BGS Science Academy & Research Centre

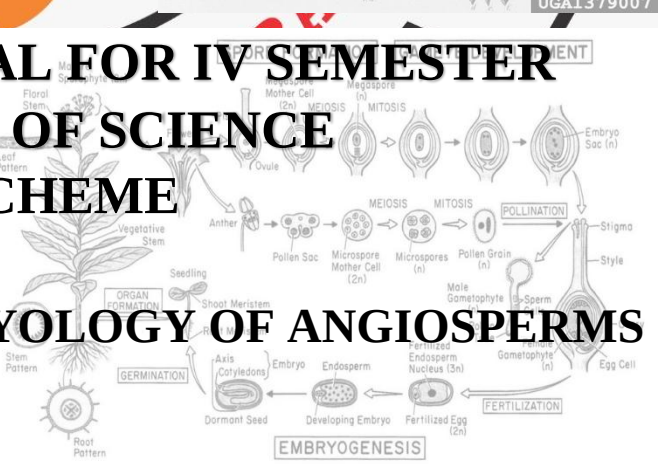
Agalagurki, Chikkaballapura

Affiliated to Bangalore North University

DEPARTMENT OF BOTANY

LABORATORY MANUAL FOR IV SEMESTER
II BACHELOR OF SCIENCE
CBCS SCHEME

GYMNOSPERMS AND EMBRYOLOGY OF ANGIOSPERMS



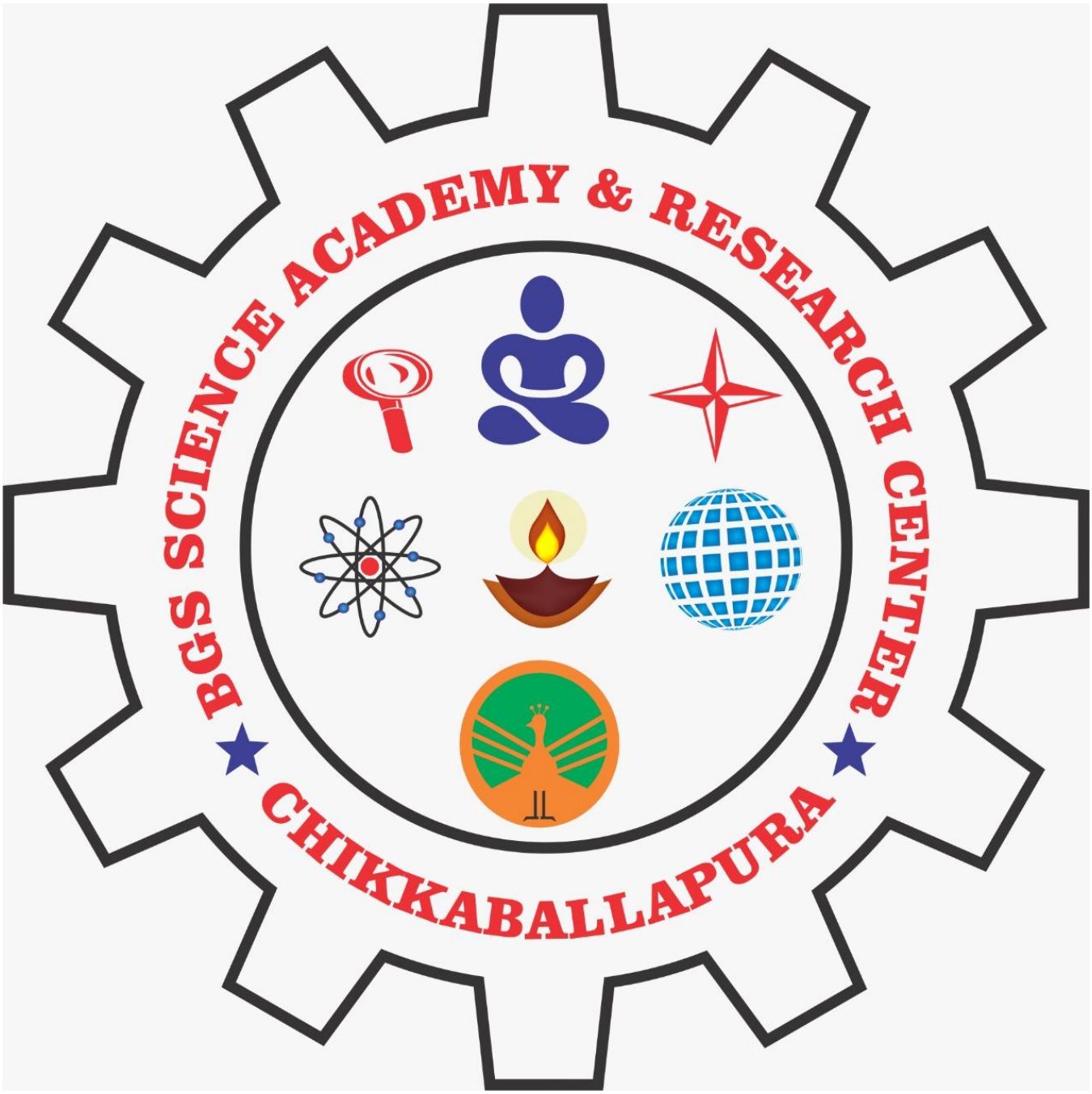
Laboratory Instructions

- Maintain discipline in lab.
- Be prepared in advance for the practicals with learning & bringing the plant or other materials of experiment.
- Do not forget to carry laboratory apron, observation book (unruled) and other required accessories- Dissection materials (New Blades- normal & surgical, 2 needles, pointed end forceps, scissors, scalpel, fine hair or camel hair brushes, piece of muslin or cotton cloth etc) Writing materials (Pen, Pencil, Eraser, Sharpner, small Permanent marker, scale, etc) .
- Handle equipments, microslides, glasswares, chemicals and specimen bottles with proper care.
- Report breakages / damages to the batch incharge or laboratory assistants.
- Keep the laboratory clean and tidy.
- Ensure to return the specimens, slides, materials, charts, instruments or other things to their respective places.
- Clean your worked area before leaving the lab.
- Follow energy saving protocols.
- Make clear pictures of obervations & verify with lab incharge teacher.

INSTRUCTIONS FOR RECORD WRITING

- In record book, Thin sheets should be used for writing notes or points & thick sheets for drawing diagrams. Scientific (characterized) well differentiated clear diagrams is mandatory, no artistic designs.
- Each experiment or topic has to be written separately without overlapping or writing behind the next experiment on the previous experiment or topic.
- Use pencil of different shades (H,HB,B) for writing diagrams.
- Write the classification on the right hand top side of the diagram page.
- Labelling of diagram should be in justified alignment & should be in Uppercase always.
- Do not use the space outside the page border.
- Submit the records in every practical class.

- Get your record binded only after the instrutions from lab incharge teacher.



Gymnosperms And Embryology Of Angiosperms

INDEX

PRACTICAL IV

GYMNOSPERMS AND EMBRYOLOGY OF ANGIOSPERMS

Total Units-13

1) Study of materials and permanent slides of gymnosperms included in theory-*Cycas, Pinus and Gnetum*. **5 units**
2) Permanent slides of microsporogenesis and male gametophyte. **1 unit**
3) Mounting of pollen grains- Grass, *Mimosa*, pollinia of *Calotropis* and pollen germination (hanging drop method). **1 unit**
4) Permanent slides of types of ovules, Megasporogenesis and embryo sac development. **1 unit**
5) Permanent slides of types of placentation- Axile, Marginal, Parietal, Basal types. Sectioning of ovary, for any two types of placentation. **1 unit**
6) Mounting of embryo- *Tridax* and *Cyamopsis*. **1 unit**
7) Mounting of endosperm – *Cucumis*. **1 unit**
8) Mini project work in groups of 3-5 students, from the following list. **2 units**

a) Study of pollen morphology of different flowers with respect to shape, colour, pores etc.
b) Pollen germination of different pollen grains and calculate percentage of germination.
c) Calculating percentage of germination of one particular type of pollen grain collected from different localities/ under different conditions.
d) Study of placentation of different flowers.
e) Any other relevant study related to Gymnosperms / Embryology

Mini project work may be carried out in groups of 3-5 students, supervised by the batch in charge. The mini project report, about 5-6 pages (type written), to be prepared in following format and certified by the teacher in charge and HOD to be submitted in practical examination.

1. Introduction
2. Aim of study
3. Materials and methodology
4. Observation
5. Conclusion

Copies to be submitted separately by individual members of the group.

PRACTICAL QUESTION PAPER- IV

GYMNOSPERMS AND EMBRYOLOGY OF ANGIOSPERMS

Time: 3 hours. **Max Marks: 35**

1. Identify and classify specimens **A**, **B** and **C** giving reasons. **3 × 3 = 9**
2. Identify the slides **D**, **E** and **F** with reasons and labeled diagrams. **3 × 3 = 9**
3. Mount the embryo/ Endosperm of specimen **G** and comment. **5**
4. Mount the pollinia / perform pollen germination of specimen **H** and comment **4**
5. Record and submission. **5 + 3 = 8**

SCHEME OF VALUATION

1. Gymnosperm materials – *Cycas*, *Pinus*, *Gnetum* (Identification and classification – **1** mark, reasons – **2** marks).
2. One Gymnosperm slide, one from T.S of young anther/ mature anther, one from megasporogenesis/ stages of embryo sac development / placentation/ types of ovules included in theory. (Identification – **1** mark, diagram – **1** mark, reasons – **1** mark).
3. Endosperm / Embryo mounting (preparation – **3** marks, comment with diagram – **2** marks).
4. Pollinia / pollen germination (preparation – **2** marks, comment with diagram- **2** marks)
5. Record and submission of mini project report (**5 + 3 = 8** marks)

Cycas

SYSTEMATIC POSITION

Division: Gymnosperm
Class : Cycadopsida
Order : Cycadales
Family : Cycadaceae
Genus : *Cycas*

Introduction

Cycas has a limited distribution. In India, *Cycas* grows naturally in Orissa, Assam, Meghalaya, Tamil Nadu, Karnataka and Andaman and Nicobar Islands. Sixteen species of *Cycas* are found in the tropical and subtropical parts of the earth. Besides *Cycas circinalis*, *C pectinata*, *C. rumphii* and *C. beddomei*, which occur in the wild state -in India, *C. revoluta* and *C. siamensis* are such species which are cultivated commonly in the Indian gardens.

Habit Structure

1. *Cycas* is a palm-like, evergreen, slow growing tree.
2. Plant body is differentiated into an underground root system and an aerial system that is distinguished into an erect stem and a crown of leaves.

2. Roots are of two types: (i) primary or normal root and (ii) secondary or coralloid root.
3. Normal root is a tap root, growing deep into the soil (positively geotropic). It is sparsely branched and sometimes grows as thick as aerial stem.
4. Secondary roots are negatively geotropic projecting above the soil surface, repeatedly dichotomously branched and appear as coralloid clusters, projecting above the soil surface (apo-geotropic).
5. The young stem is almost tuberous but when grows old, it becomes thick, columnar and unbranched (Branching is rare and is caused due to injury, etc.). The trunk is covered by persistent leaf bases.
6.The stem bears a terminal group of leaves which are dimorphic (i.e. of two types) (i) foliage leaves (green assimilatory fronds) and (ii) scale leaves (brown and hairy). These leaves alternate with one another.
7. Young foliage leaves are circinately coiled and are covered with ramenta (hairs).
8. Mature leaves are spirally arranged and pinnately compound. Each leaf has about 80- 100 pairs of pinnae that are closely arranged, opposite one another on the rachis with a decurrent base. Each pinna is tough, leathery and entire with a definite midrib but no lateral veins.
9. Scale leaves are small, simple, brown with aborted lamina and covered with hairs. These leaves cover the apex and young developing foliage leaves. Scales are also persistent, like leaf bases.

Anatomy - T.S of Rachis

1. Outline is cylindrical. It shows insertion of pinnae on the adaxial side (upper side).
2. The rachis is differentiated into epidermis, hypodermis, ground tissue and a ring of vascular bundles.
3. Epidermis is single layered, thickly cuticularized and is interrupted by stomata throughout its surface. The condition is known as amphistomatic.
4. Hypodermis is mainly composed of thick-walled cells (sclerenchyma). Intermixed with these cells are a few cells having chlorenchyma.
5. This sclerenchymatous hypodermis is 2-3 layered toward adaxial side and many layered toward abaxial side.
6. Ground tissue. The rest of the tissue that forms most part of the section is called ground tissue. It is parenchymatous.

7. Mucilage ducts are scattered throughout the ground tissue. Mucilage ducts are double layered, the inner layer being composed of epithelial cells and the outer of tangentially elongated sclerenchymatous cells.
8. The vascular bundles are arranged in an inverted omega (Q) shaped arc. Each vascular bundle is surrounded by a thick walled, single layered bundle sheath. It is conjoint, collateral and open.
9. The arrangement of xylem and phloem differs in vascular bundles at the base, middle and upper region of the rachis.

(i) Higher up and for most part of the rachis, bundles are diploxylic i.e. two types of xylem elements are present - centripetal and centrifugal xylem. The centrifugal xylem occurs in two small groups, present on both the sides of large triangular and centrally located centripetal xylem. The phloem is situated on the abaxial side of the rachis.
(ii) At the very base of the rachis, vascular bundles show only centrifugal xylem which is endarch. Phloem occupies the abaxial side of the rachis.
(iii) Little higher up the base of rachis, vascular bundles show centrifugal xylem on abaxial side and centripetal xylem on adaxial side. In the centre of these two xylem groups, lies the protoxylem. This condition is said to be mesarch.

Anatomy - T.S of leaflet (Pinna)

1. The leaflet shows a distinct midrib and the wings.
2. The midrib is swollen, while wings on the lateral sides are narrower and flattened.
3. Upper epidermis is present on the upper side. It is thickly cuticularized and single-layered.
4. Hypodermis is present below the epidermis. It is sclerenchymatous.
5. Mesophyll lies below the hypodermis and is well developed. It is differentiated into upper palisade layers and lower of spongy parenchyma.
6. Spongy parenchyma with many intercellular spaces lies immediately above the lower epidermis.
7. Transfusion tissue. On either side of the centripetal metaxylem of mid rib bundle and somewhat connected with it, are present two tracheid-like cells-transfusion tissue.
8. Accessory transfusion tissue. Between the palisade and spongy parenchyma cells, there are 3 or 4 layers of tracheid-like, long colourless cells which run transversely from the midrib to near the margin of the lamina. This is known as accessory transfusion tissue. It is connected with the xylem of the vascular bundle of midrib through the transfusion tissue.
9. Lower epidermis bounds the leaflet from lower side. It is thickly cuticularized and single layered. Sunken stomata are found in the lower epidermis in the midrib region.
10. Stomata are very much sunken in the lower epidermis in C. *revoluta*, while they are not so much sunken in C. *circinalis.*
11. Midrib bundle. In middle of the swollen portion representing the midrib lies a single vascular bundle surrounded by parenchymatous tissue (with calcium oxalate crystals). Vascular bundle has a definite and thickened, parenchymatous bundle sheath.
2. The vascular bundle is conjoint, collateral, open and diploxylic.
13. Phloem lies towards the abaxial (lower) side. In between xylem and phloem, cambium is present.
14. Xylem - It shows a large, triangular patch of centripetal xylem and two small groups of centrifugal protoxylem.

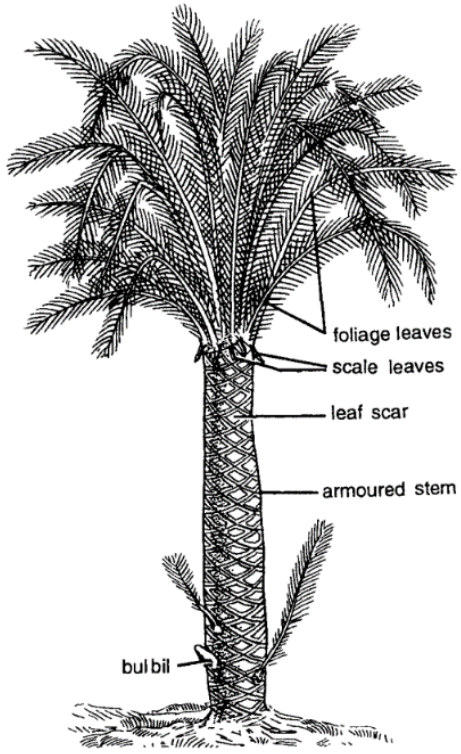


Fig. 1. *Cycas*. External features.

Cycas. A single foliage leaf.

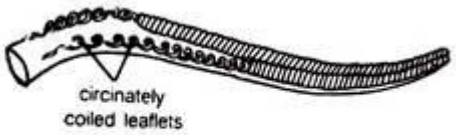


Fig. 5. Cycas. A young leaf showing circinate vernation.

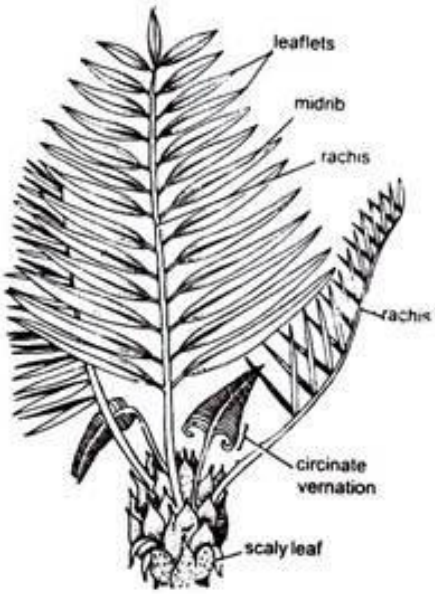
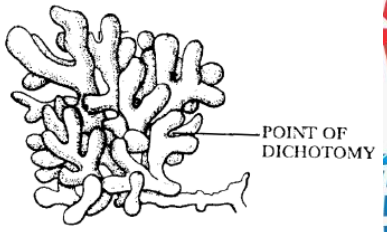
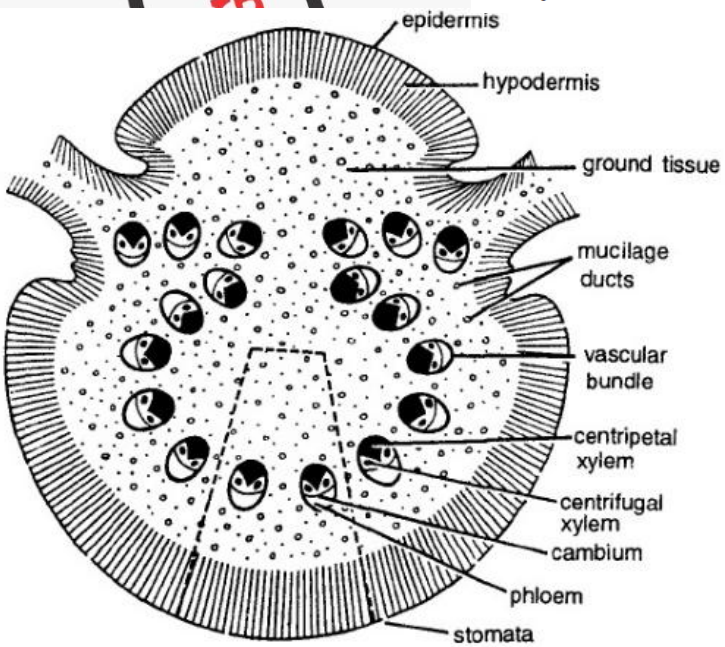


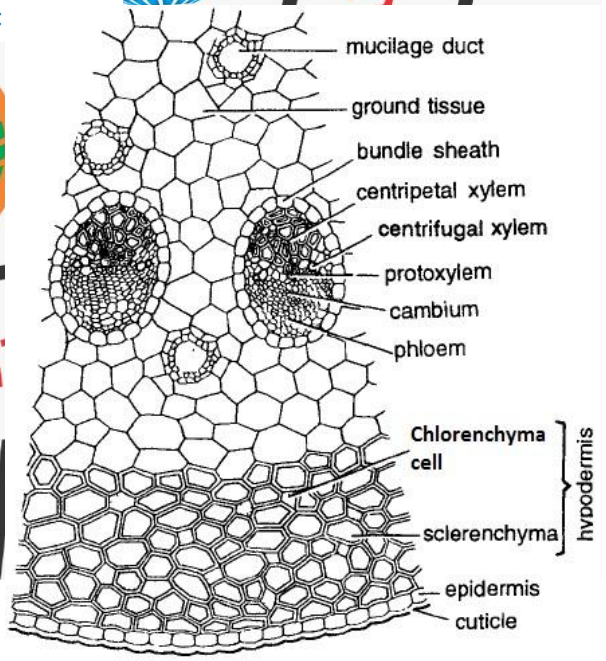
Fig. 6. Cycas. A single bulbil.



Cycas- Coralloid root



Cycas. T.s. of rachis (diagrammatic).



Cycas. T.s. of rachis (a part shown by dotted lines in Fig. 11 in details).

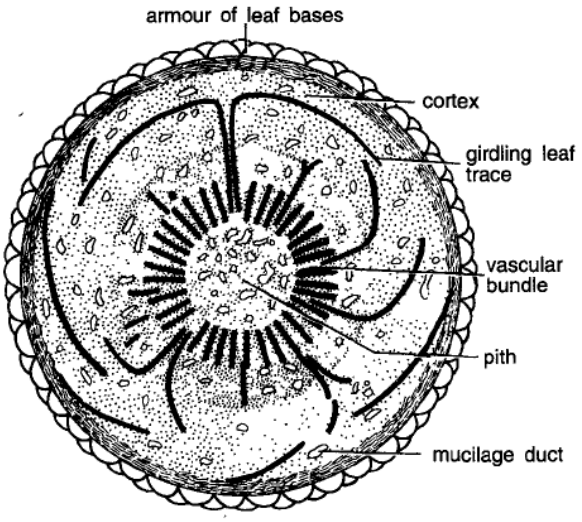
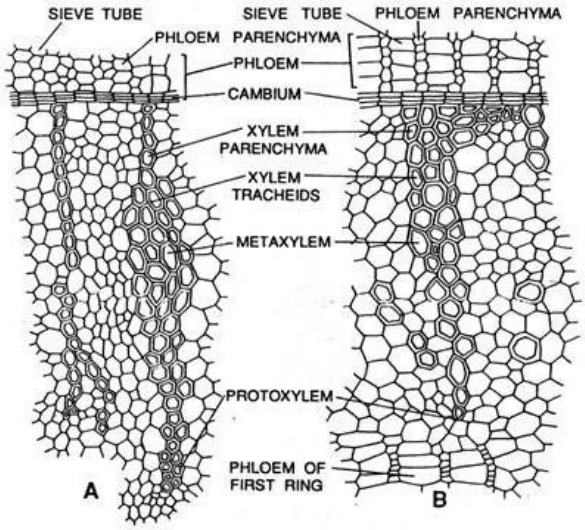
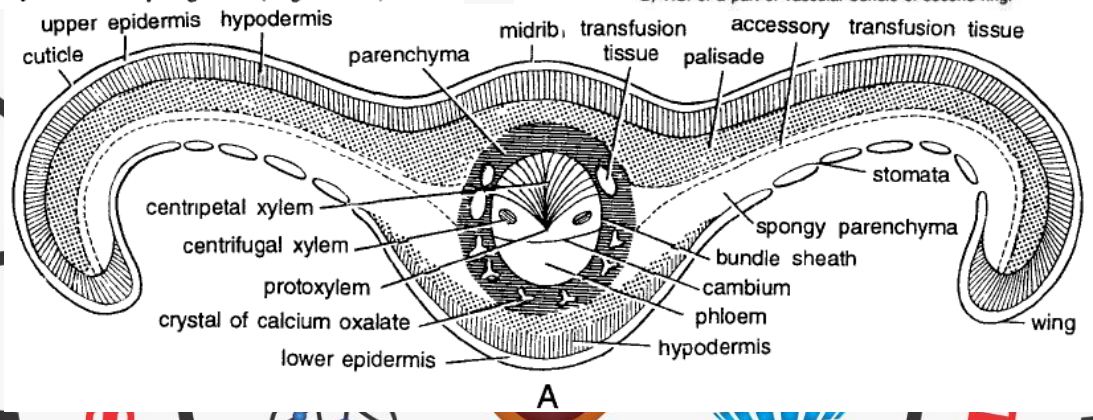


Fig. 8. *Cycas*. T.s. of young stem (diagrammatic).



Cycas revoluta. Antomy of stem. A, transverse section of a part of vascular bundle of first ring; B, T.S. of a part of vascular bundle of second ring.



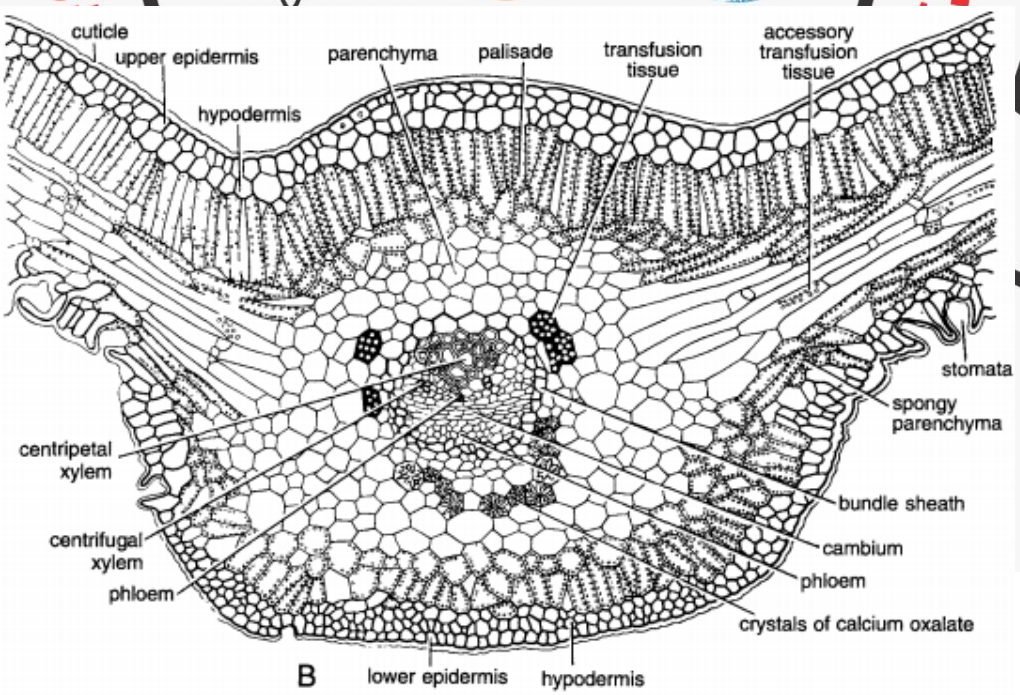


Fig. 12. *Cycas*. A. and B. T.s. of leaflet.

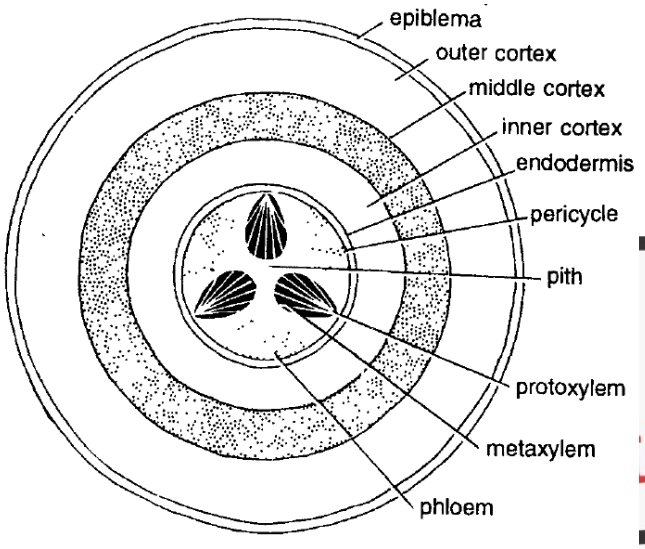


Fig. 6. ***Cycas.*** T.s. of coralloid root (diagrammatic).

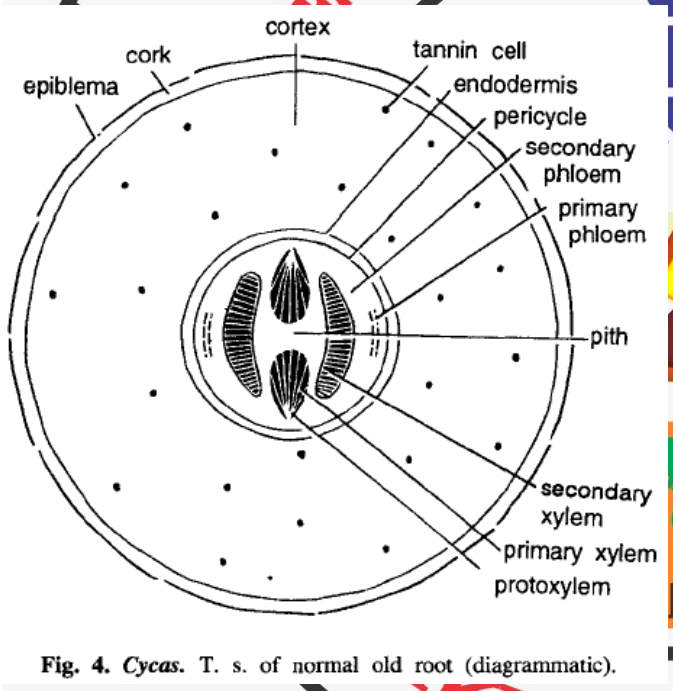


Fig. 4. ***Cycas.*** T. s. of normal old root (diagrammatic).

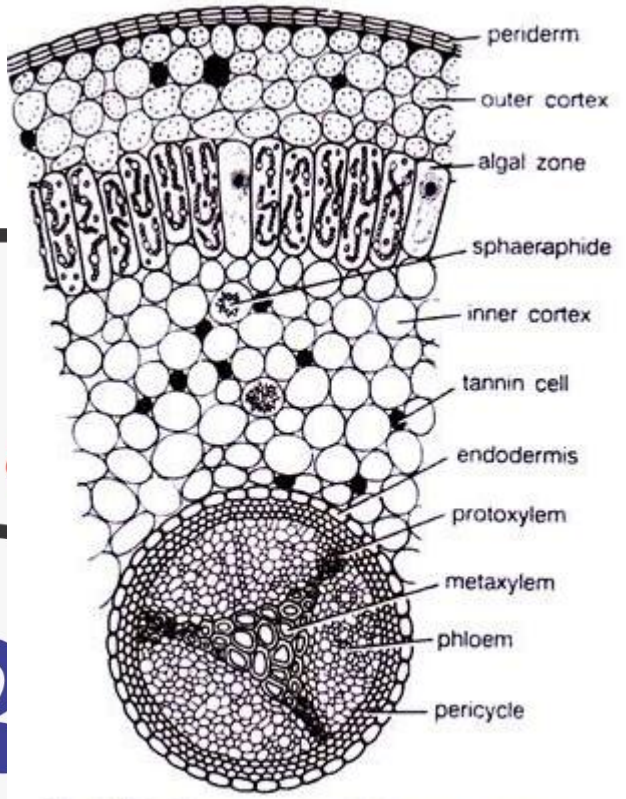


Fig. 8.18. *Cycas revoluta* T S coralloid root

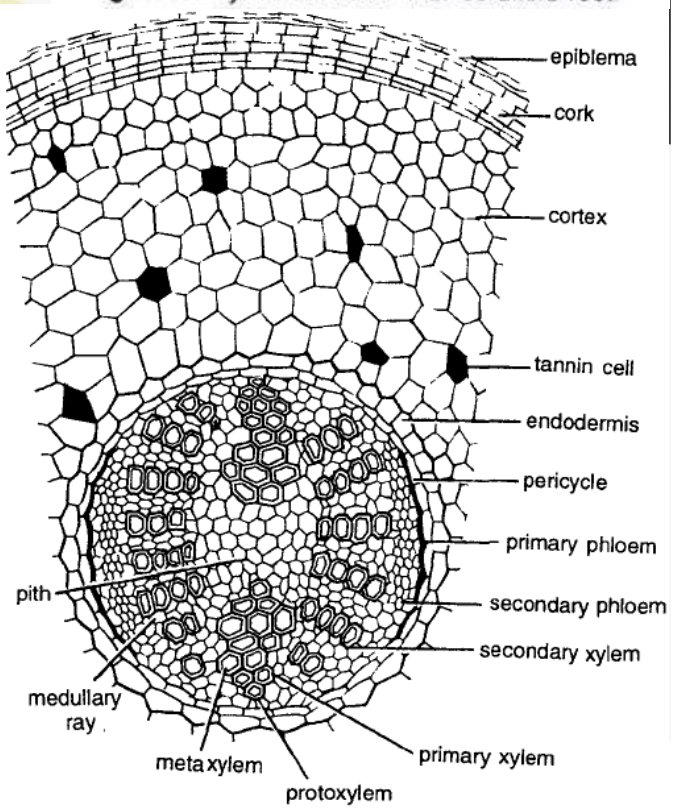


Fig. 5. ***Cycas.*** T. s. of old root (a part cellular).

Anatomy-T.S of Coralloid Root

1. The structure is almost similar to that of a normal root. It consists of epiblema, differentiated cortex and vascular tissues.
2. Epiblema is outermost and single layered.
3. The cortex is divisible into three regions -outer, middle and inner. These are similar in size. Cortex parenchymatous.
4. The middle cortex is also called algal zone. The cells are radially elongated. A blue-green alga *Anabaena cycadae* occurs endophytically in these cells. It is believed to be symbiotic and helps in nitrogen fixation.

5. Endodermis separates cortex and vascular tissues. It is single layered and followed by many layered pericyc1e. Vascular bundles are radial and xylem is triarch and exarch.
6. Secondary growth is generally absent; if present, it is very less.

Anatomy-T.S of Normal old Root

1. It shows secondary growth, rest of the structures being similar to that of a young root.
2. The epiblema is ruptured due to the thick walled cork cells found below it. Cork cells are a few layered.
3. Cortex is large, parenchymatous and multilayered. It is present below the cork. A few tannin filled cells occur scattered in the cortex.
4. Endodermis is single layered. It is followed by many layered pericyc1e.
5. Primary phloem is the outermost (near the pericycle) and is crushed during secondary growth. Secondary phloem follows this layer, the cells of which are intact.
6. Cambium arcs are found along the inner edges of phloem in the vascular region.
7. Secondary xylem is situated towards pith. The primary xylem is situated in the same region as it was before the secondary growth.
8. Medullary rays are found.
9. In the centre is a small parenchymatous pith.

Anatomy - T.S of young stem

1. Outline of the section is irregular due to the presence of numerous persistent leaf bases.
2. The structure is divisible into cortex, vascular tissue and pith.
3. Cortex - Greater part of the stem is made of starch filled parenchymatous cortex. It is traversed by many girdle-shaped leaf traces, supplying the leaves. Many mucilage ducts are irregularly scattered in this region.
4. Stele is an ectophloic siphonostele.
5. Endodermis surrounds the stele. It is single layered while underlying pericycle is few celled thick.
6. Vascular cylinder is composed of many vascular bundles arranged in a ring. Ring of vascular bundles lies near the centre and is very small in comparison to the massive cortex.
7. The vascular bundles are conjoint, collateral, endarch and open.
8. Xylem is made of tracheids only and xylem parenchyma. Vessels are absent.
9. Phloem consists of sieve tubes, phloem parenchyma and phloem fibres.
10. The young stem is monoxylic (Le. with one ring of vascular bundles only).
11. Pith - There is parenchymatous pith in the centre, with scattered mucilage canals.

Reproductive organs:

A Male cone:

1. Cycas is dioecious and as such, bears terminally, either male cone or female reproductive structures.
2. The male cone is terminal, shortly stalked, compact, large and oval or conical in shape and consists of a central cone axis around which numerous microsporophylls are spirally arranged.
3. The outer covering of the male cone is formed by closely set sterile ends of the microsporophylls usually possessing upcurved apices, apophysis.

L.S of Male cone:

1. The L.S. shows stalk and the cone.
2. Male cone is attached at the apex of the plant by a stout and broad stalk.
3. The cone itself consists of a central cone axis with many microsporophylls.
4. Each microsporophyll is attached to the cone axis. The part of microsporophylls away from the axis is upcurved and is called apophysis.
5. The upper surface of the microsporophyll is sterile.
6. The lower surface of the microsporophyll is fertile and bears many microsporangia in groups (sori).

7. Microsporophylls in the middle part of the cone are largest and get gradually smaller towards the base and the apex.

Microsporophyll with sori

1. A single microsporophyll is woody, more or less horizontally flattened and triangular structure.
2. It is differentiated into a fertile and sterile parts. Fertile part is wedge-shaped and is expanded distally from a narrow point of attachment. Sterile part is the distal part of the microsporophyll which tapers into an upcurved apophysis.
3. Lower (abaxial) surface of the fertile part of the microsporophyll bears microsporangia in groups of 3-4, forming definite sori.
4. Microsporangia are arranged in sori around central papilla. Sporangia show radial lines of dehiscence. Many hairs are distributed on this surface mixed with sporangia.

T.s. of Microsporophyll

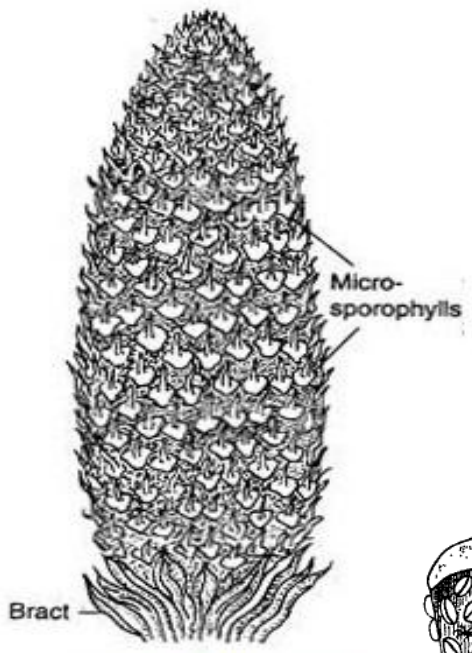
1. The section shows microsporangia attached to the abaxial (lower) surface by their short stalks.
2. A mature microsporangium has three layered wall. The outermost layer is thick and cutinized, termed as exothecium. The remaining inner layers are thin and are collectively known as endothecium and enclose a tapetum.
3. Numerous microspores remain enclosed inside the wall of the microsporangium.
4. In the microsporophyll are present many mucilage ducts, regularly scattered, among the rounded mesophyll-like cells forming the tissue of the sporophyll.

A Female reproductive structures

1. The female reproductive structures are the megasporophylls developing in place of foliage leaves. The vegetative apex continues to grow as usual.
2. The megasporophylls are smaller than the foliage leaves. They are brown or light brown in colour and are densely covered with wooly hairs.

Megasporphyll

1. Female reproductive body consists of megasporphylls arranged spirally and arising in acropetal succession on the stem.
2. Megasporophylls appear as a rosette or a crown, leaving the apical meristem unaffected to grow further. A crown of megasporophyll is formed each year. Numerically they are more than the leaves.
3. They leave their persistent bases on the stem.
4. Each megasporophyll is leaf-like and densely covered with brown hairs. It varies in size from 6 to 12 inches.
5. Each megasporophyll is distinguished into a proximal (lower) petiole, a middle ovule bearing portion and a distal (upper) pinnately dissected sterile part.
6. The middle portion of sporophyll bears ovules which are borne in two rows, one on either side. The ovules of the two rows may be opposite or alternate.
7. Ovules are generally yellow or orange or dark green coloured, shortly stalked, oval and smooth. Number and size of the ovules differ from species to species.



Cycas: A MALE CONE

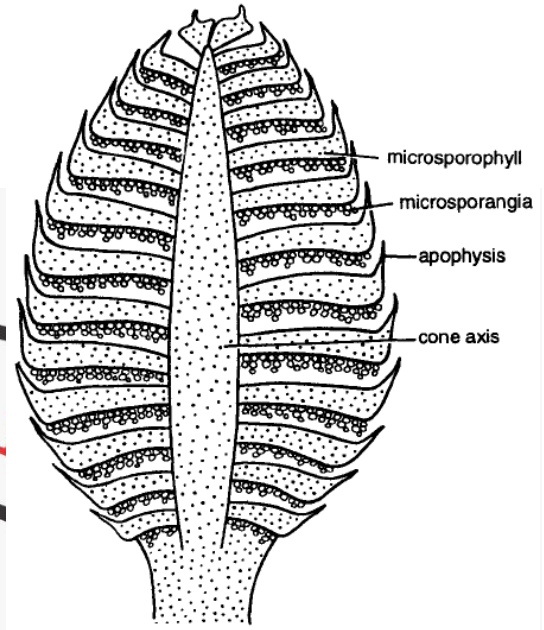
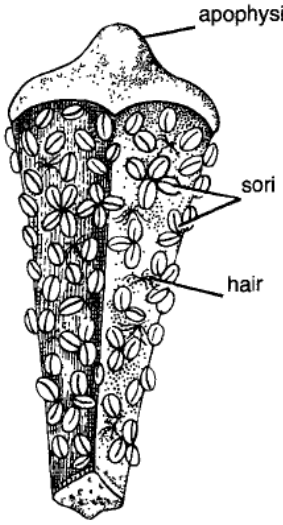


Fig. 14. ***Cycas***. L.s. of male cone.



Cycas. A microsporophyll from lower side.

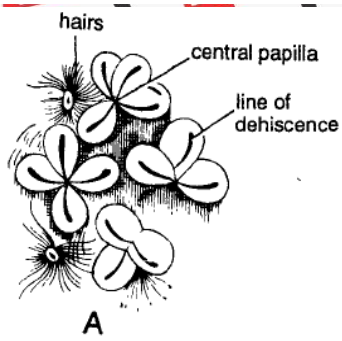


Fig. 16. ***Cycas***. Microsporangia in sori. **A.** Before dehiscence, **B.** After dehiscence.

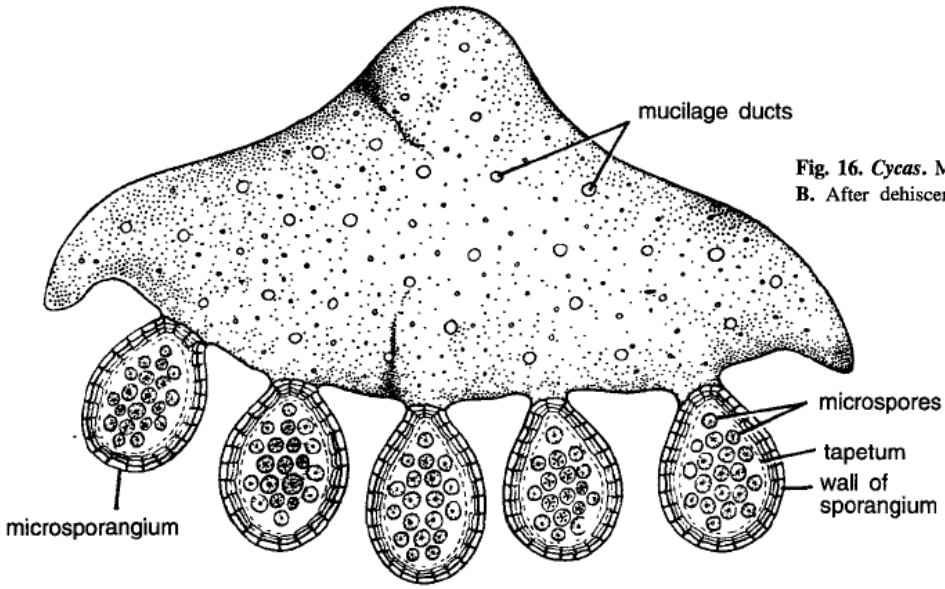


Fig. 17. ***Cycas***. T.s. of microsporophyll.

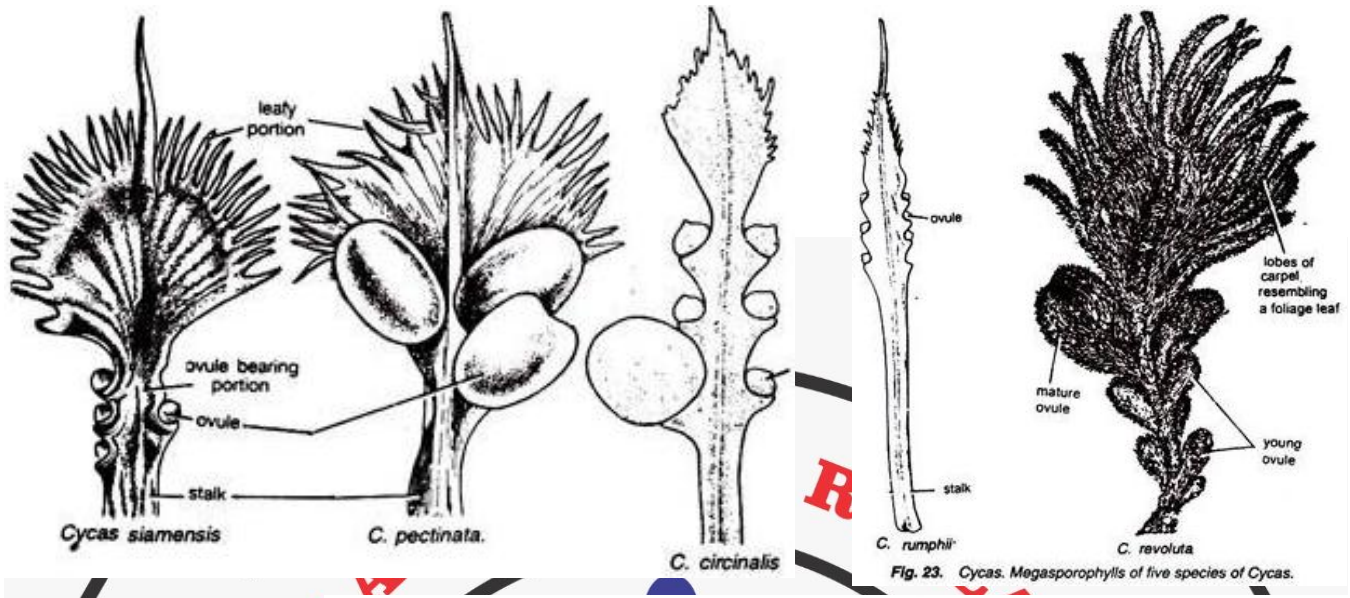


Fig. 23. Cycas. Megasporophylls of five species of Cycas.

L.S. of mature ovule

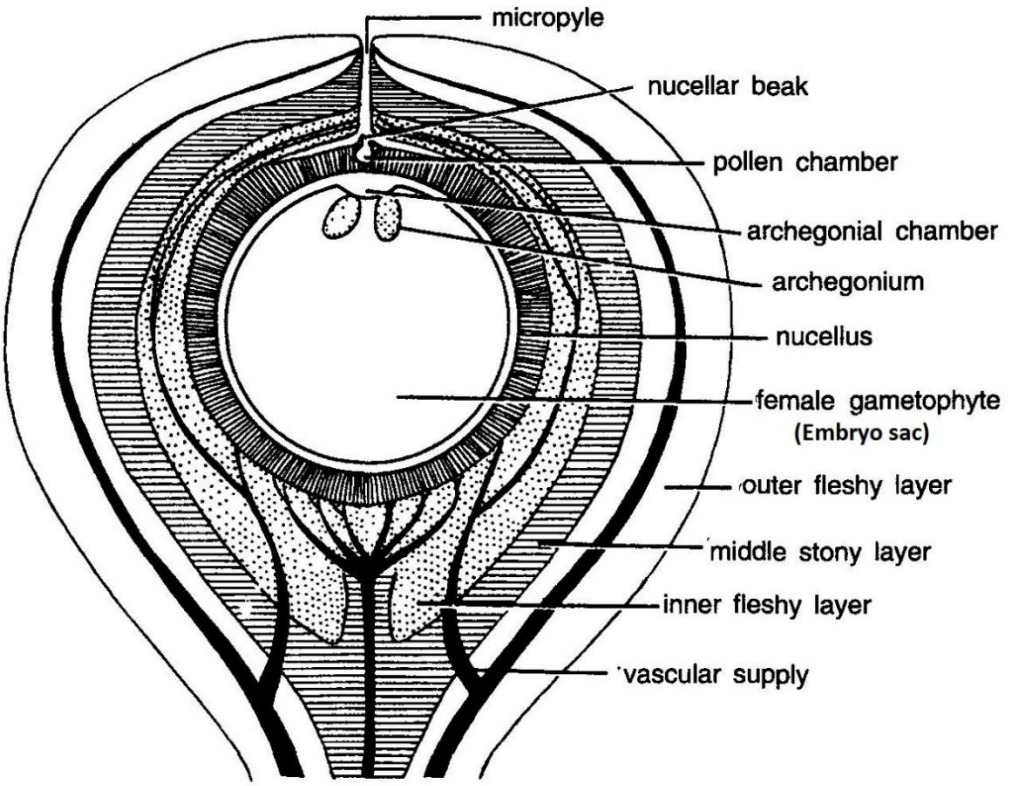


Fig. 19. ***Cycas.*** L.s. of ovule.

1. The section shows that the ovule is orthotropous.
2. It is unitegmic (possess a single integument). The integument is very thick. It remains fused with the nucellus except for the nucellar beak leaving a small and narrow micropyle.
3. The integument consists of three distinct layers an outer fleshy layer, middle stony layer and an inner fleshy layer. The outer and inner fleshy layers are supplied with vascular strands but the middle stony layer receives no vascular supply.
4. The nucellus lies just below the integument and forms a nucellar beak in the region of the micropyle.
5. A few cells of this nucellar beak dissolve themselves and form a pollen chamber that lies in the tissue in the central region of the beak.
6. Female gametophyte. The innermost region of the ovule is filled with the tissue of female gametophyte, wherein lie two archegonia, situated opposite the pollen chamber.
7. Just above the archegonia is the archegonial chamber.
8. The orange coloured, fleshy ovules are oval in shape and each shows a small point at the distal end which represents the remnant of the micropyle.

Pinus

SYSTEMATIC POSITION

Division : Coniferophyta
Class : Coniferopsida
Order : Coniferales
Family : Pinaceae
Example : *Pinus*

INTRODUCTION:

The plant *Pinus* is a large tree with worldwide distribution. They grow abundantly in the temperate regions of eastern and western Himalayas. The genus *Pinus* has about 90 living species. The common Himalayan species of *Pinus* are *Pinus wallichiana, Pinus khasya; Pinus roxburghii; Pinus excelsa, Pinus sylvestris*. Some species of *Pinus* are of great economic values.

HABIT STRUCTURE:

1. *Pinus* is a tall, perennial, erect, evergreen tree.
2. Sporophytic plant body is differentiated into roots, stem and acicular (needle-like) leaves.
3. The main trunk is unbranched.
4. Branches grow spirally and thus the plant gives the appearance of a conical or pyramidal structure.
5. A well-developed tap root with few root hair are present (it disappears soon). Later on many lateral roots develop, which help in absorption and fixation. The ultimate branches of these roots are covered by a covering of fungal hyphae called ectotrophic mycorrhizae.
6. The stem is cylindrical and remains covered with scaly bark. Branching is monopodial.
7. Two types of branches are present: long shoots (branches of unlimited) and dwarf shoots (branches of limited growth).
8. Long shoots contain apical bud and grow indefinitely. Many scaly leaves are present on the long shoot.
9. Dwarf shoots are devoid of any apical bud and thus are limited in their growth. They arise on the long shoot in the axil of scaly leaves.
10. The leaves are of two types, i.e., foliage and scaly.
11. Scaly leaves are small, thin, brown-coloured and scale like and develop on long as well as dwarf shoots.
12. Foliage leaves are present at the apex of the dwarf shoots only.

T.S. of Needle (Foliage Leaf):

1. It is circular in outline in *P.* monophylla, semicircular in *P. sylvestris* and triangular in *P. longifolia*, *P. roxburghii*, etc.
2. Outermost layer is epidermis, which consists of thick-walled cells. It is covered by a very strong cuticle.
3. Many sunken stomata are present on the epidermis.
4. Each stoma opens internally into a substomatal cavity.
5. Below the epidermis are present a few layers of thick-walled sclerenchymatous hypodermis. It is well-developed at ridges.
6. In between the hypodermis and endodermis is present the mesophyll tissue.
7. Cells of the mesophyll are polygonal and filled with chloroplasts. Many peg-like infoldings of cellulose also arise from the inner side of the wall of mesophyll cells.
8. Few resin canals are present in the mesophyll, adjoining the hypodermis. Their number is variable but generally they are two in number.
9. Endodermis is single-layered with barrel-shaped cells and clear casparian strips.

HABIT OF PINUS

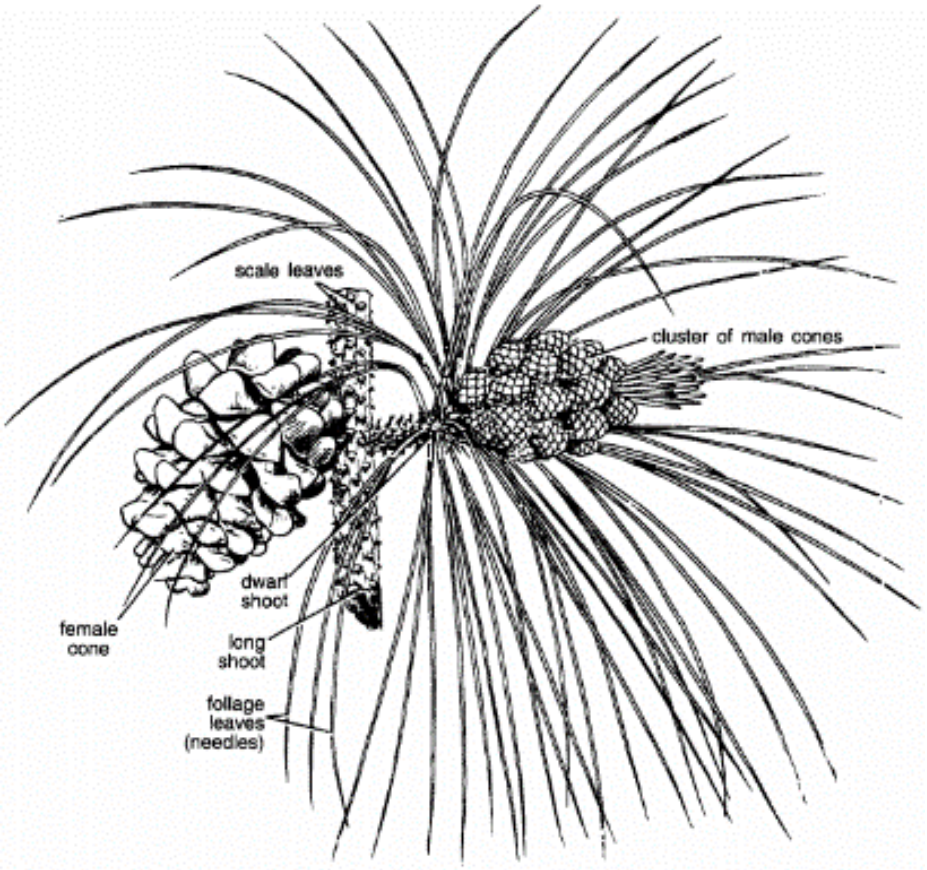
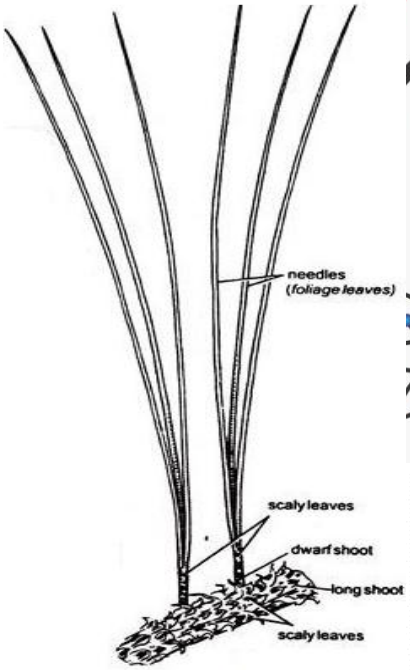


Fig. 14. *Pinus*. A twig with male and female cones.



TRIFOLIAR SPUR

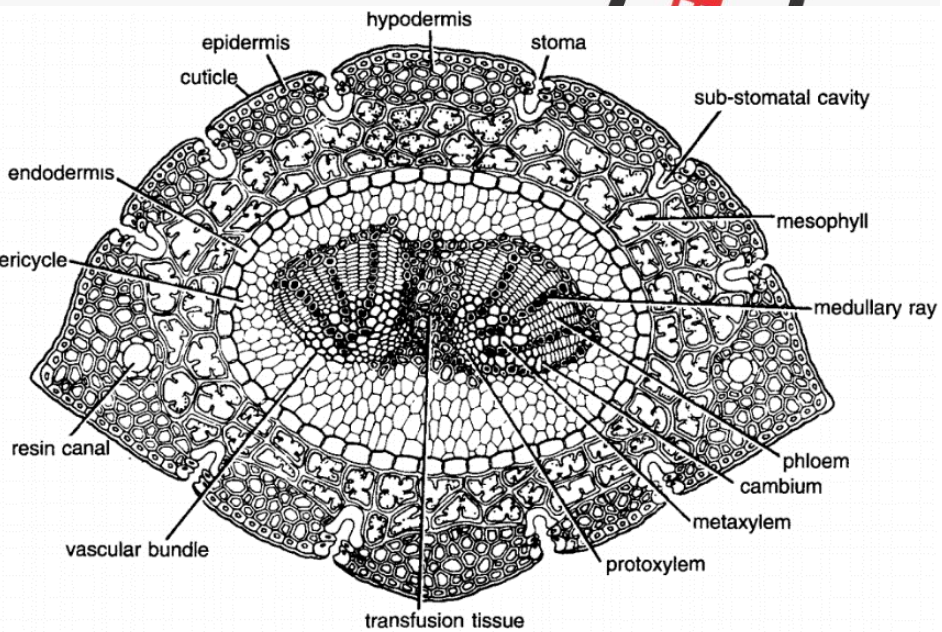


Fig. 13. *Pinus*. T.s. of needle (foliage leaf)-cellular details.

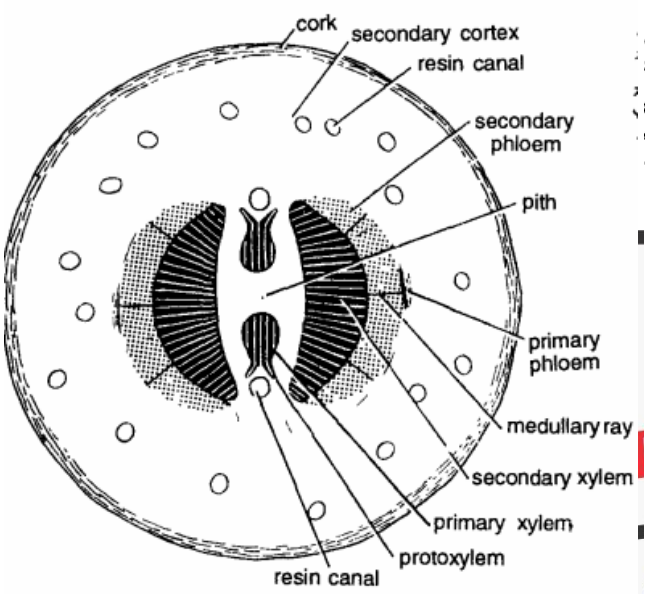


Fig. 3. *Pinus.* T.s. of old root (diagrammatic).

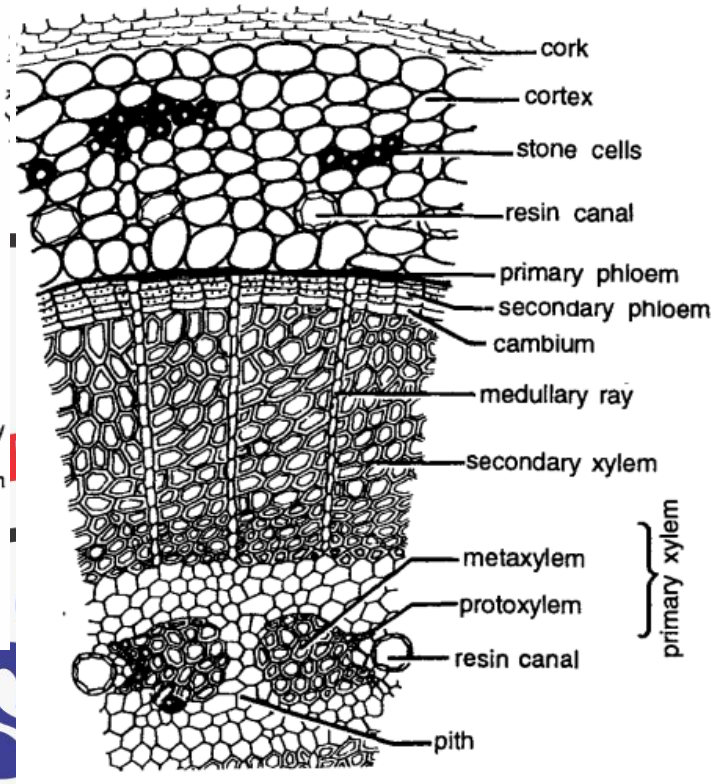
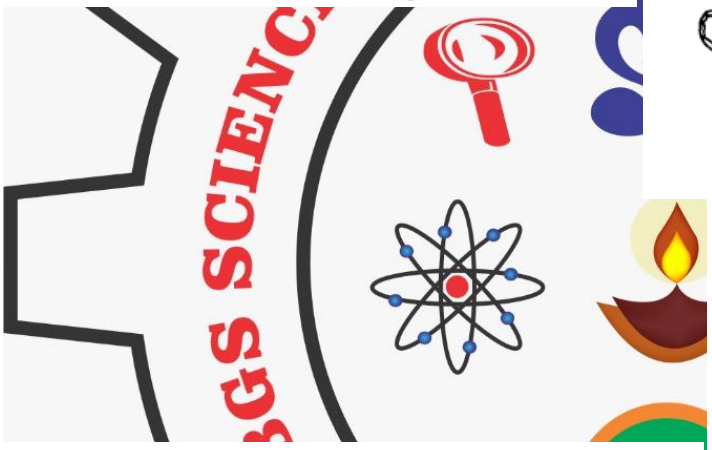


Fig. 4. *Pinus.* T.s. of old root (a part cellular).



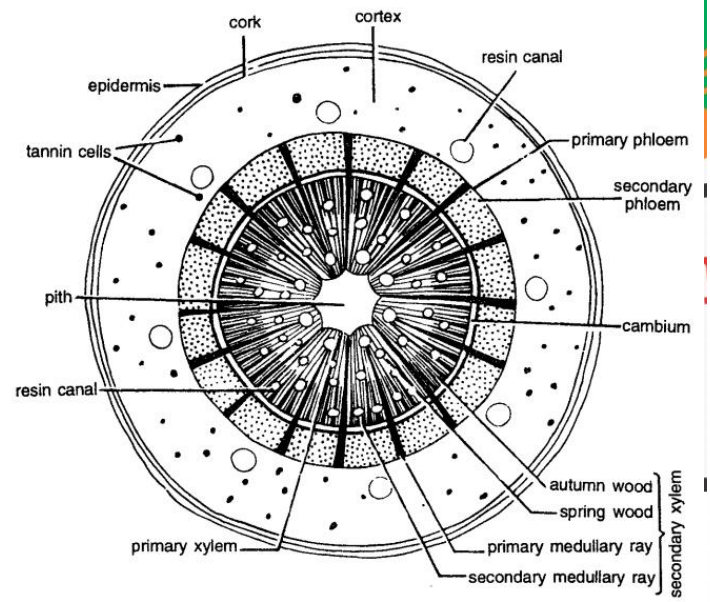


Fig. 6. *Pinus.* T.s. of old long shoot (diagrammatic).

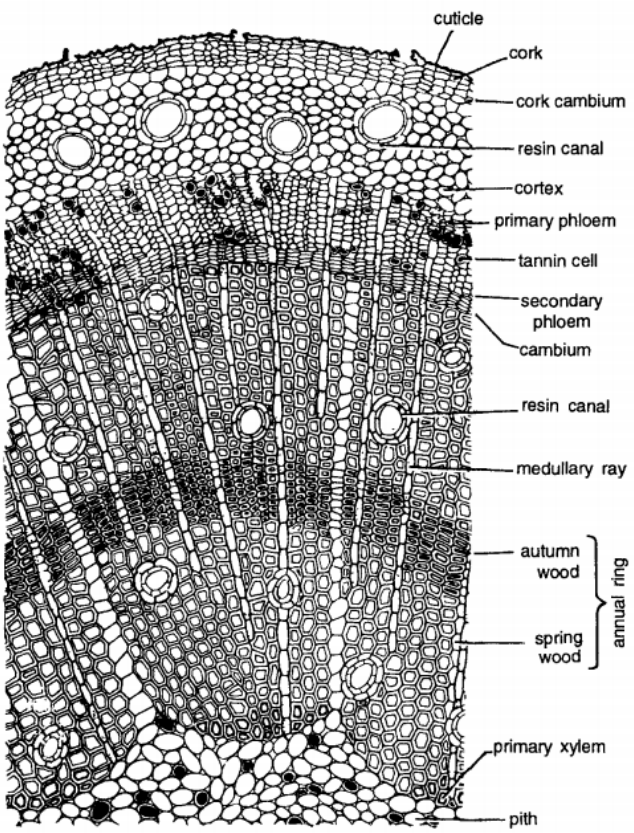
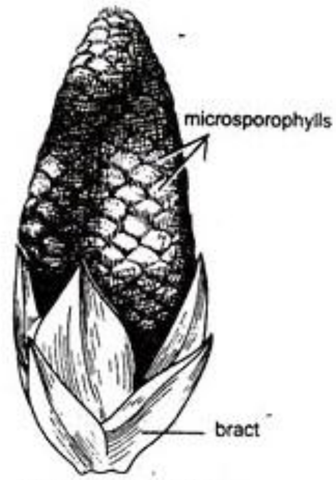


Fig. 7. *Pinus.* T.s. of old long shoot a part cellular.



A MALE CONE

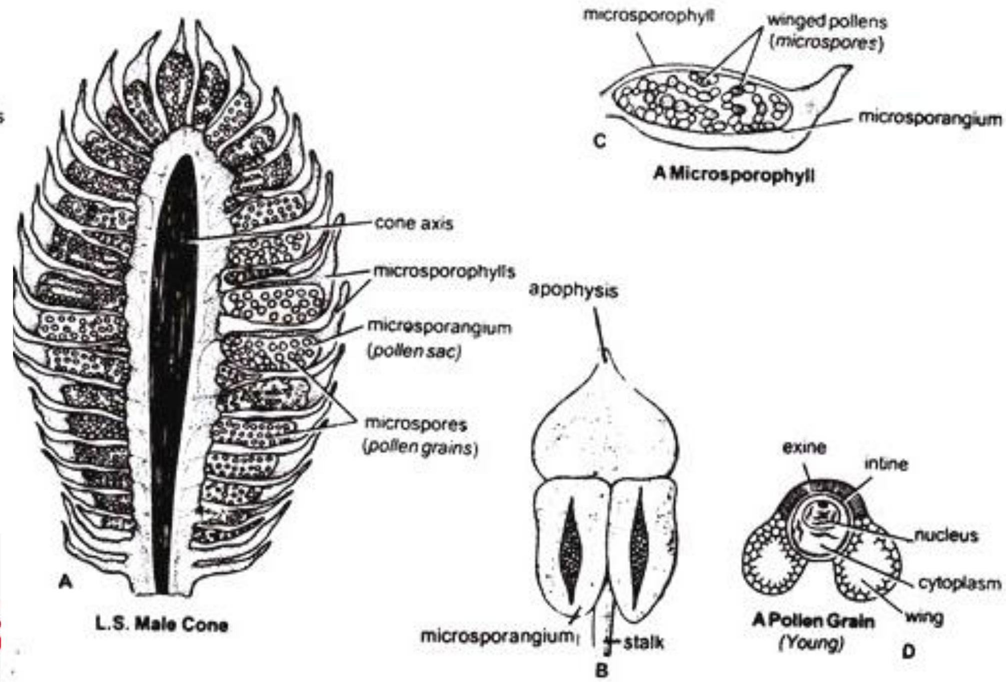
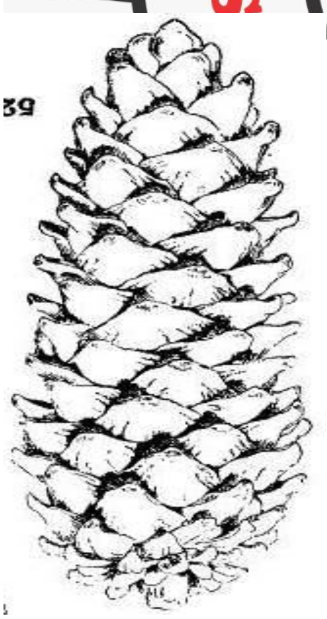
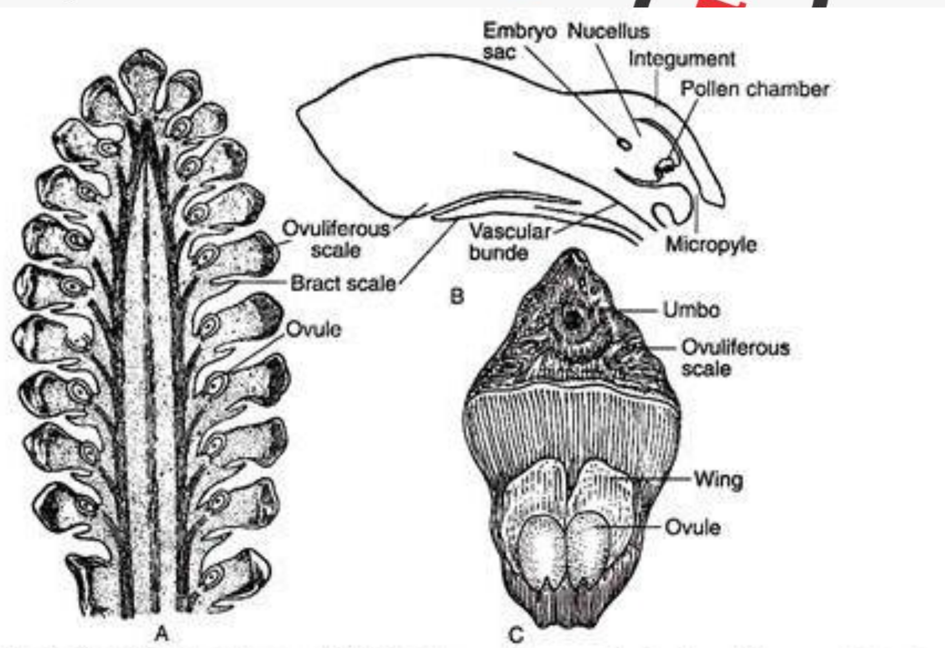


Fig. 41. *Pinus. A, L.S. male cone; B, A single microsporophyll with microsporangia in surface view; C, A microsporophyll; D, A young pollen grain.*



A FEMALE CONE



Pinus : A. Median L.S. of female cone, B. V.L.S. of an ovuliferous scale, C. An ovuliferous scale bearing two seeds (fertilized ovules)

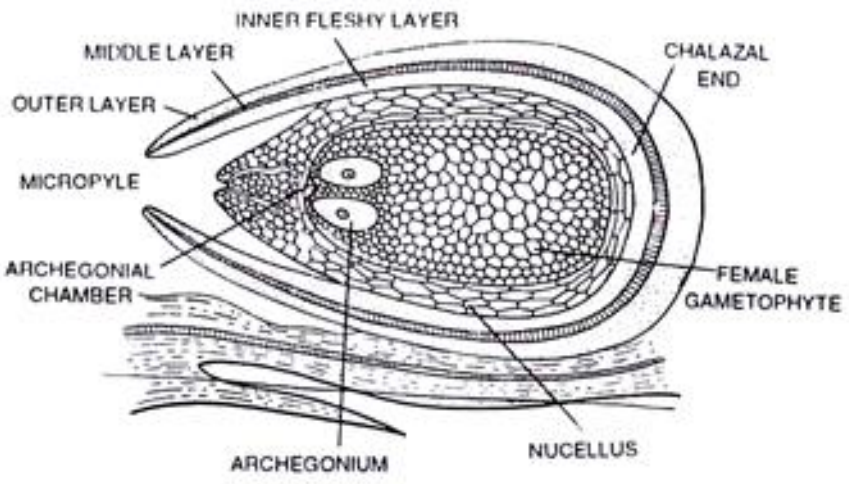


Fig. 4.39. *Pinus roxburghii*. L.S. of mature ovule showing archegonia.

10. Pericycle is multilayered and consists of mainly parenchymatous cells and some sclerenchymatous cells forming T-shaped girdle, which separates two vascular bundles. Transfusion tissue consists of tracheidial cells.
11. Two conjoint and collateral vascular bundles are present in the centre. These are closed but cambium may also present in the sections passing through the base of the needle.
12. Xylem lies towards the angular side and the phloem towards the convex side of the needle.

Anatomy : T.S. of old root

1. Outermost layer of the circular roots is thick-walled epiblema with many root hairs. On the outer side are present a few layers of cork, formed by the meristematic activity of the cork cambium.
2. Epiblema is followed by many layers of parenchymatous cortex. Cork cambium cuts secondary cortex towards inner side. Many resin canals and stone cells are present in the secondary cortex, the cells of which are separated with the intercellular spaces.
3. Inner to the cortex is present a layer of endodermis and many layers of pericycle.
4. Vascular bundles are radially arranged and diarch to tetrarch with exarch protoxylem.
5. Protoxylem is bifurcated (Y-shaped) towards the periphery, and in between each bifurcation is present a resin canal.
6. Phloem is present alternate to the protoxylem. Below the phloem patches develop cambium, which cuts secondary phloem towards outer side and secondary xylem towards inner side. Crushed primary phloem is present outside the secondary phloem. Many uniseriate medullary rays are present in the secondary xylem.
7. Pith is poorly-developed or absent.

Anatomy : T.S. of old stem

1. Many leaf bases are present on the stem, due to which it appears wavy in outline.
2. Outermost single-layered, thick-walled epidermis is heavily cuticularized and followed by multilayered cortex.
3. A few outer layers of cortex are sclerenchymatous, and some inner layers are parenchymatous.
4. In the inner layers of cortex are present many tannin-filled cells and resin canals.
5. The stele is eustelic or polyfascicular endarch siphonostele.
6. Vascular bundles are conjoint, collateral, open and endarch, and resemble greatly with that of a dicot stem. 5-10 vascular bundles are arranged in a ring.
7. Endodermis and pericycle are indistinguishable.
8. Narrow xylem rays connect the cortex and pith.
9. Endarch xylem consists of only tracheids.
10. Phloem is present on the ventral side and consists of sieve cells, sieve plates, phloem parenchyma and some albuminous cells.
11. Intrafascicular cambium is present in between the xylem and phloem.
12. Many leaf traces are also present.
13. A small parenchymatous pith is present in the centre of stem.

Secondary growth

1. Secondary growth, similar to that of a dicotyledonous stem, is present in the old stem of *Pinus* .
2. Cork cambium cuts cork towards outer side and a few layers of secondary cortex towards inner side.
3. Cambium cuts secondary phloem towards outer side and secondary xylem towards inner side
4. Primary phloem is crushed and pushed towards outer side by the secondary phloem.
5. In the secondary xylem, annual rings of thin-walled spring wood (formed in spring season) and thick-walled autumn wood (formed in autumn season) are present alternately. Such a compact wood is called pycnoxylic (Age of the plant can be calculated by counting the number of these annual rings).
6. Below the secondary xylem are present a few groups of endarch primary xylem.
7. Some of the medullary rays connect the pith with the cortex and called primary medullary rays while the others run in between secondary xylem and secondary phloem and called secondary medullary rays.
8. Resin canals are present in cortex, secondary xylem, primary xylem and rarely in the pith.

Reproductive Structures

Pinus is monoecious, and male and female flowers are present in the form of cones or strobili on the separate branches of the same plant. Many male cones are present together in the form of clusters, each of which consists of many microsporophylls. The female cones consist of megasporophylls. The male cones on the plant develop much earlier than the female cones.

Male Cone:

1. The male cones develop in clusters in the axil of scaly leaves on long shoot.
2. They replace the dwarf shoots of the long shoot.
3. Each male cone is ovoid in shape and ranges from 1.5 to 2.5 cm. in length
4. A male cone consists of a large number of microsporophylls arranged spirally on the cone axis.
5. Each microsporophyll is small, membranous, brown-coloured structure.
6. A microsporophyll is comparable with the stamen of the flower of angiosperms because it consists of a stalk (=filament) with a terminal leafy expansion (= anther), the tip of which is projected upwards and called apophysis.
7. Two pouch-like microsporangia (= pollen sacs) are present on the abaxial or undersurface of each microsporophyll. In each microsporangium are present many microspores (= pollen grains).
8. Each microspore or pollen grain is a rounded and yellow-coloured, light, uninucleate structure with two outer coverings, i.e., thick outer exine and thin inner intine.
9. The exine protrudes out on two sides in the form of two balloon-shaped wings. Wings help in floating and dispersal of pollen grains.
10. Wings help in floating and dispersal of pollen grains.
11. A few microsporophylls of lower side of cone are sterile. Sporangia are also not present on the adaxial surface of each microsporophyll of the male cone.

Female cone:

1. Female cone develops either solitary or in groups of 2 to 4.
2. They also develop in the axil of scaly leaves on long shoots like male cones.
3. Each female cone is an ovoid, structure when young but becomes elongated or cylindrical at maturity.

L.S. Female Cone:

1. In the centre is present a cone axis.
2. Many megasporophylls are arranged spirally on the cone axis.
3. A few megasporophylls, present at the base and at the apex of strobilus, are sterile.
4. Megasporophylls present in the middle of the strobilus are very large and they decrease in size towards the base and apex.
5. Each megasporophyll consists of two types of scales, known as bract scales and ovuliferous scales.
6. Bract scales are thin, dry, membranous, brown- coloured structures having fringed upper part. These are also called carpellary scales.
7. An ovuliferous scale is present on the upper surface of each bract scale.

8. Each ovuliferous scale is woody, bigger and stouter than bract scale and it is triangular in shape. A broad sterile structure, with pointed tip, is present at the apex of these scales. This is called apophysis.
9. At the base of upper surface of each ovuliferous scale are present two sessile and naked ovules.
10. Micropyle of each ovule faces towards the cone axis.
11. Each ovule is orthotropous, and it remains surrounded by a single integument, consisting of an outer fleshy, a middle stony and an inner fleshy layer. It opens with an opening called micropyle.
12. Integument surrounds the megasporangium or nucellus.
13. Just opposite the micropyle is present a pollen chamber.
14. In the embryosac or female gametophyte are present 2 to 5 archegonia.

Structure of a Mature ovule or T/S of mature ovule.

1. Ovule is elongated in shape.
2. It is unitegmic and the integument is three layered. The outermost layer is thin. The middle layer is stony and prominent. The innermost layer is fleshy and well developed.
3. Nucellus is fused with inner layer of the integument, except at its tip where it forms an elongated and slender micropyle, directed towards the cone axis.
4. In the nucellar region lies a small cavity just opposite the micropyle. It is known as pollen chamber.
5. Female gametophyte (embryosac) is differentiated from nucellus. About 2-5 archegonia are situated in this region at the micropylar end near the base of the archegonial chamber.

Gnetum

SYSTEMATIC POSITION

Division : Chlamydospermatophyta
Class : Gnetopsida
Order : Gnetales
Family : Gnetaceae
Example : *Gnetum*

Introduction:

Gnetum, represented by about 40 species is confined to the tropical and humid regions of the world. Nearly all species, except *G. microcarpum*, occur below an altitude of 1500 metres. Five species (*Gnetum contractum*, *G. gnemon*, *G. montanum*, *G. ula* and *G. latifolium*) have been reported from India. *Gnetum ula* is the most commonly occurring species of India.

Habit Structure:

1. Majority of the Gnetum species are climbers except a few shrubs and trees. Gnetum plants resemble angiosperm plants.
2. The main plant body is sporophyte and it is differentiatd into root, stem and leaves.
3. Root is branched which does not grow very deep.
4. Two types of branches are present on the main stem of the plant, i.e. branches of limited growth and branches of unlimited growth. Each branch contains nodes and internodes.
5. Stem of several species of Gnetum is articulated with prominent joints.
6. In climbing species the branches of limited growth or short shoots are generally un-branched and bear the foliage leaves. The leaves (9-10) are arranged in decussate pairs. They often lie in one plane giving the appearance of a pinnate leaf to the branch. The leaves are large and oval with entire margin and reticulate venation. Some scaly leaves are also present.

Anatomy T/S of young Root:

1. T/S of Young root show the following regions like epiblema, cortex and vascular region.
2. Epiblema – is the outermost layer composed of compactly arranged cells, some cells gives out unicellular root hairs.
3. Cortex – is present below the epiblema and it is composed of several layers of starch-filled, loosely arranged parenchymatous cells, which are large and polygonal in outline. An endodermal layer is distinguishable and is innermost to the cortex. Casparian strips are seen in the cells of the endodermis. The endodermis follows 4-6 layered pericycle.
4. Vascular cylinder - Roots are diarch, radial and exarch. Small amount of primary xylem, visible in young roots, becomes indistinguishable after secondary growth.
5. The secondary growth is of normal type. A continuous zone of wood is present in the old roots. It consists of tracheids, vessels and xylem parenchyma. Some of the xylem elements have starch grains. Phloem consists of sieve cells and phloem parenchyma.

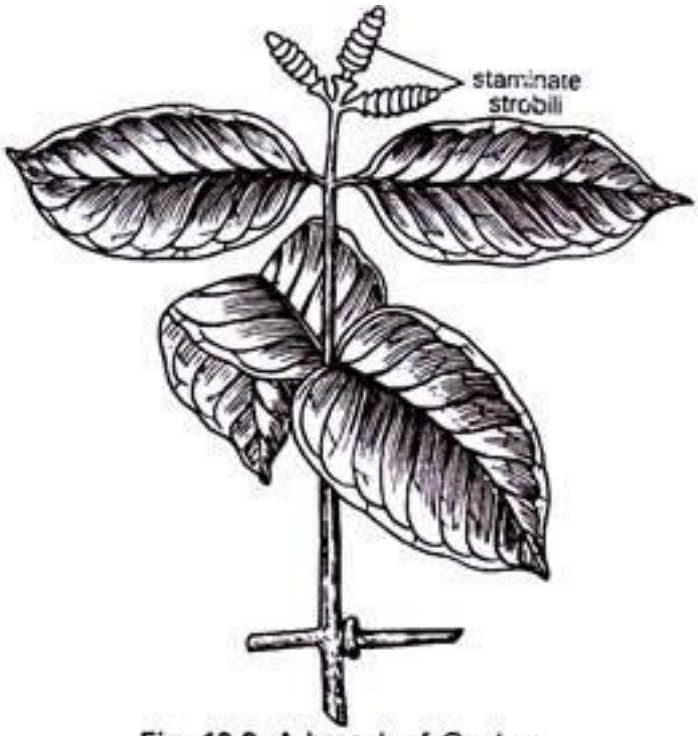
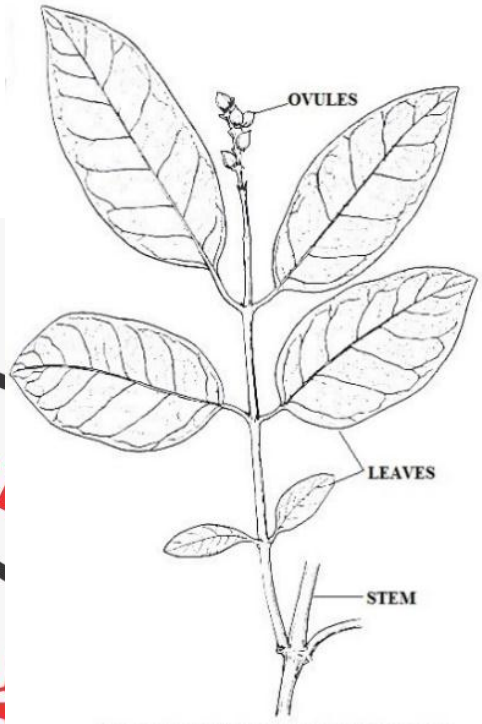
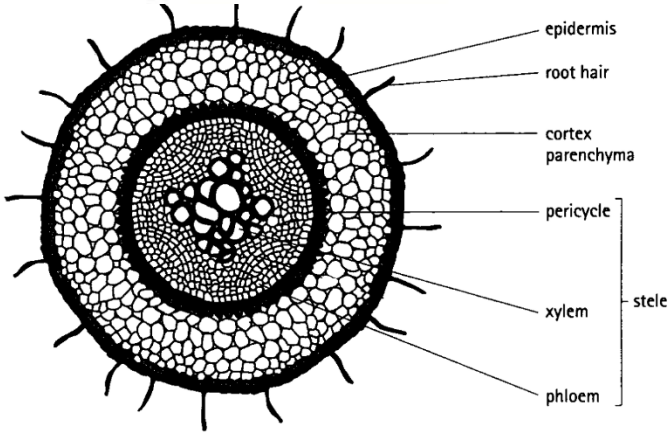


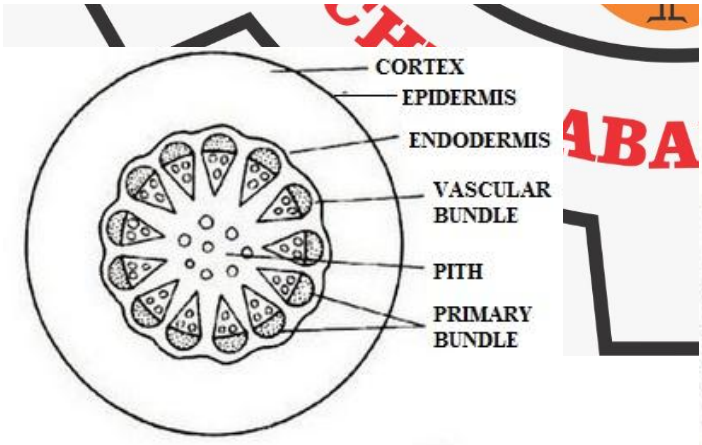
Fig. 13.2. A branch of *Gnetum*.



HABIT OF GNETUM WITH FEMALE CONE



T/S OF YOUNG ROOT - GROUND PLAN



GROUND PLAN OF T/S OF YOUNG STEM

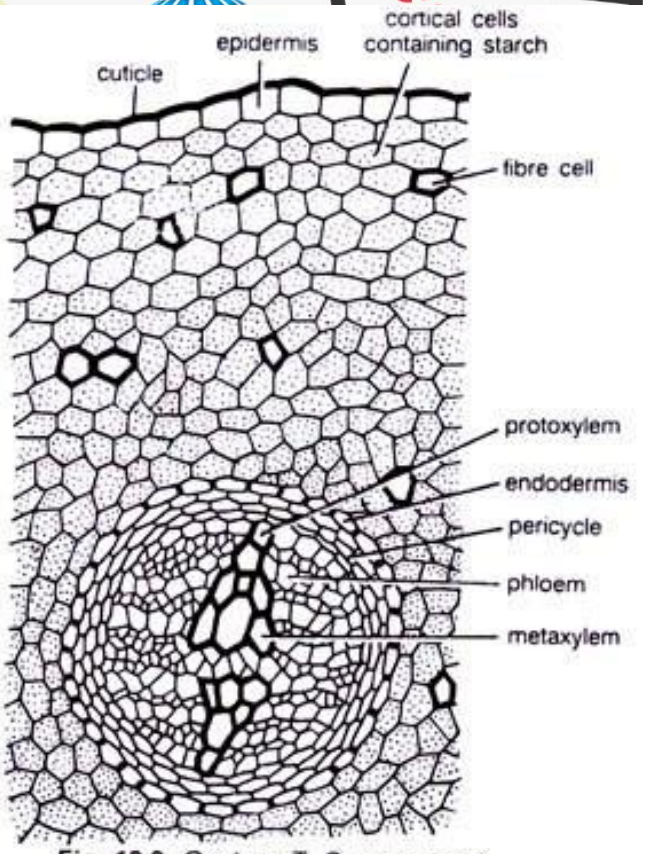


Fig. 13.3. *Gnetum*. T. S. young root.

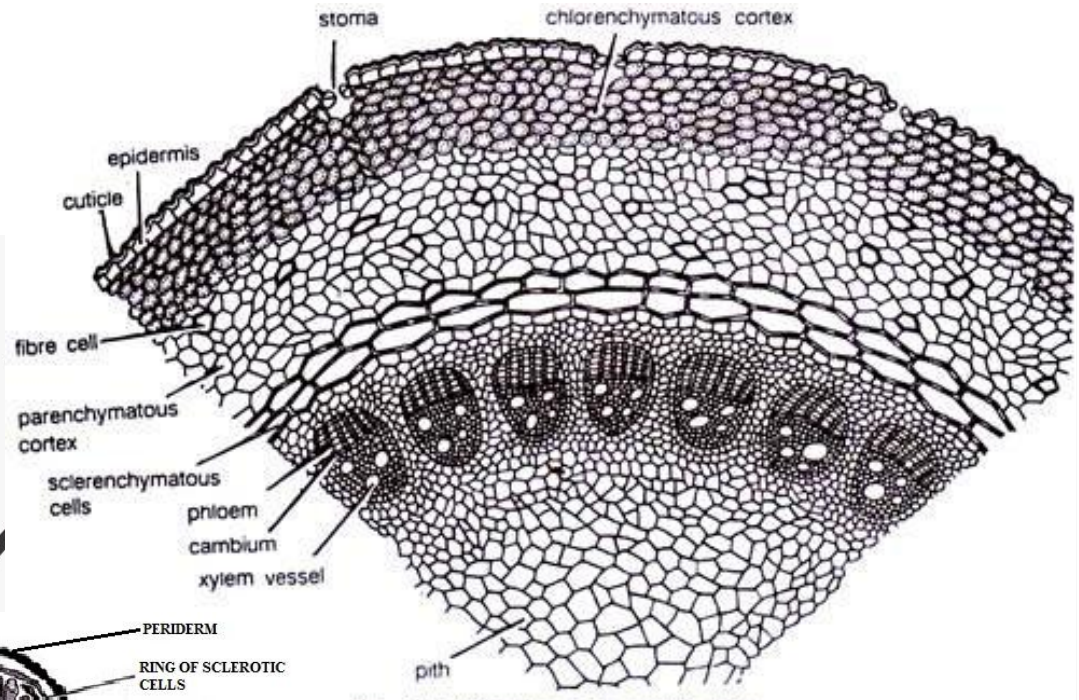
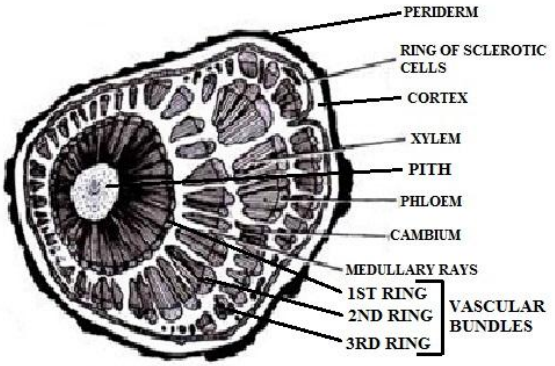
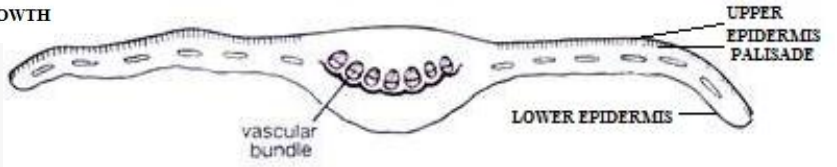
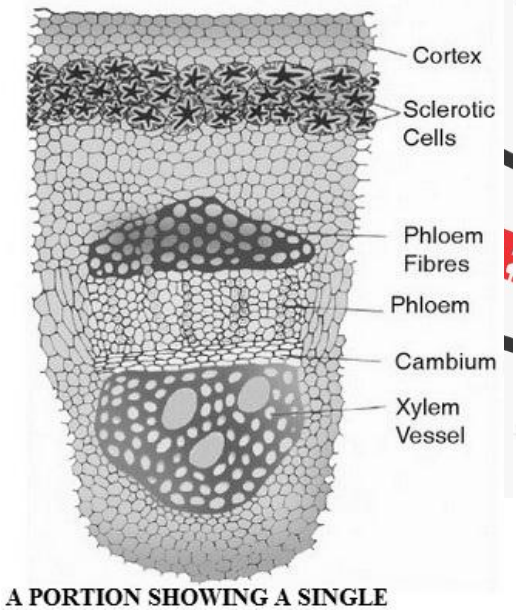


Fig. 13.5. Gnetum. T. S. young stem.

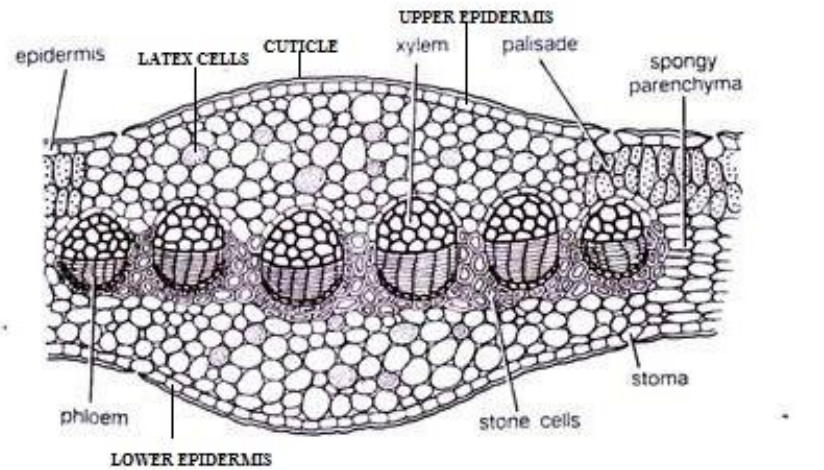


T/S OF OLD STEM SHOWING ECCENTRIC SECONDARY GROWTH



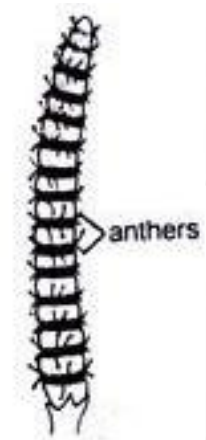


A PORTION SHOWING A SINGLE VASCULAR BUNDLE

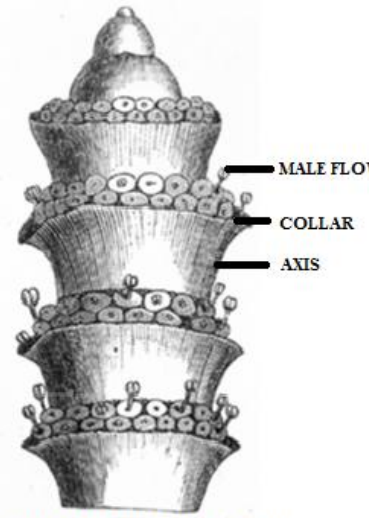


Gnetum. Upper-T.S. leaf (diagrammatic) ; Lower-T.S. leaf (a part cellular).

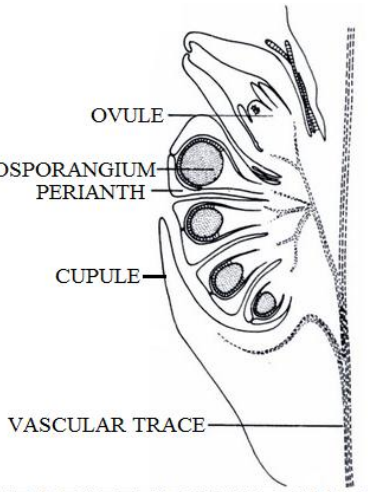
MALE CONES



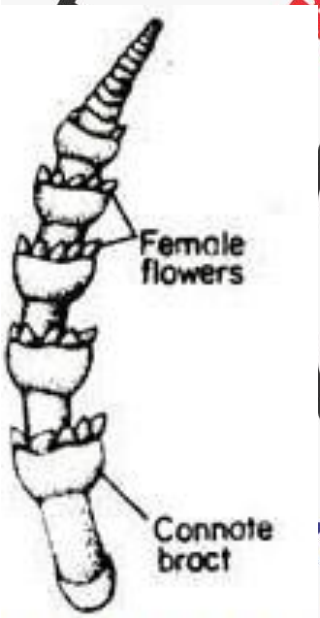
A SINGLE MALE CONE



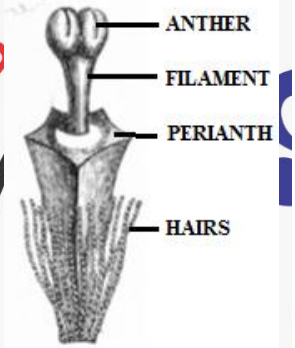
A PART OF MALE CONE



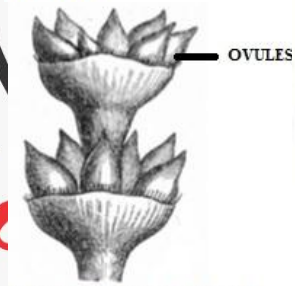
L/S OF A PART OF MALE CONE



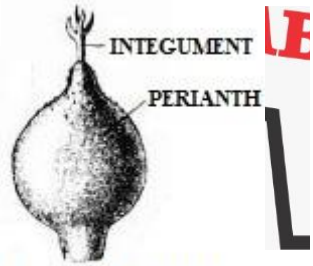
A FEMALE CONE



A SINGLE MALE FLOWER



A PART OF SINGLE FEMALE CONE



A SINGLE FEMALE FLOWER (OVULE)

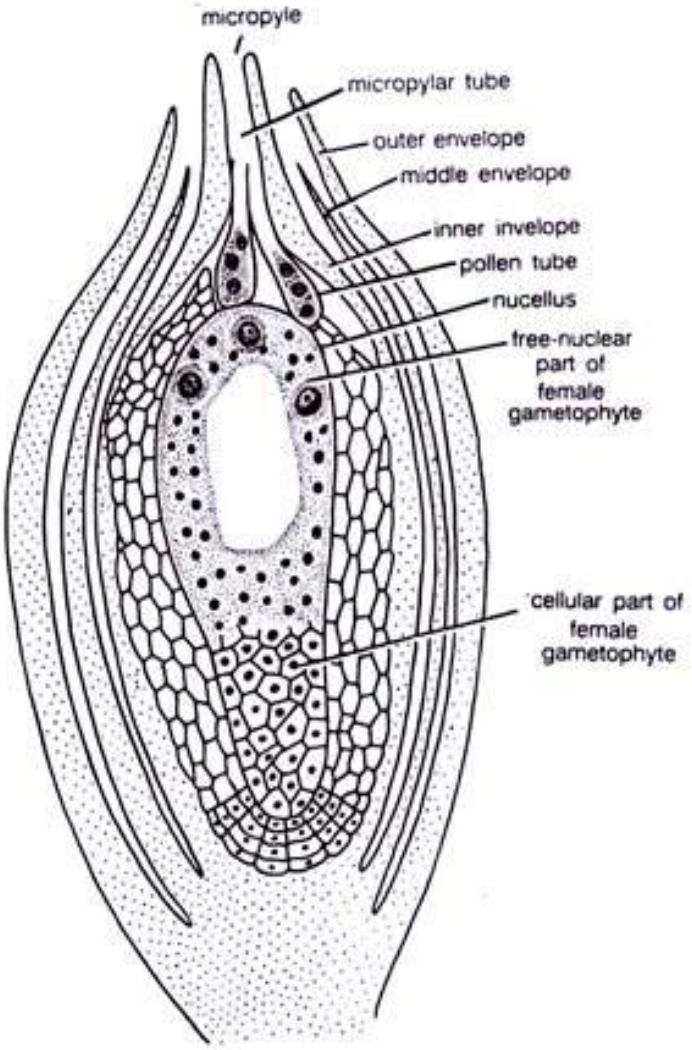


Fig. 13.16. *Gnetum*. L.S ovule.

Anatomy T/S of Young Stem: shows the following regions like epidermis, cortex and stele.

1. The young stem in transverse section is roughly circular in outline. It remains surrounded by a single-layered epidermis, which is thickly circularized and consists of rectangular cells. Some of the epidermal cells show papillate outgrowths. Sunken stomata are present.
2. The cortex consists of outer 5-7 cells thick chlorenchymatous region, middle few-cells thick parenchymatous region and inner 2-4 cells thick sclerenchymatous region. Endodermis and pericycle regions are not very clearly distinguishable.
3. Stele – is composed of 20 -24 vascular bundles. Several conjoint, collateral, open and endarch vascular bundles are arranged in a ring in the young stem.
4. Xylem consists of tracheids and vessels. Presence of vessels is an angiospermic character. Protoxylem elements are spiral or annular while the metaxylem shows bordered pits which are circular in outline. The phloem consists of sieve cells and phloem parenchyma.
5. An extensive pith, consisting of polygonal, parenchymatous cells, is present in the centre of the young stem.

Anatomy T/S of old Stem: shows the following features:

Old stems in Gnetum show secondary growth. In *G. gnemon* the secondary growth is normal. But in majority of the species (e.g., *G. ula*, *G. africanum*, etc.) the anamolous secondary growth is present. The primary cambium is ephemeral, i.e., short-lived. The secondary cambium in different parts of cortex develops in the form of successive rings, one after the other. The first cambium cuts off secondary xylem towards inside and secondary phloem towards outside. This cambium ceases its function after some time. Another cambium gets differentiated along the outermost secondary phloem region, and the same process is repeated. In the later stages, more secondary xylem is produced on one side and less on the other side, and thus the eccentric rings of xylem and phloem are formed in the wood.

The periderm is thin and develops from the outer cortex. It also possesses lenticels. The cortex also contains chlorenchymatous and parenchymatous tissues along with many sclereids. In old stems the secondary wood consists of tracheids and vessels.

Anatomy T/S of leaf: shows the following regions:

1. Epidermis: It is bounded by a layer of thickly circularized epidermis on both the surfaces. Stomata are distributed all over the lower surface except on the veins.

2. Mesophyll: is present between upper and lower epidermis. The mesophyll is differentiated generally into upper compactly arranged single-layered palisade parenchyma cells and a well-developed spongy parenchyma is present above the lower epidermis. The latter consists of many loosely-packed cells. Many stellately branched sclereids are present near the lower epidermis in the spongy parenchyma. Many stone cells and latex tubes are present in the midrib region of the leaf.

3. Vascular bundles: Several vascular bundles (5-6) in the form of an arch or curve are present in the prominent midrib region. A ring of thick-walled stone cells is present just outside the phloem. Each vascular bundle is conjoint, collateral and closed. The xylem of each vascular bundle faces towards the upper surface while the phloem faces towards the lower surface. The xylem consists of tracheids, vessels and xylem parenchyma while the phloem consists of sieve cells and phloem parenchyma.

Reproduction of Gnetum:

Gnetum is dioecious. The reproductive organs are organised into well-developed cones or strobili. These cones are organised into inflorescences, generally of panicle type. Sometimes the cones are terminal in position.

A cone consists of a cone axis, at the base of which are present two opposite and connate bracts. Nodes and internodes are present in the cone axis. Whorls of circular bracts are present on the nodes. These are arranged one above the other to form cupulas or collars. Flowers are present in these collars. Upper few collars may be reduced and are sterile in nature in *G. gnemon*.

Male Cone (Strobili) and Male Flower:

Each cone consists of elongated axis with 10-15 collars or cupules at the nodes. The male flowers are arranged in definite rings above each collar on the nodes of the axis of male cone. The number of rings varies between 3-6. The male flowers in the rings are arranged alternately. There is a ring of abortive ovules or imperfect female flowers above the rings of male flowers.

Each male flower contains two coherent bracts which form the perianth. Two unilocular anthers remain attached on a short stalk enclosed within the perianth. At maturity, when the anthers are ready for dehiscence, the stalk elongates and the anthers come out of the perianth sheath.

Female Cone (Strobili):

Each cone consists of elongated axis with 10-25 collars or cupules at the nodal regions. The female cones resemble with the male cones except in some definite aspects. A single ring of 4-10 female flowers or ovules is present just above each collar. Only a few of the ovules develop into mature seeds.

In the young condition, there is hardly any external difference between female and male cones. All the ovules are of the same size when young but later on a few of them enlarge and develop into mature seeds. All the ovules never mature into seeds.

Structure of mature Ovule or Female Flower:

The ovule of *Gnetum* is orthotropous type. Each ovule consists of a nucellus surrounded by three envelopes. The inner nucellus consists of central mass of cells. The inner envelope elongates beyond the middle envelope to form the micropylar tube or style. The nucellus is a thick massive envelope contains the female gametophyte, in which 2-3 eggs are present towards the micropylar end. There is no nucellar beak in the ovule of Gnetum. Stomata, sclereids and laticiferous cells are present in the two outer envelopes. The ovules in *G. ula* are stalked. The rudimentary pollen chamber is present at the tip of nucellus.

EMBRYOLOGY OF ANGIOSPERMS

Microsporangium or Anther

The anther is the male reproductive structure of stamen and normally consists of two lobes, each with two elongated microsporangia or pollen sacs. The anther lobes are fused together by the connective tissue. All Angiospermous anthers are bilobed and quadrilocular (i.e., formed of four micro- sporangia) at an early stage of development and this condition is seen in most mature stamens. Hence the development of anther in angiosperms is tetrasporangiate type.

Rarely, however, the anther becomes bilocular or one-chambered either by the abortion of one lobe and destruction of the portion wall between the two chambers or the destruction of the entire partition tissue separating the four chambers. Microspores i.e., the pollen grains, are developed inside microsporangia. The microsporangia are developed inside the corners of the 4-lobed anther.

The transverse section of young anther consists of two regions like anther wall and anther locule. Both anther wall and anther locule developed from the archesporial cells.

I. Development Of Archesporial Layers

During the development of the microsporangium, the anther is seen at first as a homogeneous mass of meristematic cells without intercellular space, oblong in cross-section and surrounded by an epidermis. It then becomes more or less four-lobed. Below the epidermis, at each corner, some cells become differentiated from others by their dense cytoplasm and conspicuous nuclei - archesporium or archesporial cells and four longitudinal rows of archesporial cells present.

There may be only one such archesporial cell in each of the four lobes or there may be more of them forming a plate.

Each archesporial cell then divides mitotically and forms an outer primary parietal cell towards the epidermis and a primary sporogenous cell on the inner side.

The parietal cell now divides by periclinal and anticlinal walls giving rise to several layers of cells forming the wall of the anther.

The wall cells just below the epidermis form the endothecium which later loses the cell contents, usually becomes fibrous, and forms the dry coat of the mature anther in which the epidermis becomes rather inconspicuous. Cells in the center forms the middle layer.

The innermost layer of wall cells directly abutting on the sporogenous tissue forms the tapetum which is a nutritive tissue nourishing the developing microspores.

The primary sporogenous cells either directly function

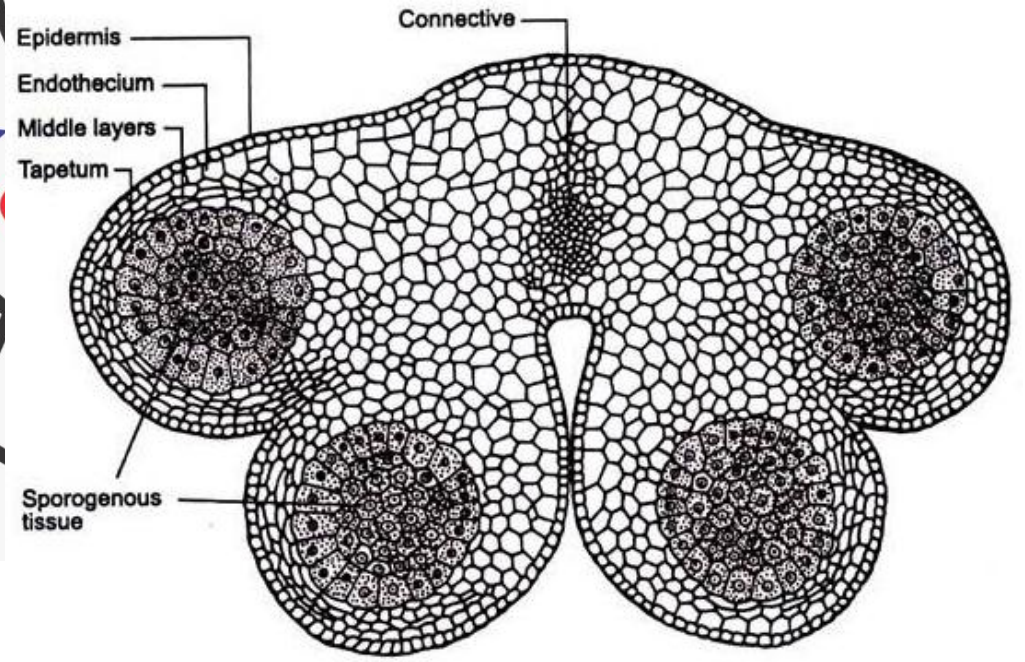


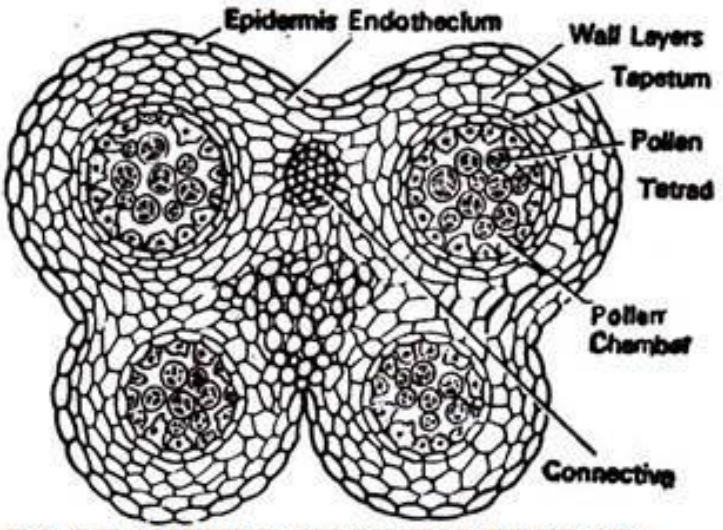
Fig. 1.1 : T.S. of the tetrasporangiate anther showing its various tissues

as spore mother cells or divide mitotically into a number of cells which function as spore mother cells. The spore mother cell undergoes meiotic division and gives rise to 4 microspores arranged tetrahedrally.

II. T.S. of Young (developing) Anther at tetrad stage and tapetum:

T/S of young anther show two regions like outer anther wall and inner anther locule.

1. It is a four-cornered structure containing a pollen sac.
2. Anther wall is the protective cover of microsporangium and it consists of the following layers centripetally and they are epidermis, an endothecial layer, one to three middle layers or wall layers and innermost layer of tapetum.
3. Epidermis is a single layer composed of compactly arranged flattened cells. Endothecium is a single layer of radially elongated cells. Middle layers composed of 2-3 layers of cells present around the tapetum. Tapetum layer consists of wedge shaped cells present around the sporogenous tissue.
4. In each pollen sac or pollen chamber are present many pollen tetrads which on separation form microspores.



T/S OF ANTHER SHOWING TETRAD STAGE AND TAPETUM

5. A joint in the form of connective is present in the centre.

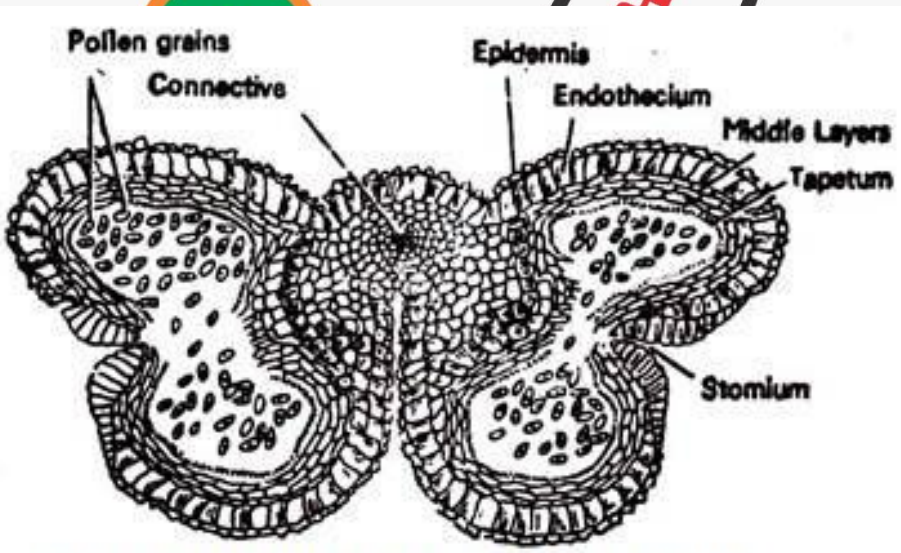
III. T.S. of Mature Anther Showing Dehiscence:

T/S of mature dehisced anther shows two regions namely anther wall and anther locule.

1. It is a four-cornered, four-chambered, multicellular body surrounded by a layer of epidermis.
2. Partition wall between the two pollen sacs or at the place of dehiscence is dissolved.
3. Endothecium, middle layers and tapetal layers are present below the epidermis.
4. Along the line of dehiscence of each lobe, thin- walled cells of endothecium form the stomium.

The cells of middle layer are crushed and not appear clearly.

5. A connective is very clear.
6. Many haploid pollen grains or microspores are present in the pollen sacs in the form of fine, powdery or granular mass.
7. Anther locule is the inner cavity of anther which are four in number at the beginning and becomes two as the anther matures.



T/S OF MATURE DEHISCED ANTHER

STUDY OF POLLEN GRAINS

"Pollen grains" or microspores are the male reproductive bodies of the flowering plants. The pollen grains do not remain united at maturity, and are dissociated into single pollen grain called monad. Sometimes rare types like dyads (two pollen grains), Octads (eight pollen grains) and Polyads (many pollen grains) are also observed. The shape of the pollen grains varies from species to species. The shape of the pollen/spores may be circular, elliptical, triangular, rectangular, quadrangular or in other geometrical shapes. Pollen grains show a great variety in their sizes. With regard to their position the apertures are polar, global or equatorial. The polar apertures are either monopolar (either in proximal or in distal pole) or bipolar (both in proximal and distal face). Global apertures are uniformly distributed over the pollen/spore surface. Equatorial apertures are meridionally arranged. The protoplasm of the pollen grains is enclosed by a wall made of intine and exine. The intine is a hyaline layer. The exine consists of an inner homogeneous layer (called endine or nexine and an outer heterogeneous layer (called ectine or sexine). Various types of ornamentation patterns are shown by exine surface. There are two types of apertures known as Pores (Porus, p1. Pori) and furrows (Colpus, p1. Colpi).

Characters of pollen grains for study

Following characters of the pollen grains are observed.

1. Polarity. The orientation of polarity is an important criterion in identification and description of pollen grains. The part of the pollen grains which is nearest to the centre of the tetrad is the proximal pole and that towards the opposite side is the distal pole. The imaginary line between the proximal and distal pole of the grain is called the Polar Axis (PA) which passes through the centre of the spore to the centre of the tetrad. The plane perpendicular to the polar axis through the middle of the grain is the equatorial plane

2. Symmetry. The pollen grains may be

(a) Symmetric- bilateral or radial.

(b) Asymmetric-without any symmetry.

3. Apertures. The exine of the pollen is often provided with apertures which are thin, more or less distinctly delimited areas formed only of a hyaline membrane. The following are the major types.

(a) lnaperturate-aperture absent.

(b) Aperturate-aperture present.

The shape of aperture. On the basis of shape these can be further divided.

(i) Those in which the outer (ectocolpium) and the inner (endocolpium) surfaces are congruent.

(1) Colpate-aperture elongated.

(2) Porate-aperture circular.

(3) Spiraperturate-aperture a continuous spiral.

(ii) Those in which outer and inner faces are incongruent.

(4) Colporate, if the outer face (ectocolpium) is elongate and the inner face (endocolpium) may be circular, longitudinally elongated (lolongate) or laterally elongated (lalongate).

(5) Pororate. When the inner face of the endocolpium is generally circular.

Distribution of apertures. On the basis of distribution following types are further recognised

(i) Zono-colpate, - porate. - colporate or - pororate. If apertures are arranged in a circular zone around the grain.

(ii) Pan- or Panto-colpate, porate, - colporate or - pororate. If pores or apertures arc distributed over the entire surface.

4. The shape of the pollen grain. It is determined by P x lOO/E formula, where P is the polar diameter and E the equatorial diameter. Some of the shape are Perbolate, Oblate, Sub-oblate, Oblate Spheroidal, Prolate - spheroidal, Sub-prolate, Prolate, Perprolate, etc.

5. Exine stratification. The wall is made of intine and exine. The intine is colourless Lind disappears during the slide preparation. Exine consists of two layers - the inner homogenous layer, the endine (= nexine) and

the outer heterogenous layer, the ectine (= sexine). The ectine is composed of radial rods, the columellae, which arc either free at their tips or are united to form a layer called tegillum (= tectum).
6. Exine ornamentation. The following are some of the patterns.
(1) The columellae forming the ectine produce pilate pattern with bright and dark areas.
(2) In some other cases columellae are arranged regularly and are fused to produce areas or lumina, the intervening areas between lumina being called muri.
(3) When a network is produced the pattern is reticulate which may be retipilate with incomplete fusion of columellae, foveolate with circular closely placed lumina, scrobiculate with circular but distantly placed lumina, or fossulate with elongated lumina.
(4) When lumina are parallel the pattern is called striate and when reticulate it is rugulate.
(5) A network with raised areas is called areolate.
(6) In some cases excrescenes such as minute granules arc present on the tegillum, the pattern is granulose, as spinulose, pointed or blunt ends, if rounded warts it is gemmate, verrucate if base of the warts is not constricted, tuberculate when tubercles are present, spinose if they arc pointed, baculate if rod shaped and clavate if club shaped.

Preparation of whole mount of pollen grains

Following are the steps in the preparation of slides for pollen study.

Collection of material

1. The pollinifcrous material (anthers) is collected fresh or herbarium sheets can also be used.
2. The anthers are picked by a clean forceps.

Preparation of material

1. The anthers are tapped by needles or glass rod on a clean slide to obtain a mass of pollen grains.
2. This mass of pollen grains is picked up by the flat end of the forceps and transferred to the centre of another clean microscopic slide.

Pre-treatment

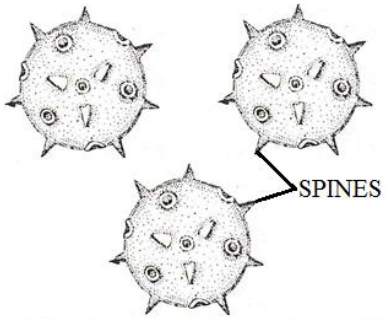
1. A drop of alcohol is added to the pollen grains. This releases oily and resinous substances in the form of a ring around pollen. Up to 3 or 4 drops of alcohol can be used.
2. The ring is wiped clean with cotton moistened with alcohol.

Mounting

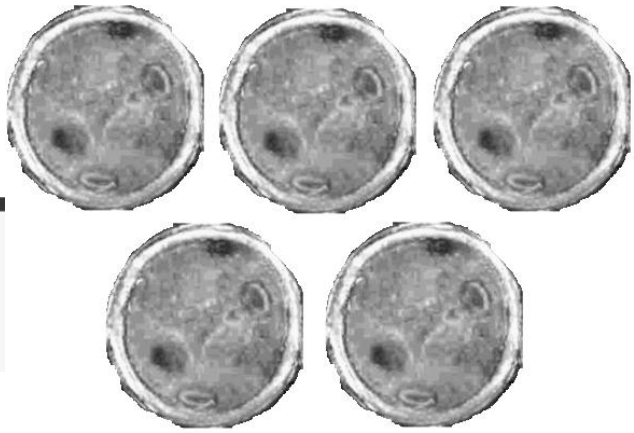
1. The grains are then stained with safranin and mounted in glycerine jelly and covered with a thin (zero number) cover slip.

Microscopic observation of pollen grains

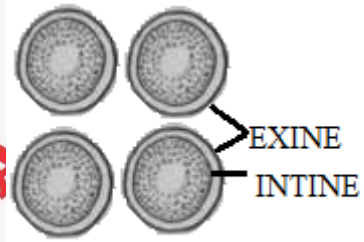
1. **Pollen Grains of *Hibiscus*:** They are spheroidal, large, thick walled. Exine wall is spiniferous and show 5 circular germ pores.
2. **Pollen grains of *Vinca*:** They are spherical, small and thick walled. They show both exine and intine, exine wall is smooth and has three germ pores.
3. **Pollen grains of Grass:** They are spherical, small, simple and shows both exine and intine walls. The exine wall is smooth, granulose and show only one pore.
4. **Pollinia of *Calotropis*:** The micropores or pollens of anther locules cling together to form an ovoid compound pollen mass called pollinia. Each pollinia has a short stalk called caudicle. The sticky glandular disc called corpusculum.
   Pollinium were removed from freshly opened flowers with the help of forceps and needle and then removed pollinium placed on slide and mounted in glycerine.



POLLEN GRAINS OF HIBISCUS



POLLEN GRAINS OF GRASS



Vinca POLLEN GRAINS

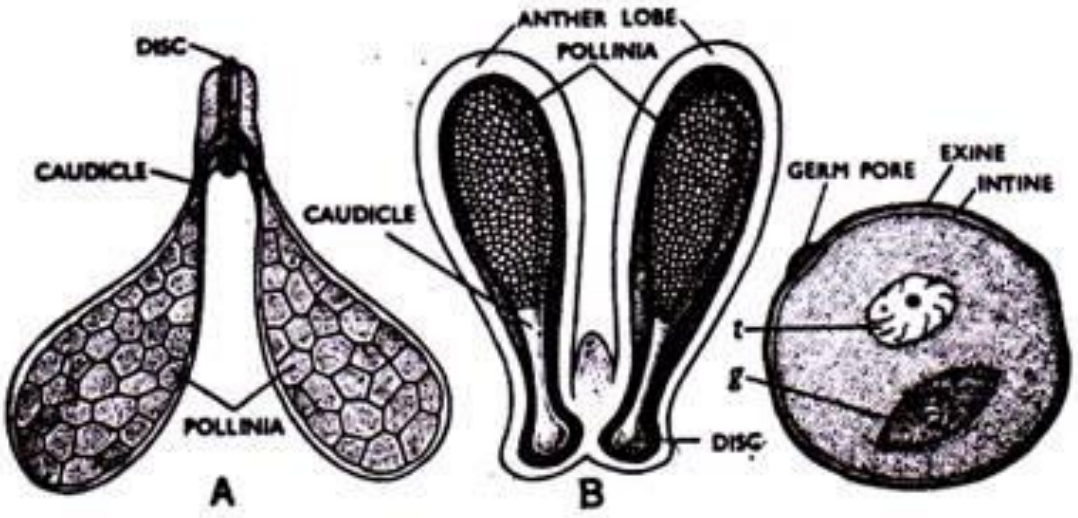


FIG. 405 FIG. 406

FIG. 405. Pollinia of A. *Calotropis* and B. Orchid. FIG. 406. A binucleate pollen showing tube nucleus (*t*) and generative cell (*g*).

GERMINATION OF POLLEN GRAINS

Aim: To germinate the pollen grains under invitro conditions by hanging drop method.

Introduction: Pollen grains germinate when they land on compatible stigmatic surface (exudate). Which contains all the requirements for the pollen to germinate. In invitro condition pollen grain germination can be done by providing the nutrient medium.

Materials required:

Mature flowers of *Catharanthus roseus* (Periwinkle), Hibiscus, Grass, *Calotropis* or any other easily available plant, sucrose, boric acid, magnesium sulphate, potassium nitrate, calcium nitrate, distilled water, beaker, microscope, measuring cylinder, dropper, slides and cover slips.

Procedure:

Preparation of Germination Medium: Take some distilled water in a measuring cylinder and dissolve in it 10 gm sucrose, 10 mg boric acid, 20 mg magnesium sulphate, 10 mg potassium nitrate and 30 mg calcium nitrate. Add some more distilled water to raise the level up to 100 ml in the measuring cylinder.

1. Clean the cavity slide and place a drop of this solution in the cavity.
2. Remove mature anthers from fresh flowers. Crush them on a slide. Collect the pollen grains with a brush from the crushed anthers. Dust the brush free of anthers in the cavity filled with solution.
3. Take a cavity slide and put a little amount of grease around its cavity. Place the inverted cover- slip on the cavity in such a way so that the germinating medium drop does not touch its surface.
4. Allow the slide to remain as such for a few hours or overnight.
5. Remove the coverslip slowly and gradually. Mount the coverslip on a fresh and clean slide in a drop of safranin. The lower side of the coverslip with germinated pollen grains should be in contact with safranin.
6. Observe the slide.

Observations

The following characters are observed.

1. Numerous germinated pollen grains are seen.
2. A pollen grain has a distinct ornamented exine with germ pores.
3. Intine lies internal to exine. It is thin and uniform.
4. Intine forms a pollen tube that comes out through one of the germ pores.
5. Pollen tube shows a vegetative nucleus and two small male gametes.

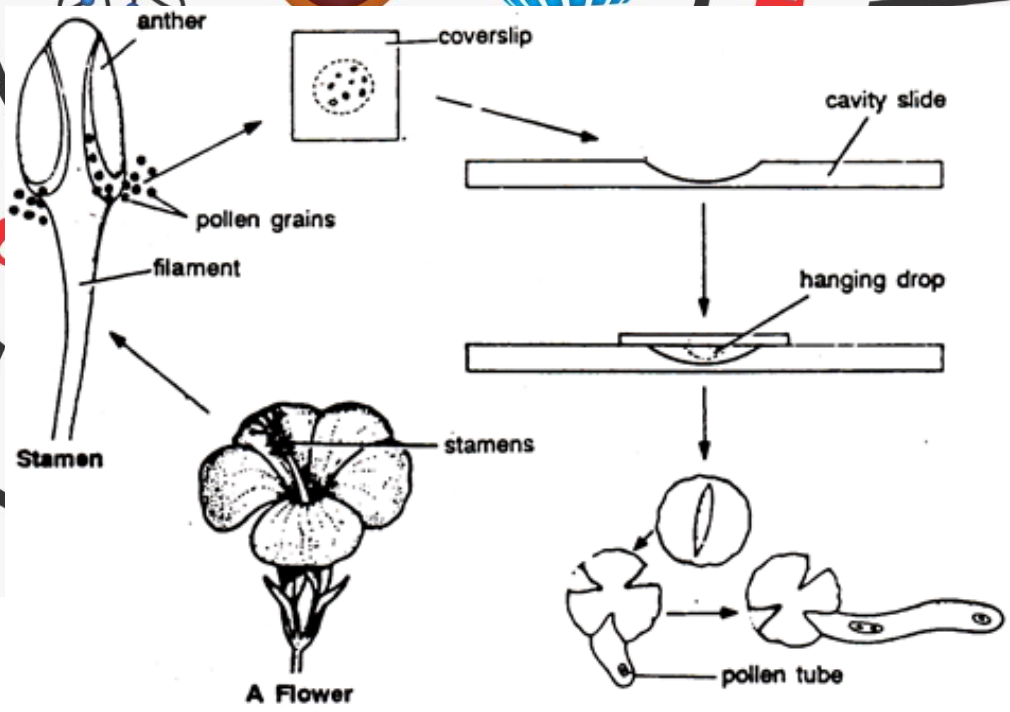
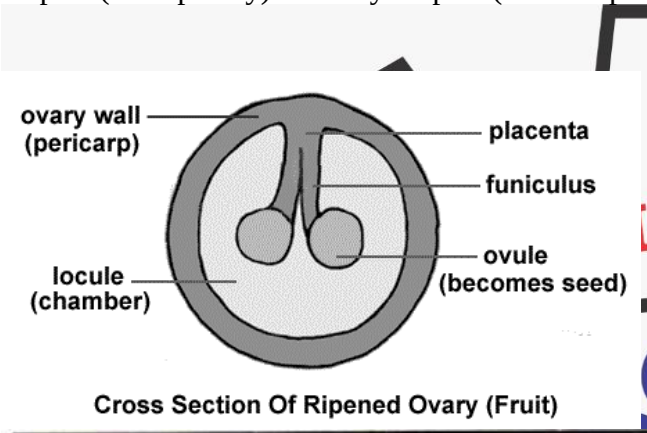


Fig. 7. *Hanging drop method of pollen germination.*

OVARY

Ovary is the basal swollen and most important part of the carpel as it contains the ovules which develop into seeds. The ovary consists of the ovary wall, the locule or locules and in a multilocular ovary, the partitions. The ovules are found to be situated on the inner or adaxial (ventral) side of the ovary wall.
It may consist of one carpel (monocarpellary) or more than one carpel, two carpels (bicarpillary), three carpels (tricarpillary) or many carpels (multicarpillary).



Cross Section Of Ripened Ovary (Fruit)

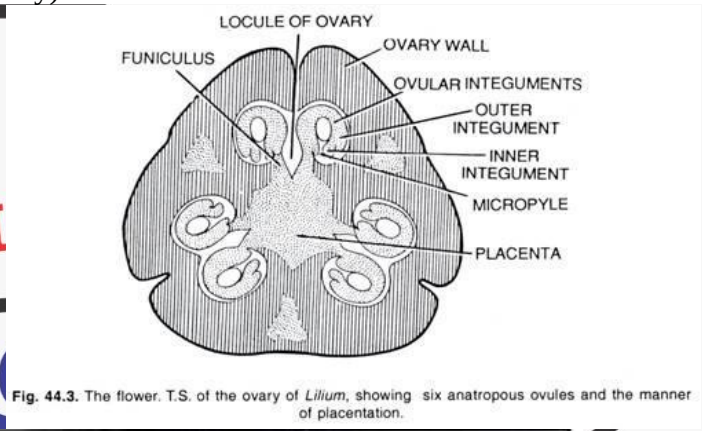


Fig. 44.3. The flower. T.S. of the ovary of *Lilium*, showing six anatropous ovules and the manner of placentation.

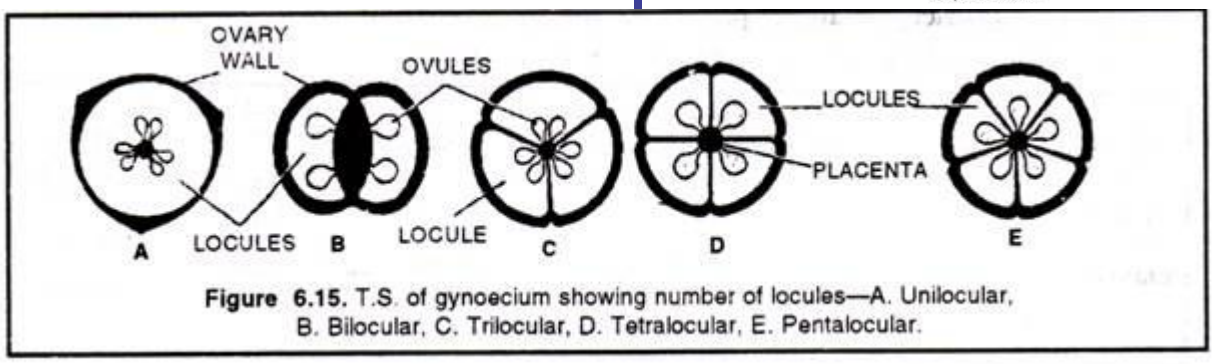


Figure 6.15. T.S. of gynoecium showing number of locules—A. Unilocular, B. Bilocular, C. Trilocular, D. Tetralocular, E. Pentalocular.

OVULE

The ovule is the megasporangium contained within the ovary. There may be one or more ovules inside an ovary and these are destined to be the seeds.

Structure of Mega-sporangium (Ovule):

When fully formed, the tissues in a typical ovule are as follows: The ovule is attached to the placenta by the funicle or funiculus which meets the ovule at the hilum, which later becomes a scar on the seed.

An ovule devoid of any funicle and directly attached to the placenta is termed sessile. Raphe is an extension of the funicle and may extend up to the chalaza which is the base of the ovule. (The ovule commonly remains reversed).

The general tissue of the ovule is called nucellus and the embryo sac. Nucellus consists of living parenchymatous cells. The nucellus serves to cover and provide nutrition to the embryo sac.

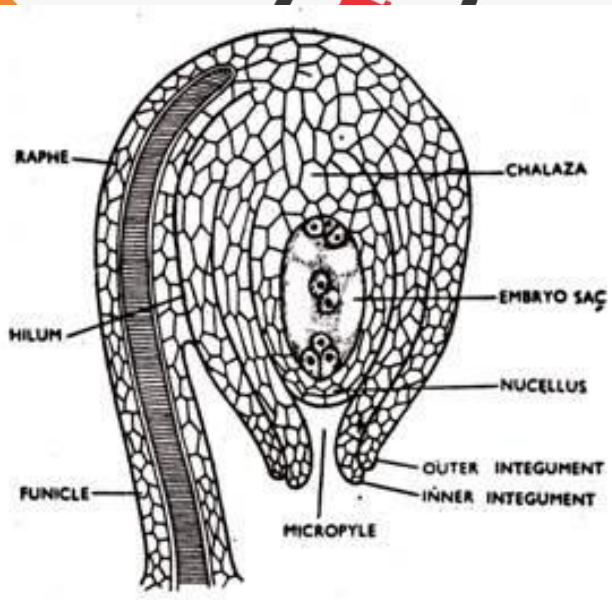


Fig. 384. Longitudinal section of a typical ovule (anatropous).

Each ovule has one or two protective envelopes called integuments (inner and outer) which encircle the ovule except at a tip where a small opening called micropyle. Abnormally, there may be a single integument or there may be no integument at all.

The basal part of an ovule just opposite to micropyle is called chalaza. Cells of the nucellus are rich in reserve food materials.

Each ovule has two distinct ends-a micropyle end (it also called opening of ovule during fertilization) and b. Chalaza end (the posterior end, opposite to micropylar end).

Externally the nucellus is covered by one or two protective covers, called integuments. These integuments arise from the chalazal end.

When only one integument is present, the ovule is called unitegmic, and if the ovule consists of two integuments, it is called bitegmic very rarely tri-tegmic (with three integuments) is present in plants like *Asphodelus*. In some plants such as *Santalum*, etc, ategmic (no integument) condition may be present.

There is generally a single embryo sac or female gametophyte located in the nucellus; it has developed from a megaspore.

(h) In mature ovules, the female gametophyte or embryo sac is present in the centre. The embryo sac consists of egg cell (female gamete), synergid cells, antipodal cells and polar nuclei.

Types of Ovules:

On the basis of position of micropyle, with respect to the funiculus, ovules are 6 types:

1. Orthotropous ovule:

It is atropous or straight, where the micropyle, chalaza and the funiculus, all are in the same line. Ex-*Cycas*, Family Polygonaceae (*Polygonum*) and Piperaceae.

2. Anatropous ovule:

It is of the most common occurrence more than 80% of angiosperm family. In this ovule, the funicle is long, whole body of the ovule is inverted, through 180°. As a result the micropyle comes close to the funicle. Ex-Most common in dicots and monocots, Sympetalae, Asteraceae (*Tridax*), Solanaceae.

3. Hemianatropous or hemitropous ovule:

In this case the body of the ovule is inverted only through 90°. As a result the funicle comes to lie at right angle to the nucellus. Micropyle and chalaza, lie in the same plane Ex-*Ranunculus*, Primulaceae.

4. Campylotropous ovule:

When body of the ovule is not completely inverted, but it is bent like-'horse shoe'. The micropyle and chalaza do not lie in the same plane (however the nucellus/ embryo-sac remain straight). Ex-Family Capparidaceae, Cruciferae (Brassicaceae), Carypohyllaceae, Fabaceae, Chenopodiaceae etc.

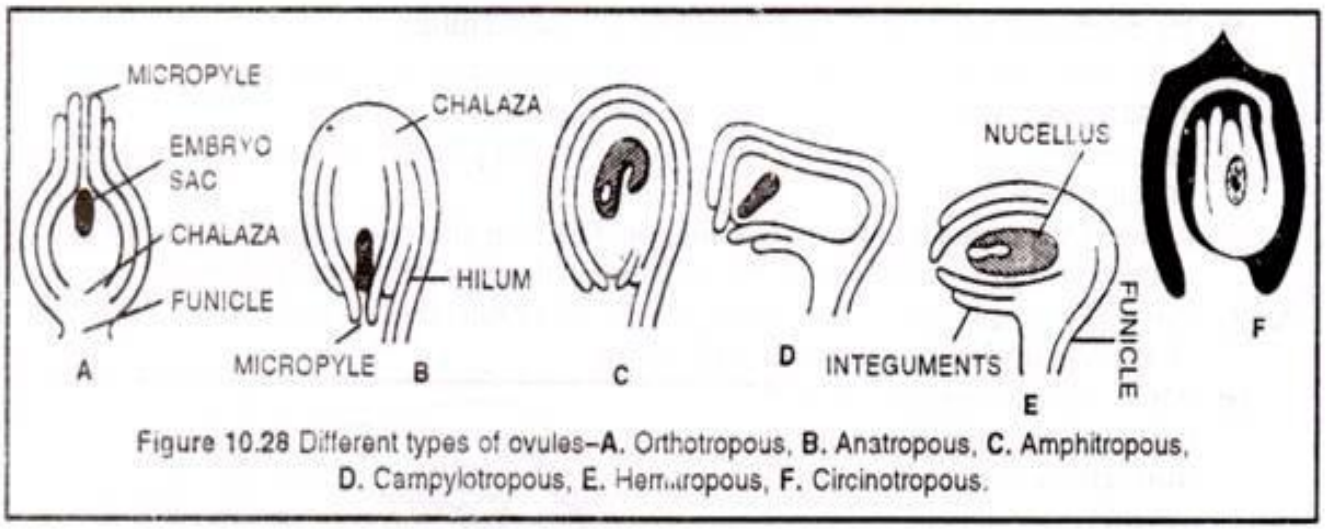


Figure 10.28 Different types of ovules–**A.** Orthotropous, **B.** Anatropous, **C.** Amphitropous, **D.** Campylotropous, **E.** Hemitropous, **F.** Circinotropous.

5. Amphitropus ovule:
It is similar to campylotropous, but in the case the nucellus/embryo-sac is also bent like 'horse shoe' Ex-Family Alismataceae (*Lemna*), Leitneriaceae.

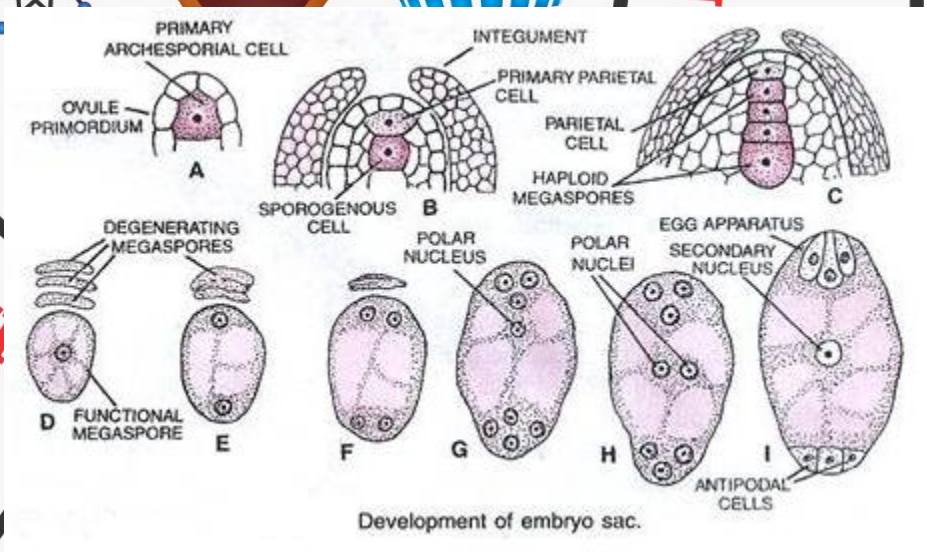
6. Circinotropous ovule:
It is of a very rare occurrence. Here the body of the ovule is bent through 360°, so that it takes a one complete turn. (Micropyle, chalaza and the nucellus are all in same plane). Ex-Opuntia

MEGA-SPOROGENESIS (DEVELOPMENT OF AN OVULE):

The formation of megaspores from Megaspore Mother Cell (MMC) is called mega-sporogenesis.
It occurs inside the nucellus of developing ovule of angiosperms. The process begins very early when nucellus is not completely surrounded by the integuments.
MMC enlarges in size and divides by the meiosis to form a tetrad of four haploid megaspores. Out of these, soon three degenerates and only one megaspore becomes functional.

Development of Female Gametophyte:
In general, the development is monosporic, e.g., in *Polygonum*. In this type of development, only one megaspore situated towards chalazal end remains functional, while the remaining three megaspores gradually degenerate and finally disappear.

Following are the different stages of development of female gametophyte:
(i) The functional haploid megaspore is the first cell of female gametophyte of angiosperm.
(ii) It enlarges in size to form the female gametophyte, also called embryo sac.
(iii) Its nucleus undergoes a mitotic division and the two nuclei move to the opposite poles, forming the 2-nucleate embryo sac.
(iv) The 2-nucleate embryo sac undergo second mitotic division giving rise to the 4-nucleate stage.
(v) The third mitotic division gives rise to 8-nucleate embryo sac, which comprises of a micropylar end and a chalazal end with four nuclei at each end.
(vi) Out of four nuclei at the micropylar pole, 3 differentiates to produce an egg apparatus consisting of 2 synergids and a female gamete egg cell.
(vii) Similarly, at the chalazal end, 3 out of 4 nuclei are grouped together and are surrounded by cytoplasm and cellular wall differentiate as antipodal cells.
(viii) The remaining nuclei, one at the micropylar end and one at the chalazal end termed as polar nuclei migrate towards the center of the embryo sac (now called central cell). They meet at the center and may remain separate until the discharge of male gametes take place and then fuse to form secondary nucleus of central cell.



Development of embryo sac.

(ix) Thus, a typical angiosperm embryo sac is 8-nucleate and 7-celled.

Placentation:
The placenta is an outgrowth of a parenchymatous tissue in the inner wall of the ovary to which the ovule or ovules (megasporangia) remain attached. The placentae usually develop on the margins of

carpels, either along their whole line of union, called the suture or at their base or apex. The manner in which the placentae are distributed in the cavity of the ovary is known as placentation.

In the simple ovary (i.e., of one carpel), there is one common type of placentation, known as marginal, and in the compound ovary (i.e., of two or more carpels united together) placentation may be axile, central and free-central, basal, parietal and superficial.

(a) Marginal:

When the gynoecium is monocarpellary apocarpous, the placentae bearing ovules are borne on the ventral suture, where the margins of the ovary wall fuse, e.g., family Leguminosae.

(b) Axile:

Ovary multilocular and ovules borne on central placenta, e.g., Hibiscus, Citrus, *Allium*, tomato, etc.

(c) Parietal:

Ovary is unilocular but pistil is syncarpous. The ovules are borne on peripheral fused margins of carpels, e.g., *Brassica*, Papaya, Gourd etc.

(d) Free central:

Ovary is unilocular and ovules borne on a central column which is not connected to the ovary wall by any septum, e.g., Dianthus, Silene, Primula etc.

(e) Basal:

Ovary is unilocular and a single ovule is borne at the base of the ovary. E.g., *Tridax*.

(f) Superficial (Laminar):

Ovary is multilocular and the ovules are borne on septa as well as all over the inner surface, e.g., *Nymphaea*.

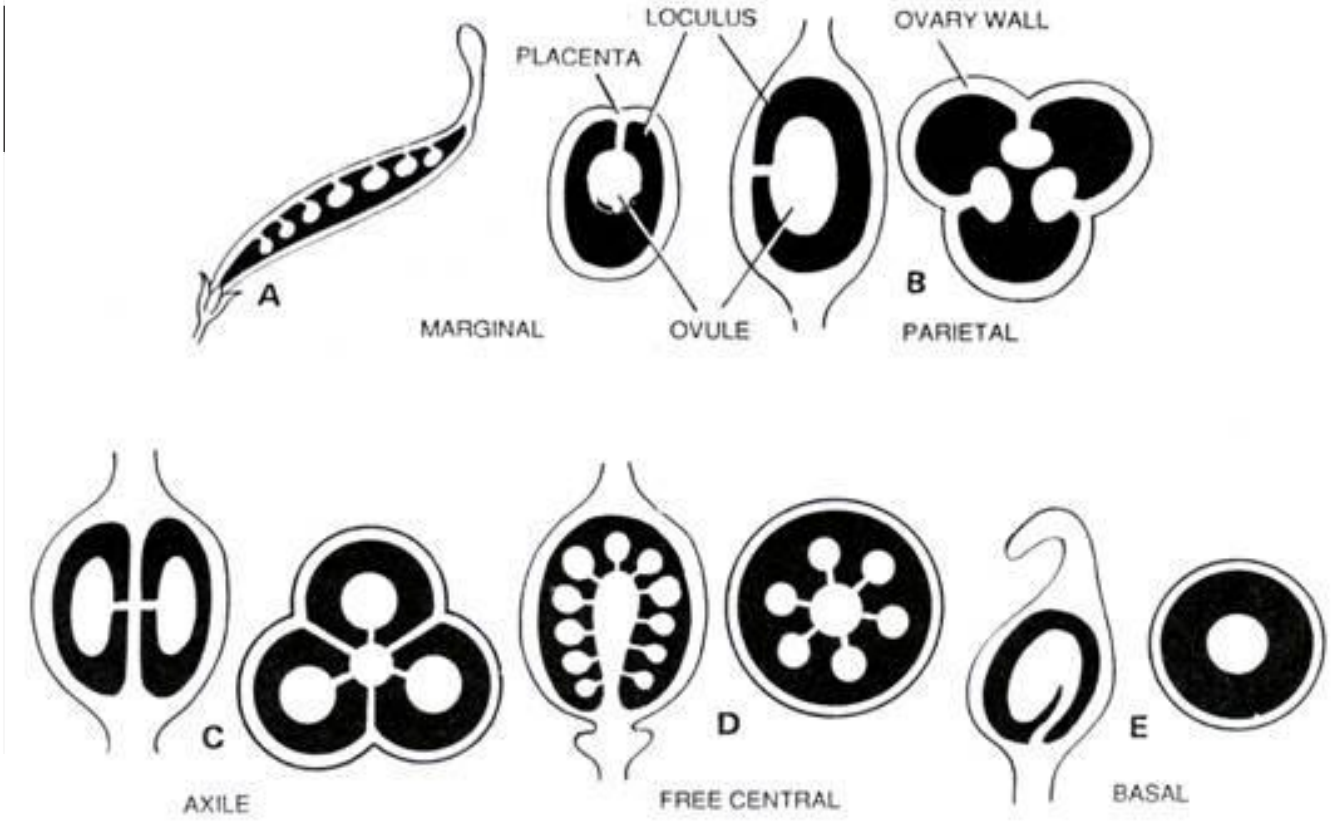
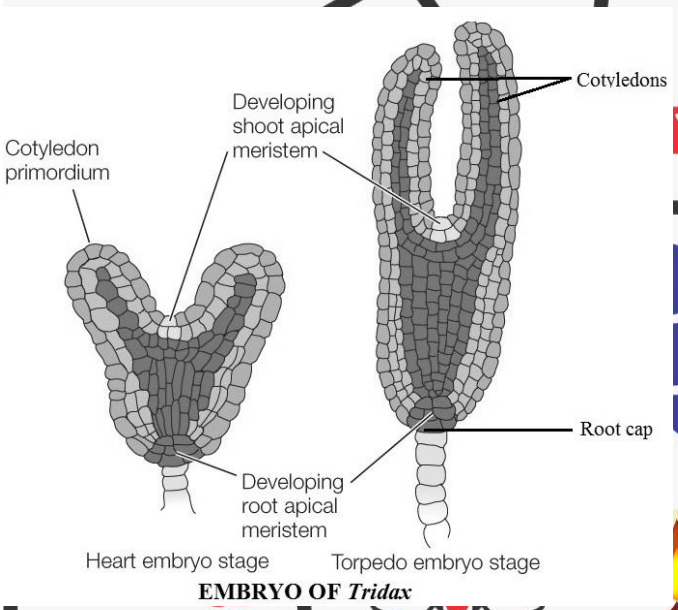


Fig. 46.14. Placentation. Different Types.

EMBRYO

After fertilization, the fertilized egg is called zygote or oospore which develops into an embryo. A series of changes occur in the ovule resulting in the formation of seed. The seed is, therefore, a fertilized ovule, consisting of seed coat and an embryo. There may also be remnant part of nucellus called perisperm and also the endosperm. Embryo consists of suspensor situated near the micropyle, cotyledons towards chalaza and a small embryonal axis called tigellum.

Embryo of *Tridax* – develops with in a few hours after fertilization. The mature embryo of *Tridax* show two cotyledons present laterally and growing apex of stem (plumule) present between two cotyledons. Radicle and root cap are present below the plumule part.



EMBRYO OF *Tridax*

Dissection of embryo

Seeds of *Tridax* are used to dissect out the embryo. The following is the procedure.

1. Place the *Tridax* seed on the stage of dissecting microscope or binocular.
2. Locate the micropyle which appears like a small opening.
3 Remove the seed coat (ovary wall) from this point, carefully with the help two sharply pointed needles.
4. Once the seed coat is removed, embryo could be seen clearly between the cotyledons.
5. Place this embryo on a slide in a drop of glycerine and observe the parts of embryo under microscope.

Monocot Embryo:

1. Only one cotyledon is present.
2. Plumule forms the stem and radicle forms the root.
3. Hypocotyle and a small suspensor are also present.

Dicot Embryo:

1. Two large cotyledones are present
2. Both the cotyledones covet a small stem apex.
3. Suspensor is swollen.
4. Near the suspensor is present the root cap.
5. Central region forms the procambium which is present in between root cap and stem apex.

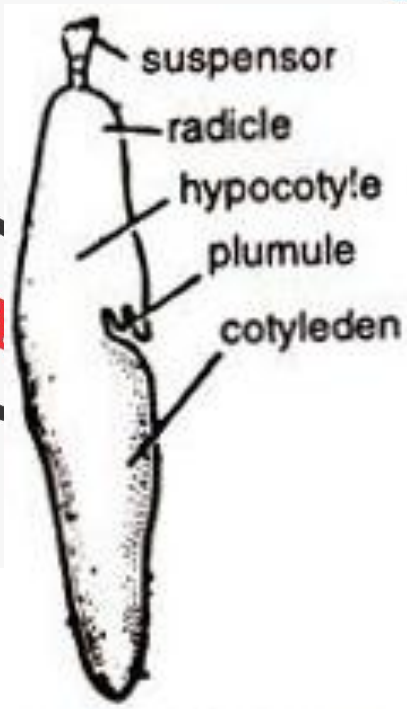


Fig.69. Monocot embryo

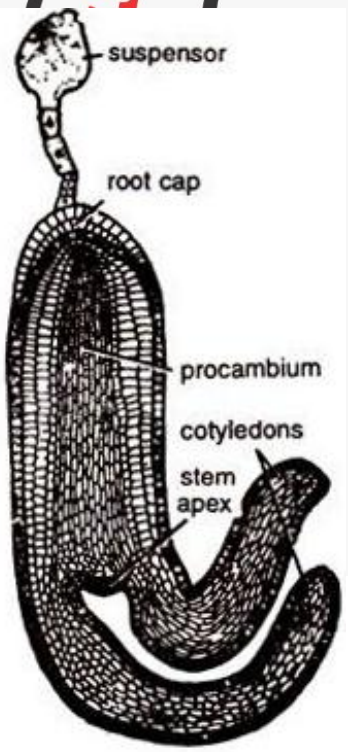


Fig. 70. Dicot embryo.

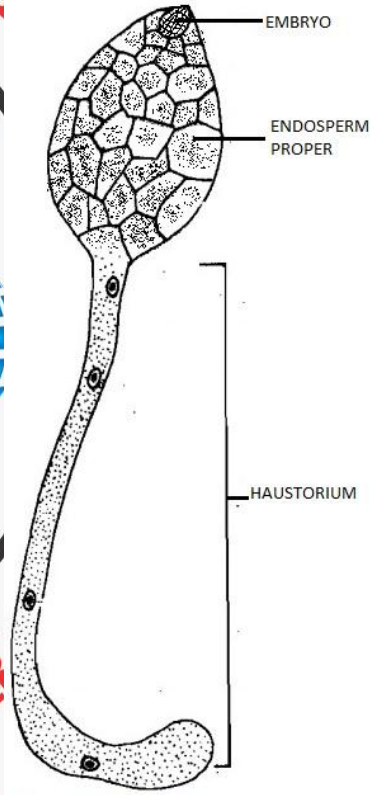
ENDOSPERM

Endosperm is the nutritive tissue for the developing embryos present in the angiosperms. It is formed as a result of divisions of the primary endosperm nucleus, which is triploid. The primary endosperm nucleus (3n) is formed due to the fusion of second male gamete (n) with the two polar nuclei (n and n) or their fusion product, the secondary nucleus (2n). Thus, the endosperm, in angiosperms, is a triploid tissue formed after fertilization.

Sometimes it is consumed by the developing embryo or it may persist in mature seed to support the growth during germination. Endosperm of some species develops a special structure called haustorium which modifies variously and tremendously to get the metabolites for developing embryo. Haustoria which develops at chalazal end are known as chalazal haustoria and the ones that develop at micropylar end are micropylar haustoria. In some plants haustoria develops at both the ends.

Endosperm of *Cucumis sativus*:

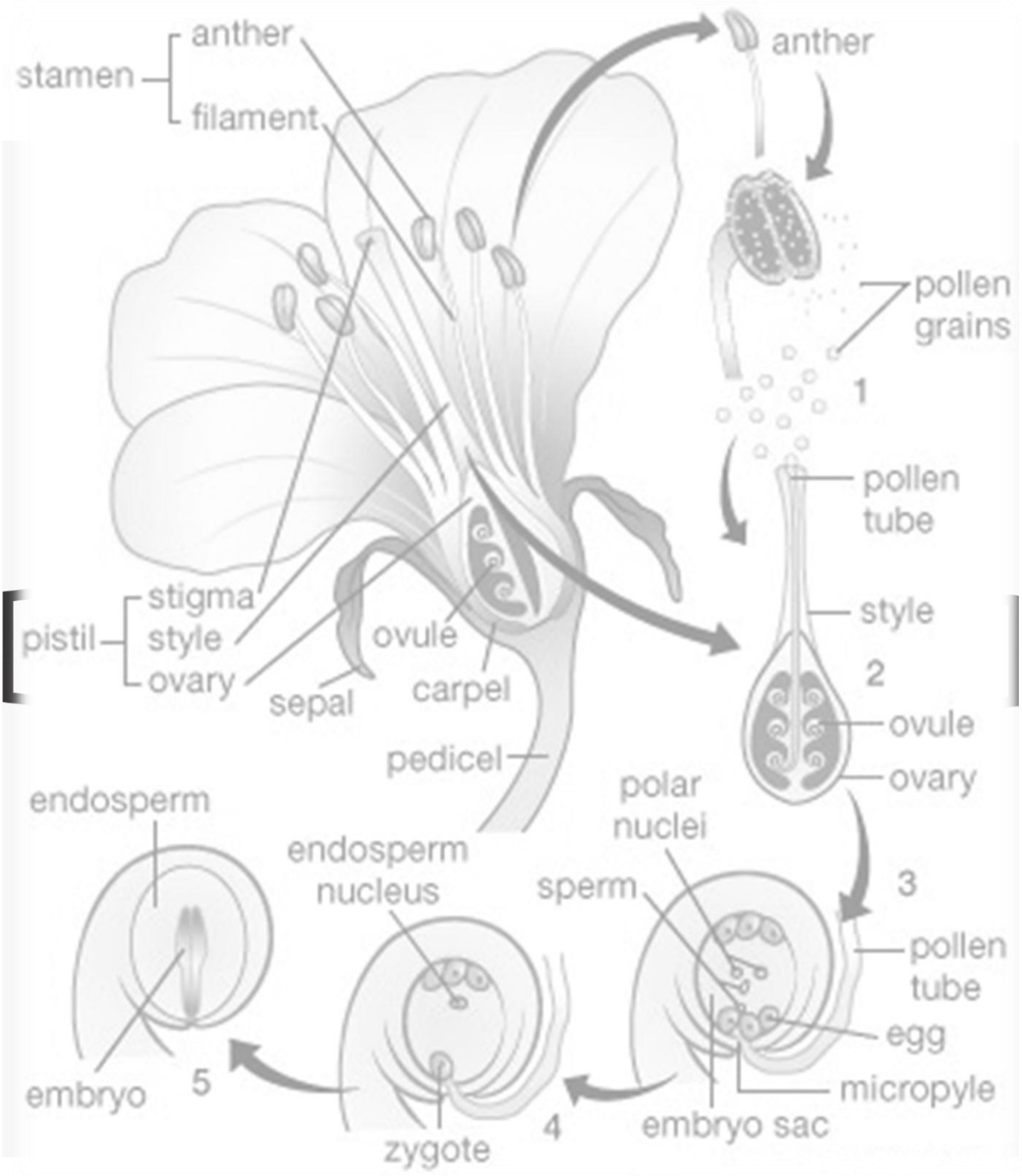
In *Cucumis sativus* haustoria develops at chalazal end. The endosperm is of nuclear type. The chalazal region extends into a long tubular haustorium with flattened spoon shaped tip for the transportation of nutrients. The haustorium consists of dense cytoplasm and number of free nuclei. The upper part of the endosperm is called endosperm proper, in which the embryo is embedded.



ENDOSPERM OF *Cucumis*

Mounting procedure for *Cucumis sativus* endosperm:

Take a young turgid *Cucumis* seed on a clean glass slide, remove the mucous present around it. Split open the seed into two parts with the help of a blade or scalpel and you will be able to see the gelatin like endosperm haustorium attached at the chalazal end without disturbing the endosperm, which is present towards the funicle part. With the help of forceps and needle gently take out the endosperm and place it on a clean slide. Add a few drops of safranin and a drop of glycerine and observe under microscope.


anther
stamen
filament
anther
pollen grains
1
pollen tube
stigma
style
pistil
ovary
ovule
sepal
carpel
pedicel
style
2
ovule
ovary
endosperm
endosperm nucleus
polar nuclei
sperm
3
pollen tube
egg
micropyle
embryo sac
embryo
5
zygote
4