1th SEM B.Sc. ZOOLOGY

UNIVERSITY OF CALICUT

ANGIOSPERM ANATOMY AND MICROTECHNIQUE

(COMPLEMENTARY COURSE)

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BOT1C01T. ANGIOSPERM ANATOMY AND MICROTECHNIQUE

Syllabus

ANGIOSPERM ANATOMY

Module – I

1 Tissues - Definition, Kinds - Meristematic & Permanent.

1. Meristematic tissues - Classification – based on origin & position; Organization of root apex and differentiation of tissue – Histogen theory; Organization of stem apex and differentiation of tissues - Tunica & Corpus theory.
2. Permanent tissues - Definition - classification; Simple tissues (Parenchyma, Collenchyma and Sclerenchyma), Complex tissues (Xylem & Pholem) Secretory tissues - Glandular tissues (Nectaries in *Euphorbia pulcherrima*, Stinging hairs in *Tragia*) Oil glands in *Citrus, Eucalyptus*; Digestive glands in *Nepenthes*; Laticiferous tissues (Non-articulate latex ducts in *Euphorbia* and articulate latex duct – latex vessels in *Hevea*), Hydathodes.

2. Vascular bundles – types: conjoint - collateral, bicollateral, concentric and radial.

Module – II

1. Primary structure of dicot and monocot root, dicot and monocot stem and leaf in dicot and monocot.

Module – III

1. Normal secondary thickening in dicot stem (*Vernonia*).

a. Intra stelar thickening: formation of cambial ring, its structure, fusiform and ray initials, storied and non - storied cambium, activity of the cambium, formation and structure of secondary wood, secondary phloem and vascular rays.

b. Extra stelar thickening: formation, structure and activity of the phellogen, formation of periderm in stem and root; bark and lenticel.

c. Growth rings, ring and diffuse porous wood, sapwood and heart wood, tyloses.

d. Normal secondary thickening in dicot root (*Tinospora*)

2. Anomalous secondary growth in *Boerhaavia*.

MICROTECHNIQUE

Module – I

1. Microtechnique - Brief Introduction
2. Microscopy: simple, compound and electron microscope
3. Microtomy: Rotary type, serial sectioning, paraffin method, significance.
4. Killing and fixing: Killing and fixing agents and their composition (Farmer's fluid and FAA.)
3. Dehydration and clearing - reagents (mention only)
4. Stains – Saffranin and acetocarmine, preparation and use.

ANGIOSPERM ANATOMY

Module-1

1. Tissues - Definition, Kinds - Meristematic & Permanent.

1. Meristematic tissues - Classification – based on origin & position; Organization of root apex and differentiation of tissue – Histogen theory; Organization of stem apex and differentiation of tissues - Tunica & Corpus theory.

Tissue is an organized group of cells which have a common origin, similar structure, and the same function

Based on location and function,

1. Meristematic tissues
2. Permanent tissues
3. Secretory tissues
4. Lactiferous tissues

Part A: Meristematic tissues - classification.

- Are group of living, immature and undifferentiated cells, which
- remain in a state of continuous division
- Cells are thin walled, isodiametric, compactly arranged, with or
- without intercellular spaces
- Formed of cellulose
- Abundant protoplasm
- Cells do not store reserve food materials

❖ **Classification of Meristems**

A. Based on Position

1. Apical meristem
2. Intercalary meristem
3. Lateral meristem

B. Classification based on origin

1. Promeristem or Primordial meristem

- Consists of meristamatic cells representing the earliest stage of a growing organ
- Location: extreme tip of stem and root
- Function: forms primary meristem and later the primary structure of the plant body

2. Primary meristem

- Consists of meristamatic cells formed from promeristem
- Location: Seen just below promeristem
- Function: forms the primary structure of the plant body
- EG: promeristem at the shoot apex give rise to protoderm, procambium, ground meristem or fundamental meristem
- Protoderm- epidermal tissue system
- Procambium- primary vascular tissues
- Ground meristem- into cortex and pith

3. Secondary meristem

- Consists of meristamatic cells formed from primary
- permanent tissues.
- Some of the primary permanent tissues regains the

- merisitamatic activity and becomes secondary meristem
- Location: Laterally placed in stem and root
- Function: responsible for secondary growth of the plant
- Example: Inter fascicular cambium and cork cambium of stem,
- cambium of root.

C. Based on plane of division

1.Mass Meristem

- In mass Meristem, cells divide in all planes forming mass of tissues.
- Eg: Development of embryo, endosperm and sporangia etc

2.Plate Meristem

- In Plate Meristem, cells divide in two planes forming a plate like structure.
- Eg: Single layered epidermis

3.Rib Meristem

- In Rib Meristem, cells divide in only one plane and forms rows or columns of cells.
- Eg: Responsible for the increase in length of organs

i. Theories on apical organisation - Apical cell theory, Histogen theory, Tunica corpus theory

1. **APICAL CELL THEORY**
   - Nageli-1958
   - Single tetrahedral apical cell in the root apex brings about growth
   - Apical cell theory is confined to vascular cryptogams only as the root apical meristem of flowering plants does not have a single apical cell.

1. **Korper – kappe theory**
   - By schuepp (1917)
   - The cells at the root apex divides in two planes
   - First transverse, then one of the daughter cell divides longitudinally

- This sequence division is called T division
- Zone with inverted T type division – Korper (cap)
- Straight T type division – Kappe (body)
- Fails to explain the differences in behavior in different species

2. Histogen Theory

- Proposed by Hanstein (1870)
- Apical root meristem consists of different meristematic zones or layers called Histogens
  1. Dermatogen
  2. Periblem
  3. Plerome
  4. Calyptrogen

ii. Organization of shoot apex and differentiation of tissues- (protodern, procambium and ground meristem should be mentioned).

- Organisation of shoot apex
- Shoot apex is the apical meristem present at the tips of stems

1. Apical cell theory

2. Histogen theory

3. Tunica corpus theory

- proposed by Schmidt in 1924
- Outer Tunica and inner Corpus
- Tunica cells divides anticlinally, so surface area increases
- Corpus cells divides anticlinally and periclinally

iii. Kopper-Kappe theory- organization of root apex in dicots- common types with three sets of initials- in monocots – Maize type with four sets of initials

1. Ranunculus type

2. Casuarina type

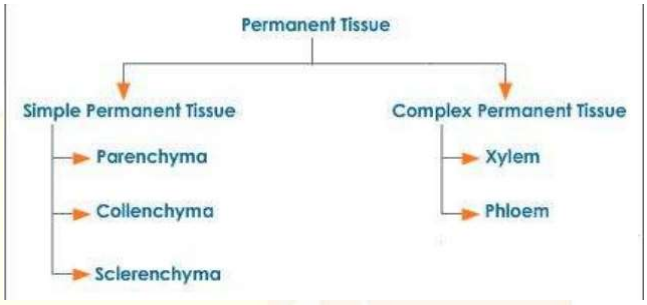
3. Common type

2. Permanent tissues - Definition - classification; Simple tissues (Parenchyma, Collenchyma and Sclerenchyma), Complex tissues (Xylem & Pholem) Secretory tissues - Glandular tissues (Nectaries in *Euphorbia pulcherrima*, Stinging hairs in *Tragia*) Oil glands in *Citrus,*

Eucalyptus; Digestive glands in *Nepenthes*; Laticiferous tissues (Non-articulate latex ducts in *Euphorbia* and articulate latex duct – latex vessels in *Hevea*), Hydathodes.

i. Simple tissues – parenchyma, collenchyma, sclerenchyma, - fibres and sclereids- structure occurrence and function.

- Simple tissues are tissues wherein the growth process has been ceased. originate from both the primary and the secondary meristematic tissue and possess a definite shape and organization, however, they lack the potential to divide



1. Paremchyma

- they are living cells and walled, soft in nature due to the presence of thin-walled cells
- The cells of parenchyma are isodiametric or polyhedral in shape. They may be polygonal, oval, round or elongated
- These cells are closely packed or may have small intercellular space
- They are made up of thin cell wall made up of cellulose, calcium pectate

➢ **Types of Parenchyma**

1. Prosenchyma: These are fibre-like elongated cells, which are thick-walled and provide rigidity and strength to the plant

2. Aerenchyma: They contain very large intercellular spaces. These are present in aquatic plants.

3. Chlorenchyma: Cells which have chloroplast and perform photosynthesis

4. Storage Parenchyma: These store various substances like water, starch, proteins etc. They act as a food and water reservoir.

2. Collenchyma

- Is the living supporting or mechanical tissue.

- Formed of living elongated thick walled cells
- Formed of cellulose and pectin-cell wall
- Elastic and extensible and are adapted for rapid growth

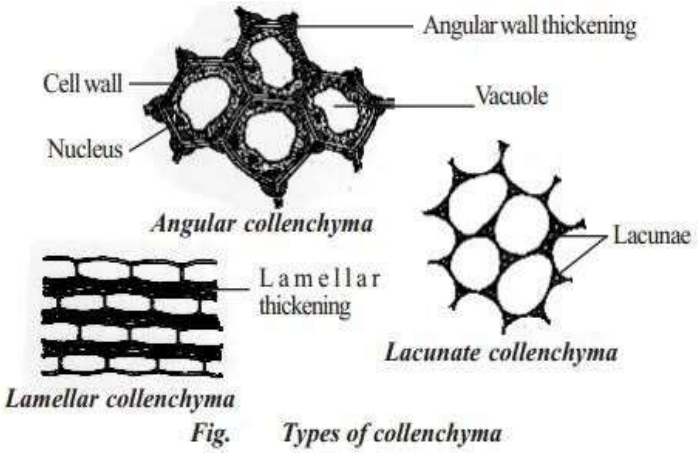
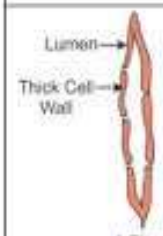
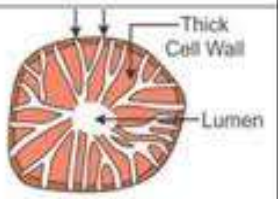
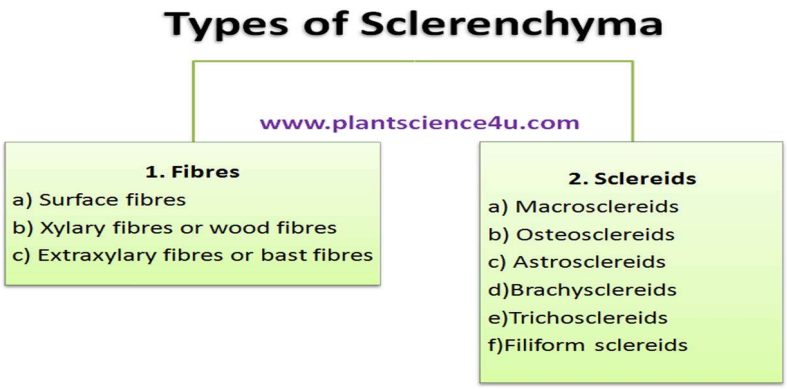


Fig. Types of collenchyma

3. Sclerenchyma

- Non living, supporting or mechanical tissue
- Cells are hard, thick walled, elastic, dead
- Compactly arranged without intercellular spaces
- Cell wall with cellulose and lignin deposits

Fibres	Sclereids
The cells of the fibres are elongate and tapering at end.	The cells of sclereids are short and of same diameter with blunt end.
Generally they are unbranched.	May be branched or maybe unbranched.
 A Fibre	 A Sclereid



ii. Complex tissues - Definition - Xylem & Phloem structure, origin and function

1. **Xylem**
    - The xylem is the principal water-conducting tissue of vascular plants. It consists of tracheary elements, tracheids and wood vessels and of additional xylem fibres. All of them are elongated cells with secondary cell walls that lack protoplasts at maturity
    - Tracheids are the chief water-conducting elements plants. Tracheids are elongated cells, closed at both ends. Tracheids look often square in cross-section, the lignified secondary wall is relatively thin. The walls are opened by numerous pits
    - The pits are often surrounded by a halo and are then called bordered pits.
    - Botanists think of wood vessels (tracheae) as the water-filled tubes of the xylem. Wood vessels are the chief water-conducting elements of plants.
    - In contrast to the tracheids the final walls of the single vessels are perforated and are therefore generally thought to be more efficient water conductors than tracheids.
2. **Phloem**
    - Movement of water through xylem is a passive process – the cells that make up xylem are dead Transport of sugars and amino acids is an active process needing energy, phloem is living tissue.
    - Movement of substances such as sugars and ions through phloem is called translocation

- The main components of phloem are sieve elements and companion cells.
- Sieve tubes Phloem is made from columns of parenchyma cells
- Each parenchyma cell is adapted to form a sieve element
- Columns of sieve elements join together to form sieve tubes
- The cross walls between successive cells (sieve elements) become perforated forming sieve plates . The cell walls are thin . Although the cell contents are living , the nucleus disintegrates and disappears . The lumen is filled with a slimy sap
- As the sieve elements mature the lose several plant cell organelles – the nucleus, ribosomes and Golgi body degenerate. This allows materials to pass through them more easily
- Sieve elements do have a cell wall, cell membrane, er and mitochondria.
- The amount of cytoplasm is very small and lines the inside of the cellulose wall.
- Companion cells -Each sieve element has at least one companion cell next to it.
- Companion cells have the normal plant cell structure with extra ribosomes and mitochondria
- Companion cells are metabolically very active
- Companion cells are linked to the sieve elements by numerous plasmodesmata .
- As might be expected, it is companion cells that enables the sieve element to stay alive.

iii. Secretory tissues - glands, glandular hairs, nectaries, hydathodes, schizogenous and lysigenous ducts, resin ducts, Laticifers –articulated and non-articulated

- The secretory structures vary greatly in structure and position.
- They may be either simple glandular trichomes or multicellular glands with vascular tissues.
- They may be external when originate from epidermis or deep seated or internal such as laticifers and resin ducts.

1. **Glandular Trichomes:**

- These trichomes consist of a stalk with a head above.
- The stalk may be unicellular or multicellular and in the latter case the cells may be arranged in several rows.
- The head is the secretory part and may be composed of single cell (ex. Pelargonium) or many cells (e.g. Callitriche).
- The head is covered with a cuticle. The secretion is accumulated beneath the cuticle.

2. **Nectary**

- Nectary can be defined as a gland or part of a flower that secretes nectar to the exterior of plants.
- They are divided into floral and extrafloral nectaries.
- The former is situated within the flower and is directly involved in pollination; the latter occurs on the vegetative organs and is not directly associated with pollination.
- The nectaries are present on the epidermis.

3. **Glands and Ducts:**

- They comprise a group of cells or sometimes a single cell that is readily distinguishable from the neighbouring cells and secretes a specific substance.
- These cells are thin walled with dense protoplasm and sometimes occur as layer surrounding a cavity, known as secretory cavity.

4. **Schizogenous glands**

- These are formed by the dissolution of middle lamella, thus separating apart the cells to form cavity.
- Example: oil glands of Eucalyptus, the secretory ducts of Rhus glabra, resin duct of Pinus etc.
- This cavity remains surrounded by a ring of intact parenchyma cells, termed epithelium, which forms a well-defined boundary of the gland.

5. **Lysigenous glands**

- These glands originate by lysis of a few cells thus forming the cavity
- (ex. glands present in the leaves and fruits of Citrus sp., that are also formed schizogenously).

6. **Laticifers**

- Laticifers can be defined as a specialized cell or a row of such cells that secrete the milky fluid termed latex.

- The word laticifer is used as a general term to denote the various latex-secreting structures — latex cell, latex vessel, latex duct, latex tube and laticiferous duct.
- The laticiferous duct is a cavity into which latex is secreted.

7. Non-articulate laticifer

- The former is derived from the enlargement of a single cell.
- This cell has the potentiality of unlimited and rapid growth, and elongates to form long latex tubes.
- The tubes may remain unbranched termed non-articulate unbranched laticifer (e.g. Vinca, Cannabis, Urtica etc.).

8. The articulate laticifers

- also termed laticiferous vessel, consist of longitudinal files of cells.
- The transverse end walls of the individual cell either remain intact or break down partly or wholly to form a continuous tube —the latex vessel.
- So the articulated laticifers are always compound in origin.
- They occur in primary or secondary phloem and may be present in cortical parenchyma.

MODULE I

Chapter 2: Vascular bundles – types: conjoint - collateral, bicollateral, concentric and radial

- **Vascular bundles**

➢ Vascular bundles are components of Vascular Tissue System
➢ Also called as ‘fascicle'
➢ Part of TRANSPORT system in plants
➢ One of the PRIMARY tissue system in plants
➢ It is a COMPLEX tissue system in plants
➢ Complex tissue = composed of MORE THAN ONE TYPE OF CELLS
➢ Vascular bundles consists of TWO main parts
➢ 1. Xylem: water conducting tissue
➢ 2. Phloem: food conducting tissue
➢ Xylem and phloem are complex tissues
➢ Components of xylem: Tracheids, Vessels, Xylem fibres & Xylem parenchyma

- Components of phloem: Sieve cells, Sieve tube elements, companion cells, Phloem parenchyma, Phloem fibres

- **Types of vascular bundles**

1. Conjoint

- Conjoint vascular bundles - Xylem and phloem are arranged together
- Xylem and phloem in same radius
- Conjoint VB are found in STEM and LEAVES
- Three types:

1. **COLLATERAL**
    - A type of conjoint VB
    - Phloem located ONLY OUTSIDE of the xylem
    - Xylem towards interior, phloem towards exterior
    - Collateral VB may be Open or Closed
    - **Cambium may be present or absent in between xylem and phloem, and so there are the following two types of collateral bundle:**
    - **(a) Closed collateral bundle:**
    - In this type cambium is absent in between xylem and phloem. Therefore stems having this type of bundle do not have normal secondary growth. Ex. Monocotyledonous stem.
    - **(b) Open collateral bundle:**
    - An open collateral vascular bundle has cambium called fascicular cambium between xylem and phloem. The bundles can increase in diameter by normal secondary growth with the help of fascicular cambium. Ex. Dicotyledonous stem.
2. **BI-COLLATERAL**
    - A type of conjoint VB
    - Phloem present in two groups
    - One outside the xylem, other inside the xylem (xylem in the middle, phloem both sides)
    - Characteristic of some Angiosperms
    - Bi-collateral vascular Bundle -Example: members of Cucurbitaceae (Cephalandra, C ucurbita)
    - Bi-collateral vascular bundles are always OPEN
3. **CONCENTRIC**

- A type of conjoint vascular bundle
- One VB element completely surrounds the other
- Either phloem surrounds xylem or xylem surrounds the phloem
- Two types: a) Amphicribal: b) Amphivasal

A). Amphicribal: A type of concentric vascular bundle

- Xylem lies at the centre, surrounded by a ring of phloem
- Example: Meristeles of ferns, small vascular traces of flowers, fruits and ovules

B). Amphivasal:

- A type of concentric vascular bundle
- Phloem lies at the centre, surrounded by a ring of xylem
- Example: Dracaena stem, Rumex, Begonia

2. Radial vascular bundles

- Radial Vascular bundle -Based on the arrangement of VB components
- Xylem and phloem are arranged separately
- Arranged alternatively in different radii
- Radial vascular bundles are found in ROOTS
- There is no primary cambium in this bundle and the secondary thickening occurs by the secondary cambium that originates at the time of secondary growth in dicotyledonous root only.

MODULE II

1. Primary structure of dicot and monocot root, dicot and monocot stem and leaf in dicot and monocot.

I. Structure of monocot root

1. Rhizodermis of epiblema
2. The outermost layer is the Rhizodermis.
3. It is made up tubular living cells. Some of the epidermal cells are protruded out in the form of root hairs.
4. These root hairs are useful in the absorption of water.
5. The epiblema gives protection to the roots.

1. Cortex:

- The cortex is broad and consists of parenchyma cells with large intercellular spaces.
- The cells are living and possess leucoplasts.
- Their function is storage.
- The last layer of the cortex is endodermis.
- The endodermal cells contain bands like structure made of suberin in their radial and transverse walls.
- These band-like structures are known as Casparian strips.
- Those endodermal cells in front of protoxylem are thin walled and known as passage cells. These passage cells conduct water from the cortex to xylem.

2. Stele:

- Stele in dicot root is differentiated into pericycle and vascular system.

3. Pericycle:

- Pericycle is a single layer of thin-walled parenchyma cells forming the outermost layer of the stele. Lateral roots arise endogenously from pericycle.

4. Vascular system:

- The primary xylem and phloem are arranged in alternate radii.
- Xylem and phloem are separated by the conjunctive tissue.
- Such a vascular bundle is said to be radial vascular bundles. Xylem occurs in the form of a solid core with a ridge like projections extending towards the pericycle.
- The number of protoxylem points is four.
- Hence the xylem is called tetrarch.
- As the first-formed xylem is pointing towards the periphery the xylem of roots is exarch.
- Phloem consists of sieve tubes, companion cells and phloem parenchyma.

- Pith is usually absent.

2. Structure of Dicot root

The transverse section of the dicot root shows the following plan of arrangement of tissues from the periphery to the centre.

1. Rhizodermis or epiblema:
   - The outermost layer is made up of single layer of parenchymatous cells without intercellular spaces. Stomata and cuticle are absent.
   - Root hairs are always single celled.
2. Cortex:
   - Cortex consists of oval or rounded loosely arranged parenchymatous cells.
   - These cells may store food reserves.
3. Endodermis
   - It is made up of single layer of barrel shaped parenchymatous cells.
   - The radial and the inner tangential walls of endodermal cells are thickened with suberin. These thickenings are known as casparian strips.
   - But these casparian strips are absent in the endodermal cells which are located opposite to the protoxylem elements.
4. Stele: All the tissues present inside endodermis comprise the stele.
   A. Pericycle
      - Pericycle is generally a single layer of parenchymatous cells found inner to the endodermis. Lateral roots originate from the pericycle.

   B. Vascular system
      - Vascular tissues are in radial arrangement.
      - The tissue by which xylem and phloem are separated is called conjunctive tissue.
      - Xylem shows exarch and tetrarch condition
      - Metaxylem vessels are generally polygonal in shape
5. Pith: Usually absent

3. Monocot stem

- In monocotyledons secondary growth does not take place. Hence, the plant body consists of only primary tissues.
- A transverse section of a monocotyledonous stem shows four regions. They are epidermis, hypodermis, ground tissue and vascular bundles.

- Monocot stem is differentiated into epidermis, hypodermis and ground tissue.
- Epidermal hairs are absent.
- Cortex is poorly developed and represented by hypodermis.
- Hypodermis is sclerenchymatous.
- General cortex is absent.
- Endodermis is absent.
- Stele is large and of advanced type.
- Stele consists of ground tissues and vascular bundles.
- Pericycle is absent.
- Vascular bundles are numerous and scattered in the ground tissue.
- Sclerenchymatous bundle sheath is present around each vascular bundle.
- The vascular bundle is oval, conjoint, collateral, closed and endarch.
- Xylem consists of few vessels (3 or 4) and arranged in the form of letter Y.
- It disintegrates and forms protoxylem lacuna.
- Phloem is small. Phloem parenchyma is absent.
- Peripheral vascular bundles are small and those situated towards centre are large.
- Medulla and medullary rays are absent.

4. Dicot stem

- In transverse section, dicot stem is differentiated into epidermis, cortex and stele.
- Epidermal hairs are present.
- Cortex is well developed and differentiated into hypodermis, general cortex and endodermis
- Hypodermis is collenchymatous
- General cortex is parenchymatous.
- Endodermis is present. It contains starch grains.
- Stele is bigger than cortex and well developed.
- Stele is differentiated into pericycle,vascular medulla and medullary rays.
- Pericycle is either completely or partly selerenchymatous.
- Vascular bundles are limited in number and arranged in the form of a ring.
- Bundle sheath is absent.
- The vascular bundle is wedge shaped, conjoint, collateral, open and endarch.

- Xylem consists of many vessels and is not arranged in the form of letter Y.
- Protoxylem does not disintegrate.
- Phloem is comparatively large and consists of phloem parenchyma also.
- All the vascular bundles are uniform in size.
- Medulla and medullary rays are distinct.

5. Monocot leaf

1. Upper Epidermis

- The upper epidermis is a single layer made up of cubical shaped cells with no intercellular spaces in between them.
- The outer surface of the upper epidermis cell is covered by a thin cuticle.
- A few cells present in the upper epidermis are enlarged to form motor cells referred to as bulliform cells.
- These cells help the leaf to roll over themselves in order to reduce the surface area exposed to sunlight during hot seasons.

2. Mesophyll

- Mesophyllis a green tissue between upper epidermis and lower epidermis.
- In monocot leaf, the mesophyll tissue is not differentiated into palisade parenchyma and spongy parenchyma with chloroplast and chlorophyll.
- The mesophyll is usually involved in photosynthesis process in the leaves of these plants.

3. Vascular Bundles

- Vascular bundles represent the veins of the leaves.
- Each vascular bundle consists of phloem and xylem tissues surrounded by a bundle sheath.
- Bundle sheath layer of the vascular bundle is made up of large barrel shaped endodermal cells.
- Xylem is usually responsible for conduction of water and dissolved minerals whereas phloem is responsible for conduction of dissolved food materials.

6. Dicot leaf

1. Epidermis

- The epidermis is usually made up of a single layer of cells that are closely packed.
- A dicot leaf consist of a lower and upper epidermis with small openings referred to as stomata.
- The upper epidermis is thicker than the lower epidermis.

2. Mesophyll

- The mesophyll usually has two regions the spongy and palisade parenchyma.

- The palisade parenchyma cells contain more chloroplasts than the spongy parenchyma cells and thus its function is photosynthesis.
- On the other hand, spongy cells are irregularly shaped and loosely arranged so as to facilitate the exchange of gases within the air spaces.

3. Vascular bundles

- The vascular bundles of a dictot leaf are surrounded by a compact layer of paranchymotous cells known as border parenchyma.
- The xylem consists of metaxylem vessels and protoxylem vessels.
- Phloem consists of sieve tubes, companion cells and phloem parenchyma.

MODULE III

Chapter 1

- **Normal secondary thickening in dicot stem**
  - Primary growth produces growth in length and development of lateral appendages. Secondary growth is the formation of secondary tissues from lateral meristems.
  - It increases the diameter of the stem.
  - In woody plants, secondary tissues constitute the bulk of the plant.
  - They take part in providing protection, support and conduction of water and nutrients.
  - Secondary tissues are formed by two types of lateral meristems, vascular cambium and cork cambium or phellogen.
  - Vascular cambium produces secondary vascular tissues while phellogen forms periderm.
  - Secondary vascular tissues are present
  - Formation of cambial ring
  - Fascicular cambium (cambium between xylem and phloem) and interfascicular
  - cambium join to form a continuous circular vascular cambial ring
  - The cambium is partly primary and partly secondary
  - The cambium produces secondary phloem to the outside and secondary xylem to the inside.
  - Cork cambium is present and produces cork to the outside and secondary cortex to the inside forming periderm.

- Primary vascular bundles are limited in number
- Vascular bundles conjoint, collateral and open
- xylem is endarch

- **Intra stellar and extra stellar thickening**
  - Formation of cambial ring
  - Fascicular cambium(cambium between xylem and phloem) and interfascicular
  - cambium join to form a continuous vascular cambial ring
  - The cambium produces secondary phloem to the outside and secondary xylem to the inside.
  - More secondary xylem is produced due to more activity of vascular cambium to the outside.
- **Extra stelar Secondary growth or Periderm formation by cork cambium**
  - The formation of more and more secondary tissues exerts a pressure on cortex and epidermis
  - The epidermis gets ruptured and is replaced by another protective tissue developed in the
  - cortex from the cork cambium is the periderm.
  - Periderm consists of meristem called phellogen or cork cambium. Cork cambium produces cork or phellum to the outside and phelloderm or secondary cortex to the outside.
  - Periderm has specialized openings called lenticels on its surface for gaseous exchange
- **Growth rings**
  - Growth ring, in a cross section of the stem of a woody plant, the increment of wood added during a single growth period.
  - In temperate regions the growth period is usually one year, in which case the growth ring may be called an “annual ring.”
  - In tropical regions, growth rings may not be discernible or are not annual.
  - Even in temperate regions, growth rings are occasionally missing, and a second, or “false,” ring may be deposited during a single year—for example, following insect defoliation.

- Growth rings are distinct if conducting cells produced early in the growth period are larger (spring, or early, wood) than those produced later (summer, or late, wood) or if growth is terminated by a layer of relatively thick-walled fibres or by parenchyma.
- In temperate or cold climates the age of a tree may be determined by counting the number of annual rings at the base of the trunk or, if the trunk is hollow, at the base of a large root.

- **Ring Porous Wood**

  - In it the vessels are of different diameter.
  - The vessels are not uniformly distributed throughout the wood.
  - Vessels with wide and smaller diameter are formed in the early and the later part of the growth season respectively.
  - Vessels with wide diameter of early wood and vessels of smaller diameter of late, summer or autumn wood are distinguishable.
  - The development of vessel is sudden and rapid.
  - The vessels are longer in length than those of diffuse porous wood.

- **Diffuse Porous Wood**
  - In it the vessels are more or less equal in diameter.
  - The vessels are uniformly distributed throughout the wood.
  - Vessels with more or less equal diameter are formed throughout the growth ring.
  - Vessels of early wood and late wood are indistinguishable.
  - The development of vessel is slow.
  - The vessels are shorter in length than those of ring porous wood.
  - The rate of transport of water in plants with diffuse porous wood is slower than those with ring porous wood.

- **Sap wood and Heart wood**
  - Sapwood is the outer light-colored portion of a tree trunk through which the water passes from the roots to the leaves, and in which excess food is often stored.

- Heartwood is the central core of the trunk. In most woods the heartwood can be distinguished from the sapwood by its darker color.

- **Tyloses**
  - Tyloses are outgrowths/extragrouth on parenchyma cells of xylem vessels of secondary heartwood.
  - When the plant is stressed by drought or infection, tyloses will fall from the sides of the cells and "dam" up the vascular tissue to prevent further damage to the plant.
- **Normal secondary thickening in dicot root**
  - Secondary vascular tissues are present
  - Formation of cambial ring
  - Cambial ring is wavy at the beginning later become circular
  - The cambium is completely secondary in origin
  - The cambium produces secondary phloem to the outside and secondary xylem to the inside.
  - Cork cambium is present and produces cork to the outside and secondary cortex to the inside forming periderm.
  - Primary Vascular bundles are radial and xylem is exarch
  - Number of xylem and phloem groups limited (2-6 or 8)
  - Common material: *Carica Papaya*

MODULE 3

Chapter 2.

1. Anomalous secondary growth - general account with special reference to the anomaly in Dicot stem – *Boerhaavia*, *Bignonia* and Monocot stem- *Dracaena*
   - Unusual position of cambium
   - Abnormal functioning of the cambium
   - Formation of more than one ring of cambia
   - Formation of extra stellar cambial ring
   - Formation of interxylary phoem
   - Formation of interxylary cork

- Vascular bundles arranged in three rings in *Boerhaavia diffusa*
- Central, middle and outer
- Central two large rings
- Middle consists of 6-14 loosely arranged bundles
- Outer consists of 15-20 small bundles
- Cap like structure of dead cells over the phloem
- Formation of new cambial ring
    - In monocotyledons
        - Secondary growth is rare because of the absence of cambium
        - Activity of specialized primary secondary thickening meristem
        - Outer epidermis, sclerencymatous hypodermis, and large number of closed collateral vascular bundles in parenchymatous ground tissue
        - Cambium behaves abnormally

Microtechnique

Introduction

- Microtechnique: is tissue preparation for microscopic examination
- There are different methods used, however the basic principles are similar

Microscopy

- Microscope – an instrument that produces an enlarged image of an object. • Biologists use microscopes to study cells, cell parts, and organisms that are too small to be seen with the naked eye.

• Microscopes magnify and show details of the image.

• Two types of microscopes:

- **Light Microscope** – light passes through one or more lenses to produce an enlarged image of a specimen
- **Electron Microscope** – forms image of a specimen using a beam of electrons rather than light

Milestones

- **Zacharias Janssen** – Dutch spectacle maker who tested several lenses in a tube and discovered that nearby objects appeared significantly enlarged.
- **Robert Hooke** – used a microscope to observe a thin slice of cork. The spaces he saw reminded him of the small rooms where monks lived, so he called them "cells," which he used to describe the smallest units of life.
- He used microscopes with two and three lenses, but they didn't produce very detailed images.
- **Anton van Leeuwenhoek** - Dutch merchant who learned how to grind lenses to make simple microscopes (have only one lens). These produced clearer and more enlarged images than Hooke's.
- He is considered "The Father of Microscopy" and built over 500 different microscopes.
- He was the first to discover microorganisms under a microscope by observing a drop of pond water filled with life. He called them "tiny animalcules."
- He also saw and studied bacteria and the blood flow through capillaries in the tail of a fish.

Parts of a Compound Light Microscope:

1. Body tube: Keeps the two sets of lenses a set distance apart.

2. Rotating nosepiece: Allows one to change between the objective lenses.

3. Objective lens: The second set of lenses in a compound microscope (usually 4x, 10x, 40x).

4. Stage clips: Hold the slide in place.

5. Diaphragm: Adjusts the amount of light that hits the slide from the light source.

6. Light source: Where light comes from to see image.

7. Ocular lens: The first lens in a compound microscope (usually 10x).

8. Arm

9. Stage: Where one puts the slide to view.

10. Coarse Adjustment Knob: Moves the stage up and down very quickly.

11. Fine Adjustment Knob: Moves the stage up and down very slowly.

12. Base

Compound Light Microscope

- Light passes through the specimen on the slide and uses two lenses to form its image.
  - o Capable of two things (below) that vary in different microscopes:
- Magnification: a measure of how much the image is enlarged
- Total magnification = (ocular lens)(objective lens being used) [The ocular lens usually has a 10x magnification, but that can vary.]
- 4x objective lens = (10x)(4x) = 40 times total magnification
- 10x objective lens = (10x)(10x) = 100 times total magnification
- 40x objective lens = (10x)(40x) = 400 times total magnification
- **Resolution**: a measure of the clarity of an image; how clear the details are

Electron Microscope

- Resolution is the limiting factor to a light microscope since the greater the magnification is, the less it is able to resolve the image. At magnifications beyond 2,000x, the image becomes blurry, but electron microscopes can be used at greater magnifications.

Characteristics of the Electron Microscope

- A beam of electrons is used to produce an enlarged image of the specimen (it does not use light). This electron beam and the specimen must be placed inside a vacuum chamber so that the electrons in the beam do not bounce off gas molecules in the air.
- Since living things cannot survive in a vacuum, the electron microscope cannot be used to view living cells.
- Much more powerful than light microscopes
- There are two types of electron microscopes:
    1. **Transmission Electron Microscope** (TEM)
        - Uses a beam of electrons transmitted through a very thinly sliced specimen. Magnets guide the beam of electrons toward the specimen, and the image is produced to view.
        - Magnification to 200,000 times.
    2. **Scanning Electron Microscope** (SEM)
        - Provides detailed 3-D images.
        - The specimen is sprayed with a fine metal coating (it is not sliced to view as in the TEM).
        - As the beam of electrons is passed over the specimen's surface, the metal coating emits a shower of electrons, and a 3-D image of the specimen's surface is produced to view.

Protocols followed in microtechniques

1. Collection & Identification
2. Labeling of the specimen with numbering
3. Fixation
4. Dehydration
5. Clearing
6. Impregnation (infiltration)
7. Embedding
8. Section cutting

9. Staining
10. Mounting

Killing & Fixation

- Killing: Sudden stopping of all living process in all cells.
- Killing agent: Chemical reagent used for killing.
- Fixation: preservation of all structural and cellular elements in as near their original state as possible.
- Fixing agent (Fixative): Chemical reagent used for fixing.
- Fixative may be a single chemical reagent of a combination of many reagents.
- "A good fixative is one that changes the cell chemistry the least and preserve the cell structures the best"

Goals for killing and fixation

- Preserve cell structures and contents in as natural form as possible
- Modify the refractive index of some cellular elements to make them better distinguishable under microscope
- Make the materials resistant and hard to reactions during further treatment in processing.
- Prepare the materials to improve upon effects of certain stains.

Significance of killing and fixation

- Harden the tissue and give them consistency
- Increase visibility of cell components
- Prevent post-mortem changes (bacterial decay and autolysis)
- Make the cell contents insoluble
- Reduce the shrinkage and distortion of cells

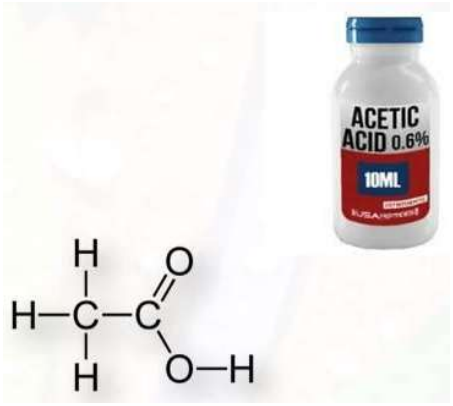
Fixatives

- Single chemical reagents used in killing and fixation
- **Ethyl alcohol (Ethanol)**
  - Miscible with water
  - B.p. 78 °C
  - Highly inflammable

- A reducing agent
- Dissolves fats and lipids
- Shrinks the tissues and hardens very much
- Precipitates proteins and nucleic acids

- **Formalin**
  - Trade name of ~40% aqueous solution of formaldehyde
  - Miscible with water
  - A reducing agent
  - No effects of lipids
  - Slow penetrability
  - Cause no shrinkage, but cause shrinkage with alcohol
  - Very great hardening effect
  - Fumes are irritating to mucous membranes and eyes

- **Acetic acid**
  - Very rapid penetration capacity
  - Miscible with water
  - No hardening effect
  - Doesn't fix cytoplasm



Fixatives (combination reagents for killing and fixation)

- Different types of fixatives according to their ingredients
- Some formulations are stable
- Stable combinations can be prepared, stored and can use at any time
- Some are unstable, they have to prepare fresh each time
- Some can store at room temperature, some at refrigerator
- Some combinations should be protected from light
- The name of fixative usually based on:
    - Names of investigators who first developed it
    - Major ingredients in the combination

I. Carnoy's Formula

Preparation (for 30 ml)

- Absolute alcohol:10 ml
- Chloroform:15 ml
- Glacial acetic acid:5 ml

- An acetic acid –alcohol mixture
- Commonly called as Carnoy's fluid
- Best used for cytological preparations-Root tips, Anther etc.)
- Nuclear and chromosomal features will be preserved
- Fixation time 10 to 15 mins
- After fixation, wash in 85% alcohol and store in 85% alcohol solution

II. Rawlin's formula-FAA (Formalin-Acetic acid-Alcohol)

Preparation (100 ml)

95% Ethyl alcohol: 50 ml

Glacial acetic acid: 5 ml

Formalin: 10 ml

Water: 35 ml

- Best for materials for histological studies
- Good for algae, bryophytes and such delicate materials
- Good hardening action

- Materials can be stored for many years
- Fixation time:18 hours
- A lower percentage of alcohol may be used for fixing delicate materials
- For woody materials, decrease the acetic acid and increase formalin content
- After fixation, wash in alcohol and store in alcohol

III. Navaschin's formula -CRAF (Chromo-acetic-Formalin mixture)

- Navaschin's formula contains two solutions, Solution A and Solution B
- **Preparation**

Solution A:	Chromic acid 1%	:	15 ml
	Glacial acetic acid	:	10 ml
	Distilled water	:	90 ml
Solution B:	Formalin	:	40 ml
	Distilled water	:	60 ml

- Mix equal volume of Sol A & Sol B just before use
- Fixation time 12 hr
- **CRAF-I Preparation**

Chromic acid 1%	:	20 ml
Acetic acid 1%	:	75 ml
Formalin	:	5 ml

- **CRAF- II Preparation**

Chromic acid 1%	:	20 ml
Acetic acid 10%	:	10 ml
Formalin	:	5 ml
Distilled water	:	65 ml

- Both CRAF I and CRAF II should be prepared just before use
- After the addition of formalin, a colour change takes places (in 2-3 hrs)
- Subsequently the color changes to olive green or deep green

- Once the green color is developed, the solution become a fixative
- Fixation time: 12 hrs
- Washing in water is not required

Technique of fixing

- Materials should be fixed as soon as possible after collection
- If possible, material should be fixed at the collection point itself
- Should know the properties of fixing reagent
- Select proper fixative according to the requirement
- Larger specimens should be cut into small pieces
- Place the specimen in a bottle with cap
- Add require quantity of fixative
- Specimens should sink in the fluid
- Required incubation time should be provided
- Wash after fixing use proper preservatives

Tissue processing

- In order to cut thin sections of the tissues, it should have suitable hardness and consistency when presented to the knife edge.
- These properties can be imparted by infiltrating and surrounding the tissue with paraffin wax, various types of resins or by freezing.
- This process is called tissue processing.
- It can be subdivided into:

  a) Dehydration

  b) Clearing

  c) Impregnation (infiltration)

- **Two types :**

1. Manual Tissue Processing

- In this process the tissue is changed from one container of reagent to another by hand
- Timings are controlled by a timer which can be adjusted in respect to hours and minutes
- Temperature is maintained around 60 ºC.

2. Mechanical Tissue Processing

- Automatic tissue processor:
- Overnight
- 12 Baths
- 16 hours

Dehydration

It is the process in which the water content in the tissue to be processed is completely removed by passing the tissue through increasing concentrations of dehydrating agents

- Tissues are dehydrated by using increasing strength of alcohol; e.g. 70%, 90% and 100%
- Water is replaced by diffusion

Clearing

- During dehydration water in tissue has been replaced by alcohol.
- The next step alcohol should be replaced by paraffin wax. As paraffin wax is not alcohol soluble, we replace alcohol with a substance in which wax is soluble. This step is called **clearing**.

Clearing agents:

- Xylene

- Toluene

- Chloroform

- Benzene

Infiltration

- In this the tissue is kept in a wax bath containing molten paraffin wax

Embedding

- **ngEmbeddi** is the process by which impregnated tissues are surrounded by a medium such as agar, gelatin, or wax which when solidified will provide sufficient external support during sectioning
- It is done by transferring the tissue to a mould filled with molten wax & is allowed to

cool & solidify

- After solidification, a wax block is obtained which is then sectioned to obtain ribbons

Sectioning

- It is the procedure in which the blocks which have been prepared are cut or sectioned and thin strips of uniform thickness are prepared
- The instrument by which this is done is called as a **Microtome**

Types of microtomes:

- Rotary microtome
- Freezing (cryostat) microtome
- Ultramicrotome

Microtome knives

- Steel Knives
- Non-corrosive Knives For Cryostats
- Disposable Blades
- Glass Knives
- Diamond Knives
- Microtome blades are extremely sharp, and should be handled with great care. Safety precautions should be taken in order to avoid any contact with the cutting edge of the blade

Rotary microtome

The knife is stationary (fixed) and the block holder is moved up and down in a vertical plane by the rotary action of the hand wheel

- It is the most commonly used
- Suitable for paraffin embedded sections
- It is suitable for cutting of small tissues
- Ideal for cutting serial sections

Ultra microtome

- U**sed for very thin section**
- The typical thickness of tissue cut is between 20 -100 nm for TEM
- Knife: Diamond or Glass

Freezing (cryostat) microtome

- Frozen tissue embedded in a freezing medium is cut on a microtome in a cooled machine called a cryostat

Staining

- Staining is a process by which we give colour to a section. Staining of the section is done to bring out the particular details in the tissue under study
- A stain is any colouring organic compound that combined with another substance imparts a colour to that substance.
- The term 'dye' is used to refer to a colouring agent that is used for general purposes, whereas the term 'stain' is used to refer to that dye which is used for biological purposes.
- The stains used for bacteria are aniline dyes they are derived from aniline ($C_6H_5NH_2$). The most commonly used aniline dyes are crystal violet, methylene blue, basic fuchsin, safranin, eosin, etc.
- There are hundreds of stains available
- The most commonly used stain in routine practice is Hematoxylin & Eosin stain
- Better visualize cells and cell components under a microscope
- Can preferentially stain certain cell components

Classification of Stains:

1. Natural dyes:
    a. Haematoxylin -----from plant
    b. Carmine ----------from female cochineal bug
    c. Orcein ----------a vegetables dye extract
    d. Synthetic: these are derived from hydrocarbon benzene
- Acid stains
- Basic stains

Staining steps:

a. rehydration

b. stain

c. dehydration

In an acidic dye:

- The basic component is colored and the acid component is colorless
- Acid dyes stain basic components e.g. eosin stains cytoplasm
- The color imparted is shade of red

In a basic dye:

- The acid component is colored and the basic component is colorless
- Basic dyes stain acidic components e.g. Hematoxylin stains nucleus
- The color imparted is shade of blue

Common stains and their uses

- **Iodine:** Stains carbohydrates in plant and animal specimens brown or blue-black.Stains glycogen red.
- **Methylene blue:** Stains acidic cell parts (like nucleus) blue. Use on animal, bacteria and blood specimens. Can be used as a substitute for Janis B green.
- **Eosin Y:** Stains alkaline cell parts (like cytoplasm) pink. Use on plants,animals and blood. Can be used as a substitute for Congo Red and Carmine.
- **Safranin :** Mainly used for sections of plant tissues, stains red
- **Toluidene blue:** Stains acidic cell parts (like nucleus) dark blue. Good to show mitosis in plant cells.
- **Wright's stain:** Stains red blood cells pink/red.
- **Leishman's stain:** Stains nucleus of WBC blue and blood cells pink
- **Crystal Violet:** Stains bacteria purple
- **Aceto-orcein:** Biological stain for chromosomes and connective tissue.
- **Sudan III:** Biological stain used as a lipid indicator.

Chromphore

- Certain chemical group introduced into benzene ring by substitution induce colour to the compound -reffered as chromphore – resultent structure –chromogen

Auxochrome

- The word ***auxochrome*** is derived from two roots. The prefix ***auxo*** is from ***auxein*****, and means *increased.*** The second part, ***chrome*** means ***colour,*** so the basic meaning of the word auxochrome is ***colour increaser.***
- This word was coined because it was noted originally that the addition of ionising groups resulted in a deepening and intensifying of the colour of compounds.
- Auxochromes are groups which attach to non ionising compounds yet retain their ability to ionise and absorbance of the resulting compound.

Mounting

- Stained section on microscope slide is mounted using mounting medium dissolved in xylene
- Examples of Mountants : DPX (Distrene Dibutyl phthalate Xylene)
- A coverslip is placed on top, to protect the sample

Metachromasia

- A dye which has the ability to change its color without changing its chemical structure is said to be metachromatic.

- The physical changes that bring about this color change are a specialized, orderly form of dye aggregation.

Mordants

- Term refers to a substance which acts as an intermediary between dye and tissue
- Advantage of dye mordent tissue complex is that it is virtually insoluble in most fluids used in biological staining.
- Mordants are used in hematoxylins which makes it a strong basic dye, cationic metal binding to both dye and tissue.