SECONDARY GROWTH IN DICOTS (*BOERHAVIA*)

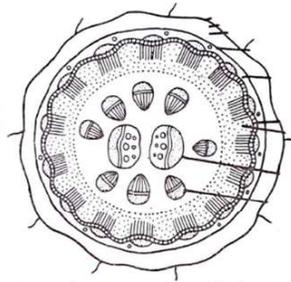
Aim: To identify and make section cutting of given plant sample and observe anomalous secondary growth in dicots.

Material: *Boerhavia* stem

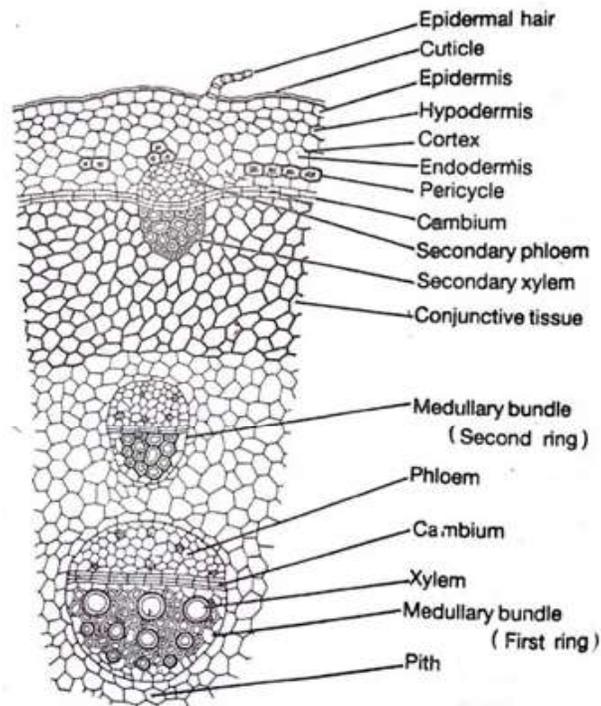
Apparatus: Compound microscope, slides, blades and brush.

Chemicals: Glycerol and Safranin.

Procedure: Take fresh stem samples of *Boerhavia* and make a thin section of the stem. Observe the sections of stem under microscope. Record the detailed features of anomalous secondary growth in dicot stem *Boerhavia*.



Anomalous Secondary Thickening
In *Boerhavia* Stem (Diagrammatic)



Anomalous Secondary Growth in Dicot Stem *Boerhavia*

Transverse Section of *Boerhavia* Stem Shows the Following Anatomical Features-

Transverse section of *Boerhavia* is circular in outline and reveals the following tissue from outside to inside:

Epidermis:

Single layered epidermis consists of small, radially elongated cells. Multicellular epidermal hairs arise from some cells. A thick cuticle is present on the epidermis. Some stomata are also present.

Cortex:

It is well differentiated and consists of few layered collenchymatous hypodermis followed by chlorenchyma. Collenchyma is 3 to 4 cells deep, but generally near stomata it is only one layered. Chlorenchyma is present inner to collenchyma in the form of 3 to 7 layers. Chlorenchymatous cells are thin walled, oval, full of chloroplasts and enclose many intercellular spaces.

Endodermis:

It is clearly developed and made up of many, tubular, thick-walled cells.

Pericycle:

Inner to the endodermis is present parenchymatous pericycle but at some places it is represented by isolated patches of sclerenchyma.

Vascular System:

Vascular bundles are present in three rings. In the innermost ring are present two large bundles; in the middle ring the number ranges from 6 to 14 while the outermost ring consists of 15 to 20 vascular bundles. Vascular bundles of innermost and middle rings are medullary bundles. **Vascular bundles are conjoint, collateral and endarch.** Two vascular bundles of the innermost ring are large, oval and lie opposite to each other with their xylem facing towards centre and phloem outwards. Middle ring consists of 6-14 small vascular bundles. Vascular bundles of inner and middle rings may show a little secondary growth. **Phloem consists of sieve tubes, companion cells and phloem parenchyma while the xylem consists of vessels, tracheid and xylem parenchyma.** Outermost ring of the vascular bundles contains inter-fascicular cambium which is absent in other two rings. Cambium develops secondarily from the pericycle and becomes active. It cuts secondary phloem towards outer side and secondary xylem towards inner side. Due to these changes the primary phloem becomes crushed and present next to pericycle. Primary xylem is situated near the pith. Inter fascicular cambium also soon becomes active and cuts internally the row of cells which become thick walled and lignified and is known as conjunctive tissue.

Pith:

It is well developed, parenchymatous and present in the centre.

Identification:

1. Presence of vessel in the xylem. (Angiosperms). 2. Cortex is well-differentiated. 3. Vascular bundles are conjoint, collateral, open and endarch. (Stem). 4. Vascular bundles are present in ring. 5. Well-developed secondary growth. 6. Well-defined pith. (Dicotyledons).

EXPERIMENT-8

ANOMALOUS SECONDARY GROWTH IN MONOCOTS (*DRACAENA*)

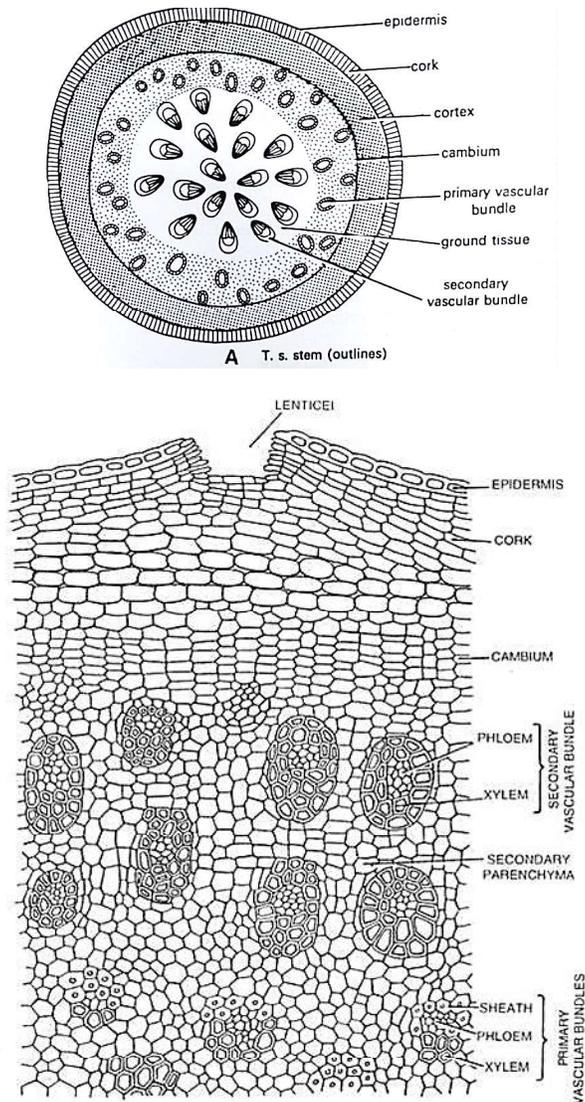
Aim: To identify and make section cutting of given plant sample and observe anomalous secondary growth in monocots.

Material: *Dracaena* stem

Apparatus: Compound microscope, slides, blades and brush.

Chemicals: Glycerol and Safranin.

Procedure: Take fresh stem samples of *Dracaena* and make a thin section of the stem. Observe the sections of stem under microscope. Note the detailed features of anomalous secondary growth in dicot stem *Dracaena*.



Anomalous Secondary Growth in Monocot Stems (*Dracaena*)

Transverse Section of *Dracaena* Stem Shows the Following Anatomical Features-

Monocotyledons normally do not show secondary growth due to absence of vascular cambium, but few plants like *Dracaena*, *Yucca*, *Aloe* etc. show anomalous secondary growth.

Primary Structure:

Dracaena is an arborescent plant, belonging to the family *Liliaceae*. The young stem shows typical monocotyledonous structure.

Epidermis:

Outer most layer made of single layer of cells with thin cuticle on the outside.

Cortex:

Several rows of parenchymatous cortex is present.

Stele:

Several vascular bundles are scattered irregularly in the ground tissue. The vascular bundles are collateral, and closed.

Anomalous Secondary Growth:

During the initiation of secondary growth, the parenchymatous cells of the cortex, external to the primary vascular bundles, become meristematic and forms the cambium in a ring. The cells of cambium divide and produce more cells towards the inner side and few cells towards outside. The cells produced on the inner side develop into vascular bundles and conjunctive tissue. Each vascular bundle develops from a single cambial initial. The initial divides first by anticlinally to form a row of two or three cells. These cells undergo periclinal division, but the division soon become irregular. This results in the formation of a group of cells. The peripheral cells develop into xylem elements, whereas the central cells differentiate into phloem elements. This leads to formation of amphivasal vascular bundles.

Formation of Cork:

After certain period of secondary growth, the parenchymatous cells below the epidermis give rise to the meristematic initials. The initials divide several times periclinally. The cells thus formed become suberised and form the cork or phellem. This cork, with suberin coated cells and radial or storied arrangement is called as storied cork.

EXPERIMENT-9
ANATOMICAL FEATURES OF DICOT LEAF (*NERIUM*)

Aim: To identify and make section cutting of dicot leaf sample.

Material: *Nerium* leaf

Apparatus: Compound microscope, slides, blades and brush.

Chemicals: Glycerol and Safranin.

Procedure:

Take fresh leaf samples of *Nerium* and make a thin section of the leaf. Observe the sections of leaf under microscope. Make a detailed note on features of *Nerium* leaf.

Transverse Section of *Nerium* Leaf Shows following Anatomical Features -

Epidermis:

Thick cuticle is present just outside the epidermis. Both upper and lower epidermal layers are multiseriate. They are composed of a few layers compactly arranged isodiametric colorless cells. There are distinct sunken stomata on the lower epidermis while upper epidermis is devoid of stomata. A good number of trichome develops from the bordering cells and remain projected within the stomatal pit.

Mesophyll:

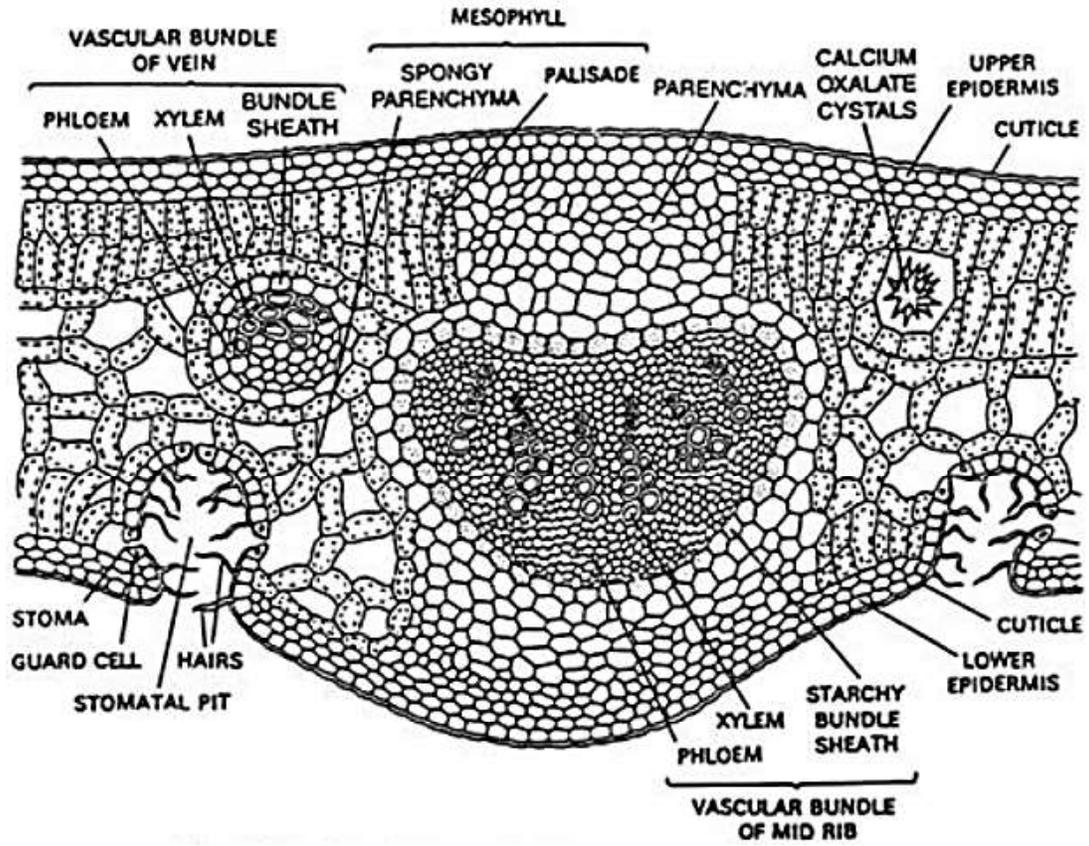
It is made up of palisade and spongy cells. In this plant palisade cells here both towards upper and lower epidermis and spongy cells are located in between them. Calcium oxalate crystals are scattered within the mesophyll cells.

Vascular Bundles:

The bundles are collateral and closed ones with xylem on the upper and phloem on the lower sides. They remain surrounded by bundle sheaths made up of parenchyma cells.

Dicotyledonous Leaf features:

- a) Leaf is bifacial.
- b) Stomata present on lower side of epidermis.
- c) Mesophyll is made up of palisade and spongy cells.



Transverse Section of Dicot Leaf *Nerium*

EXPERIMENT-10**ANATOMICAL FEATURES OF MONOCOT LEAF (*ZEА MAYS*)**

Aim: To identify and make section cutting of monocot leaf sample.

Material: *Zea mays* leaf

Apparatus: Compound microscope, slides, blades and brush.

Chemicals: Glycerol and Safranin.

Procedure:

Take fresh leaf samples of *Zea mays* and make a thin section of the leaf. Observe the sections of leaf under microscope. Note the detailed features of *Zea mays* leaf.

Transverse Section of *Zea Mays* Leaf Shows the Following Anatomical Features-**Epidermis:**

It is present on lower side as well as upper side. Stomata present on both sides. In upper epidermis a few bulliform cells can be seen, which are few, large and colourless.

Mesophyll:

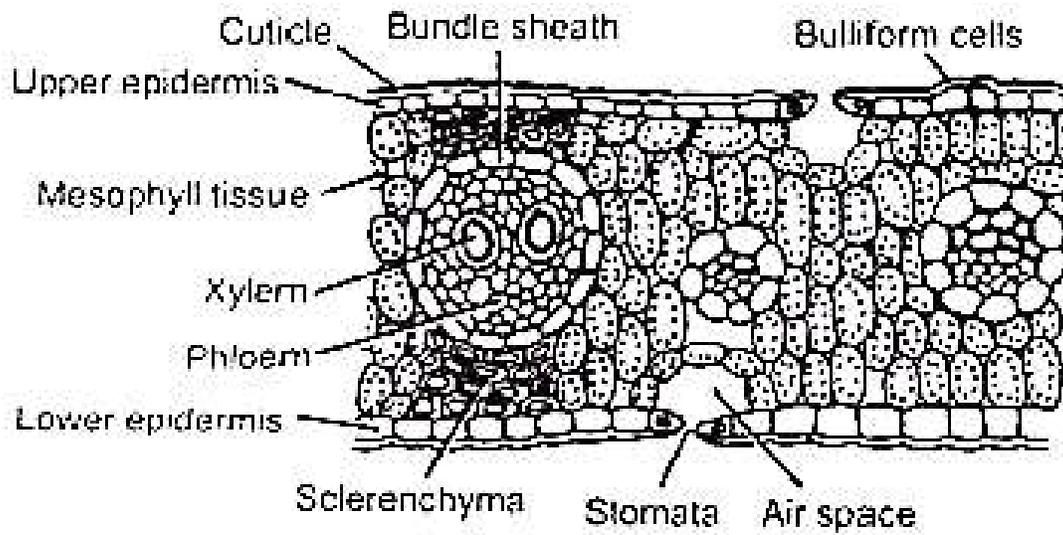
It occurs between upper and lower epidermis. Cells are isodiametric and containing chloroplasts. These are compactly arranged cells. This tissue is not differentiated into palisade and spongy parenchyma.

Vascular Bundles:

These are many in numbers and arranged in parallel series. Each bundle is collateral and closed type and surrounded by parenchymatous bundle sheath. Larger bundle has more xylem and phloem than smaller ones.

Monocotyledonous Leaf Features:

- a) Leaf is isobilateral.
- b) Stomata present on both side of epidermis.
- c) Mesophyll tissue is not differentiated into palisade and spongy parenchyma.



Transverse Section of Monocot Leaf *Zea mays*

EXPERIMENT-11**STUDY OF STRUCTURE AND TYPES OF STOMATA**

Aim: To identify and observe structure, types of stomata.

Material: Plant Sample

Apparatus: Compound microscope, slides, blades and brush.

Chemicals: Nail polish

Procedure:

- Take fresh leaf samples of Cucurbitaceae, Brassicaceae, Acanthaceae and Fabaceae.
- Clean both upper and lower surfaces of leaves under running tap water in order to remove dust particles. Gently wipe the water present on the leaves.
- Take a transparent nail polish and apply a thin layer on both the sides of the leaves using point brush.
- After five minutes carefully remove the thin layer of nail polish with the help of forceps and mount the nail polish peeling on a clean glass slide and cover the peeling with transparent cellophane tape.
- Observe the slide under microscope and identify the features of various types of stomata present in the given leaf samples.

Stomata:

There are tiny openings in certain parts in all green plants which are essential in the gas control. They are kidney like shape in dicot and dumbbell shape in monocot.

Types of Stomata:

Metacalf and Chalk recognized four types of stomata on the basis of their structure

Anomocytic Type:

In these stomata, accessory cells are absent. The guard cells are surrounded by ordinary epidermal cells, e.g., families Ranunculaceae, Cucurbitaceae, Papaveraceae and Malvaceae.

Anisocytic Type:

In these stomata the guard cells are surrounded by three accessory cells. Of these two are larger whereas one is smaller in size, family Brassicaceae.

Paracytic Type:

In these stomata the guard cells are also surrounded by two accessory cells, but their common walls are parallel to guard cells, e.g., families Rubiaceae, Fabaceae etc.

Diacytic Type:

In these stomata the guard cells are surrounded by two accessory cells. Their common walls are at right angle to the walls of guard cells, families Caryophyllaceae, Acanthaceae.

Actinocytic (Star Celled):

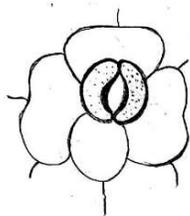
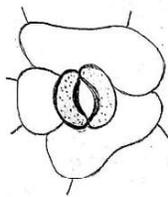
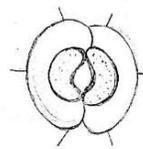
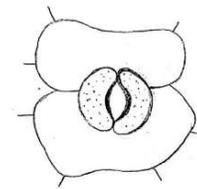
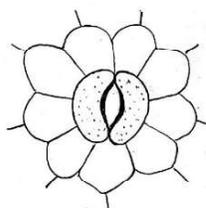
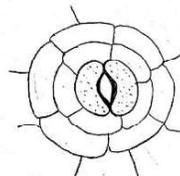
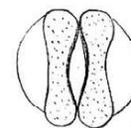
In this type, the stomata have guard cells that are surrounded by at least five radiating cells forming a star-like circle. This is a rare type of stomata that can for instance be found in the family Ebenaceae.

Cyclocytic (Ring-Celled):

This type of stomata is surrounded by four or more subsidiary cells forming a narrow ring around the guard cells. Examples: Palmae, Pandanus, Cyclanthaceae.

Gramineous (Grass-Like):

Gramineous or graminoid stomata have two guard cells surrounded by two lens-shaped subsidiary cells. The guard cells are narrower in the middle and bulbous on each end. This middle section is strongly thickened. The axis of the subsidiary cells is parallel to the stoma opening. This type can be found in monocot families including Poaceae and Cyperaceae.

**Anomocytic****Anisocytic****Paracytic****Diacytic****Actinocytic****Cyclocytic****Gramineous****Various Types of Stomata**

EXPERIMENT-12**STUDY OF ANATOMICAL DIFFERENCES BETWEEN C₄ AND CAM PLANTS**

Aim: To identify basic anatomical variations between C₄ and CAM plants.

Material: C₄ and CAM plant leaves

Apparatus: Compound microscope, slides, blades and brush.

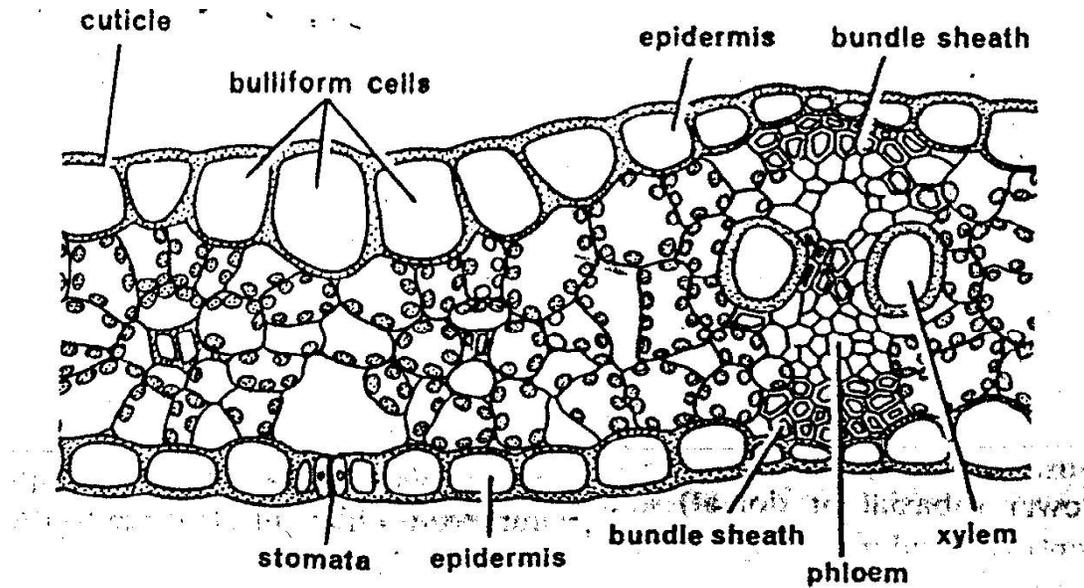
Chemicals: Glycerol and Safranin.

Procedure:

Take the section cuttings of fresh leaf samples of C₄ and CAM plants. Observe the sections of leaf under microscope. Note and compare detailed differences between C₄ and CAM plants.

Transverse Section Showing Differences between C₄ And CAM Plants-**Leaf Anatomy of C₄ Plants:**

- 1) **Epidermis:** The leaf is bound by thickly cuticularized epidermis on both sides. Stomata are found in lower as well as upper epidermis.
- 2) **Mesophyll:** This tissue is located between the two epidermal layers. It is composed of spongy parenchyma of varied shapes and sizes, leaving large intercellular spaces.
- 3) **Vascular Bundles:** The vascular bundles differ in their size. Each vascular bundle is conjoint, collateral and closed. Tightly packed thick-walled bundle sheath cells surround the bundle. A patch of sclerenchyma occurs on both ends of each vascular which extends up to the epidermis on their respective sides. The bundle sheath cells are arranged like a wreath around the bundle. This is called Kranz anatomy because kranz means wreath. Large number of chloroplasts is present in the bundle sheath cells. Xylem is located towards the upper side while phloem is present in lower side.

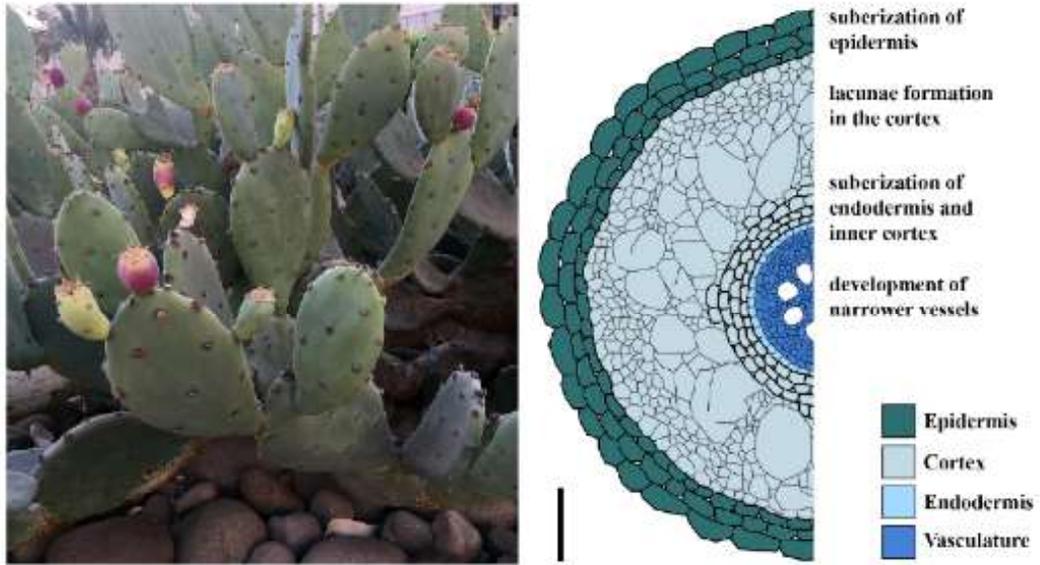


Leaf Anatomy C₄ Plants

Leaf Anatomy of CAM Plants:

CAM plants (e.g., *Opuntia*, *Kalanchoe*, *Agave*, *Aloe*) show distinctive anatomical adaptations that support nocturnal CO₂ uptake, malic acid storage, and extreme water-use efficiency.

- 1) **Epidermis:** Epidermis covered by a thick, waxy cuticle. Minimizes water loss during daytime. Often impregnated with cutin and waxes. Stomata are sunken into epidermal pits. Often fewer in number. Open at night (scotoactive stomata). Reduce transpiration during hot daytime.
- 2) **Hypodermis:** Usually multi-layered. Cells are thick-walled, sometimes sclerenchymatous. Acts as Mechanical support, additional water storage. Common in *Agave* and *Aloe*. Unlike C₄ plants, CAM plants do not show Kranz anatomy. Mesophyll is not differentiated into palisade and spongy tissues. Cells are large, thin-walled and isodiametric. Cells contain large central vacuole, peripheral cytoplasm. Vacuole stores malic acid (as malate) at night. Cell volume may increase nocturnally due to acid accumulation. Vacuoles are extremely large. Occupy 80–90% of cell volume. Chloroplasts are numerous, located in the peripheral cytoplasm. Intercellular spacers are poorly developed or reduced.
- 3) **Vascular Bundles:** Vascular bundles are relatively reduced, embedded within succulent tissue. Xylem is with narrow vessels (reduces cavitation risk). Phloem shows efficient translocation of photosynthates



Transverse Section of CAM Leaf *Opuntia*

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