PRACTICAL

DYE is one kind of coloured material which consists of two kinds of active chemical groups **1) *chromatophore***(part of colour of it's own) and 2) ***auxochromatic*** groups (part by which the dye combines with the tissue). The dye is used to impart stain. Most of the cellular compounds are colourless. In order to render them visible under microscope they must be suitably stained. If the *auxochrome* forms a salt with a base the **dye is acidic**. On the other hand If the *auxochrome* forms a salt with an acid the **dye is basic.** The section of a stain depends upon (i) the nature of the material, (ii) the type of P_H of the fixative used and (iii) the chemical reactivity of the stain.

Stains are of two types -:

Acid dyes - Acid dyes have a greater combining capacity at low P_H than basic dyes and is used for staining the cytoplasm (particularly proteins). The colour is carried by the anions, its net charge is (-) negative. Some acid dyes are- methyl orange, light green, methyl blue , acid fuchsin, eosin, etc.

Basic dyes - Basic dyes have a greater combining capacity at high P_H and are used for staining nucleus, chromosome, xylem tissue, cuticularised epidermis etc. In basic dye the colour radical is the cation, it has positive (+) charge.

Section cutting-:

T.S. and L.S. give us an idea of tissue distribution inside the plant body of the supplied portion of the plant.

Two main types of staining i.e. (1)Temporary (2) Permanent are used to study the T.S. and L.S. of supplied portion of plant.

2) **Permanent stain** - It is always accompanied by complete dehydration and clearing followed by mounting in Canada balsam or euparol etc.

Depending upon the number of stains used it is called (a) **Single staining,** (b) **double staining**.

a) **Single staining** - It is generally done in case of thallophytes and bryophytes, where lignified tissue is not present and the cell wall material is mostly composed of cellulose. Stains used are - Light greenand Bismark brown.

b**) Double staining** – it is used in case of sections having both lignified and non-lignified tissues, i.e. Pteridophytes, Gymnosperms and Angiosperms. Lignified wall turns red and cellular wall turns green. The sections with two different stains show most contrast. There are many double stains present, some of them are:-

1) Safranin and Delafield's hematoxylin stain
2) Safranin and fast green
3) Safranin and light green

One of the double staining procedure i.e. Safranin and light green staining schedule is given below-

Alcohol grades and stain used	**Period of treatment**
30% alcohol	For 5 minutes.
50% alcohol	For 5 minutes.
70%Alcohol	For 5 minutes.
2% Safranin	For 15 minutes.
De-stain with 70%Alcohol	
80 %alcohol	For 5 minutes.
90%alcohol	For 5 minutes.
Light green	1-2 min
De-stain with 90%Alcohol	
100% Alcohol	For 5 minutes.

Mounting - After staining the section is mounted on a glass slide in Canada Balsam and observed under microscope.

Precaution to be taken during staining:

1) Take a few drops of the required reagent in a watch glass and after transferring the section into it always keep it covered by another watch glass.
2) During transfer of the section from one reagent to another, always use scalpel. Brush should not be used as it transfers the reagent also. Needle should not be used for transfer as it may prick the section and damage it.
3) Fan should be switched off during staining in order to reduce evaporation of alcohol used.
4) Watch glass should be placed on a piece of white paper while transferring the sections as they can be easily located against a white background.
5) While mounting the section on Canada balsam, the cover glass should be dipped in xylol and placed over the section on the glass slide gradually with the help of a needle.
6) If the Canada balsam appears to be too thick, it should be diluted with little xylol.
7) After mounting, the section should be kept on a hot plate (37^oC-45^oC) for at least 24 hours as it helps to drive away air bubbles and dries the Canada balsam.

Different types of stains

Natural dye –

Hematoxylin – This stain is obtained from the hard wood of the plant Hematoxylin Campechianum. The dye solution itself has no affinity for tissues, unless iron or aluminium is present as a mordant. The colour effect of hematoxylin depends upon the pH of the solvent. In acidic medium colour is red whereas in alkaline medium it is blue.

Coal tar dye - (1) Azo dyes, e.g. Bismark brown
(2) Quinone amide group, e.g. Azine
(3) Phenyl methane group, e.g. Light Green (diamino triphenylmethane), Crystal violet (Triamino triphenylmethane)

Light green - It is a coal tar dye, acidic in nature and belongs to diamino triphenyl group. Dissolve 1 gm. light green in 25 ml absolute alcohol and add 75 ml clove oil. Let it stand for several hours, stirring frequently, then filter and store. The dye dissolves in 90% or 95% alcohol. It is a good stain for non lignified tissues i.e. cellulose wall, cytoplasm etc.

Safranin - It is a coal tar dye, basic in nature and belongs to the azin group. It's solubility is better in alcohol than in water. Safranin solution is made by dissolving safranin (1 gm) in 50% ethyl alcohol (99ml). It is used for permanent staining. It stains lignified suberized, cutinized and chitinized structures.

Bismark brown - This is a synthetic dye obtained from coal tar and belongs to the azin groups. It is a basic dye. The solution is generally made by dissolving Bismark (1 gm) in 70% ethyl alcohol (99ml).
It is generally used for staining relatively soft plant tissues without lignin such as bryophtes. On applying the stain, cellulose walls appear bright brown in colour.

Eosin -It is available both in blueish & yellowish form. It is coal tar dye, acidic in nature. It is used for staining cytoplasam.

Bignonia (Bignoniaceae)

Supplied material - woody, brown in colour, more or less squarish. T.S. through internode of a mature stem is taken, stained suitably (using double stain) and observed under microscope.

Study of anatomical features -

Epidermis - Uniseriate , thickly cuticularised, made up of compact cells having multicellular hairs.

Cortex- A few layered cortex follows epidermis. It is parenchymatous with many inter- cellular space.

Perivascular region - There is scattered patches of sclerenchymatous cells, particularly under ridges of the stem

Endodermis - A distinct endodermis with casparian strips is absent, instead, a parenchymatous layer is present. This layer is bounded by starch sheath layer.

Stele –It is the central region having following parts

Pericycle - It is heterogenous**.** It occurs just below the starch sheath layer in the form of few patches of sclerenchyma with intervening parenchyma cells. These patches are of different size and form almost discontinuous ring. Sclerenchyma patches are present particularly under the ridges of the stem.

Vascular tissue system -

1) Primary vascular bundles are initially arranged in a ring around a prominent pith.
2) Primary vascular bundles are conjoint, collateral, endarch and open, forming a continuous cambium ring by union of fascicular and interfascicular cambium
3) This cambium ring behaves normally in the beginning. Later it cuts off different proportion of xylem and phloem at four alternating points arranged in form of a cross. At four diagonal regions lesser amount of xylem are cut off externally.
4) These phloem masses intrudes inward forming four deep wedges of phloem in the xylem.
5) At the rest of the four patches the cambium ring cuts off greater amount of secondary xylem towards the center and lesser amount of secondary phloem outwards. So the cambium ring breaks up into number of strips.
6) Secondary xylem is notched at four places due to phloem wedges.
7) The phloem in furrows are supported by 1-3 transverse bands of phloem fibres, and transversed by narrow medullary rays.

Pith – It is conspicuous and made up of thin walled parenchymatous cells with distinct intercellular space.

Identifying characters -.
1) Multicellular epidermal hairs present.
2) Presence of starch sheath layer in inner cortex.
3) Ground tissue differentiated into intra-stelar and extra-stelar region.
4) Vascular bundles are conjoint, collateral, endarch and open, forming a continuous a cambium ring by union of fascicular and inter-fascicular cambium.
5) Pericycle is heterogenous**.** It occurs just below the starch sheath layer in the form of few patches of sclerenchyma with intervening parenchyma cells.
6) Pith is present and conspicuous.
So It is a dicot stem.
1)T. S. of mature stem is squarish
2) Cork with lenticels present.
3) There is scattered patches of sclerenchymatous cells, particularly under ridges of the stem
4) Secondary growth - It cuts off different proportions of xylem and phloem at four alternating points arranged in form of a cross. In one set of alternating points the cambium produces less amount of secondary xylem and much amount of secondary phloem, and vice versa in other set of alternating points. As a result the woody cylinder appears to have four longitudinal grooves alternating with four ridges. The ridges are wider than the grooves. As a result a peculiar structure with ridged and furrowed xylem cylinder is formed.

Hence, it shows the amomalous secondary growth in dicot stem where cambium is normal in position but shows abnormal activity.

Comment -
1)Cambium is normal in position but shows abnormal activity. Ridged and furrowed xylem cylinder is formed due to abnormal activity of cambium. At four places cambium produces greater amount of phloem on it's outer side than the amount of secondary xylem on it's inner side. This results in the formation of four deep wedges of secondary phloem which projects into the secondary xylem. This is a kind of adaptive anomaly.

As a result, the stem is twining and thus is inextensible as it hangs freely and has to bear its own weight due to unequal growth in thickness.

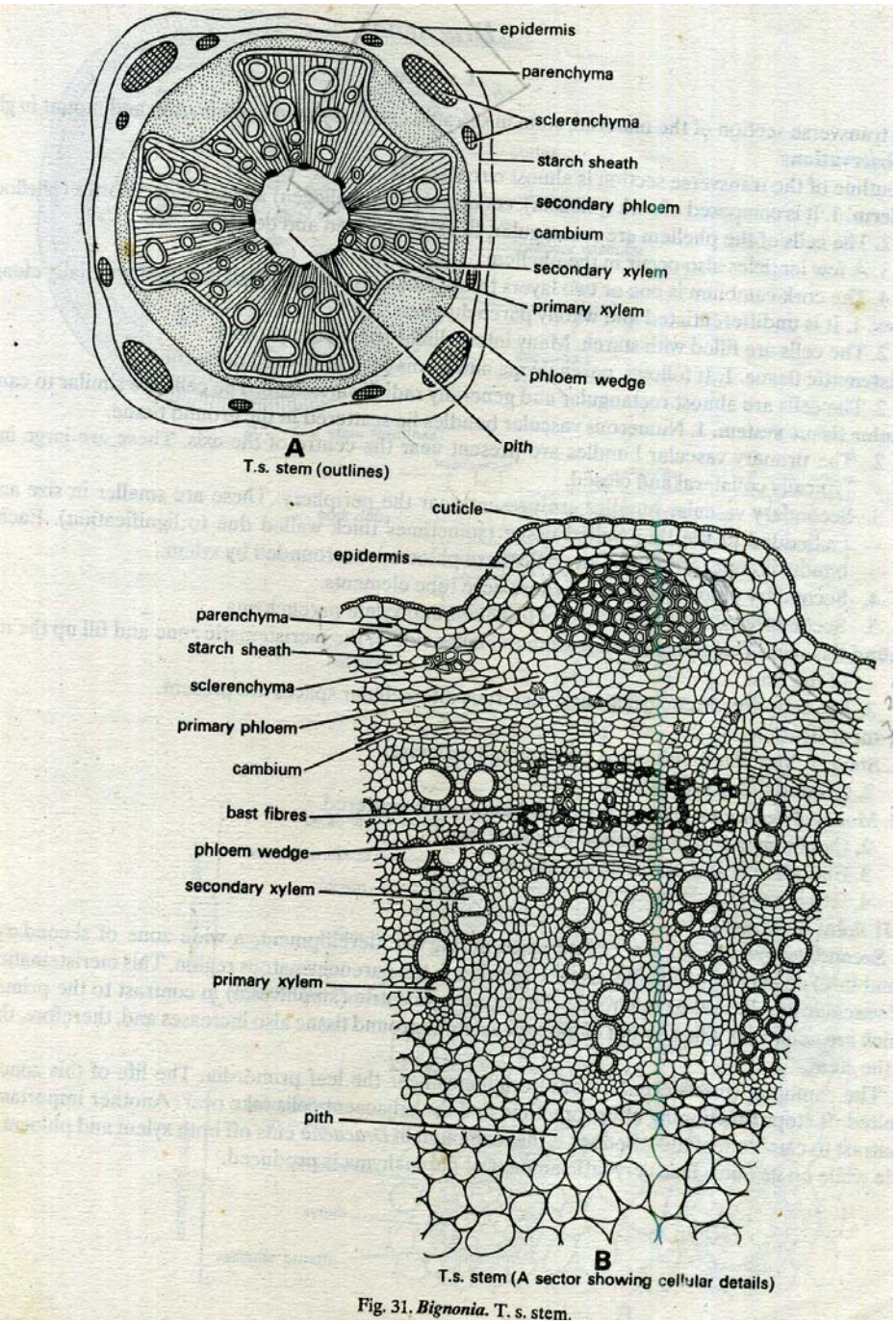


Fig. 31. *Bignonia*. T. s. stem.

Boerhaavia (Nyctaginaceae)
(refer figures from Hait vol 2 page 712,713)

Supplied material
Roundish, deep brown in colour, slightly woody, T.S. through the internode of mature stem is taken, stained (double stain) suitably and observed under microscope.
Study of anatomical features - T.S through the stem shows the following characters arranged from periphery towards center.
Epidermis -It is single layered with distinct cuticle and scattered stomata, many multicellular hair develop from epidermal cell.
Cortex - It is distinguished into three layers e.g. (a) Hypodermis- composed of collenchyma cells, (b) General cortex-composed of parenchyma cells with intercellular space, (c) Endodermis - Last layer of cortex is the starch sheath which is a wavy band made up of single row of compactly set barrel shaped parenchyma cells having starch grains.
Stele -This is the central cylinder including following-
Pericycle - Below the starch sheath few layered parenchymatous cells represent pericycle.
Vascular bundles - The Vascular bundles are arranged in three rings. Two large fairly central Vascular bundles present at the central region are called medullary bundles. Each bundle is conjoint, collateral, open and has intra fascicular cambium. Secondary growth in these bundles is very limited, no interfascicular cambium develops in the inner ring of the cambium. The middle ring consists of 6-14 loosely arranged comparatively smaller ones**,** no inter -fascicular cambium present. These bundles are conjoint, collateral and open. The third outermost ring consists of large number of much smaller bundles just beneath the pericycle. Each bundle has intra fascicular cambium which are connected by inter -fascicular cambium producing a continuous accessory cambium cylinder. These cambium produces secondary xylem on the inner side and secondary phloem opposite to secondary xylem vessels and lignified conjunctive tissue in the inter fascicular regions. So newly formed secondary bundles remain embedded in the lignified conjunctive tissue.
Pith- This region is not clearly demarcated due to the scattered distribution of primary **vascular bundles in the parenchymatous ground tissue.**

Identifying characters
1)Distinct uniseriate epidermis with multicellular hair.
2)cortex is distinguished into three layers . Hypodermis, general cortex & endodermis
.
4)Hypodermis - composed of collenchyma cells
5)Pericycle few layered parenchymatous cells represent pericycle
. 6)Vascular bundles- Vascular bundles are conjoint ,open, collateral and endarch, arranged in a ring
7)protoxylem endarch.
8)Presence of secondary growth.
Pith- This region is not clearly demarcated

So the supplied specimen is a Dicot stem.

1) T.S. is circular in outline with unisereate eridermis having stomata.
2) Hypodermis - composed of collenchyma cells .
3) General cortex composed of parenchyma cells with intercellular spaces.
4) The stele includes primary vascular bundles in three rings. Two fairly large bundles, **called medullary bundles** occur at the central region**. Each bundles is** conjoint, collateral, open and has intra fascicular cambium. Secondary growth in these bundles is very limited. No interfascicular cambium develops in this ring.The middle ring consists of 6-14 loosely arranged comparatively much smaller rings forming a loose ring which surrounds the medullary bundles. The third outer most ring consists of large number of much smaller bundles just beneath the pericycle forming a continuous cambium cylinder in the outer ring. Secondary xylem and phloem formation remains restricted in the fascicular regions only and lignified conjunctive tissue in the interfascicular regions. Scanty secondary phloem is formed outside opposite to the xylem vessels. The newly-formed bundles remain embedded in hard lignified conjunctive tissue.
Hence, it is case of anomalous secondary growth in dicot stem due to secondary cambium development which is abnormal in position and abnormal in activity.

Comment : 1)The vascular bundles are arranged in three rings; each bundle is collateral, open and devoid of bundle sheath. It indicates the dicotyledonous nature of the stem. 2) The stele shows non-adaptive type of anomalous secondary growth. 3) Production of secondary vascular tissues is

restricted to medullary bundles only. 4) In the peripheral bundles a complete cambium ring is present by the union of fascicular and interfascicular cambium. But the interfascicular cambium produces conjunctive tissue in addition to new vascular bundles. 5) Collenchyma at hypodermis and sclerenchymatous conjunctive tissues are mechanical cells. 6) The continuous band of sclerenchyma with secondary xylem acts as a cylinder and the vascular bundles form I-girders across the stem, and these protect the stem from lateral bending strain.

Tecoma stem (Bignoniaceae)
(figures Hait vol 2 page 715)

Supplied material - Greenish brown, outside wavy, slightly woody. T.S. of stem through the internode is taken, stained (double stain) suitably and observed under microecope.

Study of anatomical feature - It shows the following tissue organisation from periphery towards center.

Epidermis - It is unisereate, cuticularised , made up of compact tubular parenchymatous cells and few multicellular hairs are present.

Cortex - It is differentiated into hypodermis, general cortex and endodermis.

Hypodermis : It is the peripheral layer of cortex and composed of two to three layers of collenchyma.

General cortex : This zone is composed of many layered parenchyma cells with intercellular spaces present.

Endodermis : It is the innermost layer of the cortex and conspicuous in older stems. A few patches of sclerenchyma occur below endodermis. The inner layer is represented by starch sheath layer.

Stele - It is central cylinder including the following features-

Pericycle-There are distinct pericycle region where scattered sclerenchymatous patches are seen.

Vascular bundles -These are conjoint, collateral and open. Primary bundles are arranged in a ring around the pith. Normal cambium ring forms typical xylem and phloem inside and outside respectively. Union of fascicular and inter fascicular cambium forms a continuous concentric ring around the pith. Later, accessory cambium are formed in two arcs at the outer margin of pith and on the inner side of the normal vascular cylinder. These cambium produces secondary xylem and phloem in reverse order i.e., xylem outside and phloem inside. As a result, two arcs of vascular bundles are formed at the margin of pith, showing inverse orientation of xylem and phloem in contrast to normal bundles. Secondary phloem patches are embedded in secondary xylem .These extra patches of secondary phloem present of inside the xylem is known as intraxylary phloem. Pith in the center become gradually crushed by these intraxylary phloem.

Pith – Thin walled parenchymatous with intercellular spaces.

Identifying characters - (1) Multicellular epidermal hairs are present, (2) Ground tissues are differentiated into extrastelar and intrastelar region. Innermost layer of cortex is differentiated into a starch sheath. (3) Vascular bundles are open, collateral and endarch, arranged in a ring. (4) Presence of secondary growth. (5) Pith is conspicuous. **So the supplied specimen is a Dicot stem**.

(1) T.S.of stem is roundish in nature. (2) A starch sheath layer is present in the innermost layer of cortex. (3)The normal cambium ring forms typical xylem and phloem inside and outside respectively. (4) Later, accessory cambium are formed in two arcs at the outer margin of pith and on the inner side of the normal vascular cylinder. (5) These cambium produce secondary xylem and phloem in reverse order i.e. , xylem outside and phloem inside. As a result, two arcs of vascular bundles are formed at the margin of pith, showing inverse orientation of xylem and phloem in contrast to normal bundles. So newly formed phloem is intraxylary in nature. (6) The two patches of intraxylary phloem gradually crush the pith.
Hence, it is a case of anomalous secondary growth in dicot stem, where accessory cambium is abnormal in activity.

Comment : (1) The dicot nature of the stem is revealed by the presence of siphonostele, collateral and open vascular bundle with endarch protoxylem. (2) Epidermis and periderm are the protective tissues. (3) Collenchyma present at hypodermis is mechanical cell. (4) Stele shows both normal and abnormal secondary growth. (5) Cambium is **abnormal in position** i.e. accessory cambium are formed in two arcs at the outer margin of pith and on the inner side of the normal vascular bundles. (6) Cambium shows **abnormal activity,** i.e. these accessory cambium produce secondary xylem and phloem in reverse order, i.e., xylem outside and phloem inside. As a result, two arcs of vascular bundles are formed at the margin of pith. (7) These extra patches of secondary phloem present inside the xylem is known as intraxylary phloem and are secondary in origin. (8) Anomaly in the stele is due to the presence of intraxylary phloem. This is the case of adaptive anomaly for the purpose of climbing.

Dracaena (Agavaceae) Monocot
(figures Hait vol 2 page 708)

Supplied material - Inner region of the stem is whitish and outer layer red in colour, fibrious in nature. T.S. through the internode of mature stem is taken, stained(double stain) suitably and observed under microscope.
Study of anatomical feature-T.s. of the stem is circular in outline showing the following tissues arranged from periphery towards center.
Epidermis - Single layered remain covered with thick cuticles, ruptured here and there by lenticels. Beneath the epidermis and hypodermis, the cork cambium arises which gives rise to the cork towards outside.
Periderm - It is formed due to secondary thickening in the extra-stelar region, and, is differentiated into three layers.(A) Phellogen - 3-5 layered cells, arranged in radial rows without having inter cellular space, thickly suberised dead cells, rectangular in shape. (B) Phellem - 3-5 layers of cells formed bty the phellogen on outside and lie just below the epidermis are called phellem. They are compactly arranged in radial rows without any intercellular space. (C) Phelloderm -1-2 layered cells formed by the phellogen on inside. They isodiametric and arranged in radial rows with inter cellular space.

Cortex - It is undifferentiated, composed of parenchymatous cells with inter cellular spaces. Starch sheath is absent. It forms several layer deep zones. Endodermis is absent. Cortex is externally bounded by 3-5 layers of cork cells forming phelloderm layer and internally by a cambium layer forming conjunctive tissue.

Stele - It is central cylinder including the following tissues-

Pericycle -This peripheral layer of the stele cannot be easily distinguished.

Vascular bundles - Anomalous secondary thickening growth is brought about by a special cambium termed as secondary thickening meristem. The stele is atactostele.Numerous vascular bundles lie scattered in the ground tissue.

The primary vascular bundles are present near the centre of the axis. These are large in size, typically collateral and closed. Each bundle is circular or oval in shape and leptocentric i.e. the xylem completely surrounds phloem and there exists no intra-fascicular cambium.

Secondary vascular bundles are present.near the periphery. A multilayered secondary accessory cambium ring has developed in the inner cortical region and cambium activity is abnomal. Due to the activity of this cambium, conjunctive tissue (ground tissue) and secondary vascular bundles are formed inwardly. These are smaller in size and embedded in the thin walled ground tissue. The ground tissue is termed as conjunctive tissue. These secondary vascular bundles and conjunctive tissues surrounds the primary vascular bundles. Each vascular bundle is leptocentric (amphivasal) where phloem is surrounded by xylem.

Pith - Pith is not well defined.

Identifying characters
(1) Epidermis is uniseriate and provided with lenticel and cork. (**2)** The stele is atactostele**. (3)** Endodermis and pericycle are absent. **(4)**Vascular bundles are scattered in ground tissue. (5) Vascular bundles are conjoint, collateral and close. (6) Protoxylem is endarch. (7) Pith is lacking.
So the supplied specimen is a T.S. of monocot stem.

1) Stele is atactostele where the vascular bundles arc irregularly scattered within the ground tissue. Each vascular bundle is circular or nearly oval in outline and leptocentric, i.e., phloem is surrounded by xylem and the fascicular cambium is absent.
2) A large number of **primary vascular** bundles are irregularly distributed in the central parenchymatous ground tissues. These are large in size, typically collateral and closed.

3) At a very late stage during the development, a wide zone of secondary meristem (cambium) develops outside the vascular bundles in the parenchymatous region. This cambium produced the conjunctive tissues inwardly. These tissues are parenchymatous, thin walled with profuse intercellular spaces and are arranged in longitudinal radial rows. Secondary vascular bundles are present near the periphery. These are smaller in size, oval in outline and embedded in the thin walled ground tissue. The ground tissue is termed as conjunctive tissue.
Hence, it is a case of anomalous secondary growth in monocot stem where secondary cambium shows abnormal activity and position.

Comment:
1) Epidermis and periderm are the protective tissues. Epidermis is ruptured by lenticel.
2) The monocot nature of the stem is revealed by the presence of atactostele and leptocentric vascular bundles.
3) Anomaly in the stele is due to presence of conjunctive tissue formed by the cambium. The tissues are parenchymatous and the secondary vascular bundles remain embedded in them.
4) Stele shows anomalous secondary growth where growth occurs by a cambium which originates in the deeper layer of cortex or pericycle.
5) The primary and secondary vascular bundles are leptocentric.
6) The bundles form I-girders and provide mechanical strength against inflexibility.
7) Atactostele, and parenchymatous conjunctive tissue are the characteristic of the stem.
Here anomaly is due to abnormal activity and position of secondary cambium.

Tinospora root
(figure from Hait vol 2 page 706)

Supplied material
Very thin, brown in colour, soft, round, T.S. through the internode of a mature root is taken, stained suitably (using double stain) and observed under microscope.

Study of anatomical features
Epidermis - It is limiting outermost layer, made up of rectangular cells ruptured here and there by the formation of lenticels.

Periderm - It is produced just outside the cortex as a protective layer. It consists of tissues differentiated into three zones i.e. (i) phellem, (ii) phellogen and (iii) phelloderm
i) phellem - It is made of rectangular dead suberised cells without having inter cellular space .
ii) phellogen - It consists of radially flattened compactly arranged rectangular cells without having inter cellular space.
iii) phelloderm - consists of thin walled cells with numerous inter cellular space. These cells contains large number of chloroplast.

Cortex- (i) It consists of thin walled parenchyma with numerous inter cellular space. (ii) The cells possess large number of chloroplasts. (iii) After the formation of periderm cortex and pericycle are peeled off.
Endodermis - Internal layer of cortex is conspicuous.

Stele - It is a central cylinder including following tissues-
Pericycle - Internal to endodermis there occurs an inconspicuous cells and is in the form of a complete ring of barrel-shaped cells.
Vascular tissue - It consists of primary phloem, secondary phloem, cambium, secondary xylem, medullary rays and primary xylem
Primary phloem - It is in the form of groups which alternate with primary xylem groups near the centre of axis
Secondary phloem - It groups occur below the patches of primary phloem and are massive.
Cambium - It is in the form of wavy ring. It is unistratose to multistratose. Cambium cells produce much more xylem at higher rate than phloem, causing formation of wavy secondary cambium ring.
Secondary xylem – It lies below the cambium. It is divided into many smaller and larger regions due to wide medullary rays which pass through it. Vessels are very conspicuous due to their large diameter. Xylem is made up of tracheids, vessels and thick walled xylem parenchyma.
Primary xylem – PrimaryVascular bundle is radial, with tetra or pentarch xylem. They are found in groups at the centre obliterating the pith. They show star like appearance due to continuous secondary growth. .
Medullary rays - The characteristic feature of the root is the occurrence of wide medullary rays which run between xylem and phloem .
Pith - In the centre of the axis a small pith is present.

Identifying characters -
(1) Epidermis ruptured here and there by the formation of lenticels. (2) Cortex consists of thin walled parenchyma with numerous inter cellular space. (3) Primary vascular bundles are radial with exarch protoxylem. (4) Presence of secondary growth (5) Secondary protoxylem is endarch. (6) Pith is scanty. **So it is a dicot root.**

(1) Wavy secondary cambium ring present (2) cambium cells formed below primary phloem starts producing secondary vascular tissues with great amount of secondary xylem. (3) Phloem tissues remain in radial patches. (4) Wide medullary rays are found. These run between and through xylem and phloem dividing the secondary vascular bundles into small groups.

Hence it is a case of anomalous secondary growth in Dicot root **where cambium is abnormal in position (accessory cambium) in mature root and shows abnormal activity.**

Comment

1) Thick walled cork at the periphery is protective and mechanical cell that provides strength against inextensibility.

2) The dicotyledonous nature of root is due to the presence of radial primary vascular bundles and obliteration of pith as a result of secondary vascular tissue formation.

3) The primary and secondary xylem are mechanical cells.

4) Presence of wavy secondary cambium ring is due to production of secondary vascular tissue with large amount of secondary xylem.

5) The broad medullary rays are formed to store reserve food materials.

Identification

The slide shows the following characters (Fig 5.127-hait vol 2 –page 689)

1) Presence of single layered prenchymatous epidermis with out cuticle.
2) Hypodermia is collenchymatous in nature..
3) Cortex is made up of parenchymatous cells with air chambers scattered all over.
4) Trichoblast are present in the ground tissue.
5) Simple vascular bundles scattered all over the ground tissue.

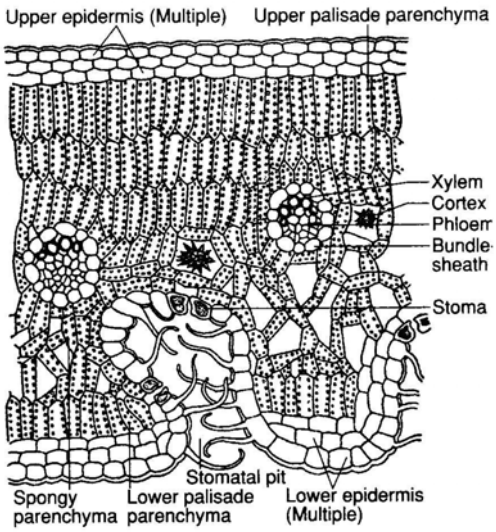
Hence,it is the hydrophytes with air chambers scattered all over.

Identifying features :—

(1) Presence of sunken stomata in lower epidermis.
(2) Presence of Pallisade and Spongy mesophyll cells.
(3) Presence of collateral, closed vascular bundles.
So, it is a dicotyledonous dorsiventral leaves.

(1) Presence of thick cuticle on upper and lower epidermis.
(2) Multilayered epidermis checks excessive transpiration
(3) Presence of stomata in lower epidermal layer, stomata are deeply sunken with trichomes projecting into the cavity to reduce water loss and protect stomata.
(4) Presence of pallisade and spongy cells.
(5) Presence of colateral, closed vascular bundles surrounded by bundle sheath cells.
(6) Presence of calcium oxalate crystals.

Hence, it is a case of dicotyledonous dorsiventral leaves with distinct xerophytic adaptive features.



Transverse section of a portion of a *Nerium* (oleander) leaf

Types of Stomata:

- **Anomocytic Type:**

(1) Each stoma consist of two kidney shaped guard cell.
(2) Each stoma is surrounded by limited number of cells which are not distinguishable from other epidermal cells
(3) In this kind of stomata subsidiary cells may be absent.

- **Anisocytic type:**

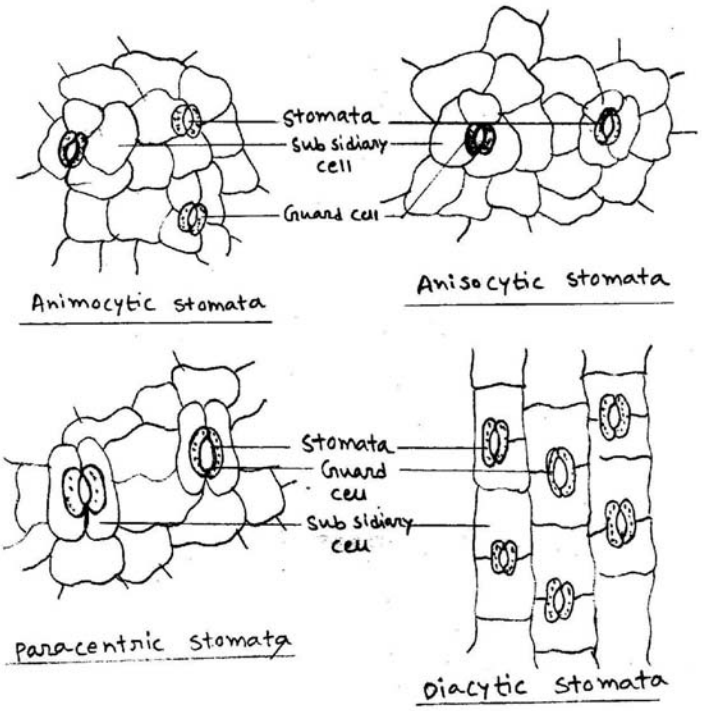
(1) Each stoma consist of ~~two~~ kidney shaped guard cells
(2) Each stoma is surrounded by three subsidiary cells which are quite different in size from epidermal cells.
(3) Among the three subsidiary cell one is much smaller than other two.

- **Paracytic type:**

(1) Each stoma consist of two kidney shaped guard cells.
(2) Each stoma is surrounded by two subsidiary cells more or less sames.
(3) The common wall of two subsidiary cells is at right angles to the guard cells.

- **Diacytic Type:**

(1) Each stoma consist of two kidney shaped guard cells.
(2) In this type the stoma has on ~~either~~ side one or more subsidiary cells.
(3) The subsidiary cells lie parallel to the long axis of the aperture or guard cells



Different types of stomata

SCLEREIDS :-

• Brachy sclereids :

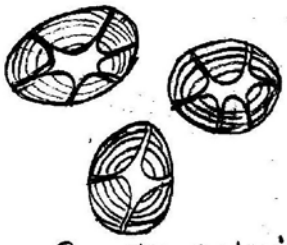
(1) The cells are irregularly scattered, thick walled.
(2) Each cell is isodiametric, ovoid or some what orbicular, lignified.
(3) Each cell posses very small central lumen from some narrow canal like simple pits radiate in all direction.

• Osteo sclereids :

(1) Each cell is thick walled, dumble shaped or bone like that means the both ends are dialated.
(2) The central lumen comparitively larger than brachy sclereids.
(3) No canal like simple pits are present.

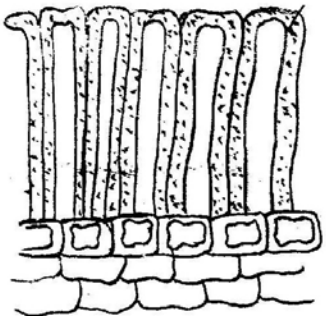
• Macro sclereids :

(1) Cells are columner, rod shaped and form a pallisade like layer.
(2) Cell wall is thick, lignified, cell lumen narrow.
(3) No canal like simple pits are present.



Brachy sclerid

Osteo sclerid



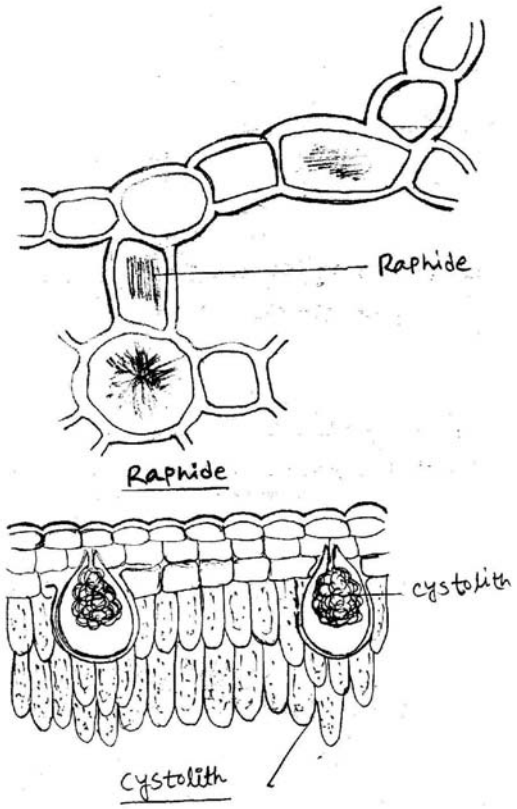
Macro sclerides

• Raphides (Colocasia):

(1) Large number of air spaces and air cavities are present.
(2) Cavities are encircled by thin walled parenchyma cell.
(3) Large boat shaped or spindle shaped idioblasts are found hanging into the air cavities.
(4) These idioblasts contain numerous parallely arranged needle shaped bunch of crystals of Calcium oxalate.
(5) Individual needle is very fine pointed at both the ends.

• Cystolith (Ficus leaf):

(1) The cell posses hanging bunch of grape like structures.
(2) The aggregated grape like bodies attached on a peg like projection, [redacted] upper epidermis which lies just beneath multiple epidermis.
(3) Aggregated bodies are composed of Calcium carbonate crystals.
(4) The aggregated structure is known as Cystolith and cell containing it, is called lithocyst.



Starch grains :

(1) Minute globular sub globular, ovoid bodies are scattered through out the cells.

(2) Each body consist of a terminal or sub terminal dark point known as hilum which is surrounded by several striations or stratifications.

(3) When the hilum lies one end of the grain called excentric grain.

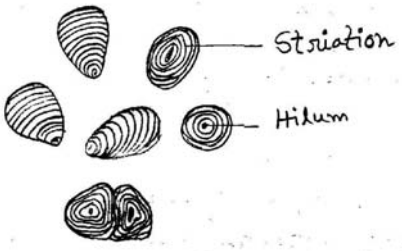
(4) When the grain posses one hilum called simple grain but when it is more than one hilum called compound grain.

Aleurone grains :

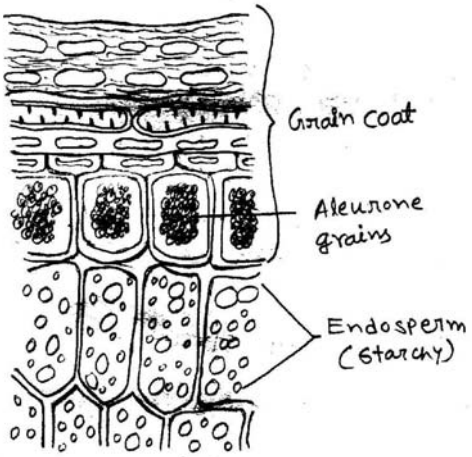
(1) The cell just beneath the grain coat is full of small round structure.

(2) The round structures are compactly arranged.

(3) Each globular body is composed of a protein matrix with one or two large crystaline bodies called crystaloidss and small round body known as globoid.



Starch grain



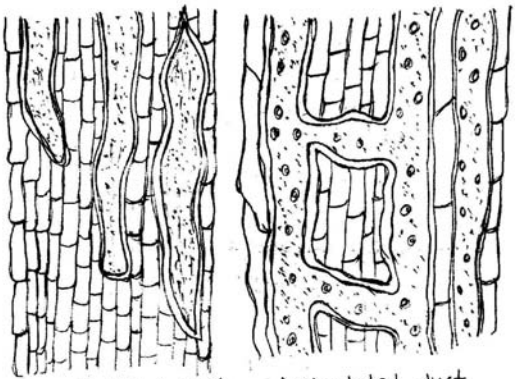
Aleurone grains

Laticiferous ducts :

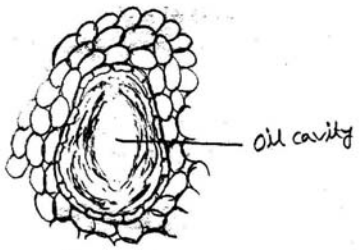
(1) Long duct like structure, either brunched or unbranched, present irregularly on tissue.
(2) The ducts either septed or non septed. when it is septed called articulated and when it is without septation called non-articulated.
(3) The ducts are filled up with granular clotted materials called latex.

Oil Glands :

(1) Numerous oval or round cavities present just beneath the epidermis.
(2) The cavity is full of essential oil and surrounded by a layer of cells.
(3) The glands are lysigenous in origin.
(4) With oil droplets ~~cavities~~ are also filled with some dead cells.



Non articulated duct Articulated duct



Oil gland