1 DEMONSTRATION OF DOUBLE STAINING TECHNIQUE

DOUBLE STAINING TECHNIQUE

Generally tissue differentiation in plant material is not clear due to single staining. Two (or) more stains are used wherever tissue differentiation requires more clarity. (Double staining).

Combination of acidic and basic dyes of contrasting colours is of general use.

This helps in the distinction (differentiation) of woody tissue from non-lignified tissue.

Some commonly used combinations are :

Haematoxylin	+	Safranin
Safranin	+	Fast green
Safranin	+	Aniline blue
Safranin	+	Crystal violet
Crystal violet	+	Erythrosine

Staining procedure for temporary preparation : The selected stained sections are taken out from watch glass and are transferred into water and then into another watch glass containing principal stains (haematoxylin; safranin; crystal violet etc). The sections are allowed to remain in the stain for sometime (for about 04-to-05; minutes).

Excess amount of stain is washed repeatedly with water untill the stain disappears from lignified (or) non-lignified tissues.

If the de staining is not complete, then the sections are washed with acid alcohols.

Washing is followed by counter stain with safranin (or) fast green (or) Erythrosine.

This stain acts on the tissues more rapidly than the principal stain; so the sections are kept in the stain for shorter duration only, (01 (or) 02 minutes).

Excess stain is removed by washing the stained sections with glycerine (15-to-20) minutes. When the sections shows demarcation between tissue systems while preserving the colour of the stain; then the section is ready for mounting.

(A) Haematoxylin + safranin :

Selection of a section

↓

Stain with Haematoxylin

↓

Wash with water

↓

Wash with ammonia water untill the stain turns to blue.

↓

Tapwater is suitable if alkaline

↓

Wash with glycerine.

section is ready for mounting (for observation)

(B) Safranin + fast green + Aniline blue :

Selection of a Section

↓

Stain with safranin (04-to-05 minutes)

↓

Washing with water repeatedly

↓

De stain with acid alcohol (if needed)

↓

Washing with water repeatedly

↓

Stain with fast green/ Anilline blue (01- minute)

↓

Wash with glycerine thoroughly.

↓

Section is ready for Mounting

2 TISSUE ORGANISATION ROOT AND TIPS STEM

TISSUE ORGANISATION IN ROOT AND SHOOT-APICES (TIPS)

(A) ROOT-TIP :

1. Tissue organisation in the root apex can be observed in its longitudinal section with the following characters.
2. Presence of terminal outer root cap with dead tissue.
3. Presence of an inactive quiescent centre above the root cap.They becomes active when other meristematic cells gets damaged.
4. Lateral appendages like leaves and branches are not formed.
5. Presence of uniform growth in length due to the absence of nodes and internodes.
6. Three (or) more groups of initials are seen in angiosperms.

   This can be observed as follows.

Terminal --- Dermatogen + Root cap (calyptrogen)

Dicots
- Middle --- Periblem
- Inner ---- Plerome

Monocot
- Outer most region form -- Root cap
- Dermatogen
- Periblem

(B) SHOOT - TIP :

1. Tissue organisation in the shoot - apex can be observed in L.S. with the following characters.

2. This is hemispherical (or) slightly flattened structure.
3. Apical promeristems are differentiated into tunica and corpus.
4. The outer tunica contains 01 (or) 5 layers of cells. They divide anticlinally and provide surface to the shoot apex.
5. The corpus contains a mass of cells and they divide randomly.
6. Rib meristem present in the centre of the corpus later develops into pith region of the stem.
7. Near the periphery surrounding the ribmeristem lies peripheral (or) flank meristem.
8. Leaf primordia arise from flank meristems. They divide rapidly and differentiate into tissues of the leaf.

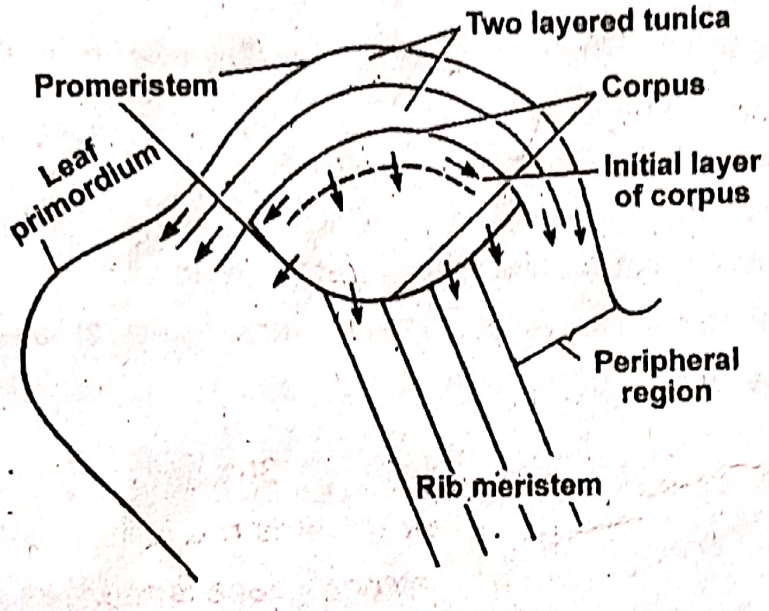


Fig : L.S. of shoot apex

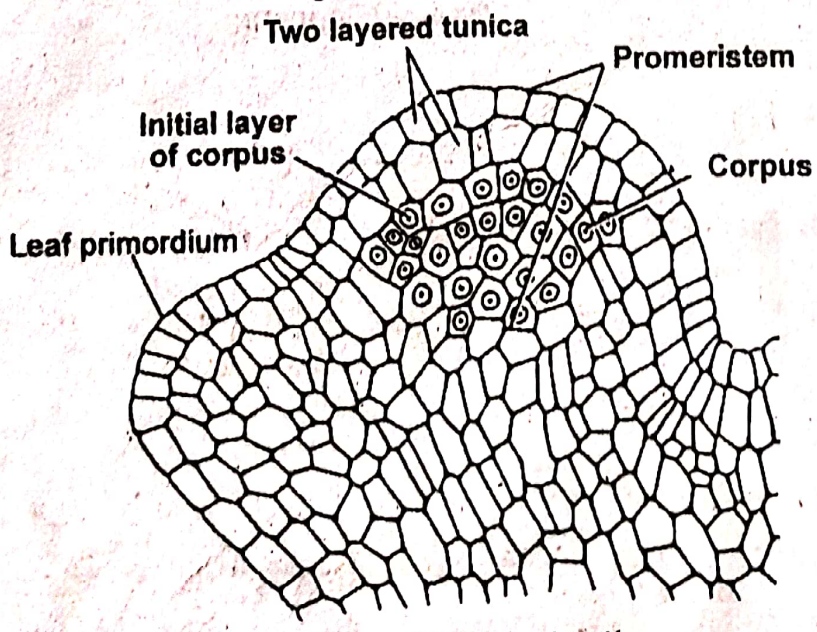


FIg : Cellular details

3 PERMANENT SLIDE PREPARATION

PERMANENT SLIDE PREPARATIONS :

These preparations can be done either with single stain or combination.

1. Stain the section with principle stain (hematoxylin, safranin or crystal violet).

2. Remove the excess of stain by repeated washing with water or acid water or acid alcohol.

3. Then pass the section through graded series of alcohol for dehydration. To begin with, transfer the section into watch glass containing 30% alcohol. Cover the watch glass with another watch glass.

4. Without disturbing the section replace the 30% alcohol with 40% alcohol using dropper. Similarly after retaining for few minutes replace the 40% alcohol with 50% alcohol. Repeat the procedure till 70% alcohol grade is reached.

5. Replace the alcohol with counter stain (e.g: Sapranin fast green or erythrosin prepared in 90% alcohol.

6. Remove the excess of stain by washing with 90% or 100% alcohol.

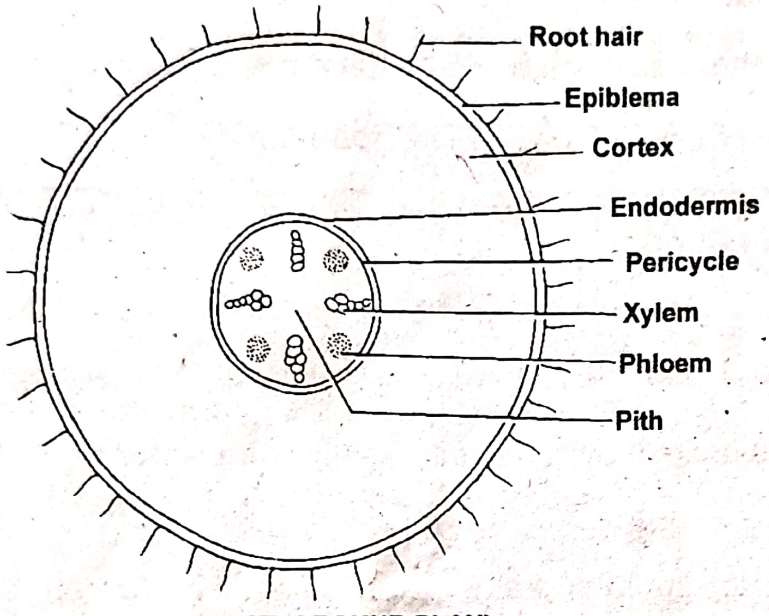
7. The section is now transferred to absolute alcohol for complete dehydration.

8. Repeat the dehydration process using graded series of xylol. Initially transfer the section in to watch glass containing 25% xylol (25cc xylol and 75 cc of absolute 1alcohol).

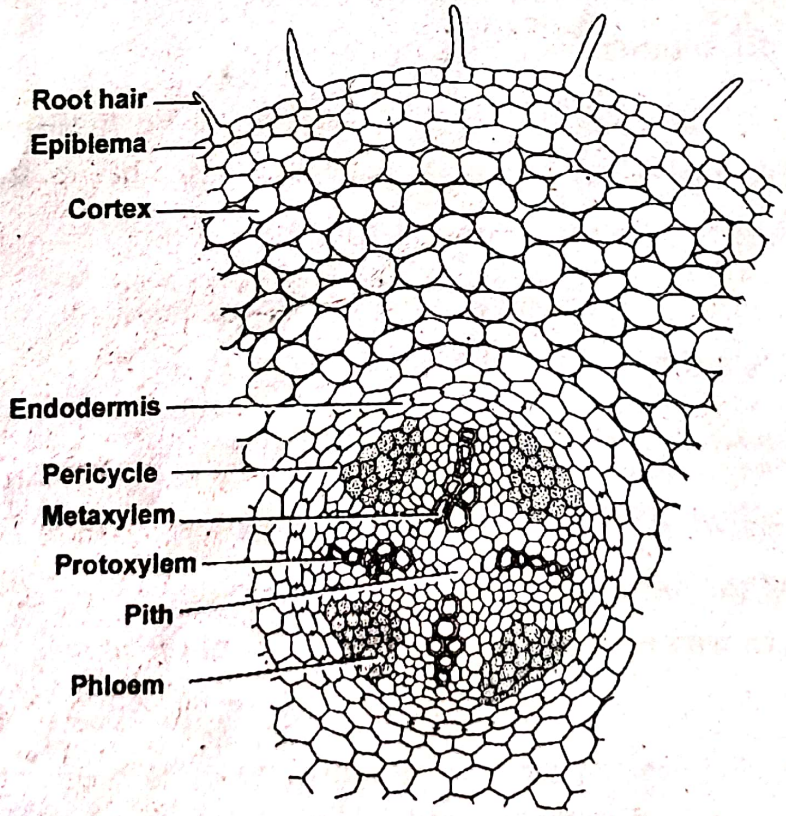
9. Similarly pass the section through 50%, 75%, 90% and finally pure xylol solutions. If pure xylol turns to white or turbid (indicate incomplete dehydration), then repeat the process in the reverse series.

(A) I. PRIMARY STRUCTURE OF ROOT STEM :

(1) Dicot Root T.S (cicer) :



T.S. ROOT (GROUND PLAN)



SECOTR ENLARGED

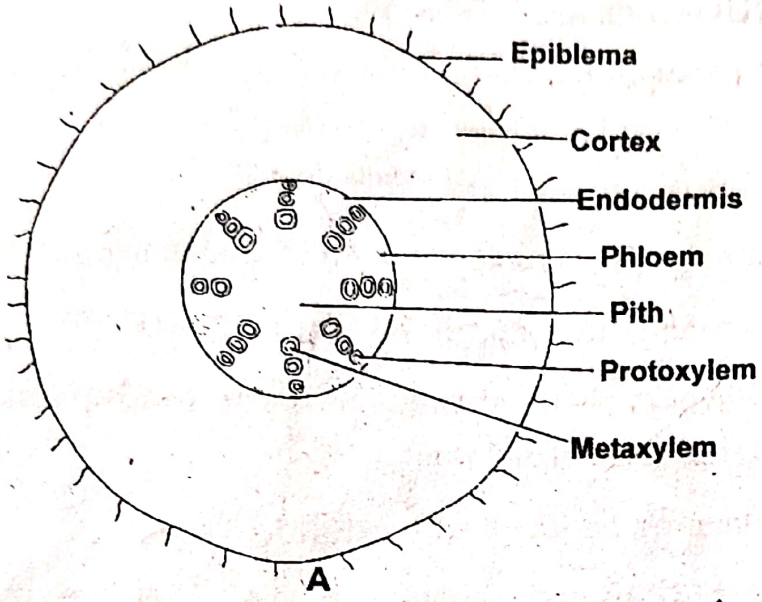
Identification characters : Eg: Cicer.

1. Presence of single layered, thin walled, barrel shaped piliferous layer (Epidermis).
2. Presence of unicellular root hairs here and there.
3. Absence of outer cuticle and stomata.
4. Piliferous layer in some roots is replaced by thick walled exodermis.
5. Presence of few layered general cortex with parenchyma.
6. Presence of single layered barrel shaped endodermis with passage cells and casparian thickinings on their radial walls.
7. Presence of single layered pericycle with parenchyma.
8. Xylem and phloem bundles are Separate, Radial, Alternate, mostly Triarch and Closed xylem is exarch.
9. The central pith may be reduced (or) absent.
10. Presence of conjunctive tissue between xylem and phloem.

(2) Monocot root T.S. (Canna) :

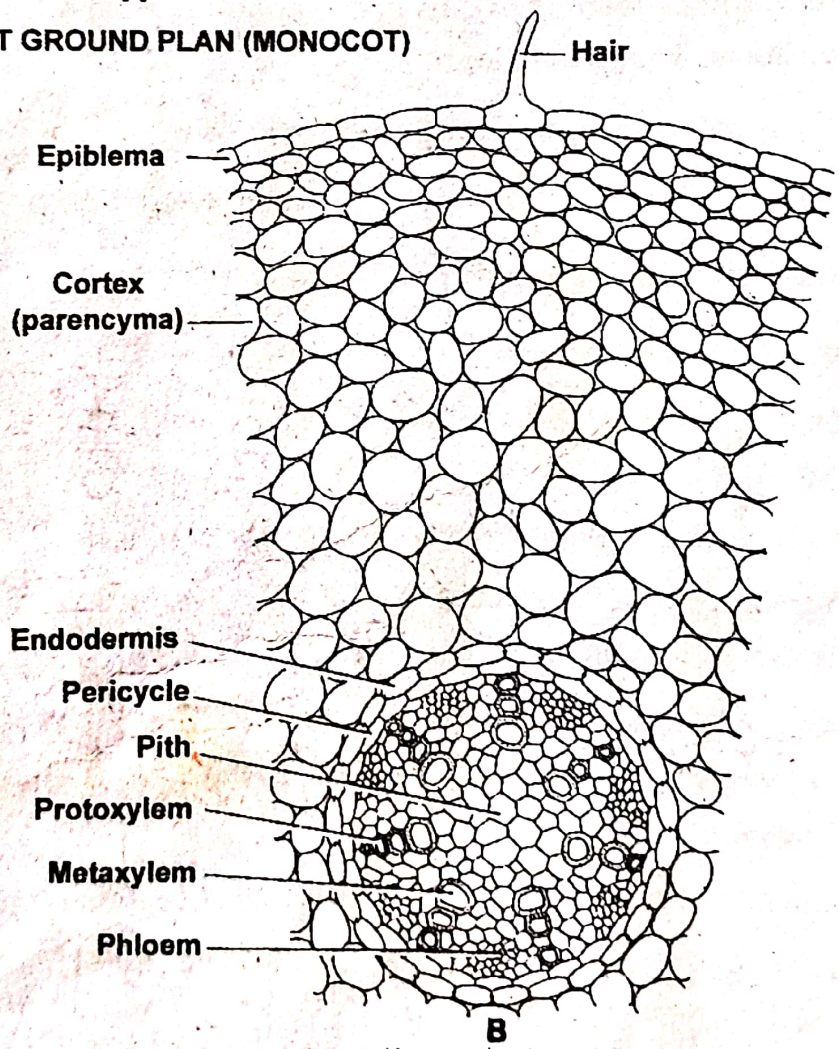
Identification characters:

1. Presence of outer, single layered, thin walled, barrel shaped, piliferous layer (Epidermis).
2. Presence of unicellular root hairs at some places.
3. Absence of outer cuticle and stomata. Piliferous layer may be replaced by exodermis with subarin cells.
4. Presence of many layered general cortex (parenchyma).
5. Presence of single layered barrel shaped endodermis with passage cells and casparian thickenings.
6. Presence of single layered pericycle with parenchyma.
7. xylem and phloem bundle are Separate, Radial, Alternate, Polyarch and Closed. Xylem is exarch.
8. Presence of large central pith or medulla with parenchymatous cells.
9. Presence of conjunctive tissue between xylem and phloem.



A

T.S. ROOT GROUND PLAN (MONOCOT)

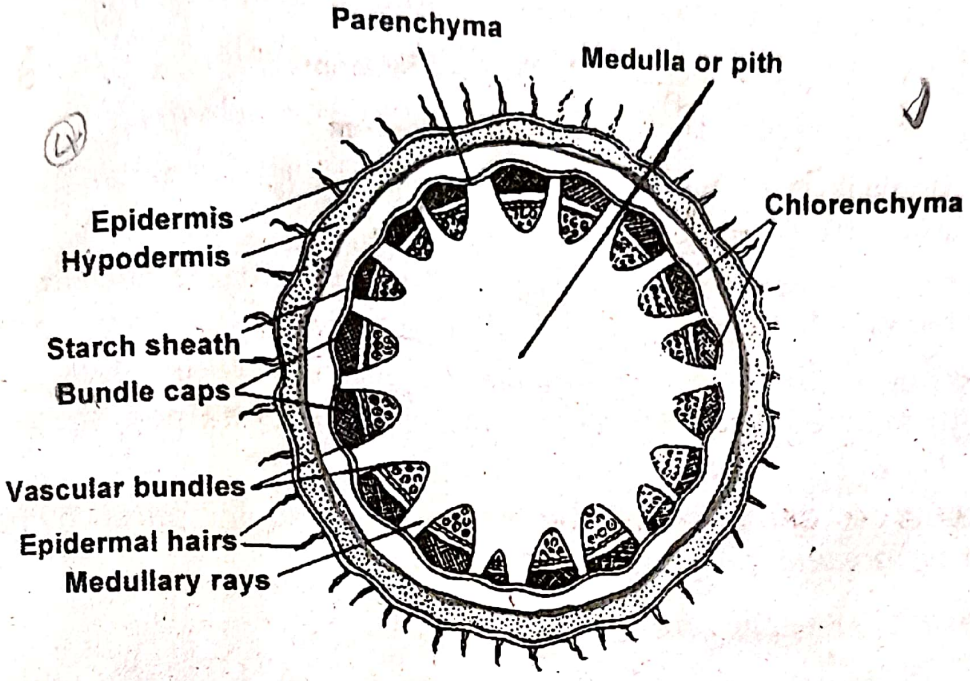


B

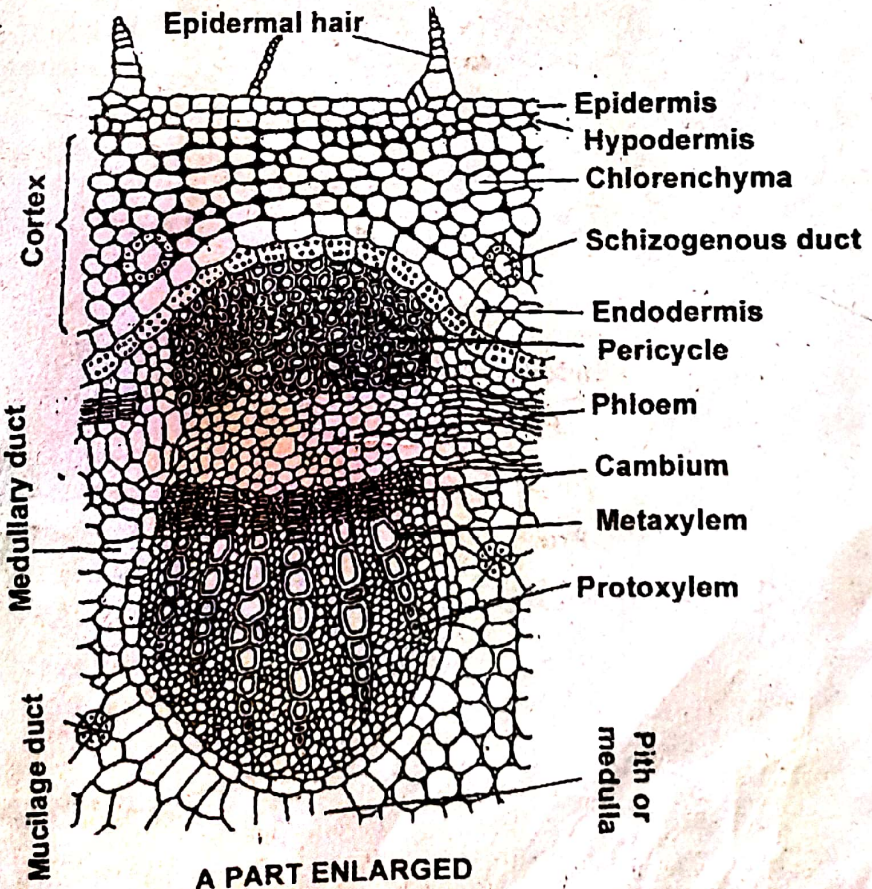
SECTOR ENLARGED

(II) PRIMARY STRUCTURES OF STEM :

(1) *Dicot stem T.S (Tridax) :*



Ground plan

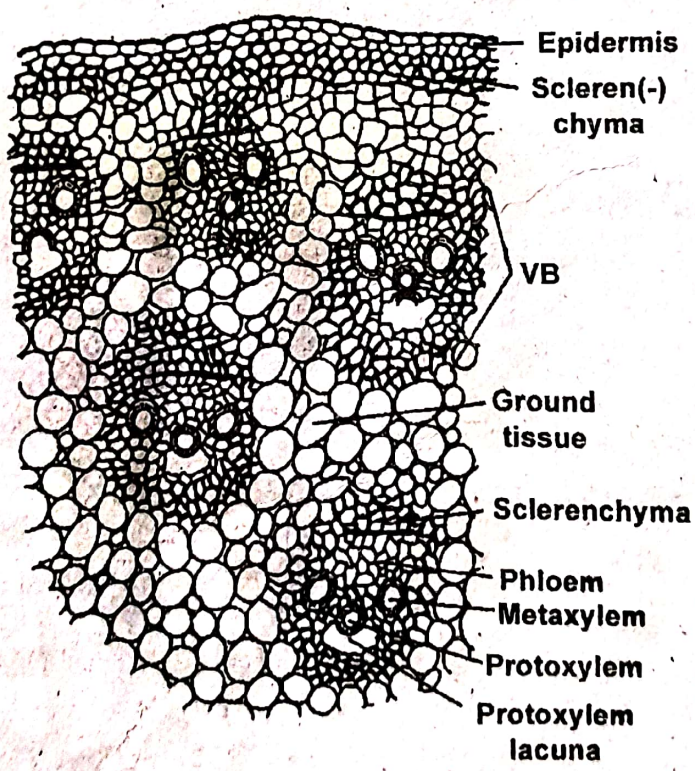


A PART ENLARGED

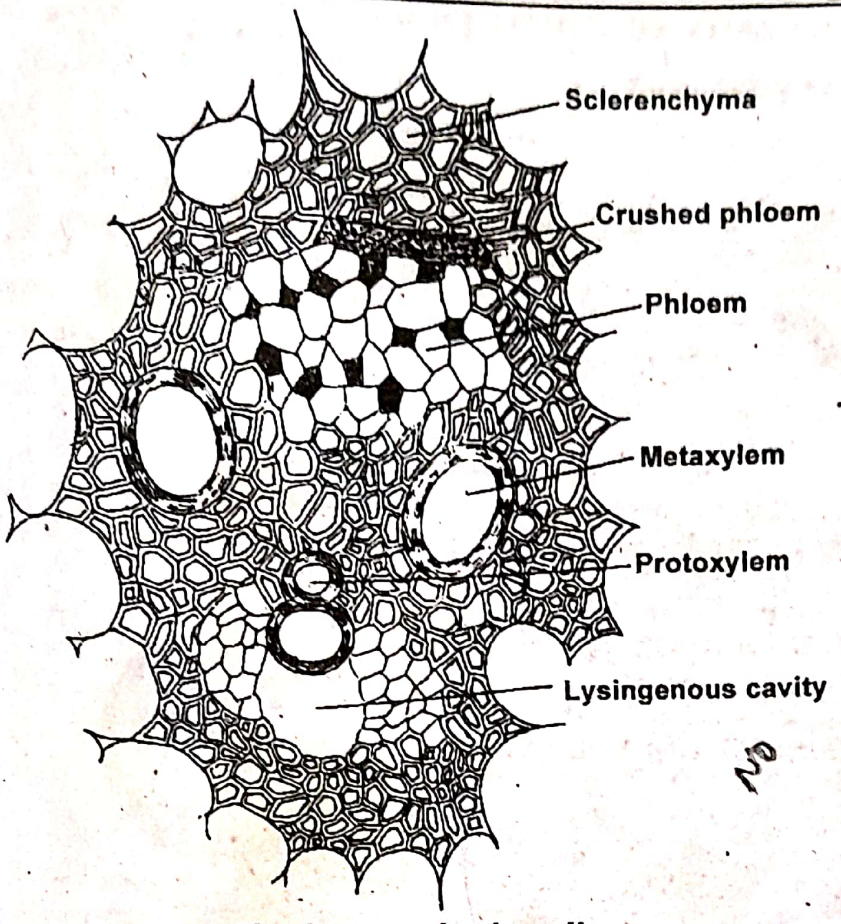
Identification characters :

1. Presence of outer, single layered barrel shaped epidermis.
2. Presence of outer, thin cuticle, stomata and multicellular, uniseriate hairs.
3. Presence of 2-to-3 layers of hypodermis with collenchyma.
4. Presence of 3-to-5 layers of general cortex with chlorenchyma.
5. Presence of single layered, barrel shaped endodermis with casparian thickenings and starch granules.
6. Presence of pericycle patches on the vascular bundles with sclerenchyma tissue; with parenchyma in between them.
7. Limited number of wedge shaped vascular bundles are arranged in "Eustelic" (Broken ring) manner. Each V.B is Conjoint, collateral and Open, the xylem is endarch.
8. Presence of large central pith at the centre with angular parenchyma, and medullary rays present in between vascular bundle.

(2) Monocot stem T.S. (Eg : Sorghum):



Part of Monocot stem



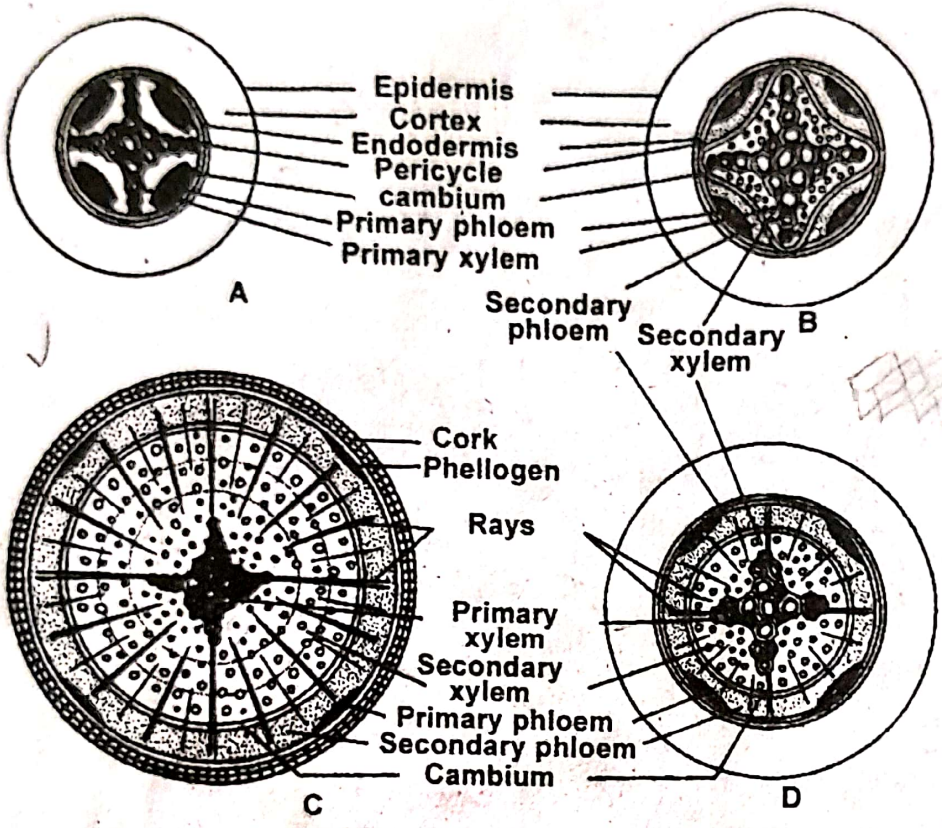
Single vascular bundle

Identification characters :

1. Presence of single layered outer, barrel shaped thick Walled epidermis with out shoot hairs.
2. Presence of outer thick cuticle.
3. Presence of hypodermis with 2 -to - 3 layer of sclerenchyma cells having chlorenchyma patches at some places.
4. The remaining central part of the stem contains angular, parenchymatous ground tissue.
5. Many, oval shaped vascular bundles are scattered in the ground tissue in "Atactostele" manner.
6. Large, few V.B.S are present at the centre and small, many V.B.S are present at the periphery.
7. Each V.B. is surrounded by sclerenchymatous bundle sheath (Fibrovascular bundle)
8. Each V.B. is conjoint; collateral and closed. Xylem is endarch with "V(or)" U "(or) "Y" shaped vessels and Contains" lysigenous cavity. (or) protoxylem lacuna".

(B) (I) SECONDARY GROWTH IN ROOT :

(1) Dicot root (Tridax) :



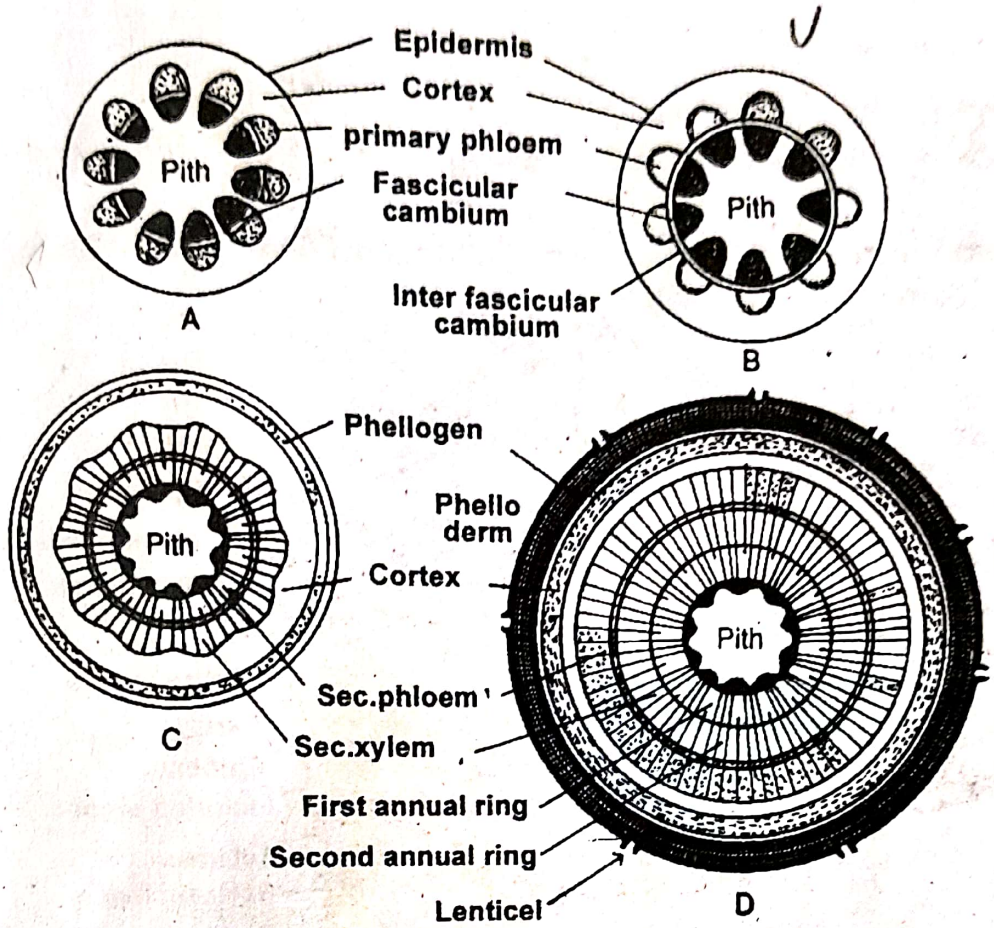
Secondary growth of a Dicot Root

Identification characters :

1. Presence of outer cork (phellem) having very few lenticels (or) without lenticels.
2. Presence of endodermis.
3. Presence of cork-cambium (phellogen) from pericycle (or) rarely from primary phloem.
4. Presence of secondary cortex (phelloderm) without chloroplasts.
5. Presence of two types of phloems (primary + secondary).
6. Presence of cambium ring.
7. Presence of secondary xylem and primary xylem at the centre and it is one of the identification character of root when compared with the stem.
8. Generally pith is totally absent but rarely it may contain thick walled cells.
9. Presence of distinct, large, primary medullary rays. Opposite to primary xylem is also the important characteristic feature of dicot root.
10. Small secondary medullary rays may also be formed.

SECONDARY GROWTH IN STEM :

(2) Dicot stem (Eg: Pongamia) :



Secondary growth of a Dicot Stem

Identification characters :

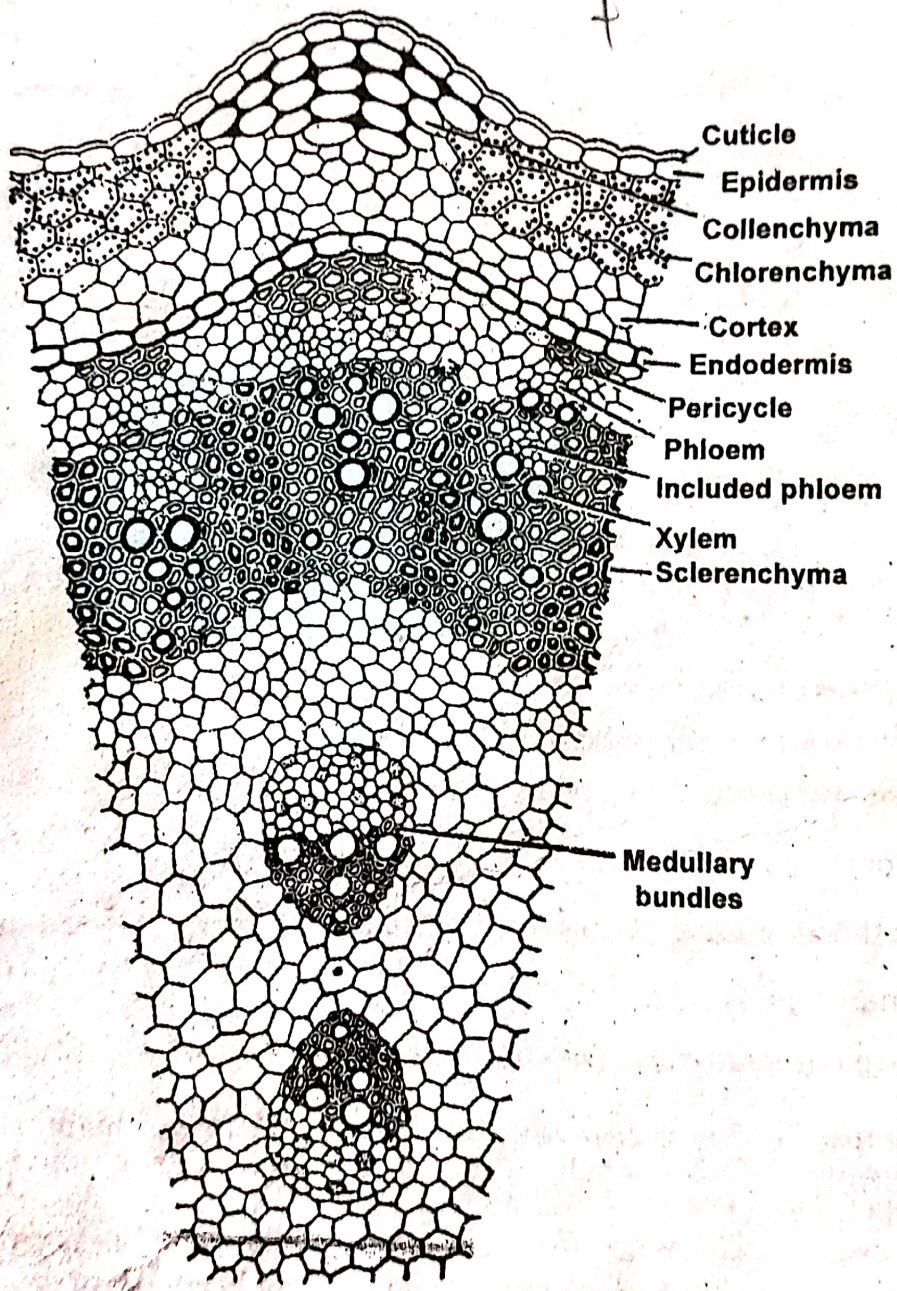
1. Presence of outer periderm with lenticels
   Periderm contain — Outer – Cork → (or) Phellem
   Middle – Cork → Cambium or phellogen
   Inner – phelloderm (or) Secondary cortex
2. Presence of dark coloured heart wood (Duramen) at the centre.
3. Presence of light yellow coloured sap wood (Alburnum) surrounding the heart wood.
4. The older stem may show growth (or) annual rings with spring wood (Early) and autum wood (late).
5. Presence of ray initials with bast rays (phloem) to the outside and wood rays (xylem) to the inner side. They are also called vascular rays. The rays may be uni (or) bi (or) multi-seriate.
6. Presence of secondary xylem and primary xylem are present in the heart wood.
7. Presence of primary phloem. Secondary phloem and secondary xylem are present in the sap wood.

(C) ANOMALOUS SECONDARY GROWTH STRUCTURE :

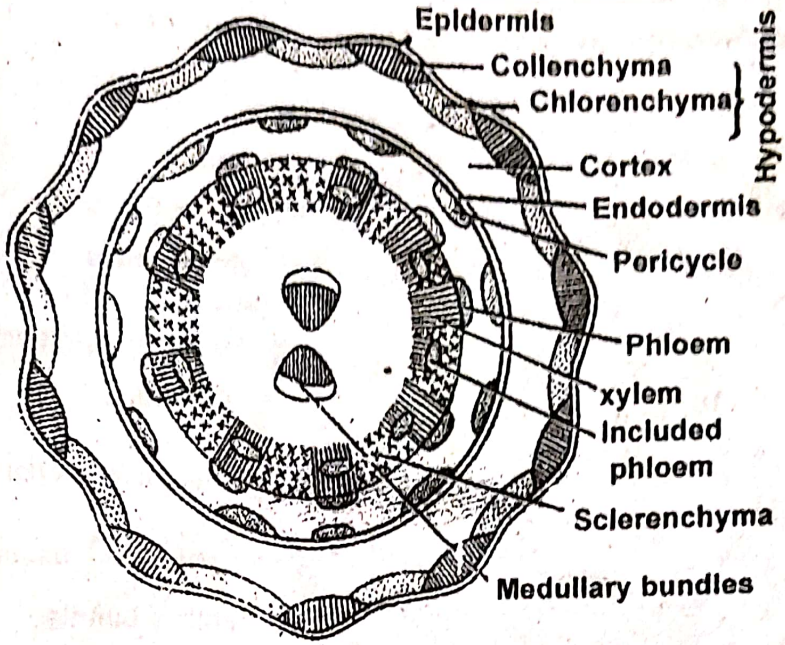
(1) T.S. of Achyranthus stem:

Identification characters :

1. Presence of ridges and furrows (grooves) in the out line.
2. Presence of single layered outer epidermis with thick cuticle and epidermal hairs.
3. The hypodermis contains collenchyma patches below the ridges and chlorenchyma in the furrows.



Sector Growth structure



Ground plan

4. Three to four layers of parenchyma cells are present below the hypodermis (General. cortex)
5. Endodermis is not clear after secondary growth.
6. Pericycle is present as patches with sclerenchyma tissue.
7. Vascular bundles are arranged in a ring. Each vascular bundle is conjoint, collateral, open and xylem is endarch.
8. The secondary vascular tissue contains.

   Primary phloem and secondary phloem.

   A ring of cambium below the phloem.

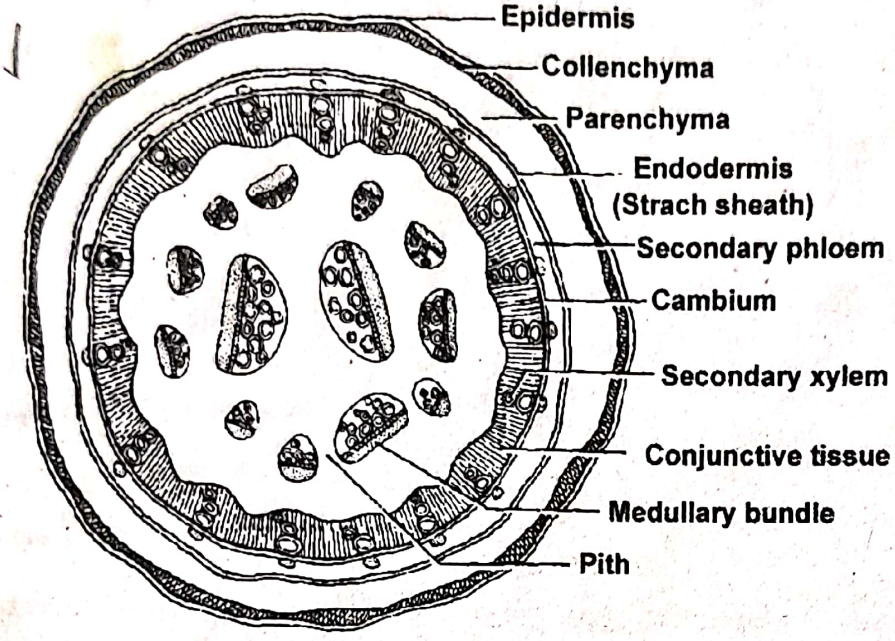
   Secondary xylem is present in the prosenchyma.

   The thick walled prosenchyma also contains inter xylary phloem.

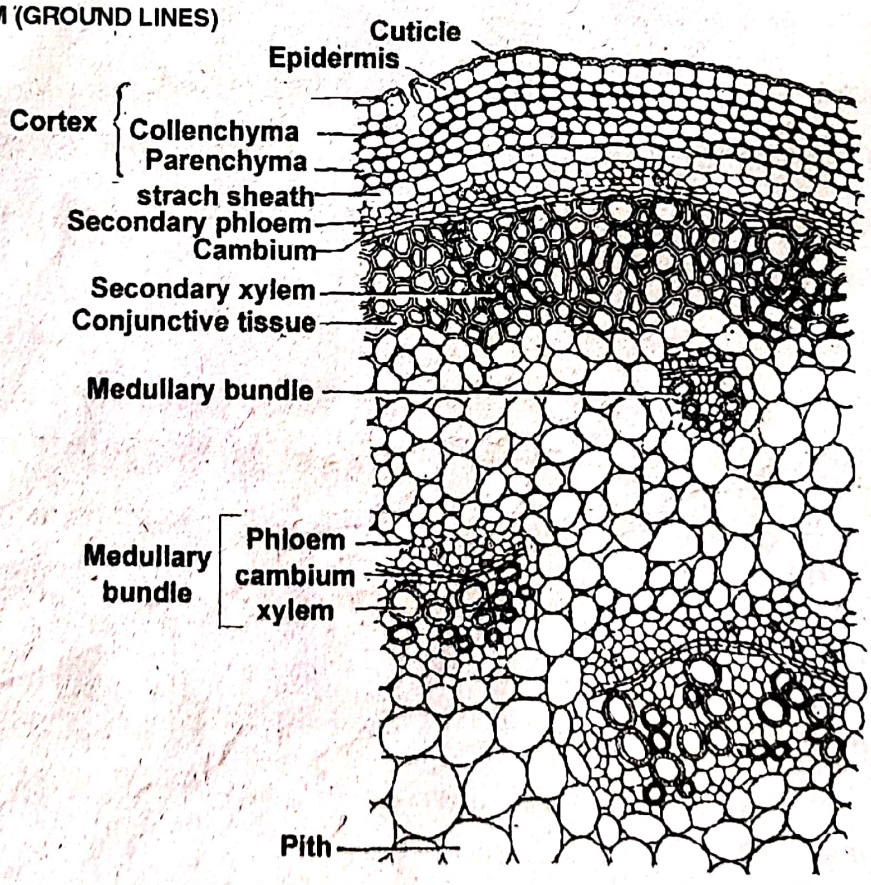
   Primary xylem is present near the pith.
9. A well developed pith with parenchyma is present at the center.

   Two medullary vascular bundles are present in the center (xylem facing one another)
10. An extra stelar cambium appear in the form of small areas in the region of pericycle. They produce secondary vascular bundles.

(2) T.S. of Boerhaavia stem :



T.S. STEM (GROUND LINES)

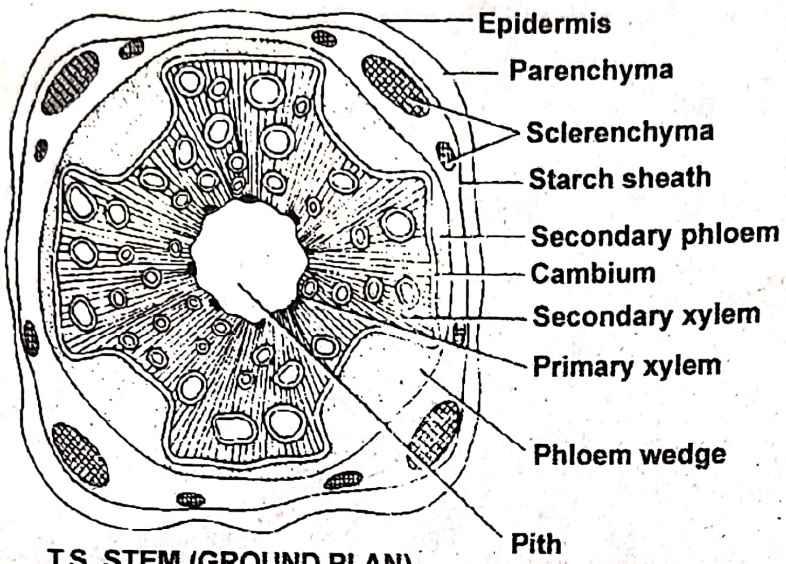


T.S. STEM (A SECTOR SHOWING CELLULAR DETAILS)

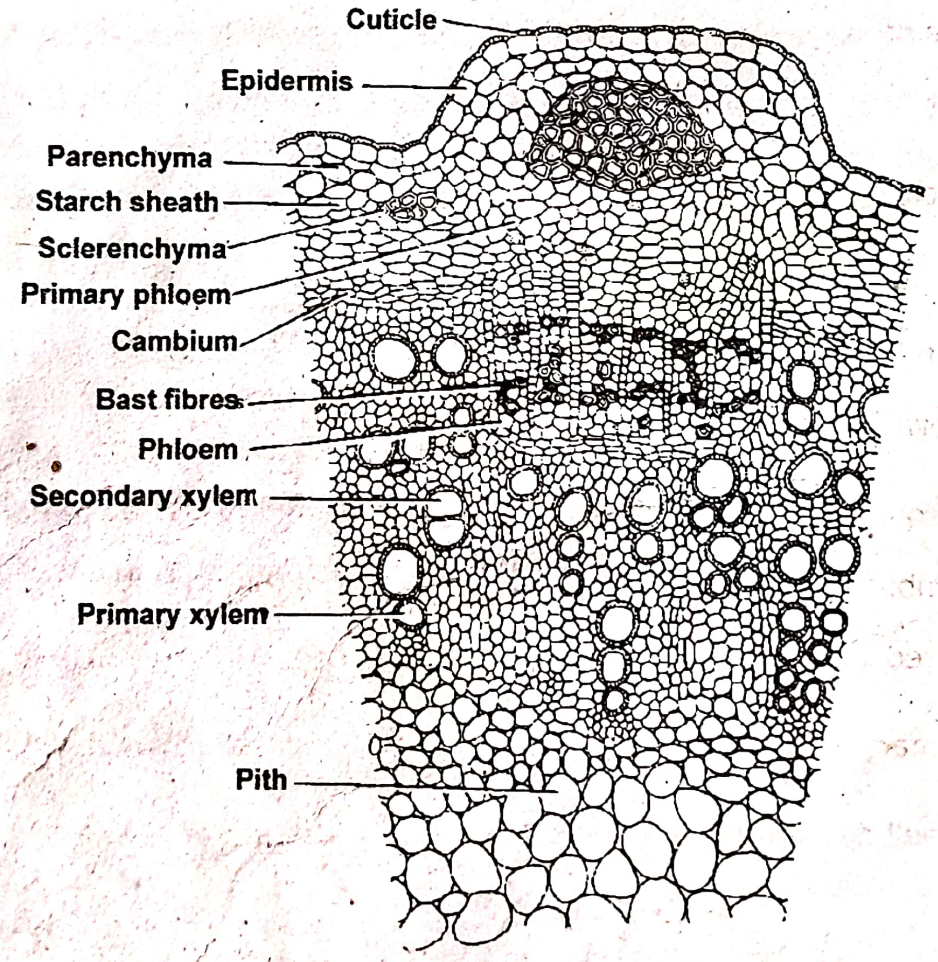
Identification characters :

1. Presence of single layered epidermis with outer thick cuticle.
2. Cortex contains 3 or 4 layer collenchyma with lower parenchymatous cells with chloroplasts.
3. Presence of distinct layers of endodermis and pericycle present below the cortex.
4. Presence of vascular bundles in two rings, wtih distinct secondary vascular tissue.
5. Due to secondary growth, phloem forms a complete cambium ring. It is present between secondary phloem and xylem.
6. Secondary xylem is present in the conjunctive tissue having prosenchyma tissue.
7. The endarch primary xylem groups are present close to the pith.
8. The inner ring contains two large vascular bundles. Each bundle is conjoint. endarch and open. Cambium (Intra) produce small amount of secondary tissue. As these bundles are close to the pith, They are also called medullary bundles.
9. Small, few bundles having the same character are present as middle ring.
10. Presence of parenchymatous pith at the center.
11. The cambium ring ceases (stop) its function after some time. Many additional cambia are produced. They produce successive-alternate zones of secondary xylem and secondary phloem - which develops concentric rings.
12. Cambia produce large amount of prosenchyma into which xylem elements are distributed without clear demarcation.

(3) T.S. of Bignonia stem :



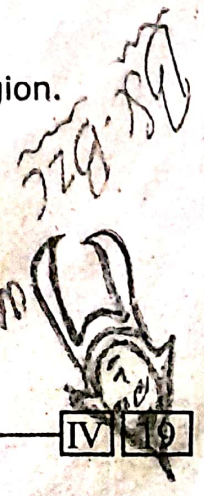
T.S. STEM (GROUND PLAN)



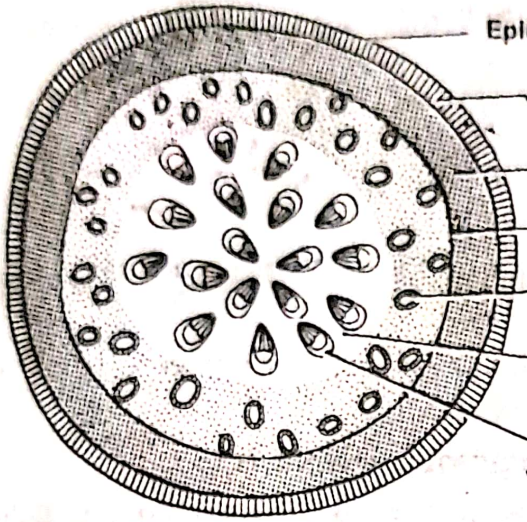
T.S. STEM (A SECTOR SHOWING CELLULAR DETAILS)

Identification characters :

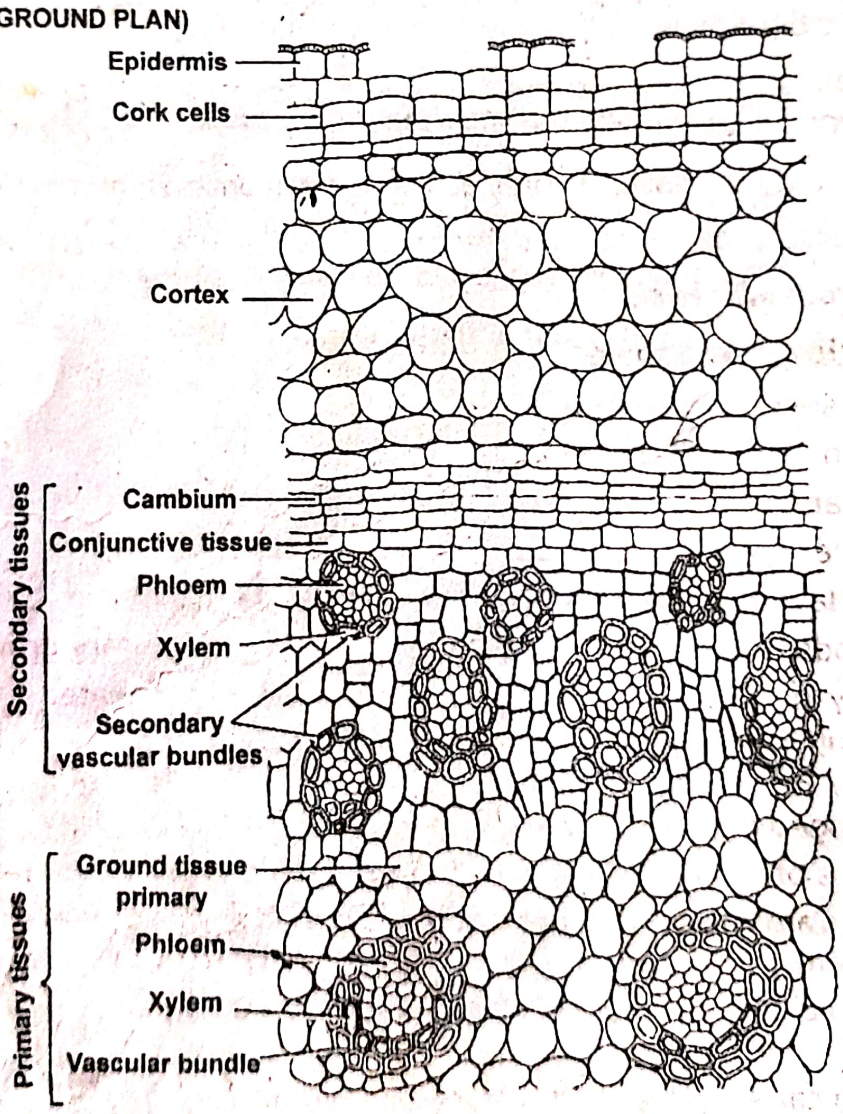
1. It is almost quadrangular in shape.
2. Presence of outer single layered epidermis with outer thick cuticle.
3. Presence of few layers of cortex with parenchyma cells.
4. Presence of sclerenchyma patches at the four corners of the stem (Hypodermis).
5. Presence of starch sheath in a single layer (Endodermis).
6. Pericycle is discontinuous with sclerenchyma patches.
7. Vascular tissue with many vascular bundles are present below the pericycle.
8. The primary phloem pushed down in the secondary tissue forming four wedges–at the four corners. They contains thick walled phloem fibres.
9. Secondary phloem is present below the primary phloem in the form of a continuous ring except at the four wedges.
10. Cambium is present below secondary phloem and shows abnormal secondary growth.
11. Secondary xylem is formed below the cambium but at the four corners the cambium produces more phloem than the xylem resulting in the formation of ridged xylem.
12. Primary xylem is endarch and present towards the central pith region.
13. A well developed parenchymatous pith is present at the center.



(4) T.S. of Dracaena stem :



T.S. STEM (GROUND PLAN)



T.S. STEM (A SECTOR SHOWING CELLULAR DETAILS)

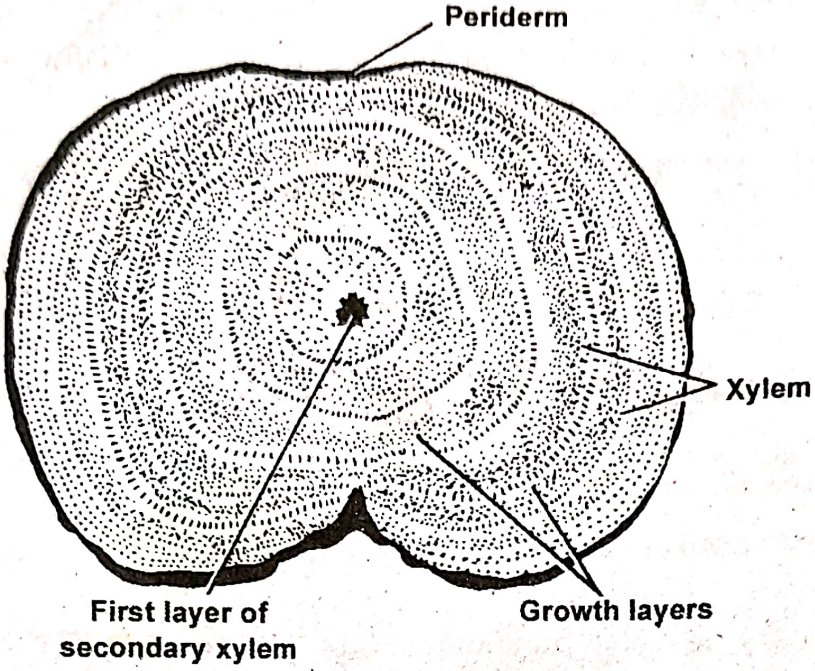
Identification characters :

1. Presence of outer periderm with cork (phollem), cork-cambium (phellogen) and secondary cortex(phelloderm).
2. The cork is discontinuous with lenticells. It contains, rectangular dead cells with suberin substance. It is covered by thick cuticularised epidermis on the outer side.
3. Cork cambium contains 1 or 2 layers of thin walled cells and tangentially elongated cells.
4. Cortex contains many layered parenchyma with strach granules and intercellular spaces.
5. Cambium with meristematic cells are present below the cortex.
6. Large primary vascular bundles present at the center are conjoint, collateral and closed.
7. Small secondary vascular bundles present at the periphery are concentric (Amphivasal= phloem at the center is surrounded by xylem).
8. The ground tissue present at the center contains inter cellular parenchyma.
9. Cambium formation is abnormal and results in the secondary growth.

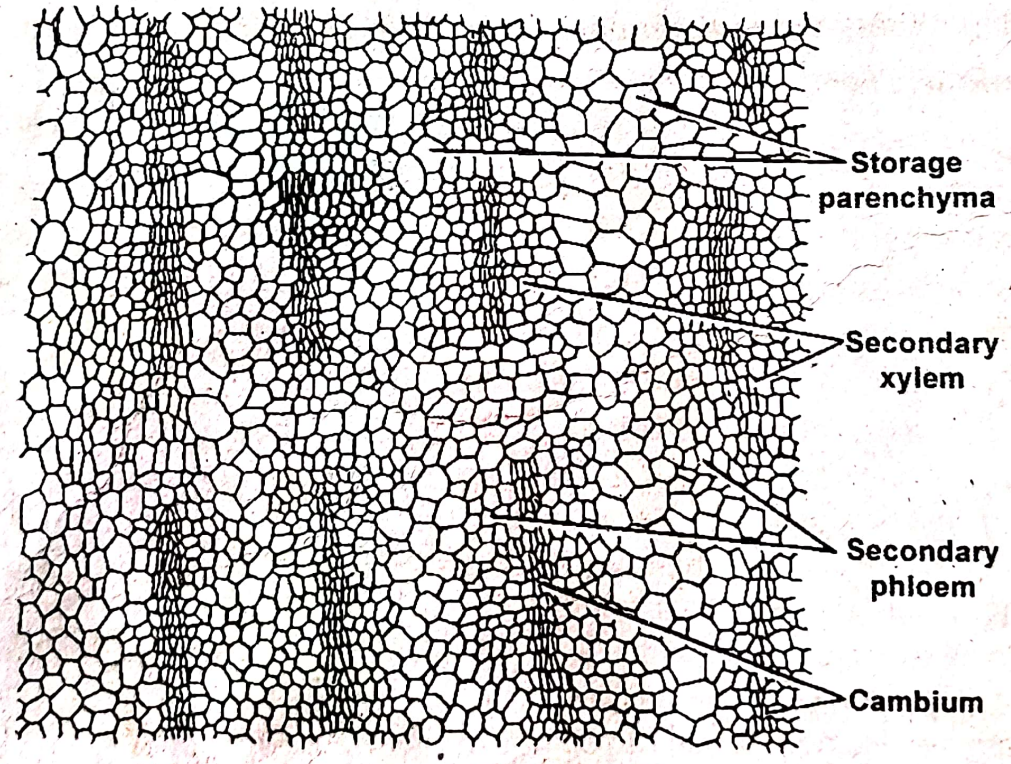
(5) Beta vulgaris root :

Identification characters :

1. The section appear as bi-lobed structure.
2. The outer periderm contains
   Rectangular, suberised, thickwalled corkcells.
   Single layered tangentially elongated cork-cambium;
   Few layers of parenchymatous secondary cortex.
3. Endodermis is not clear in the root showing secondary growth.
4. Many pericycle layers are formed due to the repeated divisions of pericycle having reserve food.
5. Many rings of vascular bundles are present. Each ring contain many closely arranged diarch and exarch xylems.
6. Bands of storage parenchyma separate the rings of vascular bundles.
7. First Cambium ring is formed from the parenchymatous cells between the xylem, phloem and from pericycle region above the protoxylem points.
8. Second cambial ring is formed from phloem parenchyma outside the first cambial ring.
9. Third cambial ring is formed from pericycle.
10. Later cambial rings are produced successively due to the division of pericycle.



T.S. ROOT (GROUND PLAN)



T.S. ROOT (A SECTOR SHOWING CELLULAR DETAILS)

4 STOMATAL TYPES USING EPIDERMAL PEELS

STOMATAL TYPES

The flollowing stomatal types are identified based on the number and arrangement of the subsidary cells.

1) **Anomocytic (or) ranunculaceous type: Eg: cucurbita**

Subsidary cells are absent (similar to epidermal cells)

2) **Anisocytic (or) cruciferous type: Eg: Brassica**

Stomata are surrounded by three unequal subsidary cells (two - large; one - small)

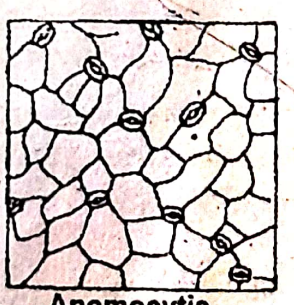
3) **Diacytic (or) caryophyllaceous type Eg:Ocimum**

Stomata are surrounded by a pair of subsidary cells. The common wall of the two subsidary cells is at right angles to the longitudinal axis ot the stomata

4) **Paracytic (or) Rubiaceous type Eg: Arachis**

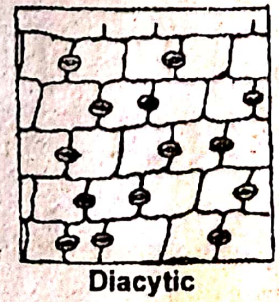
Once (or) more subsidary cells accompany each guard cell.

Their longitudinal axis are parallel to the guard cells and stomatal opening.

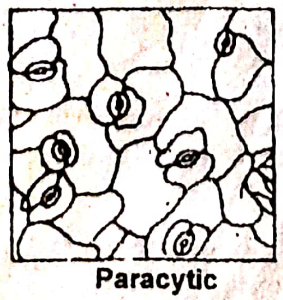


Anomocytic

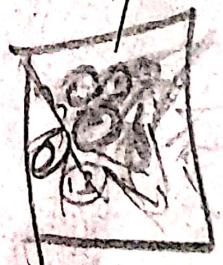
Anisocytic

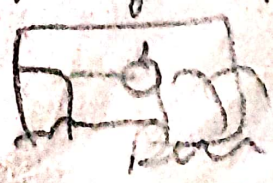


Diacytic



Paracytic





5 MICROSCOPIC STUDY OF WOOD IN T.S, T.L.S AND R.L.S

WOOD T.S ; T.L.S AND R.L.S

Microscopic study of wood (Secondary xylem):

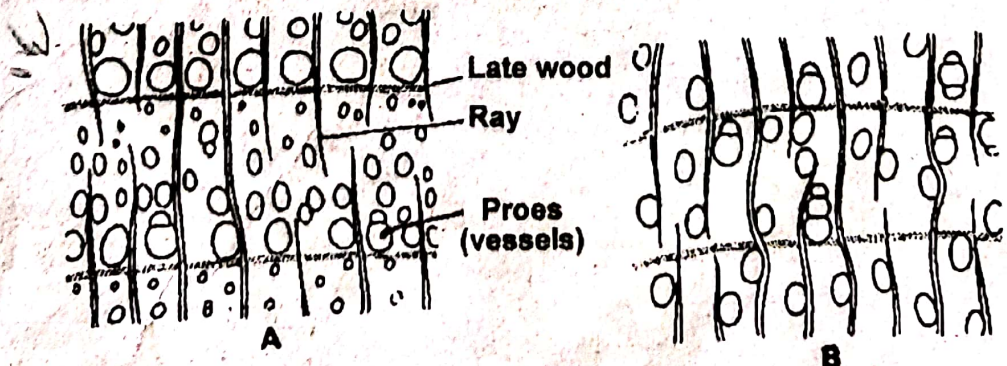
1. It is made up of tracheids, vessels, fibers and wood parenchyma.
2. In general primary and secondary xylem are almost similar.
3. The vessels are short and more in number than in secondary xylem and are usually pitted.
4. The secondary xylem of tree species constitutes the *timber* or *wood.*

Types of secondary xylem (wood)

Based on the size and arrangement of secondary xylem vessels, wood is differentiated in to two types - a) diffuse porous wood and b) ring porous wood. In a cross section of wood the vessels appear as *pores*. The pattern of distribution of these pores is different in these two types of woods. The vessels of ring porous wood are larger than those of diffuse porous wood.

Diffuse porous wood : In this wood, the vessels are of similar size and are almost uniformly distributed in the growth ring. However, there is a gradual change in size of the vessels form early to late wood. E.g. *Acer, Eucalyptus*.

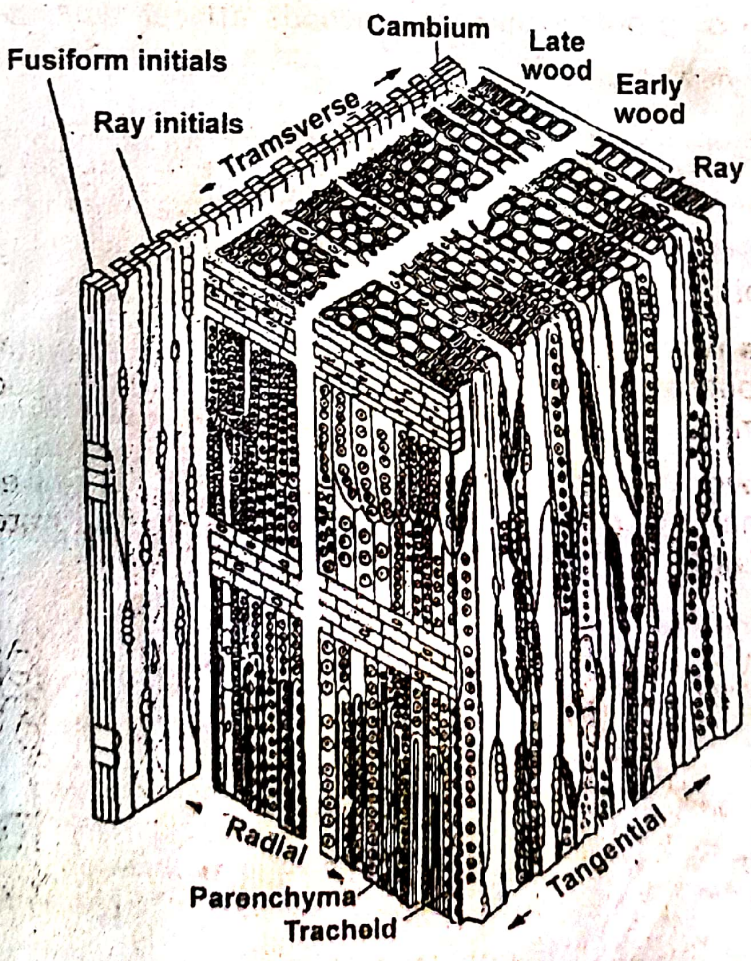
Ring porous wood : In this wood, the pores (vessels) of early wood are distinctly larger than that of the late wood. Thus, it appear as separate distinct zone in cross section. E.g. *Lagerstromia, Tectona, Morous*, etc.



(A) Ring porous wood, (B) Diffuse porous wood

1.1. S OF WOOD :

1. Take a peace of wood and cut a longitudinal section along with the tangent in the outer region.
2. Stain the section with safranin- fast green combination and make a temporary preparation by mounting with glycerine.
3. Wood section comprises of tracheids and medullary rays.
4. Tracheids show bordered pits on their radial walls. Side-view of the pit is visible in this plane. Pits appear as dome like structure with overarching borders enclosing the central disc called torus.
5. The height and width of medullary ray was clearly understood.
6. The ray may be uniseriate, biseriate or multiseriate.
7. The nature of the ray is also understood in this section.
8. Ray may be homocellular made up of single type of thin walled cells, (or) heterocellular made up of both thin walled or thick walled (lignified) cells.



THREE DIMENSIONAL DIAGRAM OF THE CAMBIUM AND SECONDARY XYLEM

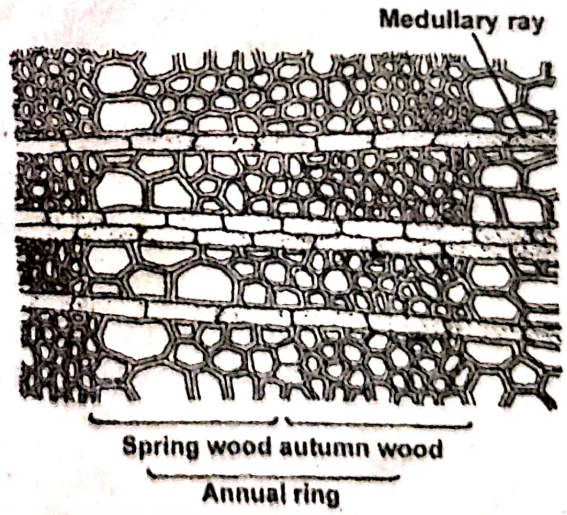
R.L.S. OF WOOD :

1. Take a peace of wood and cut a longitudinal section along with one of the radii.
2. Stain the section with safranin- fast green combination and make a temporary preparation by mounting with glycerine.
3. Wood section comprises of tracheids and medullary rays.
4. Tracheids show bordered pits on their radial walls. The surface view of bordered pits is seen in this plane.
5. Nature of the ray-homocellular (or) heterocellular can be visualized in this plane also.

T.L.S OF WOOD :

1. The wood produced during different seasons in a year can be differentiated by the nature of wood which can be observed as concentric rings in the trunk.
2. Two different types of woods are produced during the two seasons – autumn and spring.
3. **Spring wood or early wood** composed of large and thin walled xylem vessels.
4. **Autumn or late wood** composed of thick walled narrow pitted xylem vessels.
5. In cross section of a stem, these two woods appear as concentric rings. These are called **annual rings** because autumn and spring woods together constitute the wood for one year.
6. Therefore one autumn and one spring wood constitute a single annular ring.
7. Thus by counting the number of annular rings one can count the age of a woody trees.

T.S. of old stem showing rings annual



T.S. OF A PORTION SEC. WOOD SHOWING CELLULAR SMALL ORGANISATION OF SINGLE RING ANNUAL

T.S. OF OLD STEM SHOWING RINGS ANNUAL

6 STRUCTURE OF ANTHER AND MICROSPOROGENESIS USING PERMANENT SLIDES

T.S.OF ANTHER AND MICROSPOROGENESIS

1. Monothecous anther contains two chambers and dithecous anther contains four chambers in transverse section (T.S).
2. Each chamber is called microsporangium.
3. Two microsporangia meet at the stomium region where the anther liberates microspores to the outside.
4. Each microsporangium contains outer single layered epidermis; and single layered endothecium having hygroscopic fibrous thickenings.
5. One-to- five layeres of middle layers.
6. Tapetum the nutritive tissue is present inner to the middle layer.
7. The diploid sporogenous cells increases in number due to mitotic division and are called microspore mother cells.
8. Each microspore mother cell forms one microspore tetrads (pollen tetrads) by meiosis. So they are haploid in condition and the process is called microsporogenesis.
9. The microspore tetrads later separate and are called pollen grains (Haploid).

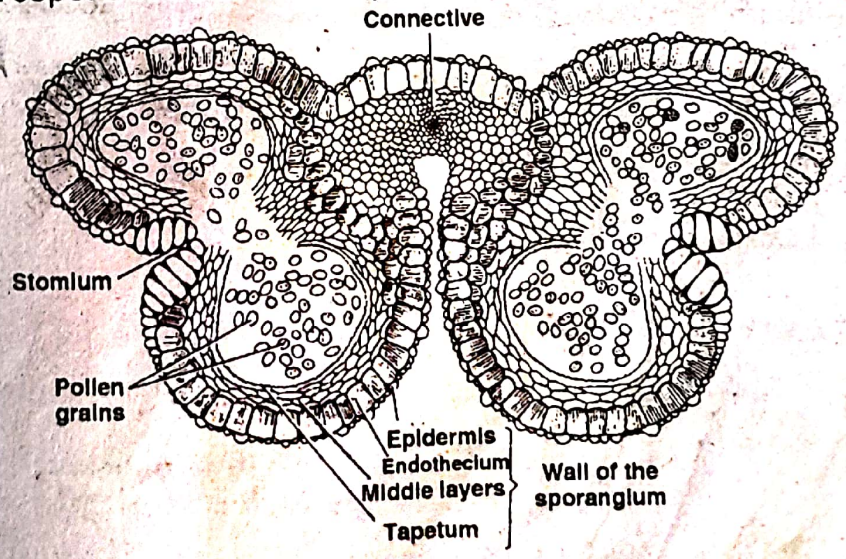


Fig: T.S of anther

7 POLLEN GRAINS OF HIBISCUS, ACACIA & GRASS

(A) Pollen grains Hibiscus sps (Family : Malvaceae)

1. Pollen grains are haploid, unicellular with sporoderm (pollen wall)
2. They show radial symmetry, isopolar character with spherical shape.
3. They contains five pores (pentaporate) and the pores are circular.
4. Exine tectate, columellae (baculae) are prominent, sexine is thicker than nexine (inner- layer).
5. The surface is hard with prominent spines (Spine base swollen and the tip is round) Rounded granules are present in betweeen the spines.

(B) Pollengrains of Acacia SPS. (mimosae) :

1. Pollen grain are haploid, Unicellular and surrounded by sporoderm walls.
2. 16 Pollen grains unite to form a polyad with spheroidal shape.
3. 8 Pollen grains are present at the center with two super-imposed pollengrains arranged as 4 grains each (4+4).
4. Exine is thick and granular. Sexine and nexine shows similar thickenings.
5. The proximal apertures always composed of pores where as the distal apertures are colporate (Compund aperture with a clopus colporate (compound aperture with a colpus and a pore). The pollen grains are heteropolar.

C) Pollen grains of Grass plant (Poaceae) :

1. Pollen grains are haploid, unicellular and surrounded by sporoderm walls.
2. Pollen grains has a circular, single pore with prominent annulus.
3. Pollen grains are radially symmetrical with spherical shape and heteropolar.
4. Exine is tectate, with smooth (or) granular surface. Sexine and Nexine shows same thickening.
5. Exine has different aperture at proximal and at distal areas.
6. Wild grass contains small pollen grains and cultivated grass plant shows large pollen grains.

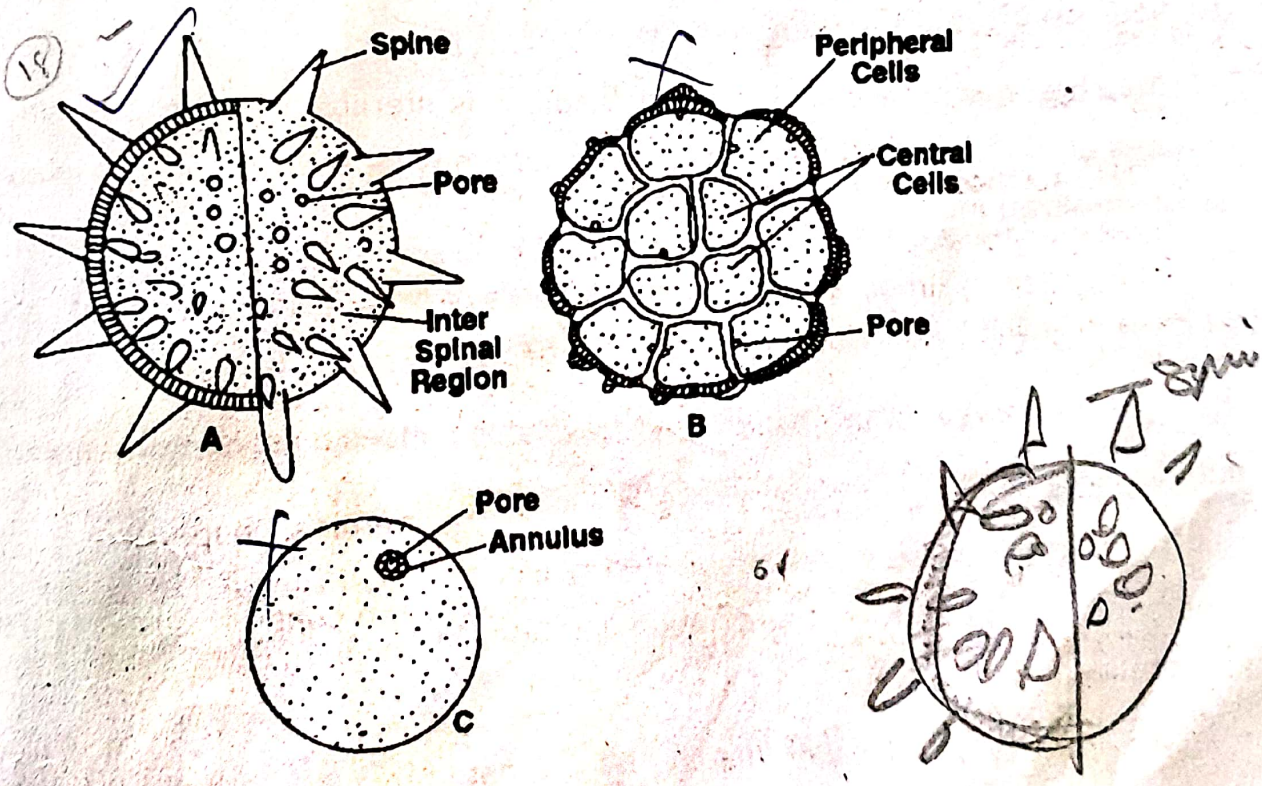


Fig: (A) Hibiscuks (B) Acacia (C) Grass

8 POLLEN GRAIN VIABILITY

The basal medium contains sucrose and organic salts with macro-nutrients (Nitrogen; Phosphorus Sulphur; Magnesium; Calcium and potassium) and micro-nutrients (Fe, Mn, Zn; B, Cu and Mo).

Amino - acids and vitamins may be added if necessary. They are dissolved in distilled water and maintain the pH as 5.8. The medium can be made semisolid with the addition of agar (5-to-8 %).

To obtain aseptic condition the medium is sterilized in the autoclave for 15 minutes at 120°C in 15 lb pressure. The medium is taken in to the petriplates having flame sterilization.

The pollen grain obtained from the anthers of catharanthus (explant) are treated with ethyl alcohol (or) chlorine water. (to get surface sterilization).

The inoculation of the pollen grain (explant) on to the nutrient medium is carried out in the Laminar air flow chamber (Aseptic environment).

The culture vessels with inoculated pollen grains are inculated under controlled conditions of temperature, illumination and humidity, pollen grains takes 3 to 4 weeks for germination.

The viability of pollen grains can be estimated by calculating the percentage of pollen grain germination on the nutrient medium.

9 TYPES OF OVULES

(1) TYPE OF OVULES :

(1) Orthotropous **:** In this type of ovule micropyle , Chalaza and funiculus are arranged on the same vertical line.

(2) Anatropous **:** It is an inverted ovule. Micropyle lies close to the funiculus. Micropyle and chalaza lie on the same straight line.

(3)Hemi-anatropous **:** The ovule body is placed at right angle to the funiculus.

(4)Campylotropous : The arrangement of the ovule body is similar as in the case Hemi-antropous ovule, but the body of the ovule bends in such away that the micropyle comes nearer to the funiculus. The embryosac is almost straight.

(5)Amphitropous : The ovule bends in such a way that the micropyle and funiculus are brought very nearer. Embryo-sac shows horse-shoe shape.

(6)Circinotropous : The funiculus is very long and coils like a watch spring around the anatropous ovule.

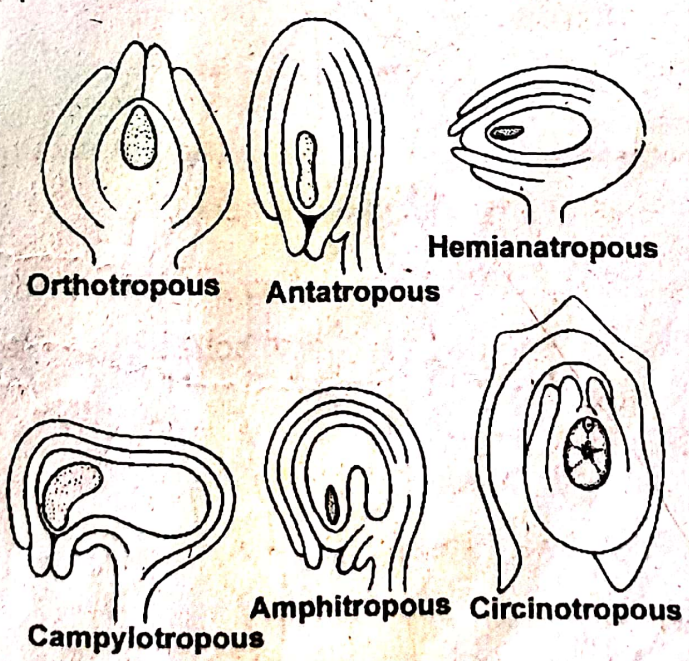


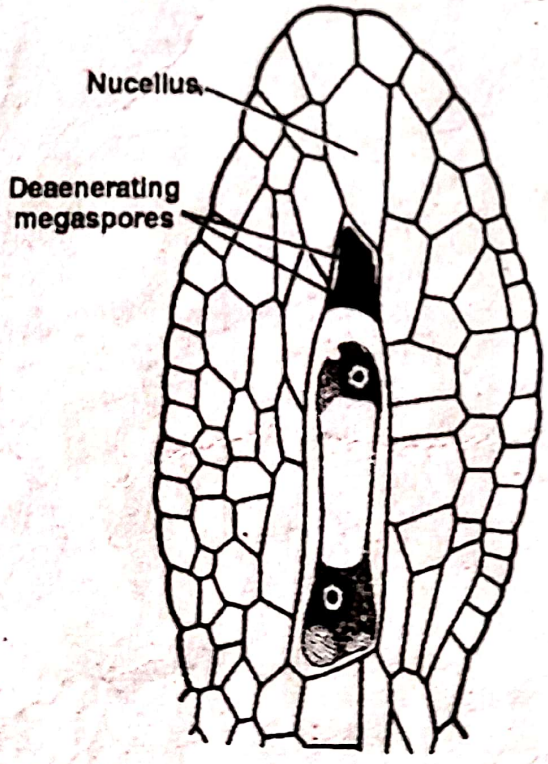
Fig: Different types of ovules

A. Orthotropous B. Anatropous C. Campylotropous D. Hemianatropous E. Amphitropous F. Circinotropous

(2) DEVELOPMENT STAGES OF EMBRYO-SAC :

(i) Bi - (2) nucleate stage of Embryo-sac:

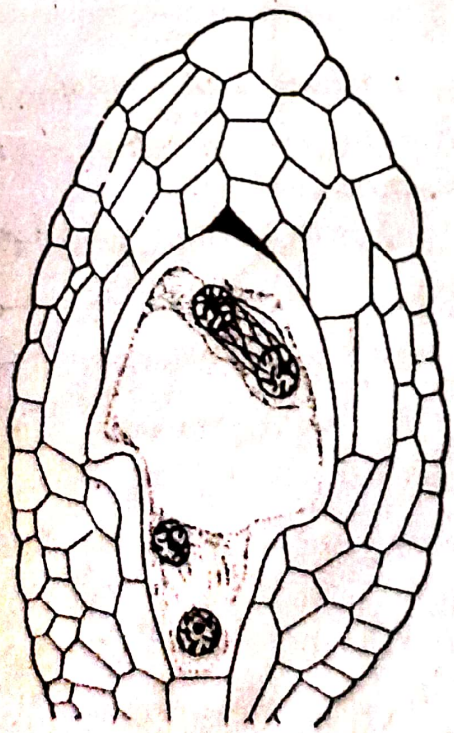
1. Ovule is a rounded structure, attached to the ovary wall at the placement with the help of a funiculus.
2. It is surrounded by one or two integuments and has a terminal micropyle and basal chalazal part.
3. The ovule contains diploid nucellus tissue.
4. One cell (2n) towards micropyle act as megaspore mother cell and undergoes meiosis to form four haploid linear megaspores (n).
5. The upper three megaspores degenerate and the lower megaspore (towards chalaza) is only functional and enlarges in size by absorbing abjacent nutritive tissue. This is called Embryo-sac (female gametophyte).
6. The haploid nucleus in the embryo-sac divides by mitosis and forms two nuclei. One nucleus moves towards the upper side (micropylar region) and the second nucleus moves towards the lower side (chalaza-region).
7. At this stage the embryo-sac is in Binucleate stage.



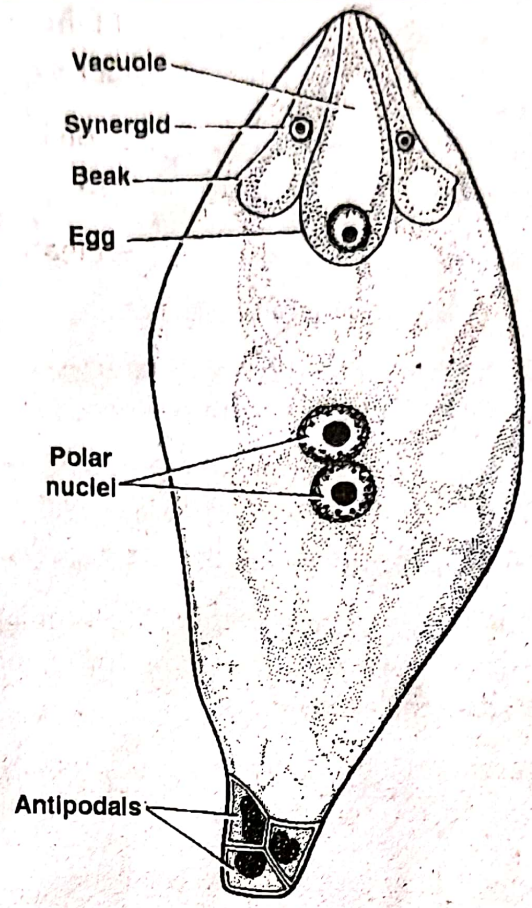
L.S. of ovule showing binucleate embryo sac.

(ii) Tetra (4) nucleate stage of Embryo-Sac :

1. Ovule is a rounded structure, attached to the ovary wall at the placenta with the help of a funiculus.
2. It is surrounded by one or two integuments and has a terminal micropyle and basal chalazal part.
3. The ovule contains diploid nucellus tissue.
4. One cell (2n) towards micropyle act as megaspore mother cell and undergoes meiosis to form four haploid linear megaspores (n).
5. The upper three megaspores degenerate and the lower megaspore (towards chalaza) is only functional and enlarges in size by absorbing abjacent nutritive tissue. This is called Embryo-sac (female gametophyte).
6. The haploid nucleus in the embryo-sac divides by mitosis and forms two nuclei. One nucleus moves towards the upper side (micropylar region) and the second nucleus moves towards the lower side (chalaza-region).
7. The two nuclei present at opposite pole (1-miicropyle; 1-chalaza) dividis by mitosis and form four haploid nuclei (2-upper side; 2-lower side).
8. At this stage the embryosac is in tetra nucleate stage.



L.S. of ovule showing tetra nucleate embryo sac.



L.S. of ovule showing 8-nucleate embyo sac

(iii) Octa nucleate stage of Embryo-sac (8-Nuclei) :

1. Ovule is a rounded structure, attached to the ovary wall at the placenta with the help of a funiculus.
2. It is surrounded by one or two integuments and has a terminal micropyle and basal chalazal part.
3. The ovule contains diploid nucellus tissue.
4. One cell (2n) towards micropyle act as megaspore mother cell and undergoes meiosis to form four haploid linear megaspores (n).
5. The upper three megaspores degenerate and the lower megaspore (towards chalaza) is only functional and enlarges in size by absorbing abjacent nutritive tissue. This is called Embryo-sac (female gametophyte).
6. The haploid nucleus in the embryo-sac divides by mitosis and forms two nuclei. One nucleus moves towards the upper side (micropylar region) and the second nucleus moves towards the lower side (chalaza-region).

7. The two nuclei present at opposite pole (1-micropyle; 1-chalaza) divides by mitosis and form four haploid nuclei (2-upper side; 2-lower side).
8. The four nuclei in the embryo sac again divides by mitosis and form eight haploid nuclei (4-Upper side; 4-lower side).
9. At this stage the embryo -sac is in octanucleate stage.

The three haploid nuclei present towards the micropyle forms egg apparatus (1-Egg cell; 2-lateral synergids) and the remaining single nucleus moves towards the center (polar nucleus).

Among the four-haploid nuclei present towards the chalaza forms three antipodals and the remaining single nucleus moves towards the center (polarnucleus).

Embryosac 8-Haploidc Embryology

- Micropyle – 3 cells (Eggapparatus) + 1 polar nucleus
- Chalaza – 3 cells - (Antipodals)+ 1 Polar nucleus.

10 ENDOSPERM TYPES

(1) TYPE OF OVULES :

1. In angiospermic plant endosperm is a post fertilization product; and developed from primary endosperm nucleus (PEN), which is triploid in condition.
2. It is formed in the embryosac due to the fusion of two haploid polar nuclei of the embryosac and one haploid male gamete (nucleus) obtained from pollen grain. This is called triple fusion.
3. The embryosac (female gametophyte) in angiosperm is formed due to megasporogenesis (i.e. after meiosis) in the ovules which are attached to the ovary wall (at placenta).
4. The development of endosperm from the triploid PEN is of the following methods and the endosperm is a nutritive tissue to the developing zygote.

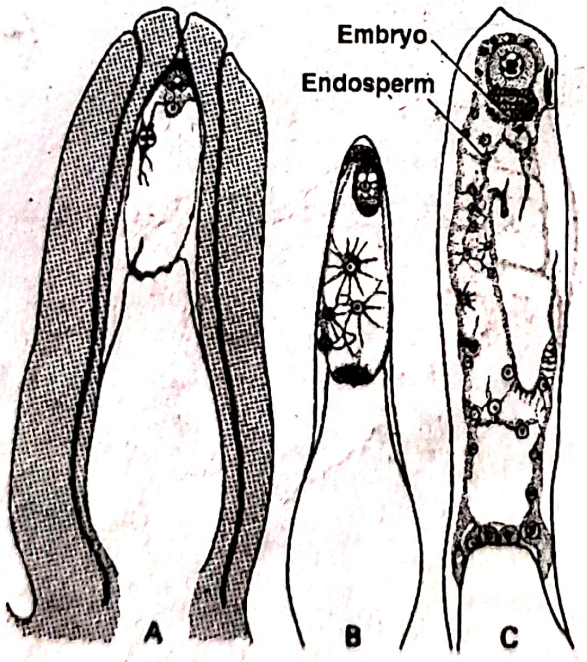


Fig: Endosperm.
A. to C. different stages in the development of nuclear endosperm

(i) Nuclear endosperm :

(A) The triploied primary endosperm nucleus divides by mitosis with out cell wall formation; and results many endosperm nuclei.

The free nuclei (3n) present at the center are pushed to the periphery of the embryosac due to the formation of a large central vacuole. As there is no cell wall formation it is called nuclear endosperm.

(ii) Cellular endosperm :

The triploid primary endosperm nucleus divides by mitosis to form many triploid (3n) endosperm nuclei. The nuclear division is always followed by cell wall formation. So the endosperm is called cellular endosperm.

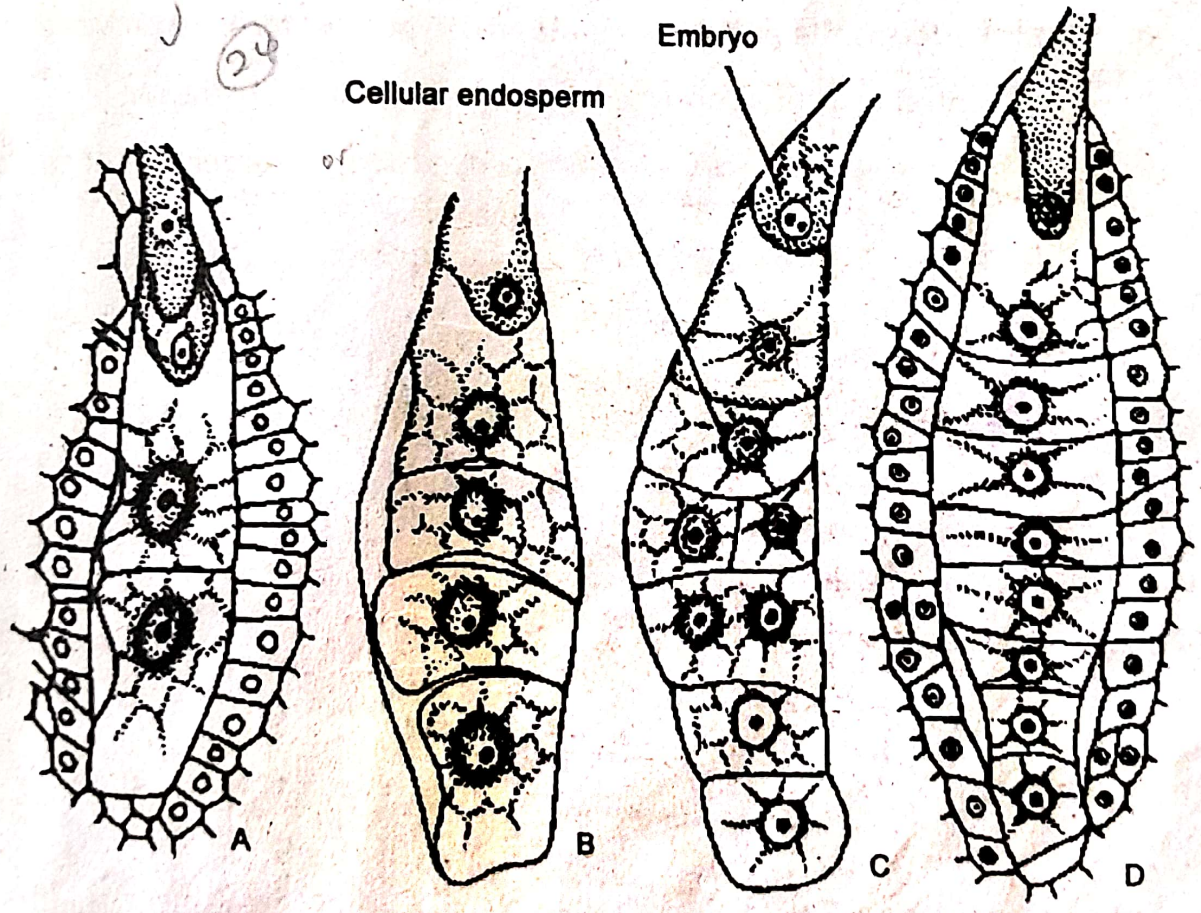


Fig: Endosperm : A–D. Different in the development of cellular endosperm

(2) STRUCTURE OF MATURE DICOT EMRBYO :

1. In angiospermic plant the embryo is formed from a diploid zygote which is formed during fertilization (Syngamy)
2. In Dicot embryo it consists of an axis and two lateral cityledons which has a terminal plumule (shoot).
3. A swollen suspensor is present at the other end (the cotyledons).
4. Embryonal axis has
   - Epicotyl-part above the cotyledons.
   - Hypocotyl - Part below the cotyledons.
5. The development of endosperm from the triploid PEN and it is a nutritive tissue to the developing zygote.
6. Epicotyl forms the plumule (shoot) and Hypocotyl forms the radical (Root).
7. The central cells of hypocotyl forms the pre-cambial strand.
8. The embryo shows a curved structure due to the enlargement of cotyledons and hypocotyl.

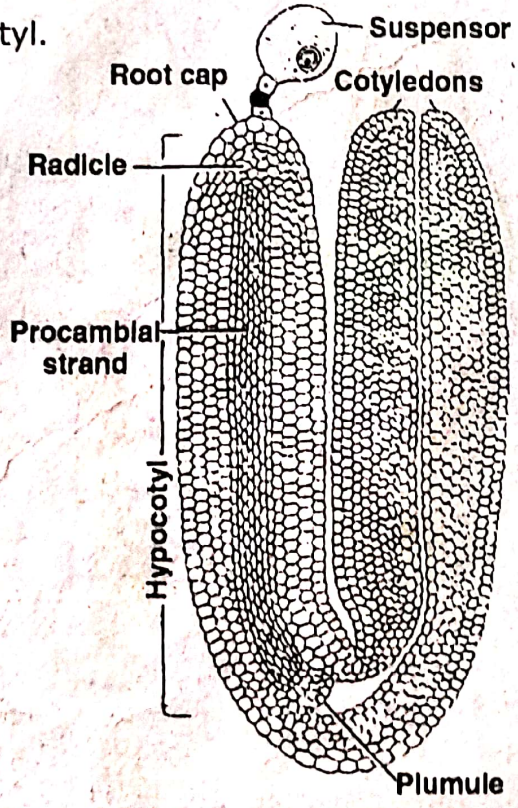


Fig: Embryo. Mature dicot embryo

Structure of monocot embryo :

1. This can be observed in the monocot fruit called caryopsis (grain).
2. Embryo occupies small part of the grain and the remaining part contains endosperm. They are separated by epithelial layer (Aleurone layer).
3. The embryo contains cotyledon called scutellum (shield shaped structure) and embryonal axis.
4. Embryonal axis contains →
   - Plumule -covered by coleoptile leaf-sheath
   - Radicle - coleorhiza root-sheath.
5. The formation of embryo is from zygote which is formed during fertilization.

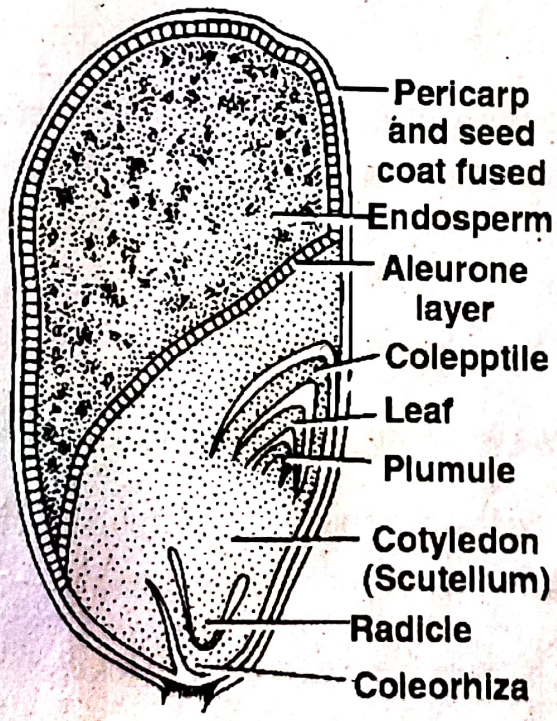


Fig: Embryo. Monocot embryo

11 ISOLATION, MOUNTING OF EMBRYO

DEVELOPED EMBRYO

The seed, formed after fertilization, consisting of an outer seed coat and an inner embryo. In addition, the remnant part of nucellus called perisperm and endosperm are also present in seed. Normally, embryo consists of a suspensor situated near the micropyle, cotyledons towards chalaza and a small embryonal axis called tigellum.

To study the structure of emryo it must dissected out of the seed and observed under binocular or dissection microscope.

Dissection of embryo:

1. Place the seed on the stage of a dissection microscope.
2. Locate the micropyle which appear like a small opening.
3. Remove the seed coat from this point, carefully with the help of two sharp pionted needles., by observing through microscope.
4. The embryo could be seen between the cotyledons.
5. Place a drop of water on the embryo and observe or mount the embryo in glycerine and observe.