B.Sc. BOTANY LAB MANUAL

2nd Semester

Prepared By
Biological Science Dept.
Botany

MIDNAPORE CITY COLLEGE

B Sc (Honours) in Botany

[Choice Based Credit System]

CP3: Mycology and Phytopathology -Lab

Fungal staining

Lactophenol cotton blue stain is used for staining fungus. This stain contains:

1. Phenol which serves as fungicides.
2. Lactic acid (clearing agent).
3. Cotton blue (stains the cytoplasm of fungus).

Requirements:-

1. Fungal culture.
2. Lactophenol cotton blue.
3. Glass slide.
4. Cover slips.
5. Bunsen burner.
6. Compound Microscope.
7. Needle.

Procedure:-

1. Take clean dry slide.
2. Transfer small amount of culture into the slide.
3. Add 1-2 drops of Lactophenol cotton blue stain into the culture.
4. Mix them gently.
5. Place the cover slip over the preparation.
6. Examine under low and high power objectives.

Result:- The fungal cytoplasm will appear as light blue region. The cell wall of hyphae, conidiophores, conidia, phialides will remain unstained.

Precaution:-1. Care should be taken to avoid trapping of air bubbles while placing the coverslip over the preparation.

Study of basidiocarp, gills, basidia and basidiospores of Agaricus

Procedure: Pluck the pileus of the mushroom after studying it externally. Observe it from the lower side under a high mangification lens. Also study a slide showing T.s. of gills.

Comments:

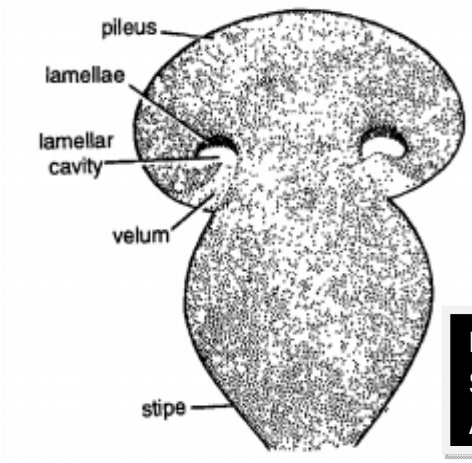
1.The mature basidiocarp consists of a stalk or the stipe, having an expanded pileus at its top.

2. With the growth of the basidiocarp, the veil or velum ruptures and in mature basidiocarp it remains in the fonn of a ring (annulus) on the stipe, just below the pileus.

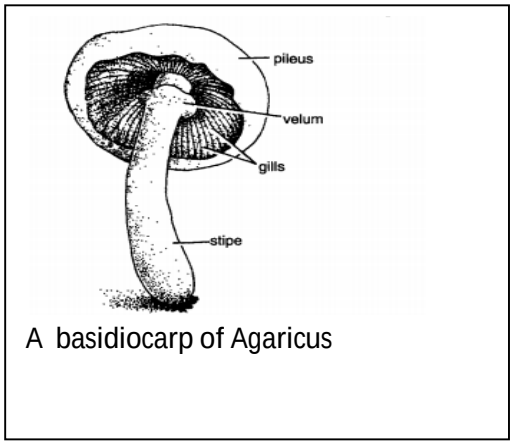
3. The upper surface of pileus is flesh coloured and tough.

4. The pileus, on the underside, bears many lamellae or gills which hang down vertically and extend almost radially from stipe to the margin of the pileus.

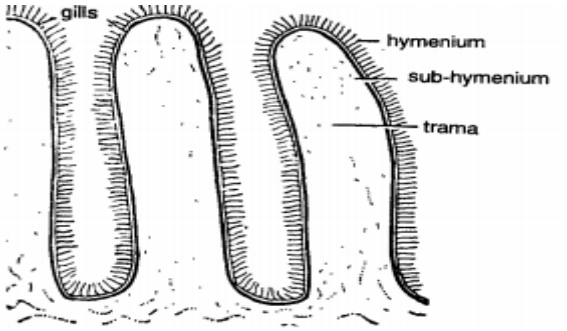
5. The gill in transverse section exhibits a trama, a sub-hymenium and a hymenium.



L.S BUTTON STAGE OF AGARICUS



A basidiocarp of Agaricus



T.S of Agaricus gills

6. The trama form s a central core of elongated sterile hyphae.

7. The hyphal cells of trama curve outwards on either side of the gill forming a more or less compact tissue of cells, the sub-hymenium.

8. Finally the hyphae terminate in elongated, clubshaped cells, forming a superficial layer of the gill, known as the hymenium.

9. The hymenium at maturity, consists of the fertile cells, the basidia, intermingled with the sterile cells, the paraphyses. (The paraphyses are undeveloped basidia).

10. Each basidium is a club-shaped structure, bearing at its top generally four but sometimes two basidiospores, on short slender stalks known as sterigmata.

11. Each basidiospore is oval in shape and uninucleate.

12.On germination, it produces the new (monokaryotic) mycelium.

Rhizopus :-Most of the species of Rhizopus are saprophytic and grow on dead vegetables or animal matter. R. stolonifer grows so frequently on bread that it is often called the 'bread mold'. It is also called 'black mold' for its black coloured sporangia and 'Pin mold' for globose sporangia at the tips of branches look like pin heads. Only a very few species viz. R. artocarpi and R. arrhizus are weak parasites. Only a few species of Rhizopus attack the plants.

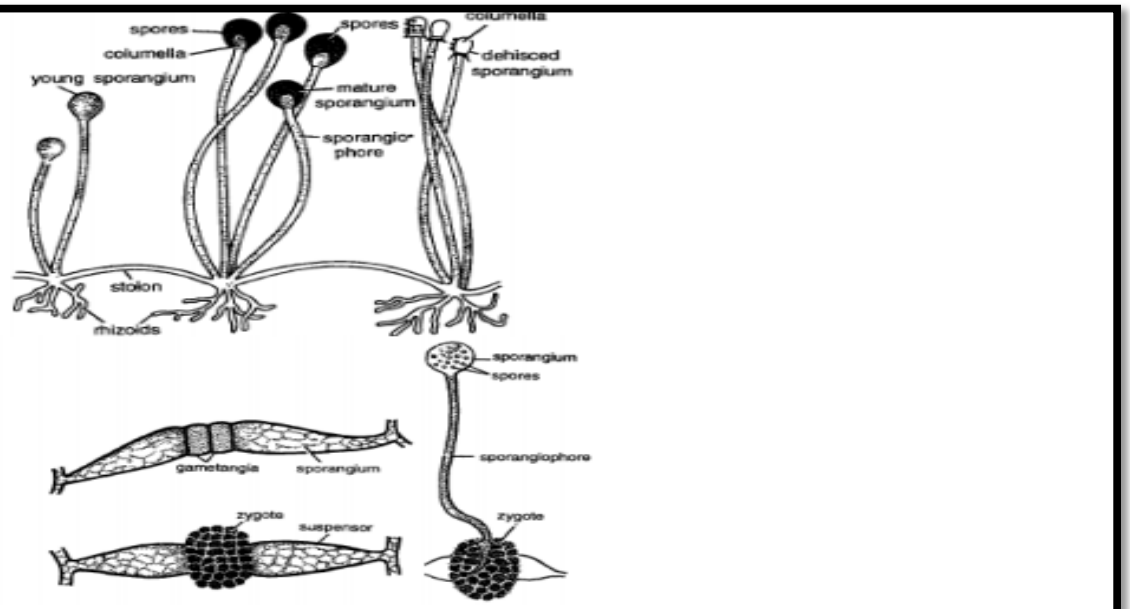
Vegetative structure:

1. The mycelium shows abundant, white cottony growth. In the older mycelium three parts of hyphae can be distinguished

(i) branched rhizoids that penetrate the substratum,

(ii) stolon or runner growing horizontally above the substratum for some distance and then bending downward, producing another group of rhizoids and

Reproductive structure:- The sporangiophores which grow upward in tufts from the point where the stolons form rhizoids



***Rhizopus*: A portion of mycelium with sporangiophores; Various stages in sexual reproduction**

2. The asexual reproductive structures are sporangia borne by the sporangiophores. Each sporangiophore is swollen at the tip and forms sporangium

3. The sporangium has a columella in the centre and the space between columella and wall of the sporangium is packed with aplanospores. This is known as the spore sac.

Identification

(1) Chlorophyll absent,

(2) Reserve food glycogen,

(3) Cell wall of fungal cellulose……. **Kingdom- Mycota**

A definite cell wall…… **Division-Eumycota**

Asexual reproduction by non motile spores, aplanospores. ……. **Sub-Division-Zygomycotina**

(1) Gametangia morphologically similar,

(2) Sexual reproduction forms a zygospore…… **Class- Zygomycetes**

(1) Mostly saprophytic,

(2) Asexual reproduction by typical nonmotile aplanospores……. **Order-**

Mucorales

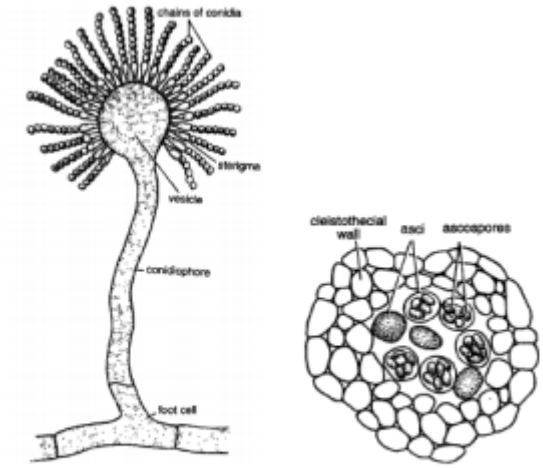
(I) Sporangia with many spores, and well developed columella,

(2) Sporangial wall relatively thin and easily breakable or deliquescent…… **Family-Mucoraceae**

Sporangiophores arise at rooting nodes of the stolon……. **Genus-Rhizopus**

Aspergillus :

Most of the species of Aspergillus are saprophytes growing on decaying vegetables, butter, bread, rice, jams, leather, cloth, fabrics, etc. However, a few species are parasites on plants and animals, including human beings. A. niger causes rot disease of pomegranates, dates and figs. In rot disease, the fruits decay, fungus enters the host through cuts and wounds in the fruits. A few hyphae could be mounted in lactophenol after staining with cotton blue.



: *Aspergillus*:A mature conidiophore with chains of conidia; Cross section of a cleistothecium showing asci

Vegetative structure: - 1. The segments of the mycelium are uni- or multinucleate. The pigments in the cytoplasm give a characteristic colour to the mycelium of various species (similarly coloured conidiophores and conidia would be present in the same species).

Reproductive Structure:-1. Conidia are asexual reproductive units borne on conidiophores. Each conidiophore arises from the foot cell of the mycelium and is long and erect hypha, terminating in a bulbous head-the vesicle.

2. The vesicle develops a number of bottle-shaped structures called the sterigmata (sing. sterigma) over its entire surface. Each sterigmata cuts off a chain of basipetally arranged conidia.

3. The conidia are coloured and the colour of the conidia depends upon the species. Mount mycelium havingcleistothecia in lactophenol after staining in cotton blue.

4. The species of Aspergillus developing perfect stage, an ascocarp i.e. cleistothecium are placed under the genus Eurotium

5. Cleistothecium consists of wall called peridium formed by somatic hyphae, enclosing many asci. Each ascus has eight uninucleate pulley wheel like ascospores with an outer sculptured epispore and an inner smooth endospore.

Identification :-

(1) Chlorophyll absent,

(2) Reserve food glycogen,

(3) Cell wall of fungal cellulose…….. **Kingdom- Mycota**

A definite cell wall present……. **Division-Eumycota**.

(1) Mycelium septate,

(2) Spores borne endogenously in the ascus,

(3) Spores definite in numbers, in multiples of two, usually eight……**Sub-division- Ascomycotina**.

Ascocarp a cleistothecium…….. **Class-Plectomycetes**

Cleistothecia sessile. …… **Order-Eurotiales**

(I) Asci lie scattered, hymenium not formed,

(2) Peridium made of closely interwovenhyphae…… **Family-Eurotiaceae**

Conidiophore unbranched…… **Genus-Aspergillus**

Penicillium (Blue Mold)

The fungus is a saprophyte and is commonly found on citrus and other fruits, jellies and other food stuffs. A few hyphae could be mounted in lactophenol after staining with cotton blue.

1. The mycelium is freely branched, septate and each cell is uni- or multinucleate.The mycelium may grow superficially on the surface of substratum or may penetrate deeply.

2. The hyphae are generally coloured due to pigments on the surface of hyphal walls.

3. The conidia are the asexual spores borne on long, erect and branched conidiophores. The branched conidiophore, with its conidia looks like a small 'Penicillus' (a brush in Latin).

4. Each conidiophore grows vertically from the mycelium and branches at its upper end. The ultimate branches are known as metulae.

5. Each branch of conidiophore ends in bottle shaped sterigmata bearing a group of conidia arranged basipetally.

6. The conidia are generally blue, sometimes green or -yellow and give characteristic colour to the colony. Prepare a slide as usual and search for cleistothecia. Apply little pressure over the coverslip to break them, so that asci and ascospores come out.

7. The fruiting body or ascocarp is called cleistothecium. It has a wall-peridium made of sterile hyphae which encloses many asci and paraphyses.

8. The globose or pear-shaped asci lie scattered inside the cleistothecium. Each ascus has eight uninucleate and wheelshaped ascospores.

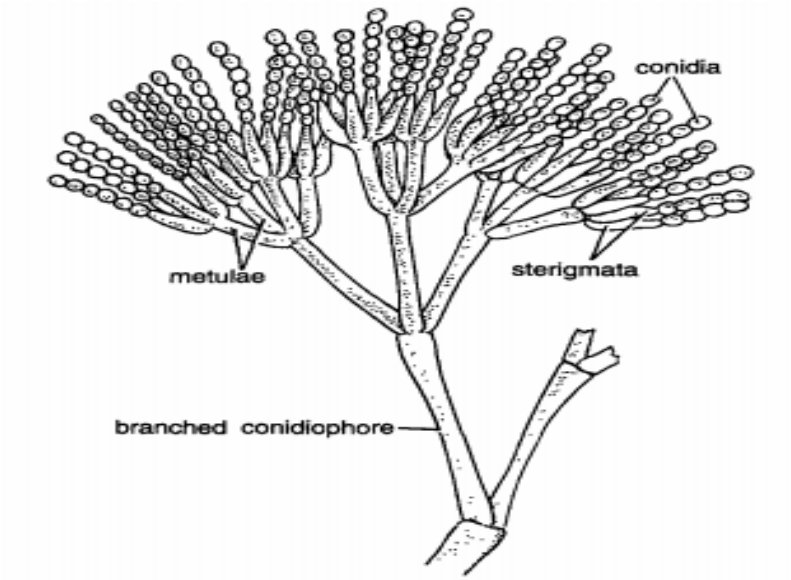


Fig. 1. *Penicillium*. Mycelium bearing conidiophore and chains of conidia.

Identification

(1) Chlorophyll absent,

(2) Reserve food glycogen,

(3) Cell wall of fungal cellulose…….. Division -Eumycota

A definite cell wall present…….. Division -Eumycota

(1) Mycelium septate,

(2) Spores borne endogenously in the ascus,

(3) Spores in definite numbers, in multiples of two, usually eight…… Sub-division-Ascomycotina

Ascocarp, a cleistothecium…… Class-Plectomycetes.

Cleistothecia sessile…… Order-Eurotiales

(1) Asci lie scattered and hymenium not formed,

(2) Peridium (outer wall of cleistothecium) of closely interwoven hyphae……. Family-Eurotiaceae.

Branched, brush-like conidiophores…….. Genus-Penicillium.

Ascobolus –

Procedure:- Pinch a small part of the ascocarp or cut a section of the sterile part of the ascocarp. Stain with cotton blue, mount in lactophenol and study.

Vegetative structure:- 1. The fungus mostly grows on the dung of herbivores and is called coprophilous. A few species (A. carbonarius) grow on burnt soils.

2. The thallus is made of richly branched mycelium that forms a complex structure and fmally a cup shaped structure.

3. The hyphal masses penetrate the substratum. These act as organs of absorption for the aerial branches.

4. The hyphae are branched and septate. Each cell is multinucleate.

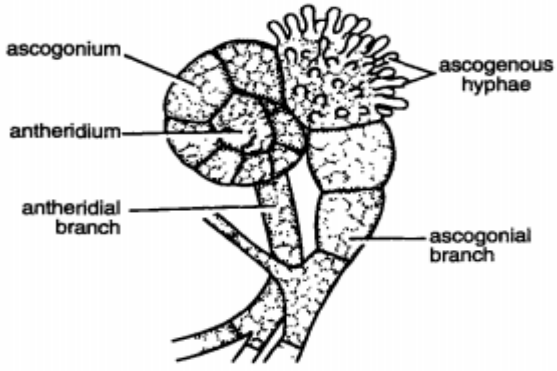
Reproductive organs: 1. The male reproductive organs are antheridia and the female reproductive organs are called ascogonia.

2. Antheridia and ascogonia are borne at the tips of separate branches.

3. Antheridium is borne at the tip of antheridial branch. It is cylindrical or clavate in shape.

4. Each antheridium is multinucleate.

5. Ascogonium is also present at the tip of the ascogonial branch. It is sub-globose in shape and is multinucleate.



Ascobolus sex organs

Fruiting body: V. S of Ascocarp shows the following structure

1. The ascocarps are apothecia and develop as a result of fertilization.

2. These are yellowish and saucer shaped.

3. Apothecium is a cup-shaped structure that is made of mycelium.

4. The section shows three zones-the outermost called hymenium, the middle called subhymenium and the lowermost called hypothecium.

5. The lowermost hypothecium, is made of sterile hyphae, loosely packed to form pseudoparenchymatous region called trama.

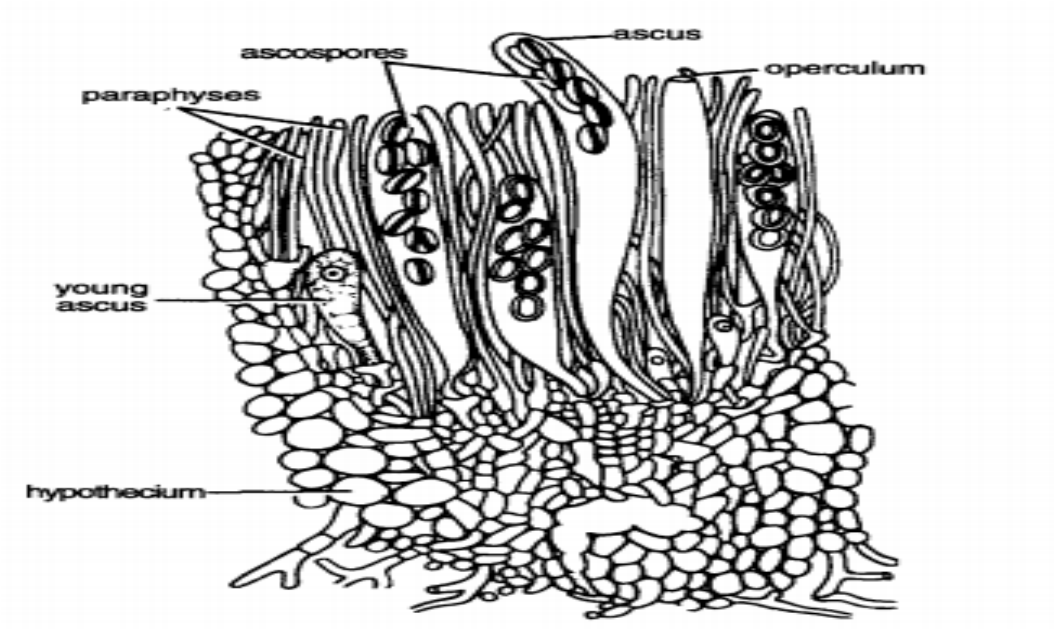
6. The moddle zone consist of a few erect hyphae. 'This later merges with hymenial layer which is called sub-hymenium.

7. The hymenium consists of asci intermingled with paraphyses.

8. The asci elongate on maturation to protrude above the hymenial surface. These are found mixed with paraphyses. Ascus is a long and cylindrical structure. It opens by a terminal pore called operculum.

9. Each ascus contains eight ascospores. Ascospore is one celled, large in diameter, purple or dark brown and the spore wall shows longitudinal colourless striations.

Ascocarp of Ascobolus



Identification

(1) Chlorophyll absent,

(2) Reserve food glycogen,

(3) Cell wall of fungal cellulose…….. Kingdom-Mycota

A definite cell wall present……. Division-Eumycota

(1) Mycelium septate,

(2) Spores borne endogenously in the ascus,

(3) spores in definite numbers, in multiples of two, usually eight. ……Sub-division-Ascomycotina

Ascocarp an apothecium…… Class-Discomycetes.

(1) Apothecia fleshy or leathery,

(2) Apothecia usually not in stroma…… Order-Pezizales

(1) Apothecia cup-shaped or discoid,

(2) Apothecia not differentiated into stipe and pileus……. F amily--Pezizaceae

(1) Apothecia upto 5 mm in diameter,

(2) Apothecia saucer-shaped and growing on dung…….. Genus-Ascobolus.

Plant pathology

Observation of Fungal Plant Pathogens and their Identification

Principle: Fungi are a large group of non chlorophyllous organisms which derive their nutrition either by being saprophytic or by being parasitic on plants, animals and even human beings. While leading a parasitic life on plants, these develop numerous symptoms which are characteristic of an infection by a particular fungus. Further identification of fungi requires the mounting of its mycelia (if sufficient growth is there), or by cutting thin sections of the infected area of the diseased plant and observing them under the microscope. Various asexual and sexual reproductive fructifications of fungal mycelium enable one to identify a fungus with certainty. Thus, the identification of fungi depends upon their habit, thallus structure and most effectively by their reproductive structures.

Materials Required: Diseased Plant samples, slides , needles, forceps, coverslips, microscopes, Fungal mounting media Lactophenolcotton blue(Lactophenol 20.0 g; Lactic Acid 20.0 g or 16 ml; Glycerol 40.0 g or 31 ml; water 20 ml , Cotton Blue dye) razor blades, Distilled Water.

Procedure: In the study of fungi it is usually necessary to make either temporary or permanent mounts of various portions of the material to be used for microscopical examination. The following are simple methods that are frequently used:

I. **Preparation of Materials for Mounting:** Many fungi may be mounted without sectioning. Portions of the somatic and reproductive structures may be removed from culture or from the natural substratum and placed in a drop of the mounting medium on a glass slide. Freehand sections of larger fungus fruiting bodies should be made for the observation of structural details. In case the fruiting body is small, or the fungus is growing on a leaf, it is frequently necessary to place a piece of split pith around the material before making sections. The material can then be sliced with a sharp razor blade and the sections may be placed on a drop of the mounting medium. There are more than one way of studying fungal Pathogens in plants.

1. Many fungi may be mounted without sectioning. Portions of the somatic and reproductive structures may be removed from culture or from the natural substratum and placed in a drop of the mounting medium on a glass slide.For a tuft of fungal mycelium that can be seen on the diseased part, few mycelia can be picked up and spread in the drop of lactophenol with the help of a needle and a forcep. This can be stained with Cotton Blue dye, if required and observe under microscope.

2. Free hand sections of larger fungus fruiting bodies should be made for the observation of structural details.

3. For dry, dusty spores (as those of Aspergillus), after putting fungus in the drop of lactophenol on slide, a glass rod tip moistened with alcohol should be touched on to it. This can be stained with Cotton Blue dye, if required and observe under microscope.

4. The affected plant part with the disease symptom can be observed only after sectioning the material into thin sections using a blade and staining and mounting in above mentioned ways.

5. It is frequently necessary to place a piece of split pith around the material which are flattened plant parts such as leaves, flower parts, etc. before making sections. The material can then be sliced with a sharp razor blade and the sections may be placed on a drop of the mounting medium.

Identification of Fungi

One may come across many forms and structures while observing fungi but there are a few common plant pathogenic fungi which can be identified on the basis of their habit, mycelia, asexual and sexual reproductive structures. Following description can be used in order to recognize a few common fungal plant pathogens.

Sign or any objective evidence of the disease or bodily disorder as shown by the plant is called symptoms of the disease. In some cases symptoms are seen on the part of the plant either due to character and appearance of the visible pathogen whereas in other appearance of the symptoms is the result of interaction between the host and the pathogen with some effect upon or change in the host plant. Symptoms due to the character and appearance of the visible pathogen or its structure or organ.

A pathogen is present in all the vegetative parts of the host plant however, they usually form visible reproductive or resting or fruiting structures either outside the plant organ or partly emerging from the host tissue. In some cases almost the entire body of the pathogen including vegetative and reproductive parts remain external to the host that can be readily seen.

Some of such symptoms are: Mildews: Mildews appear as white, gray, brownish or purplish patches of varying size on leaves, herbaceous stem or fruits. In downy mildews the superficial growth is a tangled cottony or downy layer while in powdery mildews enormous numbers of spores are formed on superficial growth of the fungus giving a dusty or powdery appearance.

Rusts: Rusty symptoms appear as relatively small red, brown, black or yellow coloured pustules of spores, usually breaking through the host epidermis.

Smuts: The affected part of the plant shows a black or purplish –black, sooty or charcoal like dusty mass. Symptoms appear on floral organs particularly the ovulary. Symptoms may also be found on stems, leaves as well roots.

White blisters or white rusts: Numerous white, blister-like pustules which upon breaking exposes white powdery mass of spores on leaves of crucifers.

Scab: Roughened or crust like lesion or freckled appearance of the diseased organ.

Sclerotia: Sclerotium is a compact, often hard, mass of dormant fungus mycelium. In some grasses, the sclerotium assumes a characteristic shape and may be buff or dark brown or purplish in colour.

Blotch: The symptom consists of superficial growth giving the fruit a blotched appearance.

Fruiting bodies: The causal fungus is identified by means of characteristic spore bearing structures (sporophores).

Exudations: Such symptoms are found mainly in bacterial diseases. Mass of bacterial ooze out to the surface of the affected organ seen as drops of various size or as a thin smear over the surface.

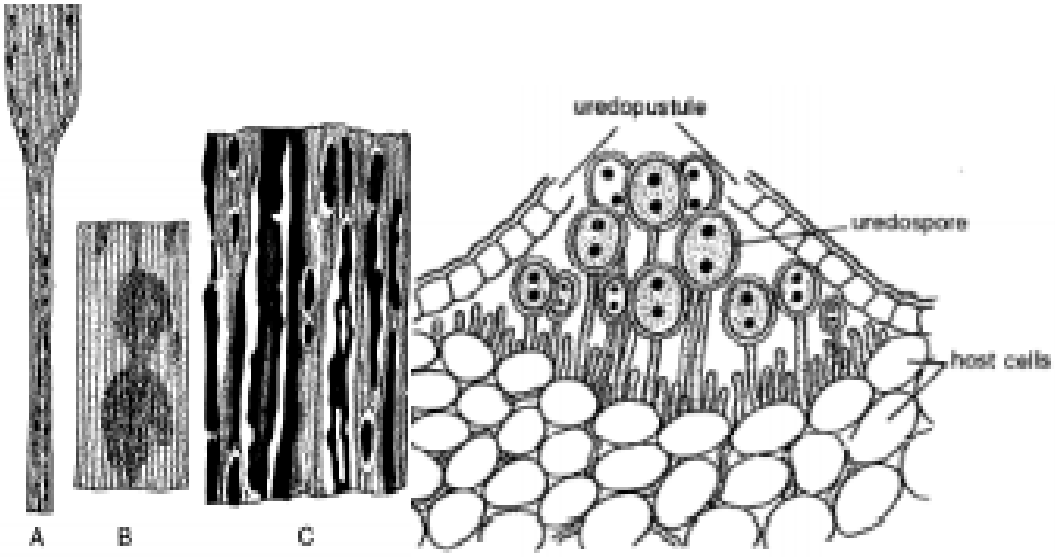
Tar spots: These are somewhat raised, black coated fungus bodies with the appearance of a flattened out drop of tar on leaves.

Puccinia (Rust Fungi) :

Species of Puccinia are known as Rusts, because the infected parts look like rusted iron. All the species are obligate parasites on some of the important cereals viz. wheat, maize and oat, millets as bajra and jowar. The pycnidial and the aecidial cups are formed only on alternate hosts such as Berberis, Thalictrum, etc. To study the mycelial structure, section of a very young wheat leaf is cut. If it shows inter- or intracellular hyphae, it is stained in cotton blue, mounted in lactophenol and studied.

1. The uredosori or uredopustules appear as red, oval or lemon shaped lesions on the leaves and leaf sheaths.

2. The uredospores are produced in massive groups from this mycelium. Each uredospore is binucleate, stalked and rounded or oblong in shape. It has an outer exine which is finely verrucose or echinulate and an has inner smooth intine.



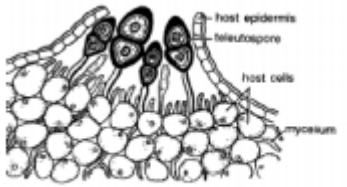
Puccinia: A, B & C Wheat leaf showing Stem Rust; T.S. of Leaf showing Uredospores.

A T.s. of the leaf showing teleutosorus could be stained in cotton blue and mounted in lactophenol.

3. The teleutosori or teleutopustules appear on leaves, leaf sheaths and stem as black, oval pustules that fuse to form patches in case of severe infection.

4. A teleutosorus in a section reveals the (dikaryotic) intercellular, branched mycelium, a bunch of teleutospores and the ruptured host epidermis.

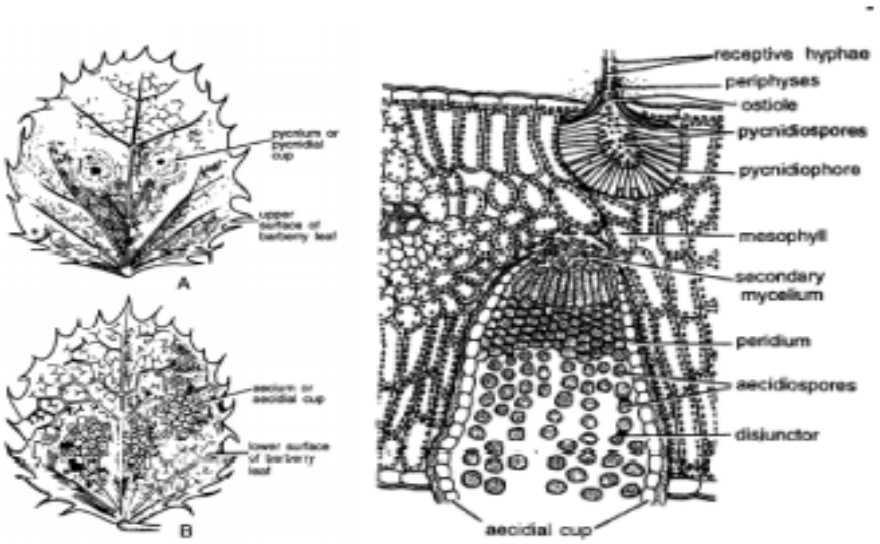
5. Each teleutospore is borne terminally by the mycelium. It is stalked, elongated and bicelled structure. The teleutospore has a very thick but smooth exine and delicate thin intine. The exine turns black at maturity



Puccinia: T.S. of Leaf showing Teleutospores

The pycnidial and the aecidial cups are formed only on alternate hosts such as barberry plant or Thalictrum. The pycnidial cups are found on the upper leaf surface of barberry.

A T.s. of leaf is cut and stained with cotton blue.



Puccinia: Berberis leaf showing Pycnial cups(A) & Aecial cups(B); T.S. showing Pycniospores & Aeciospores

6. A mature pycnidium is flask-shaped with a pore known as ostiole at its apex. The hyphae near the ostiole are unbranched, pointed and orange coloured. These are called periphysis and project through the ostiole.

7. The cavity of the pycnidium is lined by many elongated and uninucleate pycnidiophores or spermatophores. The pycnidiophores are arranged in a palisade like layer and each cuts off a chain of pycnidiospores or spermatia.

To study the aecidial cup and aecidiospores, a transverse section of barberry leaf is stained with cotton blue and mounted in lactophenol.

The aecidia are generally present on the lower surface of leaf and thus both pycnidia and aecidia can be seen in the same section of the host leaf.

8. Each aecidium is cup-like structure with an outer protective layer called peridium. At the base of aecidium there are many elongated cells known as sporophores, arranged in a palisade-like manner.

9. Each sporophore cuts off alternately, a small and a large cell. The small cell is a disjunctor whereas the latter is the aecidiospore

Identification

(1) Chlorophyll absent.
(2) Reserve food glycogen,
(3) Cell wall of fungal cellulose. …… Kingdom-Mycota

A definite cell wall present…….. Division-Eumycota.
(1) Mycelium septate,
(2) Basidium is reproductive body,
(3) Basidiospores usually four, produced exogenously……Sub-division-Basidiomycotina

(1) Basidiocarp lacking,
(2) Teliospores or chlamydospores in sori or scattered,
(3) Parasitic on vascular plants…… Class-Teliomycetes

(1) Teleutospores formed terminally,
(2) Basidiospores on sterigmata,
(3) Infected plants rusty in colour…….. Order- Uredinales.

(1) Teleutospores stalked,
(2) Teleutospores free or united but never in the form of a layer……. Family--Pucciniaceae
(1) Teleutospores bicelled,
(2) Aecia cupulate……. Genus-Puccinia

Albugo

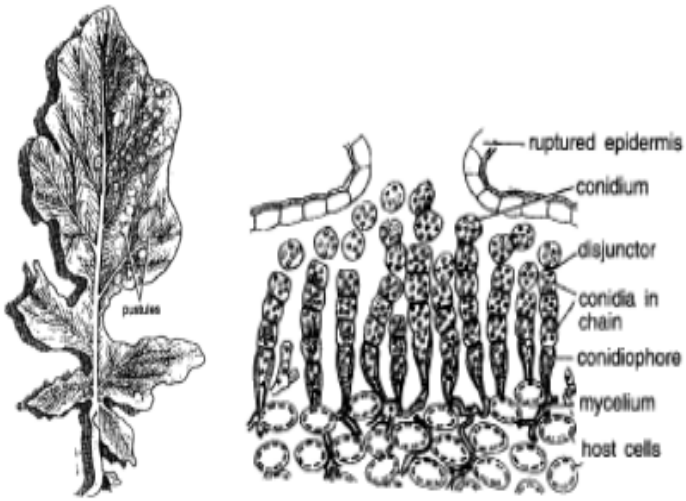
All the species of Albugo are obligate parasites on flowering plants especially the members of Cruciferae causing a common disease, the white rust of crucifers. Cut a section though a diseased patch on the leaf of radish, stain in cotton blue, mount in lactophenol and observe.

1. The mycelium is branched, intercellular, unseptate and produce globular or knobshaped haustoria.

2. Asexual reproduction takes place by conidia. The mass of intercellular hyphae beneath the host epidermis produces vertical palisade-like groups of conidiophores.

3. Each conidiophore bears at its tip a chain of conidia arranged basipetally i.e. the youngest at the base of the chain and oldest at the top. Cut a transverse section of radish or toria. Observe the presence of sex organs

4. The oogonium occurs in the intercellular spaces at the tips of mycelium. It is spherical with a central ooplasm and peripheral periplasm. The antheridium is paragynous in position and contains several nuclei. The oospore develops a thick, ornamented and three layered wall.



Albugo: Infected Leaf of Radish; T.s. of infected host leaf showing ruptured epidermis and conidia in chains

Identification

(1) Chlorophyll absent,

(2) Reserve food glycogen,

(3) Cell wall of fungal cellulose…… Kingdom-Mycota.

A definite cell wall present…… Division-Eumycota

Presence of motile spores or zoospores…… Sub-division-Mastigomycotina

(1) Usually mycelial (aseptate),

(2) Zoospores biflagellate……. Class-Oomycetes

(1) Sexual reproduction aplanogametic (gametes non-motile) and oogamous,

(2) Oogonia contain single egg surrounded by periplasm……. Order-Peronosporales

(1) Mycelium intercellular, provided with haustoria,

(2) Conidia in chains on clavate conidiophores…… Family-Albuginaceae

(1) White shining pustules on leaves,

(2) Infected parts (stem and flower) exhibit hypertrophy,

(3) Conidia arranged basipetally on conidiophores…….. Genus-Albugo

Collection of Diseased Plant Material

Objective: To know the procedure to collect and preserve the diseased plant material.

Requirement: paper bags, Scissors, Forceps, Hand lens, Napthalene balls etc.

Procedure: The field study of diseased plant material includes surveys, field trips,

- collection of diseased specimens from the field and isolating the pathogen fron the diseased parts. The diseased plant parts are to be collected with utmost care and transported to the laboratory under aseptic conditions.
- Usually the diseased plant part are collected in sterile polythene covers and on reaching lab it is surface disinfected and kept for isolating the pathogen.
- The collected plants or plant parts are needed to be immediately killed and subsequently preserved for a long time. For this purpose, a few chemicals are used which do not cause any structural disturbance or distortion of the material.
- Formalin-aceto-alcohol, formalin-propionoalcohol, Navashinfluid and Bouin's fluid are some of the common agents used. Plants are generally fixed immediately after collection or can also be fixed after bringing them to laboratory. The material must be kept completely immersed in preservatives.
- Most common preservatives used are FAAs 70% alcohol. Ethylalcohol (95%)• = 5 cc; Glacial acetic acid (95%) = 5cc and Formalin (40%) = 5cc. for FAA preparation are used.
- In lab we also maintain a collection of dried specimens or cultures. A collection of herbarium specimen is a basic requirement to store information.
- Herbarium sheet is prepared by pressing them to avoid its rotting and dehydration. Collected specimen should be dried and mount on herbarium sheet. The preserved material should be washed after 24 hrs. with water and after washing it should be preserved in 70% alcohol for longer time. Complete specimen is preserved in FAA for demonstration.
- Photographs can also be taken in field also for their study in nature.
- Sections of preserved material are cut in suitable planes from desired region.
  To distinguish different tissues, cells or inclusions specific stains are used such as cotton blue, aniline blue, crystal violet, fast green and safranin etc. These stains are used in different combinations also. For temporary and permanent preparations different techniques are used.
- Mounting is necessary to properly position an object for clear view.
  Lactophenol, glycerine and glycerine jelly are used for temporary mounting while Canada balsam is used for permanent mounting

Study the growth forms of lichens (crustose, foliose and fruticose) on different substrates.

Crustose Lichens:

1. The thallus is poorly differentiated.

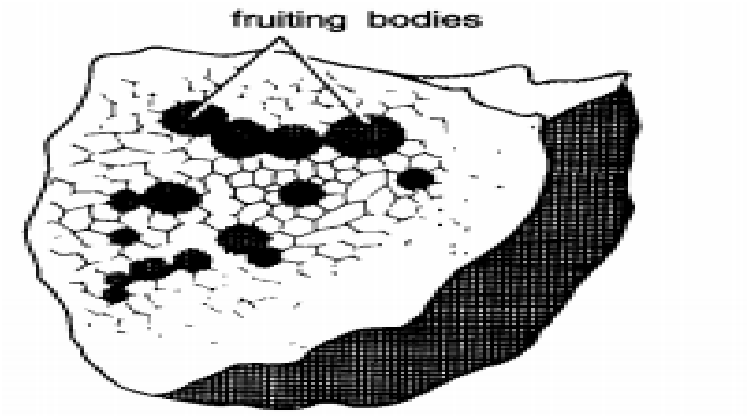
2. The tissues forming the thallus are arranged more of less in strata, one above the other.

3. The upper cortex is made of rudimentary or sometimes highly developed hyphal layer.

4. Algal layer lies just beneath this layer. The layer shows agal filaments and the fungal hyphae in close association.

5. Much below the algal layer lies the medulla composed of loose tissue of branching hyphae.

6. The lower cortex lies next to medulla. It may be well developed or entirely absent.



1. *Lecidia platycarpa*. Crustose ascolichen ;

Foliose Lichens:

1. The foliose lichens have a flat, leaf-like, lobed or deeply incised thallus.

2. It is attached to the substratum only at certain points by rhizines.

3. Rhizines are rhizoid-like outgrowths which arise from the under surface.

4. The thallus may be attached to the substratum either by a single rhizine or by several rhizines.

5. The thallus is generally greyish or brownish in colour.

6. Certain small, hard, dark and gall-like outgrowths called cephalodia may also be present. These help in retaining moisture.

7. The common examples include Parmelia, Physcia, etc.

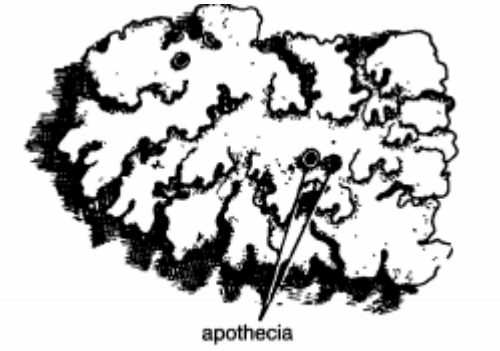


Fig. 2. *Parmelia flavicens.* Foliose ascolichen.

Fruticose Lichens:

1. These appear shrubby with cylindrical, flat or ribbon-like body.

2. It is upright, generally branched and pendulous.

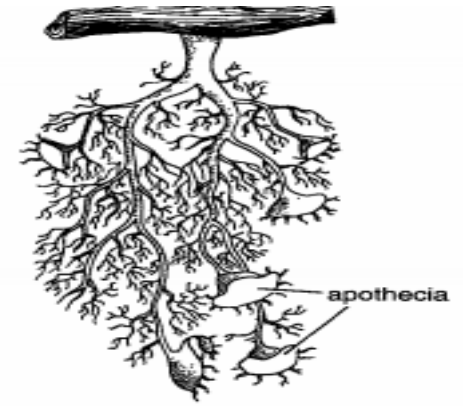
3. It remains attached to the substratum by rhizoidlike structures forming a disc.

4. The cortex is made of closely packed and interwoven fungal hyphae. Intercellular spaces are absent.

5. The algal zone consists of cell of unicellular green alga, Protococcus.

6. Medulla follows the algal zone. It. consists of algal cells loosely mixed with fungal hyphae scattered in different directions.

7. The central chondroid axis is made of longitudinally arranged, compact, thick walled and closely grouped fungal hyphae.



Usnea sp. A fruticose ascolichen t

CP4: Archegoniate-Lab

Study of external features of gametophyte of *Ricciasp.*

Work procedure

Study the external features of the gametophyte, both from dorsal and ventral surfaces.
Observe the two types of rhizoids and violet coloured scales.
Comments

1.The plant body is thalloid, dorsi ventral , prostrate and ribbon-like.

2. A rosette is fonned due to repeated dichotomies of the thalli.

3. The thallus is linear to wedge shaped with an apical notch at the apex and thickened midrib in the sagittal axis. On the dorsal side, the midrib is traversed by a mid-dorsal groove.

4. On the ventral side, scales and rhizoids are present. The scales are present at the margins. The rhizoids arise from the midrib region. Each scale is violet coloured, multicellular and one celled thick.

5. Each scale is violet coloured, multicellular and one celled thick.

6. Rhizoids are of two types--(i) smooth walled and (ii) tuberculate. The smooth walled rhizoids have inner smooth walls whereas tuberculate rhizoids produce tuber-like or peg-like ingrowths of their inner wall which project into the lumen of the rhizoids.

7. Sex organs are present in the mid-dorsal groove and are embedded in the thallus. The sporophytes, however, may be seen as black dots, when mature, under the dissecting microscope.

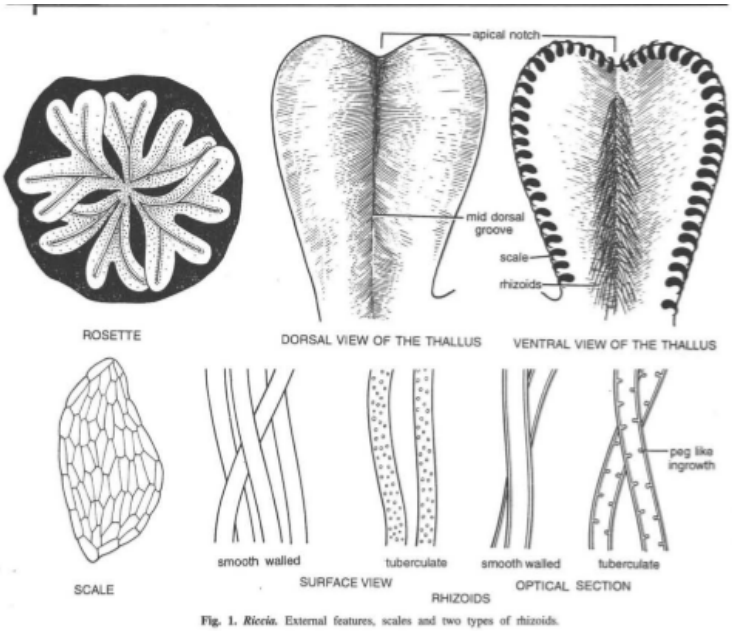


Fig. 1. *Riccia*. External features, scales and two types of rhizoids.

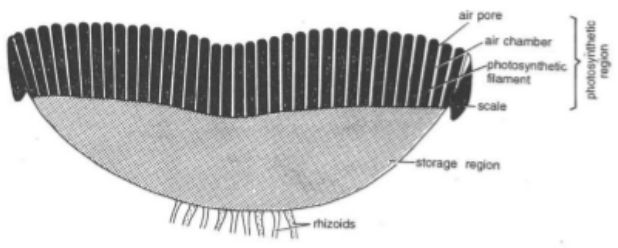


Fig. 2. *Riccia*. V.t.s. thallus (diagrammatic).

Study of external morphology of thallus of *Marchantia* sp.

Work procedure

Study the external features of gametophyte. Observe the thallus from dorsal and ventral surfaces. Remove the rhizoids, stain in safranin and study. Also remove the scales from ventral side from the mid ventral region and margins. Mount in glycerine and study.

Comments

1. Plants are thalloid, dorsi-ventral and prostrate.

2. Thallus is dichotomously branched and the apex of each branch is notched.

3. The dorsal side has a conspicuous midrib and many polygonal areas. These represent the underlying air chambers, each of which opens by a central air pore.

4. Each air pore is compound being made of 4-S superimposed tiers of 3-4 cells each.

5. Certain cup-like structures present along the midrib are known as gemma cups. These contain gemmae, the vegetative reproductive bodies.

6. The ventral surface bears scales and rhizoids along the midrib.

7. Scales are arranged in two to four rows on either side of the midrib. Scales are of two types-(i) the simple or ligulate and (ii) the appendiculate. The appendiculate scales have a sub-rotund appendage at their tips.

8. The rhizoids are of two types-(i) smooth walled and (ii) tuberculate. The inner wall of the smooth walled rhizoid is smooth, while that of the tuberculate rhizoid has tuber-like or peg-like ingrowths. These appear just like circular dots in surface view.

9. The genus is dioecious, male and female thalli being different.

1O. The sex organs are present on the stalked male and female receptacles. The male receptacle is known as antheridiophore and the female as archegoniophore. These structures arise from the growing apices of the thallus.

Study of anatomy of thallus of *Marchantia* sp.

Work procedure

Cut V.T.s. Of thallus by placing it in pith. Stain in safranin or fast green. Mount in glycerine and study.

Comments

1.Thallus is dorsiventrally differentiated into an upper photosynthetic or assirrulatory region and a lower storage region.

2. Photosynthetic region is differentiated into upper epidemis and air chambers.

3. Upper epidermis is interrupted by compound, barrel-shaped air pores which open below into air chambers.

4. Air pore is made of 4-S superimposed tiers of cells.

5. Each air chamber is filled with many branched assimilatory or photosynthetic filaments. The cell of these filaments and epidermis possess many chloroplasts.

6. Storage region is thick in the centre and gradually narrows towards margins.

7. Storage region consists of compactly arranged parenchymatous cells. A few cells are filled with oil bodies and mucilage.

8. The cells in the midrib or centre are slightly thickened to serve for conduction.

9. The lower surface of the thallus is bound by the lower epidermis, which bears scales (two types) and rhizoids (two types) in the middle region.

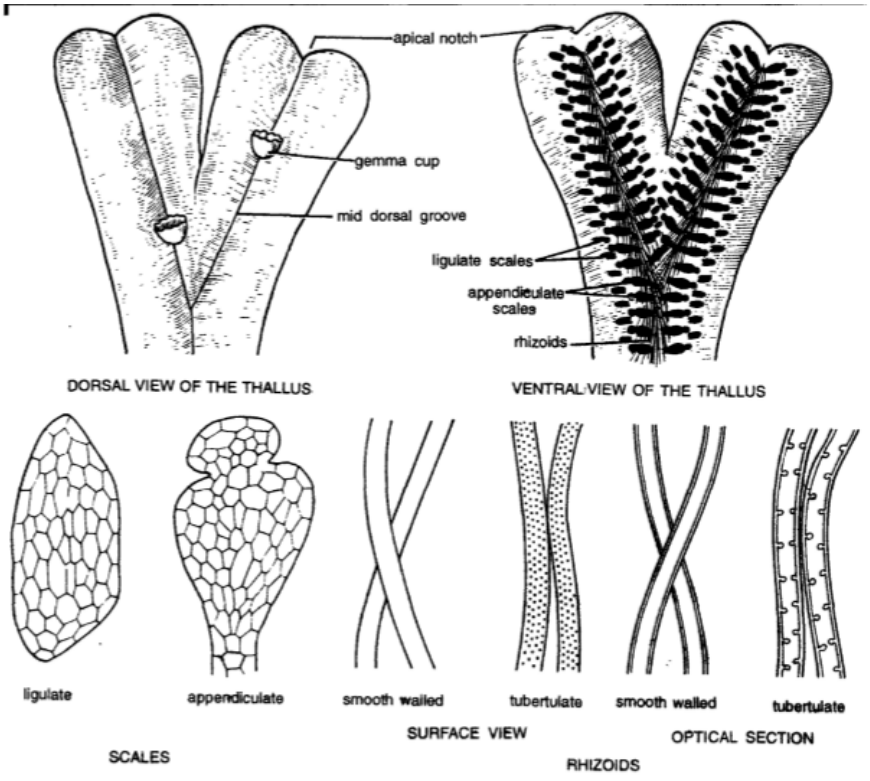


Fig. 1. ***Marchantia.*** External features; dorsal and ventral views, scales and rhizoids.

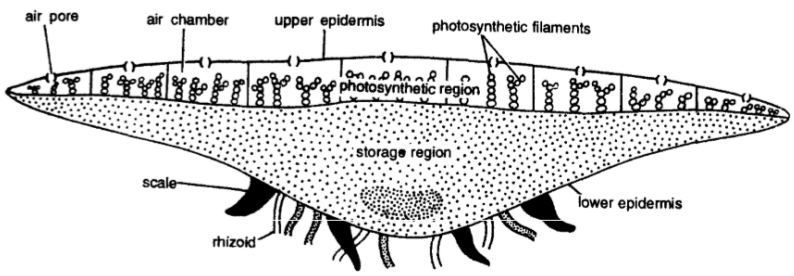


Fig. 2. ***Marchantia.*** V.t.s. thallus (diagrammatic).

Study of vegetative reproductive structure : the gemma cup of *Marchantia* sp.

Work procedure

Gemma cups are found on the upper side of thallus. Cut V.T.s. after placing the thallus in pith. Stain in safranin or fast green and mount in glycerine.

Comments

1. Outline is goblet-shaped with an outer wall and central cavity.
2. The outer wall shows outer photosynthetic region and inner storage region.
3. The internal structure of photosynthetic region and storage region is similar to that of thallus.
4. From the floor of the central cavity arise numerous discoid gemmae.
5. Intermingled with gemmae are many mucilage hairs or cells.
6. The gemma cup arises as a part of the thallus. It remains attached with the thallus by its base.
7. Gemmaeis one-celled, stalked structure. The stalk keeps gemma attached to the base of the gemma cup.
8. The discifonngemma has two shallow notches on both the lateral sides. Each notch possesses a row of apical cells.
9. Towards the periphery of the gemma colourless oil cells are present. Inner to them are the rhizoidal cells.
10. All the cells of gemma except the oil cells and rhizoidal cells contain chloroplast.

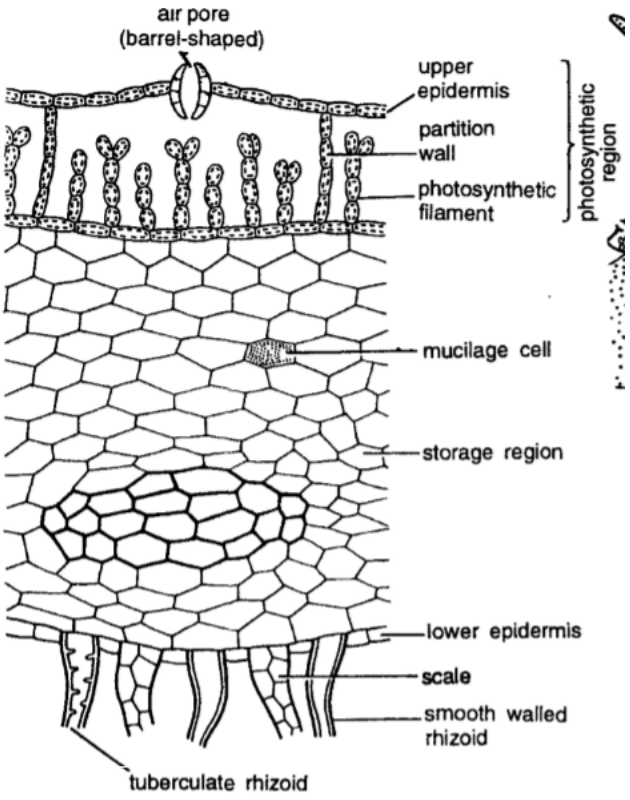


Fig. 4. ***Marchantia.*** V.t.s. thallus (a part cellular).

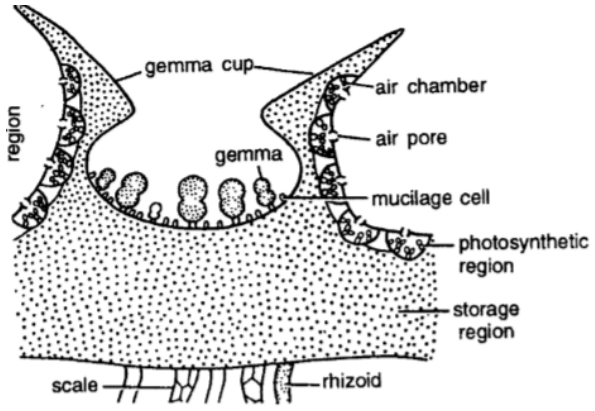


Fig. 6. ***Marchantia.*** V.t.s. of thallus through gemma cup.

Study of male sex organs

Work procedure

Cut a L.s. through antheridiophore, stain in safranin or fast green and mount in glycerine.

Comments

1. The antheridiophore consists of 0.5 to 2.0 cms long stalk, bearing at its apex one eight lobed disc.

2. The peltate disc is slightly convex. The internal structure resembles with that of the thallus.

3. Epidermis is interrupted below by barrel-shaped air pores, each opening below, into an air chamber with branched assimilatory filaments.

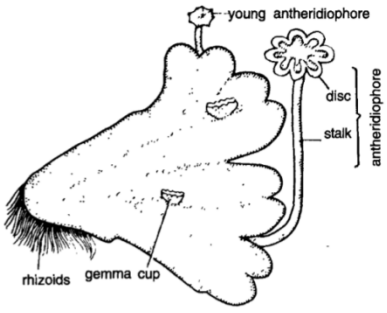


Fig. 9. ***Marchantia.*** Thallus bearing antheridiophore.

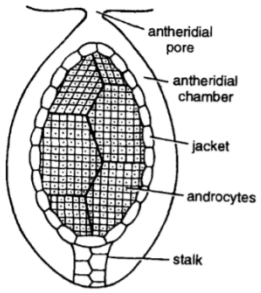


Fig. 11. ***Marchantia.*** An antheridium in anteridial cavity.

4. Alternating with air chambers, are antheridial cavities. Each antheridialcavity, that opens by an antheridial pore, has a single globulr antheridium.

5. The antheridia are acropetally arranged i.e. oldest is nearest the centre and youngest nearest the margins.

6. It has a multicellular stalk attached to the base of the antheridial cavity.

7. The globular body has a single sterile jacket layer. Many androcytes or antherozoids occupy the space inside the jacket.

Study of female sex organs.

Work procedure

Cut L.s. through archegoniophore, stain in safranin or fast green and mount in glycerine.

Comments

1. It is a stalked structure, (stalk 1 to 5 cms long) possessing a nine-rayed stellate disc at the apex. Groups of archegonia are found in between the rays. In each archegonial group, the archegonia are borne in radial rows.

2. After fertilization, sporophyte is formed in the same archegonium.

3. The peltate disc is convex. The internal structure is similar to that of thallus.

4. Outermost is the epidermis, interrupted by air pores. These open into air chambers with branched photosynthetic filaments.

5. In a young receptacle, archegonia are acropetally arranged on the upper side of the disc.

6. Due to the growth in the centre of the disc (which happens only after fertilization),

7. The nearly mature archegonium has swollen' venter and a long rieck.

8. The venter encloses fln egg cell and a venter canal cell, while the neck has 4-8 neck canal cells surrounded by six vertical rows of jacket cells.

9. The cover cells are not much distinct. 10. After fertilization perianth and involucre are developed.

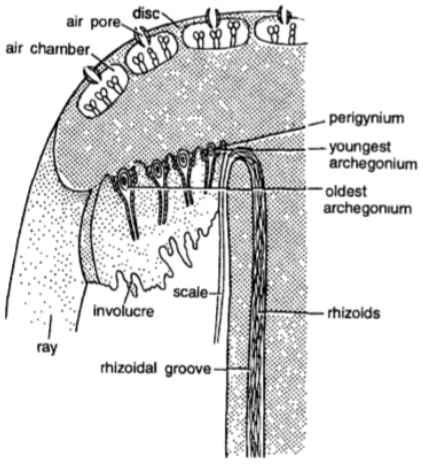


Fig. 14. ***Marchantia.*** A part of L.s. of archegoniophore.

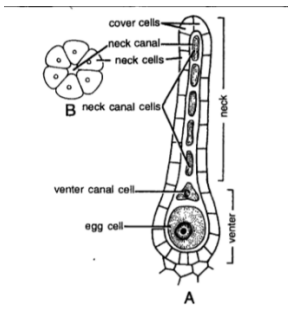


Fig. 15. ***Marchantia.*** **A.** An archegonium. **B.** T.s. through the neck.

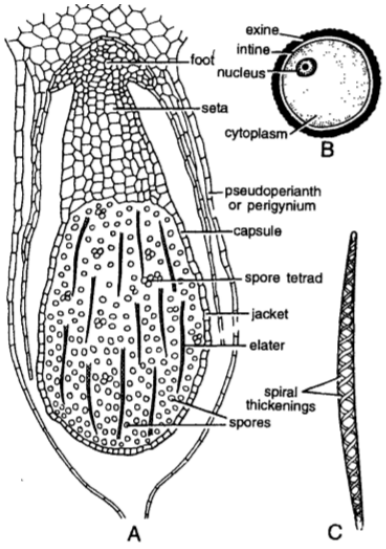


Fig. 16. ***Marchantia.*** **A.** L.s. of nearly mature sporophyte. **B.** Spore. **C.** An elater.

Study of sporophyte

Cut L.s. through disc of mature archegoniophore, stain in safranin and mount in glycerine.
Comments

1.Sporophyte develops in the same place as archegonium after its fertilization. Therefore, capsules are seen in a disc of mature archegoniophore, on the lower side. Only one sporophyte develops in one involucre.

2. The sporophyte is enclosed by three coverings (i) calyptra, (ii) perigynium (perianth) and (iii) perichaetium (involucre). It is differentiated into a foot, seta and capsule.

3. Foot is basal and bulbous. Seta is middle and short and the capsule is spherical, occupying the distal end of the sporophyte.

4. Capsule has a single layered jacket, inside which lie many spores and elaters. Spores are arranged in tetrahedral tetrads.

5. A spore has an outer thick sculptured exine and a thin uniform intine. Every spore is uninucleate with rich cytoplasm.

6. The spores are very small in size.

7. Elaters are spindle-shaped and each possesses 2 spiral thickening bands. These are hygroscopic and help in the dispersal of spores.

Study of external morphology of Pellia sp.

Work procedure

Study the external features of gametophyte. Observe the thallus from dorsal and ventral sides. Remove the rhizoids and study.

Comments

1.The plants are thalloid, prostrate, drosiventral and dichotomously branched.

2. On the dorsal side there is an indistinct midrib and one celled thick lateral wings, with somewhat wavy margins.

3. At the apex is a notch in which growing point is situated. Club-shaped mucilaginous hairs are also present at the apex.

4. The shape of the thallus depends upon moisture conditions. If the thalli grow near water, they are narrow, ribbon-like, delicate and with distinct midrib. If the thalli happen to grow on dry soil, they become shorter, thick and bear an indistinct midrib.

5. Only smooth walled rhizoids are present on the ventral side towards the midrib portion. The tuberculated rhizoids and scales being altogether absent.

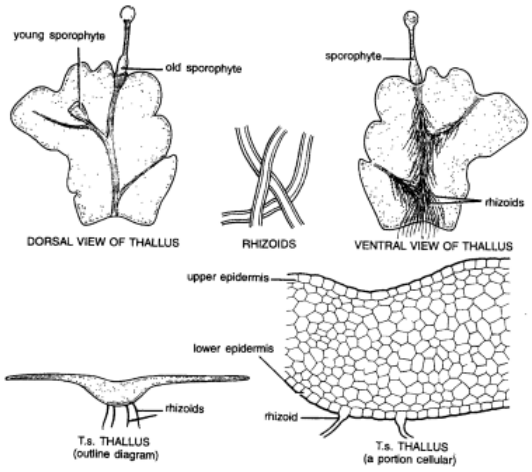


Fig. 1. *Pellia*. External features and anatomy of the gametophyte.

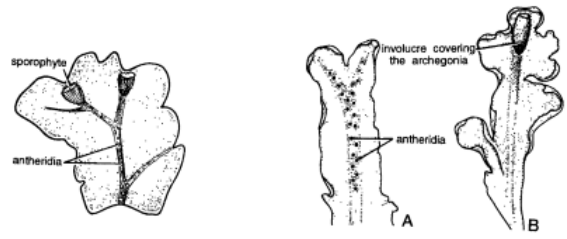


Fig. 2. *Pellia*. Fertile thalli of *P. calycina*.

Fig. 3. *Pellia*. Fertile thallus of *P. epiphylla*.

Study of male sex organs-theantheridia

Work procedure

Cut V.T.s. of thallus passing through midrib. Stain in safranin or fast green, mount in glycerine and study

Comments

1. All the species are dioecious except P. epiphyllaI .." which is monoecious and protandrous.

2. The antheridia are found just behind the growing points in two to three irregular rows in the midrib portion on the dorsal side. 3. Externally antheridia appear as wart-like projections.

4. Each antheridium is present in the antheridial cavity that opens on the dorsal side by a narrow pore known as the' antheridial pore.

5. Each antheridium consists of a short multicellular stalk and a nearly spherical body.

6. The body of the antheridium has a single-layered jacket enclosing many androcytes or antherozoids.

7. Each antherozoid is a spirally coiled, biciliate structure.

Study of female sex organs-the archegonia.

Work procedure

Cut L.s. of the thallus passing through growing point. Stain with safranin or fast green, mount in glycerine and study.

Comments

1. The archegonia are found in groups of 4 to 12, just near the apical cell.

2. Each archegonial group is surrounded by an involucre which may either be tubular, cylindrical or flap-like.

3. Archegonia are intermingled with each other without definite arrangement of older and younger archegonia.

4. A nearly mature archegonium has a short multicellular stalk, a broad venter and a long neck.

5. The jacket of a neck consists of five vertical rows of cells and encloses usually 6-8 neck canal cells. 6. The venter is two-layered thick and encloses a single venter canal cell and an egg cell.

7. The cover cells are 4 in number but are not very much distinct.

Study of sporophyte.

Work procedure

Study the external features and also cut L.s. Stain with safranin or fast green, mount in glycerine and study.

Comments

1. The mature sporophyte consists of foot, seta and capsule.

2. The foot is very prominent with its edges overlapping the basal portion of seta, thus assuming a collar-shape.

3. The seta is short when young but becomes very much elongated at maturity.

4. The capsule is covered by calyptra and involucre respectively.

5. The capsule is nearly spherical and consists of an outer jacket composed of 2 or 3 layers. 6. The jacket layers of capsule have radial thickenings except at 4 places at the top wherefrom the dehiscence takes place.

7. Inside the jacket at the base of the capsule is present a sterile tissue, known as elaterophore. On this elaterophore are attached some 20 to 100 elaters, radiating into the cavity of capsule.

8. In the remaining cavity of the capsule are present spores and elaters.

9. The spores are arranged in tetrahedral tetrads and are formed by lobing of spore mother cell.

10. Each spore is unicellular. The elater is a long, slender, spindle-shaped, structure with generally 2 but sometimes 3-4 spiral thickening bands.

Study of external features of Porella

Work procedure

Study the gametophyticthallus and note down the characters.

Comments

1. The plants are large, prostrate, dorsiventral and grow in compact greenish patches.

2. Thallus is differentiated into a branched stem and leaves, arranged in 3 rows.

3. Out of the three rows of leaves, the two are dorsal and one is ventral. These ventral smaller leaves are known as the amphigastria.

4. The dorsal leaves are incubously arranged (the anterior margin of the lower leaf is covered by the posterior margin of the leaf next above it, when seen from above) and each is divided into a larger (antical) lobe and a smaller (postical) lobule.

5. The lobe is usually ovate with rounded apex while the lobule is narrower with acute apex. 6. Many rhizoids are present on the lower side of the stem.

Study of anatomy of axis.

Work procedure

Place the thallus in pith. Cut T.s. and stain either in safranin or fast green. Mount in glycerine and study.

Comments

1.The axis is differentiated into an epidermis, cortex and medulla.

2.The cortical cells are usually small and thickwalled, in comparison to the large, thin-walled medullary cells

Study of antheridial branch and antheridium.

Work procedure

Tease out the bracts and expose the antheridia, from antheridial branches. Stain in safranin, mount in glycerine and study.

Comments

1. The genus is dioecious and the male and female plants can be externally differentiated.

2. The male plants are usually smaller with the antheridial branches projecting nearly at right angles from the main axis.

3. The antheridial branch has many closely arranged bracts.

4. In the axil of each bract, antheridium is present.

5. Each antheridium consists of a long stalk, composed of two rows of cells subtending at its apex a globular body.

6. The body has a jacket layer composed of 2-3 layers in the basal part and one layer in the upper part.

7. Within the jacket are present many androcytes which ultimately go to form the biciliateantherozoids.

Study of archegonial branch and the archegonium.

Work procedure

Tease the apex of archegonial branch. The archegonia appear between the leaves. Stain with safranin or fast green, mount in glycerine and study.

Comments

1.The genus is dioecious. The female plants are distinct from the male plants.

2. Female plants are usually larger though archegonial branches are shorter than the antheridial branches.

3.The archegonia are found at the apex of archegonial branch. 4. The fIrst few archegonia are arranged acropetally and the last archegonium is formed by the apical cell itself, thus checking the further growth of archegonial branch.

5. The archegonium consists of a broad neck and the venter.

6. The neck consists of fIve vertical rows of cells, typical of jungermanniales, and encloses 6-8 neck canal cells.

7. The venter wall is two layered. It encloses a venter canal cell and an egg cell.

Study of L.s. sporophyte.

Work procedure

Cut a thin L.s. of the capsule. Stain in fast green, mount in glycerine and study.

Comments

1.The sporophyte consists of a small foot, a short seta and a globose capsule.

2.The young sporophyte is covered by calyptra and perianth.

3. The calyptra developed from venter of archegonium is more than one layered thick. It surrounds the sporophyte until its maturity.

4. The perianth is formed by the fusion of two uppermost perichaetial bracts and is more or less dorsiventrally compressed, in the anterior region.

5. The capsule is globose and consists of a jacket layer, two or six cells in thickness.

6. Enclosed within the jacket are many spores and elaters, the latter help in dispersal of spores.

Sphagnum
(Peat Moss or Bog Moss)

Work procedure

Study the branching, the branches and arrangement of leaves on the axis.

Comments

1. The plants are aquatic, growing about the margins of small lakes and ponds or growing on dripping rocky banks.
2. The pH of water in which *Sphagnum* grows ranges from 3.7 to 4.9. Since this water accumulates year after year to form peat and hence the name peat moss.
3. The size of the plant varies from a few inches to a maximum of 7 inches.
4. The plant is erect, branched and differentiated into stem and the leaves. The colourless rhizoids are formed at the base but soon disappear. Hence, there are no rhizoids on mature gametophores.
5. At the apex of the gametophore there are a number of short branches densely crowded in a cluster, called coma.
6. In the posterior part of the stem, the branches arise in tufts in the axil of every fourth leaf and in each tuft there are 3-8 branches.
7. These branches are of two types-(i) diverging branches which are stout, short, growing outward and upward and (ii) drooping or flagelliform branches which hang downward, around and close to main stem. These are absent from submerged forms.
8. At intervals, one of the branches in the tuft grows and forms an apical cluster of branches like the main stem. This is called an innovation. It helps in vegetative propagation by separating from the main branch.
9. When first formed, the leaves are in three vertical rows or three ranked. Later the arrangement changes to 2/5 to. The leaves lack a midrib an exception to mosses.

***Object* : Study of leaf : external features.**

Work procedure

Take out a few leaves, stain in safranin and mount in glycerine and study.

Comments

1. The mature leaf is sessile, entire, acute and onecelled thick.

2. The leaf lacks a midrib and the surface view shows two kinds of cells (i) the narrow, chlorophyll containing assimilatory cells, and (ii) the large, dead, rhomboidal hyaline cells with spiral thickenings and pores.
3. The spiral thickenings provide mechanical support and keep the hyaline cells from collapsing when they are empty.
4. The pores help in rapid intake of water.The green assimilatory cells of the leaf are joined together and form a network with sinuous walls.

Funaria
(Cord Moss)

***Object* : Study of external features of gametophyte.**

Work procedure: Observe the branching, arrangement of leaves, midrib of the leaf, branched and multicellular rhizoids. Pick up a few rhizoids, stain in safranin and observe oblique septa.

Comments

1. The gametophyte shows a prostrate underground protonema and an erect leafy gametophore.
2. The gametophore that arises from protonema is differentiated into (i) rhizoids, (ii) axis or 'stem' and (iii) leaves.
3. Many rhizoids are present at the base. These are slender, branched, and multicellular. The septa are oblique. 4. Young rhizoids are colourless while mature are coloured brown. They also develop chloroplast and become green if exposed to sunlight.
5. The axis is erect and branched. It is 1-3 cms high. The branches arise below a leaf and are thus extra-axillary.
6. The stem and branches are covered with small, simple, sessile and spirally arranged leaves with 3/8 phyllotaxy.
7. The leaves at the apex of the gametophore are crowded to form a bud-like head.
8. Each leaf is nearly ovate in shape and bears a clear midrib except when young.
9. Sex organs are borne at the apices of the axis.

***Object* : Study of antheridial branch and antheridium.**

Work procedure: Tease a few tips of branches. Remove the cluster of leaves to bring out antheridia. Stain in safranin, mount in glycerine and study.

Comments

1. The sex organs are present at the apices of branches. These are enclosed by a/ group of leaves at the apex.
2. At the tip of the stem, is an antheridial branch or 'male flower' -a cluster of antheridia.
3. Intermingled with antheridia are multicellular capitate hairs, known as paraphyses.
4. Both antheridia and paraphyses are surrounded by large leaves, known as perichaetial leaves.
5. In the antheridial branch antheridia in various stages of development occur together.
6. The mature antheridium consists of massive stalk and a club-shaped body.
7. The body has a single layered outer jacket, the cells of which contain chloroplasts.
8. At the apex of the jacket is an operculum, which helps in liberation of antherozoids.
9. A dense central mass of androcytes lies within the jacket.

***Object* : Study of archegonial branch and archegonium.**

Work procedure: Tease the apex of the female branch to remove the leaves. Stain in safranin, mount in glycerine and study.

Comments

1. The sex organs are situated at the apices of branches inside the cluster of leaves.
2. The archegonia also arise in clusters at the apex of the archegonial branch.
3. Intermingled with archegonia are paraphyses.
4. The archegonia and paraphyses are surrounded by closely folding, unmodified leaves.
5. All the archegonia of this cluster are almost of the same age and developmental stage.
6. The nearly mature archegonium is a multicellular, stalked structure, with a broad venter and narrow twisted neck.
7. The wall of the venter is double layered. The neck consists of six longitudinal rows of cells surrounding a central canal.
8. In the neck there are six or more neck canal cells and the venter has one venter canal cell and one egg cell.

***Object* : Study of internal structure of sporophyte.**

Work procedure: Cut a T.s. of the capsule and stain in safranin and fast green; mount in glycerine and study.

Comments

1. L.s. of the capsule can be divided into three regions-(i) apophysis, (ii) theca proper and (iii) upper region.
2. Apophysis is the basal region. In its centre is a conducting stand in continuation with that of seta.
3. Around the conducting strand are few layers of cells with intercellular spaces and chloroplast. The epidermis in this region is ventilated (stomata present).
4. The theca proper is the fertile region. It has a central columella, the upper part of which is cone-shaped, projecting into the concavity of the operculum. On the basal end, it is connected with the central tissue of the apophysis.
5. Around the columella is a U-shaped spore sac, broken at the base, thus separating the two arms of U.
6. Spore sac has an outer wall of 3-4 layers of cells and an inner of one layer. Between these, only spores are present, elaters being absent.
7. Each spore has an inner hyaline endosporium and a coloured, almost smooth exosporium.
8. Inside the endosporium is the cytoplasm, with a nucleus, oil globules and chloroplasts.
9. Outside the spore sac, is an air space that is divided into many air cavities by green filaments which run from the external tissue of the wall to the outer wall of the spore sac.
10. The wall of the capsule is many layered. Two to three inner wall layers of the capsule in theca region are green and show intercellular spaces while outermost 2-3 layers just beneath the epidermis are compact parenchymatous and colourless.
11. The upper region consists of operculum and peristome. It is marked off by a conspicuous constriction, immediately below which is a rim and above the annulus.
12. Calyptra covers the capsule. The peristome teeth encircle the operculum.

Psilotum

***Object* : To study the spore producing organ the synangium.**

Work procedure: Study the external features of synangium, slide of T.s. and also a single spore.

Comments

1. Sporangia, the spore producing organs, are produced on the aerial branches.
2. These are borne in triads on minute appendages subtended by a bract. (Since the sporangia are fused with one another, the structure is called as synangium).
3. In a transverse section, synangium reveals 4-5 layered jacket, outer of which is made of thick walled cells. The loculi are filled with numerous spores. Interspersed among the spores are disintegrated sporocytes which serve as nutritional fluid.
4. Individual spores are bean-shaped or bilaterally symmetrical. The wall pattern is reticulate. There is a narrow slit with a median ridge.

Selaginella

(Small Club Moss)

***Object* :Study the external features of the plant.**

Work procedure: Study the plant, specimen observe the differentiation of plant body into root, stem and leaves. Study the two types of leaves, their arrangement and structure. Also observe the structure of a ligule. Note the presence of rhizophore.

Comments

1. Many species are prostrate, creeping on the ground e.g. S. *kraussiana,* others are sub-erect e.g. S. *trachyphylla* or erect e.g. S. *erythropus.* A few species climb with the help of rhizophores e.g. S. *alligans.*
2. The plant body is divided into root, stem and leaves.
3. The primary root is short lived and all other roots are adventitious.
4. On the basis of nature of stem and form of the leaves, the genus is sub-divided into two sub-genera-the homoeophyllum and the heterophyllum.
5. In homoeophyllum species *(S. selaginoides,* S. *rupestris,* etc.) the stem is upright and all leaves are alike, while in heterophyllum species (majority of the species), the stem is prostrate and dorsiventral; and leaves are dimorphic (small and large).
6. In homoeophyllum, all the leaves are alike, spirally arranged, small and simple.
7. In heterophyllous species, they are dimorphic and are borne in pairs on dorsi ventral stem. The two leaves are markedly different in size (one is larger and other smaller).
8. The smaller leaf of each pair is inserted on the dorsal side of the stem while the larger leaf is inserted on the ventral side.
9. The successive pairs of leaves are so arranged, that large leaf always alternates with the large leaf, and small leaf with the small leaf.
10. Each leaf is sessile, generally obovate with acute apex, and has a distinct midrib.
11. At the base of each young leaf, on the adaxial face, their is small tongue-like out growth, the ligule.
12. It is differentiated into basal sheath, glossopodium and the body of the ligule.

Pteridophyta

13. Whereas the cells of the sheath are tubular in shape and are dead, those of the glossopodium are vertically elongated.
14. The body of the ligule has parenchymatous cells with dense protoplasm.
15. From the point where stem branches, a cylindrical leafless organ is seen growing downward. This is known as rhizophore.

16. On reaching the ground, rhizophore terminates into roots (The morphological nature of the rhizophore is still open to question).
17. Certain vertical branches from the stem are reproductive in nature and bear strobili.

***Object* : Study of anatomy of the stem.**
Work procedure
Cut a T.s. of the stem, stain in safranin-fast green combination, mount in glycerine and study.
Comments
1. The outline of the section appears slightly wavy.
2. The section shows epidermis, cortex and the stele.
3. Epidermis is the outermost layer. It is cuticularised and lack stomata.
4. The cortex consists of parenchymatous cells, without any intercellular spaces. All the cells of the cortex are thin walled.
5. Hypodermis occurs close to epidermis. It develops from cells of outer cortex which become thick walled. In xerophytic, species (e.g. S. *rupestris*, S. *lepidophyUa)* hypodermis is more thickened.
6. The stele is generally a protostele.
7. Endodermis separates vascular tissue from the cortical region, by radially elongated endodermal cells, called as trabeculae, with conspicuous intercellular spaces between two trabeculae. In spite of their great elongation, trabeculae still retain the transverse thickenings, the casparian strips, on their radial walls, characteristic of endodermal cells. Xerophytic species lack trabeculae (e.g. S. *lepidophylla* and S. *rupestris).*
8. Pericycle is a single layer surrounding the xylem and phloem and follows endodermis.
9. Stele. The number of steles in a stem varies from 1-16 thus exhibiting a polystelic condition.
10. Single stele, when present is generally diarch and exarch.
11. In S. *kraussiana,* the commonest species, there are two steles, each with a single exarch mass of protoxylem.
12. The protoxylem masses of the two steles point in opposite directions.
13. The phloem consists of smaller cells with dense protoplasm and completely surrounds the central core of xylem, in each stele.

***Object* : Study of spore producing organs.**
Work procedure: Study the external features of the strobilus. Cut L.s. of the strobilus, stain in safranin-fast green combination, mount in glycerine and study. (Alternatively study the slide of L.s. of the strobilus).
Comments
1. The spore producing organs are sporangia, aggregated in strobili which are generally present at the apices.
2. In some cases (as exemplified by S. *patula)* the axis may grow beyond the strobilus, terminating into a vegetative shoot or even in a second strobilus.
3. L.s. of the strobilus shows a strobilar axis, around which sporophylls are spirally arranged. Each sporophyll is ligulate and similar to a foliage leaf.
4. The sporangia are of two types, borne in the axils of the sporophylls, attached either strictly to the axils or to the axis just above.
5. Selaginella is heterosporous, with megaspores (large) and microspores (small), borne in their respective sporangia, known as megasporangia and microsporangia.
6. If a micro sporangium is borne in the axil of the sporophyll, it is known as a microsporophyll but if it is a megasporangium, the sporophyll is termed as a megasporophyll.

7. Generally strobilus bears both types of sporangia but in S. *gracilis,* there are only one type of sporangia (either mega-or micro sporangia).
8. When both kinds of sporangia occur in one and the same strobilus, their arrangement differs from species to species: (i) In some species (e.g. S. *oregana)* there are only megasporangia on one side and only microsporangia on the other.
(ii) In most of the species (e.g. S. *kraussiana)* there are only one or two megasporangia at the base and the rest are microsporangia.
9. Both types of sporangia are stalked and have. two layered jackets. The outer layer of the jacket is chlorophyllous and has columnar cells, whereas the inner layer has tangentially elongated cells. It may form tapetum.
10. The cells of the outer jacket are thickened, except at the apex.
11. The two types of sporangia when ripe, differ -in their size, form, structure and colour.
12. The megasporangium is much larger, four lobed, pale green or orange in colour and has only four megaspores.
13. The microsporangium is smaller with uniform outline. It is dark brown or red in colour and has many spores.
14. The megaspores are large in size and posses a triadiate ridge at its apex. It has thick sculptured exine and thin uniform intine.
15. The microspores are pyramidal in shape, and have thick, ornamented exine and a thin, uniform intine.
16. Both types of spores have a nucleus suspended in a rich cytoplasm.

Equisetum
(Horse Tails)

***Object* : Study of anatomy of internode of aerial shoot.**

Work procedure: Cut a T.s. of the aerial shoot passing through the internode, stain in safranin-fast green combination, mount in glycerine and study.

Comments

1. The outline is wavy with ridges and grooves.
2. The tissues are organised into epidermis, cortex, stele and a pith cavity.
3. The epidermis is cuticularized with tangentially elongated and silicified cells.
4. The stomata are mostly found in the grooves. The guard cells are surrounded by two subsidiary cells, one on either side.
5. Cortex follows the epidermis and is highly differentiated. It is divided into outer and inner cortex.
6. Outer cortex, below the ridges has a group of sclerenchyma. Small patches of sclerenchyma may also occur, below the grooves.
7. Beneath the ridges radially elongated chlorenchymatous cells (palisade tissue) are present. The amount of palisade beneath the grooves is lesser.
8. The inner cortex is composed of large and thin-walled, parenchymatous cells.
9. Vallecular canals are present in the cortex. These are situated below the grooves.
10. The stele is an ectophloic siphonostele that consists of ring of vascular bundles.
11. Endodermis occurs at different positions in different species. (i) Most commonly, the endodermis forms a simple sheath, outside the ring of bundles. (ii) In some cases, in addition, there is also an internal endodermis and outer endodermis dips in between the bundles. (iii) In third condition, each bundle is surrounded by an individual endodermis.
12. Pericycle lies below the endodermis.
13. The vascular bundles are collateral and endarch, arranged in a ring and each bundle lies below each ridge.

14. Each bundle has one inner strand of protoxylem and two outer of metaxylem.
15. The protoxylem elements lie on the sides of a protoxylem lacuna, the carinal canal, formed by the disintegration of protoxylem elements.
16. The two metaxylem groups lie on two lateral sides of carinal canal (Le. on the shoulders of the bundle).
17. The rest of the tissue of the vascular strands is parenchymatous.
18. Pith cavity known as central canal lies in centre.

***Object* : Study of anatomy of rhizome.**
Work procedure: Cut a T.s. of rhizome, stain in safranin-fast green combination, mount in glycerine and study.
Comments
1. The outline is wavy with ridges and grooves.
2. Epidermis. This is the outermost thickly cuticularised layer. Stomata are absent.
3. The cortex consists of a few layers of sclerenchyma just below the epidermis and a large zone of parenchyma spread upto the ring of vascular bundles.
4. Large vallecular canals are present in the
5. Endodermis is single layered and encloses a ring of vascular bundles.
6. Each bundle is located below the ridge.
7. The bundle is conjoint, collateral and endarch.
8. The bundle has a large protoxylem lacuna, carinal canal.
9. Pith cavity. The centre has a large cavity, called pith cavity.

***Object:* Study of spore producing organs (L.s. of cone.)**
Work procedure: The spore producing organs are sporangia borne in cones, generally terminating the main axis and sometimes the lateral branches. The structure is best studied by observing L.s. of cone, single sporangiophore and spores. Study the features shown by respective slides.
Comments
1. L.s. of the cone shows cone axis and attached sporangiophores.
2. Cone axis is centrally located.
3. It bears sporangiophores in whorls which are mostly alternate though not regularly.
4. At the base of the cone is a calyx-like whorl, the annulus (which most probably represents a modified leaf whorl).
5.The sporangiophores are attached to the cone axis at right angles with its stalk.
6. The stalk holds a polypogonal peltate disc at right angles to it. The peltate discs of sporangiophores fit closely to form a protective cover for the sporangia below.
7. Sporangia appear attached on the lower side of the disc.
8. Each sporangium is elongated and sac-like. It has one-layered jacket that encloses numerous spores.

***Object* : Study of spore producing organs (T.s. of cone)**
Work procedure: Study the characters of the cone by observing various features as shown by the slide of T.s. of cone.
Comments
1. T.s. of cone shows a cone axis and sporangiophores attached to it.
2. Centrally located part is called cone axis.
3. Sporangiophores are attached in a whorl.
4. Each sporangiophore consists of a stalk and a disc.
5. Stalk keeps the disc attached to cone axis.

6. The peltate disc bears sporangia on the underside, with one layered jacket which enclose the spores.
7. Each sporangium appears elongated and cylindrical.
8. Sporangiophore is one of the units, of which cone is made of.
9. These are attached to the central cone axis in successive whorls.
10. Each sporangiophore consists of a stalk and a polygonal peltate disc.
11. The stalk is attached to the cone axis on one side and to the peltate disc on the other.
12. About 5-10 cylindrical sporangia are arranged in a ring near the margins on the lower side of the disc.
13. Sporangium has a one layered jacket with helical thickenings.
14. Numerous spores, all similar (homosporous condition) are present in the sporangial cavity.
15. A longitudinal line of dehiscence is also clearly seen.

Cycas

***Object* : Study of anatomy of normal young root.**

Work procedure: Cut a T.s. of the young part of primary root, stain in safranin-fast green combination, mount in glycerine and study.

Comments

1. The section is circular in outline. It shows an outer layer or epiblema, cortex and centrally located stele.
2. Epiblema is made of single layer of thin walled cells. Some of these cells bear unicellular root hairs.
3. Cortex is multilayered with starch filled parenchymatous cells. A few tannin filed cells are also scattered in this region.
4. Endodermis is single layered and indistinguishable. Many-layered pericycle separates the cortex from vascular tissues.
5. The central stele is made of radial and exarch vascular bundles. There are two protoxylem groups and thus condition is diarch.

***Object* : Study of anatomy of older part of normal root.**

Work procedure: Cut a T.s. of the older part of normal or primary root, stain in safranin-fast green combination, mount in glycerine and study.

Comments

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 formed below it. Cork cells are a few layered deep and are arranged in brick-like fashion.
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 pericycle.
5. Primary phloem is the outernost (near the pericycle) and is crushed during secondary growth. Secondary phloem follows this layer, the cells of which are intact.
6. Cambium arcs are formed 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 formed.
9. In the centre is a small parenchymatous pith.

Object : **Study of anatomy of rachis.**

Work procedure: Cut a T.S. of rachis from its middle region, stain in sarfanin-fast green combination, mount in glycerine and study.

Comments

1. Outline. It 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. Epidennis is single layered, thickly cuticularized and is interrupted by stomata throughout its surface. The condition is known as amphistomatic.
4. Hypodennis is mainly composed of thick-walled cells (sclerenchyma). Intermixed with these cells
are a few cells having chloroplasts chlorenchyma.
5. This sc1erenchymatous 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 (ʊ) 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 conditionis is said to be mesarch.

Object **: Study of anatomy of leaflet (pinna).**

Work procedure Cut a T.s. of leatlet, stain in safaranin-fast green combination, mount in glycerine and study.

Comments

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.
(i) In C. *revoluta* midrib is less projected than in C. *circinalis,* where it is much projected on the upper side.
(ii) Margins of wings are revolute in C. *revoluta,* and C. *beddomei* while they are straight in C. *circinalis,* C. *rumphii,* C. *pectinata* and C. *siamensis.*
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. (i) In C. *revoluta,* hypodermis is present in the midrib (near both upper and lower epidermis) and wings (below the upper epidermis). (ii) In C. *circinalis,* hypodermis in the midrib region is present on both the sides (upper and lower) while in the wings, it occupies only the comers, being absent from rest of wings.

5. Mesophyll lies below the hypodermis and is well developed. It is differentiated into upper palisade layers and lower of spongy parenchyma. (i) In C. *revoluta*, palisade is present beneath the hypodermis, both in the midrib and the wings. (ii) In C. *circinalis* palisade is absent from the
midrib region
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 *Gymnosperms*
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.
12. The vascular bundle is similar in all respects to that found in the upper region of the rachis. It 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 centripetal protoxylem.

***Object* : Study of microsporophyll and microsporangia.**
Work procedure: Take out a microsporophyll from the male cone. Study both- upper and lower surfaces. Observe the sporangia on the lower surface with a magnifying lens.
Comments
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.

***Object* : Study of** T.s. **of microsporophyll.**
Work procedure: Study the characters observed III sliok of T.s. of microporophyll.
Comments
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 micro sporangium.
4. In the microsporophyll are present many mucilage ducts, regularly scattered, among the rounded mesophyll-like cells forming the tissue of the sporophyll.

***Object* : Study of L.s. of mature ovule.**
Work procedure: Study the slide showing L. s. of mature ovule.
Comments
1. The section shows that the ovule is orthotropous.
2. It is unitegmic (possesses 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 layersan outer fleshy layer, middle stony layer and an inner fleshy layer. The outer and inner flshy 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. Archegonial chamber. Just above the archegonia is the archegonial chamber.
8. Micropyle. 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

***Object* : Study of T.s. needle (leaf)**
Work procedure: Cut a thin T.s. of a needle, stain with safranin-fast green combination, mount in glycerine and study.
Comments
1. The outline of the section varies according to the species. (Triangular if spur is trifoliar, semicircular
if spur is bifoliar)
2. The needle is differentiated into epidermis, mesophyll and stele.
3. Epidermis is single with tangentially elongated and thickly cuticularized cells.
4. Stomata are sunken. These are present on all the faces of epidermis. The needle is thus said to the amphistomatic.
5. Epidermis is followed by hypodermis. It is few layered thick at the corners and 1-2 layered in other parts. Sub-stomatal chambers occur in this region. Cells are sclerenchymatous and fibrous.
6. Mesophyll lies below the hypodermis. It is made up of polygonal parenchymatous cells, densely filled with the chloroplasts. Numerous plate-like or peg-like infoldings project into the cell lumen (cavity) from the wall of the mesophyll cells.
7. Resin canals generally occur in the sclerotic hypodermis but also occur in the mesophyll tissue.
8. Endodermis is conspicuous. Cells are barrelshaped and tangentially thickened. It is followed by a many layered, parenchymatous pericycle.

9. Generally two vascular bundles remain surrounded by this tissue. (In *P. strobus* there is only one vascular bundule).
10. The vascular bundles are separated from one another by a T-shaped thick walled transfusion tissue.
11. Each vascular bundle is conjoint, collateral and open. Protoxylem faces adaxial side. Phloem is located on the abaxial side.
12. Xylem and phloem groups are separated from one another by cambium at the base of the needle and by parenchymatous cells in the upper region.
13. Secondary growth is very little during which the medullary rays run between xylem and phloem.

***Object* : Study of L.s. of male cone.**
Work procedure: Study a slide showing L.s. of male cone.
Comments
1. It shows a cone axis bearing microsporophylls.
2. The cone axis is centrally located.
3. Microsporophylls are spirally arranged. These are scaly, triangular and expanded.
4. It is attached to the cone axis by a stalk-likebase.
5. The outer expanded part is sterile and is known as apophysis.
6. Microsporangia are present on the lower or abaxial surface.
7. Each micro sporangium has a wall that encloses a cavity.
8. The wall consists of epidermis. wall layers and tapetum.
9. The cavity shows numerous microspores in various stages of development.

***Object* : Study L.s. of female cone.**
Work procedure: Study a prepared slide of L.s. of female cone.
Comments
1. Female cone is made of centrally located cone axis and spirally arranged sporophylls.
2. Each sporophyll consists of two kinds of paired scales : (i) bract scale or cone scale and (ii) ovuliferous scale or seminiferous scale.
3. Many small and thin bract scales are arranged spirally around the cone axis. They are directly borne on the cone axis. Each of these is present on the abaxial (lower) side of the ovuliferous scale.
4. On the adaxial (upper) side of the bract scale, a thick, large, woody and triangular ovuliferous scale is present.
5. The ovuliferous scales in the middle part of the cone are the largest and get gradually smaller
towards its base and apex.
6. Ovuliferous scale and bract scale are fused for a little distance near the cone axis while free at a distance away from it.
7. Ovuliferous scale is shortly stalked and rest of the part is expanded.
8. At the base "of this expanded, triangular part, two naked and sessile ovules are present. These are situated on the adaxial, (upper) surface of the ovuliferous scale, at its base, with their rnicropyles directed towards cone axis.
9. The terminal part of the ovuliferous scale is broad and sterile and is known as apophysis.

***Object* : Study of T.L.s. of wood.**
Work procedure: Cut a thin section of wood along the tangent in the
outer region, stain in safranin-fast green combination, mount in glycerine and study.
Comments

1. Tracheids and medullary rays are cut transversely in this plane.
2. The bordered pits are cut to show overarching borders, forming a dome-like structure. It encloses in the centre a small disc, called torus.
3. Medullary rays are uniseriate. Since they are cut transversely, their height and breadth can thus be determined.
4. Each medullary ray appears to be a short row of more or less rounded cells, three or four cells high.
5. Composition of medullary ray reveals centrally placed, thin-walled and living cells-the albuminous cells (in the phloem region) and the ray cells (in the xylem region).
6. These are surrounded on the lower and upper sides by thick walled and dead cells known as ray tracheids.

***Object* : Study of R.L.s. of the wood.**
Work procedure: Cut a thin section of wood along anyone of the radii, stain in safranin-fast green combination, mount in glycerine and study.
Comments
1. It shows presence of secondary xylem, ray tracheids and medullary rays.
2. Xylem is composed of tracheids with bordered pits on their radial walls. The bordered pits in
this section are seen in surface view.
3. Bordered pits are circular areas surrounded by special cellulose thickenings called crassulae or Bars of Sanio. If pits are close to one another,
the bars fuse to form Rims of Sanio.
4. Medullary rays run horizontally. In radial longitudinal plane they are cut length-wise and their length and height can be noticed. They are uniseriate.
5. Each medullary ray is made up of ray cells, ray tracheids and parenchyma.
6. Ray tracheids are present on both the sides of the medullary ray cells, only in the region of xylem. These cells are thick, narrow and long. They show bordered pits.
7. Ray parenchyma occurs between the tracheids. These cells are thin, broad, small and living.
8. Medullary ray, in the region of phloem replaces ray tracheids with albuminous cells. They are small and contents are dense. (Ray parenchyma associated with these cells is filled with large amount of starch).