Year	Sem.	Subject Code	Title of the paper	Hours/ Week
2018 -2019 onwards	II	18MBO22C	PAPER – V- ANATOMY AND EMBRYOLOGY	7

Objectives:

1. To understand the basic principle of differentiation of cell types
2. Application of various micro techniques
3. To trace the development of male and female gametophyte
4. To highlight the physiological role of endosperm in the morphogenesis of embryo
5. To assess the process of seed development

Unit - II

Periderm: Structure, organization and activity of phellogen. Polyderm and Rhytiderm – wound periderm. Normal secondary thickening in Dicots; Anomalous secondary growth in Dicots (Amaranthaceae, Aristolochiaceae, Bignoniaceae, Piperaceae, Nyctaginaceae) and arborescent Monocots. Primary thickening in palms; Ontogeny of leaf, Structure and types of Stomata; Leaf abscission; Major nodal types; Kranz anatomy and its significance.

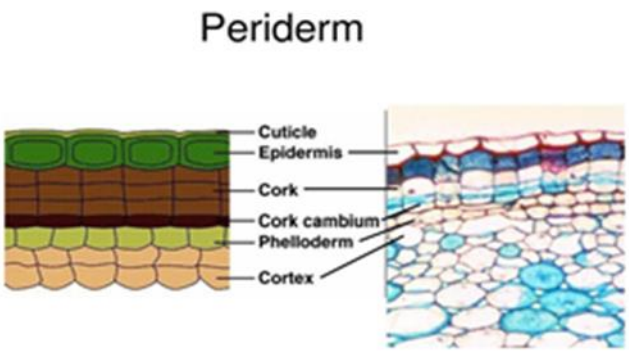
Microtechnique: Principle of killing and fixation, dehydration and rehydration of botanical specimens. Stains: Principle of double staining (fast-green and light green) of free hand sections; Protocol for serial sectioning of paraffin wax impregnated specimens; Mounting and mounting media.

UNIT-2

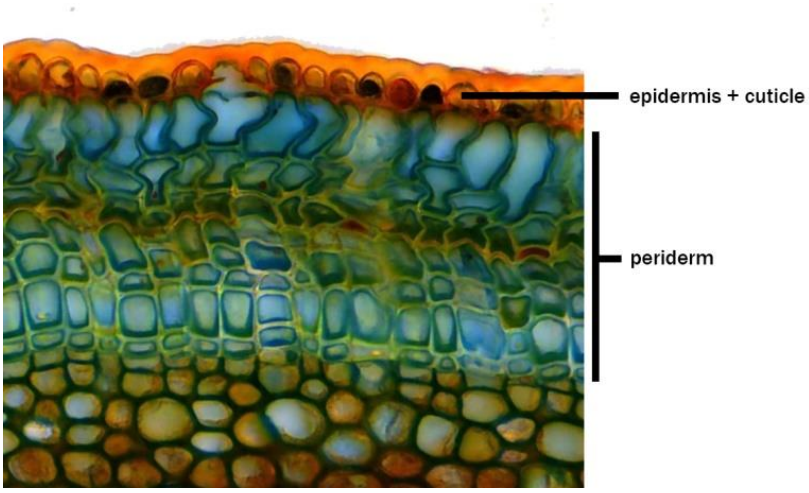
PERIDERM: STRUCTURE, ORGANIZATION AND ACTIVITY OF PHELLOGEN

PERIDERM:

- The periderm is a cylindrical tissue that covers the surfaces of stems and roots of perennial plants during early secondary growth; therefore it is not found in monocots and is confined to those gymnosperms

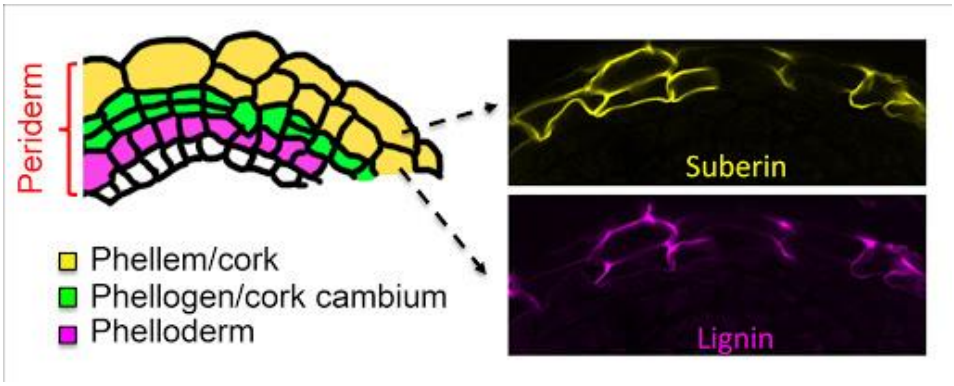


- It generally develops in gymnosperms and dicotyledonous axis and is rarely produced in leaves or monocotyledons.
- The periderm is also formed along surfaces exposed after abscission of plant parts, such as leaves or branches. It also evolves as protective layer near injured parts (wound periderm)



STRUCTURE OF PERIDERM

- Periderm is composed of the phellogen, phellem, and phelloderm. During secondary growth, the outer epidermal layer and the cortical layer are broken because of the cambium
- The periderm consists of the phellogen or cork cambium
- The meristem that produces the periderm,the cork or phellem, the protective tissue produced outside by the phellogen
- The inner cortex or phelloderm, the living parenchyma, formed inside by the phellogen.
- Because of the formation of cork, the tissues outside it usually die out.
- The phellem or cork cells are often prismatic in shape and can be elongated vertically, radially or tangentially to form irregularly shaped structures.
- These are compactly arranged and absent inter-cellular spaces. These are dead cells at maturity. The cell walls are suberized



- In many species viz., Rhododendron maximum, the phellem includes non-suberized cells, called phelloid cells together with cork. These may also be thin or thick walled. When thick walled these are known as sclereids.
- The phelloderm is a typical parenchyma which may be distinguished from other parenchyma by being present in the same radial files as the phellem cells.

ORGANIZATION OF PERIDERM

- The first periderm commonly appears during the first year of growth of stem and root. In stem most usually it originates in the sub-pepidermal layer.
- In some species, the first periderm appears rather deep in the stem, usually in the primary phloem viz., Berberis, and Vitis etc.
- In roots, the first periderm originates in the pericycle. In some cases, where the root cortex serves for food storage, it can originate near the surface also.
- The subsequent periderms may appear the same year or later in the successively deeper layers beneath the first, i.e., from the parenchyma of the phloem, including ray cells.

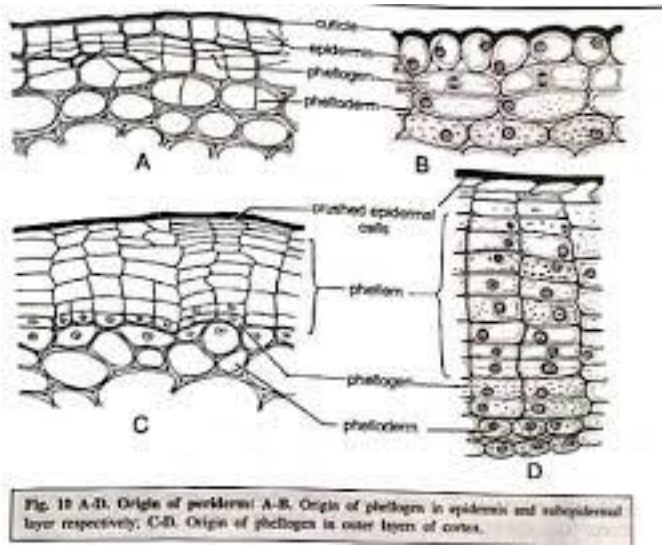


Fig. 19 A-D. Origin of periderms: A-B. Origin of phellogen in epidermis and subepidermal layer respectively; C-D. Origin of phellogen in outer layers of cortex.

- The first phellogen is generally initiated uniformly around the circumference of the axis. Sometimes, e.g., roots in localized area which becomes continued afterwards.
- The subsequent periderms appear as discontinuous but overlapping layers. These can finally appear as continuous, or partly so, layers, around the axis.
- The phellogen arises from living cells which are potentially meristematic. In these cells, the initiating divisions can start in presence of chloroplast and orgastic substances, viz., starch, tannins

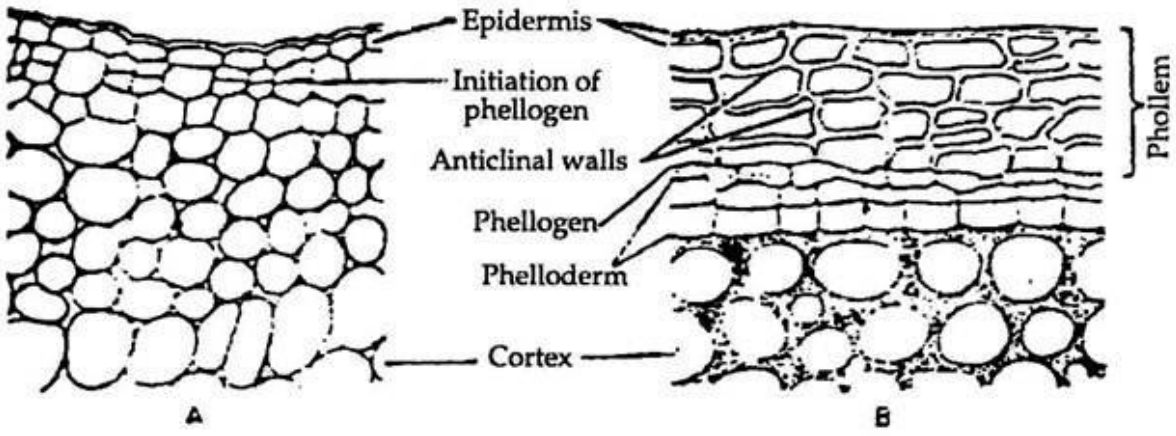


Fig. 7.2. Origin of periderm is *Pelargonium* stem as seen in cross section.

- But gradually these structures disappear. The phellogen is initiated by periclinal divisions and it forms the phellem and phelloderm by the same type of divisions.
- The activity of the phellogen is more on the outside and thus, the amount of phelloderm formed is generally very small, sometimes restricted only to few layer of cells.
- On the basis of manner of function, two kinds of barks are distinguished
  - scale bark
  - ring bark.
- The former occurs when subsequent periderms exist in restricted overlapping strata, each cutting out scale of tissue, e.g., Finns and Pyrus etc.
- Ring bark results from the formation of successive periderms approximately concentrically around axis, in the produce of sheets e.g., Vitis, and Lonicera, etc.

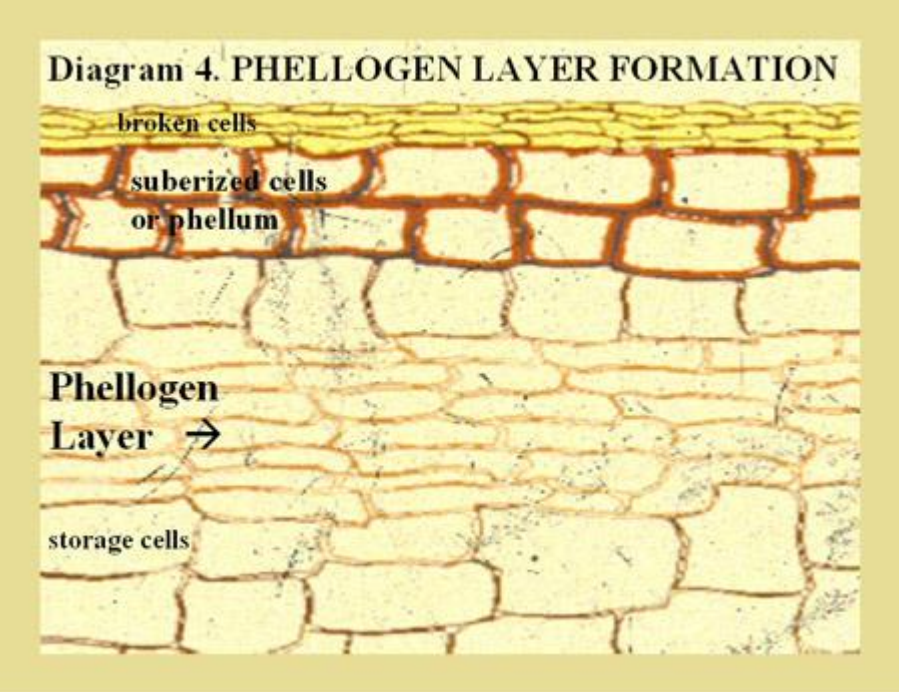
FUNCTION:

- Its main function is to protect the underlying tissues from desiccation, freezing, heat injury, mechanical destruction, and disease.
- Although periderm may develop in leaves and fruits, its main function is to protect stems and roots

ACTIVITY OF PHELLOGEN

- The Phellogen also called as cork cambium is the meristematic plant tissue responsible for the formation of the periderm (the covering of the stem, shoots and roots).

- In the inner side of the phellogen layer forms the phelloderm and its outer side forms the cork (suber) and the suber secretes suberin, an impermeable substance that impregnates the tissue.
- The phellogen is initiated from collenchyma cells of one of the three outermost layers of the cortex. The first phellogen persists for several years. The activity is periodic and slow



- A highly active phellogen is characterized by the production of large thin-walled phellem cells.
- A layer of flat, thick-walled phellem cells next to the phellogen characterizes an inactive meristem.
- The annual rhythm of phellogen activity of Robinia trees grown outdoors reveals that the phellogen is active twice a year, while under the same conditions the cambium is active once a year only.
- Under controlled conditions of temperature and day-length the phellogen was found to be active mainly under combinations of short day and high temperature or long day and low temperature.

PEIDERM:

Periderm (also called wound periderm) develops in the callus. ... Type of soil determines the number of layers formed in the wound cork of potato. It is observed in potato tuber that during wound healing suberin or cutin deposits over the damaged surface. Thus the surface is sealed and the formation of phellogen follows. Polyderm:

Periderm:

Continued secondary growth in the intrastelar region by the activity of the cambium cylinder large amount of pressure is exerted on the extrastelar tissues, which migrate ultimately to the epidermis.

As a result, the epidermis gets more stretched and ultimately tends to rupture exposing the internal tissues to the outside. In this situation, to protect the inner tissues, a new dermal tissue is formed secondarily, called periderm, in the extrastelar region.

It consists of three tissues:

1. A meristem known as phellogen or cork cambium.

2. The phellogen derived cells on the outer side, called phellem or cork cells.

3. The phellogen derived cells on the inner side, called phelloderm.

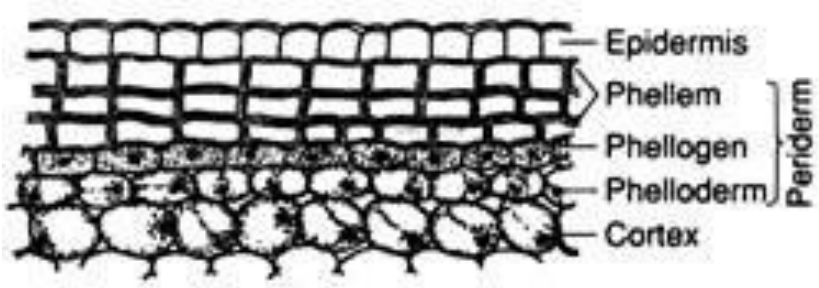


Fig. 5.136 : Diagram showing periderm formation

Polyderm:

Polyderm is a special protective tissue consisting of twenty or more alternating layers of uniseriate suberised cells and multiseriate non- suberised cells. The peripheral cells are dead and the inner cells including the suberised cells contain living protoplasts. It is found in underground stems and roots of Rosaceae, Onagraceae, Myrtaceae and Hypericaceae.

At the time of its formation, a special phellogen is differentiated at the pericycle, which forms tissues by tangential divisions in centripetal succession. The newly formed tissues consist of thin walled non-suberised parenchyma cells alternating with uniseriate endodermoid cells.

The latter are differentiated into cork cells with the formation of casparian strips on their walls that later undergo more extensive suberisation. Polyderm is formed at the pericycle and it is exposed to outside after the death of the cortical tissues. It performs the function of protection of the inner living tissues. The non-suberised cells are concerned with food storage.

Rhytidome:

Rhytidome is a special type of bark composed of successive layers of periderm as well as either the cortical parenchyma or secondary phloem. In Robinia periderm formation

continues successively by the development of phellogen in the deeper regions of stem giving rise to periderm bands due to the death of the peripheral ones. The cork cells get suberised and the successive periderm bands enclose either cortical tissues or secondary phloem.

All the periderm bands together with the enclosed cortex or secondary phloem and all the tissues present external to the innermost phellogen are collectively referred to as rhytidome. The term bark is applied to all the tissues present external to the vascular cambium. Sometimes the term outer bark is applied to rhytidome and the living part of the bark inside the rhytidome is referred to as inner bark

Periderm (also called wound periderm) develops in the callus.

WOUND PERIDERM:

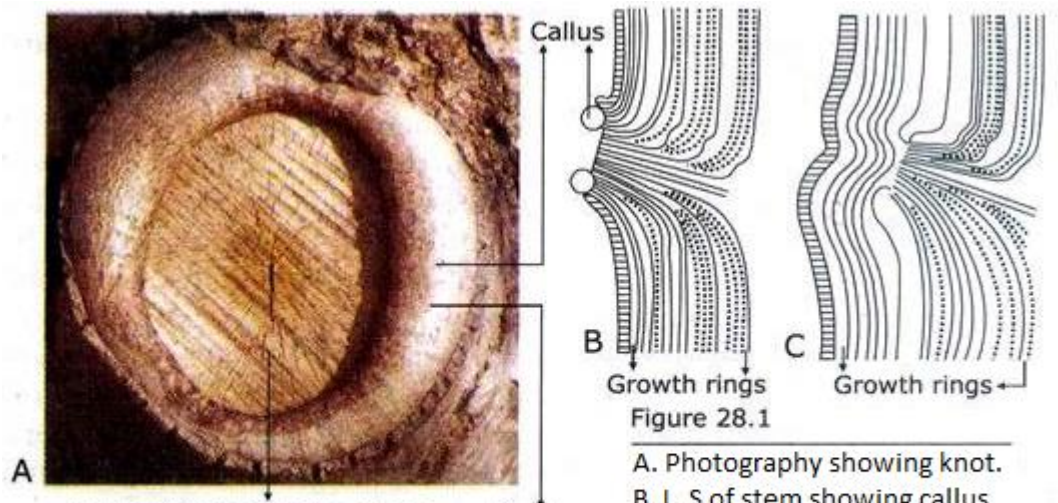


Figure 28.1

A. Photography showing knot. B. L. S of stem showing callus tissue. C. Diagram illustrating burial of wound caused due to pruning. Barks are hatched.

Wound periderm forms a protective bark that inhibits the entry of pathogen.

Plant organs often are wounded by adverse calamities of nature, grazing animals and human interference etc. As a result the living cells are exposed and they are subjected

desiccation and attack by bacteria, fungi and other pathogenic microorganisms etc. Higher plants have their own arrangement of healing of wounds.

There are different methods of healing of wounds. When the wound is superficial the exposed cells dry up and eventually die. Suberin deposits in the cells situated below the wound and thus a protective layer is formed. Some plants have laticifers and secrete latex. In plants having deep wounds callus or wound tissue is formed. Callus is composed of masses of soft parenchyma cells. It develops over and below the damaged surface of stem and root. It is formed, by the division of parenchyma cells that occur either in the phloem, in the cortex or in the vascular rays.

Most frequently cambium donates callus. During the formation of callus the cambium cells proliferate and produce masses of parenchyma. The peripheral cells of callus become suberized thus sealing the exposed surface of damaged tissue. **Periderm (also called wound periderm) develops in the callus.**

Wound periderm forms a protective bark that inhibits the entry of pathogen. Below the periderm a recognized cambium occurs and it forms new vascular tissues normally. Suberinization seems to create an internal condition that is favourable for periderm formation.

Experiments with potato tuber and sweet potato root reveal that suberinization is preceded by an accumulation of phenolic substances, particularly of chlorogenic acid. It also reveals that lignification is associated with wound healing. In potato tuber wounding stimulates mitosis and the wound healing is accompanied by suberinization and wound cork formation.

Increased temperature and high humidity are favourable for wound healing. Type of soil determines the number of layers formed in the wound cork of potato. It is observed in potato tuber that during wound healing suberin or cutin deposits over the damaged surface. Thus the surface is sealed and the formation of phellogen follows.

Experiment with potato tuber reveals that covering of cut surface is necessary for phellogen formation. In an experiment the cut surface was covered with paraffin wax. It was observed that phellogen formed without prior suberinization. Later researches reveal that in wounded potato tubers rapid increase in endogenous gibberellins like substances occur.

In trees where wound is deep, the uninjured living cells below the wound are stimulated to divide. As a result callus is formed. It overgrows the wound. Phellogen appears on the peripheral side of callus and divides to form wound cork. Callus with wound cork appears as swelling over the stem surface. Such structure is usually referred to as knot.

In trees where the wound is due to pruning of branches callus is formed on the circumference of wounds . This happens in the early growing season. Production of secondary xylem continues in the uninjured surrounding tissues by the cambium. Annual ring is also formed in undamaged area around the scar.

The base of branch dies, shrivels up and loses the capacity of growth. As more and more growth rings are formed the base of branch gets buried more and more deeply. The cambium layer becomes continuous over the scar and thus the wound is completely covered by the secondary xylem . In a timber the of branch appears as knot.

In case of deep wound where the cambium is damaged, normal callus is formed in the damaged area provided the uninjured tissues are protected from drying out immediately. In the callus a new cambium develops and it unites with the uninjured cambium. Thus the cambium becomes continuous and the production of secondary tissues continues.

The differentiation of cambium in callus and its subsequent fusion with neighboring cambium form the basis of grafting. In grafting the junction of stock and scion is filled up with callus. Within callus cambium differentiates and it fuses with the cambia of stock and scion. Thus a continuous cambium is formed and it donates normal vascular tissues.

Secondary Growth 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.

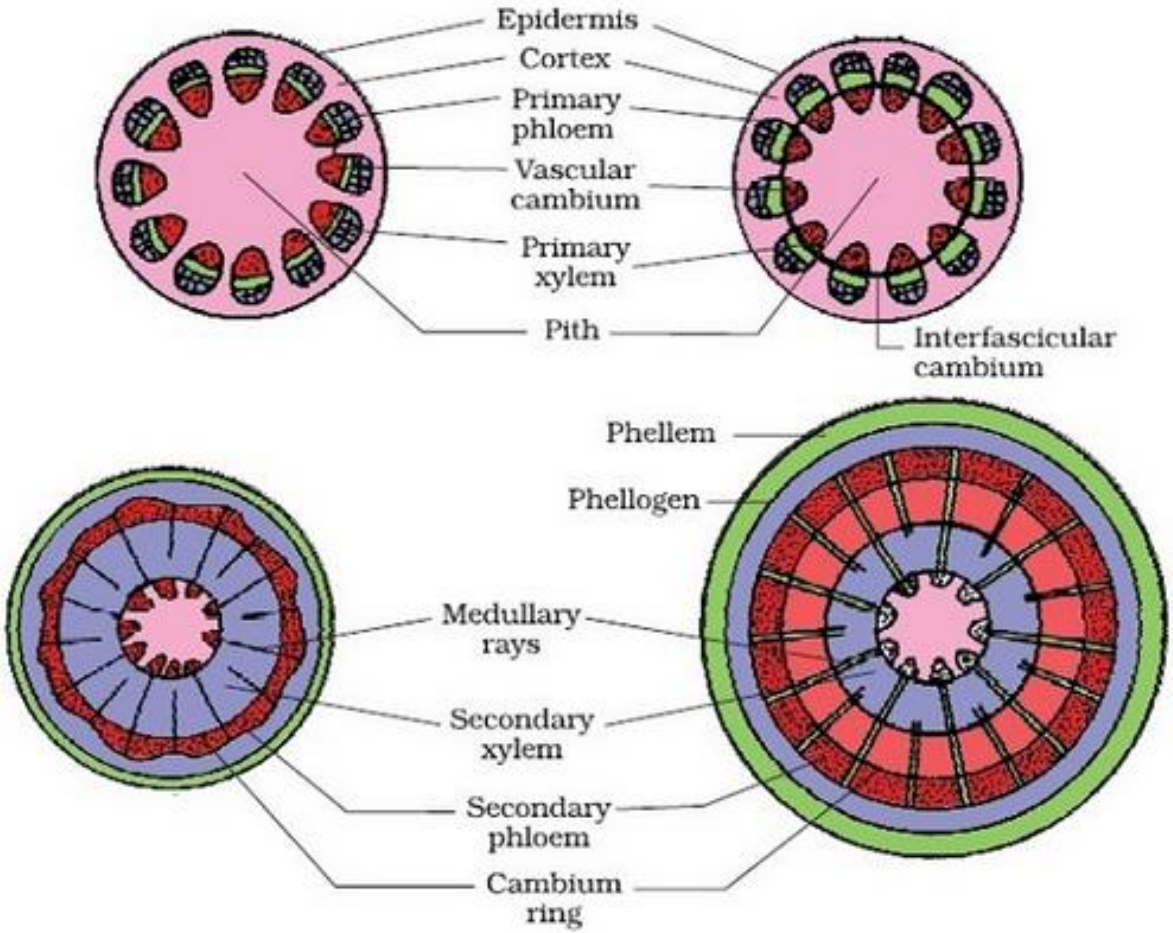
Secondary growth occurs in perennial gymnosperms and dicots such as trees and shrubs. It is also found in the woody stems of some herbs. In such cases, the secondary growth is equivalent to one annual ring, e.g., Sunflower.

The vascular tissue arises from the vascular cambium, a layer of meristematic tissue insinuated between the primary xylem and primary phloem (see above Vascular tissue). Secondary xylem develops on the inner side...Secondary growth

- Meristem is responsible for the development of primary plant body.
- Primary growth increases length of the plant as well as lateral appendages.
- However, secondary Grier increases thickness or girth of the plant by the formation of secondary tissues.
- There secondary tissues are formed by the two types of lateral meristem i.e. vascular cambium and cork cambium (phellogen).
- Secondary growth occurs in stem and root of dicots and gymnosperms.
- However, it is absent in stem and root of monocot and completely absent in leaf.
- A process of formation of secondary tissues due to activity of vascular cambium and cork cambium for increasing thickness or girth or diameter of plant is termed as secondary growth.
- On the basis of the activities of vascular cambium and cork cambium, the process of secondary growth can be discussed under the following headings:

- Activity of the vascular cambiu
  - Activity of the cork-cambium

region due to activity of the vascular cambium



i. formation of vascular cambium

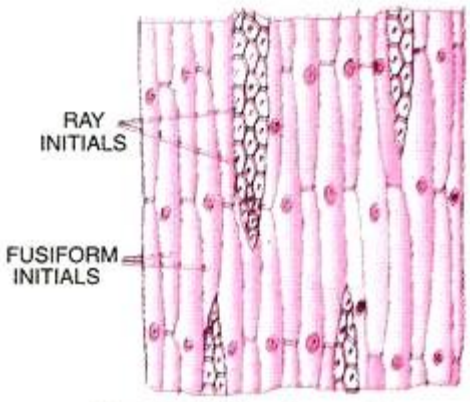


Fig. 6.29. L.S. Vascular cambium showing fusiform and ray initials.

- In vascular bundles of a dicot stem, the cambium is present in between the xylem and phloem. It is known as intrafascicular cambium.
- During secondary growth, some cells of medullary rays become active and show meristematic activity which form a strip of cambium in between vascular bundles called inter-fascicular cambium.
- Both the intra-fascicular and inter-fascicular cambium unite together to form a complete ring called the cambium ring.
- The activity of the cambium ring gives rise to secondary growth.

ii. ***Formation of the secondary tissues:***

- The cambium ring acts as a meristem which divides.
- The cambium layer consists of a single layer of cells.
- These cells divide in a direction parallel with epidermis.
- A cambial cell divides into two daughter cells, one of which remains meristematic and other differentiates into secondary vascular tissue.
- The cell formed towards inner side develops into secondary xylem.
- Likewise, the cell formed towards outer side develops into secondary phloem.
- Normally, more secondary xylem cells are formed towards the center due to which cambium ring moves towards the periphery.
- Due to the formation of secondary xylem and secondary phloem, the primary xylem and primary phloem which were initially closed, moves towards inner and outer side respectively.
- As a result, they become separated apart.
- The layers of secondary tissues gradually added to the inner and outer side of the cambium continuously throughout the life of the plant.
-

iii. ***Formation of secondary medullary rays:***

- Certain cells of the cambium instead of forming secondary xylem and phloem for some narrow bands of living parenchyma cells.
- These form two or three layers of thick radical rows of cells passing through the secondary xylem and secondary phloem and are called secondary medullary rays.
- These provide the radial conduction of food from the phloem, and water and mineral salts from the xylem.

iv. ***Formation of annual rings:***

Fig. 6.30. Part of T.S. old stem showing annual rings.

- The activity of cambium is affected by variations in temperature.
- In moderate climate, the cambium becomes more active in the spring and forms greater number of vessels with wider cavities, whereas in winter it becomes less active and forms narrower and smaller vessels.
- The wood formed in the spring is known as spring wood or early wood and that formed in the dry summer or cold winter is autumn wood or late wood.
- These two kinds of wood appear together as a concentric ring known as the annual ring or growth ring, as seen in transection of the stem and successive annual rings are formed year after year by the activity of the cambium.
- The growth of the successive years appears in the form of concentric or annual rings, each annual ring representing the one year's growth.
- The age of the plant thus, can be approximately determined by counting the number of annual rings.

v. ***Formation of heart wood and sap wood:***

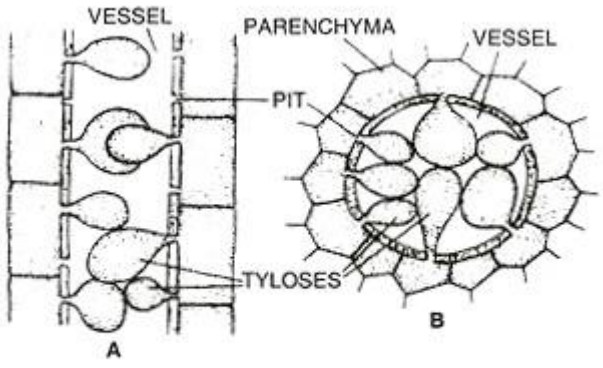


Fig. 6.32. Formation of tyloses in heartwood.
A, L.S. vessel showing tyloses.
B, T.S. vessel showing tyloses.

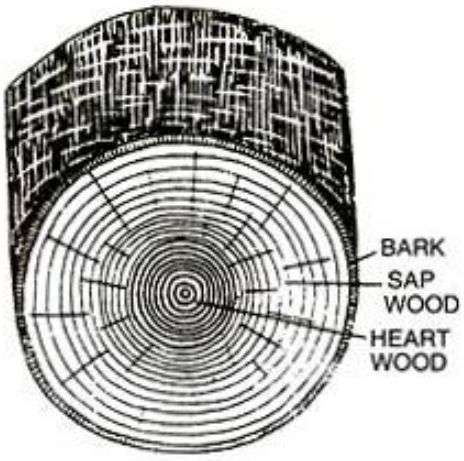


Fig. 6.33. Sapwood and heartwood in T.S. of trunk.

- In the old trees, where sufficient amount of secondary growth has taken place, the secondary wood of inner side lose the power of conduction.
- Their cells get filled with tannins, resins, gums, essential oils which makes the plant part hard and darker called the heart wood or duramen.
- The heart wood ceases the function of conducting tissue and simply provides mechanical support to the stem.
- However, the outer region of secondary wood, which consists of younger living xylem cells, remains yellow in colour called the sap wood or laburnum.
- It functions as the conducting tissue and also as the food storage tissue.

Secondary growth in extra stellar region due to activity of cork-cambium:

- The marked increase in diameter or thickness of stem brought about by the secondary thickening exerts a great pressure on the outer tissues.
- This results in the rupture of the cortex and epidermis, the outer cortical cells become meristematic and begins to divide. This is known as cork cambium or phellogen.
- The cork cambium divides to form secondary tissue on both the sides i.e. internal and external but its activity is more on the outer side than on the inner side.
- The cells formed on the outer side constitutes the phellem or cork and those on the inner side form secondary cortex or phelloderm.
- The phellogen, phellem and phelloderm together are called periderm.

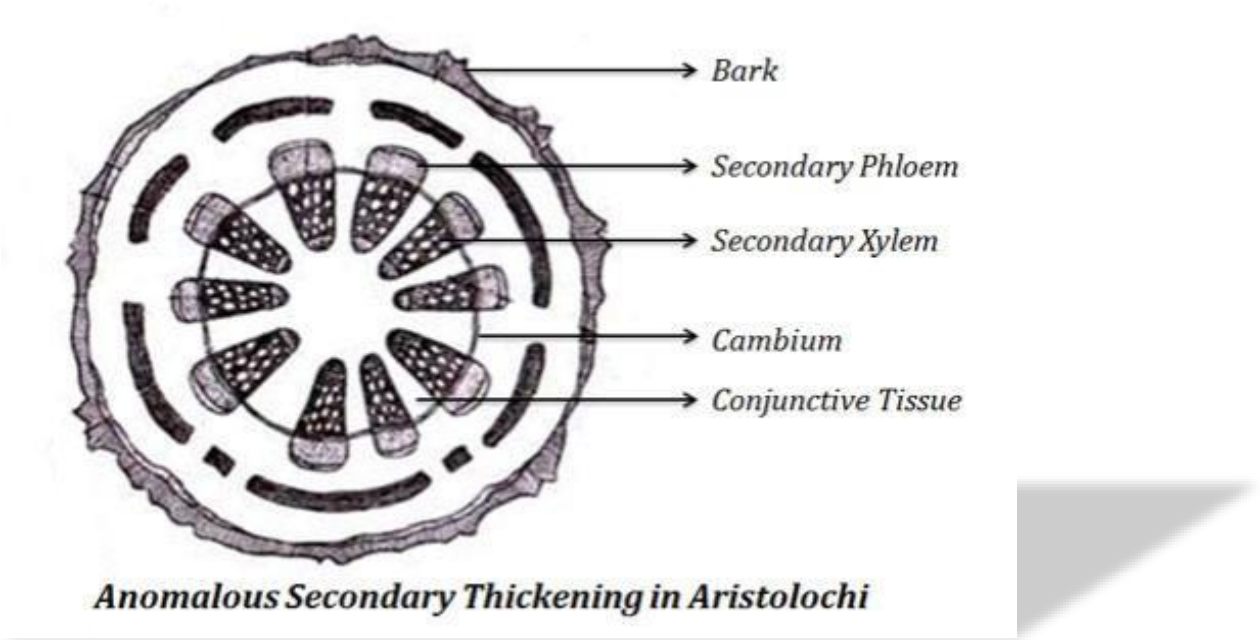
ANOMALOUS SECONDARY GROWTH IN DICOTS (ARISTOLOCHIACEAE)

Secondary Growth in Aristolochia Stem

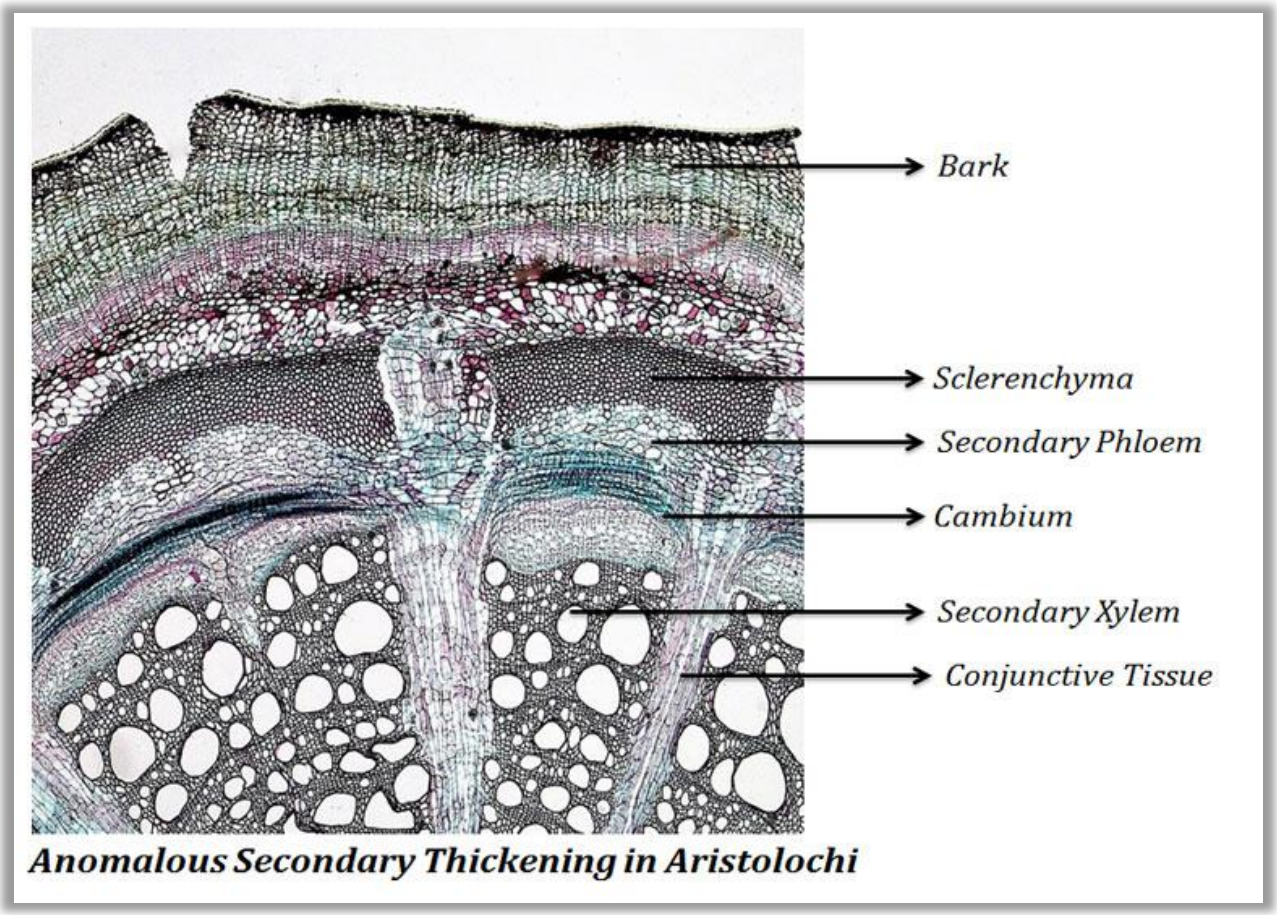
Has been stat Aristolochia that this stem which is a liane, differs from the normal ones in the process of secondary growth.

The most striking points of difference are the formation of only parenchymatous medullary rays by the interfascicular cambium, and consequent occurrence of secondary tissues in strands

ARISTOLOCHIA STEM T.S



Anomalous Secondary Thickening in Aristolochi



Anomalous Secondary Thickening in Aristolochi

In the primary condition the Aristolochia stem has the following structure. Epidermis is uniseriate with cuticularised outer walls. Cortex is differentiated into collenchymatous hypodermis, parenchymatous portion and the starch sheath.

Chloroplasts are present in both collenchyma and parenchyma cells. A continuous band of sclerenchyma with strongly thickened walls occur. They may be called perivascular fibres, or may be said to form pericycle together with the adjoining parenchyma cells.

Broad medullary rays com-posed of parenchyma cells occur between the vascular bundles. The central portion of the stem is occupied by a large parenchymatous pith. The vascular bundles remain arranged in a ring. The bundles are distinctly collateral and open ones, with xylemand phloem on the inner and outer sides and having a strip of cambium in between the two.

With commencement of secondary growth in thickness a few parenchymatous cells of the broad medullary rays become meristematic in a line with the fascicular cambium of the vascular bundles. The newly-formed meristem, a secondary meristem, is known as interfascicular cambium.

It joins up with the fascicular cambium and thus a continuous cambium ring is formed. The fascicular cambium, in fact, the cambial zone goes on dividing tangentially and produces secondary xylem and secondary phloem on the inner and outer sides respectively.

Thus the primary xylem and primary phloem are pushed apart from each other. The secondary xylem has the usual elements arranged in vertical and horizontal systems. Metaxylem elements are fairly large in size. Annual rings with early wood and late wood are formed as a result of seasonal activities of the cambium.

The secondary phloem pushes the primary one on the outer side, and the latter usually gets crushed due to the pressure. Bands of sieve

tubes and associated cells alter-nate with bands of parenchyma in the secondary phloem; fibres are absent. On the whole the vascular bundles increase enormously in size due to continued activity of the fasci-cular cambium.

The interfascicular cambium simply produces parenchyma cells on the outer and inner sides. Thus the medullary rays become increasingly more broad and long. The cells remain arranged in more or less radial rows.

The formation of the secondary tissues brings about profound changes in other portions of the stem. The central pith gets more and more reduced in extent. Distinct disruption in the continuous cylinder of sclerenchyma is caused by the increase, so that the band is ruptured here and there, commonly in front of the medullary rays.

The adjoining parenchyma cells fill up the gaps thus formed. These cells gradually undergo sclerosis and are ultimately transformed into sclereids. They, in fact, repair, so to say, the gaps caused by the onrush of the internal tissues.

The band of hypodermal collenchyma also suffers from pressure and breaks down frequently. The parenchyma cells of the cortex make their way into the breaks and thus occur as strips amongst hypodermal collenchyma.

The epidermis gets stretched and ruptures. Periderm develops in the subepidermal layers. Phellogen is formed in patches. They divide and produce a thick layer of cork cells on the outer side and considerable e on the inner. Lenticels are formed

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GAC.

ANAMALOUS SECONDARY GROWTH IN BIGNONIA

Bignonia is a member of Bignoniaceae family. The young stem shows wavy outline with prominent ridges and furrows. The most interesting anatomical feature in Bignonia is the occurrence of anomalous secondary structure. This is as follows -

Anomalous secondary thickening is due to the abnormal functioning of cambium. Cambium is normal in disposition and abnormal in function (adaptive type).

A) Presence of Phloem Wedges in the Xylem

The young stems which exhibits this type of structure when

Mature are provided with a normal ring of vascular bundles. The origin, position and

Function of cambium ring is normal in the initial stage of growth. Cambium function

Normally producing more secondary xylem towards inner side and less secondary

Phloem to the outer side. But after sometime, the cambium cut off different proportion of

Xylem and phloem in different points.

At four diagonal point arranged in cross shaped order,

Formation of secondary xylem is reduced and that of secondary phloem correspondingly

Increase, so that secondary phloem with transverse bands of bast fibre gets penetrated

Within the mass of xylem in the form of phloem pocket. Hence, the woody cylinder

Appears to have four longitudinal grooves which become increasingly deeper with

Secondary growth.

As a result of these four furrows at four equidistant points appear in the

Xylem, extending almost to the pith. The cambium breaks up into a number of strips,

Widest ones occurring opposite the four projecting ridges of wood and the narrow ones at

The base of the grooves. Cambium is not found on the radial surface. Peculiar structure

With ridged and furrowed xylem cylinder is formed (Anomalous structure in stelar

Region). The four radial groups of the phloem are united by medullary rays seen

Traversing the phloem of furrows

B) Presence of Fissured Xylem –

The fissured xylem may only be seen in fairly old

Stems. First of all wedges of phloem are formed and thereafter the xylem strand

Becomes fissured by dilation and cell division in wood parenchyma and pith

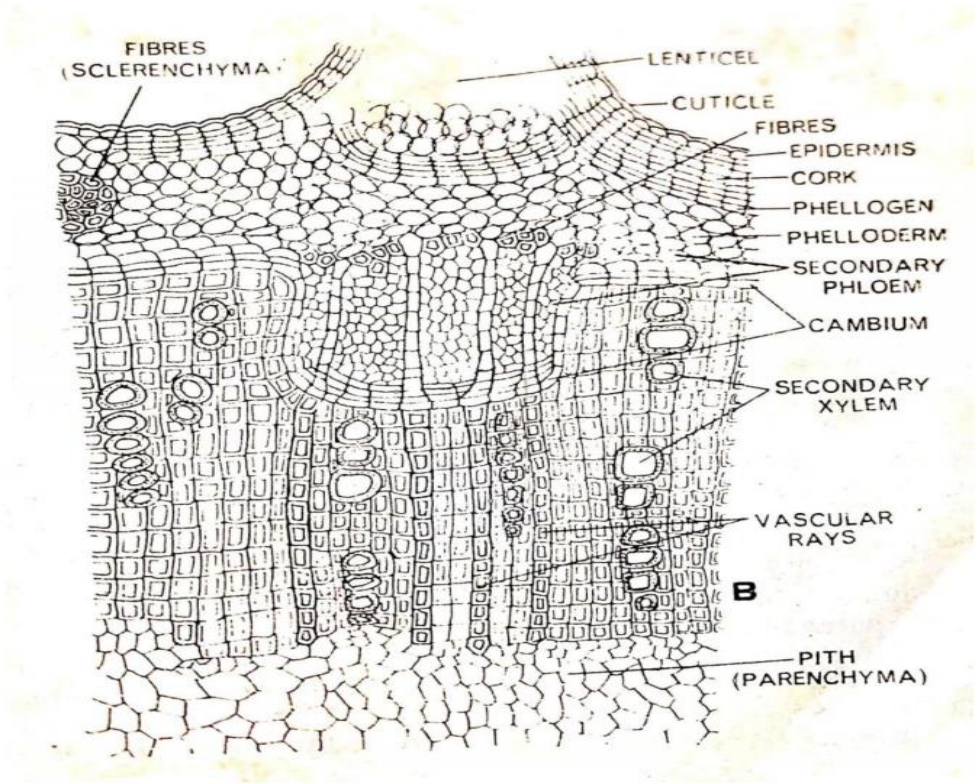
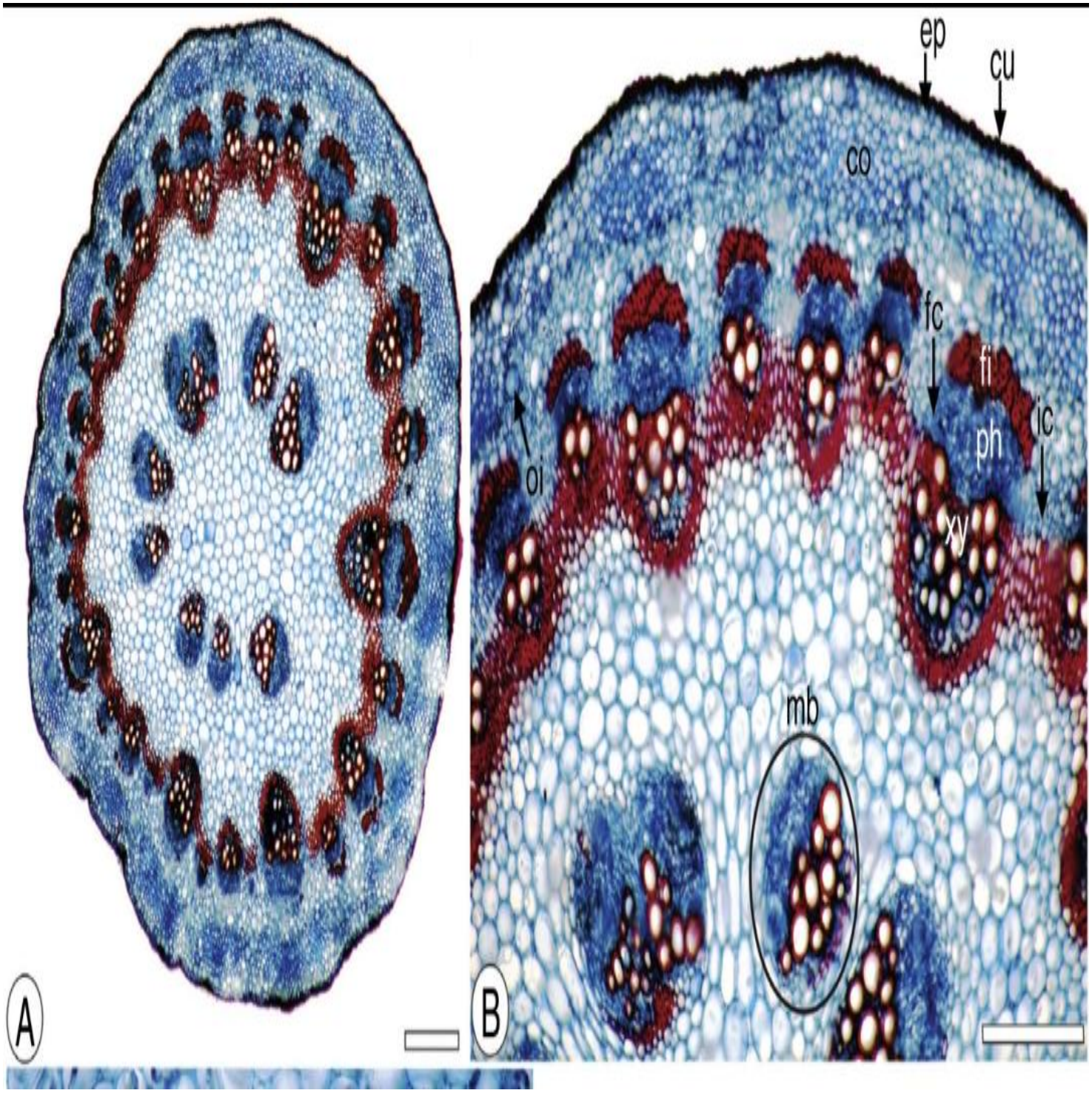


Fig. *Bignonia* Stem :- T.S. of Stem of *Bignonia* Showing An Anomalous Secondary Growth.

ANAMALOUS SECONDARY GROWTH IN PIPER

Though pepper (Piper nigrum) and Piper colubrinum are dicotyledons, their stem structure resembles that of monocotyledons. Their vascular bundles are scattered

T.S OF PIPER AMALAGO



P. colubrinum, as in many other species of Piper, exhibits a dimorphic branching pattern. The orthotropic shoots show monopodial:growth and the plagiotropic (flowering) branches sympodial growth. During the course of development, vegetative apical .buds of the latter modifY into spikes and vegetative growth is continued by axillary buds. The spikes therefore appear leaf opposed.

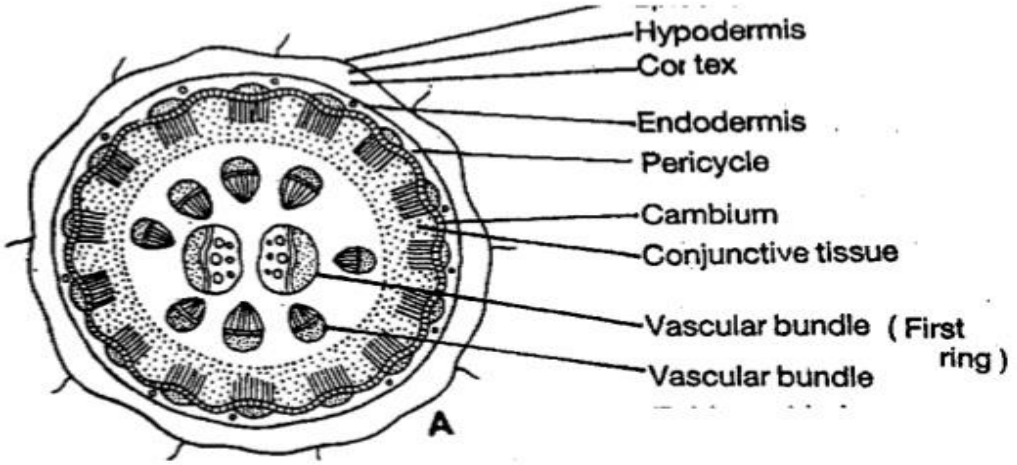
Vascular structure of P. colubrinum
Ravindran & Remashree
stem is basically similar to that of other Piper species. There are 5-10 medullary bundles and 39 to 49 peripheral bundles in a 4-5 mm thick stem. Secondary thickening is restricted to the peripheral vascular bundles (Fig. 1 A). The epidermis of both plagiotropic and orthotropic stems are single layered having a thick cuticle (Fig. 1 B).

The
cortex possesses three types of cells - chlorenchyma, collenchyma and parenchyma (Fig. 1 B). Mucilage canals are present in outer cortex while there is no central mucilage canal as in p, nigrwn (Fig. 1 C). The cortex possesses 15 to 17 layers of cells. Endodermis with casparian strips and peri cycle are distinct (Fig. 1 D). Vascular bundles are conjoint, collateral and open. In each peripheral vascular bundle, a continuous wavy band of 3-5 layered sclerenchymatous conjunctive tissue is observed and the lower part of xylem region gets merged with these layers (Fig. 1 C). The medullary bundles are large and 2 or 3 layers of sclerenchymatous sheaths are present on the

upper side of the bundles during maturation (Fig. 2 A). The vascular bundles in the peripheral ring consists of small and large bundles arranged alternately Phloem consists of sieve tube elements, companion cells, phloem parenchyma and phloem fibres (Fig. 2 B). Cambium is two to three layered and produces xylem towards inside and phloem outside (Figs. 1 C & 2 B). Xylem is highly lignified but the newly produced metaxylem elements are unlignifed (Fig. 1 C). Xylem consists of xylem vessels, tracheids and fibres, having pitted thickening and simple perforation plates. Tracheids are of scalariform and annular types. Libriform fibres are also present.

Anomalous Secondary Growth In Boerhaavia

Boerhaavia is a member of family, Nyctaginaceae.



TS in Boerhaavia stem

Herbaceous plant.

Boerhavia Stem

Transverse section through the young stem of Boerhaavia show following

Tissues : -

Epidermis –

1) Epidermis is single layered and consists of small, radially elongated

Parenchymatous cells.

2) Multi-cellular epidermal hairs arise from some cell.
3) A thick cuticle is present on the epidermis.
4) Some stomata are also present.

Cortex –

1) Cortex is well differentiated and consists of few layered collenchymatous

Hypodermis followed by parenchyma.

2) Collenchyma is 3-4 cells deep, but generally near stomata it is only one

Layered.

3) Parenchyma is present inner to collenchyma in the form of 3 to 7 layers.
4) Parenchymatous cells are thin walled, oval, full of chloroplast and enclose

Many intercellular spaces.

Endodermis is Clearly developed and made up of many tubular thick walled cells.

Pericycle – Inner to the endodermis is present parenchymatous pericycle but at some

Place it is represented by isolated patches of sclerenchyma.

Pith – Well developed , parenchymatous, present in the centre.Vascular System –

Vascular bundles are collateral, conjoint and open with endarch xylem and

Are arranged in three rings –

1) Two large centrally placed medullary vascular bundles.
2) A middle ring of 6 to 14 loosely arranged and medium sized vascular

Bundles.

3) The outer ring of 15 to 20 small vascular bundles just beneath the

Pericycle.

Anomalous Structure In Boerhaavia –

a) Primary Anomaly – Presence of two large central medullary vascular bundles

Encircled with a second ring of 6 to 14 loosely arranged vascular bundles lying in the

Ground tissue.

b) Non-adaptive type Anomaly – Normal indisposition of cambium with its unusual

Activity.

Anomalous Secondary Growth –

The stem of Boerhaavia contain well defined anomalous secondary

Growth which is characterized by the presence of successive rings of xylem and phloem

(vascular bundles).

After primary growth, the secondary growth is limited in the inner (Two medullary

Vascular bundles) and the middle ring of vascular bundles (6 to 14) by a fascicular

Cambium . As a result they slightly increase their size.

Two vascular bundles (medullary vascular bundles) of the inner most ring are Large,

Oval and lie opposite to each other with their xylem facing towards centre and phloem

Outwards.

In the stem , the secondary growth occurs by the activity of a complete cambium ring

Formed in the outer ring of the vascular bundles in the normal position. Outer most ring of

The vascular bundles contain inter-fascicular cambium which is absent in other two rings.

The outer ring consists of 15-20 vascular bundles, here the inter fascicular and intra

Fascicular cambium strips join together to form a continuous cambial ring. The cambial

Ring is functionally segmented into fascicular and intra fascicular region. This cambial

Ring produce –

Internally : - Secondary xylem in the intra fascicular region and lignified

Conjunctive tissue in the inter fascicular region on the inner side.Externally :- Secondary phloem in the intra fascicular region and parenchyma

From the inter fascicular region opposite the conjunctive tissue.

After the formation of secondary tissue the cambium ceases its activity and a new fresh

Cambial ring is arises by the joining of secondary parenchyma cells opposite to the

Conjunctive tissue and the cells of pericycle outside the phloem. This accessory cambial

Ring functions in a similar manner to the previous cambium. It produce secondary xylem

Alternating with lignified conjunctive tissue on the inner side and secondary phloem

Opposite to the secondary xylem and parenchyma above the conjunctive tissue.

Sometime, the activity of this cambium also cease.

One or more cambium gets differentiated which also functions similar manner.

The process, several such cambial rings may be formed. As a result of these successive

Cambial differentiation several concentric rings of vascular bundles get embedded .

Thick walled lignified conjunctive tissue separated by thin walled parenchymatous

Give the appearance of growth rings.

The cambium is composed of fusiform initials only which gives to ray-less secondary

Vascular tissue. Each successive ring of cambium is originated from the outer most

Phloem parenchyma cell. This Anomalous type of secondary growth thickness takes

Place by the means of successive ring of collateral vascular bundles.

ARBORORESCENT

MONOCOTS AND PRIMARY THICKENING

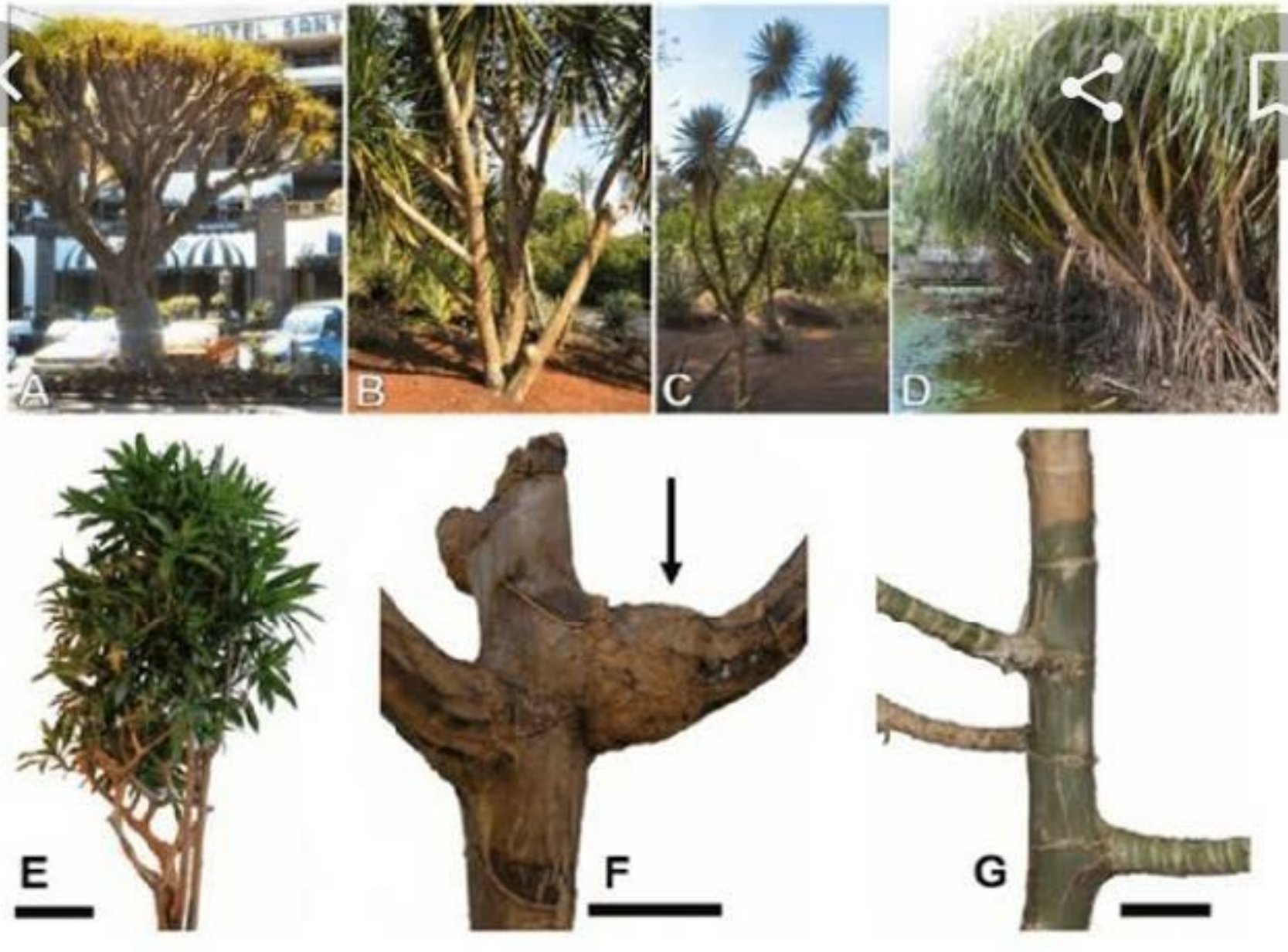
IN PLANT

ARBORORESCENT MONOCOTS

Some **monocots** have become **arborescent** (treelike) by growing upward and developing thick, fibrous trunks without conventional secondary growth. In most **arborescent monocots** (palms, screw pines), the thickening growth of the stems occurs at the growing tip, in what is known as the primary thickening meristem.

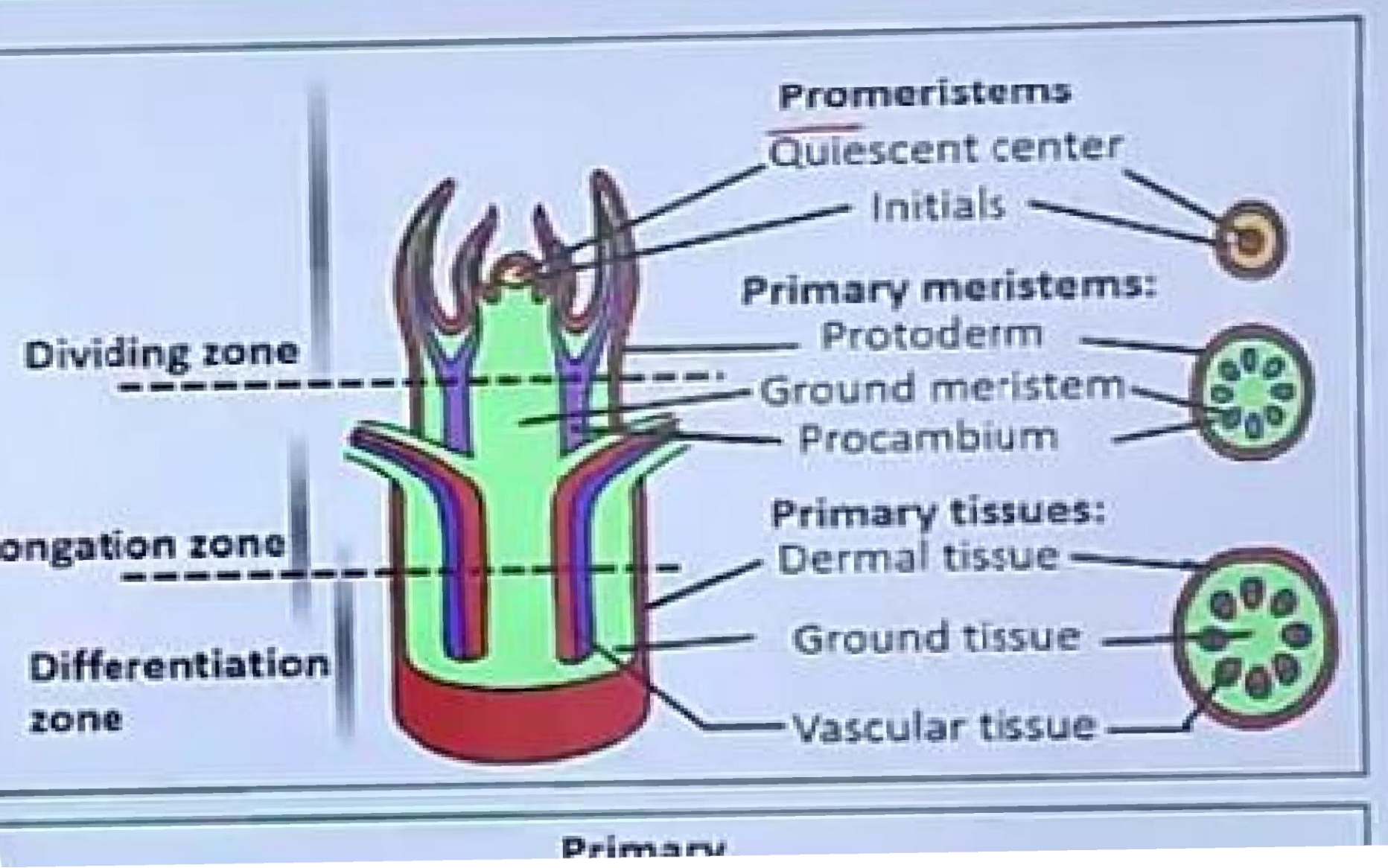
The Monocotyledones, or monocots, are a large and very distinctive class of angiosperms or flowering plants, phylum Anthophyta, consisting of some 133 families, 3,000 genera, and 65,000 species. Monocotyledones form one of the two major subdivisions of angiosperms, the other being the Eudicotyledones (eudicots), with about 165,000 species.

Typical monocots have a single cotyledon (seedling leaf), stems with scattered vascular bundles, root systems composed entirely of adventitious roots (arising directly from stem tissues), leaves with parallel venation and sheathing bases, and flower parts in threes. Monocots lack the ability to produce secondary growth (wood).

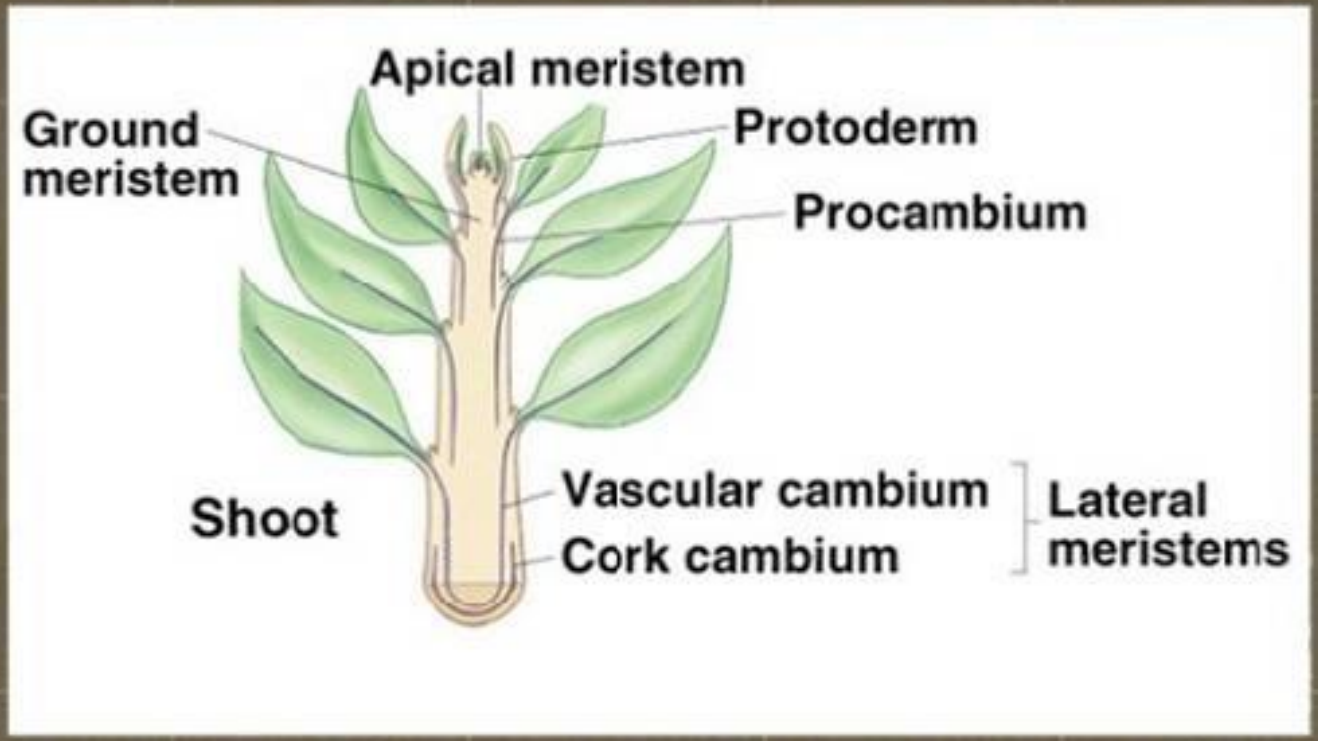

A
B
C
D
E
F
G

The **primary thickening** meristem is responsible for **primary thickening** of a stem axis. ... The list ofplants in which the PTM has been described supports the generalization that monocotyledonous species which are rosette **plants**, i.e., which have wide stems with very short internodes and crowded leaves, have a PTM.

- Appear early in life of plant.
- Derived from embryonic meristem or promeristem.
- Contribute to formation of primary plant body and help in growth in length (except intrafascicular cambium).
- They persist throughout life.
- They show their presence at apices of root and stem and primordia of leaf. Examples: Protoderm(form outer covering of plant), procambium (form vascular tissue) and ground meristem.


Promeristems
Quiescent center
Initials
Primary meristems:
Protoderm
Ground meristem
Procambium
Primary tissues:
Dermal tissue
Ground tissue
Vascular tissue
Dividing zone
ongation zone
Differentiation zone

Meristems


Apical meristem
Ground meristem
Protoderm
Procambium
Shoot
Vascular cambium
Cork cambium
Lateral meristems

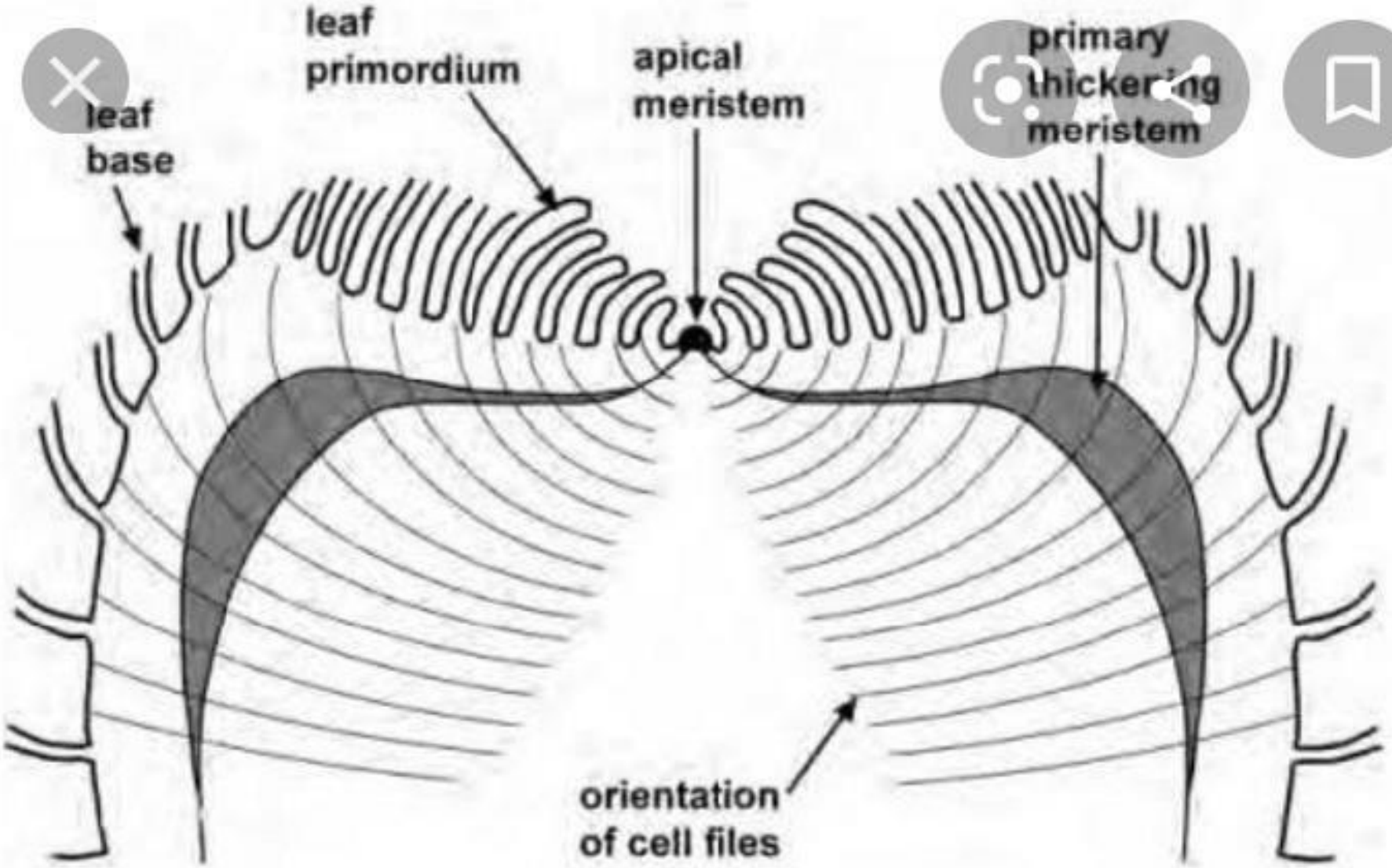
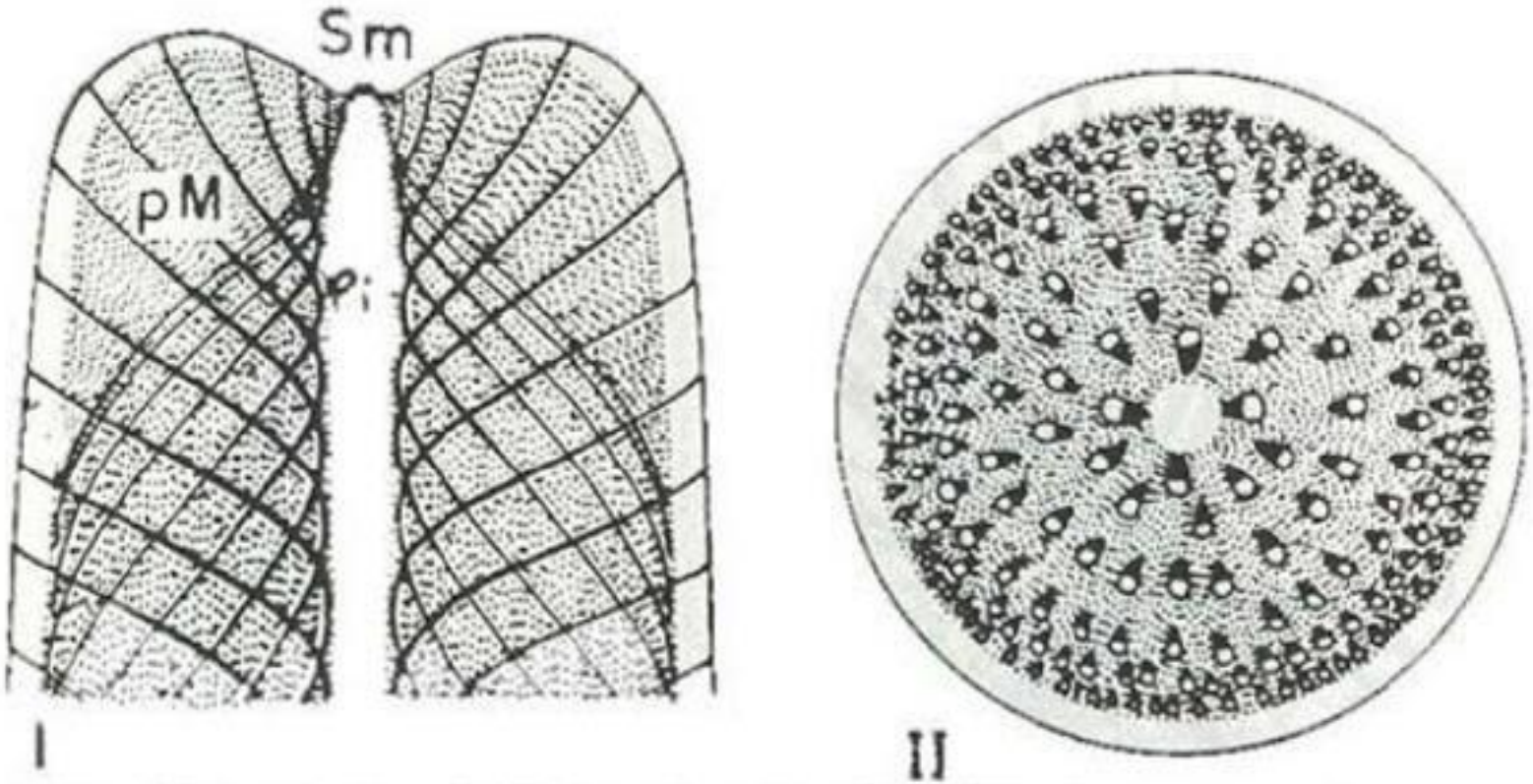


Figure 2.13 Primary thickening meristem (PTM): diagram of longitudinal section of the crown of a typical thick-stemmed monocot, showing orientation and extent of radial PTM derivatives. Vascular strands not shown. (Adapted from DeMason 1983).

Primary Thickening Meristem



I. After thickening, longitudinal section. II. After thickening, cross section.
Sm = shoot meristem, Pi = pith, C = cortex, pM = primary meristem.

Nodal Anatomy of Angiosperms: Unilacunar, Trilacunar and Multilacunar Node with Examples

Anatomy of Nodal and Inter-nodal Region are Different:

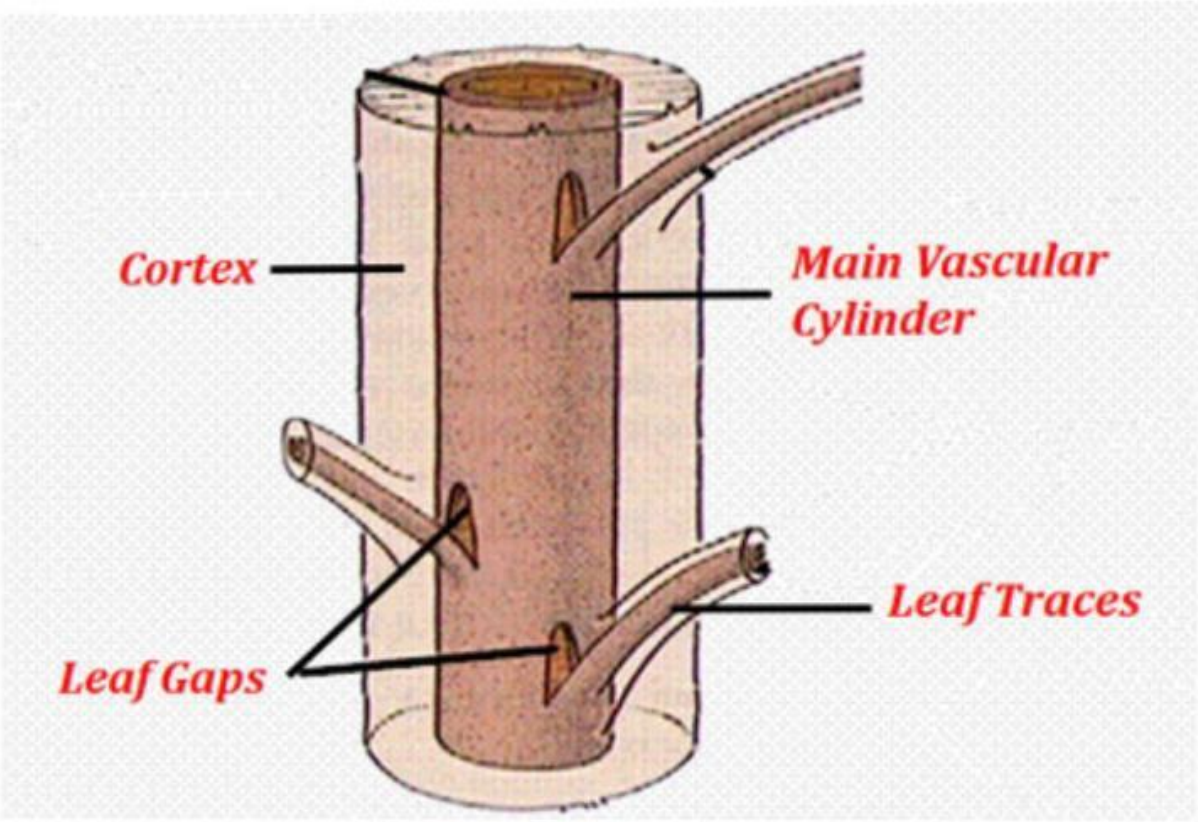
- The stem of plants is differentiated into nodes and internodes. The anatomical features of the nodal region are quite different from that of the inter-nodal region.
- This anatomical difference is due to the presence of **Vascular Supply** to the leaves and branches from the main vascular cylinder of the stem.

Nodal Region of Higher Plants Posses Leaf Gaps and Leaf Traces

- Each leaf that originates from the node, of higher plants possesses vascular tissue and these vascular tissues of the leaves are connected to that of the stem.
- A vascular strand that extends between the vascular cylinder of stem and the base of the leaf is called **Leaf Trace** or **Foliar Trace**.

- Even if the leaf trace possesses both xylem and phloem, the relative amount of xylem will be more in the leaf trace than phloem.
- Moreover, the proximal portion, (portion near the vascular cylinder of the stem) contains only the xylem.

- Whereas, the distal end of the leaf trace (near to the leaf base) contains both xylem and phloem. Leaf trace helps to transport water and minerals from the xylem to the leaf lamina for photosynthesis.
- The circulation of photosynthetic products from the leaf lamina to the phloem of the stem is also facilitated by the phloem strands in the leaf traces.



Leaf Gaps and Leaf Traces

- Even though the leaf trace is an extension of the vascular cylinder to the leaf, they are not a continuous supply from the main vascular cylinder. In the vascular cylinder just above the point of departure of the leaf trace, a small parenchymatous patch occurs. This parenchymatous region between the main vascular cylinder and the leaf trace is called **Leaf Gap** or **Lacuna**.

- *The leaf gap is situated on the upper side of the leaf trace. In a cross-section through the leaf gap region, it is visualized as a zone of parenchyma inside the vascular cylinder. Due to the presence of leaf gap, the cortical parenchyma becomes continuous with that of pith during the early stages of secondary growth.*

Nodes are classified on the basis of Number of Leaf Gaps and Leaf Traces

The number and nature of leaf gaps and leaf traces varies in different plants. On the basis of the number of leaf gaps and leaf traces,

THREE main nodes are described in Angiosperms.

1). Unilacunar

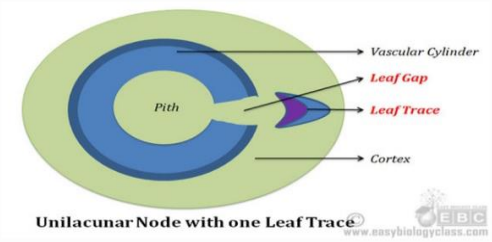
(2). Trilacun(3). Multilacunar

(1). Unilacunar Node:

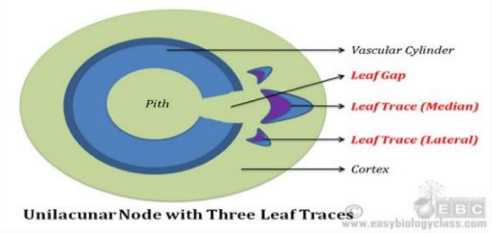
A unilacunar node possesses only a single leaf gap to a leaf. Each leaf may possess one or two or three leaf traces.

Example: *Nerium*, *Lantana camara*, *Justicia* (with one gap and one trace); *Clerodendron splendens* (two traces); *Chenopodium album* and *Withania somnifera* (with one gap and three traces).

2). Trilacunar Node:



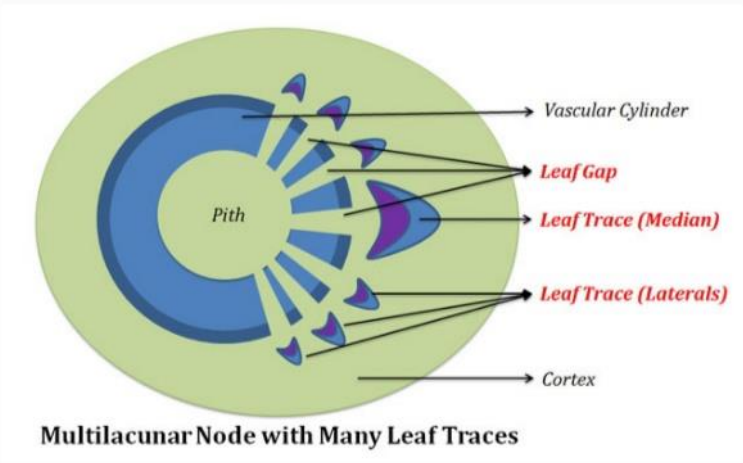
Unilacunar Node with one Leaf Trace



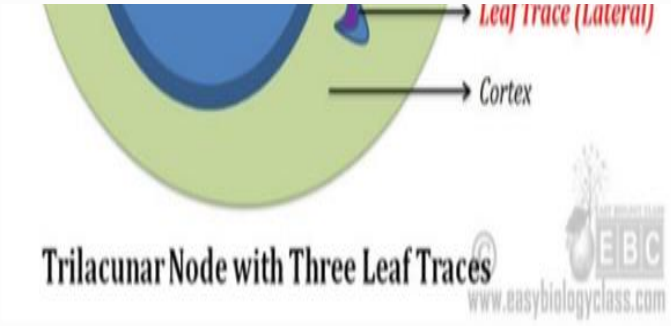
Unilacunar Node with Three Leaf Traces

A trilacunar node possesses three leaf gaps and three leaf traces. Among these three leaf traces, the middle one (median) will be larger and the other two laterals will be smaller.

Example: **Azadirachta.**



Multilacunar Node with Many Leaf Traces



Trilacunar Node with Three Leaf Traces

(3).Multilacunar Node:

A multilacunar node possesses many leaf gaps and many leaf traces. Among the many traces, the median one (middle) will be larger and all others will be smaller.

Example: **Polygonum plebeium, Coriandrum sativum, Aralium**

Nodal Anatomy shows the Phylogenetic Primitiveness or Advancements

A unilacular node with two traces is considered to be the most primitive type of node among Angiosperms. One trace unilacunar node and trilacunar node is considered to be evolved from a trilacunar condition. During the evolution of nodal region, fusion, deletion and additions of leaf traces have occurred in the remote past. Apart from the phylogenetic

significance, the nodal anatomy is a good taxonomic character used in the systematics of higher plants.

Kranz Anatomy:

The word Kranz means “wreath” or “ring”. Kranz anatomy is a specialized structure in C_4 Plants where the mesophyll cells are clustered around the bundle-sheath cells in a ring-like fashion. The number of chloroplasts in the bundle-sheath cells is more than that in the mesophyll cells. This is found in C_4 grasses such as maize and a few dicots. The Kranz anatomy is developed in three different steps:

- Initiation of procambium
- Bundle sheath and mesophyll cell specification
- Chloroplast development and integration of the C_4 cycle

Also read: Photosynthesis in Higher Plants

Kranz Anatomy in C_4 Plants

The light-dependent reactions and the Calvin cycle are separated in the C_4 plants. The Calvin cycle occurs in the bundle-sheath cells and the light-dependent reactions occur in the mesophyll cells.

The atmospheric oxygen is fixed first to form 4-carbon compound oxaloacetate in the mesophyll cells, catalyzed by PEP carboxylase.

Oxaloacetate is converted to malate which is transported to the bundle-sheath cells.

Malate dissociates in the bundle-sheath cells to release carbon dioxide.

Rubisco fixes the carbon dioxide and converts it into sugars.

Carbon dioxide is constantly pumped into the bundle sheath cells by the mesophyll cells, the carbon dioxide concentration around Rubisco is always higher. This reduces photorespiration.

In the majority of plants, carbon dioxide is fixed into a 3 carbon compound by the action of Ribulose bisphosphate carboxylase oxygenase (Rubisco). Rubisco can also catalyze a reaction with oxygen giving a wasteful process known as photorespiration. To overcome this, the C4 pathway fixes atmospheric carbon dioxide using the enzyme phosphoenolpyruvate carboxylase. Carbon dioxide is then released for refixation by Rubisco.

In C4 grasses such as maize, the mesophyll cells surround the bundle sheath cells, and the bundle sheath cells surround the veins.

Also read: C_3 and C_4 Pathway

Differences Between Mesophyll Cells and Bundle-Sheath Cells

Mesophyll Cells	Bundle-Sheath cells
Many well-developed and large grana are present.	Grana are very small and poorly developed or might be absent.
RuBP carboxylase is absent. No C3 cycle occurs.	RuBP carboxylase is present in high concentration. C3 cycle occurs.
High activity of photosystem II.	Low activity of photosystem II.
No starch grains present.	A lot of large starch grains present.
Key enzymes for starch synthesis are absent.	Key enzymes for starch synthesis are present.

Define Kranz anatomy:

Kranz anatomy is a special structure in the leaves of C4 plants where the tissue equivalent to spongy mesophyll cells is clustered in a ring around the leaf veins outside the bundle sheath cells.

plants exhibit Kranz anatomy:

Kranz anatomy is exhibited by C4 plants. They consist of two photosynthetic cell types. These include bundle sheath cells that surround the vascular bundles and the mesophyll cells that surround the bundle sheath cells.

The function of vascular bundles:

Vascular bundles are a part of the transport system in vascular plants. The vascular tissues exist in two forms, xylem and phloem, which facilitate the transport of water and minerals. Both these tissues are present in vascular bundles.

C3 plants have Kranz anatomy:

No C3 plants do not have Kranz anatomy. In these plants, the bundle sheath cells do contain chloroplast and carbon dioxide fixation occurs only once.

The difference between C3 and C4 plants:

The main differences between the C3 and C4 plants are that the bundle sheath cells of C3 plants do not contain chloroplast whereas the bundle sheath cells of C4 plants do. Carbon dioxide fixation in C3 plants takes place only once, whereas that in C4 plants takes twice.

MICROTECHNIQUE

KILLING AND FIXATION

- Killing is the sudden stopping of all living process in all the cells of a collected biological specimen such as a piece of stem, leaf, flower bud etc.
- Killing agent is the chemical reagent used for killing the plant specimens.
- Fixation is the preservation of all structural and cellular elements in a biological specimen in as near their original state as possible.
- Fixing agent is the chemical reagent used for fixing the plant specimens.
- Usually the killing and fixing are done by a single fluid called Fixative.
- The 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 preserves the cell structures the best”

KILLING AND FIXATION

DEHYDRATION

- Water is present in the cells. Water from the cells should be removed for the permanent slide preparations, because:-
- $. Water will decay the specimen.
- $. Water is not miscible with paraffin wax used for embedding.
- $. Water will not mix with the usual media of staining.
- $. What is not miscible with permanent mountant.

DEHYDRATION

- **Dehydration**: “The process of removal of water from biological samples before impregnation or final mounting is called dehydration”
- Ø **Dehydrant**: The reagent used for dehydration is called dehydrant.
- **Process of dehydration:**
- Ø Dehydration of the biological specimens cannot be done quickly, it should be done gradually.
- Ø The dehydration is done by treating the material with a series of solutions containing progressively decreasing concentrations of water and a progressively increasing concentration of dehydrant.
- Ø Specimens are kept in the dehydrant for specific time interval.
- Ø The time interval is determined by the size and nature of the specimen.
- Ø Long interval in low concentration makes tissue soft.
- Ø Long interval in high concentration makes tissue brittle.
- Ø Dehydrating reagents are changed by decanting from the material.
- Ø After decanting, specimens are immediately filled with next grade fluid.
- Ø Materials should not be dried at any stage.

CLEARING OR REHDRATION

CLEARING OR REHDRATION

- **Reagents used in clearing**
- Commonly used reagents in the clearing are:

- 1. Xylene (xylol)
- 2. Trichloroethylene
- 3. Chloroform
- 4. Benzene

STAINING

- **Double Staining:**
- Double staining is resorted to in case of all sections having both lignified and non-lignified tissues, i.e., in case of pteridophytes, gymnosperms and angiosperms. One of the two stains is specific for lignified tissues and the other stains the non-lignified tissues, i.e., mainly cellulose. The sections with two different stains show more contrast. There are numerous double staining schedules.

DOUBLE STAINING

- **Safranin and Fast Green Staining:**
- 1. Keep the sections in 30% alcohol for 5 minutes.
- 2. Stain in a 1% solution of safranin in methyl cello solve 50% alcohol for 2 to 24 even 48 hours (Prepare safranin solution by dissolving 4 g of safranin in 100 ml each of 95% alcohol and distilled water followed by 4 g of sodium acetate and 8 ml of formalin The acetate intensifies the stain and formalin acts as a mordant.).

hours, or • 3. Wash off the excess stain with running water for a few moments.

.

- 4. Differentiate and dehydrate with 95% alcohol to which 0.5% picric acid has been added. Ordi-narily, about 10 seconds of treatment is sufficient for differentiation.
- 5. Stop action of the acid by immersing the slide in 95% alcohol to which 4 to 5 drops of ammonia per 100 ml of alcohol has been added. This treatment should not be continued for more than 2 minutes, as alcohol extracts stain
- 6. Dehydrate in absolute alcohol for 10 seconds.

DOUBLE STAINING

- 7. Counterstain in fast green for not more than 15 seconds (Prepare a nearly saturated solution of fast green in equal parts of methyl cello solve and absolute alcohol and 75 parts clove oil.). This stain can be used repeatedly.
- 8. Pour the fast green stain back into dropping bottle and rinse off the excess stain with clove oil. Used clove oil may be used.
- 9. Clear in a mixture of 50 parts clove oil, 25 parts absolute alcohol and 25 parts xylol — for a few seconds (Actually this reagent mixture, after use, is collected in a bottle and used in step 8.).
- 10. Remove the clearing mixture by treating for a few seconds in xylol (Add 3 to 4 drops of absolute alcohol to this xylol to take care of any moisture that may be inadvertently brought over.).
- 11. Give two changes in pure xylol for at least 10 minutes' interval and then mount in Canada balsam.
- The safranin appears a brilliant red in chromosomes, nuclei and ii} lignified and cutinized cell walls; while the fast green gives a bright green colour to cellulose cell walls and cytoplasm. Both the colours are permanent and they persist for many years.

PREPARATION OF PARAFFIN WAX BLOCK

- Preparation of Paraffin Block | Plant Microtechnique
- Preparation of paraffin block.
- Transfer the tube or tubes containing the material to a high temperature paraffin bath (60°C), along with some paraffin of suitable melting point taken in a beaker.
- Suitable melting point refers to the melting point of paraffin which will give ideal ribbons of sections (see section cutting). This has to be learnt by experience. In India, 56-58°C paraffin is suitable for summer and 52-53°C paraffin for winter. In other seasons, a mixture of the two proves convenien
- When paraffin of both the tube and the beaker melts, pour off the supernatant paraffin of the tube and add some fresh paraffin from the beaker. Repeat the process 2-3 times at ½hr intervals till the smell of chloroform is completely removed.
- Prepare a paper-tray as shown in Fig. 3.2. Place it on a rectangular glass plate. By using a glass plate the lower surface of the block is made smooth; it also makes the removal of the block easier. Keep a burner nearby and make a needle and a scalpel sufficiently hot. Pour some paraffin from the beaker into the paper tray.

PREPARATION OF PARAFFIN WAX BLOCK

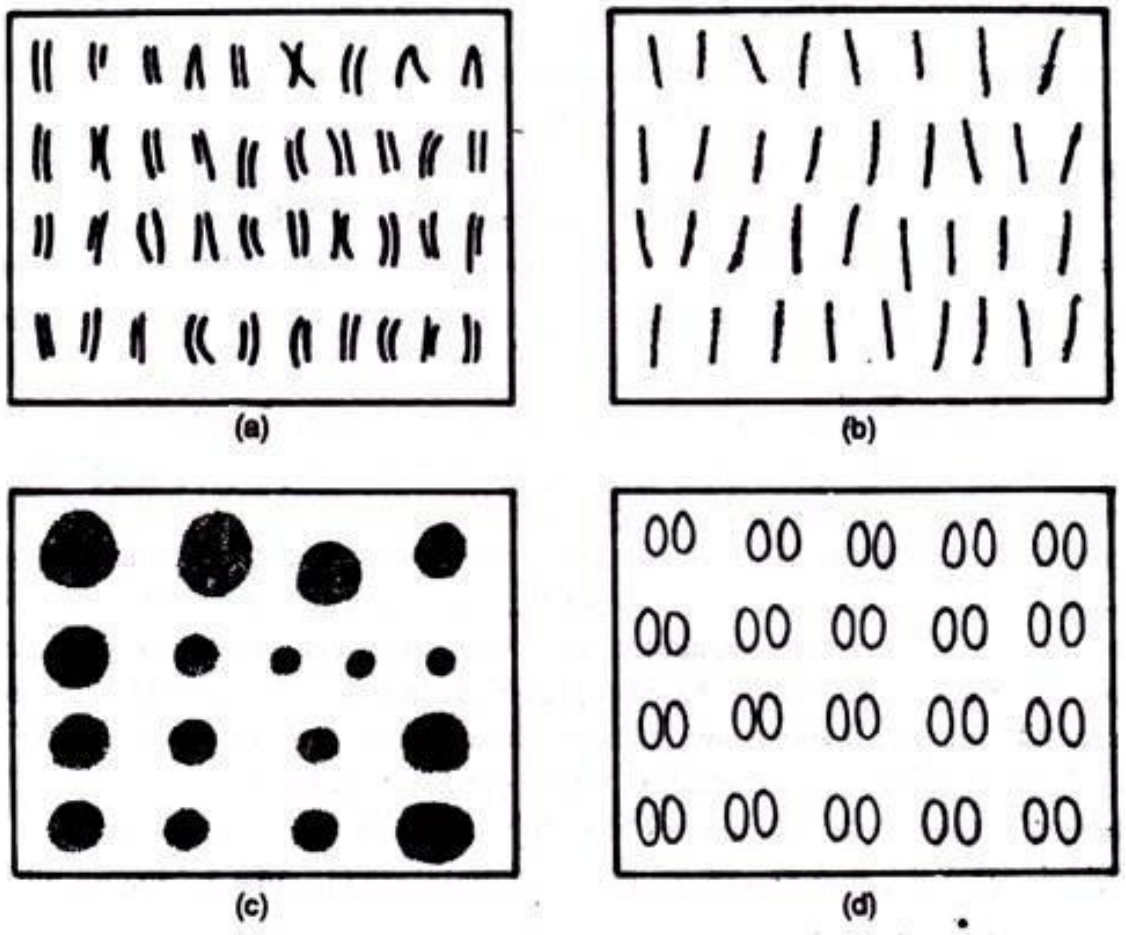


Fig. 3.3 : Arrangement of materials in paraffin blocks — (a) Roots in pairs, (b) Single roots, (c) Flower buds in clusters, (d) Flower buds in pairs

PREPARATION OF PARAFFIN WAX BLOCK

- The depth of paraffin need not be more than 1 cm. Take out the tube, stir it well and pour down the contents into the paper tray in one action so that all the buds or root-tips fall into it. Take the needle and the scalpel and with these arrange the buds or root-tips in straight lines as shown in Fig. 3.3. The materials are arranged in groups of 2 or 3. In case of root-tips, care must be taken to keep the tips at the same level.
- Preparation of good blocks requires experience. The whole operation has to be completed very swiftly, as the upper layer of paraffin solidifies quickly in contact with cool air. To prevent this the hot scalpel is to be regularly rotated in the paper tray.
- When paraffin starts solidifying around the needle and the scalpel, they are again heated. If all the material cannot be arranged in line,

MOUNTING MEDIA

What are mounting media?

Mounting medium is the medium that your sample is in while it is being imaged on the microscope. The simplest type of mounting medium is air, or a saline-based buffered solution, such as PBS. Because most people use the term mounting medium when referring to fixed-cell imaging performed with immunofluorescence labeling, during live-cell imaging, the term imaging medium is more often used to refer to the medium that samples are in while they are being imaged.

There are several reasons to place your fixed-cell sample in a mounting medium while you image:

To help hold a specimen in place while you are imaging

To prevent your sample from drying out

To more closely match the refractive index for the objective you will use

To prevent photobleaching

To preserve your sample over time for long-term storage

The choice of mounting medium is largely dependent on your sample type, how you will image, and which fluorophore or fluorescent proteins you use. There is a wide variety of mounting media to choose from, whether you buy commercially available versions or want to “brew” your own, and they can differ widely in composition. Some are based on organic solvents such as toluene or xylene, others are water-based or aqueous mounting media.

MOUNTING MEDIA

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- **Some types of mounting media can help protect and preserve samples**
- Formulations of mounting media that can add favorable properties such as optimizing the refractive index to match that of glass, preventing photobleaching, or preserving samples for long-term storage are widely available. Keep in mind that some require time to “cure” or harden. For mounting media that need time to cure, it’s important to let the sample fully harden before imaging so that you don’t inadvertently damage or destroy your sample by moving it around on the slide or cause photobleaching of your stained cells. Typically, the refractive index of the mounting medium will not reach its specified value until after it has fully cured, and its photoprotective properties will increase during the curing process. The degree of hardness after the mounting medium has fully cured will also vary; some will set like jelly while others will set like hard plastic.