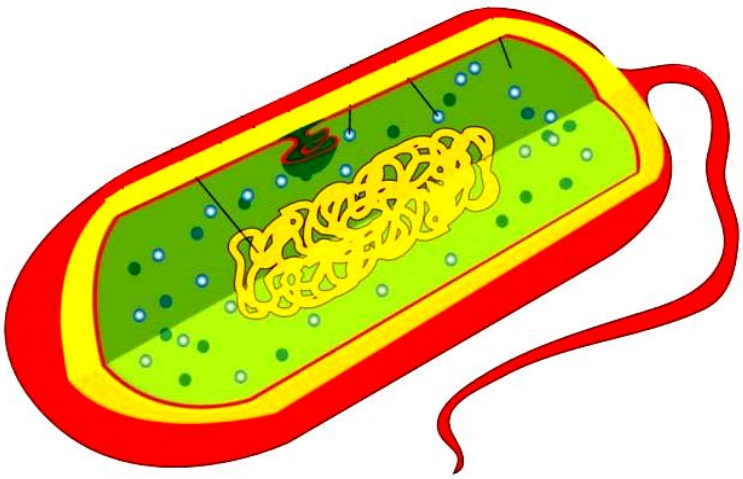

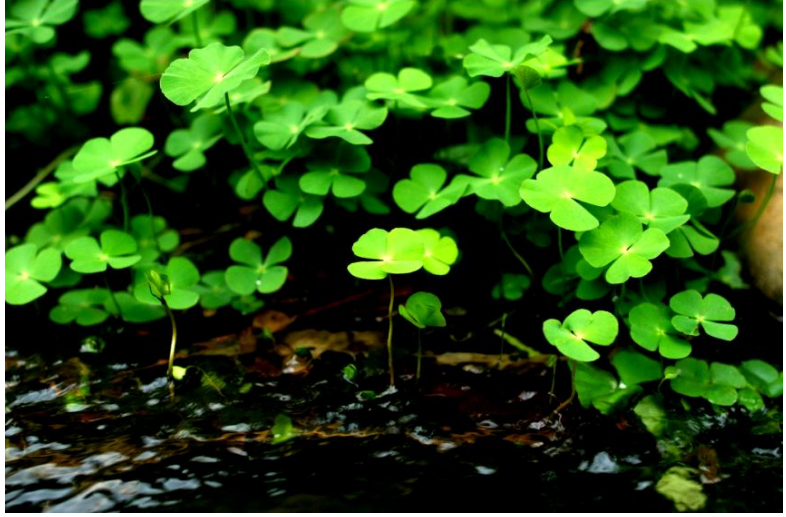

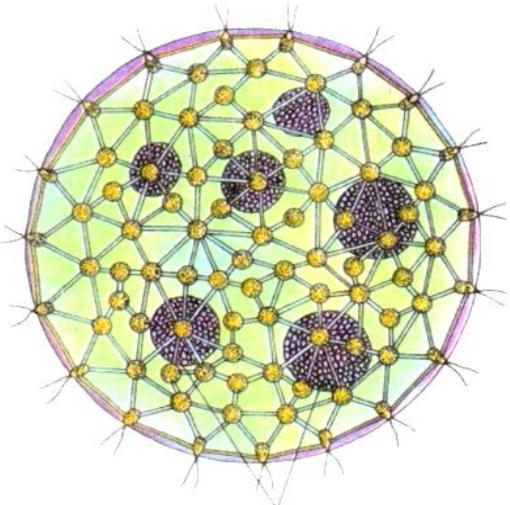

A Text book of unified syllabus of Practical Botany (B.Sc. 1st year)

S. Saad, S. Bushra, A.A. Khan

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Dedicated

to my be loved parents and brother
Syed Saad
Akil A. Khan

to my Ammi (Late Mrs. Mudassir Fatima)
who nurtured me as a small plant and shaped me as a strong tree, and always remained with me as a strong pillar during the long duration of my study. Their countless blessings have no horizon for me.
Sayyada Bushra

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Preface

The present book “A Text book of unified syllabus of Practical botany of B.Sc. 1st year” is written strictly in accordance with the UGC model curriculum and revised syllabi of various Indian Universities. It written as an aid to the students in their laboratory work, science then the emphasis on practical work kept on increasing. It caters to the need of undergraduate first year students. An attempt has been made to provide all the informations necessary to understand and appreciate the problems which students have been facing. This book is also designed to meet the examination needs of our readers. Hence all important aspects of practicals have been discussed and suitable illustration provided. The aspects that have perceived special attentation are Introduction, Fungi, Microbiology, Algae, Bryophyta, Lichens, Pterodophyta, Gymnosperms and palaeobotany. The special features of the present book are self explanatory labelled diagrams, easy to understand language, incorporation of recent developments in the subject. It is hoped that it will also benefit the postgraduate students and teachers as well. Though every possible efforts have been made to make the book flawless, chance are still there for further improvement. So, colleagues and students are requested to bear with me and to mail their valuable suggestions to give this book a new look next time.

Acknowledgements

The authors of this book also wish to acknowledge the valuable suggestion they have received from their learned colleagues especially Dr. Azahar Sajjad and Dr. M. Sayeed Akhtar. Authors are extremely thankful to Professor Nafees Ahmad Khan, Chairman, Department of Botany, Aligarh Muslim University, Aligarh for their moral support and encouragement. Above all, we are thankful to the Almighty.

Proposed Syllabus for B.Sc. Botany

Paper I - Diversity of Viruses, Bacteria and Fungi

Paper II - Diversity of Algae, Lichens and Bryophytes

Paper III - Diversity of Pteridophytes and Gymnosperms

Paper I: Diversity of Viruses, Bacteria and Fungi

Unit - I

History, nature and classification of Viruses, Bacteria and Fungi. History of virology and bacteriology; prokaryotic and eukaryotic cell structure (bacteria, mycoplasma and yeast); structure, classification and nature of viruses; structure (gram positive and gram negative) and classification (based on cell structure) of bacteria; classification, thallus organization and reproduction in fungi; economic importance of fungi.

Unit - II

Viruses: Symptoms of virus infection in plants; transmission of plant viruses; genome organisation, replication of plant virus (tobacco mosaic virus); techniques in plant viruses - purification, serology and electron microscopy; structure and multiplication of bacteriophages; structure and multiplication of viroids.

Unit - III

Bacteria: Nutritional types of bacteria (based on carbon and energy sources), metabolism in different nutritional types (basics only) and nitrogen cycle; bacterial genome and plasmids; bacterial cell division, variability in bacteria - mutation, principles of genetic recombination; techniques in sterilisation, bacterialculture and staining; economic importance.

Unit - IV

Fungi: The characteristics and life cycles of the following:
Mastigomycotina: *Albugo, Pythium,*
Ascomycotina: *Saccharomyces, Aspergillus; Ascobolus;*
Basidiomycotina: *Ustilago, Puccinia, Polyporus, Agaricus;*
Deuteromycotina: *Fusarium*

Paper II - Diversity of Algae, Lichens, and Bryophytes

Unit - I

General characters. Range of thallus organization, classification, ultrastructure of eukaryotic algal cell and cyanobacterial cell, economic importance of algae. Lichens, classification, thallus organization, reproduction, physiology and role in environmental pollution.

Unit - II

The characteristics and life cycles of the following:

Cyanophyta: *Microcystis, Oscillatoria* **Chlorophyta:** *Volvox, Hydrodictyon, Oedogonium, Coleochaete, Chara;* **Bacillariophyta:** *Navicula;* **Xanthopyta:** *Vaucheria;* **Phaeophyta**; *Ectocarpus;* **Rhodophyta:** *Polysiphonia*

Unit - III

Bryophytes, general characters, classification, reproduction and affinities. Gametophytic and soporophytic organization of:

Bryopsida: *Pogonatum;* **Anthocerotopsida:** *Anthoceros*

Unit - IV

Gametophytic and sporophytic organization of ***Hepaticopsida***: *Riccia, Marchantia.*

Paper III – Diversity of Pteridophytes, Gymnosperms and elementary Palaeobotany

Unit - I

Pteridophytes: General features, classification, stellar system and its evolution. Comparative study of morphology, anatomy, development, vegetative and reproductive systems of following:
Lycopsida - *Lycopodium, Selaginella;* ***Psilopsida*** **-** *Rhynia*

Unit - II

General and comparative account of gametophytic and sprophytic system in **Filicopsida -** *Pteridium, Nephrolepis, Marsilea.* Heterospory and seed habit

Unit - III

Gymnosperms: General characters, classification. Comparative study of morphology, anatomy, development of vegetative and reproductive parts in **Cycadales:** *Cycas*

Unit - IV

Study of morphology, anatomy, development and reproductive parts in:
Coniferales – *Pinus*; **Gnetales –** *Ephedra.* Affinities and relationship of Gymnosperms, evolutionary significance. Elementary Palaeobotany: general account, types of fossils, methods of fossilization and geological timescale.

Table of Contents

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1. INTRODUCTION TO LABORATORY

Preamble

Science is a systematised study based on facts and observations. It involves curiosity, inquisitiveness and unbiased analysis. Most of the scientific work is done in a laboratory. It provides an opportunity to a person with scientific frame of mind to see and study various aspects of an object under observation. Hence, a biology student too is obliged to attend laboratory work-out with utmost sincerity, honesty and inquisitiveness.

Laboratory Etiquette

The study of living things in laboratory requires that facilities provided are properly used. One is expected to complete the assigned work within a specified time. This requires proper utilization and planning of time. One should; therefore, keep busy with own work and wherever necessary consult the teacher alone. Laboratory provisions should be handled with utmost care. At the end of the laboratory period, working place should be left clean and in order. Laboratory exercise to be performed should be read in advance and one is expected to arrive to the class theoretically prepared.

Work Plan

1. Listen and understand the instructions and information given by teacher-in-charge.
2. Work out or observe the materials carefully.
3. Mount to prepare slides as per requirements.
4. Study the preparations or specimen carefully.
5. Draw suitable diagrams in a proper sequence and label them in your practical record.
6. Write down the observations, sequentially and watch carefully if variations occur.
7. Get your work checked by teacher-in-charge and make necessary corrections.

Instruments

Keep with you these important instruments when you entered in the lab, a pair of forceps, two fine, long handle, dissecting needles, glass droppers, good and sharp razor, safety blade, a fine hair brush, a pair of sharpened pencils, pencil eraser, a clean and soft handkerchief and practical record with cover file and spare pages, etc.

Microscope

It is the most indispensable instrument in a biology laboratory, so much so that it comes to be called `The primary instrument of the biologists'. It helps to increase the- resolving power (property to distinguish objects lying very close as separate bodies) of human eye which fails to recognise objects lying closer between 0.01 to 0.25 mm.

Some common types of microscopes are listed below -

1. Dissecting microscope
2. Compound microscope
3. Binocular microscope
4. Phase contrast microscope
5. Electron microscope

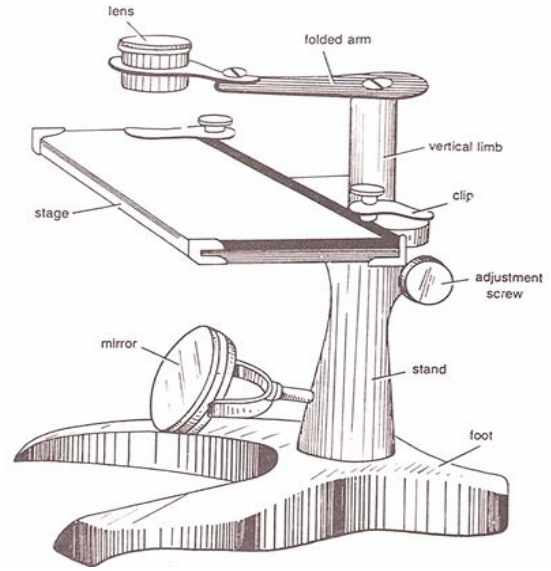
Of these, dissecting microscope and compound microscopes are very commonly used by the students.

[1] Dissecting microscope

It is actually a simple microscope that consists of one lens unit. This lens unit may even be and ordinary magnifine glass. It is used either for dissecting the material or for less magnification that is only 6x, 12x or rarely 20x, it is mainly used for embryo sepration and taxonomic studies etc.

Construction

It consists of basal foot, a vertical limb, stage and a lens. The basal foot is a stand. The limb has an attached stage made of glass plate. A folded arm which can be moved vertically holds the lens. A mirror is attached at the base of the limb.



The material to be viewed or dissected is placed on the stage. The eye is placed closed to the lens. Folded arm is tilted to bring the lens over the material. Light is adjusted by the movement of the mirror present below the stage. Focussing is done with the help of adjustment screw.

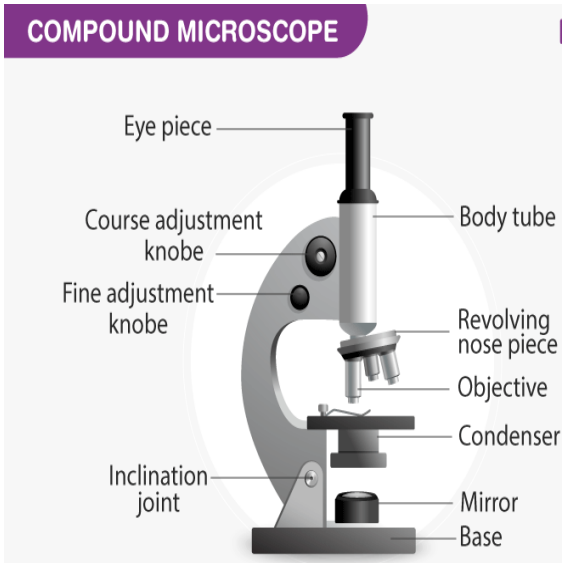
[II] Compound microscope

It is one of the most commonly used and by far the most suitable microscope in the Botany Laboratory. At one time, it employs one ocular (eye piece) and one objective, in working position. As such, it is also known as monocular-mono-objective microscope.

Construction

The microscope is built around a strong basal foot and a vertical limb. The foot supports the vertical limb. A round, rectangular or square stage is fixed to the limb. It is provided with spring clips to hold the slide in position.

A movable or fixed sub-stage is situated directly below the stage. It is provided with an iris diaphragm and condenser lens. Iris diaphragm is a wheel-shaped metal disc to regulate the aperture, through which light rays reach the condenser and are passed to an object. Condenser is a system of two or more lenses under the stage which receives parallel light rays from mirror and converge them at the level of stage.



A movable concave mirror is fixed at the lowermost part of the limb to focus a converging cone of rays at the level of specimen. Whether day or artificial light is used as a source, concave mirror converge the light if there are no condensing lenses.

Body of the microscope is composed of a tube. At the upper end of the tube, is an ocular (eye piece) which can be changed for lower or higher values. of magnifications. At the lower end of this tube is a revolving nose-piece with about three objectives viz. low power, high power and oil immersion. These magnifications range from 3.2x to 100x. The conventional low power objective is 10x.

Tube of the microscope is vertically movable with the help of coarse and fine adjustment screws on the limb, operated by a rack and pinion system. Coarse adjustment moves the tube rapidly, while fine adjustment screw does it gradually.

Mechanical operation

1. Microscope is placed in maximum diffuse light. Direct sunlight is harmful, for the eyes. The northern light is most suitable. If light source is artificial, filter (preferably blue coloured) is used.
2. Light is adjusted by turning the mirror towards the source of light and also by moving the sub-stage up and down, as well as with the help of iris diaphragm.
3. A prepared slide is placed on the stage. Object is adjusted just over the stage aperture.
4. The object is located and focussed with a low-power objective using coarse adjustment.

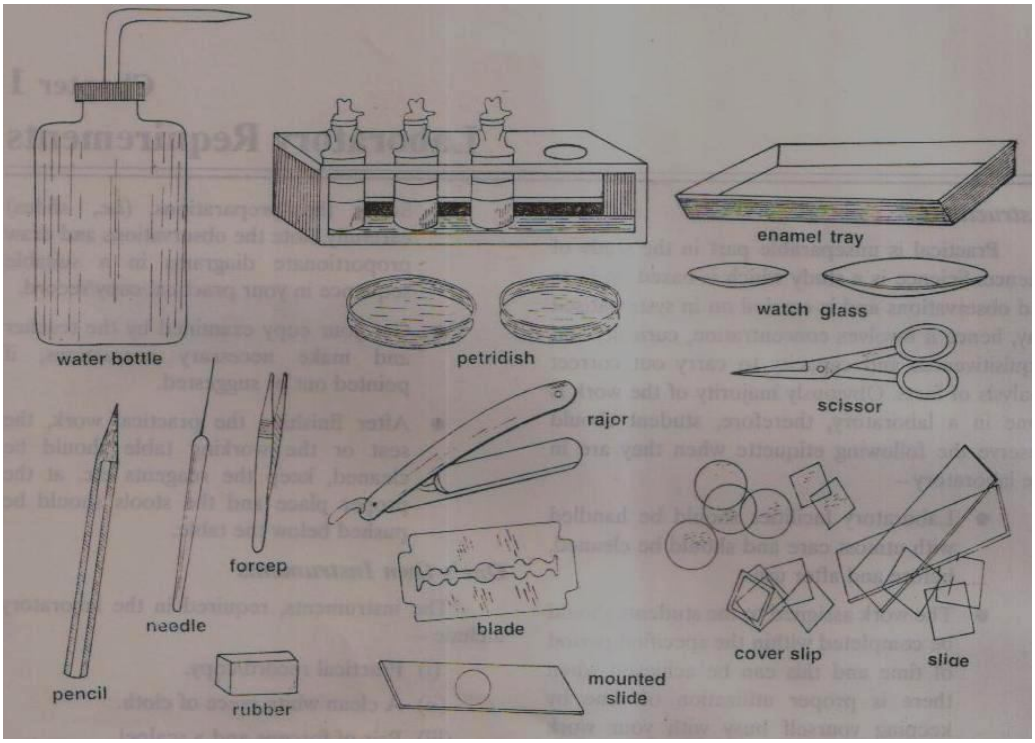
5. If higher magnification is desired, nose-piece is turned to next higher power. Fine adjustment can be used freely at this stage, while the use of coarse adjustment is avoided.
6. High power objective and subsequent higher powers are used only when object is properly mounted under coverslip.
7. The object should always be observed with both eyes open.

Precautions

1. Before and after the use, all the lenses and metal parts including stage should be cleaned. The lenses are cleaned with tissue paper, muslin cloth or clean and soft handkerchief.
2. Microscope is kept covered when not in use. Proper wodden box, plastic bags, bell jars or even a clean cloth can be used.
3. Objectives should not be ordinarily removed from the nose-piece.
4. Operating screws, condenser, iris diaphragm, mirror and stage or stage clips should always be handled carefully.

LaboratoryProvisions

Provision available on the working seat in biological laboratory include staining rack with dropping bottles, slides, coverslip, watchglasses, petridishes, beakers, enamel trays, plasticwash bottles, sprit lamp, duster and leather strap for sharping the razor. Brief description is given below.



1. Staining rack. It is mostly made of wood to hold the dropping bottles. The capacity of number of bottles per rack varies.
2. Dropping bottle. The stains, chemicals, mounting media, etc., are stored in these bottles. This glass bottle has a narrow mouth fitted with a slotted cock. Cock is provided with a beak that permits the liquid to flow out in drops.
3. Slides. The size of slides is mostly 3" x 1" (25 mm x 75 mm). It is about 1 mm thick. These are used to mount the material under study.
4. Cover glasses. The cover glasses are mounted on the object when the preparation is finally ready. These may be either square or round shaped. The thickness of the coverslip is 0.17 mm.

Fixing Agents and Preservatives

In botanical studies the plants or plant parts, collected fresh need 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. Carnoy's fluid, Formalin-aceto-alcohol, Formalin-propiono-alcohol, Randolph's modified Navashin fluid and Bouin's fluid are some of the common agents used. Plants are generally fixed immediately after collection but these can also be fixed after bringing them to laboratory. The collected material must always be kept completely immersed in preservatives. Formalin-Acetic-Alcohal is the most commonly and effective fixative used in the laboratory. Its composition is as-70% or 90% alcohol 90 ml glacial acetic acid and 5 ml formalin. After fixing the plant material in F.A.A. It is being transferred to 70% or 90% alcohol after some time or some days. The collected and fixed plant material must always be kept completely immersed in preservatives.

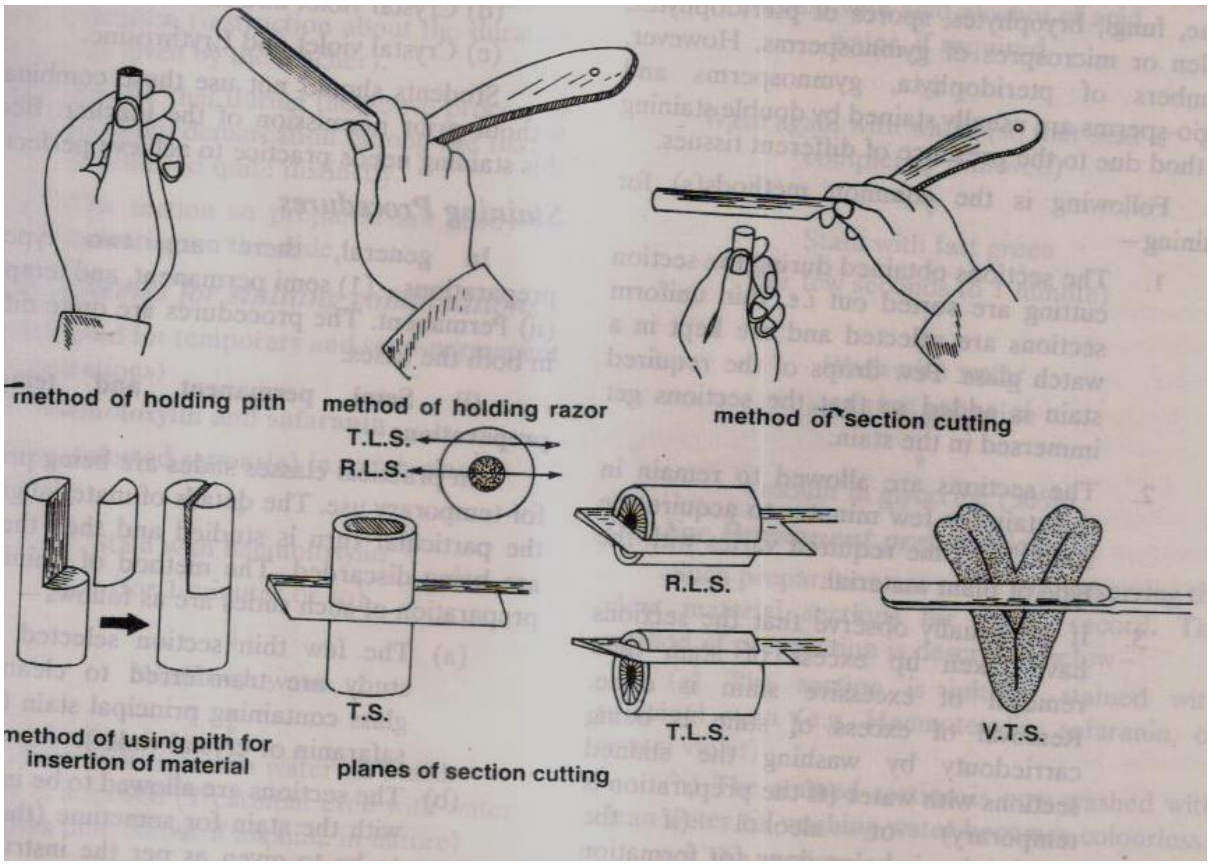
Laboratory Techniques

Section cutting

Sections of preserved material are cut in suitable planes for histological and ecological studies. Razor is suitable for cutting the sections in laboratory.

Honing and stropping

Razor should be sharp and free from nicks. Hence, it should be sharpened on a hone (fine-grit stone). Oblique, uniform and slow strokes are carefully given to the razor with edge foremost on this stone.



After honing, uniform strokes are given on the strop (a smooth leather belt). The leather side of the belt is first slightly oiled and then razor is moved over. This should be done more frequently than honing, to maintain razor edge in good condition.

Planes. The following are a few commonly needed planes-

In case of cylindrical organs: (e.g., sterns, roots, etc).

Transverse. The section is cut by passing razor's edge at right angles to the longitudinal axis.

Longitudinal. The section is cut by passing razor's edge at right angles to the transverse axis. Two sections are possible in this plane.

(i) Radial Longitudinal section (R.L.s.) if it passes along one of the radii.

(ii) Tangential Longitudinal section (T.L.s.) if section is cut along one of its tangents.

In case of dorsiventral organs (e.g. leaf, thallus of liverwort, etc.), transverse section is cut. It is known as vertical transverse section (being cut in vertical plane).

Method. Following steps would be useful for section cutting.

1. Soft, thin and small materials are placed in pith either by piercing a hole with a needle or by splitting it longitudinally with a blade. The pith used includes carrot root and radish root, potato tubers, etc.
2. A razor must be held properly to cut the section. The handle and the blade of the razor should be at right angles to one another. The handle should remain free while the index finger is placed on the hooked end of the razor; 1st, 2nd and 3rd fingers pressed against the thick back edge of the razor and thumb against the milled surface of the thick shank of blade.
3. The material or the pith with embedded material is held between the thumb and the fingers of the left hand.
4. The material in the left hand and the razor's edge should form right angle.
5. The razor is now moved quickly over the material and the stroke is completed in one action only.
6. More and more uniform strokes are used till desired quality and number of sections is obtained. Care is taken to keep the material and the razor flooded with water.
7. Sections float in water on the razor's edge. These are carefully lifted by a fine camel hair brush and then transferred to a watch glass containing water.
8. After the section cutting is over, the razor is tapped dry and cleaned without disturbing the edge. It is honed, stropped and encased.
9. The sections which float on water in the watch glass are considered to be thin.
10. These sections are lifted by a hair brush, placed on a slide in a drop of water and observed through microscope. A thin and uniform section is selected for staining.

Stains and staining

The selected sections need to be stained. The stains help to distinguish different tissues, cells or inclusions from one another by developing specific colours. Acetocarmine, Aniline blue, Crystal violet, Erythrosine, Hematoxylins, Fast green, Light green and Safranin are some of the commonly used stains.

1. Specificity. Most of the stains are specific in reaction and are purposely used so that definite structures or substances are stained. The following are some of the stains used for staining different structures.

Achromatic figure

Aniline blue

Erythrosine

Fast green

Light green

Cellulose cell wall

Aniline blue

Delafield hematoxylin

Fast green

Light green

Lignifled cell wall
Crystal violet

Safranin

Suberised cell wall

Safranin

Cytoplasm
Aniline blue

Erythrosine

Fast green

Light green

Cutinised cell wall
Crystal violet

Erythrosine

Safranin

Callose
Aniline blue

Chitin
Safranin

Proteins
Safranin

Mitochondria
Crystal violet

Plastids
Crystal violet

Iron hematoxylin

Nucleus
Crystal violet

Hematoxylin

Safranin

Chromosomes
Hematoxylin

Safranin

2. Single stains. Safranin or fast green is used alone to stain filaments of algae, fungi, sections of bryophytes, spores of pteridophytes, pollen grains of gymnosperms, etc. Aniline blue or safranin is suitable for algae.

Following is the common method of staining

1. The material is kept in a watch glass. A few drops of stain are added so that the material is immersed in the stain.
2. The material is allowed to remain so for a few minutes and allowed to take stain. The time required varies with materials.
3. After the stain is taken up, the excess of stain is washed off in water. The washing is repeated till stain stops coming out.

4. In some cases, excess stain is removed by acid water or acid alcohol if water alone fails to do so.

5. The stained material is ready for mounting.

Fungi are stained in cotton blue as given below.

1. A drop of cotton blue (prepared in lactophenol) is placed on a slide.

2. Fungal hyphae is now placed in this drop.

3. The slide is run over the flame of the spirit lamp so that the stain is warmed up.

4. The preparation is now ready for mounting.

3. Combinations. Commonly two or more stains are employed wherever tissue differentiation is found. Combination of acidic and basic dyes of contrasting colours is of general use. This permits the distinction of woody tissue from non-woody tissue. The following few combinations are commonly recommended-

1. Hematoxylin and safranin,

2. Safranin and fast green,

3. Safranin and aniline blue,

4. Safranin and crystal violet and

5. Crystal violet and erythrosine.

4. Staining procedures. There are two types of preparations-semi-permanent and permanent. The procedures differ in both the cases. These are given below.

(a) For semi-permanent and temporary preparations. Certain preparations are made for temporary use. The material is studied and the slide is then discarded. The method for staining them is given below.

1. The selected sections are transferred from watch glass containing water to another watch glass containing principal stain (e.g. hematoxylin, safranin or crystal violet).

2. The sections are allowed to remain in the stain for sometime (for about 4-5 minutes).

3. Excess amount of stain is removed by washing the sections repeatedly with water. (This can be seen under the microscope. The stain should be taken either by lignified or non-lignified tissues. Otherwise the section should be washed till the stain disappears from one type of tissue).

4. If destaining is not achieved, sections are washed with acid alcohol. In this case, further washing with water is necessary till traces of acid are removed.

5. This is followed by transfer of sections to a watch glass containing counter-stain (e.g., safranin, fast green, erythrosine). This stain acts on the tissue more rapidly than the principal stain. Therefore, section is kept in this stain for short period (about a minute or two).

6. Excess of stain is removed by washing stained sections with glycerine (15-20%). The section should distinctly bring out demarcation between tissue system while preserving the colour of the stain.

7. The section is now ready for mounting.

(b) For permanent preparations. In certain cases preparations need to be stored permanently as future record. The method of preparation followed is described below-

1. The section is first stained with principal stain (aqueous hematoxylin, safranin or crystal violet).

2. The section is then washed' with water till no more stain dissolves -and water remains colourless.

3. Section is passed through a graded series of alcohol for dehydration. A watch glass is filled with requisite amount of alcohol, (beginning with 30% alcohol) and the section is transferred to it. This watch glass should always be covered with another larger one. In order not to disturb the section, used alcohol is removed by glass dropper. All the 30% alcohol is replaced with 50% alcohol. This procedure is repeated till 70% of alcohol grade is reached.

4. At this stage, counterstain is employed (e.g. safranin, fast green or erythrosine prepared in 80% or 90% alcohol).
5. This stain acts quickly and as such section is washed immediately after the requisite time is over.
6. Destaining is done by washing sections with 90% or 100% alcohol.
7. The section is now transferred to absolute alcohol to complete the dehydration.
8. Clearing now begins with 25% of xylol (25 cc of xylol and 75 cc of absolute alcohol). The sections are gradually passed through xylol series of 25%, 50%, 70%, 90% and finally transferred to pure xylol. If dehydration is not complete, pure xylol turns white or turbid. At this stage section should be passed through reverse series.
9. Pure xylol is the last stage of clearing. Section is now ready for mounting.
10. Mounting is done in Canada balsam.

Specific Schemes for Staining Combinations
(for temporary and semi-permanent preparations)

1. Hematoxylin & safranin

Select a section
↓
Stain with hematoxylin
↓
Wash with water
↓
Wash with ammonia water till stain turns blue
↓
(tap water is suitable if alkaline)
↓
Wash with water
↓
Wash with safranin
↓
Wash with glycerine
↓
Mount in glycerine

2. Safranin & fast green or aniline blue

Select a section
↓
Stain with safranin (for 4-5 minutes)
↓
Wash with water
↓
Destain with acid alcohol if necessary
↓
Wash repeatedly with water
↓
Stain with fast green or aniline blue
↓
Wash with glycerine
↓
Mount in glycerine

[III] Mounting an object

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.

1. Mounting media. Following are some of the common media.

(a) Canada balsam. It is a resin obtained from a conifer - Abies balsamea, most suitable for permanent slide preparation. The material to be mounted should come through alcohol (dehydration) and xylol (clearing) series.

(b) Lactophenol. It is a mixture of equal parts of phenol crystals, lactic acid, glycerine (sometimes two parts) and distilled water. Stains may be mixed with this medium (e.g. cotton blue in lactophenol used to stain fungi) or copper acetate is added to preserve green colour of the pigment.

Specific Schemes for Staining Combinations (for permanent preparations)

1. Hematoxylin & Safranin

Select a section
(If necessary use mordant)
↓
Stain in hematoxylin
(If necessary destain
with mordant)
↓
Wash in ammonia water
or tap water
↓
Dehydration with 30% alcohol
↓
50% alcohol
↓
70% alcohol
↓
Stain with safranin
↓
Destain with 70% alcohol
↓
90% alcohol
Absolute alcohol
↓
Clear with 25% xylol
↓
50% xylol
↓
70% xylol
↓
90% xylol
↓
Pure xylol
↓
Mount in Canada balsam

2. Safranin & fast green & safranin
3. Crystal violet & erythrosine

Aqueous safranin / crystal violet

Water change, until colourless
↓
Dehydration with
30% alcohol
↓
50% alcohol
↓
70% alcohol
↓
90% alcohol
↓
Stain with fast green/erythrosine
↓
Destain with 90% alcohol
↓
Absolute alcohol
↓
Clearing or de-alcoholizing
with 25% xylol
↓
50% xylol
↓
70% xylol
↓
90% xylol
↓
Pure xylol
↓
Mount in Canada balsam

(c) Glycerine. Pure glycerine diluted to 15-25% is widely used. Semi-permanent and temporary preparations are mounted in glycerine.

(d) Glycerine jelly. Jelly is also used for mounting. It is made of gelatin 1 : glycerine 7 : water 6. Warm the gelatin for two hours by adding water. Phenol (1%) is added later. Add crystals of safranin if desired. Allow the solution to cool and settle into jelly.

Many other mounting media like cedar oil, dammar, balsam, venetian turpentines and synthetic resins are also used.

Care. Following care should be taken during mounting-

1. Object should be mounted in the centre of the slide. A simple method may prove suitable for this purpose. Take a piece of thick and white cardboard sheet larger than the size of the slide. Place the slide over it. Draw lines along all the four edges. Join all the four corner points diagonally by two lines. The point, where these two lines cross, gives the centre of the slide. While mounting an object, place the slide over this drawn sheet and an object on the central point.
2. No air bubbles should enter the medium while mounting. This results in drying of medium and preparation is spoiled. To avoid air bubbles, touch one side of the coverslip to the drop of mounting medium on the slide. Support the coverslip by needle and lower it gradually before finally removing it.
3. Use the necessary small quantity of mounting medium so that it does not flow on to the slide. If so, use little lesser quantity for the next preparation. The extra amount can be soaked by touching a piece of blotting paper to the edge of the coverslip.
4. Prepration should be calm hence the edges of slide and coverslip alone should be held between the fingers.
5. Labels are pasted uniformly on one side of the prepared slide. It should carry the name of the division or generic and specific names, the part mounted and the section's plane. At the bottom, the name of the student who had prepared the slide be written.

3. Sealing the coverslip: Temporary preparation can be sealed with canada balsam, gums, dammer and nail polish etc. Such a preparation is called a semi-permanent preparation.

Sealing is done by simply painting the edges of the coverslip with sealing agent in such away that the space between the slide and the coverslip gets filled with the agent. It should prevent mounting medium from drying.

Paper I - Diversity of Viruses, Bacteria and Fungi

Fungi

The term 'Fungi' is used for those plants which are non-chlorophyllous and heterotrophic. Being the members of ***Thallophyta***, their plant body is simple and not differentiated into root, stem and leaves. The word **fungus** has been taken from **Latin**, which means **Mushroom**. **Professor Karam Chand Mehta** (1892-1950), known as father Indian mycology**. Heinrich Anton de Bary** (1831-1888) was a German surgeon, botanists, microbiologist, mycologist and he was considered as a founding father of plant pathology and modern mycology.

The study of fungi is known as '**Mycology**' and the scientists as '**Mycologists**'. It included the study of mushrooms only to begin with. The credit for laying the foundation of present day mycology goes to Italian Botanist **Pier' Antonio Micheli** who included his researches on fungi in a book-Nova Plantarum Genera published in 1729. Fungi include 100,000 species, of which 40,000 are known to be valid species leaving 60,000 to be investigated and described in future. The earlier fungal taxonomists included bacteria also in this group, the practice which is now completely discarded. The members of this heterotrophic group exist either as parasities or saprophytes. Most of the members are pathogenic for other living organisms. Special nutrition absorbing organs called haustoria are found. Thallus is either coenocytic (without partition wall/aseptate) or septate forming compact structures like mushrooms. Hyphal system (filamentous) forms a thallus, called **Mycelium**. The cell wall is typically made of chitin while the reserve food mainly in the form of glycogen.

Both asexual and sexual methods of reproduction are known in fungi. Asexual reproduction is through different types of spores like zoospores, conidiospores, basidiospores, and chlamydospores etc. Sexual reproduction in fungi is extremely reduced. In lower members distinct sexual reproduction is present but is

gradually reduced in higher members and finally it is found to be absent in Fungi Imperfecti (*Alternaria* and *Fusarium* etc).

The diseases caused by this group have rendered its sufficient economic importance. The members of this group attack plants as well as animals including the human beings. Since the fungi are principal agents of decay, so through the decomposition of organic matter, they play an essential role in the nutrition of the green plants. Contrary to these harmful activities, fungi serve as food, used in preparation of medicines and antibiotics, and are employed in many industrial processes.

Fungi have been variously classified by numerous mycologists, time and again. Basically the group was divided into five classes-Myxomycetes, Phycomycetes, Ascomycetes, Basidiomycetes and Deuteromycetes. This classification was the most convenient. However, the trend now is to treat fungi as a kingdom rather than as a sub-group of Thallophyta equal in rank to algae. One of such classifications was proposed by Aexopoulos and Mims. It is being used in this chapter with certain modifications. Only those taxa have been described which are included in the syllabus and the classification as given by **Alexopoulos and Mims** (1979).

Classification of fungi by Alexopoulos and Mims

First comprehensive classification was given by **Alexopoulos and Mims (1979)**, they placed all fungi in a kingdom **Myceteae** in which cell wall is made up of chitin and they all were heterotrophic. They divide kingdom myceteae into 3 divisions, 8 sub-divisions and 11 classes and one form class. Outline of their classification as follows,

Kingdom – Myceteae = Achloropyllous,

Division sub – divisions Class Order Genus

1-Gymnomycota	(a)Acrasiogymnomycotina.	1.Acrasiomycetes,	X	X
	(b)Plasmodiogymnomycotina	1.Myxomycetes	X	X
2-Mastigomycota	(a)Haplomastigomycotina.	1.Chytridiomycetes		
		2.Hyphochytridio-mycetes	X	X
	(b)Diplomastigomycotina.	3.plasmodiophoro-mycetes	X	X
		1-Oomycetes	Peronosporales	Pythium, Alugo,
3-Amastigomycota	(a)Zygomycotina	Zygomycetes, Trichomycetes.	X	X
	(b)Ascomycotina,	Ascomycetes.	Endomycetales Aspergillales or Eurotiales Pezizales	Saccharomyces Aspergillus or Eurotium. Ascobolus.
	(c)Basidiomycotina,	Basidiomycetes.	Ustilaginales Uredinales. Agaricales. Aphyllophorales	Ustilago. Puccinia. Agaricus. Poyporus.
	(d)Deuteromycotina	Deuteromycetes.	Moniliaes	Fusarium.

Symbols used in above classification: X = not in syllabus,

Some common fungi and name of fungal diseases in plants

Name of Fungus	Name of disease
Synchytrium endobioticum	wart disease if potato
Pythium aphenodermatum	stem rot of papaya
Phytophthora infestans	Late blight of potapo
Erysiphe polygoni	powdery mildew of peas
Erysiphe graminis	powdery mildew of wheat
Peronospora pisi	Downy mildew of peas
Albugo candida	white rust of crucifers
Sclerospora graminicola	Green ear of bajra
Puccinia graminis tritici	black rust of wheat
Urocystis tritici	Flag smunt of wheat
Ustilago tritici	Loose smut of wheat
Ustilago hordei	Covered smut of barley
Ustilago nuda	Loose smut of wheat
Ustilago kolleri	Covered smut of oat
Ustilago scitamineae	whip smut of sugarcane
Sphacelotheca sorghii	Grain smut of jowar
Tolyposporium penicillari	Smut of bajra
Alternaria solani	Early blight of potato
Cercospora perspnata	Tkka disease of groundnut
Colletotrichum falcatum	Red rot of sugarcane
Pyricularia oryzae	Blast disease of rice
Fusarium udum	Wilt of arhar
Plasmodiophora brassicae	Clup root of disease of crucfers
Protomyces macrospores	Stem gall of coriander
Haemelia vastatrix	Leaf rust of coffee

***Albugo* / Cystopus (White Rust)**

Classification

Kingdom Mycetae

Division Mastigomycota

Sub-division Mastigomycotina

Class Oomycetes

Order Peronosporales

Family Albuginaceae

Genus *Albugo*

Occurrence: The genus *Albugo* is represented by about 30 species, distributed in most part of the world. It occurs as an obligate parasite on higher plants. The species of Albugo infect the members of the families Cruciferae, Convolvulaceae, Compositae, Amaranthaceae etc. *A. candida* is the most common species of the genus and it parasitises the members of the cruciferae like cabbage, mustard, radish, turnip etc. and causes white rust of crucifer.

Exercise 1

Study of hosts, diseases and symptoms

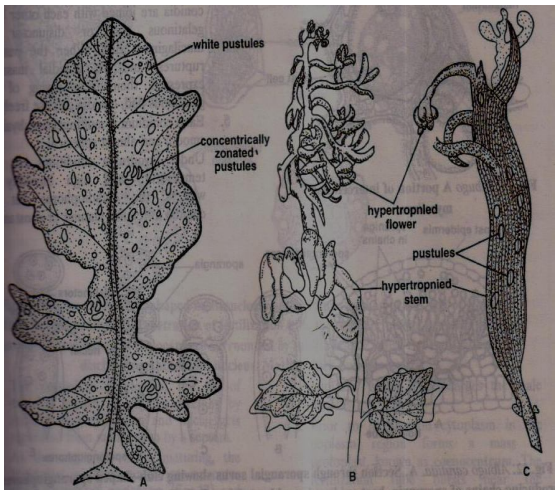
Work procedure

Collect the diseased parts of hosts from the list given below. Preserve the specimen in F.A.A. or mount them dry on herbarium sheets.

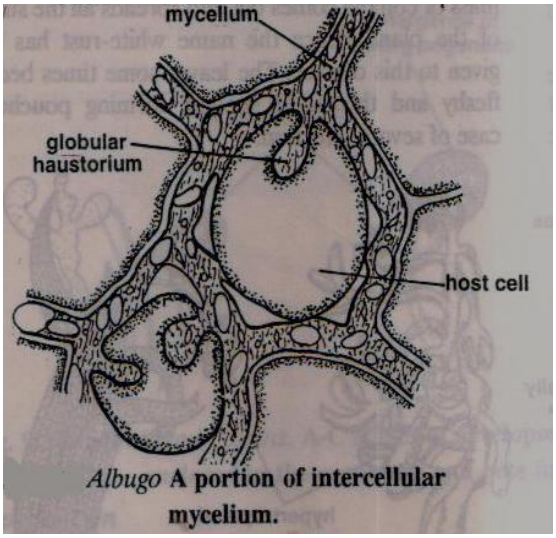
Comments

All the twenty five species of Albugo are obligate parasites on flowering plants. The fungus attacks many flowering plants especially the members of Cruciferae.

A common disease, the **white rust of crucifers** is caused by *A. candida* (*Cystopus candidus*) in crucifers. The fungus attacks wild as well as the cultivated plants of economic importance. The most important being cabbage (*Brassica oleracea* var. *capitata*: vern. patta gobhi), cauliflower (*Brassica oleracea* var. *botrytis*: vern. phool gobhi), mustard (*Brassica campestris*: vern. sarsaun), radish (*Raphanus/satives* var. *catirus*; vern. mooli) and toria (*Eruca* vern. tarra) etc. The fungus may attack all parts of the plant with the exception of root. The symptoms of disease appear on the leaves in the form of **white shining pustules** which later on coalesce to form patches.



When epidermis is ruptured by the pressure of underlying conidia, white powdery mass is seen on the underside of leaf, in the diseased white patch. The leaves in some cases become fleshy and thickened. Inrolled and in case of severe infection reduced in size and the entire plant may remain dwarfed. The flowers and stems when infected, show various malformations and enormous **hypertrophy** (hypertrophy is the abnormal enlargement of the host tissue). The membranous petals become very thick and fleshy. Due to abnormal growth the flowers become sterile. In case of systematically infected plant the entire plant shows stunting or dwarfing. *A. ipomoeae-pandoranae* attacks Ipomoea species (fam. Convolvulanceae) and forms appreciable galls on the stem due to hypertrophy. *A. bliti* is common on members of Amarantaceae.



Albugo **A portion of intercellular mycelium.**

Exercise 2

Study the vegetative structure

Work procedure

It can be studied by cutting the section of diseased part specially leaves where pustules are formed.

Comments

1. The mycelium is branched, intercellular, aseptate and produce globular or knob-shaped haustoria.

2. Haustoria penetrate the host cells and absorb the food material.

Exercise 3

Study of the asexual reproductive structures- sporangia

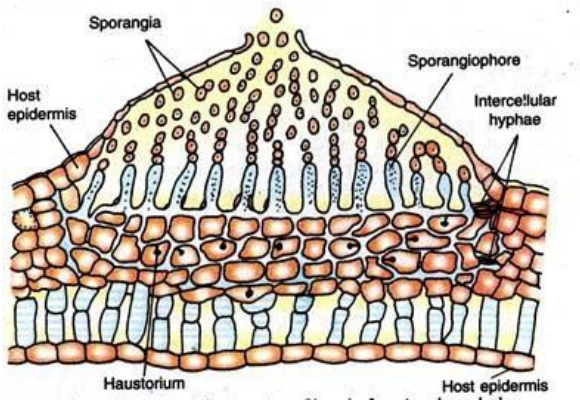
Work procedure:

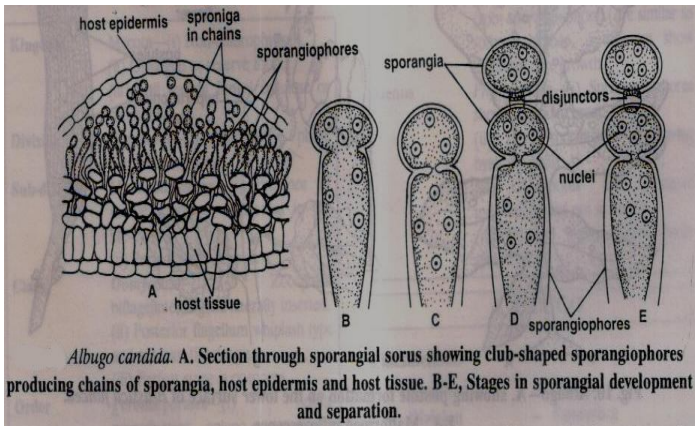
Cut a T.s. of the infected leaf through a diseased patch. It can be stained in safranin and fast green combination and mounted in glycerine.

Comments

Asexual reproduction takes place by sporangia

1. The mass of intercellular hyphae beneath the host epidermis produces vertical palisade-like groups of sporangiophores.
2. Each sporangiophores bears at its tip a chain of sporangia arranged basipetally i.e. the youngest at the base of the chain and oldest at the top.
3. In a chain, two sporangia are joined with each other by a gelatinous pad called disjunctor.
4. Each sporangium is multinucleate (5-8 nucleate), hyaline, smooth and spherical.
5. The sporangiophores constantly cut off sporangia forcing underlying epidermis to rupture.
6. The sporangia are disseminated by wind and germinate either directly (when conditions are unfavourable) by producing a germ tube or may form zoospores (when conditions are favourable).
7. The kidney-shaped biflagellate zoospores also germinate to form the new mycelia.





Albugo candida. A. Section through sporangial sorus showing club-shaped sporangiophores producing chains of sporangia, host epidermis and host tissue. B-E, Stages in sporangial development and separation.

Exercise 4

Study of sexual reproductive structures-antheridia and oogonia

Work procedure

Cut a transverse section of radish or toria. Observe the presence of sex organs. Stain with safranin and fast green combination. Mount in glycerine and study.

Comments

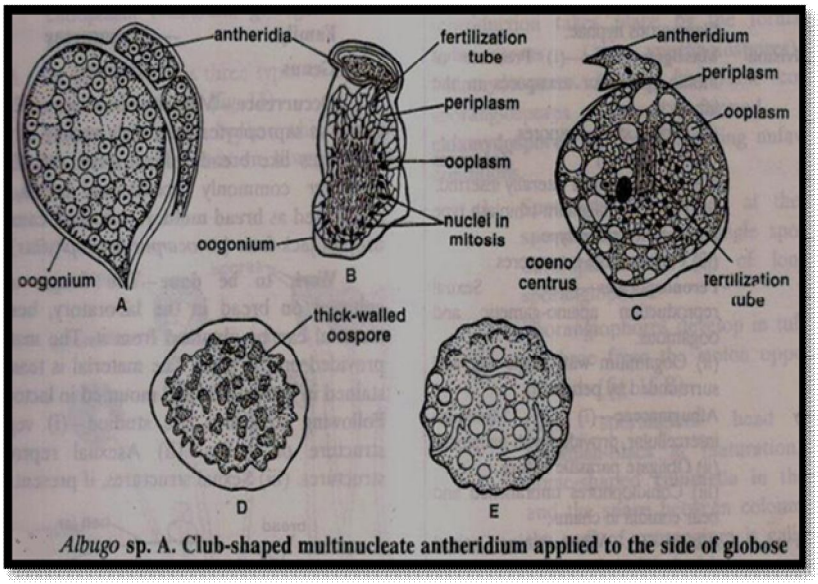
1. Sexual reproduction is oogamous. The sex organs are generally formed in the stem towards the end of the growing season of the host.

2. The oogonium (female sex organ) occurs in the intercellular spaces at the tip of mycelium. It is spherical with central ooplasm and peripheral periplasm. In *A. candida* ooplasm is uninucleate.

3. The antheridium (male sex organ) is paragynous in position and contains several nuclei.

4. The antheridia and oogonia are separated from remainder of the mycelium by a septum.

5. A mature antheridium develops a slender fertilization tube which grows through the oogonial wall and the periplasm and penetrates deeply into the ooplasm or oosphere.



Albugo sp. A. Club-shaped multinucleate antheridium applied to the side of globose

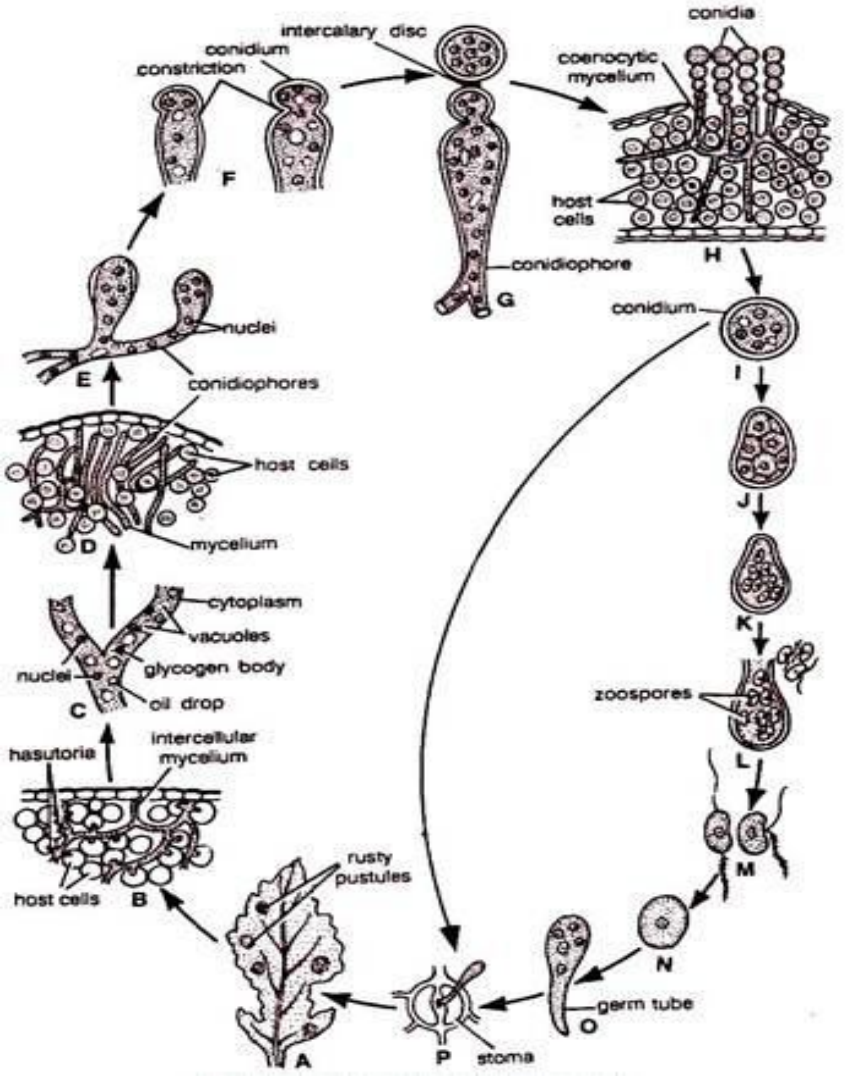
6. A single male nucleus enters and fuses with nucleus in the oosphere thus effecting fertilization.

7. In some species (e.g. *A. bliti*), the oosphere ooplasm is multinucleate and these nuclei get fertilized by the entry of equal number of male nuclei. The resulting oospores or zygotes are thus multinucleate.

8. The oospore develops a thick, ornamented three layered wall.

9. The nucleus of the oospore divides meiotically. A vesicle is produced in which 40-60 biflagellate zoospores are formed. Zoospore germinates into a new mycelium.

In this life cycle conidia is replaced by sporangia and conidiophores by sporangiophore



Life cycle of Albugo candida

***Aspergillus* (Eurotium)**
(Black Mould or Blue Green fungus)

Classification

Kingdom	Mycetece
Division	Amastigomycota
Sub-division	Ascomycotina
Class	Ascomycates
Order	Aspergillales/ Eurotiales
Family	Aspergillaceae/ Eurotiaceae
Genus	*Aspergillus/Eurotium*

Occurrence: The genus *Aspergillus* is generally found as saprophytic fungus occurring over a wide range of habitat. Species of this fungus are found growing on the stored food, vegetables, pickles, james, jellies and other food stuffs. Some species are formed growing on paper, leather, textie and soils. It causes aspergilloses and mycosis in men and animals showing its parasitics nature. The name *Eurotium* represents the perfect stage of the fungus. While the name Aspergillus represents the conidial stage.

Exercise 1

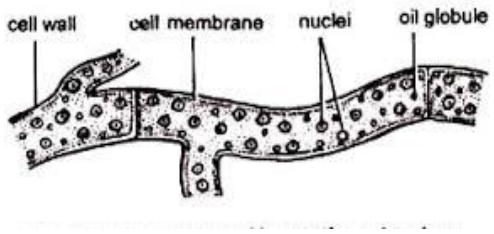
Study of hosts, diseases and symptoms
Work procedure
A list of hosts is given below to identify and collect species of *Aspergillus*. Hosts should be preserved in F.A.A. or alcohol 90% or dried for mounting on herbarium sheets.

Comments
Most of the species of *Aspergillus* are saprophytes growing on decaying vegetables, butter, fruits, pickles, bread, rice, jams, leather, cloth, fabrics, etc. A few species are parasites on plants and animals, including human beings. Some species are found growing on paper, leather, textiles, soil etc. It causes as **aspergillosses** and mycosin man and animals showing its parasitic nature.

1. *A. niger* causes rot disease of pomegranates (*Punica granatum*; vern. anar; fam. Punicaceae), dates (*Phoenix dactylifera*; vern. pind khajoor; fam. Palmae) and figs (*Ficus carica*; vem. Anjir; fam. Moraceae). In rot disease, the fruits decay, fungus enters the host through cuts and wounds in the fruits.
2. *A. fumigates, A. flavus and A. niger* attack animals including human beings and cause a group of lungs diseases collectively known as Aspergilloses. The symptoms of Aspergilloses closely resemble to those of tuberculosis.
3. The name *Eurotium* represents the perfect stage of the fungus, while the name *Aspergillus,* represent conidial stages. Some species of *Aspergillus* infect human ear and cause Otomycosis.

Exercise 2

Study the vegetative structure
Work procedure
A few hyphae could be mounted in lactophenol after staining with cotton blue.



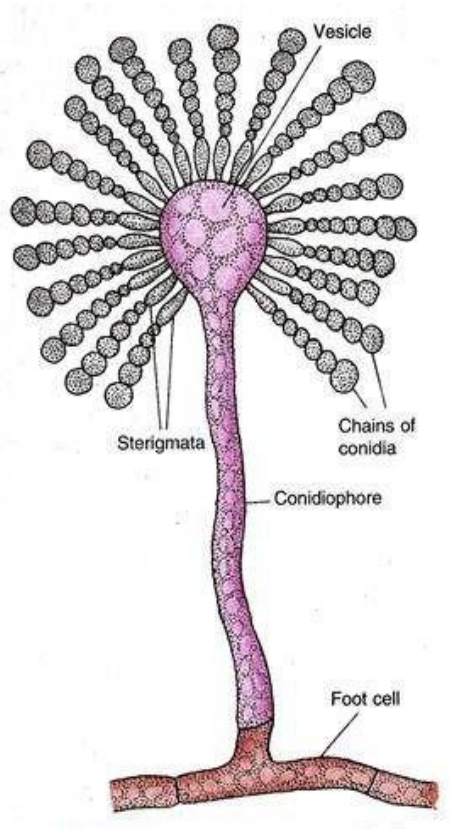
Comments
1. The mycelium is well developed, profusely branched and septate.

2. 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).

3. Some of the hyphae spread superficially over the substratum while others penetrate deep into the substratum. The latter absorb food for the mycelium.

Exercise 3

Study of the asexual reproductive structures
Work procedure
Mount mycelium showing conidia in lactophenol after staining in cotton blue.



Comments

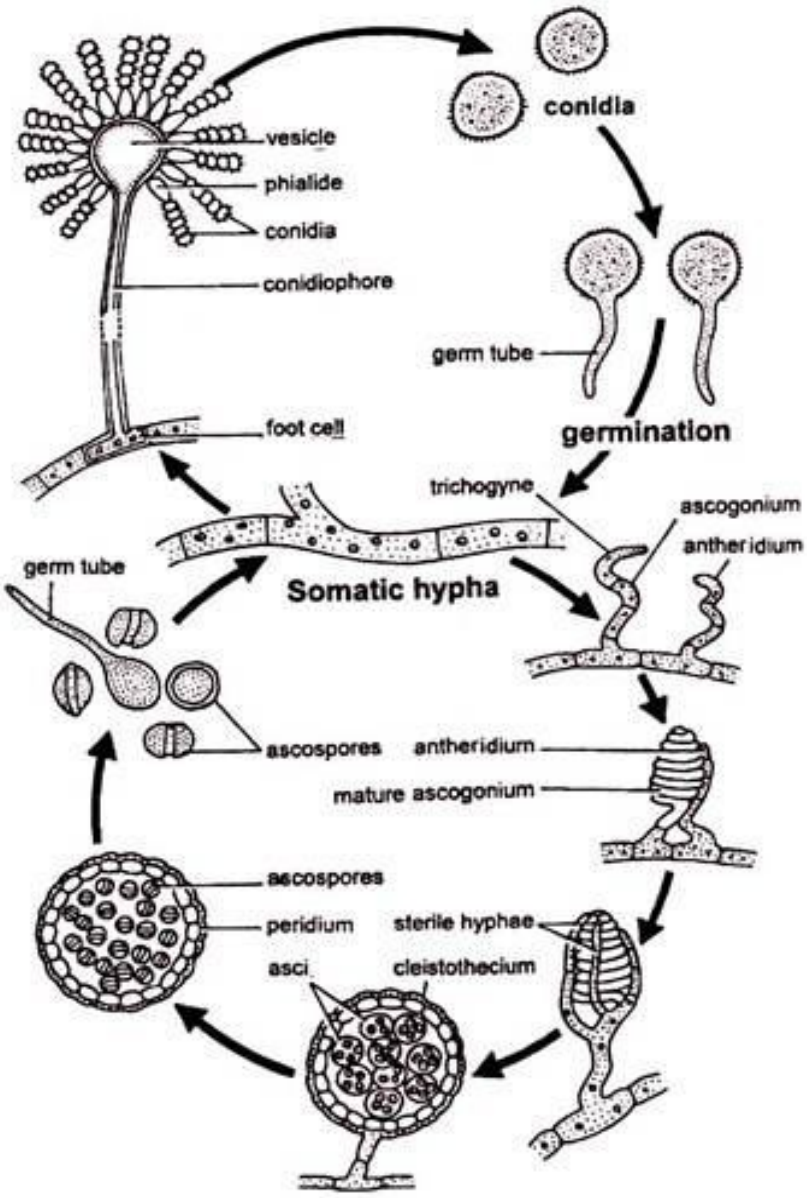
1. Conidia are asexual reproductive units borne on conidiophores.
2. Each conidiophore arises from the foot cell of the mycelium and is long and erect hypha, terminating in a bulbous head—the vesicle.
3. The vesicle develops a number of bottle-shaped structures called the sterigmata (sing. sterigma) over its entire surface.
4. In some species two layers of sterigmata one above the other, are formed. In such a case those of the lower layer are called primary sterigmata and those of the upper, secondary sterigmata. The conidia are borne only by secondary sterigmata.
5. Each sterigmata cuts off a chain of basipetally arranged conidia.
6. The conidia are coloured and the colour of the conidia depends upon the species.
7. Each conidium is uninucleate with an outer finely spiny epispore and inner smooth endospore.
8. A conidium germinates to form mycelium if it happens to fall on a suitable substratum.

Exercise 4

Study of ascocarp, asci and ascospores
Work procedure:
Mount mycelium having cleistothecia in lactophenol after staining in cotton blue.

Comments

1. The species of *Aspergillus* developing perfect stage i.e. cleistothecium are placed under the genus *Eurotium*.
2. The ascospores are produced after reduction division of ascospore mothercell in a sac-like cell - the **ascus**.
3. The asci are developed inside a fruiting body - the **cleistothecium**.
4. Cleistothecium consists of wall called peridium formed by somatic hyphae, enclosing many asci.
5. Each ascus has eight uninucleate pulley wheel-like ascospores with an outer sculptured epispore and an inner smooth endosporeand germination of each ascospore gives rise to a haploid mycelium.



Life cycle of Aspergillus

***Peziza* (Cup Fungus)**

Classification

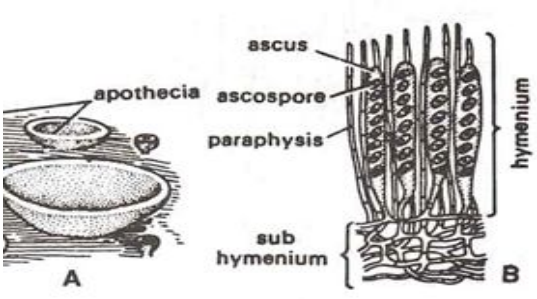
Kingdom	Myceteae
Division	Amastigomycota
Sub-division	Ascomycotina
Class	Ascomycates
Order	Pezizales
Family	Pezizaceae
Genus	*Peziza*

Occurrence: It is commonly known as cup fungi and is found as saprophyte on humus rich soil, animal dung, bark of trees, rotting logs. Science it grows on dung hence it is coprophitons in nature.

Exercise 1

Study of vegetative structure

Work procedure:
Take apothecium tear off a part it to study mycelium.



Peziza. A, Some fruiting bodies; B, A few asci, ascospores and paraphyses.

Comments

1. It is a common saprophyte growing on rich humus soils and decaying woods. Sometimes it becomes coprophilous (i.e. grows on dung).
2. The mycelium is a complex system that penetrates the substratum.
3. The mycelium is profusely branched, septate and the cells are multinucleate.
4. The mycelium becomes visible only in the form of apothecial cups above the ground surface.

Exercise 2

Study of conidia/chlamydosprores (Asexual reproductive structure)
Work procedure:
Study the mycelium for the presence of conidia/chlamydospores.

Comments

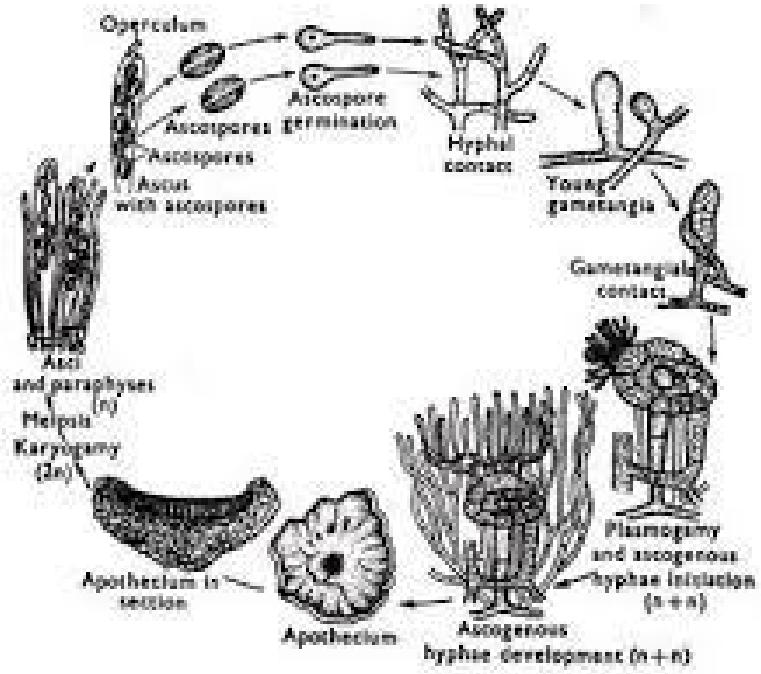
1. The conidia are the asexual reproductive bodies.
2. Conidia are hyaline to lightly coloured and elliptical.
3. In some species, thick walled and intercalary chlamydospores are produced singly or in series on mycelium.
4. On germination chlamydospore produces a new mycelium.

Exercise 3

Study of life cycle (ascocarp, asci and ascospores)
Work procedure
Cut a V.s. of apothecial cup, stain with cotton blue and in lactophenol and study.

Comments

1. The ascocarp is an apothecium. It is fleshy, shortly stalked, about 5 cm in diameter with a bright red or bright grey lining.
2. A vertical section of ascocarp shows a cup-shaped structure made up of mycelium. It shows 3 regions-hymenium, hypothecium and excipulum.
3. Hymenium consists of asci and paraphyses arranged vertically in orange-red-coloured palisade-like layer.
4. The hypothectum consists of thin and lightly coloured hyphae that run parallel to hymenium.
5. Excipulum forms a basal large part of loosely interwoven hyphae of apothecium.
6. The hymenium is encircled by densely interwoven hyphae forming the wall of the apothecium-the peridium.
7. Ascus is elongated with a single row of eight ascospores, arranged obliquely.
8. Each ascospore is uninucleate, hyaline or faintly coloured, elliptical, surface smooth or coarsely reticulate and ellipsoidal. It germinates to form new mycelium.



Ascobolus **(**coprophillous fungus**)**

Classification

Kingdom	Myceteae
Division	Amastigomycota
Sub-division	Ascomycotina
Class	Ascomycates
Order	Pezizales
Family	Pezizaceae
Genus	*Ascobolus*

Occurrence: It is coprophilaus fungus, growing an old dung of cow and other herbivorus animal. Some species that is *A. carbonarius* grow on old bonfire sites.

Exercise 1

Study of the vegetative structure

Work 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.

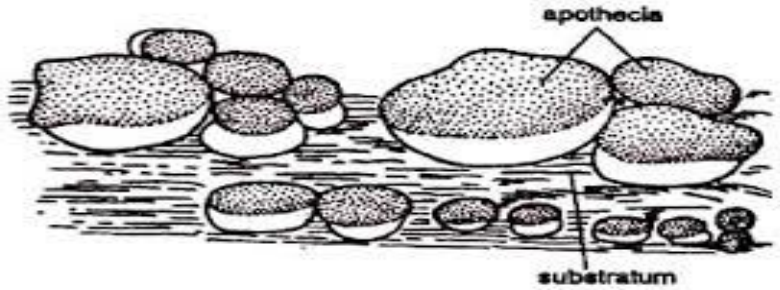


Fig. 1. *Ascobolus* : Aerial apothecia.

Comments

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 finally 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.

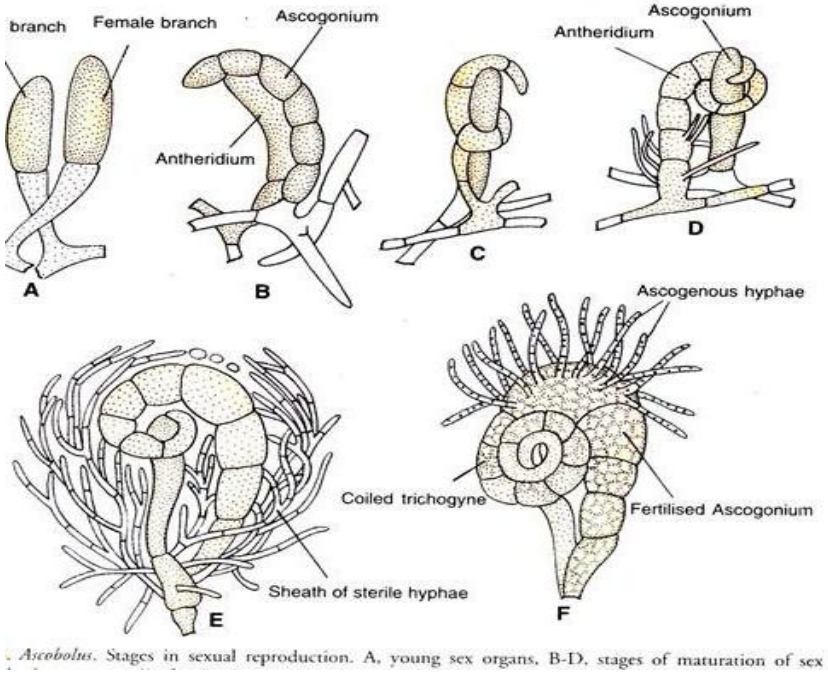
Exercise 2

Study the sexual reproductive structures
Work procedure:
The mycelium shows sex organs just before apothecia appear fully formed. The mycelium at this stage is stained with cotton blue and mounted in lactophenol and sex organs are searched.

Comments
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 multinucicate.
5. Ascogonium is also present at the tip of the ascogonial branch. It is sub-globose in shape and is multinucleate.
6. The tip of ascogonium is drawn into a long and terminal organ called trichogyne. It reaches the tip of an antheridium.

Exercise 3

Study the life cycle (ascocarps, asci and ascospores)
Work procedure:
Cut a V.s. of apothecium, stain in cotton blue, mountin lactophenol and study.



Ascobolus. Stages in sexual reproduction. A, young sex organs, B-D, stages of maturation of sex

Comments
1. The ascocarps and apothecia develop as a result of fertilization. These are yellowish and saucer shaped.
2. Apothecium is a cup-shaped structure that is made of mycelium.
3. The section shows three zones - the outermost called hymenium, the middle called sub-hymenium and the lowermost called hypothecium.
4. The lowermost hypothecium, is made of sterile hyphae, loosely packed to from pseudoparenchymatous region called trama.
5. The middle zone consists of a few erect hyphae. This later merges with hymenial layer which is called sub-hymenium.
6. The hymenium consists of asci intermingled with paraphyses.
7. The asci elongate on maturation to protrude above the hymenial surface. These are found mixed with paraphyses. It opens by a terminal pore called operculum. Each ascus contains eight ascospores. Ascospore is one celled, large in diameter, purple or dark brown and the spore wall shows longitudinal colourless striations.

***Ustilago* (Smut Fungi)**

Classification

Kingdom	Myceteae
Division	Amastigomycota
Sub-div	Basidiomycotina
Class	Basidiomycates
Order	Ustilaginales
Family	Ustilaginaceae
Genus	*Ustilago*

Occurrence: The genus ustilago commonly known as smut fungi, it form black rusty mass of spores which resembles black spot .It occurs parasitically in the form of deep brown or black powdery mass on many members of Graminae or poaceae (that is wheat, oat, barley, sugarcane, doob grass etc). Smut complete its life cycle on single host, various speeies of ustilago are parasite in their nature habitat but many of them are capable of growth as saprophyte.

Exercise 1

Hosts, diseases and Symptoms

Most of the species infect members of the grass family (Poaceae) and cause enormous loss. The cereal smuts are classified into **loose smut** and **covered smut**. Both are seed borne types.

i. In **loose smut**, the spores occur in the grains which are exposed only on maturity of the ear and are easily blown away by wind (infection air borne).

ii. In **covered smut**, spore masses remain covered by the wall of grain and glumes (not exposed). These are liberated when wall is ruptured under pressure (infection seed borne).

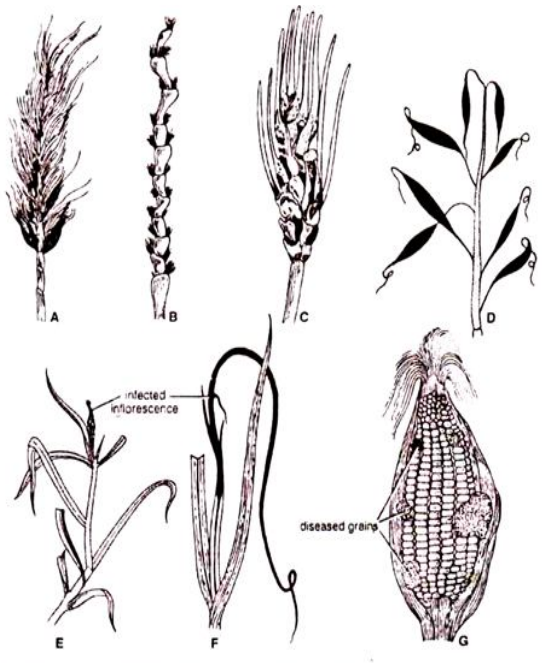


Fig. 1 (A–G). *Ustilago* symptoms. (A) Loose smut of wheat caused by *U. nuda* var *tritici*, (B) Bare rachis; (C) *U. hordei* on barley; (D) *U. kolleri* on oat; (E) Loose smut of doob grass; (F) Whip smut of sugarcane; (G) Gall smut by *U. maydis* on maize

Only the inflorescences are attacked in all the cases. The disease in general, caused by species of *Ustilago*, on various hosts, is known as **smut disease** because of production of black dusty mass of spores. The following are common hosts and diseases.

1. *U. tritici* infects wheat (*Triticumvulgare*; vern. gehun) and causes loose smut of wheat.
2. *U. nuda* and *U. hordei* infect barley (*Hordeum vulgare*; vern. jau) and cause loose smut and covered smut of barley, respectively.
3. *U. scitaminea* attacks sugarcane (*Saccharum officinarum*; vern. ganna) causing whip smut of sugarcane. The whip smut of sugarcane causes the floral axis of infected plant to become long and whip-like, covered by black sooty spores.

4. *U. maydis* attacks maize (*Zea mays*; vern makka) and result into a disease called, common smut of maize. In common smut of maize, large galls are produced on the cob which is sometimes as large as man's fist. These galls partly consist of host tissue and partly of fungus tissue.
5. *U. cynodontis* infects grass (*Cynodon dactylon*; vern. doob ghas) and causes loose smut of grass.

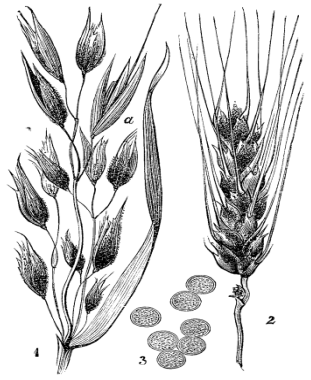
Exercise 2

Vegetative Structure

If vegetative structure or thallus is to be studied, then young infected ear (spikelet) is sectioned or teased and stained with cotton blue.

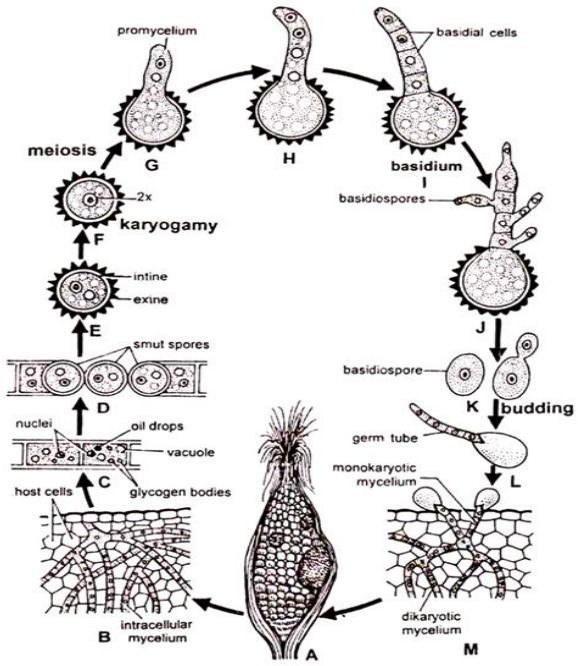
1. The mycelium is septate, branched and generally intercellular. However, sometimes it becomes intracellular also.
2. The mycelium present in infected plant parts is dikaryotic (n+n), because only dikaryotic mycelium is able to infect the host and produces symptoms. Monokaryotic mycelium is incapable of causing infection and symptoms.

Teleutospore or Chlamydospore: For studying smut spores or teleutospore or chlamydospores, the infected grains with black shooty mass is gently taped on the slide having lactophenol-glycerine drop. These spores are shed in the drop, which is mounted with coverslip and examined.



1. Chlamydospores are formed by dikaryotic mycelium. During the blossom period of the host, the mycelium reaches the floral parts and become more active and infects the embryonic spikelets of complete spike. The mycelium then aggregates in the young ovaries and forms dense masses of hyphae.
2. The hyphae acquire food from the ovary tissue and get matured. Now these hyphae are sporogenous hyphae which by dissolution of the connecting wall between the dikaryotic cell of the hyphae get separated and each dikaryotic segment, round off, develops its own double wall and finally are converted into binucleate (n+n) smut spores. Complete hyphal mass is converted into smut spore, hence the grain is converted into spores bag, which externally look blackish in colour.

Chlamydospore or Smut spores



1. Each smut spore is globose, unicellular, dikaryotic (n+n). The spore wall is differentiated in to an outer thick exine and inner thin intine. The exine may be smooth (e.g. *U. hordei*) or ornamented (e.g. *U. maydis, U. tritici*).
2. The spores are disseminated by wind and before germination they become diploid (as the two nuclei fuse) and produce basidium and basidiospores.

***Puccinia* (Rusts)**

Classification

Kingdom	Myceteae
Division	Amastigomycota
Sub-div	Basidiomycotina
Class	Basidiomycates
Order	Uredinales
Family	Pucciniaceae
Genus	*Puccinia*

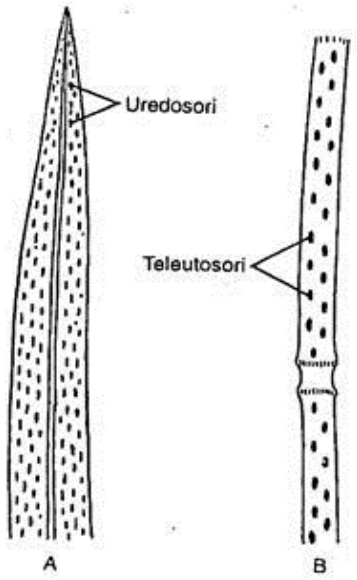
Occurrence: It is distributed practically in all part of the world. It causes serious rust disease in crope like wheat, barley, oats, maize and numerous wild and cultivated grasses. Species of Puccinia are known as Rusts because the infected parts look like rusted iron. Rusts are known from very ancient times, because of the enormous loss caused by them to the crop.

Exercise 1

Study of host, diseases and the symptoms

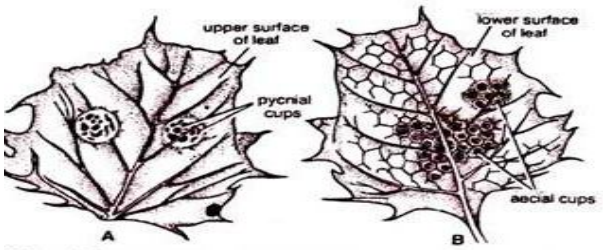
Work procedure

Numerous species of *Puccinia* occur on different hosts. A list is given below so that specimen could be collected and preserved in alcohol or as dried herbarium sheets.



: *Puccinia graminis* : Uredosori on leaf (A) and Teleutosori on Stem (B) of wheat

All the species of *Puccinia* are obligate parasites on some of the important cereals (fam. Graminae or Poaceae) viz. wheat, maize and oat, on millets as bajra and jowar, and on other plants as *Berberis* and *Thalictrum.* All the species of *Puccinia* are polymorphic. Some species such as *P. graminis* are **heteroecious** (i.e. they complete their life cycle on two different hosts), while others such as *P. butleri* are **autoecious** (i.e. those which complete their life cycle on one host only). In almost all the heteroecious rusts, uredo- and teleutostages are found on primary host while pycnidial and aecidial stages are found on alternate host. Some of the hosts, their causal organisms and diseases are as follow: 1. *P. graminis tritici* and *P. striformis* (=*P. glumarum*) infect wheat (*Triticum* sp., vern. gehun), the primary host and barberry (*Berberis vulgaris* sp.; fam. Berberidaceae) the alternate host. They cause **Black rust or Stem rust and Yellow or Stripe rust** respectively on wheat. The symptoms of the disease are seen on leaves, leaf sheaths and sometimes on stem also. The floral organs are generally not affected (cf. *Ustilago*). In Black or Stem rust, (*P. graminis tritici*) dark brown or black, oblong to linear lesions are produced on leaves, leaf sheaths, coalesce to form large patches.



) *Puccinia* **graminis** *tritici* **: Symptoms on barberry plant. (A) Dorsal of leaf showing the pycnial cups, (B) Ventral surface of the leaf showing the aecial cups**

2. P. recondita (*P. triticina*) infect wheat, the primary host and *Thalicutrum* sp. (fam. Ranunculaceae), the alternate host and causes Orange leaf rust of wheat. It shows round to slightly oblong, orange coloured irregularly scattered pustules or lesions or form clusters on the leaf blades. They are never found in rows or strips. The alternate host, Thalictrum, shows small brown specks in clusters on leaves.

3. *P. purpurea* parasitizes *Sorghum* (vern. Jowar, fam. Graminae or Poaceae), the primary host and *Oxalis* (vern. Khatti butti, fam. Oxalidaceae), the alternate host. The disease is known as Leaf rust.
4. *P. sacchari* causes leaf rust on sugarcane (*Saccharum officinarum* varn. Ganna, fam. Graminae or Poaceae).
5. *P. butleri* is an autoecious rust, attacks *Launea* sp (fam. Compositae) and causes leaf rust. In case of the other leaf rust orange to black pustules are seen on leaves and sometimes also on leaf sheath.
6. *P. coronata* parasitizes oats (*Avena sativa*, vern. Jaii, fam. Graminae), the primary host, causing Crown rust and *Rhamnus* (fam. Rhamnaceae), alternate host.

Exercise 2

Study of Vegetative structure
Work procedure
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.

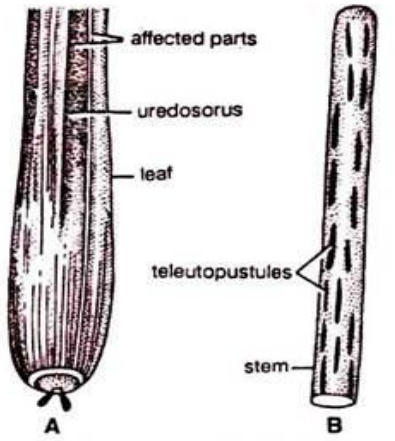


Fig. 1 (A, B). *Puccinia gaminis tritici*: Symptoms on wheat plant. (A) Uredosorus on leaf; (B) Teleutopustules on stem

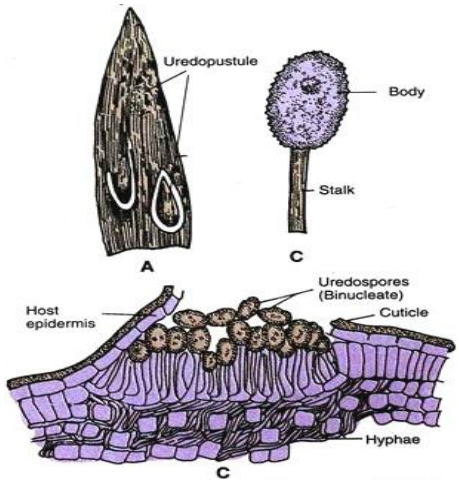
Comments
1. The mycelium is well developed, branched and septate. It is generally intercellular and sometimes shows globular haustoria also.
2. The mycelium is called dikaryotic because it possesses two nuclei of different stains in each cell. The description mostly applies to heteroecious rusts. The uredosori and telutosori are developed on primary host.

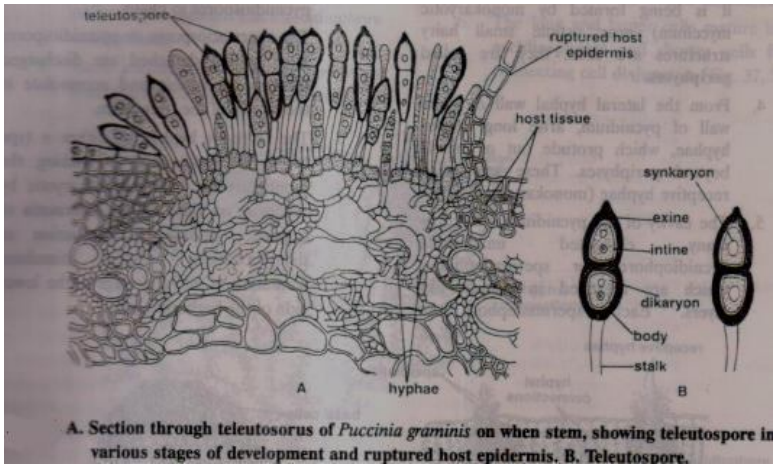
Exercise 3

Study of uredosorus and uredospores
Work procedure:
Diseases are found on wheat leaf. A section is through the pustule, stained in cotton blue mounted in lactophenol or stained in fast green combination for permanent mounting.

Comments
1. The uredosori or uredopustules appear as red, oval or lemon shaped lesions on the leaves and leaf sheaths.
2. The uredosorus in section reveals the ruptured host epidermis due to the pressure of underlying uredospores.
3. The (dikaryotic) intercellular and branched mycelium is aggregated beneath the epidermis.
4. The uredospores are produced in massive groups from this mycelium.
5. Each uredospore is binucleate, stalked and rounded or oblong in shape.
6. It has an outer exine and an inner intine layer.
7. Each uredospore has four equatorial germ pores.

8. The uredospores get disseminated by wind and infect the fresh wheat plants.





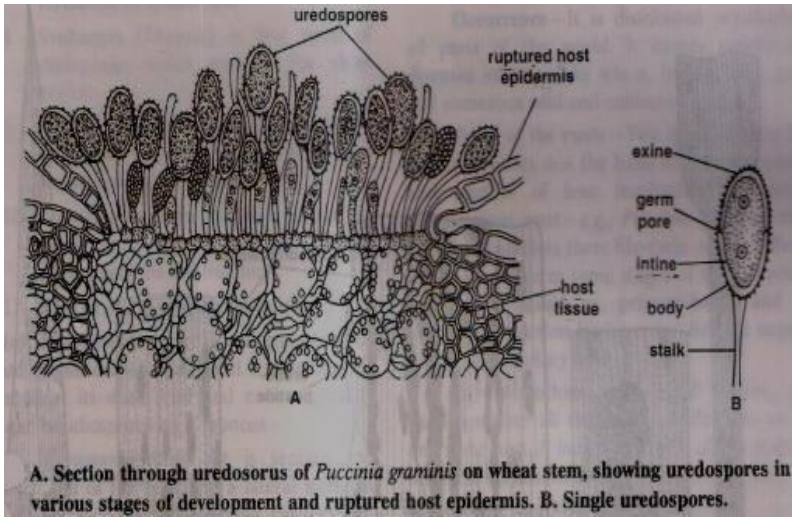
A. Section through teleutosorus of *Puccinia graminis* on when stem, showing teleutospore in various stages of development and ruptured host epidermis. B. Teleutospore.

Exercise 4

Study of teleutosorus and teleutospores

Work procedure

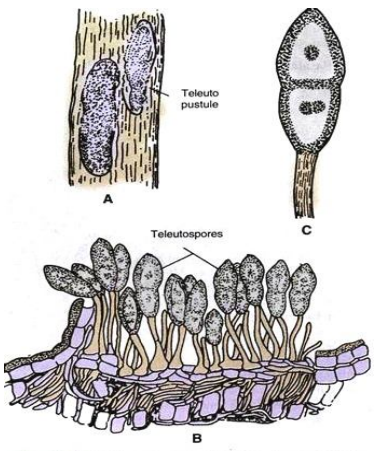
These are found on wheat leaves. A T.s. of the leaf showing teleutosorus could be stained in cotton blue and mounted in lactophenol for temporary preparation. Safranin-fast green combination is used if permanent preparation is to be made.



A. Section through uredosorus of *Puccinia graminis* on wheat stem, showing uredospores in various stages of development and ruptured host epidermis. B. Single uredospores.

Comments

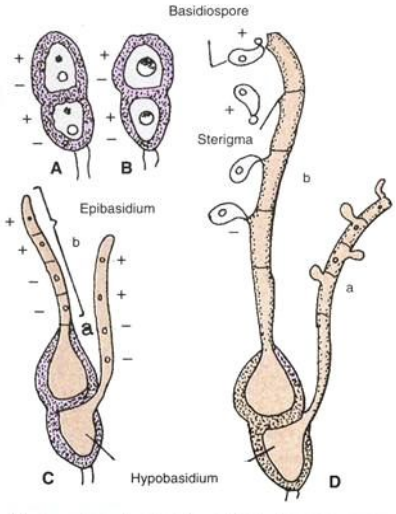
1. The teleutosori or teleutopustules appear on leaves, leaf sheaths and stem as black, oval pustules that fuse to form patches in case of severe infection.
2. A teleutosorus in a section reveals the (dikaryotic) intercellular, branched mycelium, a bunch of teleutospores and the ruptured host epidermis.
3. The teleutospores are formed by the same mycelium which earlier produced uredospores.



4. Each teleutospore is borne terminally by the mycelium. It is stalked, elongated and bicelled structure.
5. The apex of the teleutospore may be rounded or pointed as in *P. graminis* or it may be nearly flat as in *P. recondita* and *P. striiformis*.
6. The teleutospore has a very thick but smooth exine and delicate thin intine. The exine turns black at maturity.

Young teleutospores, mature teleutospore and formation of basidiospores

7. At first each of the two cells of the teleutospore is binucleate but later on, the nuclei fuse making each of them uninucleate.
8. Each cell of the bicelled teleutospore has a single germ pore. The



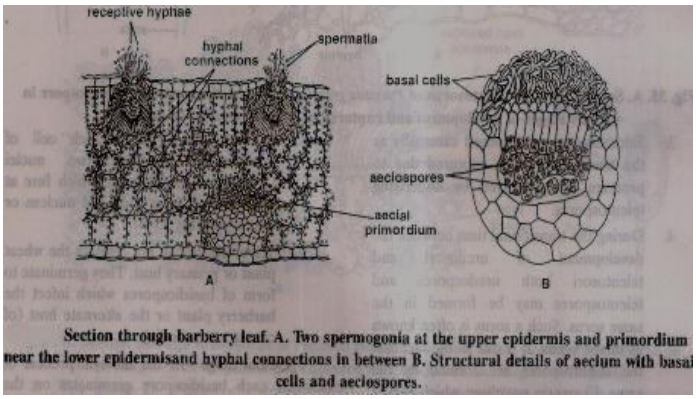
teleutospores are incapable of infecting the primary host (wheat plant). They germinate to form the basidiospores which infect the barberry plant or *Thalictrum* etc (the alternate host). The pycnidial and aecidial cups are formed only on alternate host.

Exercise 5

Study of pycnidial cup and pycnidiospores

Work procedure:

These are found on the upper leaf surface of barberis. A T.s. of leaf is cut and stain with cotton blue or safranin and fast combination for temporary or permanent preperations respectively.

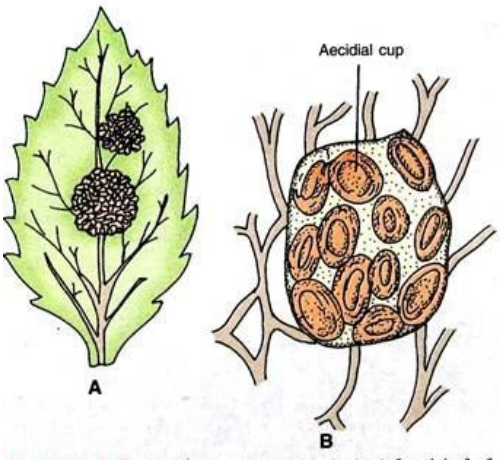


Section through barberry leaf. A. Two spermogonia at the upper epidermis and primordium near the lower epidermisand hyphal connections in between B. Structural details of aecium with basal cells and aeciospores.

Comments

Each basidiospore germinates on the leaf of alternate host producing (monokaryotic) mycelium that ultimately forms the pycnicial cup or pycnidium.

1. The pycnidia are generally present on the upper surface of the leaf and may be best studied in a transverse section of the host leaf.
2. A mature pycnidium is flask-shaped with a pore known as ostiole at its apex.
3. The hyphae near the ostiole are unbranched, pointed and orange coloured. These are called **periphysis** and project through the ostiole.
4. Some of the periphyses are branched and thin walled. These are called receptive hyphae (or flexous hyphae). They project through the ostiole far beyond the periphyses.



5. The cavity of the pycnidium is lined by many elongated and uninucleate pycnidiophores or spermatophores.
6. The pycnidiophores are arranged in a palisade-like layer and each cuts off a chain of pycnidiospores or spermatia.
7. The pycnidiospores or spermatia are discharged through the ostiole and help in producing the dikaryotic mycelium.

Exercise 6

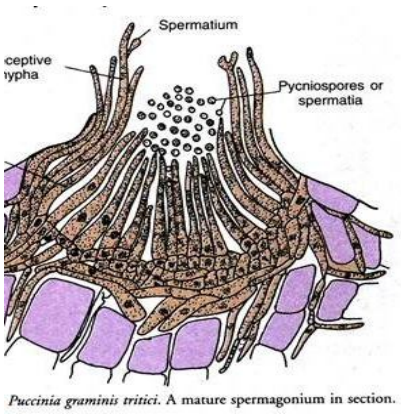
Study of aecidial cup and aecidiospores

Work procedure

A T.s. of barberry leaf is stained with cotton blue and mounted in lactophenol. If permanent preparation is to be made safranin-fast green combination is useful.

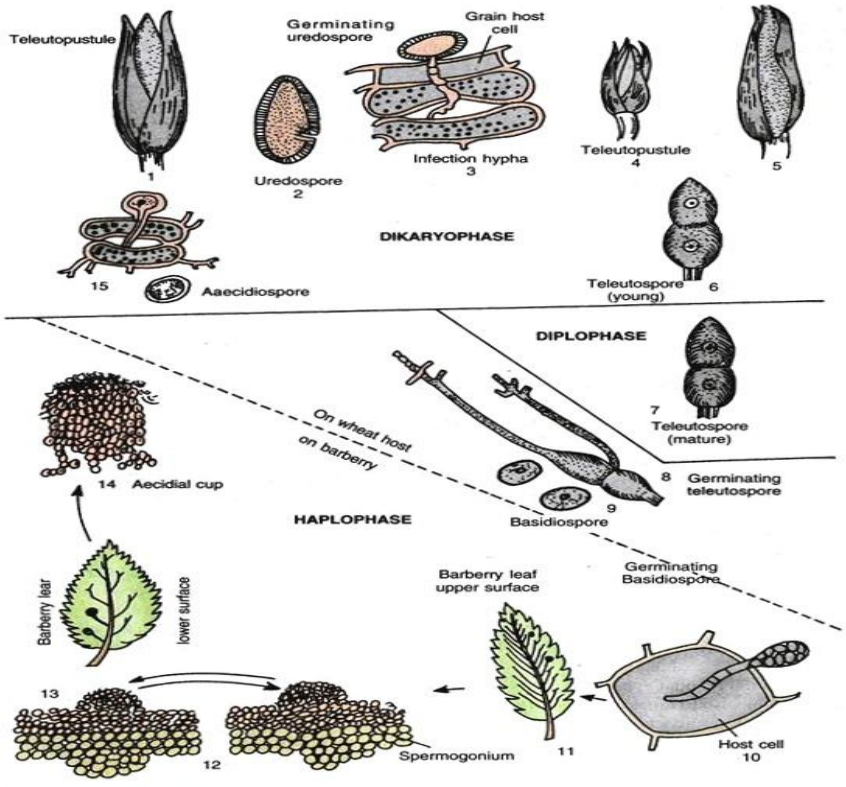
Comments

1. The aecidial cup or aescidium can only be formed by a dikaryotic mycelium.
2. 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. Each aecidium is cup-like structure with an outer protective layer called peridium.



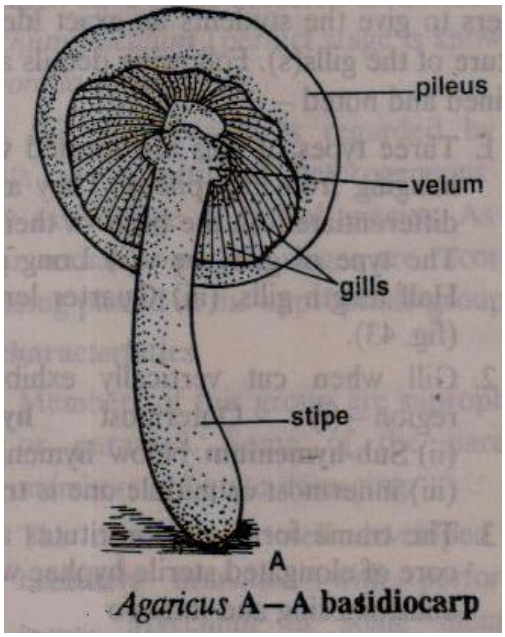
Puccinia graminis tritici. A mature spermagonium in section.

3. The developing aecidium elongates and is pushed through the host epidermis.
4. At the base of aecidium there are many elongated cells known as sporophores, arranged in a palisade-like manner.
5. Each sporophore cuts off alternately, a small and a large cell. The small cell is a disjunctor whereas the latter is the aecidiospore.
6. In younger conditions, aecidiospores are hexagonal and are held in chains by the disjunctor cells. The spores round off as soon as they get separated.
7. Each aecidiospore is a binucleate structure with a thick and smooth wall.
8. The aecidiospores are blown away by wind and infect wheat plant (primary host). They are not capable or reinfecting barberry (the alternate host).



Life cycle of puccinia graminis-tritici

***Agaricus* (Mushroom)**



Agaricus A—A basidiocarp

Classification

Kingdom	Myceteae
Division	Amastigomycota
Sub-division	Basidiomycotina
Class	Basidiomycates
Order	Agaricales
Family	Agaricaceae
Genus	*Agaricus*

Occurrence: It is an obligate saprophyte which is found growing on dead decaying leaf and wood logs in rainy season. This fungus is locally known as Kukarmutta or khumb or Dhingri. *A. compestris* (known as field mushroom) and *A. bisporus* (cultivated mushroom) are common edible mushroom. In nature, the basidiocarps grow in a circle and form fairy ring.

Exercise 1

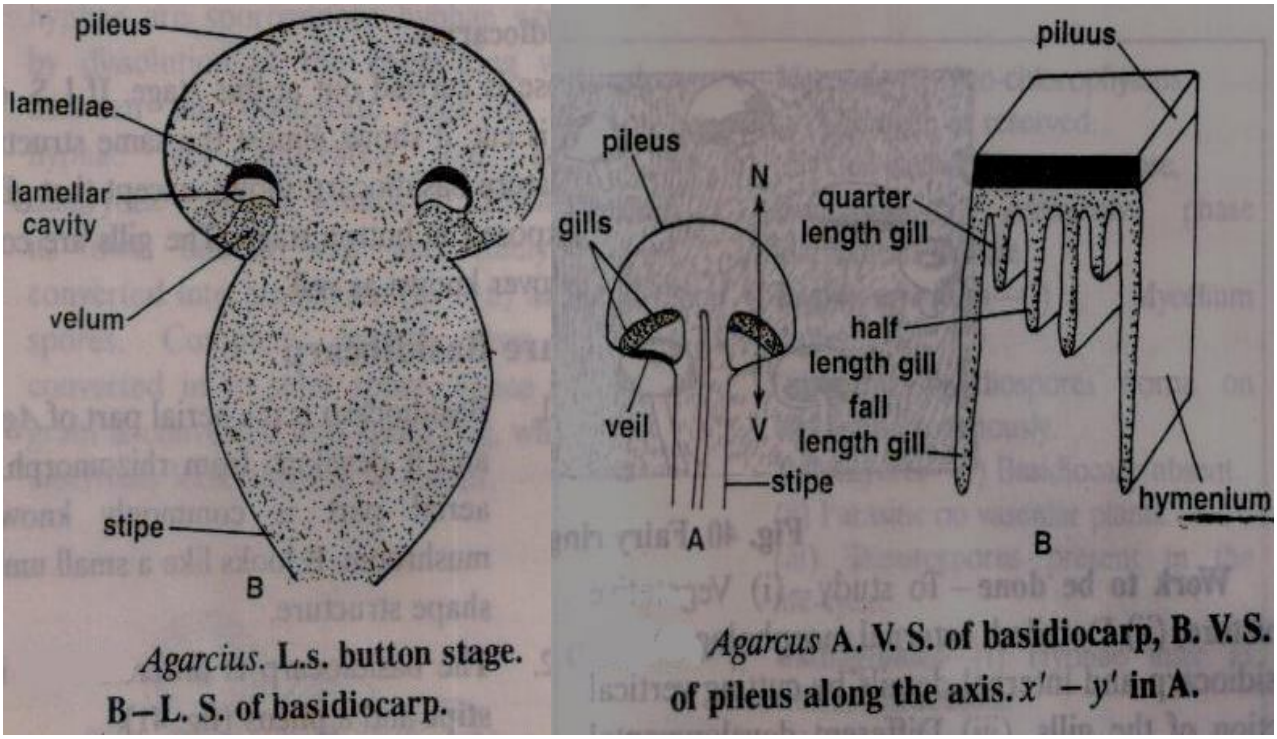
Study the vegetative structure
Work procedure
Collect white cotton growth on decaying matter, stain in cotton blue, mount in lactophenol and study.

Comments
1. The mycelium is underground and consists of much branched hyphae, anastomosing at their points of contact, forming a network in the substratum.
2. Hyphae are septate, dikaryotic with granular protoplasm and prominent oil globules.
3. The aerial portion constitutes the fruiting body. (Fruiting body is formed only by the dikaryotic mycelium).

Exercise 2

Study of button stage
Work procedure:
Study a small and young mushroom. Cut L.s. stain with safranin or fast green and mount in glycerine to study.

Comment
1. This is a developmental stage of the basidiocarp.
2. It is formed above the ground in the form of a small, globose body.
3. In a longitudinal cut, it reveals a small stipe surmounted by the pileus.
4. In - between pileus and stipe, there is a constriction.
5. At the level of this constriction are seen two lamellar cavities or chambers, one on either side.
6. The lamellar cavities or chambers have small lamellae or gills.
7. The edge of the pileus is connected to the stipe by a thin sheet of tissue - the veil or velum.



Agarcius. L.s. button stage. B–L. S. of basidiocarp.

Agarcus A. V. S. of basidiocarp, B. V. S. of pileus along the axis. $x' - y'$ in A.

Exercise 3

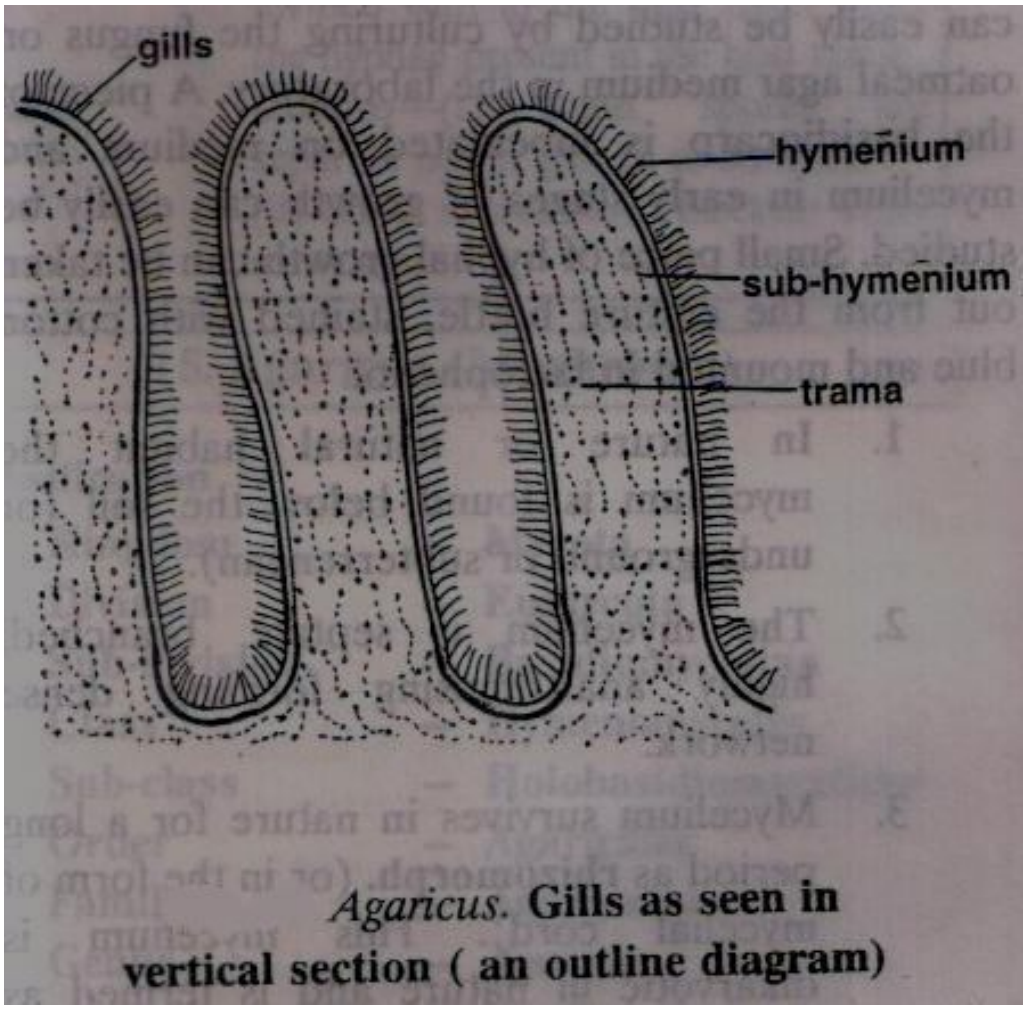
Study of basidiocarp, gills, basidia and basidiospores

Woark procedure:

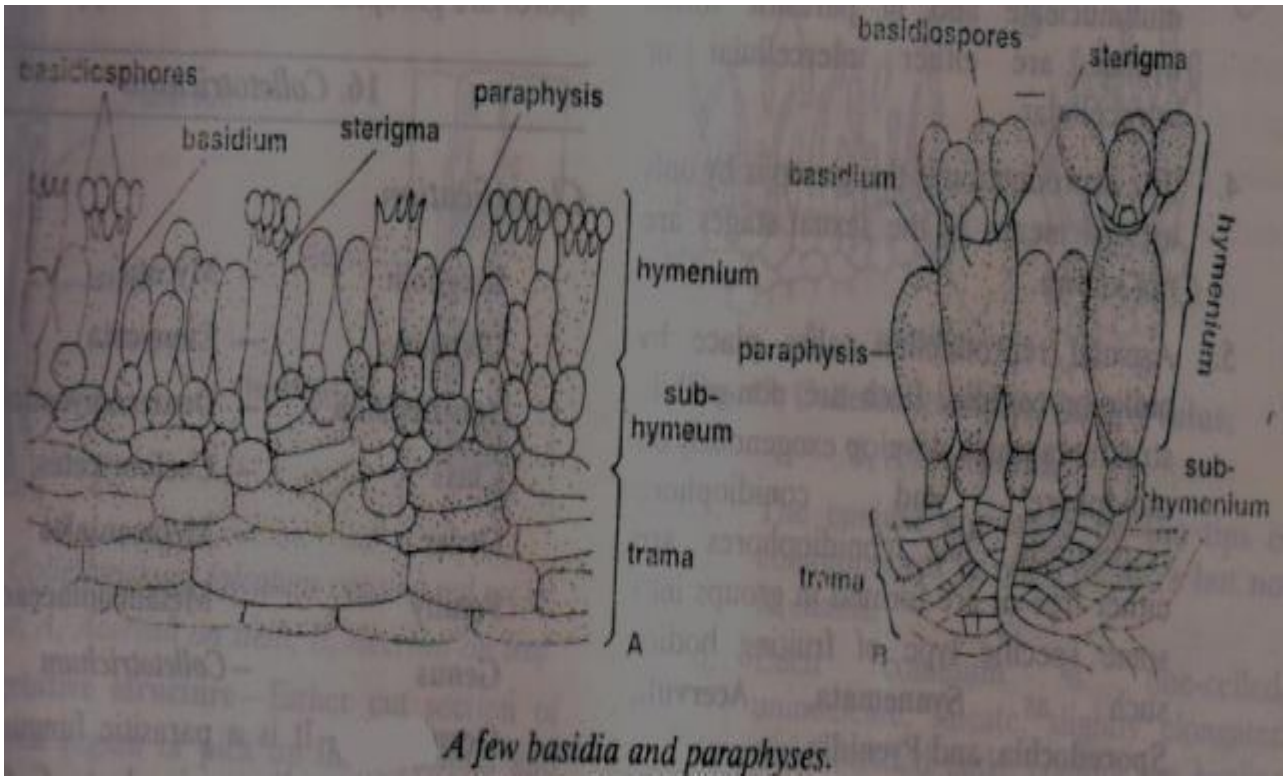
Pluck the pileus of the mushroom after studying it externally. Observe it from the lower side under a mangification lens. Also study a slide showing T.S. of gills.

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 form 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.
6. The trama forms 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, cup shaped cells, forming the superficial layer of the gills, known as hymenium.
9. The hymenium at maturity consists of the fertile cell, the basidia, intermingled with the sterile cells, the paraphysis. (The paraphysis are undeveloped basidia)
10. Each basidium is a cup shaped structure, bearing at its top generally four but sometimes two basidiospores, on short slender stalk known as sterigmata.
11. Each basidiospore is oval in shape and uninucleate.
12. On germination, it produces the new (monokaryotic) mycelium.



Agaricus. **Gills as seen in vertical section (an outline diagram)**



A few basidia and paraphyses.

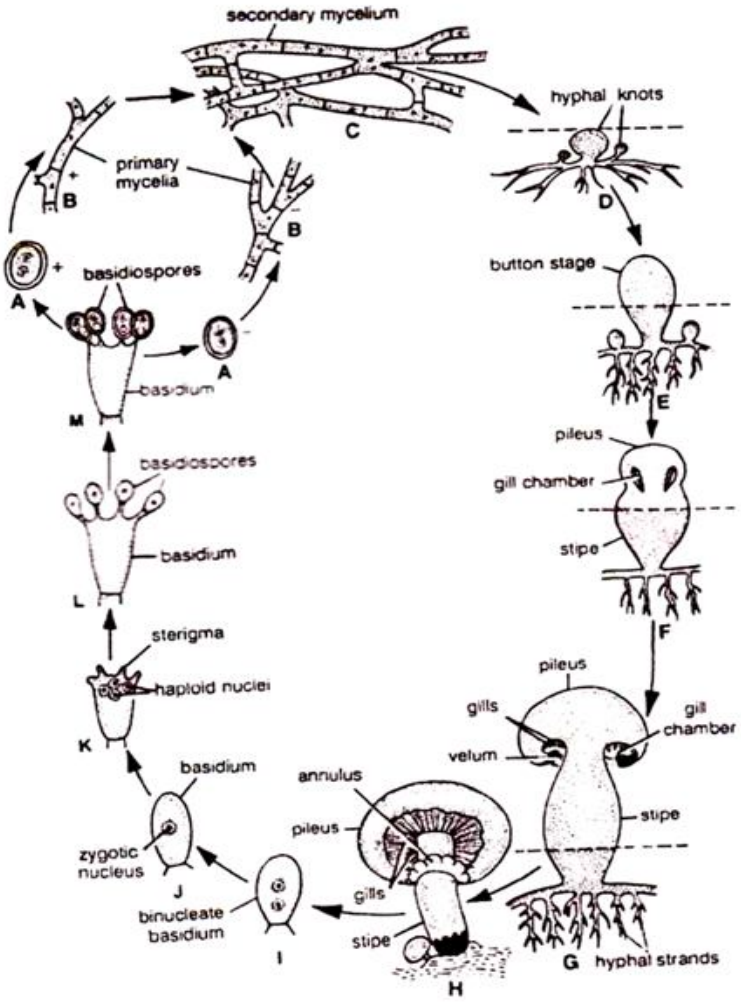


Fig. 8 (A–M). *Agaricus* : Diagrammatic life cycle

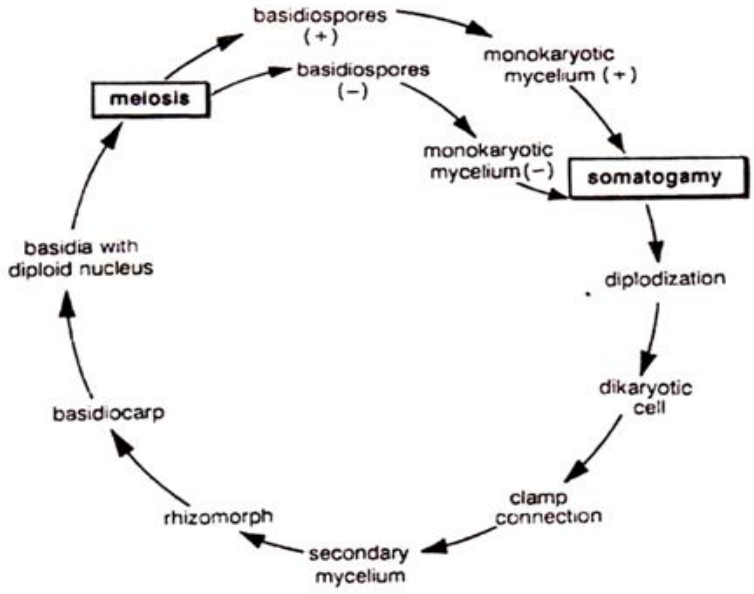


Fig. 9. *Agaricus* : Graphic life cycle

***Polyporus* (Bracket Fungus)**

Classification

Kingdom	Myceteae
Division	Amastigomycota
Sub-division	Basidiomycotina
Class	Basidiomycates/ Hymenomycetes
Order	Polyporales
Family	Polyporaceae
Genus	*Polyporus*

Occurrence: It occurs commonly as a parasite on roots, tree trunk and timber like oak, Dalbergia, Albizzia etc.

Exercise 1

Study of hosts and diseases
Work procedure:

A list of various hosts is given below. Collect this bracket fungus in polythene bags.

Comments

1. Many species of the genus are destructive parasites and cause disease in forest and other shade trees. Many others grow on lumber and destroy it.
2. *P. sulphureus* (sulphur mushroom) causes Wood rot of oaks (Quercus sp.; fam. Fagaceae).
3. *P. squamosus* causes a serious Heart rot in elms (*Ulmus* sp.; fam. Ulmaceae) and other trees.
4. *P. versicolour* grows on various woods and is known as Wood rotten
5. *P. betulinus* is very common on birch (Betula sp.; fam. Betulaceae).

Exercise 2

Study of vegetative structure
Work procedure:
Pick up a part of bracket with forceps or cut a thin section, stain in cotton blue and mount in lactophenol.

Comments

1. The mycelium is well developed, branched and septate.
2. Generally the mycelium grows within and below the bark but in case of severe attacks, it completely invests the central wood cylinder.

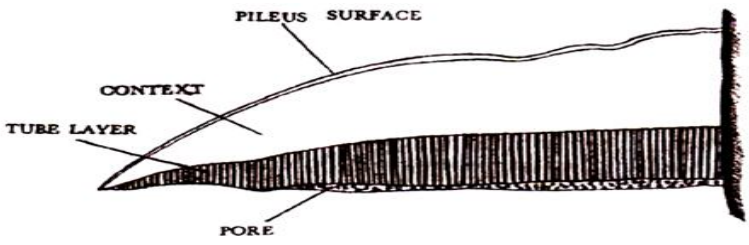


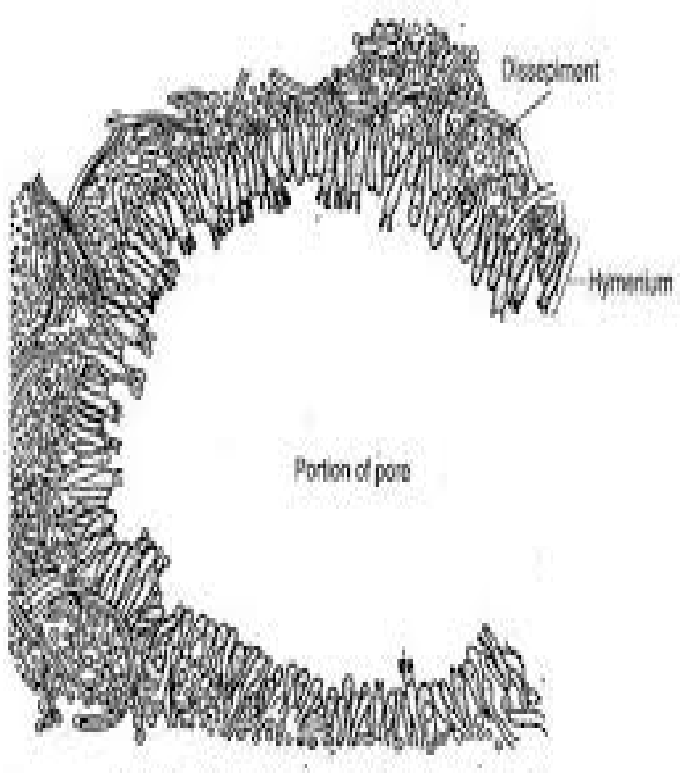
Fig. 2. *Polyporus*: Diagramatic representation of the vertical section of mature basidiscarp.

Exercise 3

Study of basidiocarp, basidia and basidiospores

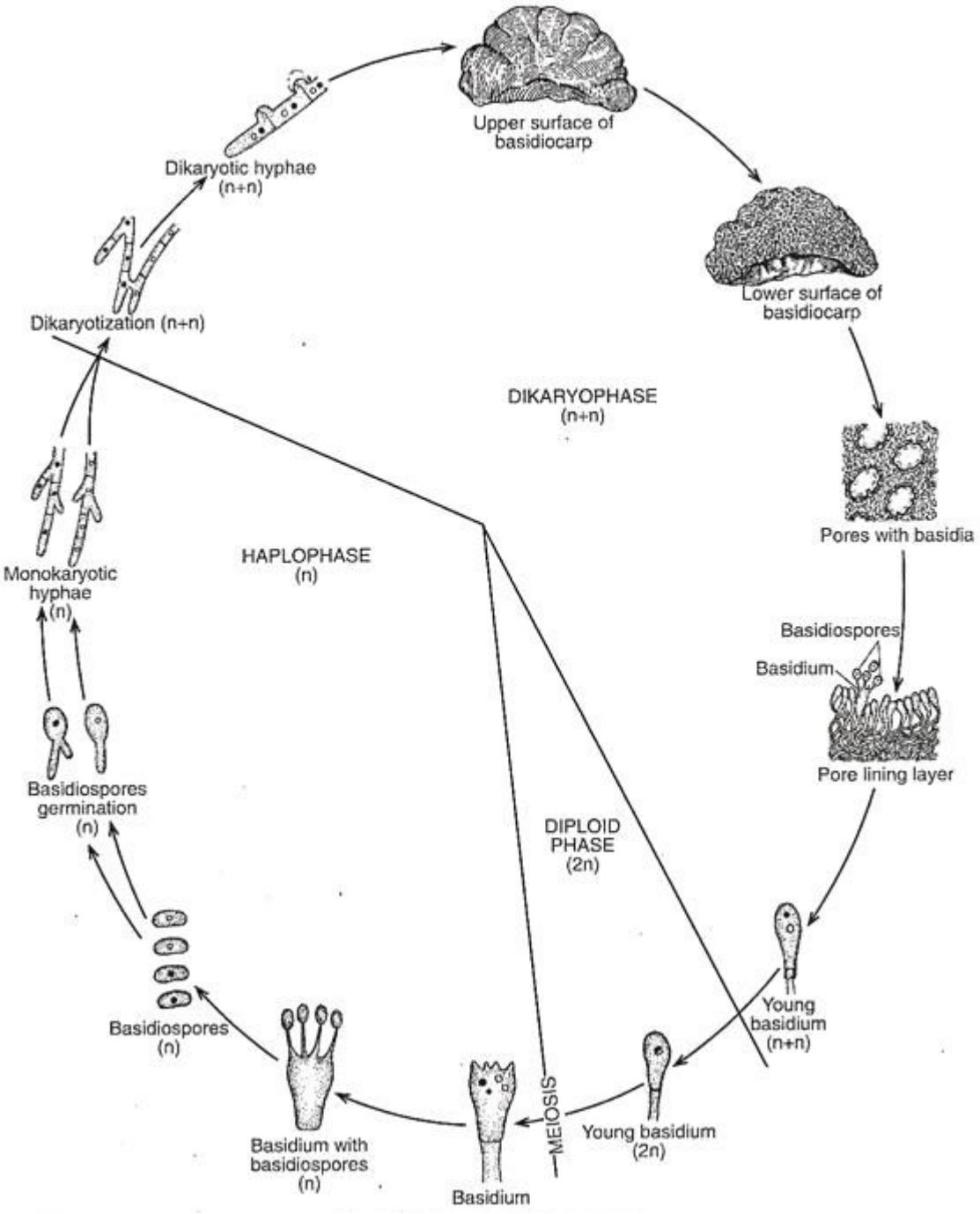
Work procedure:

Study the external features of a fungal organization, cut a T.s., stain in cotton blue, mount in lactophenol and study.



Comments

1. The mycelium (dikaryotic) forms a more or less flat fruiting body, the basidiocarp.
2. The basidiocarp is characteristically shelf-like, shortly stalked and arises from the tree trunks.
3. It is leathery, corky or woody, whitish or slightly grayish or brownish in colour.
4. The upper surface is generally smooth, sometimes rough, often undulating, while the lower surface is porous. In some species the upper surface is distinctly striated.
5. The section of basidiocarp shows an outer context, trams, pores and hymenium.
6. The context is the outer fibrous part made up of thick walled hyphae.
7. The trama is a loose mass of much branched, septate and anastomosing hyphae.
8. The pores or tube extend from below the context to the lower surface.
9. The hymenium is made up of a distinct layer of basidia, lining each pore or tube.
10. The basidia are club shaped, somewhat larger than the sterile cells of the hymenium and project slightly into the cavity of the pore.
11. Each basidium has four short sterigmata at its free end, terminating into a basidiospore each.
12. The large number of basidiospore discharged from the pore.
13. Each basidiospore is small, oval and is uninucleate.
14. The basidiospore on germination gives rise to the mycelium (momokaryotic).



Life cycle of polyporous

Microbiology

Microbiology is the study of micro-organisms.

Antonie van Leeuwenhoek is commonly known as father of microbiology.

Microbiology is mainly concerned with the morphology, reproduction, physiology and identification of micro-organisms. It also includes the study of their distribution in environment, their interrelationships, their association with other living beings their beneficial and harmful effects on man, and the changes they bring to their environment. The micro-organisms include algae found most commonly in aquatic environments or in damp soil; bacteria and fungi found in practically all natural environments; single celled protozoans which cause diseases in man and other animals: rickettsiae obligate intercellular parasites found in many instects

which transmit them to man and other animals; and the fascinating viruses-the well known pathogens of plants, animals and bacteria because of the small size of the micro-organisms, they are studied under microscope. Viruses are so small that they can be visualized only by electron microscope. One can find micro-organisms wherever they exist, know about their transmission and evolve methods for prevention of their sporadic growth. Thus the information emanated from this discipline does well to help identify micro-organisms in the preservation of food. In the industries, in space laboratories to prevent their spread and then the protection against various diseases.

Exercise 1

To culture or cultivate bacteria (and micro-organisms)
Requirements:
Potato, dextrose, sucrose, sodium nitrate ($NaNO_3$), potassium hydrogen phosphate (K_2HPO_4), potassium chloride (KCI), hydrated magnesium sulphate ($MgSO_4$, $7H_2O$), ferrous sulphate ($FeSO_4$), sodium chloride (NaCI), sodium hydroxide (NaOH), sodium bicarbonate ($NaHCO_3$), hydrochloric acid (HCl), alcohol, agar-agar, water, beef, etc.

Conical flasks (250 ml, 500 ml, 1 lit.) culture tubes, petri dishes, beakers (500 ml), 100 ml pipette, 500 ml graduated measuring cylinder etc.

Cotton, potato peeler, Bunsen burner/spirit lamp, wire test tube, basket, wax pencil, forceps, lime' inaculating needles, towel, etc. Autoclave/pressure cooker, inoculation chamber, staining rack, balance, weights, etc.

Work procedure
Cultivation of bacteria and their storage in laboratory is a process that involves sterile conditions and subsequent care. Therefore, precautions must be taken to avoid contamination by other micro-organisms. The major steps are -

(1) Preparation of culture medium, (2) Sterilization (3) Filling test tubes, and petri dishes, (4) Inoculating or transfer of organisms to culture medium and (5) Storage.

[I] Preparation of culture medium
A few common culture media and their methods of preparation are given below.

(a) Meat broth or meat extract broth
Beef extract 3 g

Peptone 10 g

NaCI 5 g

Distilled water 1000 ml

Agar agar 15 g

Meat infusions or beef extracts are prepared by soaking meat or beef in water for many hours. Add beef extract, peptone and sodium chloride together and heat to 65°C, stirring until the materials are completely dissolved. Filter and adjust the pH to 7.2 to 7.6 by adding a pinch of sodium bicarbonate. Add agar to the broth. Heat slowly until agar is dissolved. Pour the medium in large conical flasks to be used as stock.

(b) Potato dextrose agar
Potato (peeled) 200 g

Dextrose 20 g

Distilled water 1000 ml

Agar agar 15 g

Weigh about 200 g of peeled potato. Wash it thoroughly. The pieces are boiled in flask for about 30 minutes. Allow to cool down and decant the supernatant potato extract. Add agar to the extract. Heat the extract slowly till agar agar is completely dissolved. Dextrose is now added and the whole mixture is agitated till it

becomes homogeneous. pH of the medium is adjusted to around 6.4 by using 1/10 N HCl or 1/10 N NaOH. The medium is now poured into flasks as per the requirement. This is the stock to be used in future.

[II] Sterilization

Conditions must be sterile or completely aseptic when bacteria are cultured. There should be no chances of contamination. There are many methods to achieve this. However, following methods would 'be more practical.

1. Dry sterilization: Glassware including petri dishes, test tubes, flasks, etc. may be sterilized by putting them in an oven at a temperature of 160° to 190°C for at least one hour.

2. Steam under pressure (Autoclave): Sterilize all the glassware, cotton, culture media, inoculating needles, etc. in an autoclave set at 15 pounds pressure for 15 minutes. Test tubes, petri dishes, etc. be kept in wire baskets. All the other 'glassware that could be useful in further procedure is also being placed in the autoclave. The flasks containing stock medium be plugged with cotton. For sterilization of small quantities of medium and glassware, pressure cooker is more practical.

[III] Filling the test tubes

If stock medium is prepared and stored in large conical flasks, these flasks must be sterilized before use. The cotton plug of the flask is opened under complete sterile conditions and close to the flame of the burner, to avoid any contamination. The culture medium is now poured in the test tubes in such a way that it does not stick to the sides of the tubes and when the medium contains agar, it must be poured quickly so that agar does not have time to solidify. Fill the tubes 1/2 or 2/3 and plug them with sterile cotton. Medium may similarly be poured in the petri dishes from the large conical flasks with stock medium. Finally sterilize all the test tubes and petri dishes by placing them in wire baskets and putting them in an autoclave for 20 minutes at 30 pounds pressure. Remove the test tubes from the autoclave and tilt the half full test tubes and set the medium at an angle so that the medium when set forms a slant (angle).

[IV] Transfer of microorganisms or bacteria to the medium:

The pathogen or bacteria are collected from the diseased host. More often, bacteria are found growing along with other micro-organisms. Hence, a culture prepared from this natural source would have a mixed population of different micro-organisms. Later, if pure cultures are desired, a colony that belongs to a desired bacterium is transferred to a fresh medium. This is generally known as sub-culturing and would permit the growth of one type of bacterium (or a micro-organism) alone (pure culture). In order to do this, the first step is to transfer the organism directly from the natural source. Large number of bacteria removed from the tissues or substances like milk, curd, salvia, urine, etc. are diluted with distilled water. This mixture is kept in a petri dish.

The table top is cleaned with spirit or with 90% alcohol. Bunsen burner or a spirit lamp is lighted. A test tube containing nutrient agar is placed in hot water bath for melting the agar. It is now taken out and cotton plug is removed bringing the mouth of the test tube across the flame to eliminate contaminants around the mouth of the tube.

The mixture of bacteria or inoculum is now transferred to the agar slants or to the petri dish either by simply pouring a drop of distilled water or by using a dropper or pipette.

To inoculate or transfer to a fresh culture medium, following procedure is generally used. Take inoculating needle and heat it over the flame of the lamp. Let the needle cool before being used again. Remove the cotton plug from the sterile tube or flask and hold it in hand, the mouth the tube be placed close to the flame of spirit lamp. The sterile and cooled inoculating needle is inserted into the old culture growing in the tube. A small part of the colony is removed by the tip of the needle. This needle is now inserted into the sterile test tube with agar slant but without bacterial or any other culture. The needle is streaked along the entire length of sterile agar surface. The needle is flamed once again before putting it aside. Also flame the mouth of the test tubes before placing cotton plugs.

When petri dishes are streaked, the cover of petri dish is slightly raised and the needle is streaked over the surface in three equally distant level lines. The cover of the petri dish is replaced quickly. The dish is sealed with adhesive tape, its contents are labelled and the dish is stored.

Storage
A special room is generally marked for the storage cultures. The temperature of this room is maintained at 27^0C. The cultures are ready for examination after about 2-3 days.

Exercise 2

To isolate micro-organisms from mixed culture and grow a pure culture
Requirements
Stock solution of meat extract medium/beef extract medium/PDA (Potato Dextrose Agar)/any other suitable nutrient medium: conical flasks, culture tube, petri dishes, cotton, Bunsen burner/spirit lamp, test tube basket, wax pencil, inoculating needles; autoclave/pressure cooker, inoculating chamber, etc.

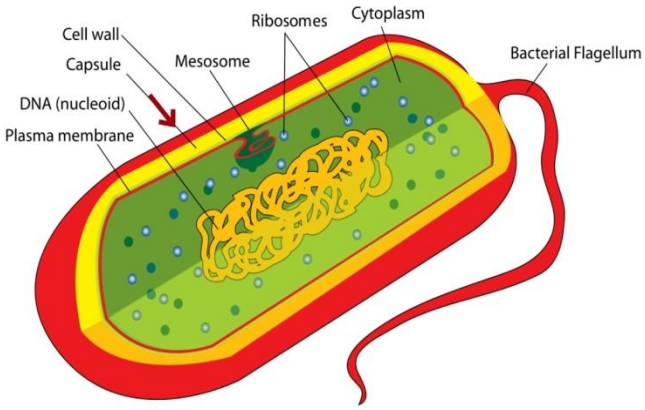
Work procedure
The initial or original inoculum from the natural source has generally a mixed population of microorganism, hence when cultured it grows into a mixed or contaminated culture. If an individual organism is to be studied, it must be isolated from the mixed culture and then grown again into a pure culture (a culture containing individuals or one species only).Many methods are available, however, the following is the simplest that can he adopted.

1. Observe different kinds of colonies growing in mixed culture.
2. Note different characteristics of colonies like size, form, elevation, margin, contour, surface, texture, colour, radial growth, etc., to identify an organism wherever possible.
3. The transfer of the desired colony is now done under the sterile conditions. The working table is wiped clean by cotton dipped in 90% alcohol.
4. The inoculating needle is heated red hot on a flame. It is then allowed to cool. The needle is generally not placed on the table.
5. The test tube with mixed culture is held in left hand and the 'sterilized needle in the right. The cotton plug of the tube is pulled out in between the fingers of the right hand. The open mouth of the culture tube is passed over the flame of the spirit lamp. The needle is now inserted into the tube and the colony or a part of it is lifted by the loop of the needle. The mouth of the tube is once again passed over the flame and the cotton plug is quickly replaced.
6. A fresh sterilised test tube with nutrient medium is taken out of wire test tube basket. The cotton plug is now pulled out between the fingers of the right hand. The open mouth is passed over the flame. The loop of the inoculating needle carrying colony (or a part of it) is now inserted into the tube and streaked along the surface of the agar slant. The mouth of the tube is passed over the flame once again and then plugged quickly.
7. Freshly inoculated tube (sub-culture) is now stored under suitable temperature
8. The colonies appear within two to three days. (Blue-green algae take about 20-30 days to appear).
9. The colonies are suitably stained and the organisms studied under the microscope.

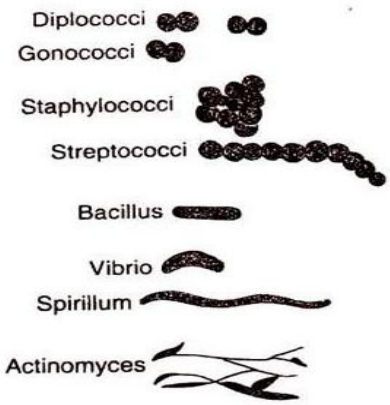
Exercise 3

To stain and study bacteria (micro-organisms)
Requirements:
Crystal violet (aq. 0.5%), Iodine, alcohol 95%, Safranin (aq. 1%), microscope, needles, blotting papers, slides, glass dropper, etc.

A Bacterial cell



classification of bacteria



Work procedure

(i) Preparation of bacterial film

1. Take a clean slide and gently warm it over the spirit lamp. Let the slide cool.

2. Take drop of broth culture or pick up a part of colony by a needle loop.

3. Place it over the slide and spread evenly to form a film. Let the film dry.

(ii) Fixing the bacterial film

1. Pass the film quickly 3-4 times over the flame of the spirit lamp.

2. Let the slide cool so that the film sticks firmly to the slide.

(iii) Staining the bacterial film:- Gram's stain is generally used to stain bacteria. The method is described separately. A summary is given below.

Stain with crystal violet for about I min

Wash with water

Put a few drops of Gram's iodine on the film and allow it to stand for about 1 minute

Rinse the slide in water

Destain with 95% alcohol for 10-30 sec.

Wash with water

Counterstain with safranin for 30 sec.

Wash with water and dry with blotting paper

Put a drop of glycerine on the film and cover with a cover slip

Now examine the slide under oil immersion objective of the microscope.

Observations

Gram (+) bacteria are stained violet or blue and Gram (-) bacteria are stained pink to reddish. Also observe the shape of the bacterial cells, the flagella if present, the capsule etc.

Exercise 4

To measure the bacterial cell /other cells

Requirements:

Ocular micrometer (also known as oculometer), stage micrometer, slide of bacteria or any other structure, which is to be measured.

Work procedure

[I] Coinciding the scales

Stage micrometer: It is a slide with engraved scale. It is generally ruled into tenths and hundredths of a millimeter. Thus each of the hundredth part or one division of stage micrometer represents 0.01 mm or 10 µ (1 mm = 1000 microns or µ).

Ocular micrometer: It is a disc of glass with engraved scale. It is does not have any standard value and hence needs to be standardised with each microscope and the lenses (objective and eye piece) of different powers.

1. Place a stage micrometer slide on microscope stage. Focus the scale.
2. Place the ocular micrometer correctly on the metal diaphragm inside the eye piece. The lines of the micrometer should be sharp and clearly visible.
3. Move stage micrometer slide while looking through eye piece in a way so that both ocular and stage micrometer scales appear side by side.
4. Now coincide the divisions of the stage micrometer slide with those of ocular micrometer.
5. In this case, for example, 72 divisions of stage micrometer slide coincide with 50 divisions of ocular micrometer.

[II] Standardisation of ocular micrometer scale:

1. The set of lenses used is-objective 45x and eye piece 10x.
2. The value of stage micrometer is-one division of stage micrometer is equal to 0.01 mm or 10 microns or µ.
3. Therefore, 72 divisions = 0.72 mm or 720 microns or µ.
4. Since 72 divisions of stage micrometer = 50 divisions of ocular micrometer.
5. 0.72 mm or 720 microns or µ = 50 divisions of ocular micrometer.
6. Therefore, 1 division of ocular micrometer = 0.72 mm/50 or 720 µ/50 = 0.144 mm or 14.4 microns or µ.
7. The value of one division of ocular micrometer is, therefore, 0.144 mm or 14.4 microns or 14.4 µ
8. The following formula can be used.

1 division of ocular micrometer

10 x divisions of stage micrometer

divisions of ocular micrometer

After substituting the values as given above

10 x 72 = 720

50 x 50 = 14.4 microns (µ)

[III] Measuring an object

1. Remove the stage micrometer slide. The ocular micrometer should, however, be allowed to remain in the eye piece.
2. Place the bacteria or any other object under the objective lens. A slide with a cover glass should be used. Move the eye piece or rotate, so that the ocular micrometer scale is adjusted over the object to be measured.
3. If the cell diameter measures 2 divisions of ocular micrometer, then the diameter would be 2 x 14.4 µ = 28.8 µ
4. In this way different dimensions of different objects can be measured.

Exercise 5

Microscopic examination of curd
Requirements:
Milk, dropper, methylene blue, glycerine, slides, coverslips, etc

Work procedure
1. Place a drop of milk on the slide and prepare a smear.
2. Let the smear dry.
3. Stain the smear with methylene blue.
4. Dry the smear again and place a drop of glycerine.
5. Mount a coverslip and study.

Observations
Numerous bacterial cells are seen scattered. These generally belong to the genera *Lactobacillus* or *Streptococcus*.

[1] Lactobacillus
1. These are rod-shaped bacteria. The rods are long and slender.
2. The rods generally occur in chains.
3. The bacterium shows peritrichous flagella but motility is rare.
4. These are Gram+ bacteria but may become Gram - with increasing age and acidity.
5. Besides dairy products, the bacterium is also found in grains and meat products, water, sewage, beer, wine, fruits and fruit juices.
6. Metabolism is generally fermentative but some are strict anaerobes.

[II] Streptococcus
1. Cells spherical to ovoid, less than 2µm in diameter.
2. The cells occur in pairs or chains.
3. Occasional motile strains are also found.
4. These are gram+, chemo-organotrophs, facultative anaerobes.

Botany second paper

Algae (plural)/Alga (singular)
The word algae (Sing. Alga) is derived from a Latin word alga, which means Sea Weeds. The science that deals with their study is called algology. The Greek word foe alga is phykos and therefore, their study is called phycology, (phykos=Sea weeds, logos=study). The branch which deals the study of algae is called **Phycology/algology** and the scientists related to this branch are known as **Phycologists/algologists. F.E. Fritsch** is known as the father of algae of the world and Professor **M.O.P. Iyenger** as father of Indian algology.

Algae and fungi differ from one another mainly due to presence of chlorophyll in the former and its absence in the latter. This makes algae autotrophic and fungi remain heterotrophic. Reserve food in algae is the form of starch while fungi store glycogen. Algal cell walls are made of cellulose and those of fungi are composed of chitin. The present day delimitation of algae is due to A.L. de Jussieu (1789). Algae is mostly found in water though some are terrestrial and some are even parasitic (*Cephaleuros virescence*, a green alga causes **Red Rust of Tea**). They are as small as bacteria and as large as *Macrocystis* (a brown alga, 196 feet long).

They are mostly chlorophyll containing autotrophic thalloid plants. They occur in a variety of habitats but majority of them are aquatic. Their plant body does not show differentiation into various tissues system. They have mostly unicellular sex organs and without sterile jacket. Embryo formation does not takes place after gametic fusion.

In different system of classification of plant, algae have been differently ranked as an order (Linnaeous, 1753), a class (Eichler, 1886), a class (Fritsch, 1935, 1945), or a group (chapman, 1962). They may be defined as an assemblage of chlorophyll bearing autotrophic thallophytes, bounded by a cell wall, made up of pure or mixed carbohydrates. According to the estimated of B.N. Prasad (1984), out of a world total of 2475 genera and 28305 species of algae, 666 genera and 5136 species are represented in India. This amount is to a proportion of 28.3% of global generic representation and 18.1% of world species.

They found in fresh water (e.g. *Spirogyra, Oedogoniun*), sea water marine alga (e.g *Sargassum, Laminaria*), on soil or terrestrial alga (e.g. *Fritschiella, Vaucheria*), on rockland and stone or lithophytic alga (e.g. *Batrachospermum, Entromorpha*), in highly concentrated salty water (e.g. *Dunaliella*), on sand or Psammophytic algae (e.g. *Phormidium, Vaucheria*), in very hot water or thermal algae (e.g. *Onconema, Synechococcus*), on ice or snow or cryophytic algae (e.g. *Chlamydomonas nivalis*), on other plant or epiphytic algae (e.g. *Oedogonium, Bulbochaete*), inside other plants or endophytic algae (e.g. *Nostoc* inside the thallus of *Anthoceros, Anabaena*), in the coralloid roots of cycas, on animal or epizoic algae (e.g. *Cladophora crispate* on the shells of mollusces), inside the animals or endozoic algae (e.g. *Stigeoclonium* in the nose of the fish, as parasite (e.g. *Cephaleuros* on the leaves of tea plant), and also in the combination with fungi in the form of lichens.

The thallus organization in algae also varies greatly and shows a clear range. They may be motile colonial (e.g. *Volvox*), palmelloid (e.g. *Tetraspora*), dendroid or tree like (e.g. *Ecballocystis*), coccoid or non-motile (e.g. *Chlorella*), filamentous (e.g. *Oedogonium, Cladophora*), heterotrichous (e.g. *Ectocarpous*), siphon like or siphonaceous (e.g. *Vaucheria*), unisexual (e.g. *Batrachospermum*), multiaxial (e.g. *Polysiphonia*) or even parenchymatous (e.g. *Sargassum*). The size of the algal members is also highly variable from 0.5µ (e.g. *Dunaliella, Chlamydomonas*) to as much as 30 meters or more (e.g. *Macrocystis*). There is an unsubstantiated record of a plant of *Macrocystis pyrifera* up to 700 feet (i.e. 213 meter), the largest plant in the world (Prescott, 1969).

Vegetative reproduction takes place by fragmentation, fission (*Desmids*), akinets (*Oedogonium*), tubers *(chara*) or hormogones (Myxophyceae). Asexual reproduction takes place by the formation of zoospores (*Chlamydomonas*), synzoospore (*Vaucheria*), aplanospore (*Vaucheria*), hypnospore (*Vaucheria*), autospore (*Chlorella*) and tetraspores *(Dictyota*).

Sexual reproduction takes place by isogamy (*Chlamydomonas*), anisogamy (*Ectocarpus*) or oogamy (*Oedogonium*). Life cycle may be haplontic (*Chlamydomonas*), diplontic (*Sargassum*), diplo-haplontic showing isomopic types of alternation of generation (*Cladophora*) or heteromorphic types of alternation of generation (*Laminaria*), haplobiontic or diphasic (*Batrachospermum*) and haplodiplobiontic or triphasic *(Polysiphonia*)

Classification of algae

Chlorophyll is present in all groups of algae, but in some groups pigments other than chlorophyll are dominant and mark the green colour of chlorophyll. On the basis of their colour following four groups were recognized.

(1) **Myxophyceae** or **Cyanophyceae** (Blue-green algae): dominant pigment **c-phycocyanin**.

(2) **Chlorophyceae** (Green algae): dominant pigment-**chlorophyll a and b**.

(3) **Paeophyceae** (Brown algae): dominant pigment-**fucoxanthin**.

(4) **Rhodophyceae** (Red algae): dominant pigment-**r-phycoerythrin**.

Algae have been variously classified by numerous phycologists, their views always differing. The most simple and practical classification was proposed by British phycologist **F.E. Fritsch in 1935.**

Classification proposed by Fritsch

First most comprehensive and authorative classification of algae given by F.E. Fritsch (1935, 48) in his book '**The structure and Reproduction of the algae'**. His classification was based on such criteria as pigmentation, type of flagella, assimilatory products, thallus structure and methods of reproduction. He divided algae into the following 11 classes:

1. **Class - Chlorophyceae** (All green autotrophic algae). All the green algae of the world include in this class and the dominant pigments are **chlorophyll a** and **b**. The class Chlorophyceae has been divided into following orders**:**

(i) **Volvocales** (e.g, *Volvox*)

(ii) **Chlorococcales** (e.g *Hydrodictyon, Chlorella*)

(iii) **Ulotrichales** (e.g, *Cladophora*)

(iv) **Cladophorales** (e.g, *Cladophora*)

(v) **Chaetophorales** (e.g, *Fritschiella*)

(vi) **Oedogoniales** (e.g, *Oedogonium*)

(vii) **Conjugales** (e.g, *Zygnema*)

(viii) **Charales** (e.g, *Chara*)

2. **Class Xanthophyceae** (yellow green algae). In this class the dominant pigmentsare **chlorophyll a, e, β carotene and xantophylls.** The member of xanthophyceae are simple, chromatophores are yellow-green due to the predominance of 13-carotene. Pyrenoids are usually absent. Oil or leucosin is present instead of starch. Cell wall is mostly composed of two equal or unequal halves, overlapping one another. Motile cells are flagellated. Flagella are borne anteriorly and are unequal in length (and, therefore, this group is also known as Heterokontae). A few members produce aplanospores instead of zoospores: Sexual reproduction is rare and if present, is generally isogamous. This class has been divided into four orders:

(i) **Heterochloridales** (e.g, *Heterochloris, Chloramoeba*),

(ii) **Heterococcales** (e.g, *Myxochloris, Halosphaera*)

(iii) **Heterotrichales** (e.g, *Tribonema, Microspora*)

(iv) **Heterosiphonales** (e.g, *Botrydium*)

3. **Class Chrysophyceae (brown-orange algae).** In this class the dominant pigments are phycocrysin which imparts brown or orange colour of this algae. Their motile cells have two equal or unequal flagella and in their life cycle sexual reproduction is rare and it is only Isogamous type. The class includes following three orders:

(i) **Chrysomonadales** (e.g, *Chrysococcus, Chrysodendron, Chromulina*)

(ii) **Chrysosphaerales** (e.g, *Chrysophaera, Echinochrysis*)

(iii) **Chrysotrichales** (e.g *Nematochrysis, Chrysoclonium*)

4. **Class. Bacillariophyceae** (**Diatoms, yellow or golden-brown algae**). In this class the dominant pigments are fucoxanthin, diatoxanthin and diadinoxanthin. Some members of this group are known as diatoms. Diatoms very commonly occur as planktons. The wall is made of two overlapping valves. The walls show either radial or bilateral entry. Each cell has one to many yellow or golden-brown matamorphoses. The reserve food product is in the form of fats volutin grains. The common method of multiplication is cell division. Some special reproductive structure are formed. The cell walls of diatoms form a large and huge deposit of diatomaceous earth or 'siliceous earth'. It is an important food for the aquatic animals. The class includes two orders:

(i) **Centrales** (e.g *Cyclotella, Chaetoceras*)

(ii) **Pennales** (e.g, *Grammatophora, Navicula, Pinnularia, Denticula, Epithemia*).

5. **Class. Cryptophyceae (brown-red algae).** In this class the dominant pigments are xanthophylls which imparts brown or red colour. Their motile cells have two unequal flagella, sexual reproduction is rare and only isogamous type. The class includes two orders:

(I) **Cryptomonadales** (e.g, *Crytomonas*)

(II) **Crytococcales** (e.g *Tetragonidium*)

6. **Class. Dinophyceae** (Peridinieae). In this class the dominant pigments are xanthophylls which imparts brown or red colour. Mostly saprophytes and show holozoic mode of nutrition.In this class six orders are included:

(I) **Desmomonadales** (e.g, *Desmocapsa, Pleromonas, Desmomastix*)

(II) **Thecatales** (e.g, *Exuviaella, Prorocentrum*)

(III) **Dinophysiales** (e.g., *Dinophysis, Ornithocercus, Phalacroma*)

(IV) **Dinoflagellata** (e.g., *Amphidinium, Blastodinium, Ceratium, Heterocapsa*)

(V) **Dinococcales** (e.g., *Dinastridium, Cystodinium, Dissodinium*)

(VI) **Dinotrichales** (e.g., *Dinothrix, Dinoclonium*)

7. **Class. Chloromonadineae.** In this class the dominant pigments are xanthophylls. This is a small class with only one order comprising of highly specialized unicellular flagellates.

Chloromonadales (e.g. *Trentonia, Vacuolaria*)

8. **Class. Euglenineae.** This is a small class, comprising of highly specialized unicellular green flagellates. Sexual reproduction is known only in few species and it is only isogamous type. **This class includes three families:**

(i) **Euglenaceae** (e.g., *Amphitropis, Ascoglena, Euglena*)

(ii) **Astasiaceae** (e.g., *Astasia, Distigma, Menoidium*)

(iii) **Peranemaccae** (e.g., *Anisonema, Entosiphon, Petalomonas*)

9. **Class. Phaeophyceae** (brown algae). In the members of this class fucoxanthin is present as dominant pigments which impart brown colour of these members. Motile reproductive cells have two unequal lateral flagella, reserve food is in the form of mannitol and laminarin. The class comprises of following nine orders:

(i) **Ectocarpales** (e.g., *Ectocapus, Halothrix,Punctaria*)

(ii) **Tilopteridales** (e.g., *Tilopteris*)

(iii) **Cutleriales** (e.g., *Cutleria*)

(iv) **Sporochnales** (e.g., *Sporochnus, Carpomitra*)

(v) **Desmarestiales** (e.g., *Desmarestia, Arthrocladia*)

(vi) **Laminariales** (e.g., *Laminaria, Lessonia, Macrocystis*)

(vii) **Sphacelariales** (e.g., *Sphacelaria*)

(viii) **Dictyotales** (e.g., *Dictyota, Padina*)

(ix) **Fucales** (e.g., *Fucus, Sargassum, Durvillea*)

10. **Class. Rhodophyceae** (Red algae). Most of the members of this class are marine water and very few are found in fresh water. Dominant pigments are r-phycoerythrin and r-phycocyanin which impart red colour. Reserve food material is in the form of floridean starch. Reproductive cells are non motile and sexual reproduction is advance oogamous type. The Class is divided into seven orders:

(i) **Bangiales (e.g.,** *Bangia, Porphyra, Porphyridium***)**

(ii) **Nemalionales** (**e.g.,** *Batrachospermum, Lemanea, Nemalion, Chaetangium, Naccaria*)

(iii) **Gelidiales (e.g.,** *Gelidium***)**

(iv) **Cryptonemiales**

(v) **Gigertinales**

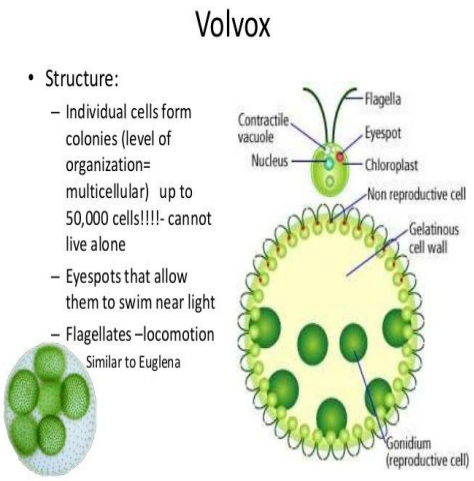
(vi) **Rhodymeniales**

(vii) **Ceramiales (***Polysiphonia***)**

11. **Class. Myxophyceae/ Cyanophyceae** (Blue- green alae)**.** In blue-green algae the dominant pigment is **c-phycocyanin**. Popularly known as cyanophyceae, cyanobacteria or blue-green algae, the group is said to be highly successful since they inhabit almost every habitat. Predominance of pigments, c-phycocyanin (a blue pigment) and c-phycoerythrin (a red pigment) imparts characteristic blue-green colour. The group is marked from all others, in its absence of definite nucleus and instead DNA strands are present (prokaryotic character) in the centroplasm. Pigments are found diffused in the form of lamellae in the peripheral part of the protoplasm, called chromatoplasm. The plastids and pyrenoids are absent. The reserve food product is cyanophycean starch and glycogen. They are capable of nitrogen fixation. Sexual reproduction being absent, only methods of reproduction are by asexual means, cell division endospores, hormogonia, akinetes, etc are few of the methods by which reproduction takes place. In this class following orders are include.

1. **Chrococcales** (*Microcystis*)
2. **Chamaesiphonales**
3. **Pleurocapsales**
4. **Stigonematales**
5. **Nostocales** (*Nostoc, Oscillatoria*)

Volvox (Coenobial colonial motile colonial, Volvox = to roll)



Classification

Sub-division Algae

Class Chlorophyceae

Order Volvocales

Family Sphareallaceae or Volvocaceae

Genus *Volvox*

Occurrence: Commonly occurs in fresh water, pools and ponds in the rainy season, in the form of small balls, of the size of a pin-head. Some common Indian species are *Volvox globator, V. prolificus, V. africanus* and *V. merely etc.*This is a small class with onlyone order comprising of highly specialized unicellular flagellates.

Exercise 1

Study of thallus

Work procedure

Stain a few colonies in safranin, wash in water and mount in glycerine to study the external features of the colony and structure of a cell.

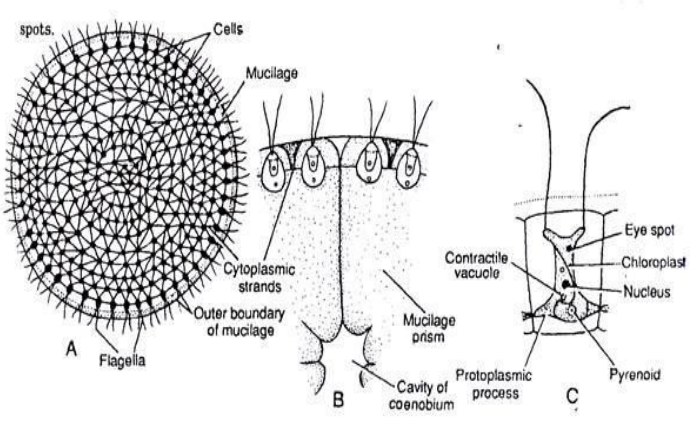
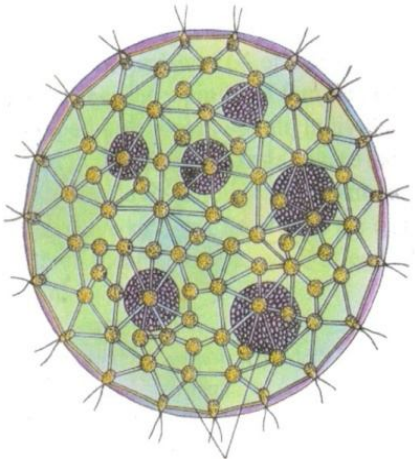


Fig. 1. (A-C) *Volvox*. A. A colony; B. A part of colony; C. Single cell.

Comments

1. Alga is commonly known as "**coenobial motile colonial**" green multicelllular alga.
2. Colonies are mostly spherical, rounded or oval in shape.
3. It is hollow in the centre and cells are arranged in a single layer towards the periphery.
4. Layer of cells is surrounded by a gelatinous mass which forms the outer and firm limiting layer.
5. The number of cells in a colony varies from 500-60,000 according to the species.

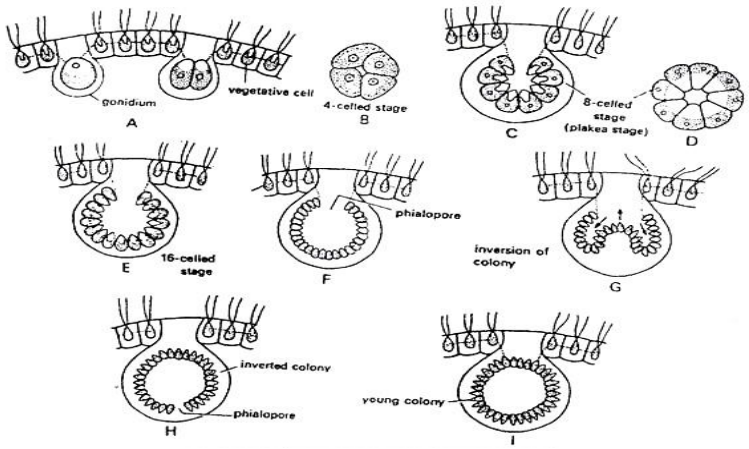


6. Each cell of the colony is connected with a few of the neighboring cells by thin and delicate cytoplasmic strands.
7. Each cell is enveloped by an individual gelatinous sheath.
8. All the cells of a colony are typically Chlamydomonas (unicellular green alga) in shape, size and structure.
9. Each vegetative cell is biflagellate, motile and ovoid. The two flagella are anteriorly inserted. A contractile vacuole is situated one each at the base of a flagellum. Cup-shaped chloroplast occupies much of the posterior part in which is situated a single pyrenoid. In the cavity formed by cup-shaped chloroplast lies a single nucleus, surrounded by cytoplasm. Cytoplasm is rich in volutin grains. Eyespot or stigma occupies anterio-lateral position.

Exercise 2

Study of asexual reproduction (Asexual reproduction by daughter colony)
Workd procedure:
Study the slide showing daughter colonies

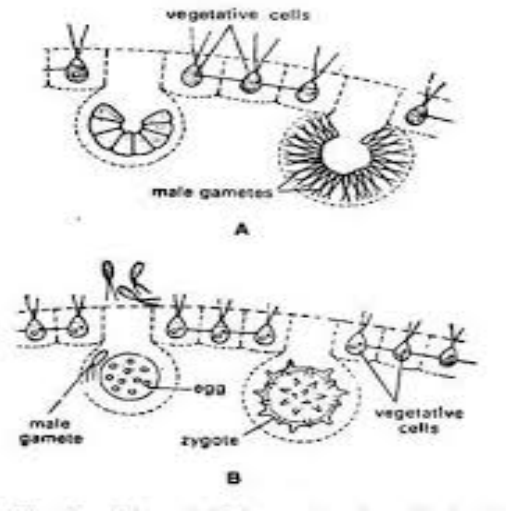
Comments



1. Asexual reproduction takes place by the formation of daughter colonies.
2. Daughter colonies are formed mostly in the posterior part of the parent colony.
3. Many small daughter colonies remain embedded in the parent colony. These appear similar to parent colony except its smaller size.
4. Daughter colonies are liberated by the gelatinization of the wall of the parent colony.

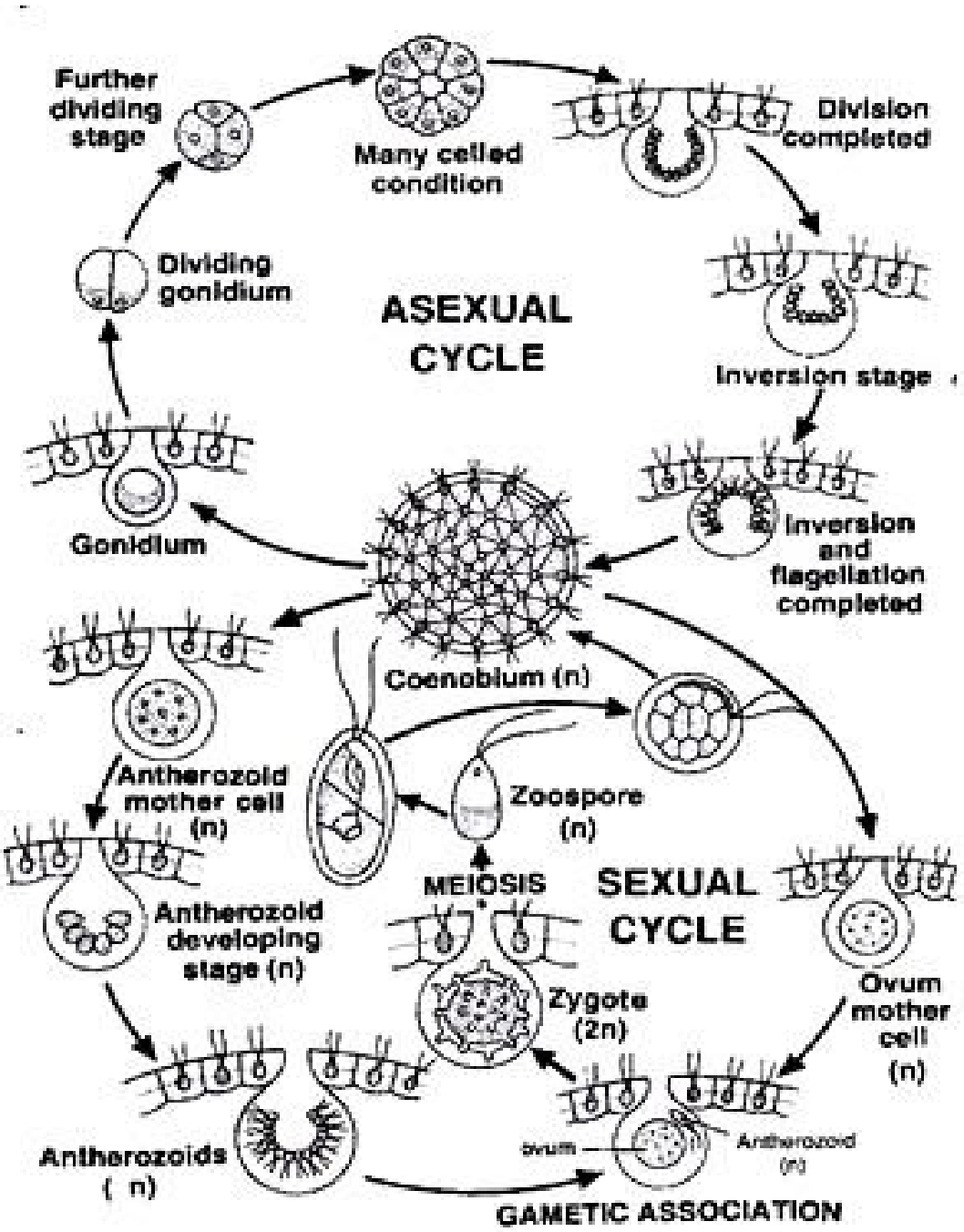
Exercise 3

Study of sexual reproduction
Work procedure
Study the slide showing sex organs and the zygote.



Comments

1. Colonies may be monoecious or dioecious.
2. Sexual reproduction is oogamous.
3. Antheridia and oogonia are developed mostly in the posterior part of the colony.
4. Antheridium produces biflagellate antherozoids while non-motile oogonium develops a single egg.
5. As a result of fertilization oospore or zygote is produced.
6. Zygote is orange-red in colour because of the presence of haematochrome.
7. Zygote is a thick-walled structure. The wall is made of two or three layers. Outermost layer is thick and may be smooth or ornamented.



Life cycle of *Volvox*

***Hydrodictyon* (water net or non motile coenobial alga)**

Classification

Sub-division	Algae
Class	Chlorophyceae
Order	Chlorococcales
Family	Hydrodictyaceae
Genus	*Hydrodictyon*

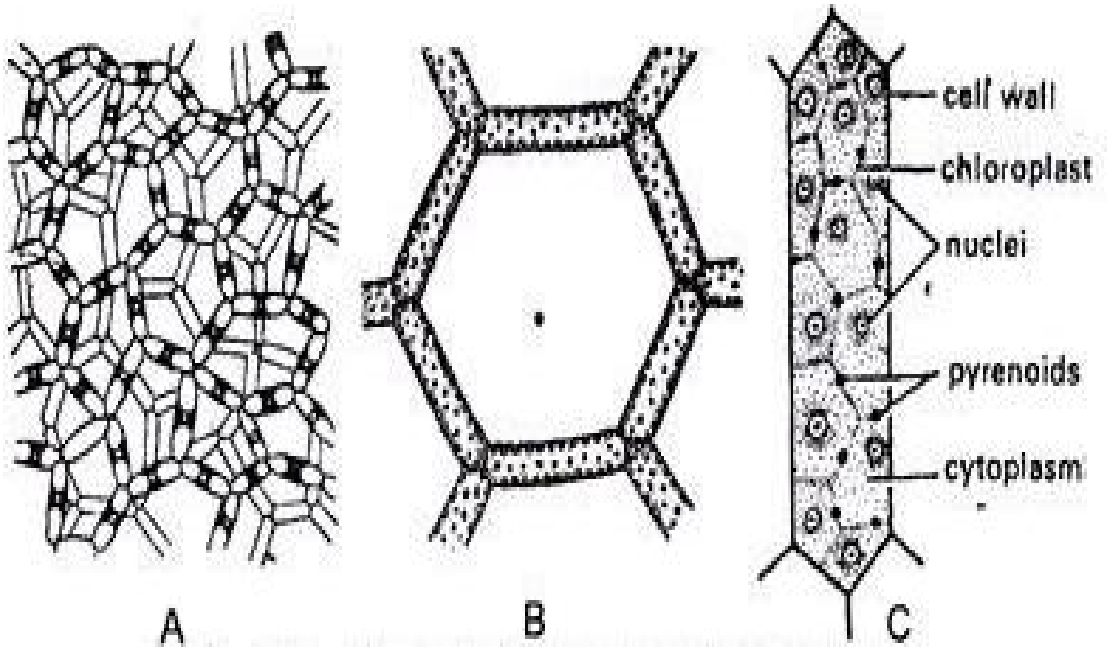
Occurrence: It occurs in calm and quite water. Some common Indian species are *Hydrodictyon indicum, H. reticulatum*.

Exercise 1

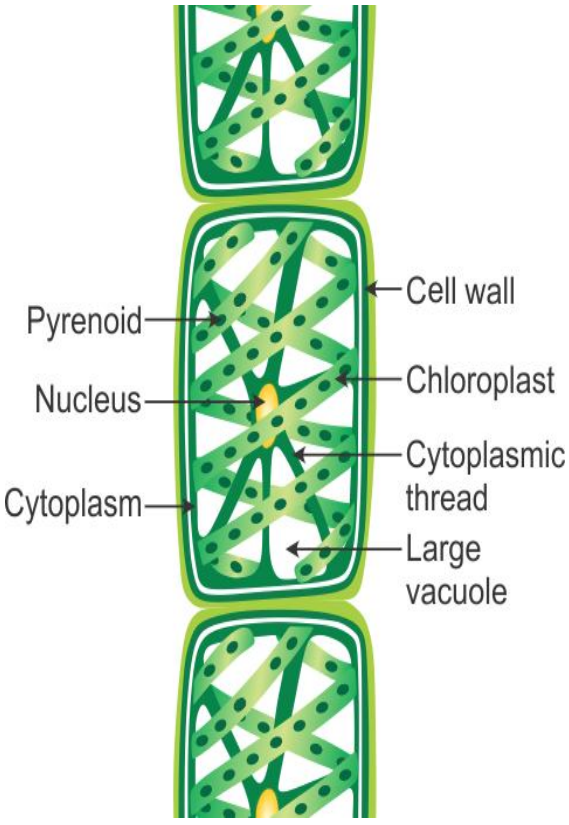
Study the thallus and a single cell

Work procedure:

Take a small part of net-like thallus, stain in safranin, wash with water and mount in glycerine. Take care to spread the net-work in a way that cells do not get overlapped.



Comments



1. The thallus is a multicellular colony forming a net-like structure.
2. Colony is a hollow and sac-like or saucer-like (saccate), cylindrical network, closed at both the ends.
3. The spaces of the reticulum are bound by five or six cells (this number varies between 3-10 cells) which form a pentagonal or hexagonal structure.
4. The cells are cylindrical. End walls are angular to facilitate the formation of a mesh.
5. A cell has a large central vacuole.
6. Cytoplasm lies towards the periphery.
7. Cells are multinucleate. The young cells are however, uninucleate.
8. Cell is called a coenocyte because of its multinucleate nature and presence of large central vacuole.
9. Young cells have zonate or entire chloroplast. Little mature cells possess reticulate chloroplast. However, during older stages chloroplast may assume discoid shape and diffuses throughout the cytoplasm.
10. Chloroplast contains large number of pyrenoids.

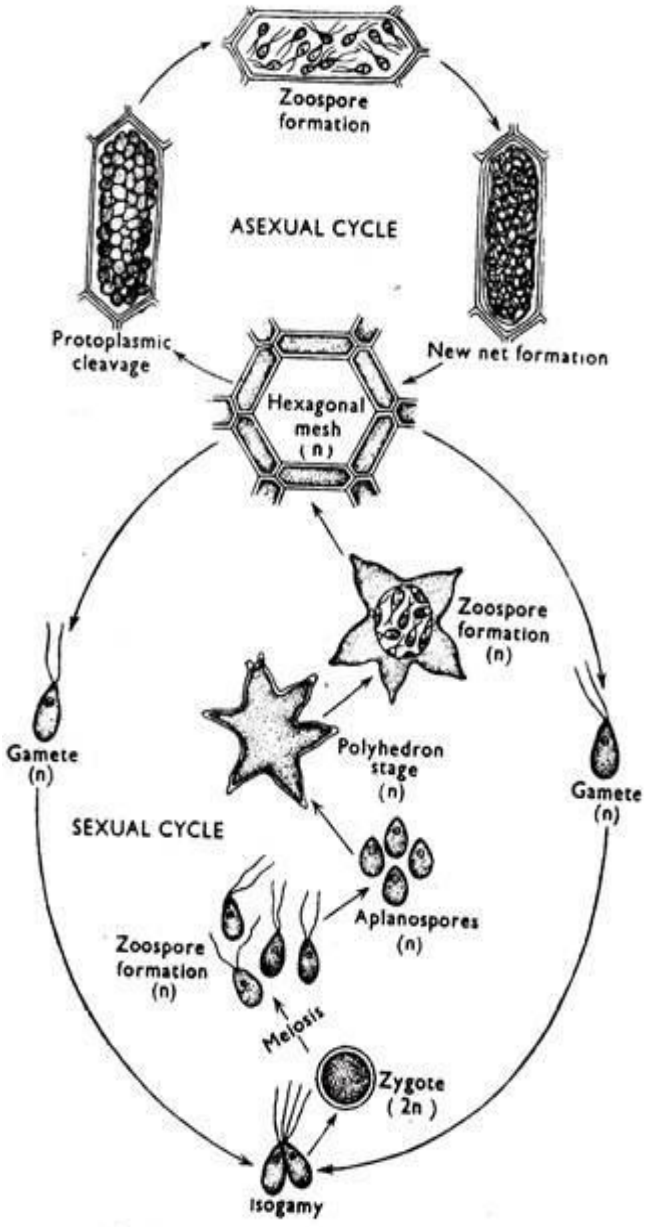


Fig. 40. Life cycle of *Hydrodictyon reticulatum.*

Life cycle of hydrodictyon

Oedogonium

(Unbranched filamentous alga, Oedos= swelling, gonas= fruit)

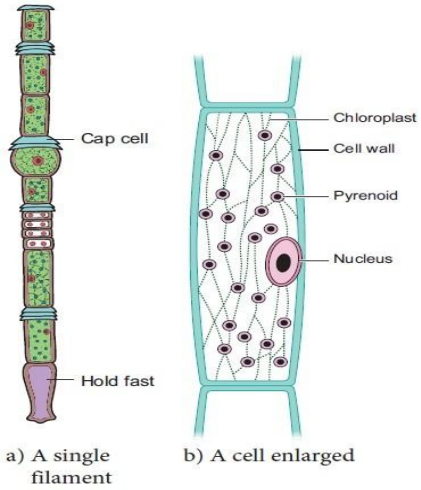
Classification

Sub-division	Algae
Class	Chlorophyceae
Order	Oedogoniales
Family	Oedogoniaceae
Genus	*Oedogonium*

Occurrence: Fresh water alga generally found as epiphyte on many hydrophytic plants. Generally, young filaments are attached and the mature filaments are free floating. Some common Indian species are *Oedogonium sociale, O. intermedia, O. vaucherii, O. perfectum*

Exercise 1

Study of thallus
Work procedure
Stain a few filaments in safranin, wash with water and mount in glycerine. Study the external features and structure of a cell.

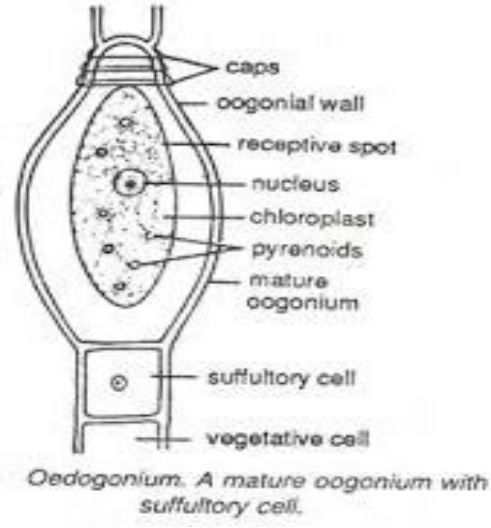


Comments

1. Thallus is multicellular, filamentous and unbranched.
2. A filament is differentiated into three types of cells according to their position: (i) basal, (ii) Intercalary (iii) apical
3. The basal cell of a filament functions as a holdfast. The lower part of the holdfast is either disc-like or finger-shaped. The upper part is mostly broad and rounded. The basal part of the cell generally lacks green pigment and, therefore, is non-green unlike other cells of, the filament.
4. A cell at the tip of the filament is known as apical cell. It is rounded at its free surface.
5. The cells present between basal and the apical cells are intercalary cells. These show typical cell structure.
6. The typical cell is cylindrical.
7. Cell wall is thick and three layered.
8. Inner to cell wall is a reticulate chloroplast that runs parallel to the long axis of the cell. Many pyrenoids are present in the chloroplast.
9. The cell is uninucleate. The nucleus is situated near the cell wall and is held by thin and delicate cytoplasmic strands.
10. Mature and old cells show 'cap cells' at their upper end, these are characteristic of the Oedogoniale.

Exercise 2

Study of Oogonium
Work procedure
Place a few filaments and observe a filament with oogonia. Stain such filaments with safranin, wash in water and mount in glycerine. Study the oogonia.



Comments

b. Oogonia are intercalary or terminal in position.
c. Oogonium may be solitary or occur in a row of 2-3 or even more.
d. Oogonium generally shows one or more cells at its upper end, indicating its development from a comparatively older cell.
e. It is mostly spherical or oval in shape and larg than a vegetative cell.
f. At the base of each oogonium lies a small flat daughter cell, known as supporting suffultory cell.
g. Oogonium encloses a single large ovum.
h. The wall of the oogonium has a small pore one side, "known as receptive pore.

i. Just opposite the receptive pore, protoplast of the oogonium has a hyaline area-receptive spot.

j. Uninucleate protoplast is rich in reserve food.

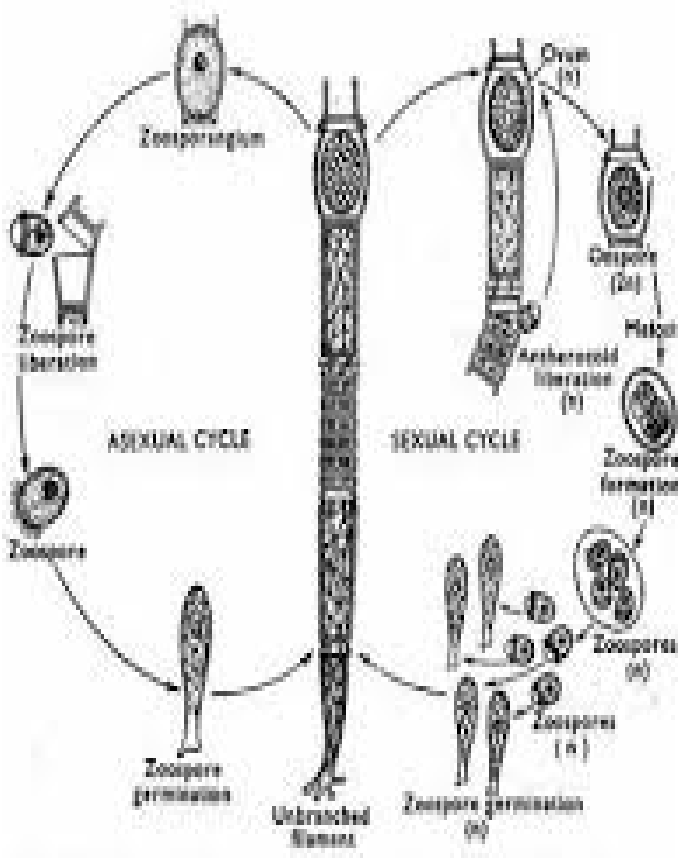
Exercise 3

Study of antheridium
Work procedure
Place a few filaments on a slide in a drop of water serve under the microscope for the presence of chain of antheridia. Isolate such filaments, stain in safranin, wash with water and mount in glycerine. Study the antheridia of **macrandrous** species.

Comments
1. Antheridia are mostly intercalaryin position.
2. Numerous antheridia form a long chain being arranged in a series.
3. An antheridium is a small and flat cell.
4. Each antheridium has two nuclei lying side by side, surrounded by dense cytoplasmic contents. Each of such protoplasmic groups later on metamorphoses into a multiflagellate antherozoid.

Exercise 4

Study of dwarf male or nannandrium
Work procedure
Isolate few filaments bearing dwarf males and stain in safranin, wash in water, mount in glycerine and study. Draw the diagram.

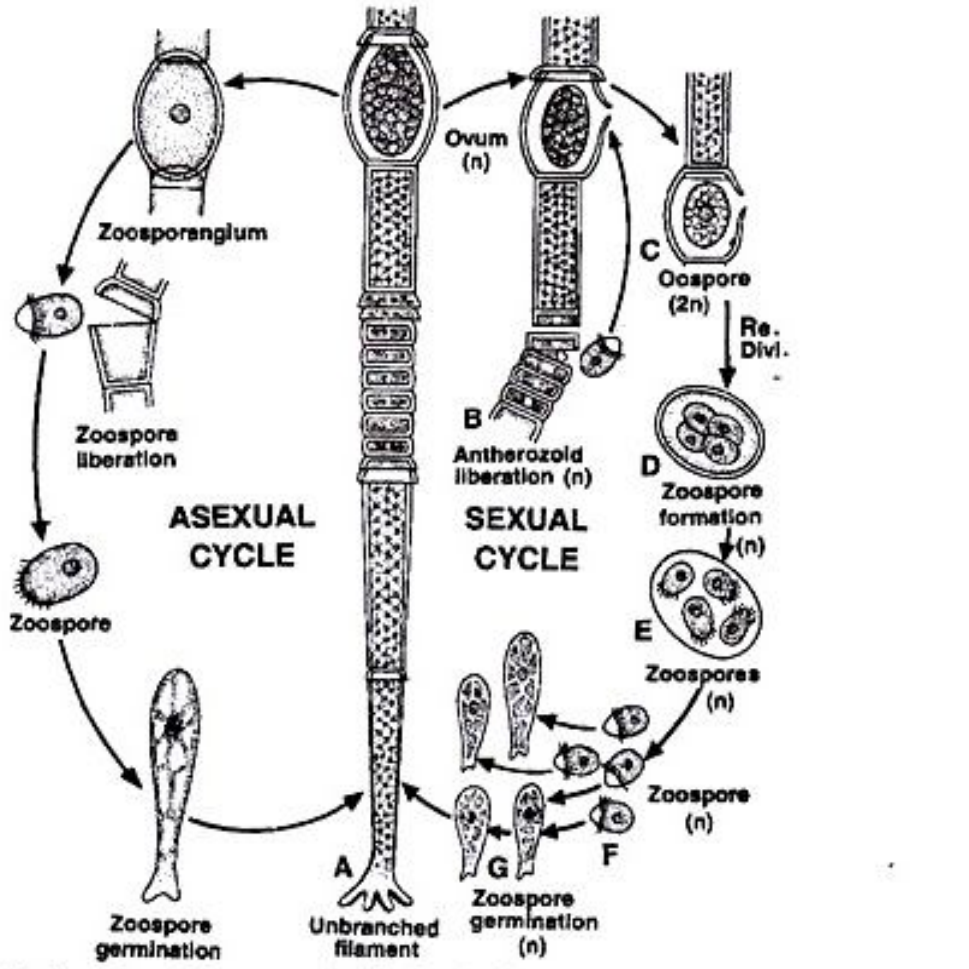


Comments
1. The dwarf male (or nannandrium) is characteristic of nannandrous species.
2. The dwarf male is produced by the germination of androspore.
3. Androspores are formed inside the androsporangia.
4. Androsporangia forma long chain of small and flat cells in intercalary position in the filament.
5. Each androsporangium develops a single multiflagellate androspore (in contrast per antheridium two antherozoids are produced).
6. Androspore germinates to produce a dwarf male or nannandrium which remains attached either to the wall of the oogonium or to the suffultory cell.
7. A dwarf male is made of a stalk cell and a terminal row of 2-3 cells.
8. Stalk cell is at the base by which the dwarf male is attached to the filament. It has a disc-like or finger-like structure at its base.
9. The terminal row has 2-3 small, flat and narrow antheridia.
10. Each anteridium has two multiflagellate antherozoids.

Exercise 5

Study of zygote
Work procedure
Study a slide showing zygotes.

Comments
1. Zygote is thick-walled post-fertilization structure. The wall is generally three layered. The layer outside the innermost may be smooth, ornamented or verrucose. Zygote develops red colour due to the accumulation of reserve food in the form of reddish oil drops.



Oedogonium. Diagrammatic life Cycle of monoecious macrandrous species.

Life cycle of Oedogonium

Chara (Stone-Wort)

Classification

Sub-division	Algae
Class	Chlorophyceae
Order	Charales
Family	Characeae
Genus	*Chara*

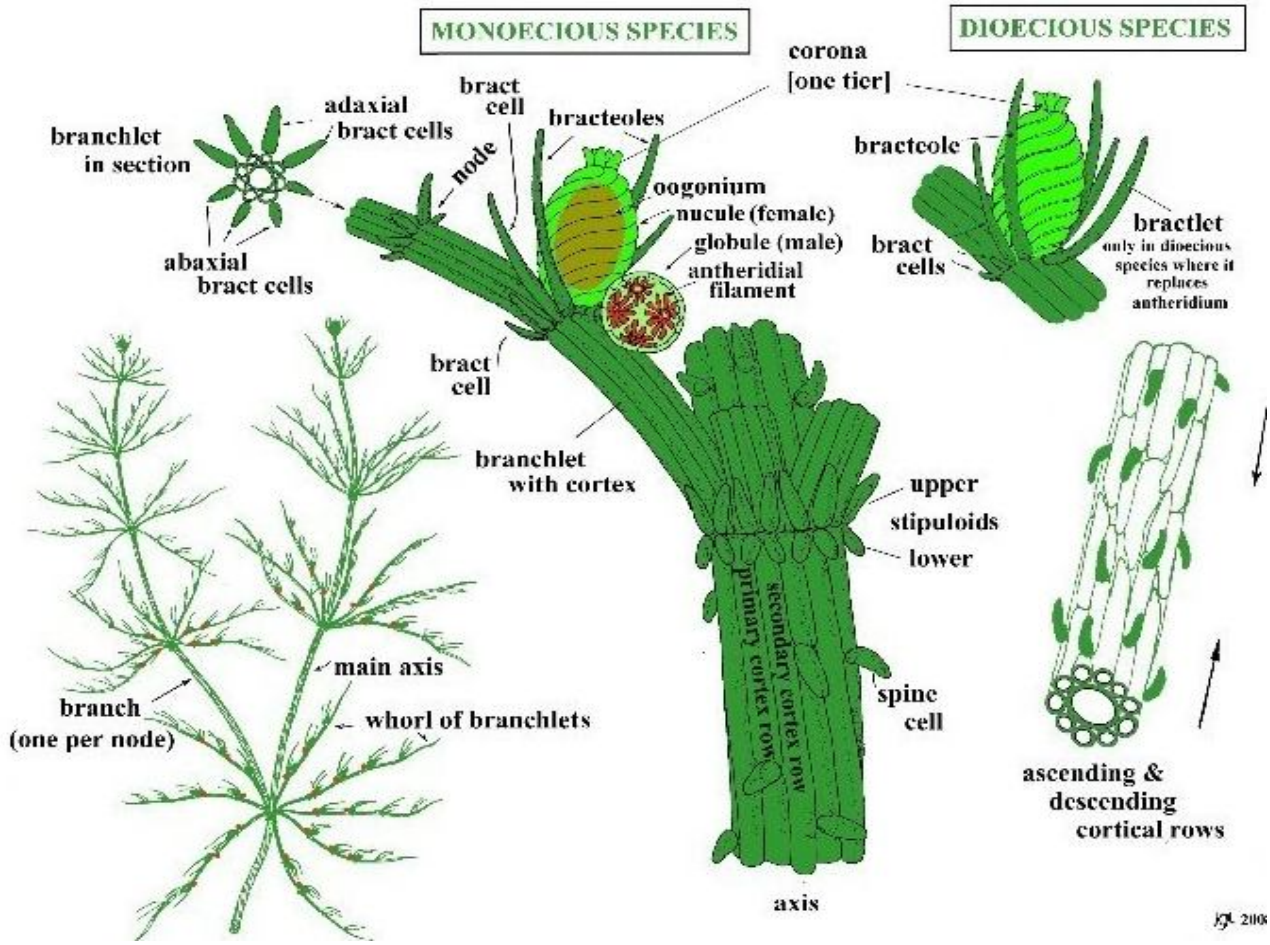
Occurrence: It is common in fresh water, ponds. Some species prefer salty water. Some common Indian species are *Chara coralline, C. zeylanica* and *C. wallichii.*

Exercise 1

Study the external features of thallus
Work procedure

Study a slide showing mounted part of the thallus or study a preserved specimen. Magnifying lens or dissecting microscope would be very useful.

Comments



1. Thallus is macrocopic, branched and multicellular. Calcium carbonate is deposited all over.
2. It remains attached to the substratum by multicellular rhizoids which bear an erect and branched main axis above.
3. Multicellular rhizoids are branched.
4. These are borne by the lower nodes of the main axis.
5. Rhizoids possess oblique septa. The rhizoids are not differentiated into nodes and intemodes.
6. The cytoplasm of a rhizoidal cell has a nucleus situated towards the upper side of the cell.
7. At the septum of a rhizoidal cell, the ends are protracted in opposite directions to form knotted part.
8. At this place, signifying a node, a plate of four cells or even more is formed which gives rise to rhizoidal branches. This part is known as rhizoidal plate.
9. Main axis is composed of long intemodes alternating with small nodes.
10. Long internode is composed of a single cell enveloped by many corticating threads.
11. A node is a group of regularly arranged cells.

12. It bears two types of branches-(i) lateral branches of limited growth (short laterals) and (ii) lateral branches of unlimited growth (long laterals).
13. Laterals of limited growth are borne in whorls around the nodes of the main axis.
14. Each short lateral is divided into nodes and intemodes.
15. The internodes of short laterals are small compared to those of the main axis.
16. Short laterals borne by the nodes of the main axis are also termed as primary laterals of limited growth.
17. From the nodes of the short laterals, second short laterals are produced which are usually small, unicellular and are variously termed as stipules or leaves.
18. Laterals of unlimited growth are borne by the nodes of the main axis only. These are situated in the axils of short laterals.
19. Long laterals possess the same characteistic as those of the main axis.
20. Long laterals are differentiated into long, corticated and unicellular intemodes and small and multicellular nodes.
21. Nodes of the long laterals bear short laterals which in their turn give out stipules or leaves at their nodes.

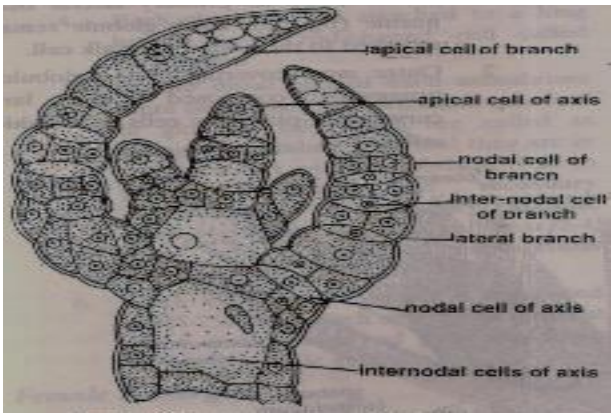
Exercise 2

Study of a cell
Work procedure
Study the slide showing T.s. of internodal cell.

Comments
1. In the centre is a large central, axial or internodal cell.
2. It is surrounded by corticating threads on all sides.
3. Internodal cell shows a typical cell structure.
4. Centre of the cell has a big vacuole surrounded by cytoplasm.
5. In the cytoplasm lies a single nucleus held by thin and delicate cytoplasmic strands.
6. Many discoid chloroplasts without pyrenoids are scattered in the peripheral cytoplasm.
7. The cell has an outermost, thick and firm cell wall.

Exercise 3

Study L.s. of apex
Work procedure
Study the slide showing L.s. of apex.



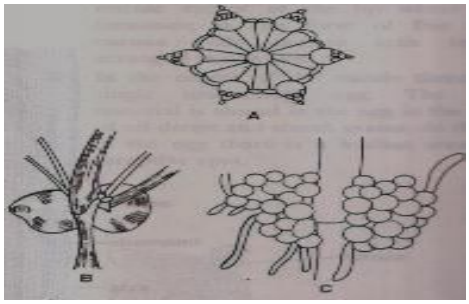
Comments
1. The cell situated at the top is an apical cell. It contributes to the development of the main axis and lateral axes.
2. It cuts off a longitudinal series of cells below.
3. Upper biconcave cell of a series is a nodal cell and lower biconvex one is an internodal cell.
4. Biconcave nodal cell in the lower part divide to produce a mass of peripheral cell which surrounds centrally located nodal cell.
5. Peripheral cell act as initials of the laterals of limited growth.

6. From peripherial cells of node, laterals are produced which shows similar arrangements of nodal and intermodal cells alternating with one another.
7. Biconvex internodal cell does not divide but in the lower part simply elongates many times.

Exercise 4

Study of Bulbils and vegetative reproductive structure
Work procedure:
Study a slide of a thallus bearing bulbils.

Comments

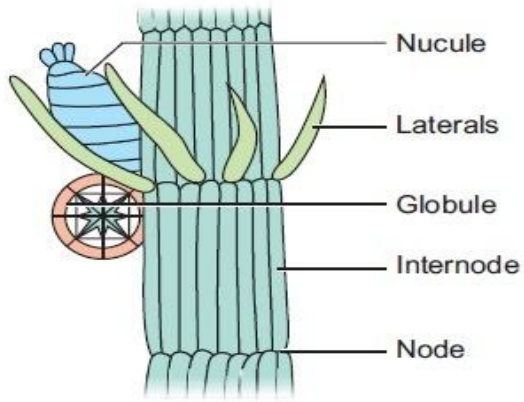


1. Bulbils are present on the knotted part of the rhizoidsor the basal nodes of the main axis.
2. These remain buried under the soil.
3. These are oval, tuber like out growths.
4. Bulbils are rich in starch and hance also called amylum stars.
5. These are the organs of vegetative propagation.

Exercise 5

Study of Sex organs
Work procedure:
Study a slide or a part of thallus bearing sex organs.

Comments



1. Most of the species are homothallic (monoecious) while a few are heterothallic (dioecious).
2. The male reproductive organ is called globule while the female reproductive organ a nucule.
3. Both the sex organs are borne at one point on the nodes of short lateralswhich bear stipules.
4. The characteristic feature genus *Chara* is the position of nucule at the node above the globule.

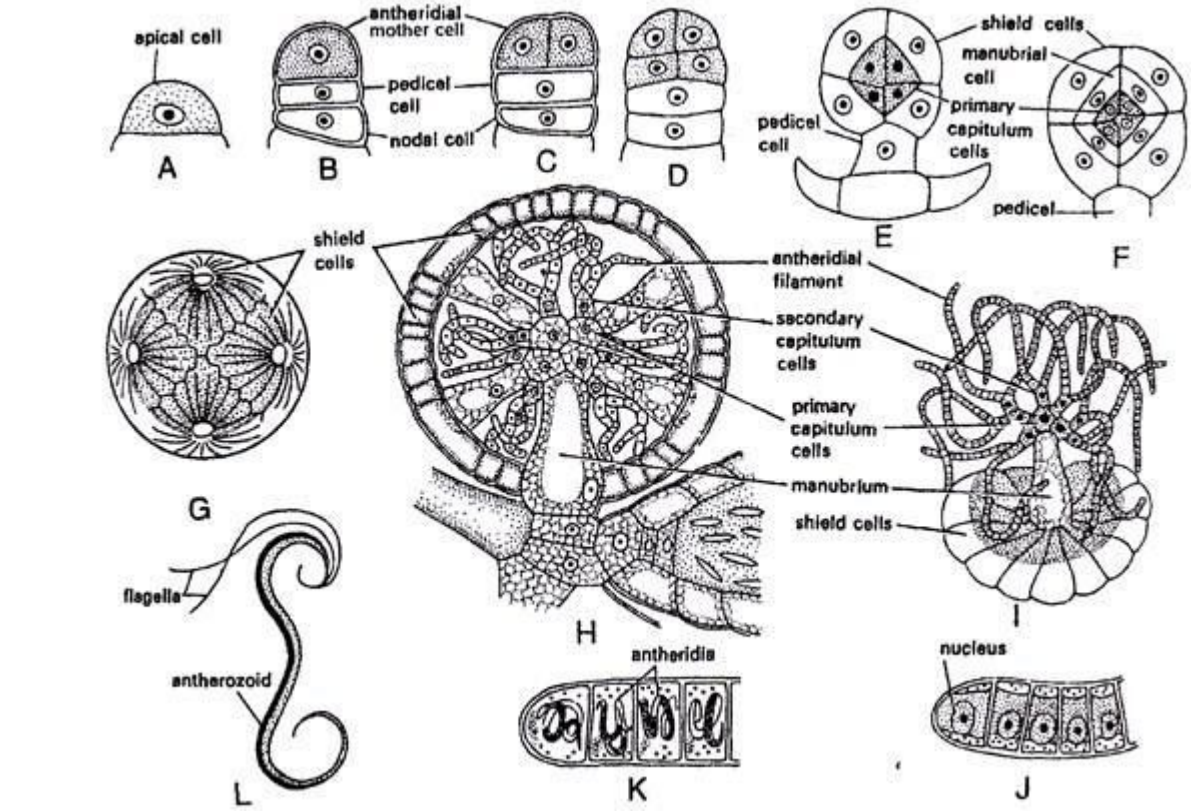
Exercise 6

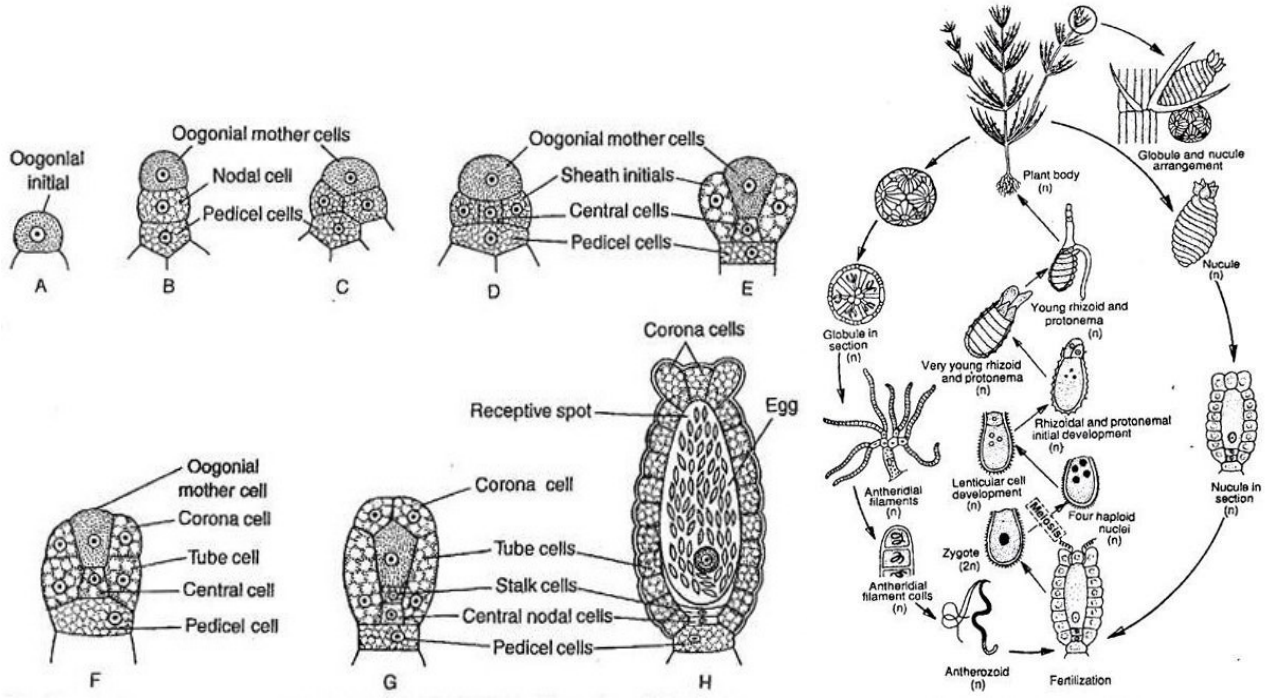
Study of Male sex organs
Work procedure
Study the external feature of globule, than press it a little to observe internal structure. Also pierce through it by a needle to separate the shield cells and associated structure. Slides of L.s. of globule would also show almost the same structure.

Comments

1. A globule is a small, spherical and conspicuously red or yellow structure attached to the node by a long stalk cell.
2. Outermost wall of the globule is ornamented, composed of eight large, curved and plate like cells, shield cells.
3. Each shield cell is attached to a long handle or rod shaped cell-manubrium.
4. At the tip of each manubrium are two groups of six cells each. The group directly in contact with the manubrium is primary capitulum while the next is secondary capitulum.
5. Each secondary capitulum bears 2-4, long and unbranched antheridial filaments.

6. Each antheridial filament is composed of 100-230 small cells.
7. Each of these, cell is an antheridium and produce a single biflagellate male gamets.





Exercise 7

Study of Female sex organs
Work procedure:

Study the whole mounts of the nucule and a zygote.

Comments

1. Nucule is oval in shape and is situated above the globule at the node.
2. It is enveloped by spirally coiled (coiling clockwise) cells- tube cells.
3. At the apex of the nucule is a corona of five small cells arranged in one tier and attached at one point.
4. Oosphere is single celled where nucleus lie surrounded by the cytoplasm.
5. It is rich in food reserves which are in the form of starch and oil.
6. After fertilization the nucule gets modified into a zygote or oospore.
7. The coronal cells at the top of the oospore appear separated.
8. The oospore wall is thick and ornamented. It has a deposition of calcium.

Vaucheria

(Siphonacious or Coenocytic alga, Named after J.P. Vaucher)

Classification

Sub-division	Algae
Class	Xanthophyceae
Order	Heterosiphonaies
Family	Vaucheriaceae
Genus	*Vaucheria*

Occurrence: It occurs in fresh water, on soil (terrestrial) and also in sea water. During winter it occurs as green matty structure on damp soil. Some common Indian species are *Vaucheria geminate, V. sessilis, V. uncinata, V. terrestris* and *V. walzi.*

Exercise 1

Study of thallus

Work procedure

Study the whole mount showing external features stain the thallus in safranin and mount in glycerin, Observe the characters.

Comments

1. Thallus is unicellular, multinucleate, flamentous and branched (coenocytic).

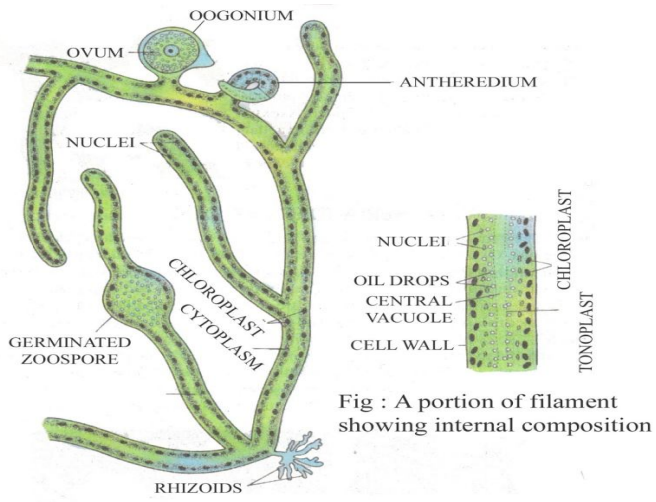


Fig : A portion of filament showing internal composition

2. Filaments are profusely branched. The branching is lateral but appears dichotomous
3. Filaments are without any septation (asepta filaments).
4. If terrestrial in habitat, a few colourless rhizoidal branches are given out which penetrate in the soils.
5. Cell wall is two layered. Outer layer composed of pectose while inner is that cellulose.
6. In the centre lies a big vacuole, continuous throughout the length of the filament.
7. The cytoplasm lies between vacuole and the

cell wall.

8. Many small nuclei are scattered in the cytoplasm near the vacuole.
9. Small chromatophores are also scattered in the cytoplasm. They are circular or elliptical in shape. Pyrenoids are absent. The reserve food material is in the form of small oil droplets.

Exercise 2

Study of Gongrosira stage
Work procedure
Study the slide showing Gongrosira stage

Comments
1. This stage of asexual reproduction develops under extreme conditions of desiccation or low temperature.
2. It is called Gongrosira because it looks similar to another algal member-Gongrosira.
3. In this stage, filament is divided into many, short and thick-walled parts.
4. Each of these parts is called akinete or cyst.
5. Akinetes are thick walled and rich in oil contents.
6. Akinetes occur in long chains, alternating with a part of filament.

Exercise 3

Study of antheridia
Work procedure
Study a slide showing anteridia.

Comments
1. Filaments are mostly monoecious but a few species are dioecious.
2. Sexual reproduction is oogamous.
3. Male reproductive bodies are antheridia and female reproductive bodies are oogonia.
4. The antheridia and oogonia are borne side by side on the same filament. Sex organs are generally sessile but a stalk-like structure is present in a few species.
5. Antheridia are terminal. These are strongly curved, hook-like and cylindrical.
6. Antheridia are cut off from the main filament by a transverse septum at its base.
7. Protoplast accumulates towards the centre. It produces many biflagellate antherozoids.
8. Antherozoids are liberated through a small pore at the tip of antheridium.

Exercise 4

Study of Oogonia
Work procedure
Study a slide showing oogonia.

Comments
1. More than one oogonium is present at the tip of the stalks which once again branch at their tips.
2. Oogonia are oval or spherical and terminate into a short beak.
3. The entire protoplast forms a single oosphere.
4. In younger stages, oosphere is multinucleate but at maturity it is always uninucleate.
5. Near the beak, in the apical part, the protoplasm leaves a small colourless area, known as receptive spot.
6. Protoplast is rich in food reserve which is in the form of oil droplets.

Exercise 5

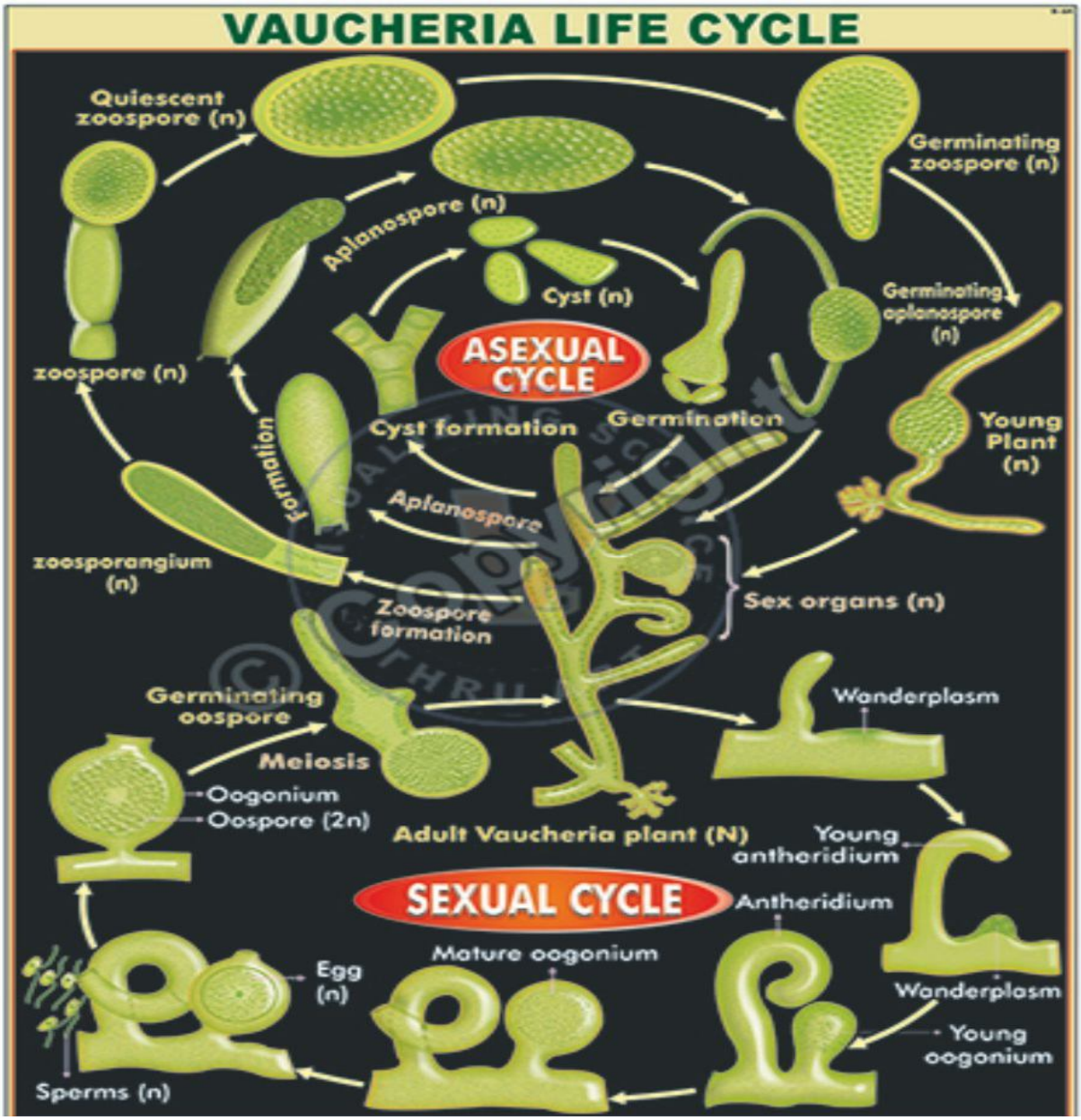
Study of zygote

Work procedure

Study a slide showing zygote

Comments

1. Zygote is the result of fertilization
2. Zygote is present inside the oogonium.
3. It is a thick walled structure being made of 3-7 layers.
4. The protoplast of a zygote is very dense
5. Numerous oil droplets are scattered throughout the protoplasm.
6. It is considered to be a diploid structure, as such there is said to be possibility of zygotic meiosis.
7. It is liberated through the oogonial beak.



Ectocarpus

(Monosiphonous brown alga, Ecto=external, Carpus=fruit)

Classification

Sub-division	Algae
Class	Phaeophyceae
Order	Ectocarpales
Family	Ectocarpaceae
Genus	*Ectocarpus*

Occurrence: Exclusively marine algaa, common in littoral and sub littoral zones of colder sea. Many species are lithophytic while some are epiphytic as members of Fucales, Laminariales etc. It is common in India in the sea shores of Mumbai, Dwarika and Porbander. Some common Indian species are *Ectocarpus confervoides, E. crinitus, E. Maculans, E. secundus, E. terminalis*

Exercise 1

Study of external features of thallus

Work procedure:

Mount a few filaments in glycerine after staining in safranin. Study the structure of thallus and also single cell.

Comments

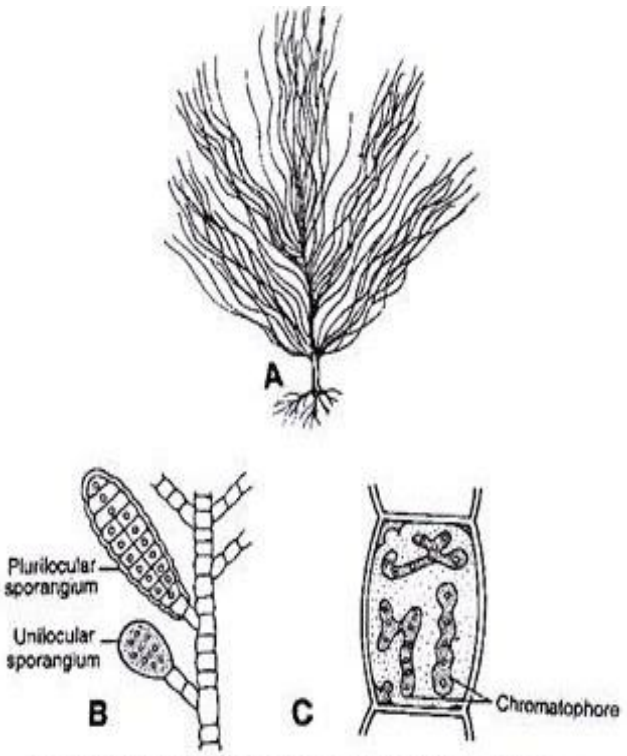


Fig. 1 (A–C). *Ectocarpus* thallus, (A) External features, (B) Part of thallus, (C) A cell

1. Thallus is multicellular, filamentous and branched.
2. Filaments are heterotrichous and differentiated into (i) prostrate portion and (ii) an erect portion.
3. In some species prostrate portion is irregularly and profusely branched or altogether absent. If present it remains attached to the substratum.
4. Erect portion is a crowded tuft of branch. Main axis is broad from which lateral branches arise just beneath the septum and taper into a point. Ultimate branches give an appearance of a hair.
5. The branches and the main axis are uniseriate.
6. The erect branches have intercalary meristem just below the terminal hair. It results in trichothallic growth.
7. The cells are squarish to cylindrical and uninucleate.
8. The cell wall is double layered. Outer is gelatinous and inner is firm and cellulosic.
9. There may be one or many chromatophores varying from irregular to band-like to discoid. Pyrenoids are absent.
10. Reserve food products occur as shining fucosan granules.
11. The cell is filled with cytoplasm in which lies a single nucleus.

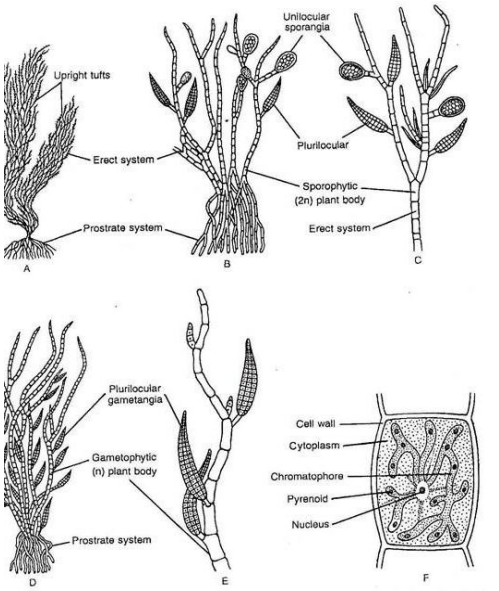
Exercise 2

Study of unilocular sporangium

Work procedure

Search for fialments with unilocular sporangia, stain in safranin, mount in glycerine and study.

Comments



Ectocarpus sp. : A. Habit, B. Sporophytic (2n) plant body with both erect and prostrate system bearing unilocular and plurilocular sporangia, C. Portion of erect filament of sporophytic (2n) plant, D. Gametophytic (n) plant with both erect and prostrate system bearing plurilocular gametangia, E. Portion of erect filament of gametophytic (n) plant and F. Cell structure

1. Unilocular sporangium is a structure of asexual reproduction, always present on diploid plants.
2. The sessile or stalked sporangium is situated terminally on lateral branches.
3. The shape varies from globose to ellipsoidal.
4. It is single celled and uninucleate when young but becomes multinucleate later.
5. Many biflagellate zoospores are produced when unilocular sporangium matures.

Exercise 3

Study of plurilocular sporangium

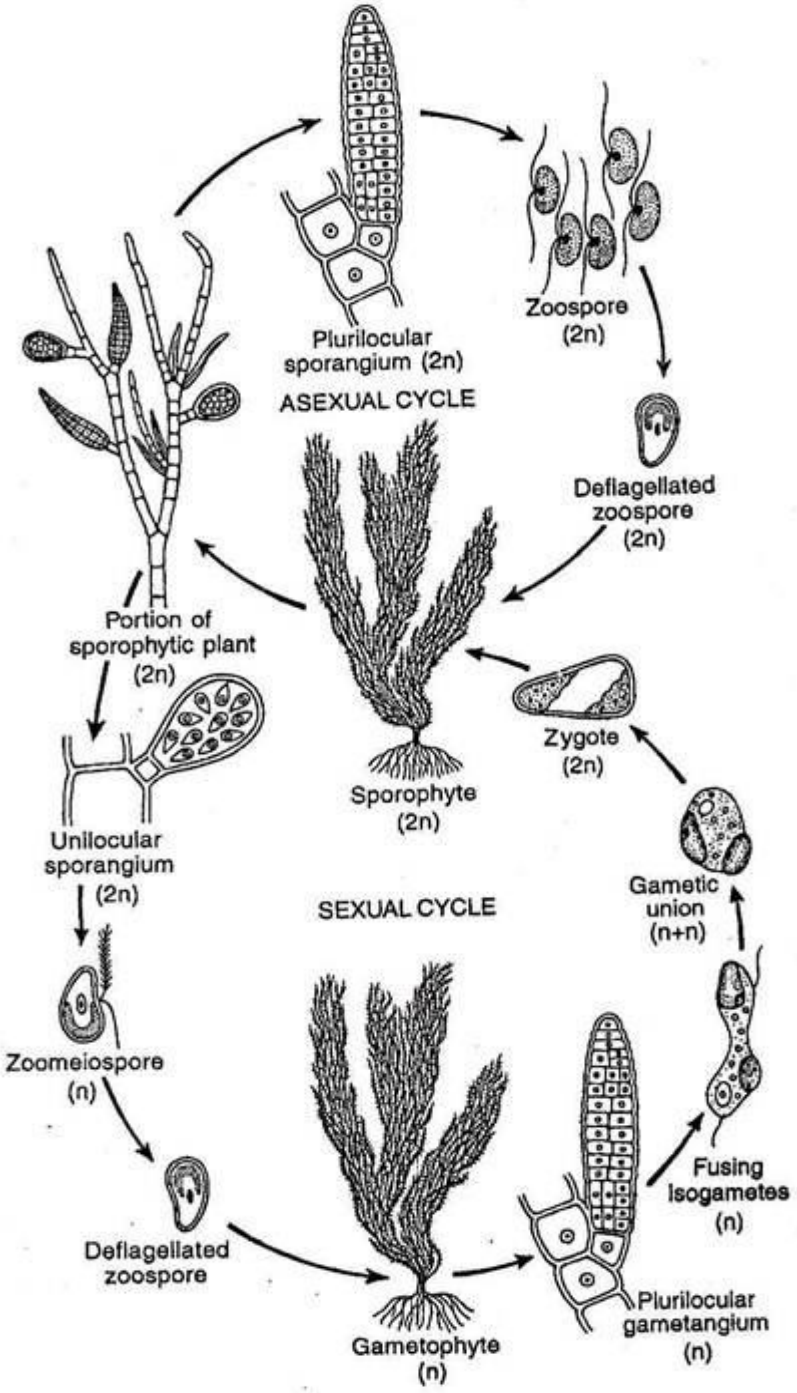
Work procedure

Search for filaments with plurilocular sporangia, stain in safranin, mount in glycerine and study.

Comments

1. These occur on both haploid and diploid plants.
2. The structure when borne by haploid plant serves as a gametangium whereas on diploid plant it functions as a sporangium.
3. Sporangia situated laterally may be sessile or stalked. These may be ovate to siliquose.
4. Plurilocular sporangium is divided into large number of cells.
5. A mature sporangium produces biflagellate swarmers, one each from every cell.
6. If plurilocular sporangium is borne on a haploid plant, the swarmers behave as gametes and if borne on diploid plant, these act as haploid zoospores.

(It is, therefore, suggested that instead of plurilocular sporangium it be called as neutral sporangium when zoospores are produced and spores as neutral spores and if it produces gametes on a haploid plant, it should be called gametangium and swarmers as gametes). The life cycle exhibits typical alternation of generations (isomorphic).



Life cycle of *Ectocarpus*

Oscillatoria

(Blue-green filamentous alga, Oscillare=to swing)

Classification

Sub-division	Algae
Class	Myxophyceae
Order	Nostocales
Family	Oscillatoriaceae
Genus	*Oscillatoria*

Occurrence: Common fresh water blue green alga, occurs on damp soil, road side ditches, fresh water channels, bottom of sewer and also in stagnant water. Some common Indian species are *Oscillatoria princeps, O. anne, O. subtilissima, O. martini, O. nigra, O. mougeotii.*

Exercise 1

To study the structure of a filament and single cell
Workd procedure:
Take a few filaments, stain in safranin, mount in glycerine and study the structure.

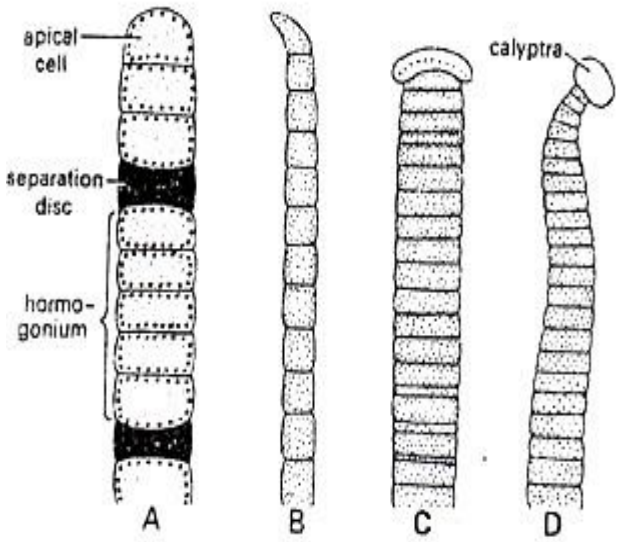


Fig. 1 (A–D) : *Oscillatoria* : Trichomes.

Comments

1. Filaments occur either singly or interwoven to form a flat or spongy, free swimming mat.
2. Filament consists of an inconspicuous and barely recognizable sheath enclosing unbranched trichome.
3. Trichome consists of a single row of cells.
4. The apical cell of the trichome may have calyptras-thick wall on its outer free face.
5. The cells show typical myxophycean cell structure. It has no definite nucleus, no chloroplasts or no membrane bound organdies (prokaryotic cell).
6. The cell shows many shining cyanophycean granules.
7. Floating species show numerous gas vacuoles.

Exercise 2

Study of reproductive structures
Work procedure
Place a few filaments in safranin, wash in water, mount in glycerine and study the reproductive structures-hormogones.

Comments

1. The only method of reproduction is hormogone formation.
2. The hormogones (small pieces of trichome with one to many uniform cells) are formed as a result of the death of intecalary cell or by the formation of special biconcave separation discs.
3. Hormogones are liberated by the disintegration of discs.
4. Each hormogone develops into a new filament.

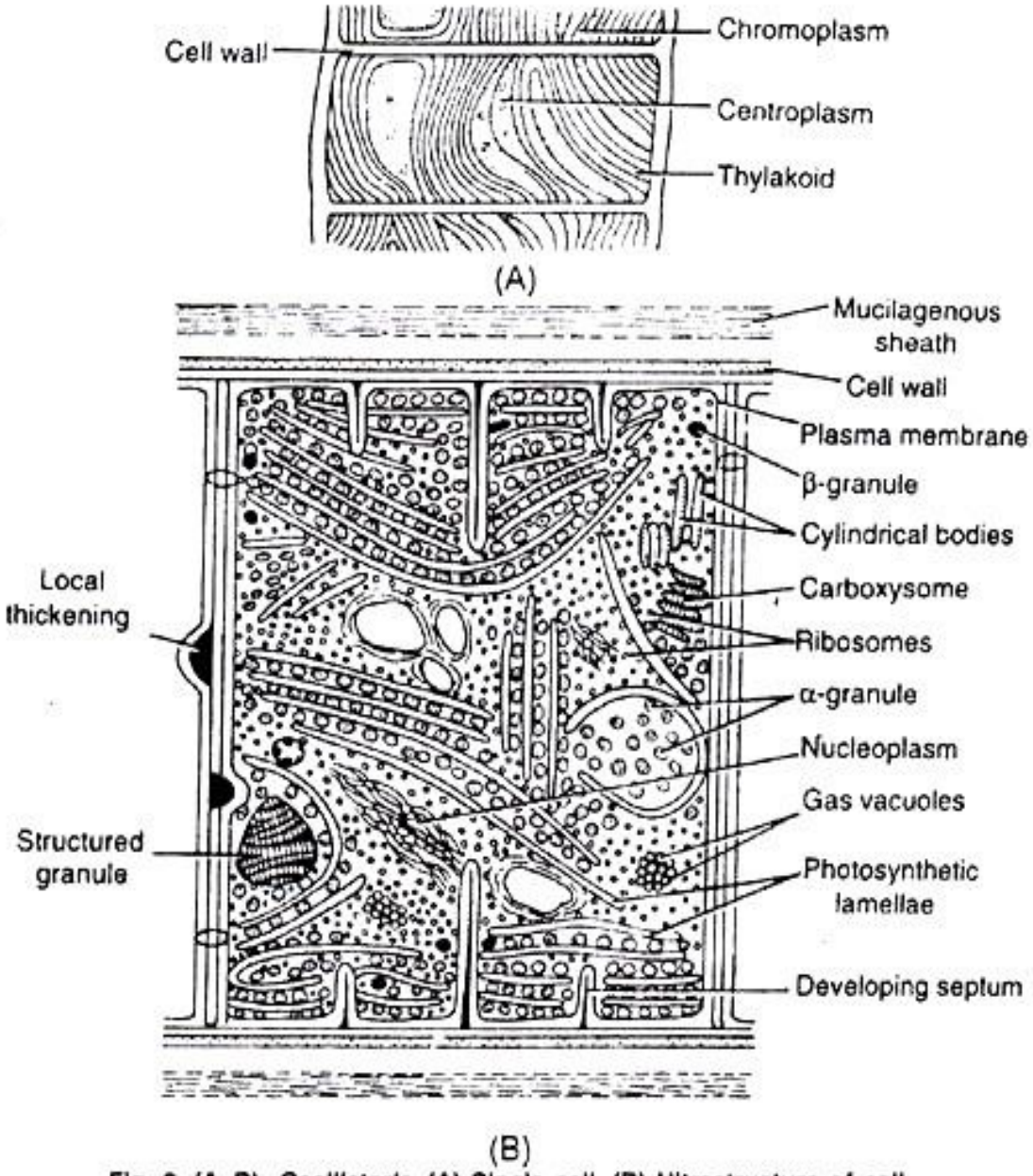


Fig. 2. (A–B). ***Oscillatoria.*** **(A) Single cell, (B) Ultrastructure of cell.**

Nostoc **(Blue-green alga)**

Classification

Sub-division	Algae
Class	Myxophyceae
Order	Nostocales
Family	Nostocaceae
Genus	*Nostoc*

Occurrence: It occurs commonly in fresh water pools, ponds, ditches etc., in the form of balls. Some species occurs epiphytically while other endophytically (as in coralloid root of Cycas and thalli of Anthoceros). It is one of the common associations of lichens. Some common Indian species are *Nostoc commune, N. rivulare, N. calcicola, N. hatei, N. sphaericum.*

Exercise 1

Study of external features of thallus

Work procedure

Place a part of colony on the slide, press it little by another slide so that it spreads flat, stain in safranin and mount in glycerine.

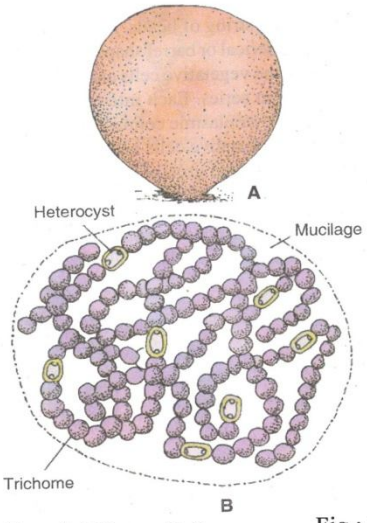


Fig : (A) Nostoc Ball, (B) Portion of filament as seen under microscope

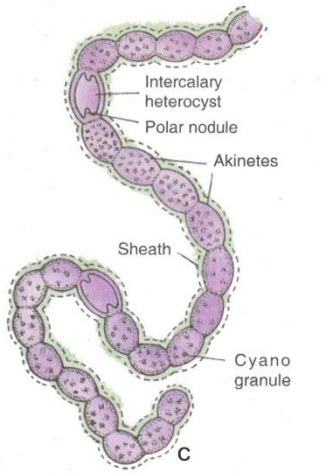


Fig : (C) A single filament with a trichome.

Comments

1. Thallus is colonial. Young colonies are microscopic, spherical and solid.
2. Mature colonies become irregular and hollow.
3. Colonial envelope encloses many filaments. These are much twisted, curved and entangled with each other.
4. A filament has diffluent gelatinous sheath.
5. The trichomes are unbranched. Each trichome is made of cells of uniform size and shape except those called heterocyst which occur throughout.
6. Structure of a cell is typically cyanophycean. It has a centrally located centroplasm, nucleus being altogether absent. Peripheral cytoplasm shows diffused pigments. A few shining cyanophycean granules are also present in this region.
7. Heterocysts are intercalary. These are double walled, pale yellow coloured with two shining polar granules, one each near the neighbouring cell on either side. Heterocysts are much of the same size of slightly bigger than the vegetative cells.

Exercise 2

Study the akinetes

Work procedure

Press a piece of mature colony to spread the filaments. Stain in safranin, mount in glycerine and study.

Comments

1. Akinetes are developed only in a mature colony. These occur in large number, in series between two heterocysts. Usually all vegetative cells between two successive heterocysts develop into akinetes.
2. Akinetes are thick walled, sometimes ornamented, rich in food reserves and cyanophycean granules.
3. Akinetes are liberated due to decay of colonial sheath. These germinate to form a new thallus.

Bryophyte

Bryophyta the term is derived from two Greek words; **Bryon**=moss; **phyton**=plants; these are recognized as the simplest and primitive plants and they were first include in emryophyta they are commonly known as **plant amphibians.** This group includes mosses, liverworts and hornworts - both living as well as fossils, and numbering about 24,000 species distributed in approximately 900 genera. They are fundamentally terrestrial plants, growing in tufts and usually occupying moist shady places such as grounds, walls, rocks and their crevices. On the other hand some found in aquatic environment and some others show epiphytic habitat. The largest bryophyte is probably an Australian genus **Dowsonia** which attains a length of about 40-70 cms and smallest (microscopic) bryophyte is **Zoopsis**. This group stands between Algae and Pteridophyta and shows a sharply defined heteromorphic alternation of generations. A noteworthy point about Bryophytes is that the

gametophyte bearing the sex organs is an independent plant body and long lived, whereas, the sporophyte, bearing the spores is dependent on the gametophyte for nutrition and is short lived. The true roots are absent and their function has been taken up by rhizoids which are unicellular and unbranched (liverworts) or multicellular and branched (mosses). Reproduction is oogamous and gametes are produced in multicellular sex organs provided with an outer sterile jacket. The antheridium (male sex organ) consists of stalk and a club-shaped or spherical body which latter produces the antherozoids (male gametes). The archegonium (female sex organ) is also stalked and a flask-shaped organ, with a swollen basal venter and slender elongated neck. The former is occupied by a large basal egg cell and a small upper venter canal cell, whereas the latter contains many neck canal cells. Water is essential for fertilization and the embryo is retained within the archegonium whose basal portion enlarges to form calyptra as protective covering. The simple sporophyte is not differentiated into stem, leaves and roots but in bryophytes it usually consists of foot, seta and spore producing terminal capsule. The haploid spores are produced by a reduction or meiosis division of spore mother cells which represent the last stage of sporophytic generation. The spores germinate to form a filamentous germ tube which later forms the thallus as in liverworts or they germinate into filamentous or thallose protonema from which many erect gametophores arise as in mosses. Bryophytes have been variously classified many times. No single classification has yet been considered satisfactory, though the classification proposed by Campbell (1936) is generally useful.

Outline Classification of bryophyte

Division – Bryophyta – 3 classes

Class1. Hepaticopsida-(4 orders)	Class 2. Anthocerotopsida-(1 order)	Class 3. Bryopsida
Marchentiales *Riccia, Marchantia* **Jungermanniales** **Sphaerocarpales** **Calobryales**	**Anthocerotales** *Anthoceros*	**Sphagnales** *Sphagnum* **Funariales** **Polytrichales***Polytrichum and Pogonatutn*

The Distinguishing Characters of Taxa

DIVISION BRYOPHYTA

(1) Gametophytic plant body, amphibian nature and presence of single layer around antheridium and true roots absent.

(2) Presence of antheridia and archegonia.

(3) True vascular tissues absent.

Class 1. HEPATICOPSIDA

(1) Thalloid plant body, pyrenoids absent, scales and rhizoids present and rhizoids are without septa.

(2) Chloroplasts without pyrenoids.

(3) Capsule lacks columella.

Following orders are include in this class

Order 1. Marchantiales

Family 1. Ricciaceae - *Riccia*

Family 2. Marchantiaceae - *Marchantia*

Order 1. Marchantiales

(1) Presence of scales.

(2) Two types of rhizoids (smooth walled and tuberculate) are present.

Family 1. Ricciaceae

(1) Simple air pores are present which open internally in photosynthetic region.

(2) Sex organs in mid-dorsal groove

(3) Sporophyte differentiated into capsule while foot and seta are absent (*Riccia*).

Family 2. Marchantiaceae:
1. Barrel shaped air pores are presnt.

2. Air chambers have photosynthetic filaments

3. Sporophyte is differentiated into foot, seta and capsule.

Order 2. Jungermanniales
1. Gametophytic plant body is thalloid or foliose.

2. Only smooth walled rhizoids are present and tuberculate rhizoids and scales are absent.

3. The thallus is not differentiated into photosynthetic and storage region.

CLASS 2. Anthocerotopsida (Hornworts).The members of this class are commonly known as **Horn worts**. This group is represented by 6 genera and about 300 species which are widely distributed in both tropical and temperate regions. Only smooth walled rhizoids are found and tuberculate rhizoids and scales are absent.

Order 1. **Anthocerotales**. Family Anthocerotaceae (*Anthoceros and Notothylas*)

1. Each cell contains at least single chloroplast and pyrenoids.

2.Thallus is not differentiated into photosynthetic and storage region.

3. In some members blue green algae is found in lower mucilaginous cells.

CLASS 3. Bryopsida (Musci). It is one of the largest and most advance group of bryophytes, it includes 660 genera and 14,500 species. The gametophyte has two phases Protenema stage and leafy or gametophore stage, the axis is differentiated into a central conducting tissue and peripheral cortex.

Order 1. Sphagnales (*Sphagnum*)

Order 2. Funariales

Order 3. Polytrichales (*Polytrichum and Pogonatutn*)

***Riccia* (liver wort)**

Classification

Division	Bryophyta
Class	Hepaticopsida
Order	Marchantiales
Family	Ricciaceae
Genus	*Riccia*

Exercise 1

Study of external features of Gametophyte (n)
Word procedure:
Study the external features of the gametophyte, from dorsal and ventral surfaces. Observe two types of rhizoids and violet coloured scales

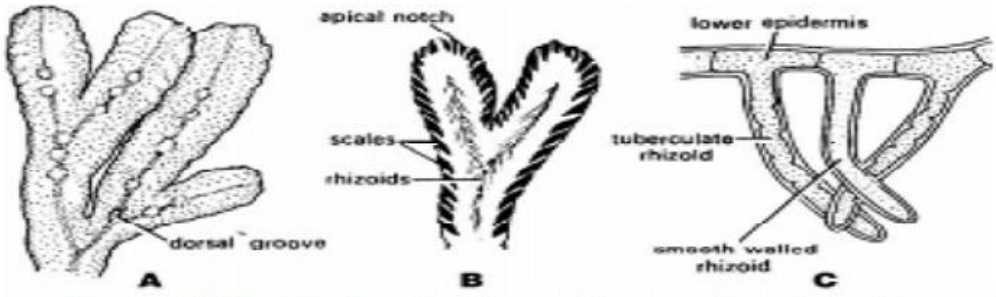


Fig. Riccia thallus (a) dorsal surface (b) ventral surface (c) smooth walled and tuberculate rhizoids

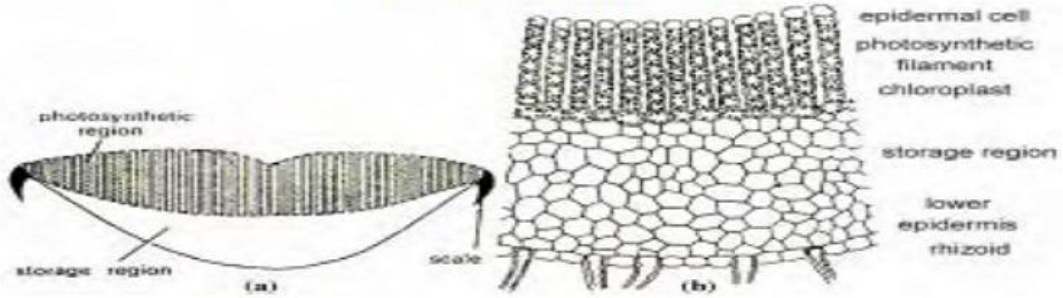


Fig. Riccia thallus internal structure (a) transverse section of thallus (diagrammatic), (b) a part of T.S. of thallus showing cellular details

Comments

1. The plant body is thalloid, dorsiventral, prostrate and ribbon-like.
2. A rosette is formed 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.
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.

Exercise 2

Study of anatomy of thallus

Work procedure

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

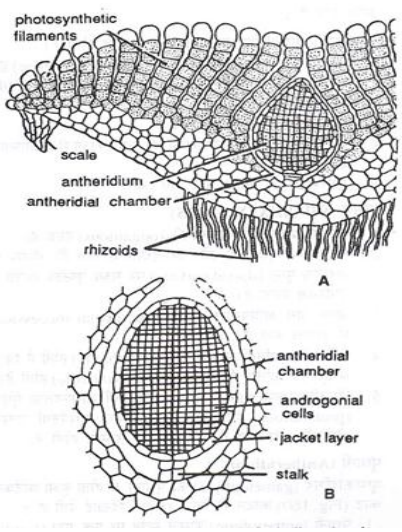
Comments

1. The thallus is boat-shaped in a vertical transverse section.
2. It is thick in the midrib region and gradually thins out towards the margins.
3. The thallus is dorsiventrally differentiated into an upper green photosynthetic region and a lower colourless storage region.
4. The lower epidermis bounds the storage region on the lower side and bears the usual two types of rhizoids (smooth walled and tuberculate) in the centre.

5. The storage region consists of compactly arranged parenchyma. These cells contain starch.
6. The photosynthetic region consists of vertical rows of unbranched assimilatory filaments, separated by narrow air chambers. The cells of the filaments are barrel-shaped and each possesses numerous chloroplasts.
7. The air chambers open to the outside through simple air pores which are the intercellular spaces between the upper epidermal cells.
8. The uppermost cells of the assimilatory filaments are somewhat large. They lack chloroplasts and are thus colourless. These form an ill-defined upper epidermis.
9. On the two margins of the boat shaped section, violet coloured scales are present.

Exercise 3

Study of antheridium
Work procedure:
Cut L.s. of thallus through mid-dorsal groove. Stain in fast green, mount in glycerine and study the antheridia.

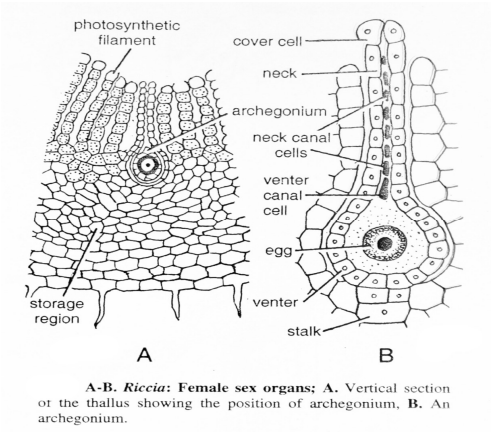


Comments
1. The thallus is monoecious, both the sex organs being situated in the mid-dorsal groove. (*R. bischoffi and R. curtisii* are dioecious).
2. The antheridium is present inside a cavity called antheridial chamber which opens outside by antheridial pore.
3. The antheridial chamber with an antheridium, lies embedded partly in the tissue of the photosynthetic region and partly in the tissue of the storage region.
4. A mature antheridium consists of a small stalk and a globular or club-shaped body.
5. The stalk is short and few celled. The body is composed of a central mass of either androcytes or antherozoids, surrounded by a single layer of sterile jacket. The cells of the jacket are tangentially elongated.

Riccia A, a part of V.T.S. thallus showing position of antheridium; B, A mature antheridium

Exercise 4

Study of archegonium
Work procedure
Cut L.s. of thallus through mid-dorsal groove, stain the section in fast green, mount in glycerine and study the archegonium.



A-B. *Riccia*: Female sex organs; A. Vertical section of the thallus showing the position of archegonium, **B.** An archegonium.

Comments
1. The thallus is monoecious and both the sex organs are situated in the mid-dorsal grrove.
2. A nearly mature archegonium is flask-shaped.
3. Archegonium is shortly stalked and consists of a broad venter and a long neck.
4. Wall of the venter is one celled. The venter has one venter canal cell and an egg cell.
5. The neck consists of 6 vertical rows of cells and is 6-9 cells in height. It possesses 4 neck canal cells.

6. The neck is surmounted by four cover cells.
7. Before fertilization, all the axial cells except the egg cell degenerate and the cover cells spread open to facilitate the entry of anthrozoids.

Exercise 5

Study the structure of sporophyte

Work procedure: Cut L.s. of the thallus through mid-dorsal groovestain in safranin or fast green, mount in glycerine and study the sporophyte.

Comments

1. The sporophyte is embedded in the tissue the thallus. It is present in the venter of fertilized archegonium.
2. Sporophyte is represented only by the capsule, foot and seta being absent.
3. The young capsule has a jacket layer and 2-layered calyptra, derived from venter.
4. The mature sporophyte has spore tetrads arranged tetrahedrally (except *R. pearsonii*) or spores. These remain surrounded only by outer layer of calyptra, the inner layer of calyptra and the jacket disintegrates.
5. The spores are discharged only after the disintegration of the thallus.
6. Each spore ranges from 0.05 to 0.012 mm in diameter and consists of spore wall, enclosing within a rich cytoplasm and a nucleus.
7. The spore wall is three layered. The outermost layer is the exosporium which is thin and cutinized. The middle mesosporium is thick and the innermost endosporium is thin and homogenous. The entire spore wall is irregularly thickened and folded.

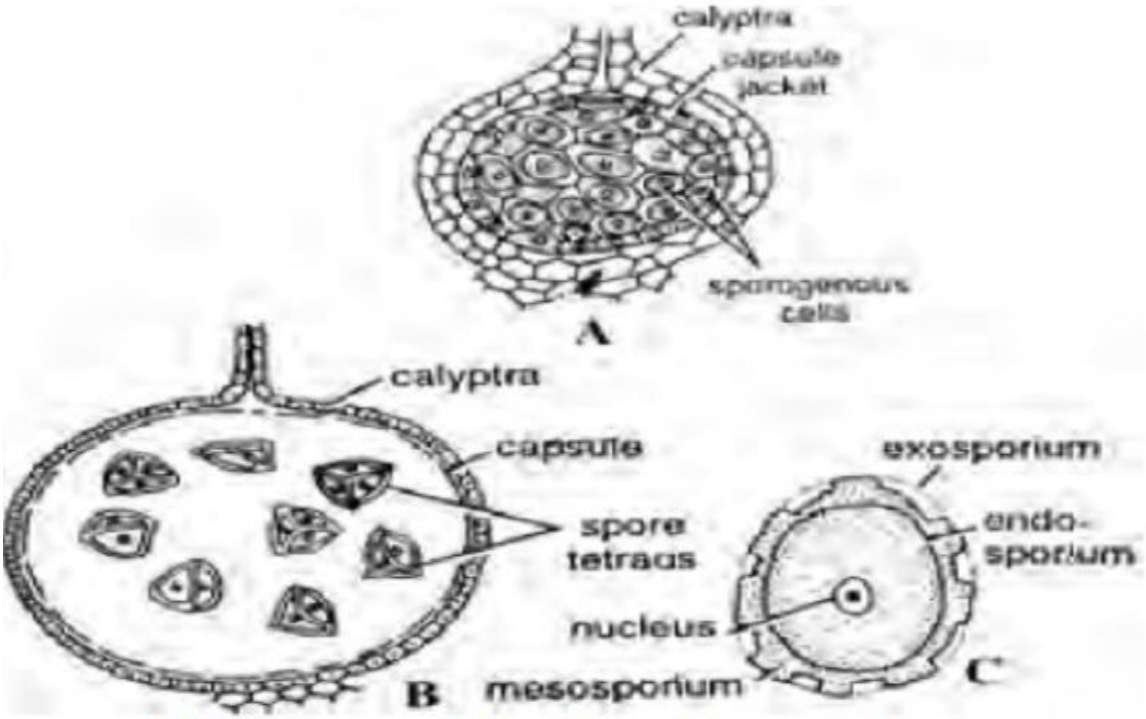
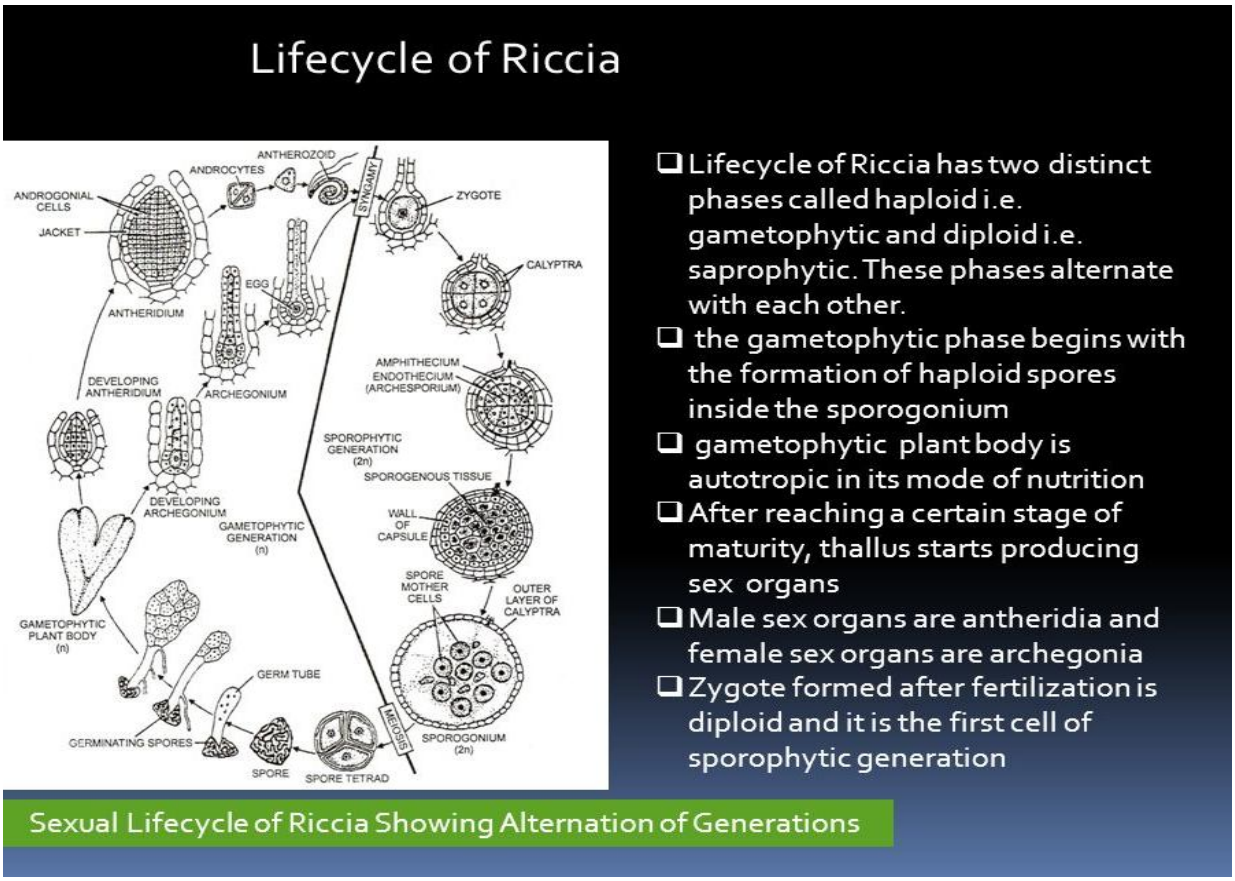


Fig. Riccia - Sporophyte
A, Developing sporophyte. B, Mature sporophyte. C, Single spore



Marchantia *(liver worts)*

Classification

Division	Bryophyta
Class	Hepaticopsida
Order	Marchantiales
Family	Marchantiaceae
Genus	*Marchantia*

Exercise 1

Study of external morphology of thallus

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 4-8 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 i.e. male and female thalli being different.
10. 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.

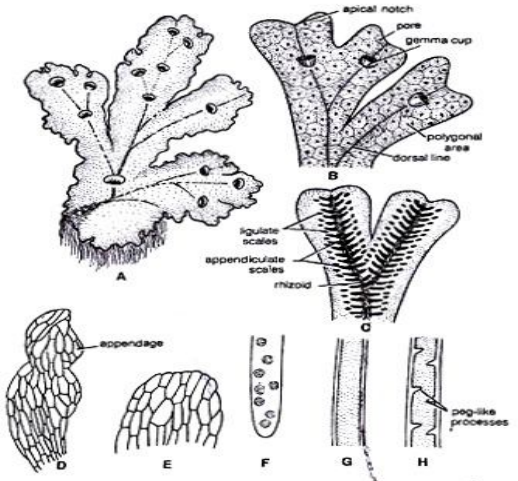


Fig. 1. (A–H). *Marchantia*. Thallus structure (A) Vegetative thallus, (B) Dorsal surface, (C) Ventral surface, (D) Appendiculate scale, (E) Ligulate scale, (F) Tuberculated rhizoid (surface view), (G) Smooth-walled rhizoid, (H) Tuberculated rhizoid showing internal view.

Exercise 2

Study of anatomy of thallus

Work procedure:

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

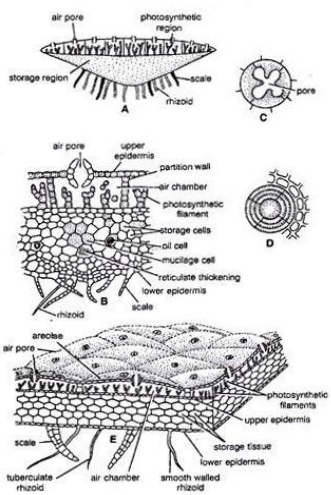


Fig. 2. (A-E). *Marchantia*. Internal structure of the thallus. (A) Vertical Transverse Section (V.T.S.) of thallus (diagrammatic), (B) V.T.S. of thallus (a part cellular), (C) Air pore as seen in the ventral view, (D). Air pore as seen in the dorsal view, (E). V.T.S. of thallus in three dimensional view.

Comments

1. Thallus is dorsiventrally differentiated into an upper photosynthetic or assimilatory 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 4-8 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.

Exercise 3

Study of vegetative, reproductive structure, the gemma cup
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.

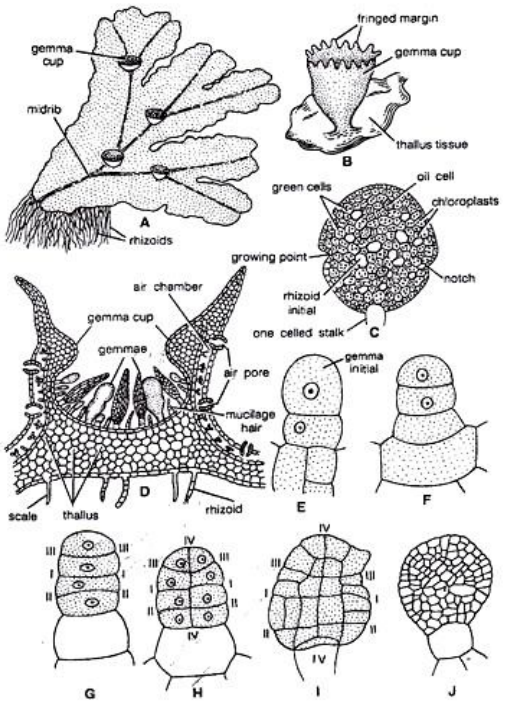


Fig. 3. (A-J). *Marchantia*. Gemma cup. (A) Thallus showing gemma cup on the dorsal surface. (B) A gemma cup, (C) Gemma, (D) Gemma cup in a vertical section, (E-J). Different stages in the development of Gemma.

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. Gemmae is one-celled, stalked structure. The stalk keeps gemma attached to the base of the gemma cup.
8. The disciform gemma 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, rhizoidal cells contain chloroplast.

Exercise 4

Study of stalk of the receptacle
Work procedure
Sex organs are found on stalked receptacles known as **antheridiophore** (male) and **archegoniophore** (female). T.s. of stalks of any one of the recepticle is cut. It is stained in safranin or fast green and mounted in glycerine.

Comments
1. The stalk is dorsiventrally symmetrical.

2. It shows 2 rhizoidal grooves on the lower side, situated one on either side. It contains two types of rhizoids.
3. Upper side has photosynthetic region divided into many air chambers. It is similar to photosynthetic region of the thallus.
4. Stalks of both male and female receptacles are similar in structure.

Exercise 5

Study of male sex organs
Work procedure:
Cut a L.s. through antheridiophore, stain in safranin or fast green and mount in glycerine.

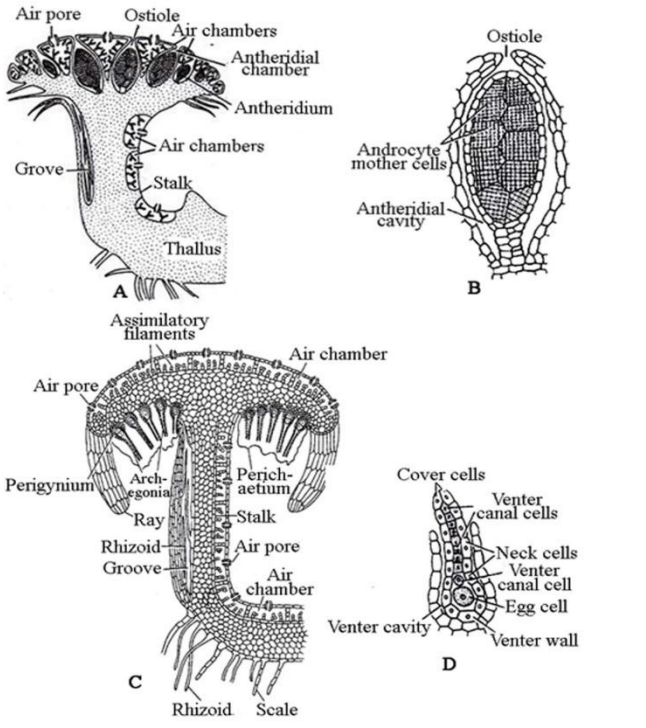


Fig: (A) Antheridiophore, (B) Antheridium), (C) Archegoniophore, (D) Archegonium.

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.
4. Alternating with air chambers, are antheridial cavities. Each antheridial cavity, that opens by an antheridial pore has a single globular 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.

Exercise 6

Study of female sex organs
Work procedure:
Cut a 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 pores. These open into air chambers branched photosynthetic filaments.
5. In a young receptacle, archegonia acropetally arranged on the upper side of disc.
6. Due to the growth in the centre of the (which happens only after fertilization archegonia finally become shifted towards the lower side.
7. The nearly mature archegonium has swollen venter and a long neck.
8. The venter encloses an 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.

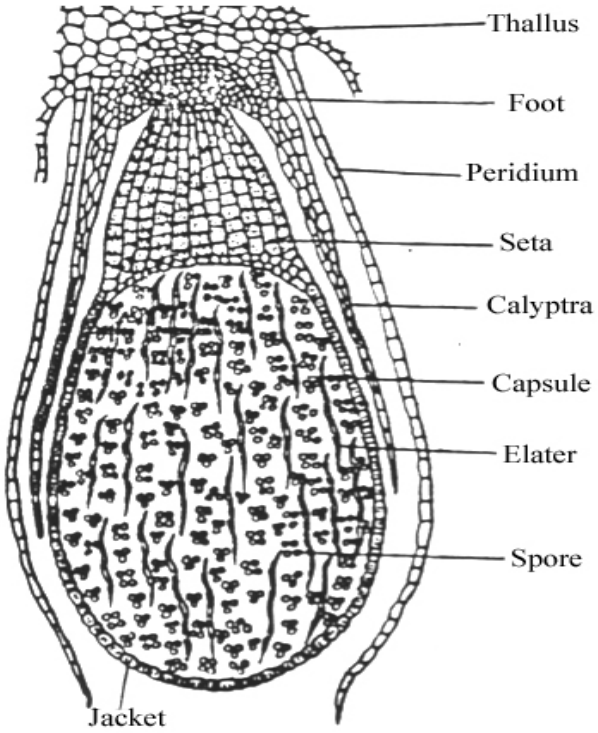


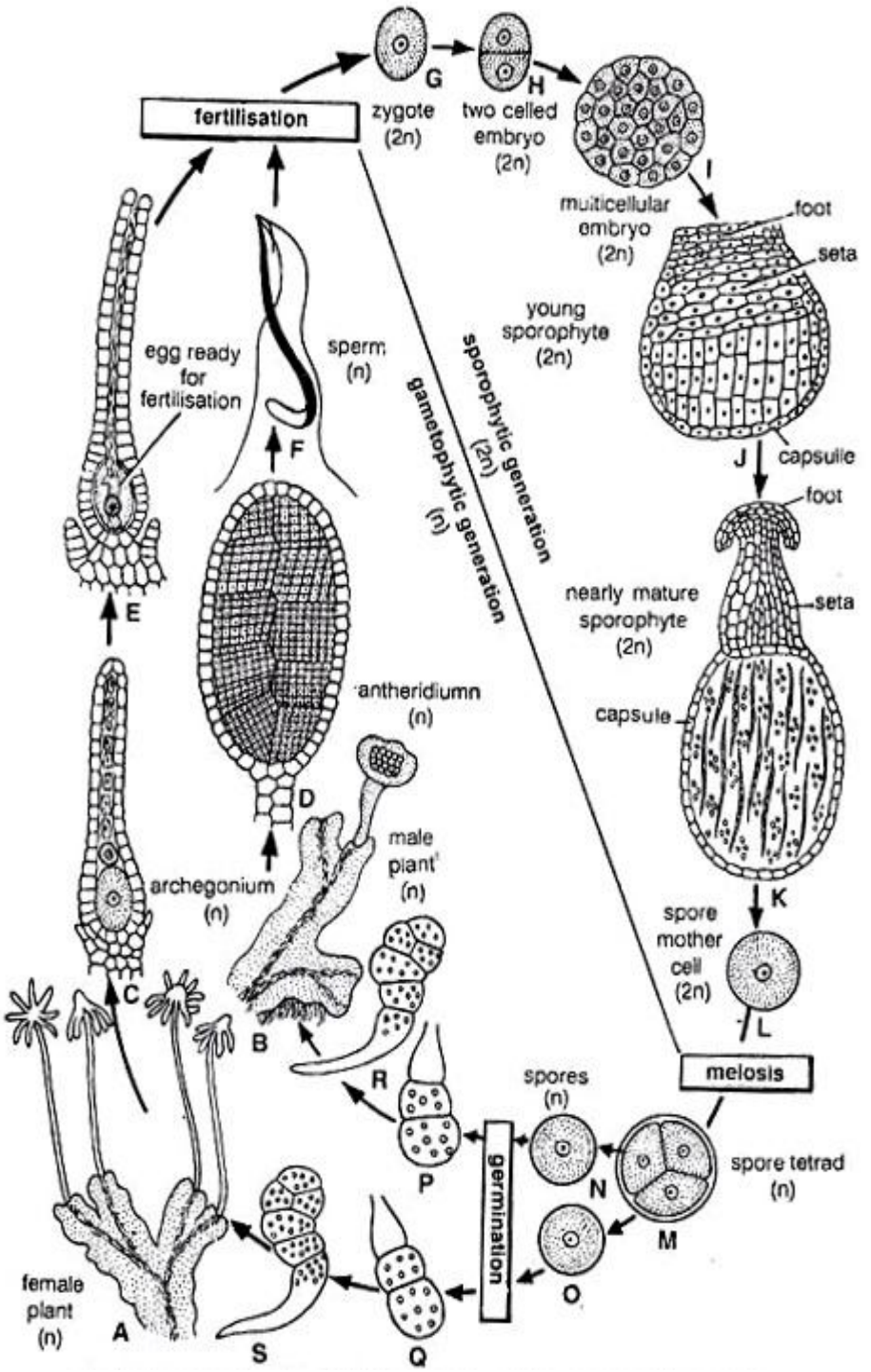
Fig: *Marchantia spp.* Structure of mature sporophyte.

Exercise 7

Study of sporophyte
Work procedure:
Cut a 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 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 eaters. 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.



Life cycle of Marchantia showing heteromorphic alternation of generation

Anthoceros (Horn Wort)

Classification

Division Bryophyta

Class Anthocerotopsida

Order Anthocerotales

Family Anthocerotaceae

Genus *Anthoceros*

Exercise 1

Study of external features

Work procedure

Study the external features of the gametophyte from dorsal and ventral sides. Note the absence of scales and presence of only smooth walled rhizoids.

Comments

1. Some common Indian species of horn wort are *A. erectus, A. himalyansis, A. crispulus, A. dexiti, A. longi* and *A. gollani* etc.
2. Plant body is thalloid, somewhat lobed or radially dissected and generally suborbicular.
3. The thallus is less often dichotomously branched and lack a definite midrib.
4. The dorsal surface of the thallus is generally smooth, velvety or rough.
5. The ventral surface bears smooth walled rhizoids only.
6. On the ventral side, a few bluish spots are seen indicating the presence of filaments of blue-green-alga (viz. *Nostoc* or *Anabaena*).
7. Sex organs are situated on the dorsal side and are embedded in the tissue of the thallus.
8. The sporophyte, however, is linear and elongated structure, arising from the dorsal side.

Exercise 2

Study of anatomy of thallus

Work procedure

Place the thallus in pith. Cut V.t.s. and stain in fast green. Mount in the glycerine and study.

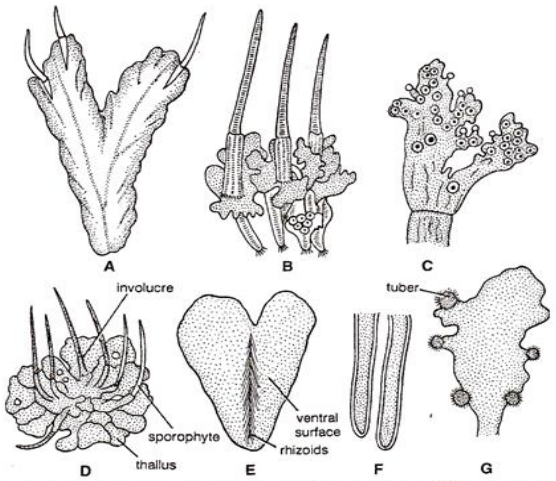


Fig. 1. (A-F). *Anthoceros.* External features (A) *A. himalayensis*, (B) *A. erectus*, (C) *A. gemmulosus*, (D) *A. crispulus* (dorsal surface), (E) Ventral surface, (F) Smooth–walled rhizoids, (G) Thallus with tubers.

Comments

1. Thallus is few cells in thickness in middle and becomes thinner towards margins.
2. Internal structure is homogeneous (i.e. all are alike). Air pores and air chambers absent.
3. On upper side is present upper epidermis lower epidermis on the lower side.
4. Parenchymatous tissue lies between these layers.
5. Each parenchymatous cell has a distinct nucleus and a chloroplast.

6. Each of the cells has a large chloroplast with single pyrenoid except the cells of lo epidermis producing rhizoids. There are two chloroplasts in the cells of *A. pearsonii and four in A. hallii.*
7. On the ventral side there are certain mucilag filled cavities which open by slime pores, through the ventral epidermis.
8. The endophytic algae *Nostoc* or *Anabaena* present in the mucilage cavities enter through these slime pores.
9. Rhizoids are smooth-walled and arise in the middle region of the thallus from the lower epidermis.

Exercise 3

Study of tuber
Work procedure
Study a thallus with tubers. It can be stained with safranin and mounted in glycerine.

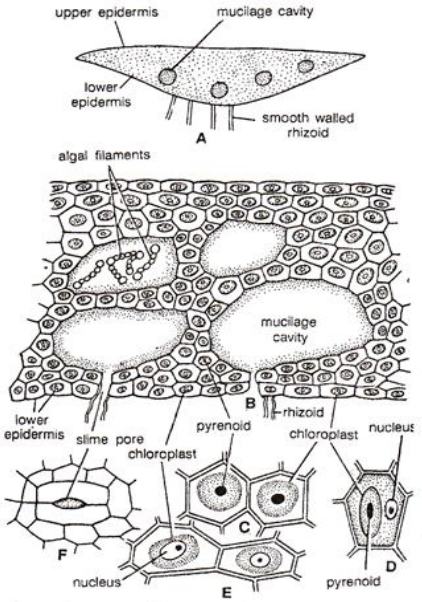


Fig. 2. (A-F). *Antheceros.* Internal structure of the thallus. (A) Vertical transverse section (V.T.S.) of thallus (diagrammatic), (B) V.T.S. of thallus (a part cellular), (C) Cells showing chloroplast and pyrenoid, (D) cells showing chloroplast, pyreroid and nucleus, (E) Parenchymatous cells with chloroplast and nucleus, (F) Surface view of slime pore.

Comments

1. The tubers are vegetative reproductive structures.
2. Tubers are formed under unfavourable conditions, on the dorsal side along the margins of the thallus.
3. A tuber in section shows outer 2-3 corky layers, protecting the inner tissue, containing the reserve food material.
4. On return of the favourable conditions, tubers develop into new thalli.

Exercise 4

Study of antheridium
Work procedure
Cut L.S. of thallus, select a section showing antheridia, stain in fast green, mount in glycerine study the antheridia.

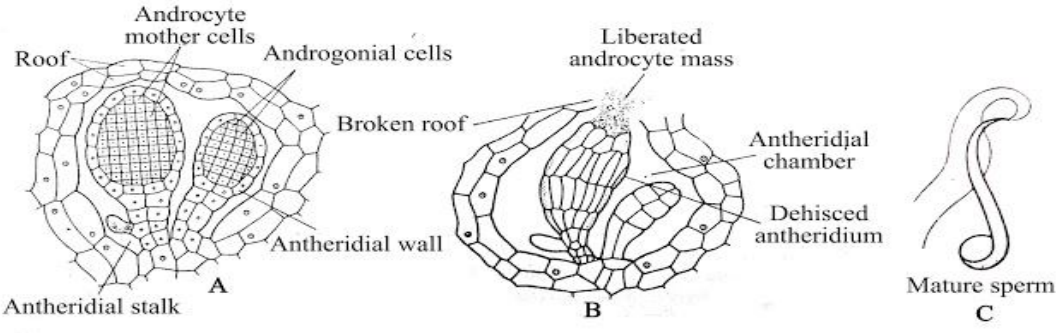


Fig: *Anthoceros.* (A) Mature antheridium inside the antheridial chamber; (B) Dehisced antheridium and (C) A mature sperm

Comments

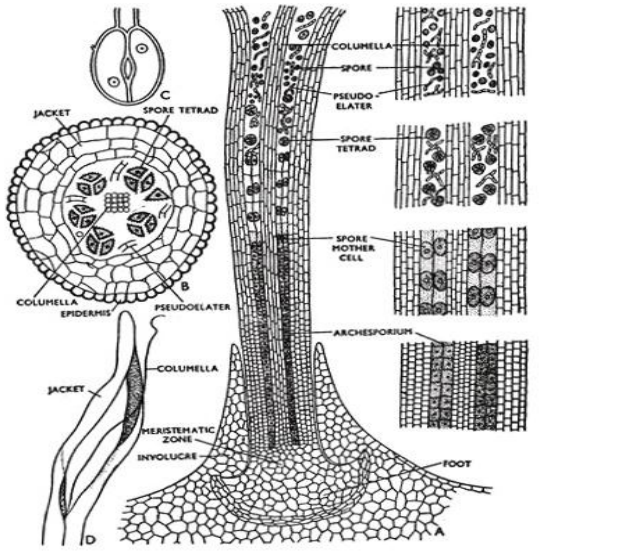
1. Both antheridia and archegonia remain embedded in the dorsal region of the thallus and are acropetally arranged.
2. Few species arc monoecious (homothallic), but some are dioecious (heterothallic).
3. Monoecious species are frequently protandrous (antheridia maturing first).
4. The antheridia are present in the antheridial cavity or antheridial chamber with a sterile roof of 2-3 layers.
5. Each antheridial cavity contains about 1-4 or more primary antheridia. Secondary antheridia arise from the stalks of primary antheridia and ultimately there may be as many as 25 in each antheridial cavity.
6. The mature antheridium is a stalked, club-shaped structure with single layered jacket. Each cell possesses a prominent plastid.
7. Inside the jacket there are large numbers of androcytes.

Exercise 5

Study of archegonium

Work procedure

Cut L.S. of thallus, select a section that shows archegonia, stain in fast green, mount in glycerine and study archegonia.



Mature sporophyte of *Anthoceros*. A. L.s. (semidiagrammatic). Different portions are agnified on the right-hand side. B. T.s. at the middle of the sporophyte. C. A stomata in the jacket epidermis. D. Ruptured tip of a mature sporophyte capsule.

Comments

1. The thalli are generally monoceious and protandruos (antheridia maturing first).
2. The archegonia are embedded in the thallus and only the cover cells project beyond the general surface of the thallus.
3. They are in direct contact with the vegetative cells, lateral to them.
4. Archegonium consists of a neck and a swollen venter.
5. The nearly mature archegonium has 4 cover cells, 4-6 neck canal cells, one venter canal cell and one egg cell.

Exercise 6

Study of sporophyte

Work procedure

Study the external features of the sporophyte. Cut a L.s. stain in safranin and fast green, mount in glycerine and study the internal structure.

Comments

1. The sporophytes are linear, 2-3 cm elongated structure, arising from the dorsal side of the thallus.
2. The base of each sporophyte is enclosed by an involucre, made of gametophytic tissue. (The internal structure of the sporophyte can be understood by studying the transverse and longitudinal sections).
3. The mature sporophyte is made of a lower foot, middle meristematic zone and upper capsule.

4. The foot is bulbous and is deeply rooted in the gametophytic tissue.
5. The seta is absent and instead is present the meristematic zone.
6. In the centre is columella, composed of 16 vertical rows of cells, extending from the base to the tip of the capsule.
7. Surrounding the columella is a cylinder of sporogenous tissue which extends from the base to the tip of the capsule where it over-arches the central columella. It reveals sporogenous series, from one layered archesporium at the base, to mature spores and pseudo-elaters at the tip.
8. At the base of the capsule is a single to double layered archesporium, while little higher up, archesporium is differentiated into alternately placed fertile cells (spore mother cells) and sterile cells (which form pseudo-elaters). A part of sporogenous tissue, upper to this region shows tetrahedral tetrads of spores and many pseudo-elaters. In the upper most region of the capsule are present the separated spores and pseudo-elaters. The wall of the capsule is 4-6 layered.
9. The outermost layer forms a well defined epidermis, ventilated with stomata.
10. The cells of the layers beneath the epidermis possess generally two chloroplasts each and these chloroplasts make the sporophyte partially self-sufficient with regard to food.
11. Each spore is uninucleate and has a thick ornamented exospore and a thin endospore.
12. Pseudo-elaters consist of 2-3 cells, joined end to end, in a simple or branched structure. (These do not possess characteristic spiral thickenings of true elaters and, therefore, are called pseudo-elaters).

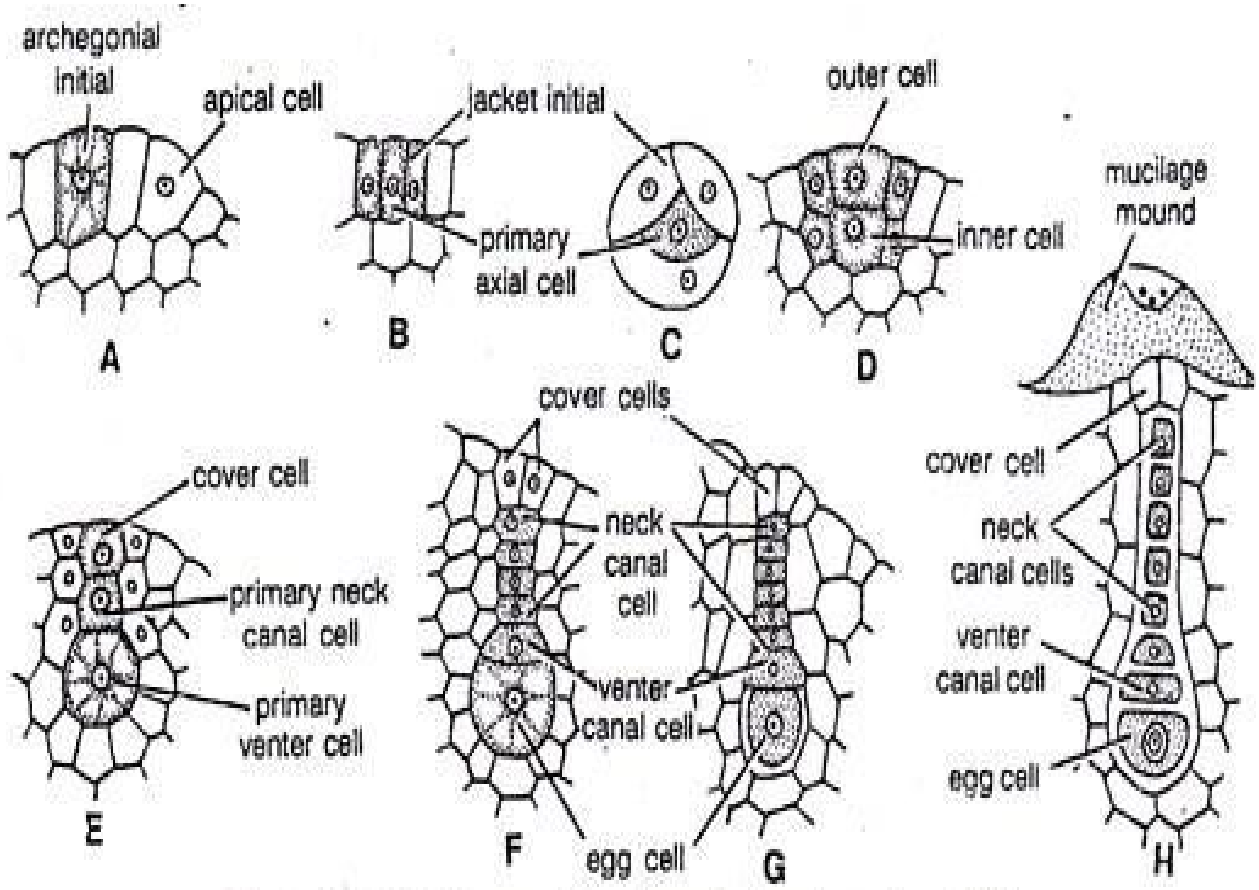


Fig. 6. (A-H). *Anthoceros*. Development of archegonium. (A-H). Successive stages in the development of archegonium.

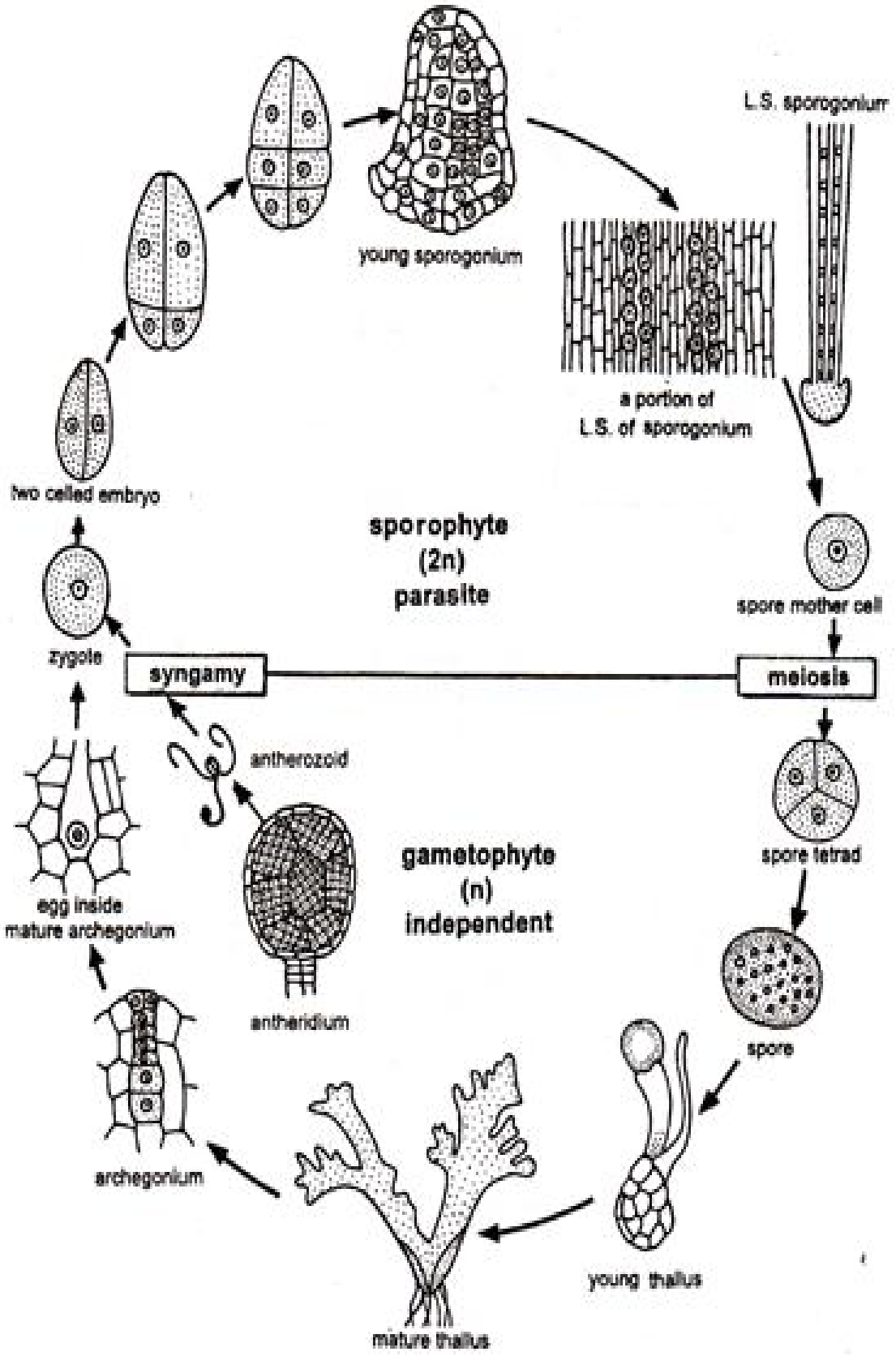


Fig. 11. *Anthoceros*. Diagrammatic life cycle.

Pogonatum (Truemoss or hair cup moss)

Classification

Division	Bryophyta
Class	Bryopsida
Sub-class	Bryidae
Order	Polytrichales
Family	Polytrichaceae
Genus	*Pogonatum*

Exercise 1

External structure of thallus (morphology)

Pogonatum is the largest genus of polytrichaceae with about 158 species distributed in temperate and tropical regions of the world. In India the genus is represented by 23 species mainly growing in Himalayan regions, out of them *P. aloides, P. microstromum* and *P. jhugnianum* etc.

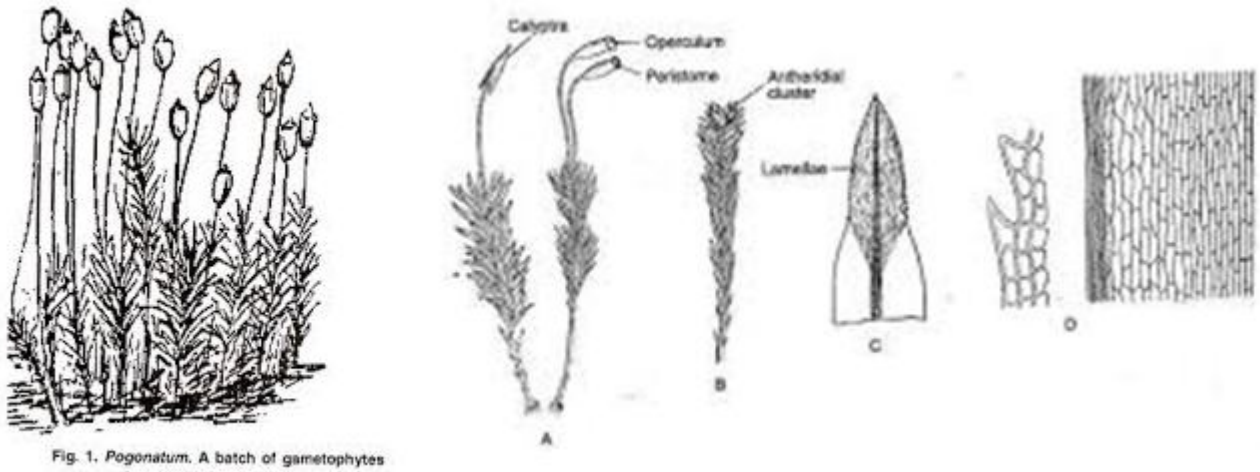


Fig. 1. *Pogonatum*. A batch of gametophytes bearing sporophytes.

Work procedure

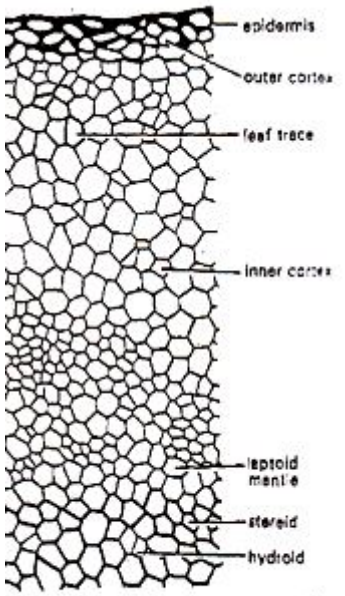
Observe the external features of the plant rhizome axis and the leaves.

Comments

1. The gametophyte is differentiated into rhizoids, stem or axis and leaves.
2. The basal rhizomatous part of the erect stem is slightly stouter and stiffer. It bears rhizoids.
3. The rhizoids are thick walled and multicellular. The septa are oblique. Many rhizoid twists together in a rope-like manner forming strong cable-like strings.
4. The aerial part of the stem bears leaves. The leaves on the lower part are very small, scale-like and much paler in colour.
5. The upper leaves are crowded together spirally, spreading out from the stem and are rather stiff.
6. The leaves are sessile, pale in colour with sheathing broad base. The upper part of the leaf is deep green to brown or reddish brown in colour. The leaves gradually taper towards the apex and the margins are serrated.
7. A leaf has a distinct thick midrib in the centre with narrow wing-like lamina on either side.
8. The upper surface of the midrib is completely covered by parallel longitudinal vertical plate-like structures called lamellae.

Exercise 2

To study anatomy of axis
Work procedure:
Cut a thin transverse section of the axis, stain in safranin, mount in glycerine and study.



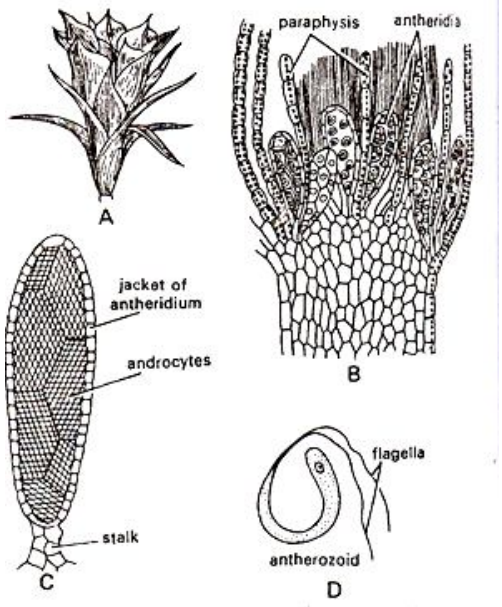
. *Pogonatum*. Transverse section of stem : a part enlarged.

Comments

1. The transverse section shows epidermis, cortex, leptoid mantle, steroids and hydroids.
2. The outermost single layer of slightly thick walled cells is the epidermis. It is broken in the upper part of the stem due to presence of leaves.
3. A wide zone of cortex follows.
4. It shows outer and inner regions. The cells of the outer cortex are thick walled, elongated and deeply coloured.
5. The inner cortex is wider than the outer. The cells are thin walled parenchymatous, lighter and compact.
6. A few thick walled leaf traces are also found in the cortex.
7. Inside the cortex is a zone of elongated cells with protoplasm but no starch. The cells are considered to be similar to sieve tubes and show oblique intervening cells like sieve plates, cells are leptoids. This zone is called as mantle.
8. The centre of the stem shows hydrom cylinder which is made up two types of cells the elongated thick walled cells with living contents called steroids and similar cells devoid of living content called hydroids.

Exercise 3

Study of the male plant and antheridium
Work procedure
Study the position of antheridia on the male plant or dissect the antheridial head, and study an anthcridium.

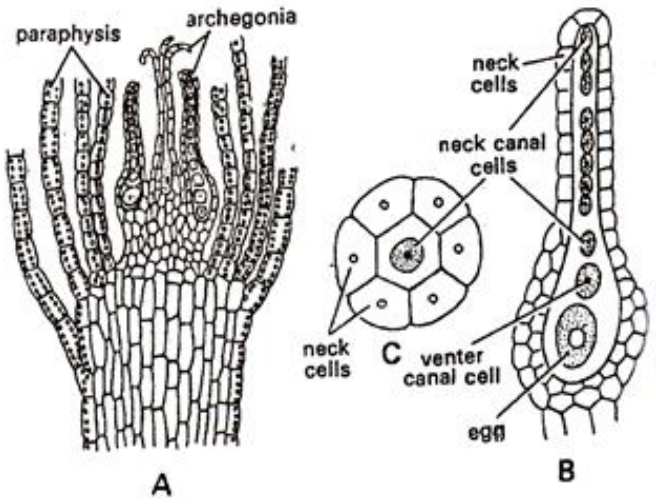


Comments

1. Antheridia are borne at the apex of the branch of male plants.
2. Antheridia are surrounded by specialized leaves, the perichactial leaves at the apex. These are coloured red or orange and form a flower head.
3. The group of these leaves enclose a cluster of antheridia and paraphyses.
4. The antheridia are borne in such a way that the center of the axis remains free for further growth.
5. Each antheridium is a club-shaped structure with a short stalk.
6. The stalk is multicellular and holds the body of the antheridium.
7. The body of antheridium is made of a single layered jacket that surrounds many androcytes.
8. Many paraphyses occur scattered amongst the anteridia. Paraphyses are long multicellular, hair-like structures with rounded apical cell. Each cell has many chloroplasts.

Exercise 4

To study the female plant and the archegonia
Work procedure
Study the position of archegonia on the female plant, or dissect the archcgonial head or 'flower' and study the archegonium.



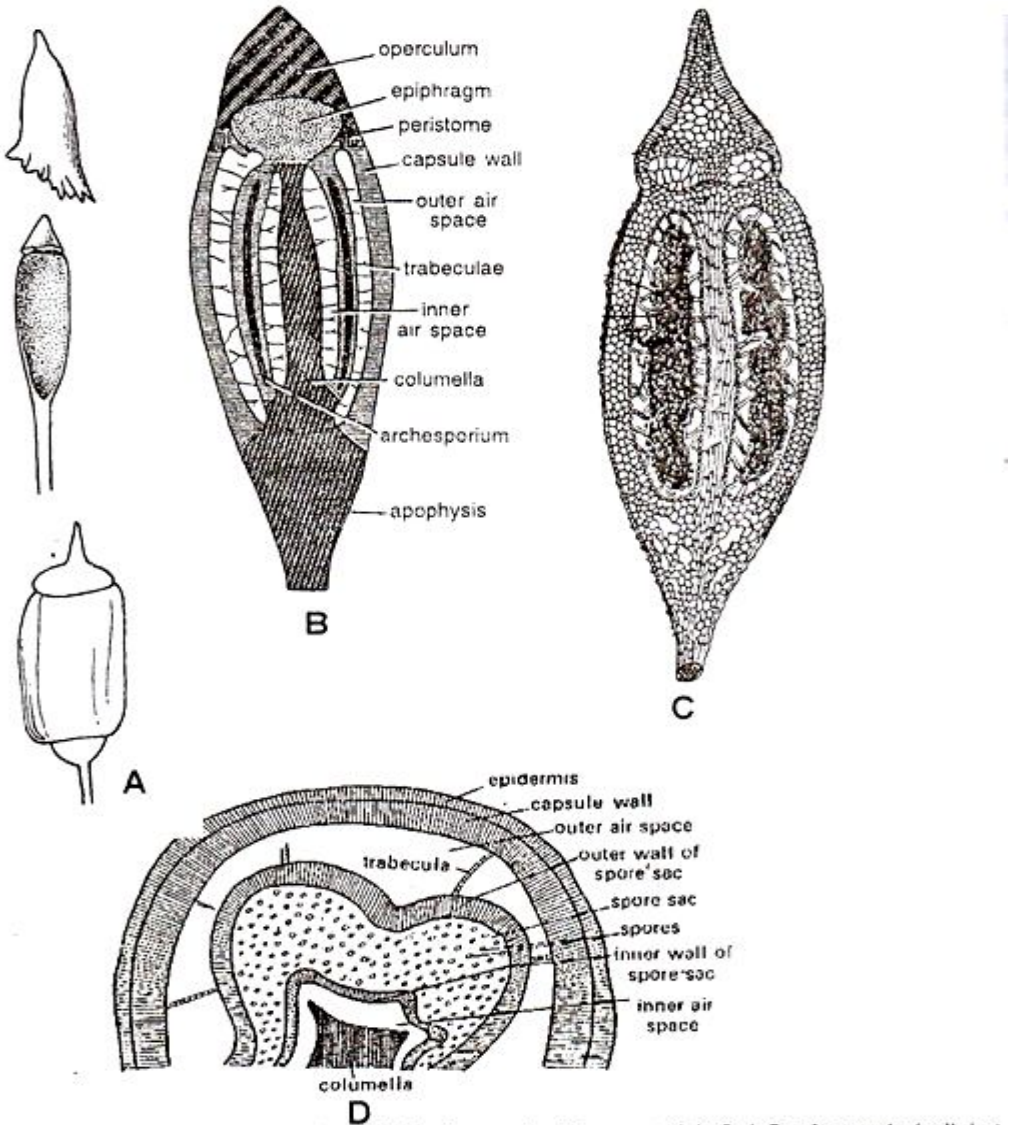
Comment
1. An archegonial cluster develops at the tip of the plant.
2. Each cluster consists of archegonia and paraphyses, surrounded by perichaetial leaves.
3. Perichaetial leaves are specially modified leaves located at the apex.
4. During the formation of archegonia, apical cell is used up and hence the axis stops any further growth.
5. The archegonium is attached to the stem apex by a short multicellular stalk.
6. The archegonium consists of a neck and a ventre. The neck consists of six vertical rows of cells, two cover cells and 8-12 (or more) neck canal cells. The ventre is made of venter canal cell and an egg cell. The wall of the venter is made of 2 layers of cells.
7. The paraphyses occurring among the archegonia are multicellular, long, hair-like structures made of single row of cells. The cells are rich in chloroplasts.

Exercise 5

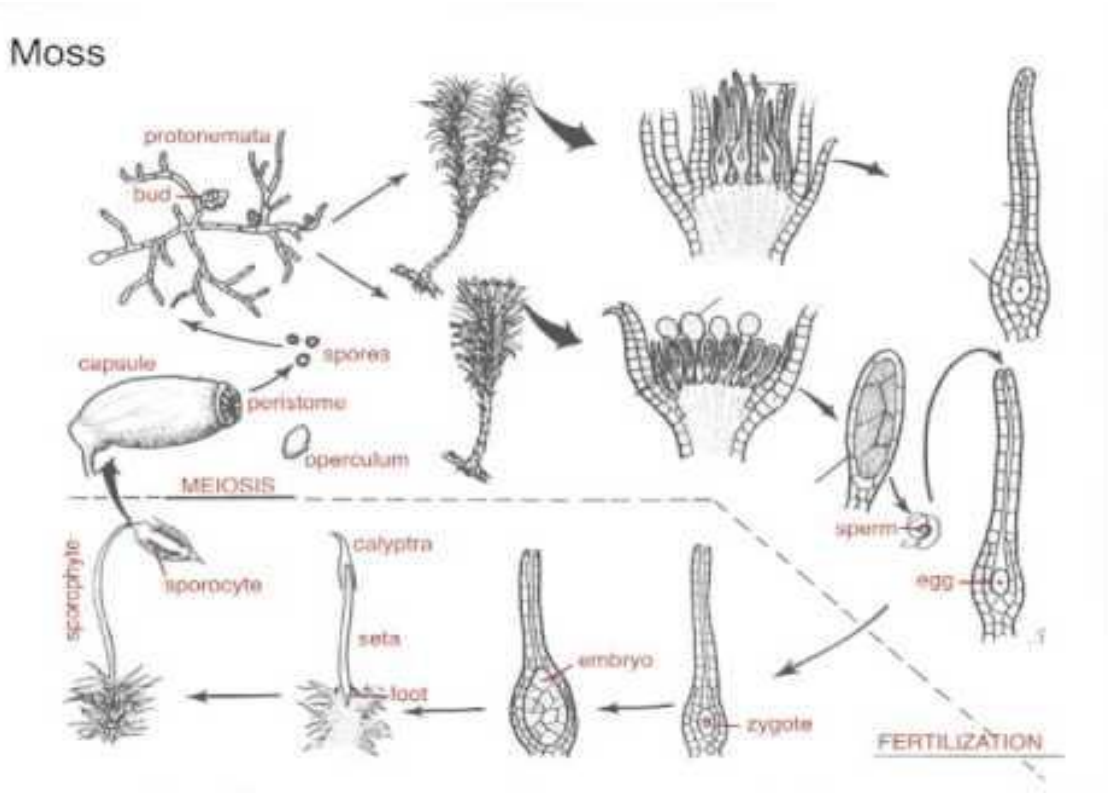
To study the sporophyte
Work procedure
Study the external features of the sporophyte and the internal structure by studying the slide of L.s. of the sporophyte.

Comments
1. The mature sporophyte consists of a foot, seta and a capsule covered by a calyptra.
2. L.s. of the capsule shows a lower stalk, middle fertile region and the upper operculum.
3. The stalk consists of a parenchymatous tissue that merges with columella. It is surrounded by chlorophyllous cells.
4. The capsule lacks a basal apophysis with stomata.
5. The fertile region of the capsule shows the following structure.
6. One layered epidermis with thick outer walls.

7. Several layered wall of chlorophyllous cells.
8. The spore sac has an outer one layered and inner two layered wall consisting of walled cells.
9. The spore sac is usually two layered thick which shows archesporial tissue when young spores when mature.
10. Inner spore wall is followed by another space (inner lacuna).
11. The green filaments of inner air space connected with centrally parenchymatous columella.
12. Columella passes into opercular region swells up to form drum-like roof of the capsule called epiphragm (tympanum).
13. Above the epiphragm is a conical and beaked operculum which is connected to the capsule by a ring-like diaphragm. Organised annulus is absent.
14. Above the diaphragm is a ring of 32 short peristomial teeth. These are formed of the bundles of fibrous cells.
15. The teeth are hygroscopic and control the dispersal of spores.



A–D. *Pogonatum*. A. An entire capsule. B. L. S. of capsule (diagrammatic), C. L.S. of capsule (cellular). D. T.S. capsule at the region of sporogenous tissue (diagrammatic).



Lichens

Lichen is a small group of curious plants. They are composed of algal and fungal components living together in a symbiotic relationship. The fungal partner is called a **mycobiont** and the algal partner as **phycobiont**. The phycobiont generally belongs to cyanophyceae or sometimes to chlorophyceae. The mycobiont is generally an ascomycete but in rare cases it is a basidiomycetes. The lichens were first discovered by **Tulasne** in 1892. The relationship between the two partners is a matter of controversy. Some hold it to be a typical case of symbiosis whereas others consider it to be as parasitism. However, it is now considered to be a case of helotism (a type of symbiotic association) where the fungus has an upper hand. The lichens grow on a variety of habitats, and are common on rocks and bark of trees etc. Many of them grow under extreme conditions of cold, humidity and drought. On the basis of their structure of thallus and nature of attachment to the substratum, lichens are classified into following three categories-(1) **Leproselichen** (2) **Crustose or crustaceous** (3) **Foliose or foliaceous** and (4) **Fruticose or filamentous lichens**.

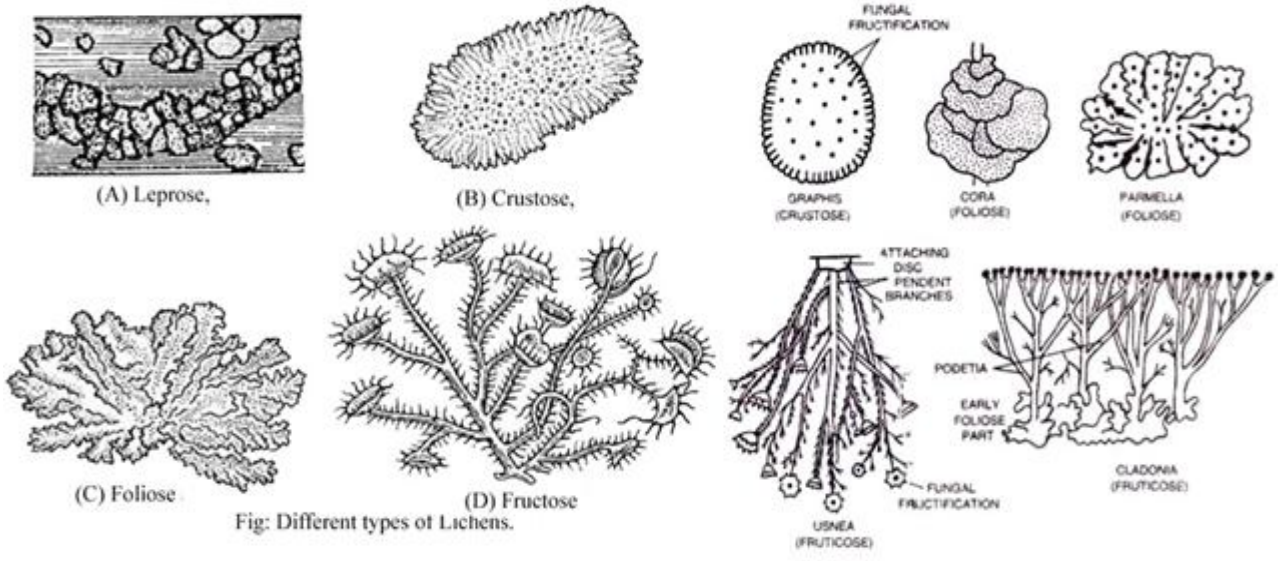


Fig: Different types of Lichens.

Depending upon the fungal component in the lichen, the group is divided into (1) **Ascolichen**, if the fungal component is the member of ascomycetes (2) **Basidiolichen**, if the fungal component is the member of basidiomycetes. Lichens have been used as food by man e.g. *Cetraria islandica* (Iceland moss) is eaten by man in Iceland. Lichens like *Cladonia, Stereocaulon* are fodder lichens being eaten by reindeers. Lichens are also useful in brewing and distillation, cosmetics, perfumes, tanning and dyeing etc. They reproduce asexually by the formation of different types of spores such as **oidia** and **pycnidia**. Only fungal component involves in sexual reproduction. Female reproductive structure is called **carpogonium** and male as **spermogonium**. Their disc shaped fruiting body is called as **apothecia** and flask shaped as **perithecia**.

ASCOLICHENS
(The fungal component belongs from ascomycetes)

According to the external shape and habitat, ascolichens are divided into three principal groups, crustose lichens, foliose lichens and fruticose lichens.

Crustose Lichens

Exercise 1

Study of external features
Study the specimen provided

Comments
1. The crustose lichens are hard, granular crusts on rocks and bark of the trees.
2. These adhere very closely and firmly to the substratum.
3. The thalli are partially or completely embedded in the substratum.
4. These lichens are generally ash-coloured. However, the colours may vary.
5. The surface of the thallus is generally divided into polygonal areas called areolae
6. Some of the commonest examples include Lecidea, Graphis, Lecanora.

Exercise 2

Study of internal structure
Work procedure
Study the slide showing internal structure.

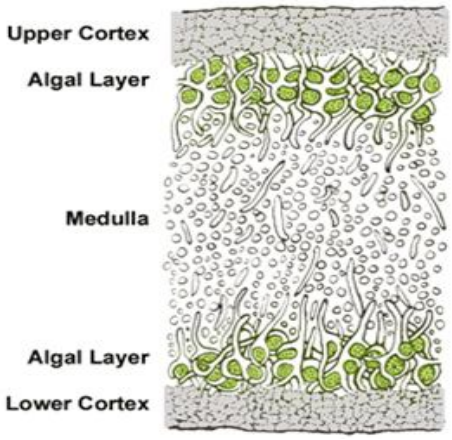
Comments
1. The thallus is poorly differentiated.
2. The tissues forming the thallus are arranged more of less in strata, one above the other.
3. The upper cortex is made of rudimentary or sometimes highly developed hyphal layer.
4. Algal layer lies just beneath this layer. The layer shows algal filaments 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.

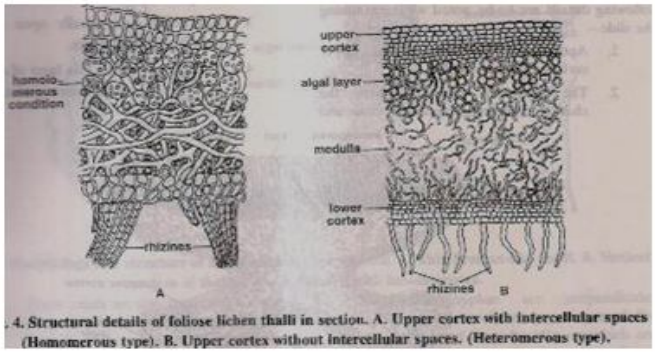
Foliose Lichens

Exercise 1

Study of external features
Work procedure:
Study the specimen provided.

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





4. Structural details of foliose lichen thalli in section. A. Upper cortex with intercellular spaces (Homomerous type). B. Upper cortex without intercellular spaces. (Heteromerous type).

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 grayish 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.

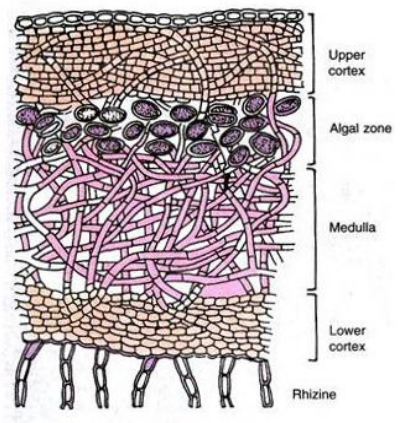
Exercise 2

Study of internal structure of homoisomerous thallus
Work procedure
Study the slide showing homoiomerous structure e.g. Collema, Leptogium, etc.

Comments
1. These types of thalli are not very common.
2. Internal structure is not much differentiated.
3. The algal cells are irregularly scattered throughout the fungal hyphae.
4. Both algal cells and fungal hyphae are enveloped in a gelatinous matrix or the ground substance.

Exercise 3

Study of internal structure of heteromerous thallus
Work procedure
Study the section of heteromerous thallus of a lichen e.g. *Parmelia*, *Physcia*, etc.



Comments
1. Internal structure of the thallus shows four distinct regions or zones. These are upper cortex, gonidial layer or algal layer, medulla and the lower cortex.
2. Upper cortex may or may not be bounded by an epidermis-like layer or hyphae.
3. Certain breathing pores may also be present in the epidermis. These help in gaseious exchange.
4. The upper cortex itself consists of vertical hyphae without intercellular spaces or with such spaces filled with gelatinous materials.

5. Beneath the upper cortex is algal or gonidial layer. It consists of numerous algal cells held together in the network of fungal hyphae.
6. Below the gonidial layer is the medulla made of very loosely interwoven hyphae.
7. On the lower side is the lower cortex, consisting of compact cells, lying either parallel or perpendicular to the lower surface.
8. Cells of the lower cortex produce rhizines.

Exercise 4

Study of pycnidiospores
Work procedure
Study a section passing through pycnidial cup or pycnidium.

Comments
1. Pycnidia or pycinidial cups are produced on the upper surface of the thallus.
2. Pycnidium is a flask-shaped cavity with a small opening at its apex, an ostiole.

BASIDIOLICHENS

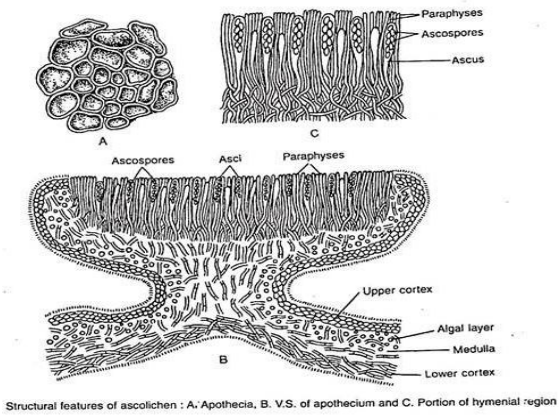
Exercise 1

Study of external features of the thallus
Work procedure
Study the characters of a specimen provided.

Comments
1. The basidiolichens are tropical in distribution and are found growing upon bare soil, rocks and trees.
2. There are only three genera of basidiolichens and out of these *Cora pavonia* is the best known.
3. The thallus is much lobed, internally attached to the substratum by rhizines and resembles superficially with 'bracket fungi'.

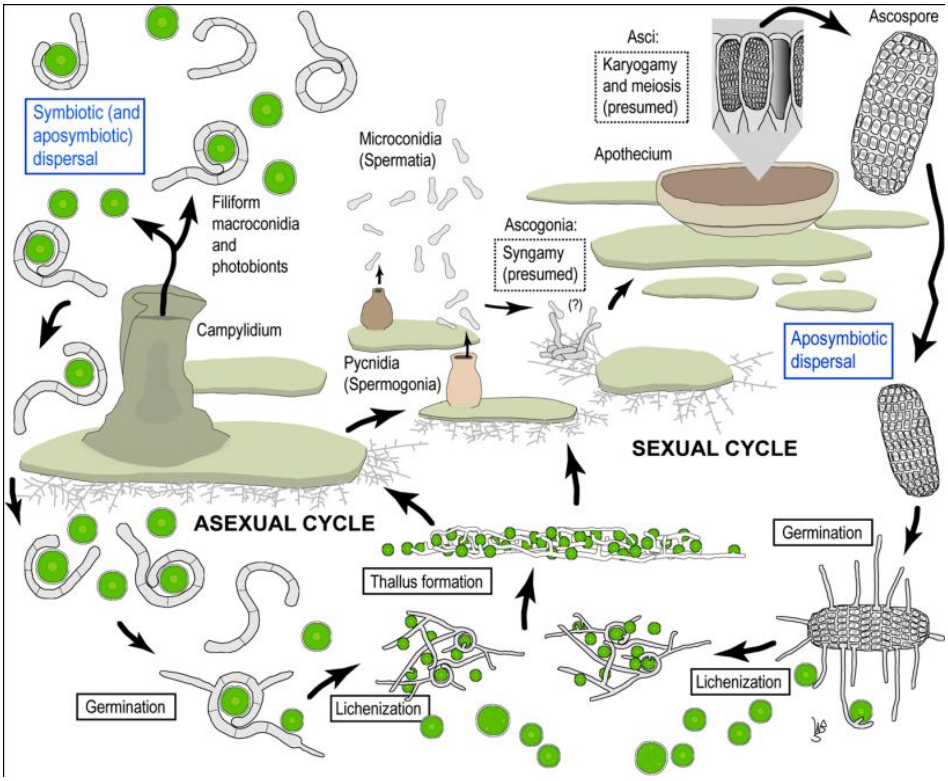
Exercise 2

Study of internal structure of thallus
Work procedure: Study the section of the thallus as seen in slide.



Structural features of ascolichen : A. Apothecia, B. V.S. of apothecium and C. Portion of hymenial region

Comments
1. As seen in a vertical cut, the thallus is differentiated into three layers-the superior player, the algal or gonidial layer and the inferior layer.
2. The superior layer is uppermost and consists of loose felt of more or less perpendicular hyphae.
3. The algal layer is the middle one. It is made of algal cells (Chroococcus species) intermixed with loose hyphae.
4. The inferior layer is the lowermost which is a dense felt of hyphae running in all directions.
5. The lowest surface bears concentric outgrowths of more or less perpendicular hyphae.
6. Each outgrowth is known as sub-hymenium and its lower face bears a palisade-like layer of basidia.
7. Each basidium bears four terminal sterigmata with basidiospores.
8. Basidiospores are thus shed from the underside of the lichen. These on contact with an alga form lichen.



PTERIDOPHYTA

Pteridophyta (vascular cryptogams) The term pteridophyta is derived from two Greek words; pteriophytes are commonly known as "**Vascular cryptogames**" because of the presence of vascular bundles (xylem and phloem).In general, pteridophytes have been referred to as an assemblage of flowerless, seedless, spore bearing vascular plants. This group include not only a large number of present day genera but also many fossil types. Many of the genera which at the present day are quite small plants are descended from ancient groups which in the days. **Pteron=feather; Phyton=Plants** means the plants bearing feather like leaves like fern. The members of this group vary greatly in their form and habitat. They may aquatic or terrestrial and xerophytic. Their main sporophytic plant body is differentiated into root, stem and leaves. Their range varies from small annuals e.g; *Azolla* to large tree like perennials e.g. *Angiopteris*. The reproductive organs are generally borne either on the leaves or in the axils between the leaves and the stem. They are made up of little capsules called sporangia in which they developed the spores. All the spores may be of the same size and the plant is said to be the **Homosporous**, or they may be of the two different sizes and the plant is called as **Heterosporous** plant. In heterosporous types the smaller spores are named as **microspores** and they are developed in microsporangia, while larger spores are called **megaspores** or **macrospores** which are generally produced in megasporangia or macrosporangia.

Some very common characters are as follows

1. Their main plant body is sporophytic (2n), which is differentiated into root, stem and leaves.
2. There vascular bundles (xylem and phloem) remain present in a definite central column of roots and stem, called **stele**.
3. The simple stele is known as **protostele**, which may be of four types on the basis of shape of xylem and position of phloem i.e., **haplo protostele**, **actino protostele**, **plecto** and **mixed** protostele.
4. Most advance and complex stele is known as **siphono stele** it may be of two types (depending upon position and number of xylem and phloem) i.e., **Ectophloic** and **amphiphloic** siphonostele.
5. In their life cycle asexual reproduction takes place in sporophytic (2n) generation by spores and sexual reproduction in gametophytic (n) generation and only oogamous type.
6. Sex organs (antheridium and archegonium) are always found on gametophytic (haploid) plant body.

Classification of Pteridophytes

Riemers proposed a classification of pteridophytes which was pulished in 1954, edition of Engeler's ***Syllabus der pflanzenfamilien***. Sporne (1966) also followed the same classification of pteridophytes. An outline of Sporne's classification is as follows:

Division **Pteridophyta** is classified into five sub-divisions.

Psilophytopsida	Psilotopsida	Lycopsida	Sphenopsida	Pteropsida
Order1. Psilophytale Family. Rhyniaceae Genuus. *Rhynia*	**Order1**. Psilotales Family. Psilotaceae Genuus. *Psilotum*	**Order1**. Lycopodiales Family. Lycopodiaceae *Lycopodium*	**Order1**. Hyniales	**Order1**. Maratiales
		Order2.Selaginellales Family. Selaginellaceae *Selaginella*	**Order2**. Sphenophyllales	**Order2** Cladoxylales
		Order3. Isoetales Family. Isoetaceae *Isoetes*	**Order3**. Calamitales	**Order3** Osmundales
		Order4. Lipododendrale Family. Lepododendraceae *Lipododendron*	**Order4**. Equisetales	**Order4**. Marsiales Family. Marsileaceae *Marsilea*
				Order 5 Slviniales Family Salviniaceae *Salvinia*
				Order 6 Filicales

***Lycopodium* (Club Moss)**

It is commonly known as club moss/ground pine/or trailing evergreen

Classification

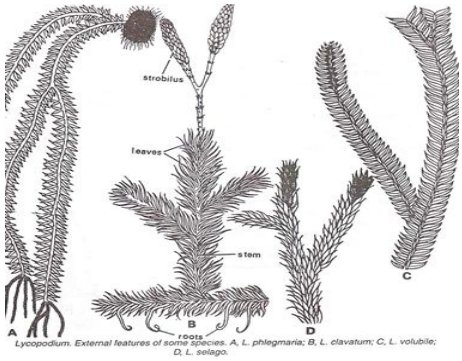
Division Pteridophyta

Sub-division Lycopsida

Order Lycopodiales

Family Lycopodiaceae

Genus *Lycopodium*



Lycopodium. External features of some species. A, L. phlegmaria; B, L. clavatum; C, L. volubile; D, L. selago.

Lycopodium's species are widely distributed throughout the world from arctic to tropical regions, however the species of this genus are commonly found in tropical and sub-tropical forest. Most of the species are grow in moist and shady places, all the species are **homosporous.** In some very common species of *Lycopodium, L. phlegmaria, L. suuarrosuum L. corinatu*m (epiphytic and terrestrial species), *L. cernuum, L. clavatum, L. densum* (creeping stem) and *L. complanatum* (erect with aerial stem) and *L. volubile* (climer) are included.

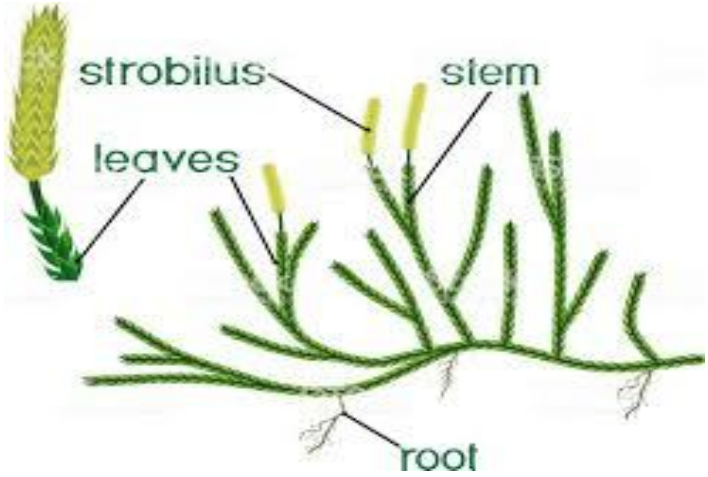
The genus *Lycopodium* is divided into two sub genera - Urostachya and Rhopalostachya

Urostachya

1. Plant body erect or pendent.
2. Branching rare, if present always dichotomous.
3. The roots take their origin only from the basal part of the stem.
4. Organized strobili are rarely found.
5. Sporophylls are almost similar to foliage leaves, the only difference is in their size.
6. The spores posses a pitted surface without any external outgrowth.

Rhopalostachya

1. Plant generally trailing or creeping
2. Branching is dichotomous at the base but in the upper region only one of the branches grows more prominently.
3. Adventitious roots may arise from any part of the stern.
4. Strobili are always well organized and borne on long stalks.
5. Sporophylls are different from foliage leaves. They are pale yellowish and chaffy.
6. Margins of sporophylls are toothed.
7. Spores possess reticulate surface.



Exercise 1

Study of external morphology

Work procedure

Study the specimen. Observe the differentiation of the plant body into root, stem and leaves. Study the leaves and the stomata.

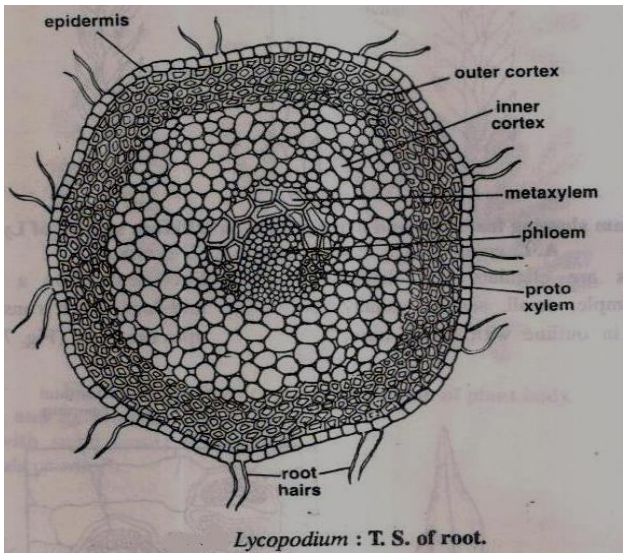
Comments

1. The plant body consists of creeping rhizome which gives off slender, elongated aerial branches from the upper side and adventitious roots from the lower. The aerial branches vary from 3-8 inches in length. *L. cernum* is exceptional in attaining a height of 2 feet or more.
2. Most of the species are terrestrial and the sporophyte may either have an upright stem or a horizontally creeping stem. Some species grow as epiphytes on higher plants which show pendent habit e.g. *L. phlegmaria and L. squarrousum.*
3. The branching is mostly dichotomous but in some species it may be monopodial also.

4. The stem and its branches are densely covered with small leaves present in close spirals or whorls.
5. The leaves are entire, small and membranous rarely, exceeding 1 cm. in length. Each leaf is supplied by a single mid-vein which runs almost unbranched right up to the apex.
6. The walls of the epidermal cells of the leaf are sinuous.
7. Stomata are more or less parallel to the midrib. These are equally distributed on both of the leaf surfaces.

Exercise 2

Study of anatomy of root
Work procedure
Cut a T.s. of the root, stain in safranin and fast green combination, mount in glycerine and study.



Lycopodium : T. S. of root.

Comments
1. The root is differentiated into an epidermis, cortex and the stele.
2. The epidermis is single layered and gives rise to root hairs, the latter occur in pairs.
3. The cortex is several layered and in older roots a few of the outer layers become sclerified. The inner cells are thin walled and parenchymatous without any intercellular spaces.
4. The stele ranges from monarch to tetrarch but generally it is diarch with two protoxylem masses.
5. The xylem is C or U shaped and is so oriented that the opening of C or U faces away from the stem.
6. The protoxylem is present at the tips of C or U and the intervening portion consists of metaxylem.
7. The phloem is present in between the arms of C or U.

Exercise 3

Study of anatomy of stem
Work procedure
Cut T.s. of the stem and stain in safranin-fast green combination, mount in glycerine and study.

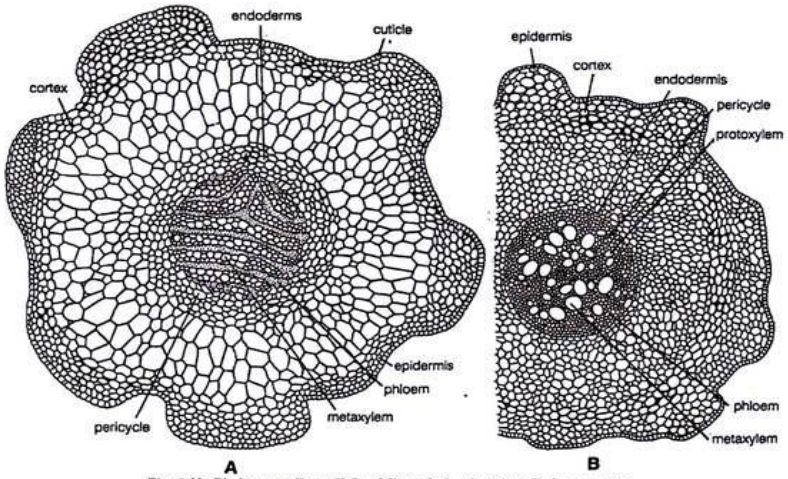


Fig. 2 (A–B). *Lycopodium* : T. S. of Stem A. *L. clavatum*, B. *L. cernuum*

Comments
1. A transverse section of the stem shows an epidermis, a wide cortex and a stele.
2. Epidermis is single layered and is provided with stomata.
3. The stem has certain ridges and grooves. Chlorenchyma is present in the ridges.
4. In cortex, the structure varies from species to species. In some species, it is parenchymatous throughout, in others the inner and other portions are thick

walled, and in still others the entire cortex is thick walled.

5. Endodermis is single layered and lies inner to cortex. In older stems, however, the endodermis may not be well defined.
6. The endodermis followed the pericycle, which is composed of 3 to 6 cells.
7. The centre is occupied by a protostele. Three different types of steles are found in species of *Lycopodium*.

(i) In some species viz. *L. clavatum* and *L. complanatum*, it is definitely organised into a plectostele i.e. xylem and phloem occur in alternating bands that are more or less parallel.

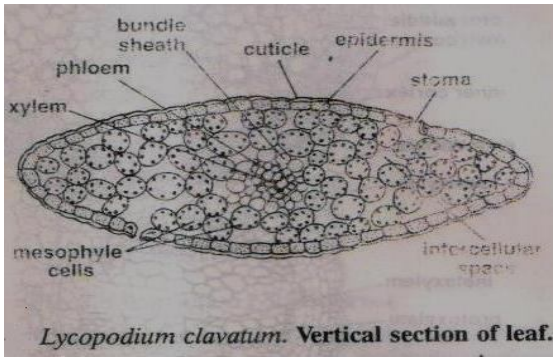
(ii) In other species viz. *L. seratum and L. phlegmaria*, it is star-shaped with 4 arms in which grooves are occupied by phloem. This is known as actinostele.

(iii) In still other species, as examplified by *L. cernum*, it is a haplostele in which the xylem strands lie scattered in phloem.

8. The xylem is exarch with protoxylem facing towards the periphery and metaxylem towards the centre.
9. The tracheids in metaxylem are scalariform while in protoxylem they are spiral or annular.
10. The phloem consists of unicellular sieve tubes with numerous sieve plates and phloem parenchyma. The companion cells absent. Leaf traces are seen to traverse the cortex.

Exercise 4

Study the anatomy of leaf
Work procedure:
Place the leaf in pith cut, T.s., stain in safrain- fast green combination, mount in glycerine, study.



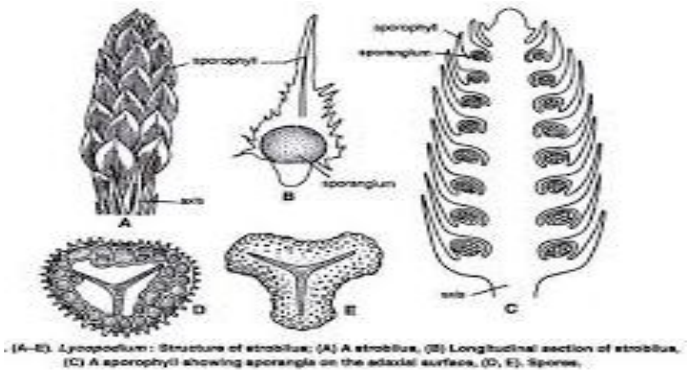
Lycopodium clavatum. **Vertical section of leaf.**

Commments

1. The epidermis is single layered. The stomata are equally distributed on both the sides.
2. The leaf has a single vascular strand which is concentric with xylem in the centre.
3. The cells between the epidermis and the vascular strand form spongy parenchyma.

Exercise 5

Study of spore producing organ
Work procedure
Study the strobili, L.s. of strobilus and the spores: observing a prepared slide.


(A–E). *Lycopodium*: Structure of strobilus; (A) A strobilus, (B) Longitudinal section of strobilus, (C) A sporophyll showing sporangia on the adaxial surface, (D, E). Spores.

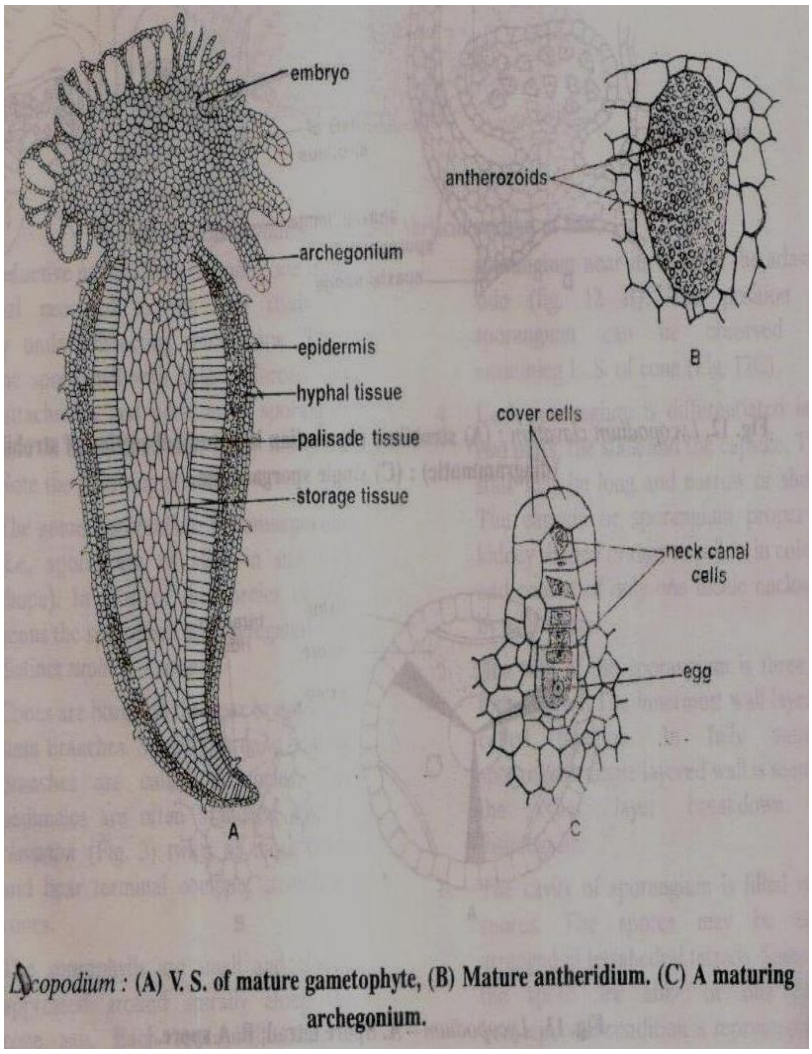
Comments

1. Sporangia are the spore producing organs. These are grouped to form strobili which are situated at the apices of branches. In *L. selago* distinct strobilus is lacking and the vegetative and reproductive regions alternate each other.).
2. L.s. of the strobilus shows a central strobilar axis with spirally arranged sporophylls.

3. Each sporophyll bears a sporangium near its base on the adaxial side.
4. A sporangium is a black, kidney shaped structure, with a long or short massive stalk.
5. Sporangium consists of a wall and the cavity with spore.
6. The wall of sporangium is several layered thick. Tapetum forms the innermost layer.
7. The cavity has many spores, arranged in tetrahedral tetrads. Since all the spores are of one type, that plant is called **homosporous**.
8. Each spore is a minute structure with a triradiate ridge. It has a thick and spiny exine and a thin and membranous intine.
9. The spores germinate to form the prothallus.

Exercise 6

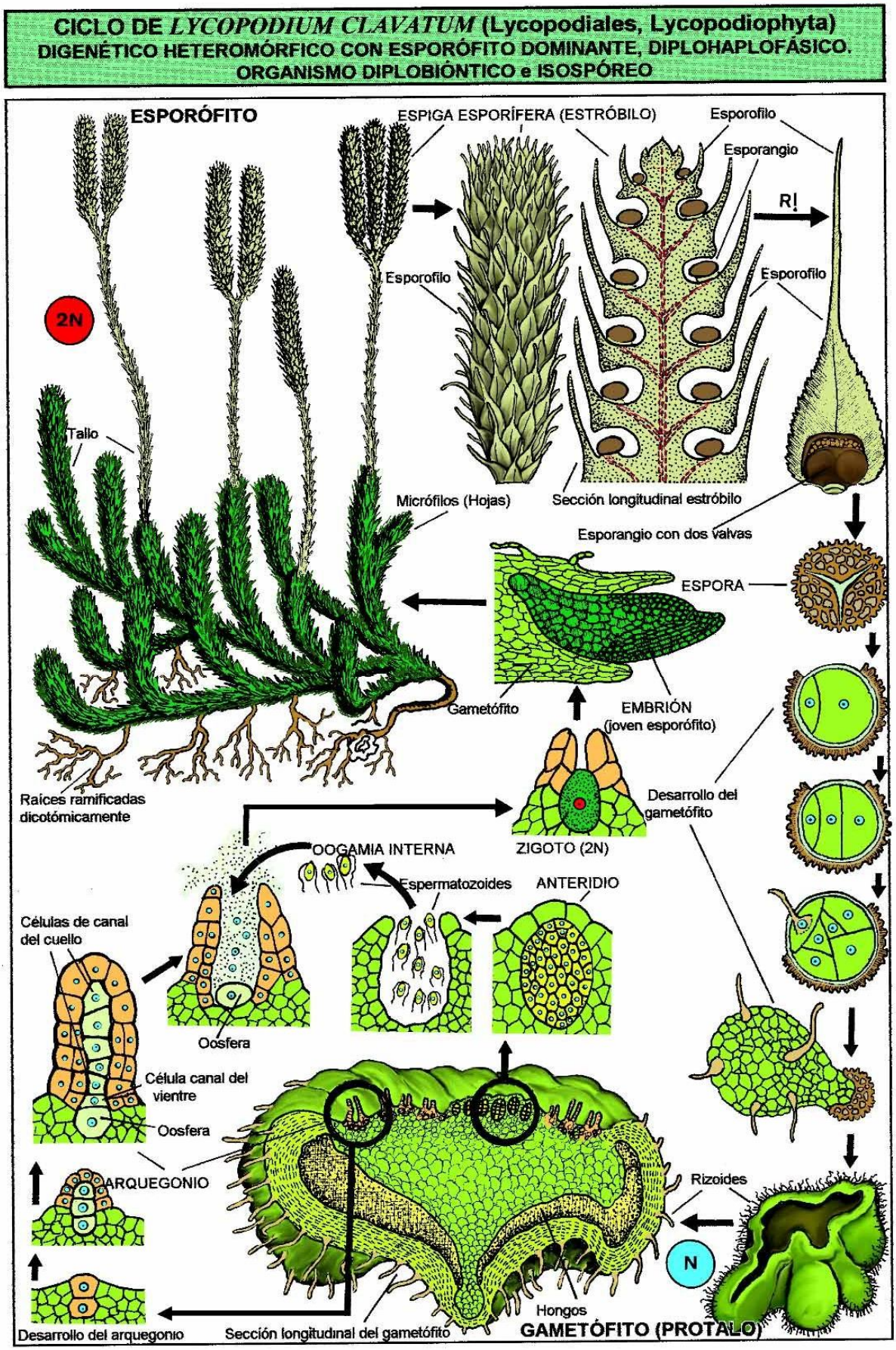
Study of gametophyte
Work procedure:
Study the slide showing gametophyte. Observe the structure of sex organs.



Lycopodium : (A) V. S. of mature gametophyte, (B) Mature antheridium. (C) A maturing archegonium.

Comments

1. The gametophytes may be subterranean or sub-aerial.
2. The sub-aerial type is green, about 2 or 3 mm in length and bears the sex organs.
3. The subterranean type is non-green and is bigger as compared to the sub-aerial type. It generally consists of a tuberous body with a lobed crown that bears the sex organs.
4. The prothallus is monoecious and the sex organ lie almost wholly embedded in the tissue of the prothallus except the uppermost portion.
5. The antheridium is spherical with a single layer of jacket, containing within, a number of antherozoids or antherozoid mother cells. The antherozoids are fusiform and biciliate.
6. The archegonium is a narrow elongated structure. It has a narrow venter with an egg and venter canal cell and a long neck generally containing 4-6 neck canal cells.


CICLO DE *LYCOPODIUM CLAVATUM* (Lycopodiales, Lycopodiophyta)
DIGENÉTICO HETEROMÓRFICO CON ESPORÓFITO DOMINANTE, DIPLOHAPLOFÁSICO.
ORGANISMO DIPLOBIÓNTICO e ISOSPÓREO
ESPORÓFITO
ESPIGA ESPORÍFERA (ESTRÓBILO)
Esporofilo
Esporangio
MEIOSIS
Esporofilo
Esporofilo
2N
Tallo
Micrófilos (Hojas)
Sección longitudinal estróbilo
Esporangio con dos valvas
ESPORA
Gametófito
EMBRIÓN
(joven esporófito)
Raíces ramificadas
dicotómicamente
Desarrollo del
gametófito
ZIGOTO (2N)
OOGAMIA INTERNA
Espermatozoides
ANTERIDIO
Células de canal
del cuello
Oosfera
Célula canal del
vientre
Oosfera
ARQUEGONIO
Rizoides
N
Hongos
Desarrollo del arquegonio
Sección longitudinal del gametófito
GAMETÓFITO (PROTALO)

Selaginella
(Small Club Moss/ spike moss)

Classification

Division	Pteridophyta
Sub-division	Lycopsida
Order	Selaginellales
Family	Selaginellaceae
Genus	*Selaginella*

Most of the species of *Selaginella* are cosmopolitan in distribution, species are found in damp and shaded forest of tropics but some species *(S. densa, S. rupestris* and *S. lepidophylla)* are growing in xerophytic habitats. It is the only living genus of the order Selaginellales, comprising about 700 species, distributed all over the world, about 70 species have been reported from India. All the species of *Selaginella* are heterosporous (having two different types of spores, micro and megaspores). They are asexually reproduce by spores and spores developed in sporangia (micro and megasporangia) and sporangia are found in special types of leaves called, **sporophylls** (micro and megasporophylls). The strobilus or cone of *Selaginella* is composed of micro sporophylls and mega sporophylls. Some common Indian species are *S. repanda, S. biformis, S. denticulate, S. monospora, S. semicordata, S. adunca* etc.

Exercise 1

Study of external features of the plant
Work procedure
Study the plant, specimen and observe the differentiated plant body into root, stem and leaves. Study the types of leaves, their arrangement and structure. 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 are 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.

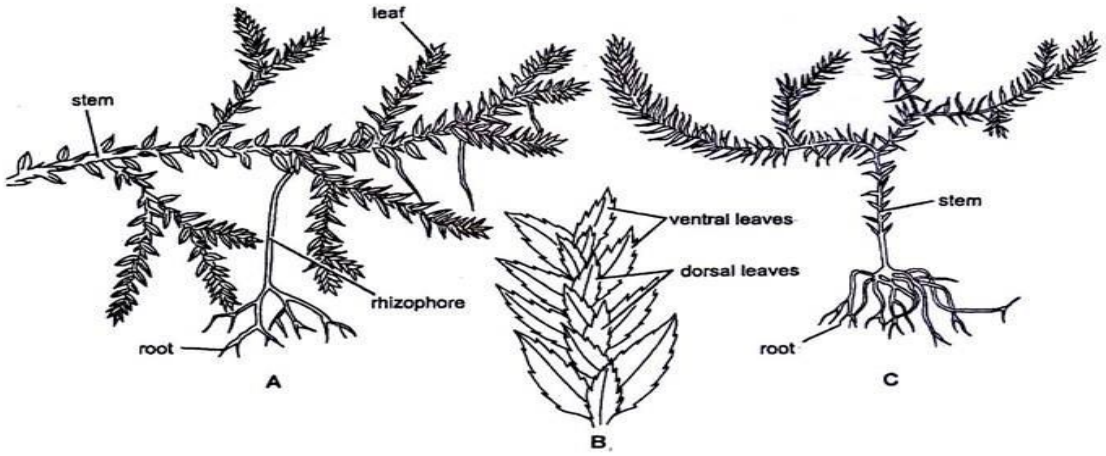


Fig. 1 (A–C). *Selaginella*. External features : A. *S. kraussiana*, B. Leaf arrangement in a branch of *S. kraussiana*, C. *S. spinulosa*

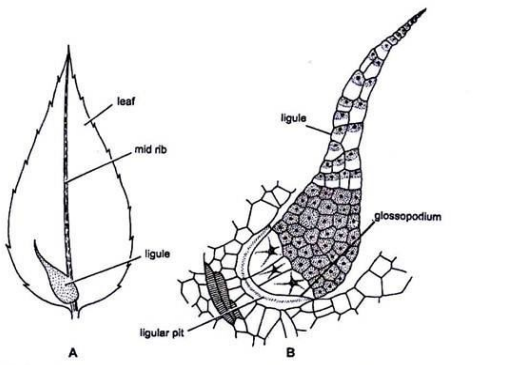


Fig. 2 (A, B). *Selaginella*. Structure of ligule : A. Leaf with ligule, B. Longitudinal section of ligule

5. In homoeophyllum species (*S. selaginoides and 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 dorsiventral 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 ovate with acute apex and has a distinct midrib.
11. At the base of each young leaf, on the adaxial face there is small tongue-like outgrowth the ligule.
12. It is differentiated into basal sheath, glossopodium and the body of the ligule.
13. Whereas the cells of the sheath are tubular 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 rhizophore is still open to question).
17. Certain vertical branches from the stem reproductive in nature and bear strobili.

Features of special Interest

1. Presence of rhizophore.
2. Dimorphic leaves (in heterophyllous species).
3. Presence of ligule.

Exercise 2

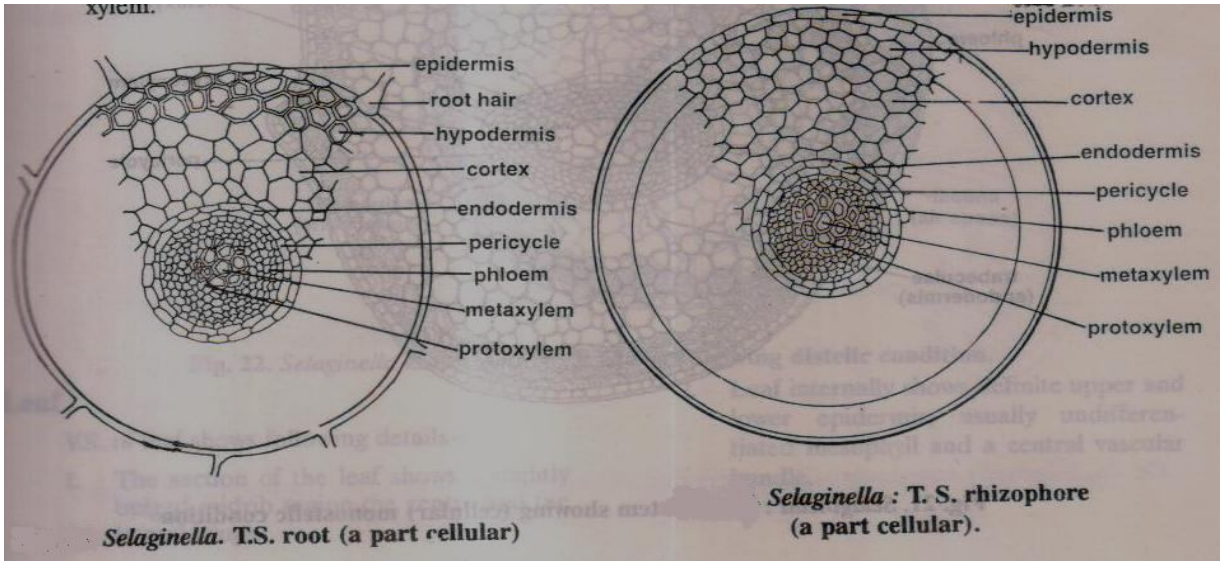
To study anatomy of the root

Work procedure

Cut a T.s. of the root, stain in safranin-fast combination, mount in glycerine and study.

Comments

1. The section is almost circular in outline.
2. The tissues are differentiated into epidermis, cortex and stele.
3. The epidermis is single layered and its cells are tangentially elongated. Few of these cells give rise to the root hairs.



Selaginella. T.S. root (a part cellular)

Selaginella : T. S. rhizophore (a part cellular).

4. Cortex may either be made of parenchymatous (thin walled) cells or the outer layer of cells may form a sclerenchymatous (thick walled) hypodemis.
5. The stele lies in the centre. It is protostelic monarch and exarch.
6. Endodermis is one layered and generally indistinct.
7. Pericycle is one to three layered.
8. Xylem forms only one group. Protoxytem is situated towards the periphery.
9. Phloem surrounds the centrally located xylem.

Exercise 3

Study of anatomy of rhizophore

Work procedure

Cut a T.s. of the rhizophore, stain in safranin-fast green combination, mount in glycerine and study. Anatomically, the structure of the rhizophore is similar to that of root, with some minor differences which occur on account of its environment.

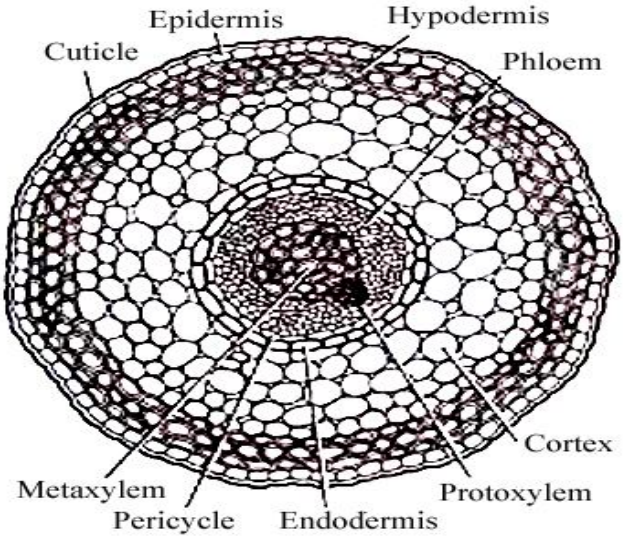


Fig: *Selaginella spp.* T.S of rhizophore.

Comments

1. The outline of the section is almost circular.
2. The section shows epidermis, hypodermis, cortex, endodermis and stele.
3. The epidermis is cuticularised.
4. Hypodermis that follows is 2-3 celled thick.
5. Cortex is few celled and parenchymatous. It occupies most of the part of section.
6. Endodermis is present between the stele and the cortex. It is followed by a single layered parenchymatous pericycle.
7. The stele is a protostele. It shows monarch and exarch condition. In some species (e.g. *S. atroviridis*) the metaxylern is lunar shaped and many protoxylem groups are situated on the concave adaxial side.

Exercise 4

Study of anatomy of 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 (towards periphery) but thick walled cells form hypodermis.

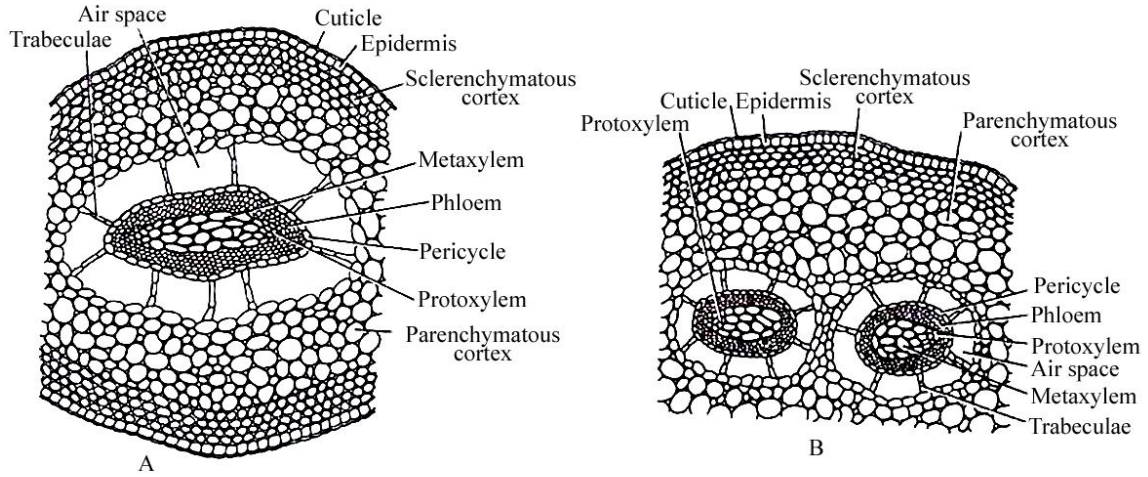


Fig: *Selaginella spp.* T.S of Stem. (A) T.S of monostelic stem; (B) T.S of distelic stem.

5. Hypodermis occurs close to epidermis. It develops from cells of outer cortex which become thick walled. In xerophytic species (e.g. *S. rupestris* and *S. lepidophylla*) 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. 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.

Features of special interest

1. Presence of modified endodermis in the form of tube like trabeculae.
2. Presence of more than one stele i.e. polystelic condition.

Exercise 5

Study of leaf anatomy
Work procedure:
Cut a T.s of leaf, stain in safranin-fast green combination, mount in glycerine and study.

Comments
1. The section shows a slightly bulged midrib in the centre and the wings.
2. It shows definite upper and lower epidermis usually undifferentiated mesophyll and a central vascular bundle.
3. The epidermis is one layered. The stomata are generally present on the abaxial surface (lower) but may also be present on the adaxial surface (upper) or on both the surfaces.
4. The mesophyll is usually not differentiated into palisade and spongy parenchyma. It shows many conspicuous intercellular spaces.
5. The cells of mesophyll contain chloroplasts, each of which has several pyrenoid-like bodies.
6. The vascular bundle is concentric with xylem surrounded by phloem and is bounded by a bundle sheath.

Exercise 6

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.

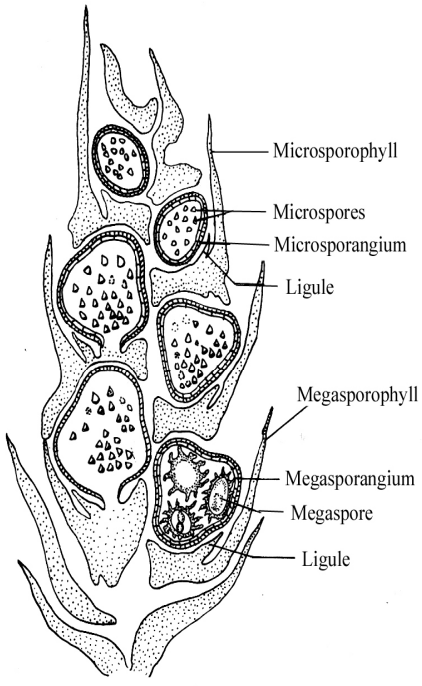
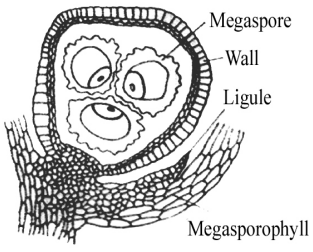
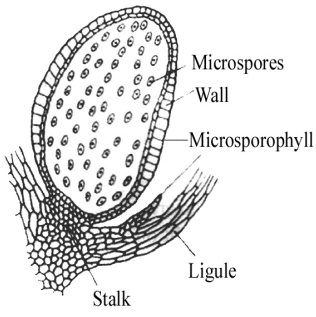


Fig: *Selaginella spp.* L.S of strobilus.



Mature megasporangium



Mature microsporangium

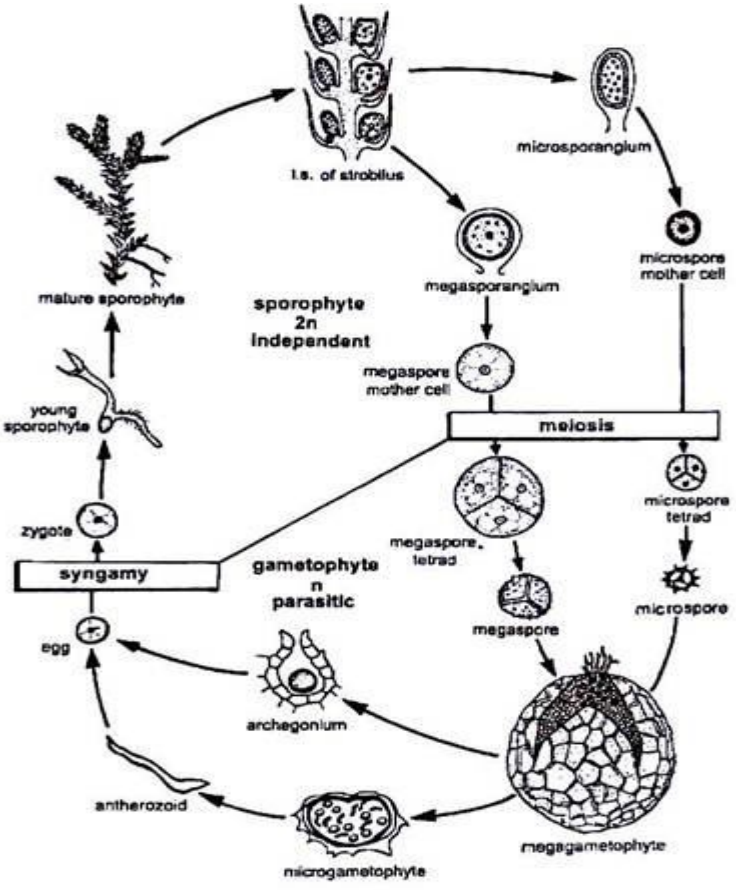
Comments
1. The spore producing organs are sporangia, aggregated in strobili which are generally present at the apices.
2. In some cases (*S. patula*) the axis may grow beyond the strobilus terminating into a vegetative shoot or even in a second strobilus.
3. L.s of the strobilus shows a strobilar axis, around which sporophylls are spirally arranged. Each sporophyll is ligulate and similar to a foliage leaf.
4. The sporangia are of two types (micro and mega sporangia), borne in the axils of the sporophylls attached either strictly to the axils or to the axis just above.
5. *Selaginella* is a **heterosporous** with megaspores (large) and microspores (small), borne in their respective sporangia, known as megasporangia and microsporangia.
6. If a microsporangium 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*, only one type of sporangia (either mega-or micro sporangia) are found in a cone.
8. When both kinds of sporangia occur in one or on the same strobilus, their arrangement differs from species to species:

(I) In some species (e.g. *S. oregana*) 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. Mature sporangia, 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.



Nephrolepis (Sword Fern)

Nepros-kidney, lepis-the indusim, kidney shaped and scale like, is represented by about 30 species. These species are distributed in the tropics of the entire world. About five species *N. cordifolia* (fish born fern), *N. exaltata* (sword fern), *N. valubilis*, *N. acuta* and *N. ramose*are found in India. Majority of the species are terrestrial but a few species e.g. *N. ramose* are found climbing on the tree. N. *cordifolia* is found throughout the Indian region upto 500 feet elevation. Some species are also grown as ornamental e.g. *N. tuberosa* and *N. acuta* etc.

Classification

Division	Pteridophyta
Sub-division	Pteropsida
Class	Leptosporangiatae
Order	Filicales
Family	Polypodiaceae
Genus	*Nephrolepis*

Exercise 1

Study of external morphology

Work procedure

Study the characters of roots, rhizome and leaves of a potted plant or a museum specimen.

Comments

1. The plant body is a sporophyte (2n). It is differentiated into roots, rhizome and leaves.
2. The rhizome gives out adventitious roots from its underside. These adventitious roots are small and branched.
3. The stem is modified to rhizome. It is subterranean, short and erect. The rhizome produces elongated slender stolons. Peltate scales cover the rhizome.
4. In *N. tuberosas* rhizome bears tubers. These are reservoirs of carbohydrates and water.
5. The leaves are long, narrow, sub-coriaceous and unipinnate.
6. The pinnae are sessile or shortly petioled. They have a usually rounded or cordate base. Each pinna has articulation with a pouch-like structure at the base.
7. The veins are prominent and the veinlets are branched with open ends. The tips of veinlets are gland dotted and they extend up to the margins.

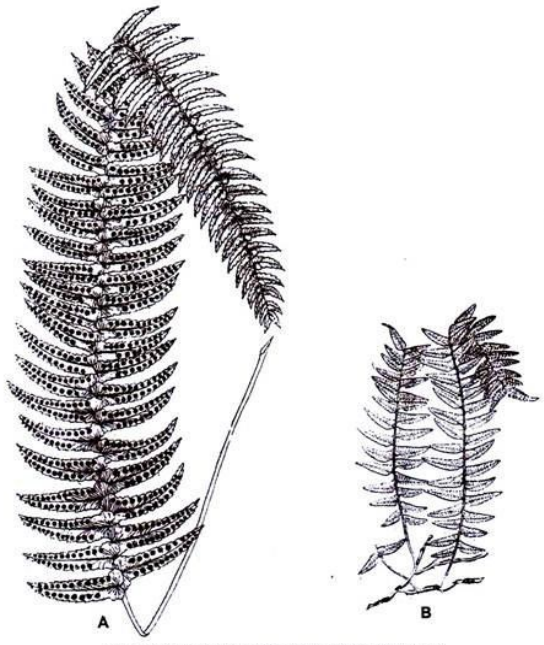


Fig. 2 (A, B). Leaves of *Nephrolepis*. (A) *N. cordifolia*, (B) *N. ramosa*

Exercise 2

Study of anatomy of the root
Work procedure
Cut a thin transverse section of the root, stain with safranin-fast green combination. Mount in glycerine and study.

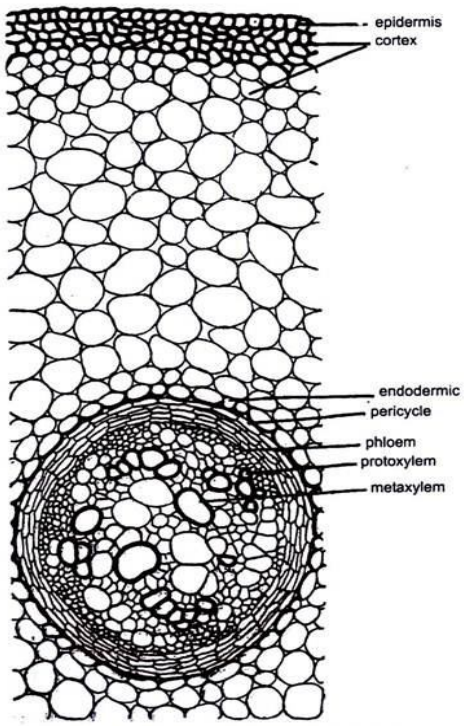


Fig. 5 *Nephrolepis.* Transverse section of runner

Comments
1. The outline of the section is almost circular.
2. The tissues are differentiated into-epiblema, cortex and the vascular cylinder.
3. Epiblema is the outermost single layer of cells. The cells are unicellular and thin walled. A few root hairs are produced by this layer.
4. Cortex forms the major part of the section. It shows outer parenchymatous region and the inner small sclerenchymatous region.
5. Endodermis follows the cortex and separates it from the vascular tissues. The cells of endodermis show casparian strips.
6. Endodermis is followed by I or 2 layered parenchymatous pericycle.
7. Vascular cylinder is represented by a radial diarch and exarch vascular bundle.

Exercise 3

Study of anatomy of rhizome
Work procedure:
Cut a thin transverse section of rhizome, stain in safranin-fast green combination, mount in glycerine and study.

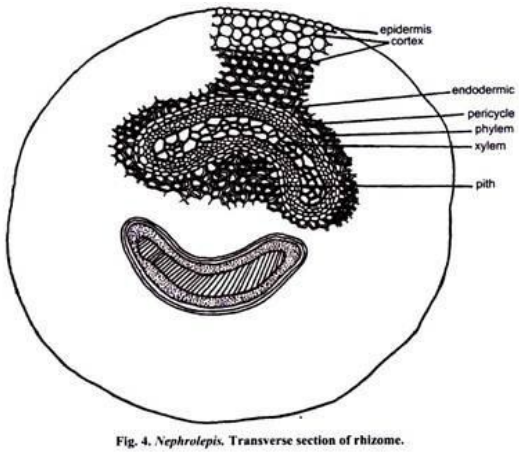


Fig. 4. *Nephrolepis.* Transverse section of rhizome.

Comments
1. The outline of the section is almost biconvex.
2. The section can be divided into epidemis, hypoderrnis, ground tissue and the stele.
3. Epidermis is the outermost single layer of thickly cuticularised cells.
4. Hypodermis that follows epidermis is made of a few sclerenchymatous layers.
5. Rest of the tissue is called ground tissue. It is parenchymatous with numerous starch grains in the cells.
6. The structure of the stele varies with the age of the rhizome.

i. In youngest part of rhizome, it is protostele.
ii. In a few weeks old plant with a few leaves, the rhizome shows ectophloic siphonostele.

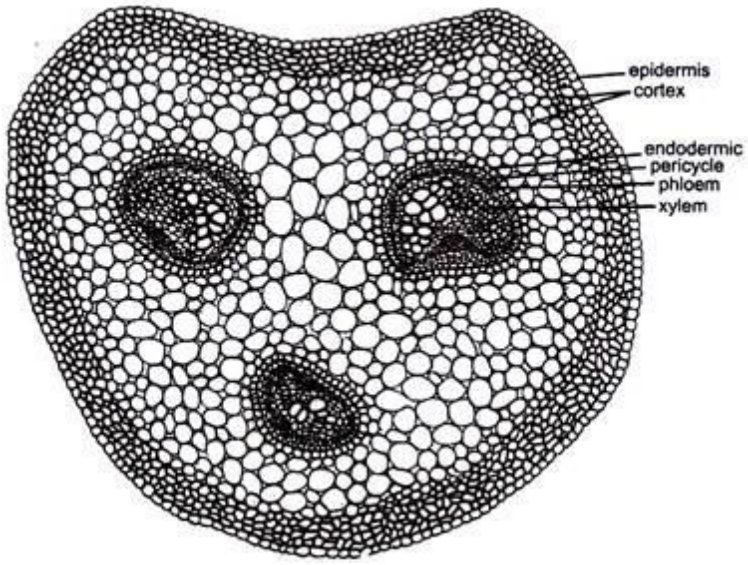
iii. The old part of rhizome shows a dictyostele.

7. Dictyostele is made of two rings of meristele, separated by two sclerenchymatous bands.
8. Meristele has its own endodermis and pericycle. The centre is occupied with xylem which is completely surrounded by phloem on all its sides.

Exercise 4

Study of anatomy of rachis
Work procedure
Cut a thin and uniform transverse section of the rachis, stain with safranin-fast green combination, mount in glycerine and study.

Comments



1. The section appears horse-shoe shaped.
2. It shows epidermis, hypodermis, ground tissue and the stele.
3. Epidermis is made of single layer of thickly cuticularised cells.
4. Hypodermis lies below the epidermis. The cells are sclerenchymatous.
5. The rest of the parenchymatous region extending throughout the section is called ground tissue.
6. In the ground tissue is situated U-shaped or horse-shoe shaped stele.
7. The stele is surrounded by a single layered endodermis followed by a few layered thick pericycle.
8. Centrally located xylem is surrounded by phloem on all sides.
9. The structure of the stele differs at various levels of rachis—

I. In younger parts, there is a single U-shaped stele.

II. Little above the base, the U-breaks at the bottom, thereby producing two stele.

III. In mature plants, dissection of the stele results in many meristeles.

Exercise 5

Study of life cycle (structure of the sporophyll)
Work procedure:
Observe the underside of the sporophyll, cut a transverse section of pinnae passing through a sorus. Stain in safranin-fast green combination, mount in glycerine and study.

Comments
1. The leaf bearing sori is called sporophyll.
2. The sporangia are present on the lower side of mature pinnae. These occur in groups called sori.
3. Sori are superficial and form definite rows, one on either side of the vein.
4. The sorus appears semi-rounded, and arises at the tip of the veinlet.
5. The sori are indusiate. The indusium is reniform (kidney-shaped) roundish or sub-orbicular.

6. Each sporangium has a stalk and a capsule.
7. The stalk is long, slender and multicellular.
8. The wall of sporangial capsule is one celled thick. A ring of thick walled cells called annulus is present. A few thin walled cells forming stomium are situated in the ring.
9. The capsule wall encloses 32 or 64 spores. All the spores are similar, the fern being homosporous.

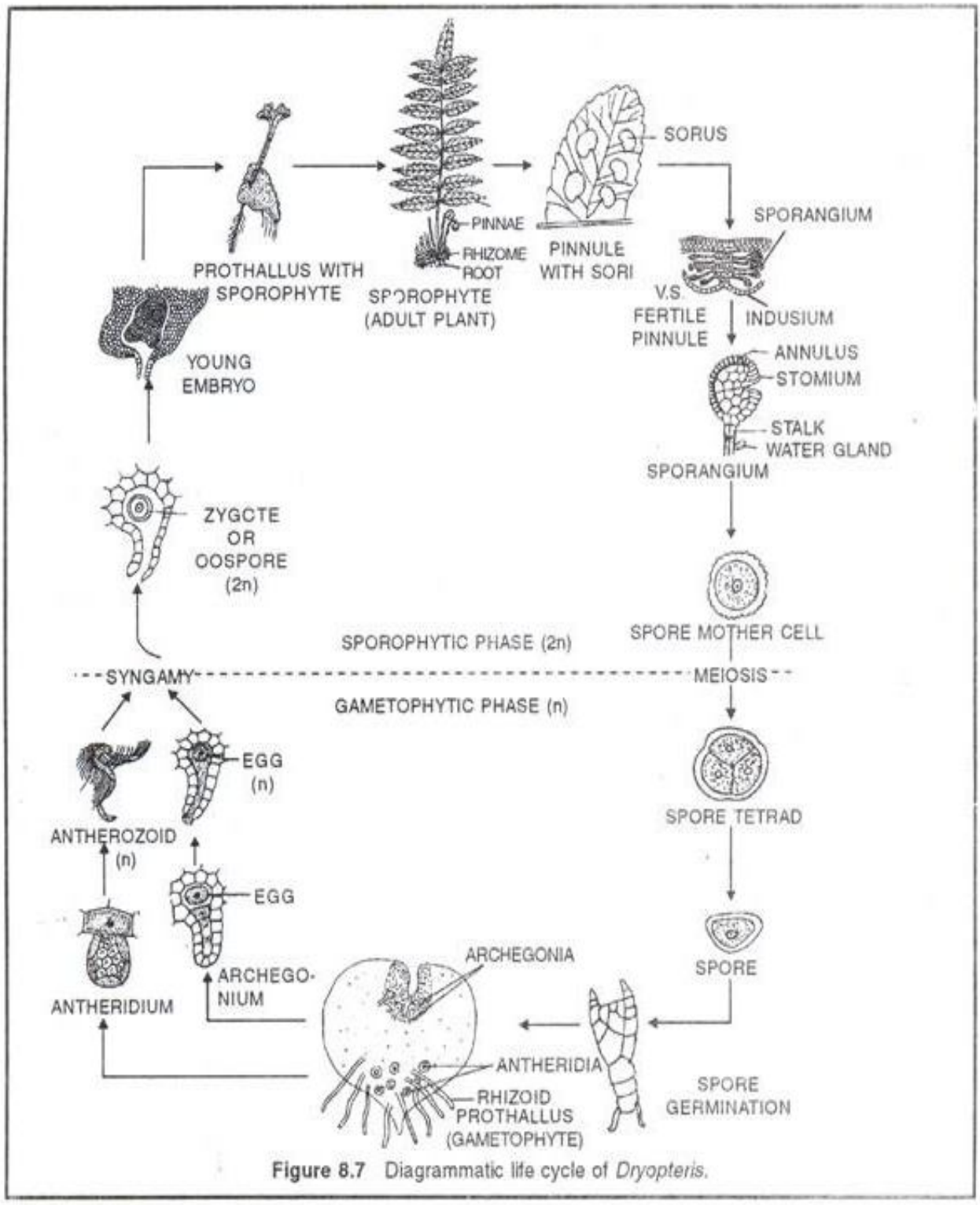


Figure 8.7 Diagrammatic life cycle of *Dryopteris*.

Pteridium
(Bracken Fern)

Classification

Division	Pteridophyta
Sub-division	Pteropsida
Class	Leptoporangiatae
Order	Filicales
Family	Polypodiaceae
Genus	*Ptridium*

Pteridium is monotypic germs, represented by a single species *P. aquilinums.* Reimers, 1954 is one of the oldest known fern. It is cosmopolitan in distribution and occurs in both temperate and tropical region of the world. In India it is commonly found at an elevation of 2000 to 3000 feet. It is one of the primary colonizers of the land and once it is established, it does not allow other fern to grow in that area.

Exercise 1

Study of external morphology of plant
Work procedure:
Study a fresh plant or a preserved specimen, the differentiation of plant body into roots, rhizome and leaves.

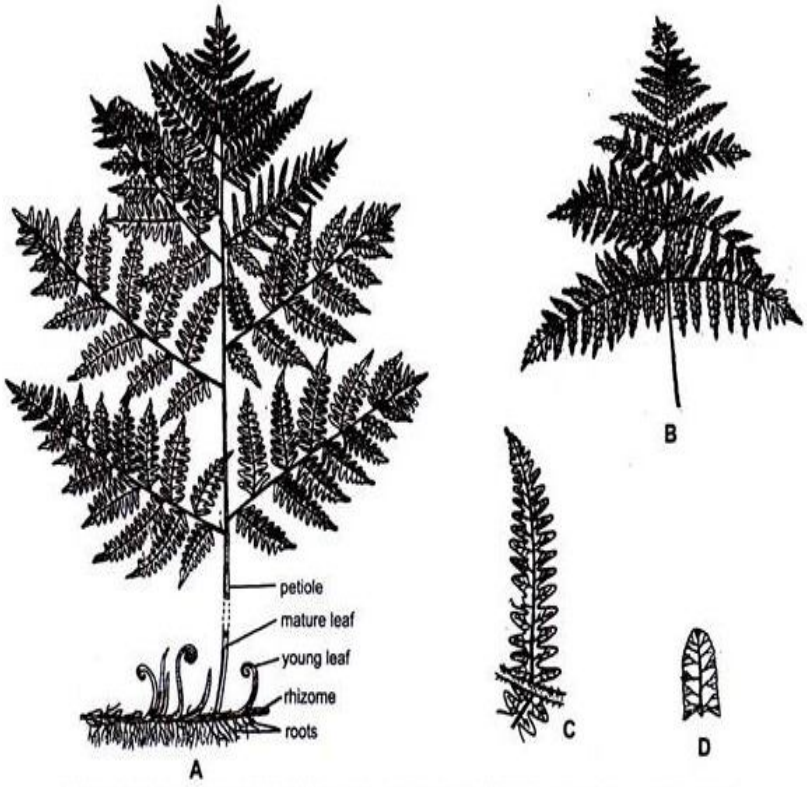


Fig. 1. *Pteridium* (A-D). A. complete sporophyte ; B. A tripinnate leaf ; C. A pinnule enlarged ; D. The ultimate pinnule.

Comments

1. The plant body is a sporophyte, it is differentiated into roots, rhizome and leaves.
2. Stem is modified to rhizome, It is subterranean, the rhizome is long, slender and dichotomously branched. It is covered with brown and multicellular hairs called ramenta.
3. The rhizome gives out adventitious roots on its underside. These are small and branched.
4. The leaves are borne alternately on the upper side of the rhizome at the nodes.
5. The young leaves are circinately coiled. The rachis is covered with 'ramenta.
6. Each leaf is tripinnately compound. Each pinna is sessile. It has a distinct midrib that gives out lateral branches.

Exercise 2

Object: Study of anatomy of the root
Work procedure
Cut a thin transverse section of the root, Stain with safranin-fast green combination. Mount in glycerine and study.

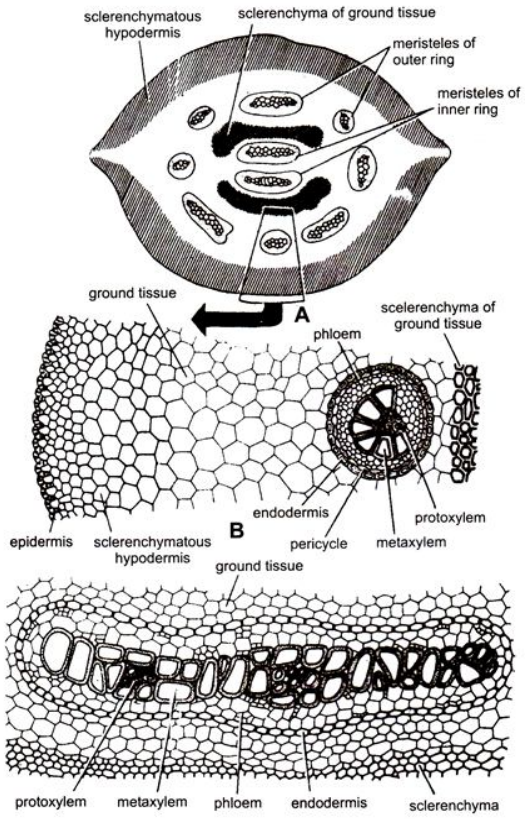


Fig. 2 (A – C) *Pteridium*. A. T.S. of rhizome (diagrammatic); B. A part magnified; C. A meristele.

Comments

1. The outline of the section is almost circular.
2. It shows three regions—epiblema, cortex and the vascular cylinder.
3. Epiblema is the outermost single layer of cells. The cells are thin walled and produce unicellular root hairs.
4. Cortex occupies most part of the section. It is differentiated into outer and inner regions.
5. The outer region is parenchymatous, while the inner few layers are sclerenchymatous.
6. Endodermis follows the cortex. The radial walls of endodermal cells are characterized by casparian thickenings.
7. Pericycle is situated inner endodermis. It is 1 or 2 layered and parenchymatous.
8. Vascular cylinder shows radial, diarch and exarch conditions.
9. The xylem consists of two central metaxylem tracheids with groups of small protoxylem elements on their both sides.
10. Phloem is present on both the sides of xylem plate.

Exercise 3

Object: Study of anatomy of rhizome
Work procedure
Cut a transverse section of rhizome, stain in safranin-fast green combination, mount in glycerine and study.

Comments

1. The outline of the section appears almost like a biconvex lens.
2. The tissues are differentiated into epidermis, hypodermis, ground tissue and the stele.
3. Epidermis is the outermost single layer of cells. The cells are thickly cuticularized.
4. Hypodermis lies below the epidermis. The cells are sclerenchymatous, which often show pitted walls. It is generally interrupted on the lateral sides by parenchyma.
5. Ground tissue follows the hypodermis. It is parenchymatous and is spread up to the centre of the section. The cells are filled with starch grains.
6. The structure of the stele varies with the age of the rhizome.

i. In just formed rhizome, condition is protostelic.

ii. In a few weeks old plant with 2-3 leaves, the rhizome shows ectophloic siphonostele.

iii. In mature plant, the old pant of rhizome shows a dictyostele.

7. Dictyostele is made of meristeles arranged in two rings, separated by two selerenchymatous bands.
8. Meristele is surrounded by its own endodermis, which is followed by one or two layers of parenchymatous pericycle.
9. The centre of the meristele is occupied by xylem, which is completely surrounded by on all sides.

Exercise 4

Study the anatomy of rachis
Work procedure
Rachis is thin and wiry, hence a sharp blade or razor would be required to cut a section, cut transverse section, stain in safranin-fast combination, mount in glycerine and study.

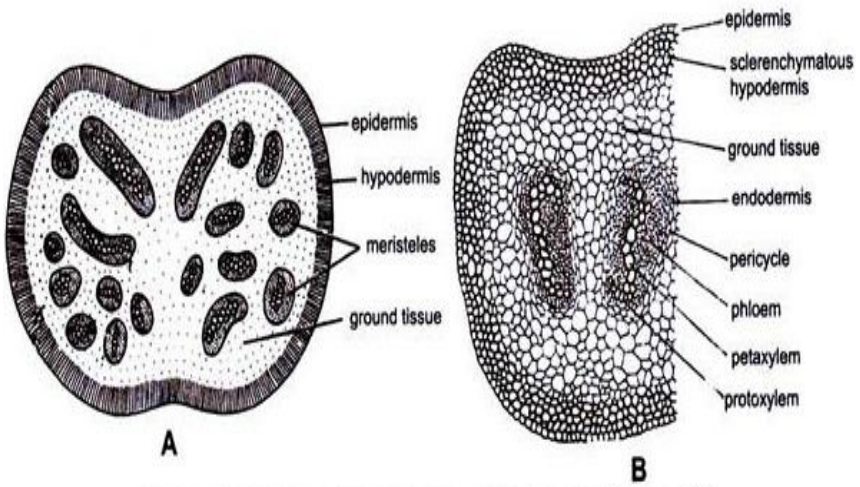


Fig. 3 (A, B) *Pteridium* A. T.S. petiole (diagrammatic); B. A portion magnified.

Comments
1. The outline of the section appears horse-shoe shaped or hemispherical.
2. The tissues of the section are differentiated into epidermis, hypodermis, ground tissue and the stele.
3. Epidermis which is the outermost single of cells is thickly cuticularised.
4. Hypodermis is present below the epidermis. It is 2 to 3 layered thick. The cells are sclerenchymatous.
5. Following the hypodermis is large region of parenchyma called ground tissue.
6. In the ground tissue, is situated U-shaped or horse-shoe shaped stele.
7. Stele surrounded by a single layered endodermis, followed by a few layered parenchymatous pericycle.
8. The centre of the stele is occupied by a massive xylem. Metaxylem is present in centre with protoxylem located at two of its ends.
9. The region between xylem and pericycle is filled by phloem.
10. The nature of the stele varies with the maturity of the rachis.

i. In younger parts, stele is U-shaped.
ii. Little above the base, it gets dissected two large meristeles.
iii. In mature plant, many meristeles are present as a result of further dissection of original stele.

Exercise 5

Study of structure of the sporophyll
Work procedure
Cut a vertical transverse section of the pinnule that has sori on the lower side. Stain in safranin and mount in glycerine. Study the characters of sorus and the sporangium.

Comments
1. The leaf bearing sori is called sporophyll.

2. The sporangia occur in groups called sori on the lower or abaxial side of pinnules. The sporangia form a continuous linear sorus along the margins. Such a confluent sorus is called coenosorus. The identity of sorus is thus lost and only one long sorus appears along the two lateral margins of the fertile pinnules.
3. The sorus is protected by indusium. It is made of upper indusial flap formed by the incurved margins of the pinnule and the lower true indusial flap that is poorly developed.
4. The sporangia in the sorus occur mixed. The development is leptosporangiate.
5. Each mature sporangium is differentiated into a stalk and a capsule.
6. The stalk of the capsule is made of three rows of cells. It is long and slender.
7. The capsule is ovate or biconvex. The sporangial jacket is single layered thick. A ring of thick walled cells forms .the annulus. A few thin walled cells of the ring form the stomium. The capsule wall encloses 32 or 64 spores.
8. All the spores are similar, the fern is homosporous. Spores are haploid and uninucicate. The wall is two layered. The outer thick layer is called exine and the inner thin layer is called intine.

Exercise 6

Study of structure of prothallus

Work procedure

Study a slide of prothallus showing sex organs. Note the positions of sex organs and also the young sporophyte.

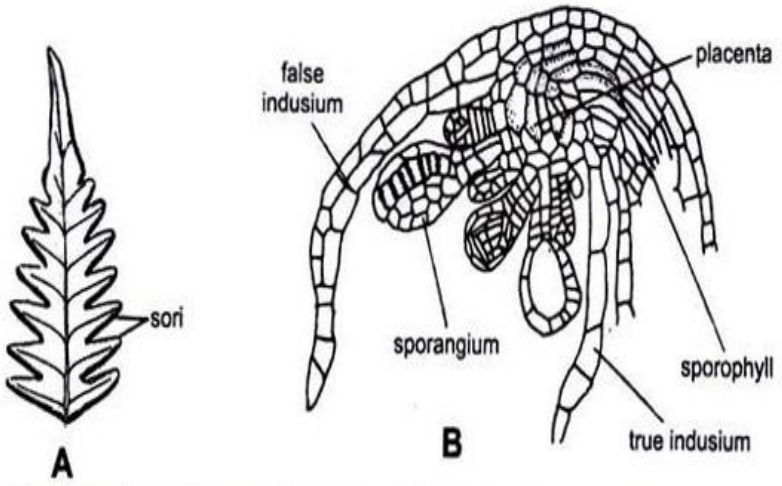


Fig. 6. (A, B) *Pteridium.* (A) A fertile pinnule; (B) Vertical section of sporophyll showing sporangia and indusium.

Comments

1. The prothallus is a gametophyte, formed as a result of spore germination.
2. It is dark green, heart-shaped and single layered sheet of cells. The midrib region becomes a cushion of several cells. It remains attached to the substratum by rhizoids produced on the lower side in the central region.
3. The antheridium is surrounded by the cells of the prothallus. Each antheridium consists of walls of three rings of cells. It encloses 30-40 multiflagellate antherozoids at maturity.

Archegoina develop near the apical notch. Each is made of neck and venter. The neck is 5-7 celled high with a single binucleate neck canal cell. The venter has a small venter canal cell and a large egg

Marsilea (water fern)

Classification

Division	Pteridophyta
Sub-division	Pteropsida
Class	Leptosporangiatae
Older	Marsileales
Family	Marsileaceae
Genus	*Marsilea*

Plants of *Marsilea* are distributed worldwide, both in the temperate as well as in the tropical region. Most of the species e.g. *M. minuta, M. quadrifolia* are aquatic or semiaquatic hydrophytes grows in ponds and puddules after the rain. They grow partially or entirely submerged in water with their roots in the soil or mud and produce sporocarp under water. Some species e.g. *M. vestita, M. aequptiaca* are amphibious and grow in temporary water reservoirs but produce sporocarps under dry terrestrial condition, when water dries up. *M. condensata* and *M. rajasthanensis* are xerophytic in nature.

Exercise 1

Study of external morphology

Work procedure:

Study external characters of the plant. Observe various features of root, rhizome and leaves. Note the circinate venation of young leaves and the characteristic leaf venation.

Comments

1. The plant body is differentiated into a rhizome, roots and leaves.
2. The rhizome is slender, creeping and branched. It may either grow in water or attached by roots in the damp soil.
3. It bears nodes and internodes. The leaves and roots occur in acropetal succession (youngest towards the apex of rhizome) on the nodes. The adventitious roots grow downwards and the leaves grow upwards. Young leaves are circinately coiled, a characteristic of most ferns.
4. The leaves present at the nodes occur in two rows, (two ranked) one on either side of the mid-line of the rhizome.
5. Each leaf consists of a long petiole, bearing at its top generally four leaflets or pinnae, apparently arising from one common point. In *M. quadrifolia*, a common Indian species, six leaflets are found. (Puri and Garg. 1953, call the leaflets as pinnules).

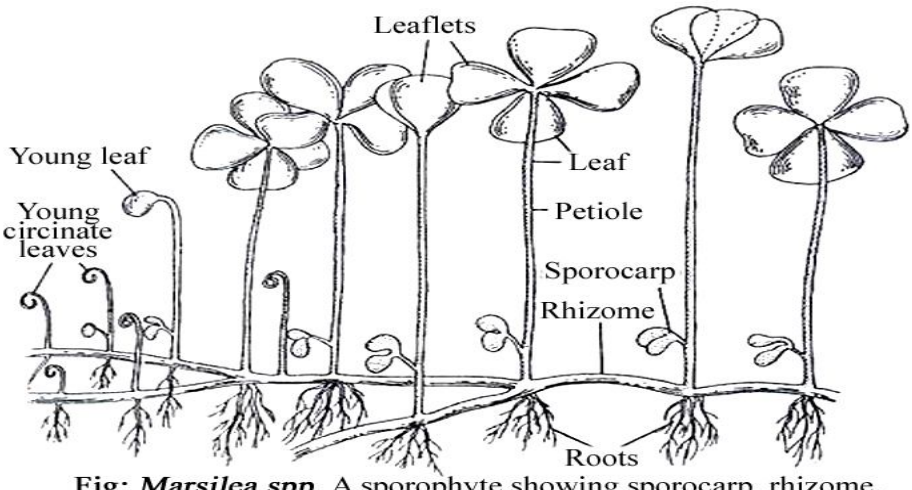
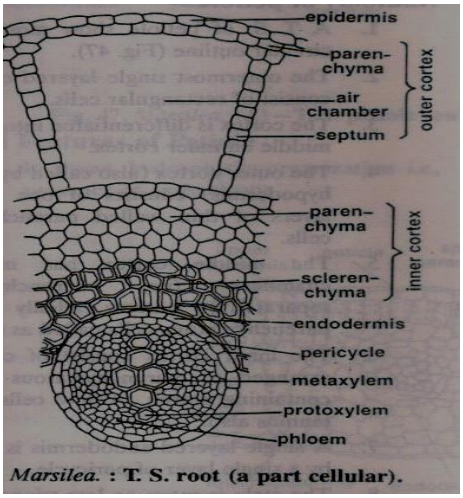


Fig: *Marsilea spp.* A sporophyte showing sporocarp, rhizome, leaves and roots.

6. The division of lamina into four pinnae is the result of three dichotomies, close to each other. Therefore, out of the four leaflets, two form a distal pair while the lower two are alternate. The leaflets, thus give a false impression of arising from one common point.
7. Each leaflet is obovate. The venation is dichotomous with several cross connections. The free veinlets at the apex of the leaflet are tied up with marginal loops.
8. Leaflets fold up in the night or early morning, thus showing sleeping movements.
9. The plant when grows in water, has long, flexible petioles and the leaflets float on the surface of the water but when it grows on mud or damp soil, the petioles become short and rigid, is interesting to note that when in a pond in which *Marsilea* is growing, water level rises, the petioles are also seen to increase in length. Contrary to it, when level goes down, the petioles are found to coil, as such in both the conditions, the leaflets float on water surface).
10. The spore bearing structures known as sporocarps are commonly borne laterally near the base on the petiole, but sometimes higher up. The two common Indian species, *M. minuta* and *M. quadrifolia* show variation in the number of sporocarps from one to four.

Exercise 2

Study of anatomy of the root
Work procedure
Cut a T.s. of the root, stain in safranin-fast green combination, mount in glycerine and study.



Marsilea. : T. S. root (a part cellular).

Comments
1. The outline of the section appears almost circular.
2. The epidermis is single layered with tangentially elongated cells.
3. The cortex is differentiated into an outer and an inner cortex.
4. The outer cortex has many air chambers separated by radial septa.
5. The inner cortex has either all the parenchymatous cells or some of the cells towards the inner side may become thick walled and sclerenchymatous.
6. Endodermis is single layered. It is followed by one layered pericycle. These surround vascular bundle.
7. Xylem is diarch and exarch. It is situated in the centre. The protoxylem elements are situated opposite one another.
8. The phloem has smaller cells and forms two bands, one on either side of the xylem mass.

Features of special interest
The root shows aerenchyma in the outer cortex (hydrophytic character).

Exercise 3

Study of anatomy of rhizome
Work procedure:
Cut a T.s. of rhizome, stain in satranin-fast green combination, mount in glycerine and study.
Comments
1. The outline of the section appears almost circular.
2. The section shows three regions—epidermis, cortex and stele.
3. The epidermis is single layered without stomata. The epidermis of aquatic plants lacks cuticle but that of terrestrial individuals has a distinct cuticle.

4. The cortex is differentiated into three regions—the outer, the middle and the inner.
5. The outer cortex has well developed air spaces, separated by radially arranged parenchymatous cells (aerenchyma). The outer most cells of the cortex contain chloroplast.
6. The middle cortex is thick walled, made of sclerenchymatous cells and is only a celled thick.
7. The inner cortex is composed of thin walled parenchymatous cells containing starch.
8. The stele is an amphiphloic solenostele.
9. Stele shows a central xylem ring. On its outer side is outer phloem ring, outer pericycle and outer endodermis. On the inner side, towards the pith are present inner phloem ring, inner pericycle and inner endodermis.
10. Protoxylem groups may or may not be distinct. They are generally exarch, but in some cases mesarch too.
11. Pith lies in the center. In aquatic plants, it is parenchymatous and in terrestrial plants it is sclerotic.

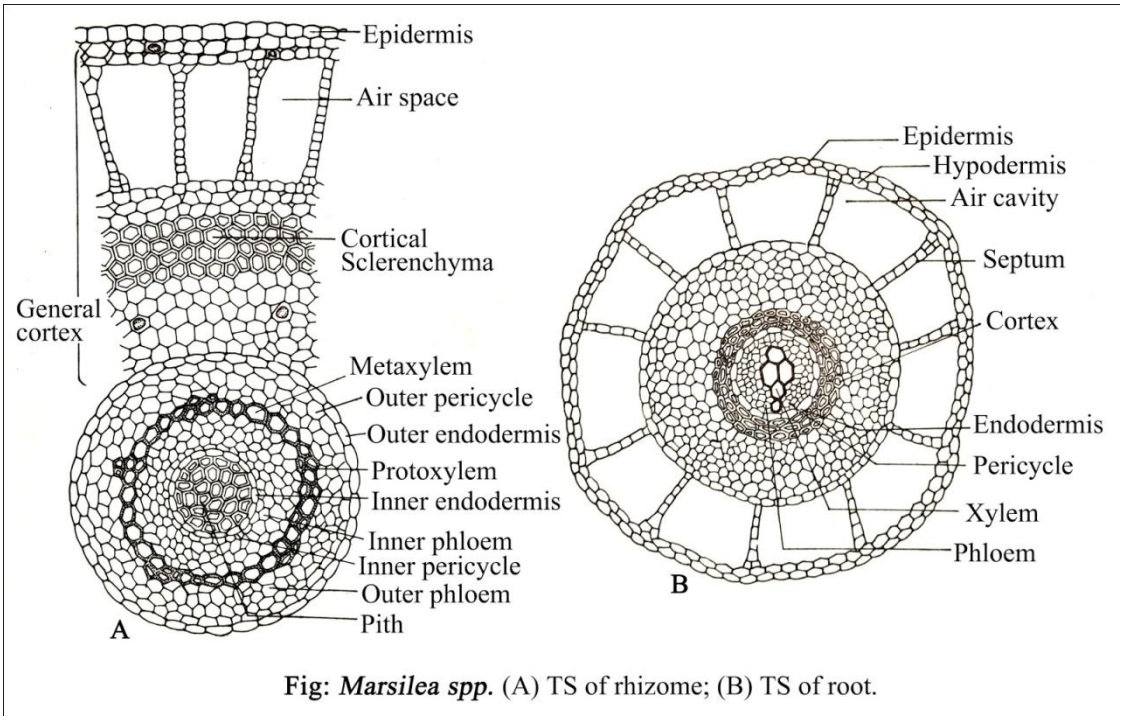


Fig: *Marsilea spp.* (A) TS of rhizome; (B) TS of root.

Features of special interest

1. It shows hydrophytic character viz. presence of aerenchyma in the cortex as well as xerophytic characters viz. thick walled middle cortex and sclerotic pith
2. Presence of amphiphloic solenostelc.

Exercise 4

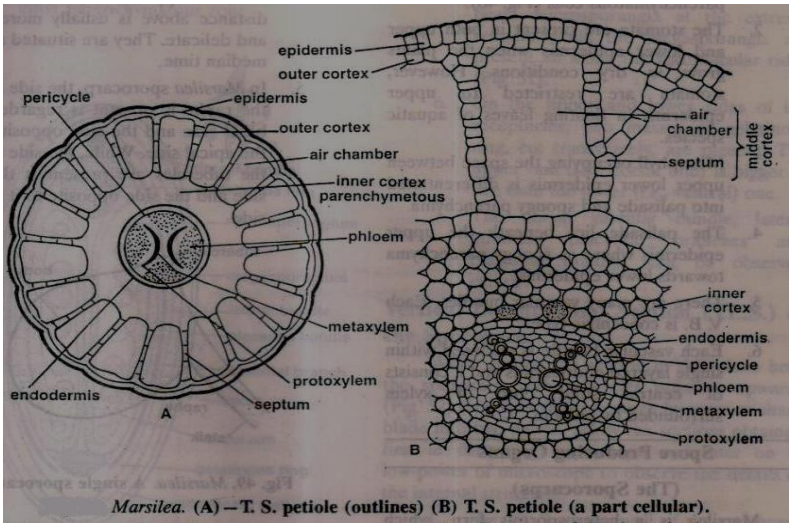
Study of anatomy of petiole

Work procedure

Cut a T.s. of petiole, stain in safranin-fast combination, mount in glycerine and study.

Comments

1. The outline of the section is circular.
2. Epidermis is the outermost layers of rectangular cells.
3. Hypodermis is sometimes present below epidermis. It is one or two layered.



Marsilea. **(A)—T. S. petiole (outlines) (B) T. S. petiole (a part cellular).**

4. The cortex is differentiated into an outer and an inner zone.
5. The outer cortex has many air chambers, separated by narrow radially arranged parenchymatous cells (aerenchyma).
6. The inner cortex has parenchymatous cells containing starch. A few cells contain tannins also.
7. The stele is a protostele.
8. Endodermis is single layered. It is followed by a single layer of pericycle.
9. **The xylem is V shaped' with** exarch protoxylem. The two arms of 'V' are slightly curved and separate. Each arm has generally one or two large tracheids in the middle and smaller tracheids towards both the ends. The open end of V always points towards the adaxial side of the petiole (towards the axis).
10. Phloem surrounds the xylem.

Features of special interest

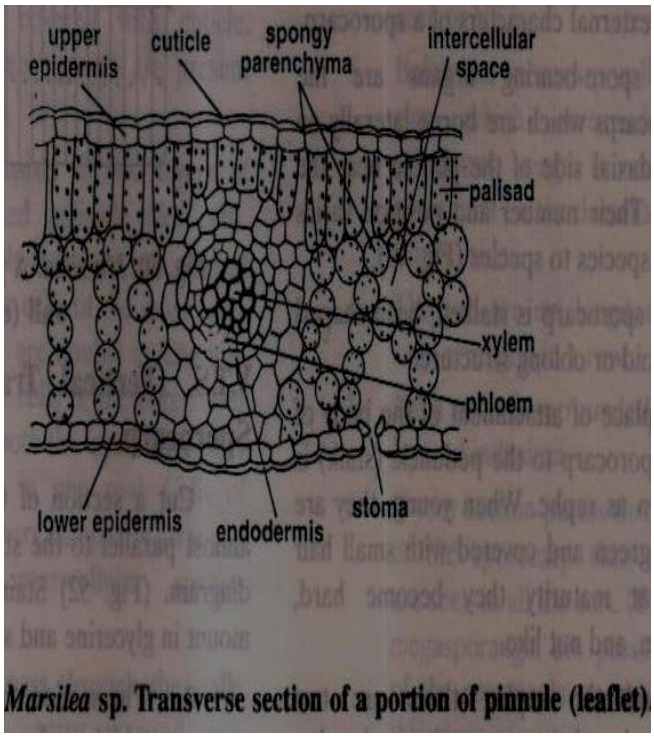
1. Shows hydrophytic character viz. presence of aerenchyma in the outer cortex.
2. Presence of V-shaped xylem.

Exercise 5

Study of anatomy of leaflet

Work procedure

Cut a T.s. of the leaflet, stain with safranin-fast green combination, mount in glycerine and study.



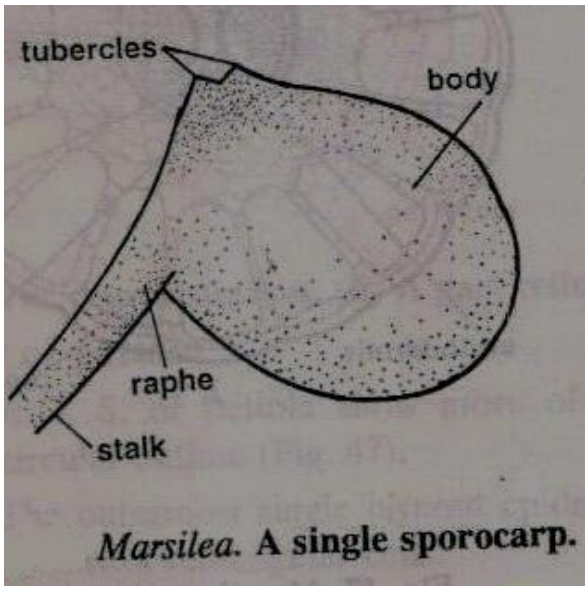
Marsilea **sp. Transverse section of a portion of pinnule (leaflet).**

Comments

1. The section shows an upper and lower epidermis, mesophyll and a vascular bundle.
2. The stomata are found on both upper and lower epidermis if the plant is terrestrial but they are found only on upper epidermis if leaves float on water surface.
3. Mesophyll is differentiated into palisade and spongy parenchyma.
4. Palisade is arranged in one layer near the upper epidermis. Spongy parenchyma is located near the lower epidermis. It is loosely arranged to form large air spaces and is called aerenchyma.
5. There are many vascular bundles. Each bundle is concentric with centrally located xylem surrounded by phloem.
6. The distinct endodermis is present just outside the vascular bundle.

Exercise 6

Study of external features of sporocarp
Work procedure
Study the external characters of a sporocarp.



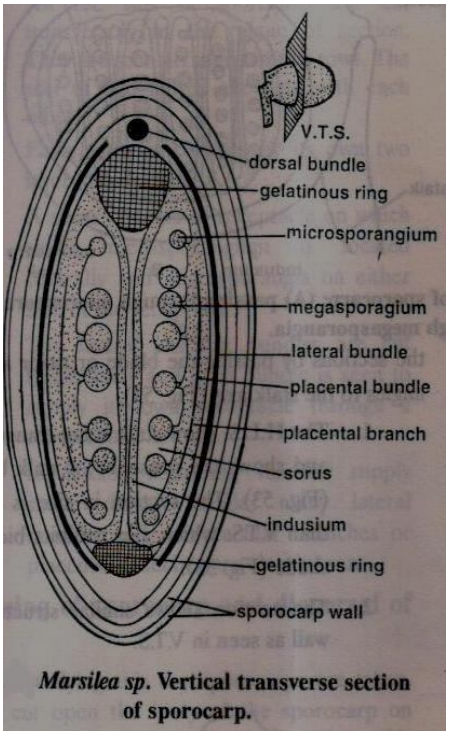
Marsilea. **A single sporocarp.**

Comments

1. The spore-bearing organs are the sporocarps which are borne laterally on the adaxial side of the petiole. Their number and positions varies from species to species.
2. Sporocarp is stalked, bean-shaped or ovoid structure.
3. The place of attachment of the body of the sporocarp to the peduncle (stalk) is known as raphe.
4. Beyond the raphe, there are two projections known as teeth or tubercles, one tooth being lower than the other.
5. The lower tooth is usually stouter and more prominent while the upper tooth, which lies a short distance above, is usually more slender and delicate.
6. The side on which the raphe is present is the basal side and the side opposite to it is the apical side. The side on which the tubercles are present is the dorsal side and the opposite of it is ventral side.

Exercise 7

Study of V.T.S. of sporocarp
Work procedure
Cut a section of the sporocarp in a plane at parallel to the stalk as shown in reference diagram. Stain the section in safranin, mount in glycerine and study.



***Marsilea sp.* Vertical transverse section of sporocarp.**

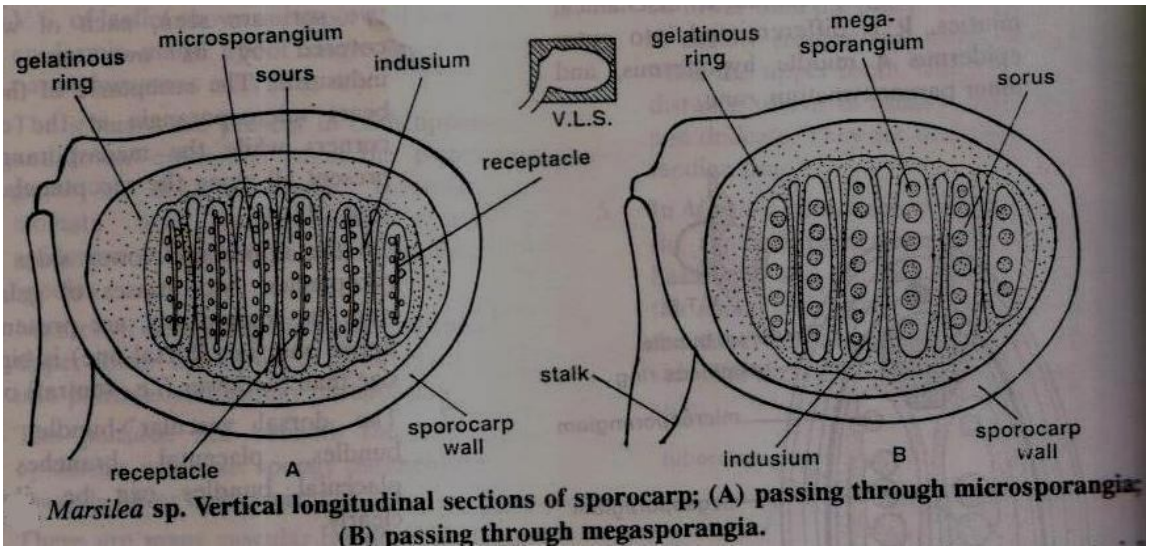
Comments

1. The section shows wall of the sporocarp which encloses sori.
2. The wall is made of outer epidermis followed by hypodermis.
3. Epidermis consists of thick walled cells. Numerous stomata are present in the epidermis.
4. Hypodermis consists of two layers of radially elongated cells. The cells of the inner layer are double in length as compared to the cells of the outer layer.
5. All the cells of both the layers have their nuclei arranged in one row.
6. Receptacles are cut longitudinally. Only two Sori are seen, each of which is covered by its own two layered indusium. The receptacle of the sorus bears microsporangia at the corners and megasporangia all along the receptacular ridge.
7. On the upper and lower sides of the receptacles, two masses of gelatinous ring, cut transversely, are present. The upper one is bigger in size than the lower.
8. The dorsal bundle, lateral bundles, placental branches and placental bundles are seen.

Exercise 8

Study of V.L.S. of the sporocarp
Work procedure
Hold the sporocarp with tubercles pointing upwards. Split the sporocarp by a sharp blade in two halves. Study the section under dissecting microscope.

Comments
1. The section shows wall of the sporocarp enclosing sori embedded in a gelatinous wall.
2. The outermost is the sporocarp wall. It is made of an epidermis with stomata and two layered hypodermis.
3. Below the sporocarp wall is a gelatinous ring which surrounds sori. It is relatively more prominent on the dorsal side than on the ventral.
4. The sori are cut longitudinally and appear in a row.
5. Each sorus is surrounded by its own indusium.
6. If the section passes through the centre, then megasporangia are seen in all the sori. Since the megasporangia are present at the apex of the receptacle, no receptacle is seen.
7. If the section is not perfectly median, then microsporangia are seen attached on either sides of the receptacle in each sorus.
8. In this section the stalk bundle and cut lateral bundles are seen.



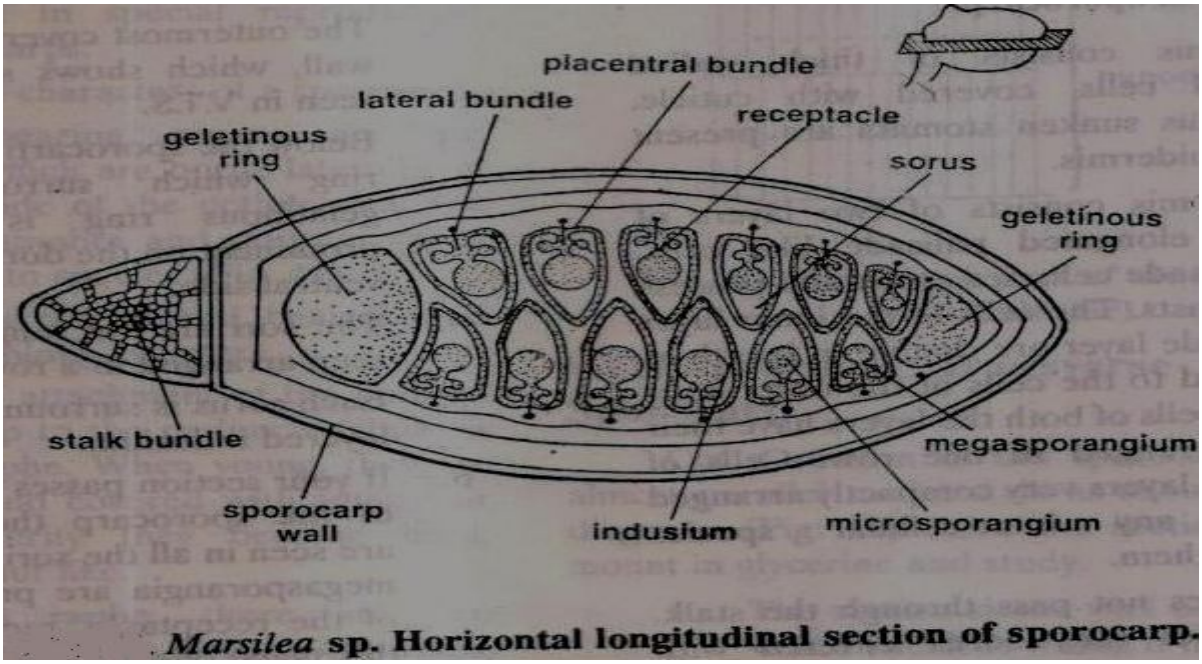
***Marsilea* sp. Vertical longitudinal sections of sporocarp; (A) passing through microsporangia; (B) passing through megasporangia.**

Exercise 9

Study of H.L.S of the sporocarp
Work procedure
Hold the stalk between the thumb and the index finger. Cut a section by passing a blade at right angles to the stalk axis (see reference diagram). Stain in safranin, mount in glycerine and study.

Comments
1. The section shows transversely cut stalk, wall of the sporocarp and two rows of sori.
2. Transversely cut stalk appears on one side. It shows the stalk bundle.
3. The wall of the sporocarp is made of epidermis with stomata and two layered hypodermis.
4. Gelatinous ring shows two patches, heavier on the dorsal side than on the ventral.

5. There are two rows of sori, one row alternating with the other.
6. Each sorus is covered by its indusium.
7. A sorus consists of a receptacle. Megasporangium is present at the apex receptacle while microsporangia are present the sides.
8. The lateral bundles are cut transversely and each is seen to supply its own receptacle by receptacular or placental branch.
9. Thus, in this section, dorsal bundle, many lateral bundles and receptacular branches (placental branches) are seen.



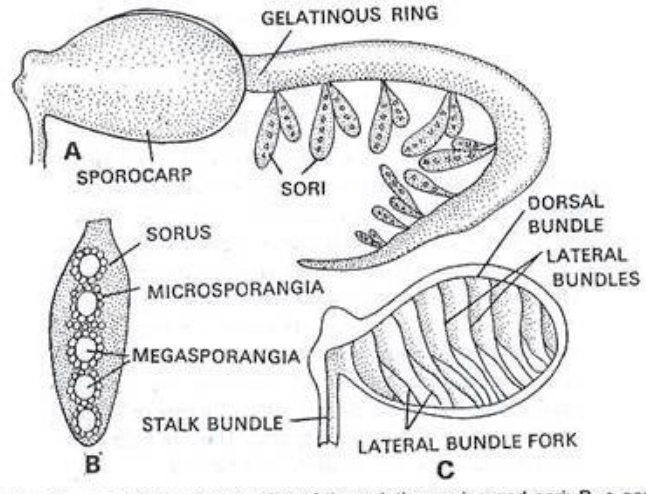
Marsilea sp. Horizontal longitudinal section of sporocarp.

Exercise 10

Study of dispersal of spores

Work procedure

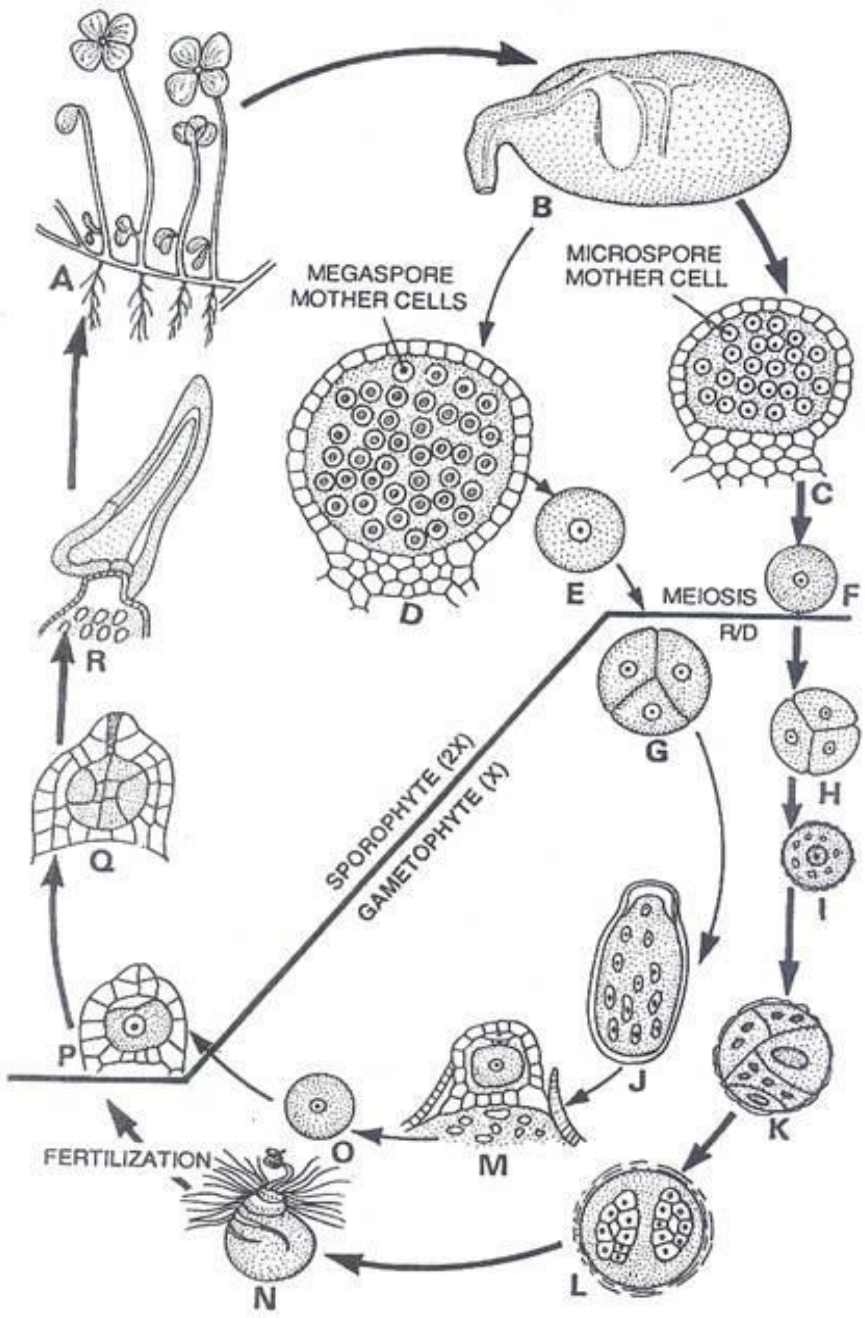
Cut open the body of the sporocarp on ventral side by a sharp blade or scalpel. Place in water for some time. Gelatinous ring with sporangia attached to it comes out.



Marsilea quadrifolia, A, extrusion of the gelatinous ring and sori; B, a sorus surrounded by indusium; C, vascular supply of the sporocarp in lateral view.

Comments

1. The sporocarp is hard and resistant to unfavorable conditions.
2. It opens through its ventral margins.
3. It imbibes water and the gelatinous ring inside swells up.
4. This ring ultimately comes out of the sporocarp wall.
5. Gelatinous ring bears two rows of sporangia, one on each side, alternating with one another



Gymnosperms

In division **Spermatophyta** (**Sperma** = seed; **phyton** = plants) all seed bearing plants are include. It has been divided into two sub-divisions i.e**. Gymnosperm** and **Angiosperms**. Gymnosperm term is derived from two Greek words **Gymnos** = naked, **Sperma** = seed; which means all the plants having naked seeds are included in this group. They are characterized by naked ovules developed on the megasporophylls. They are commonly known as "**Phanerogams without ovary**"**.**

Gymnosperm is a large group of evergreen slow growing plant. Though true seed are formed, the group differs from other group of seed-bearing plants the angiosperms, in possessing naked ovules and in the absence of true vessels and companion cells. The leaves of some gymnospermic plants (Cycadales order) resemble with higher cryptogams, while higher gymnosperms such as **Gnetales** and **Coniferales**, resemble with members of Angiosperms. The sub–division phanerogams (flowering plant) i.e. gymnosperm is represented by over 60 genera and 700 species of which about 16 genera and 53 species have been reported from India.

Gymnospermic plants are woody, perennial with shruby or tree like habit and show xerophytic characters. Their sporophytic plant body is differentiated into root, stem and leaves. Tap root system is exarch and diarch to polyarch. They have two types of leaves i.e., foliage leaves and scaly leaves. The leaves are pinnately compound in *Cycas* and are needle like in *Pinus*. The vascular bundles are conjoint, colletral open and endarch in stem. Due to presence and activity of cambium, secondary growth is present. Wood becomes closely packed or pycnoxylic in conifers due to the much reduced nature of pith and cortex. It is monoxylic or loose in cycas due to the prescence of well-developed pith and cortex. Xylem is composed of tracheids with bounded pith, companion cells are absent in phloem. Reproductive parts are generally arranged in the form of compact and hard cones or strobilis. The cones are generally unisexual. Polyembryony is present in the members of e.g *Pinus*, true fruits are lacking, plant show alternation of generation. Sixteen genera of Gymnosperm reported from India are *Cycas, Pinus, Gnetum, Ephedra, Abies, Cedrus, Piecea, Larix, Tsuga, Cupressus, Cephalotaxus, Juniperus, Podocarpus. Libocedrus, Taiwania* and *Taxus*. They vary in size from small plants to large gigantic plants. *Sequoia semperviren*(red wood tree), grows up to a height of about 150 meters (California) and *Taiodrum maxiconum* has a mink with the enormous diameter of about 17 meters and *Zamia pygmaea*is the smallest gymnosperm with and underground tuberous stem. Reproduction takes place in sporophytic plant body by spores, which developed in sporangia and sporangia developed in sporophylls. All the gymnosperms are heterosporous having two different types of spores (micro and megaspores). The male and female cones differ in shape and size, whereas the male cones are usually smaller and short lived and female cones are quite larger and long lived. Considerably the gametophytic generation is even more reduced than pteridophyta. The gymnosperms are also important from economic point of view. Some conifers are valuable timber. Some medicine is also extracted from a conifer trees. Last but not the least gymnosperm plant also proved themselves for their food value viz. sago palm (*Cycas revoluta*, yield sago or sabudana) and the very familiar fruit of chilgoza is the seed of *Pinus gerardiana*.

Classification of Gymnosperms

In 1827 first time Robert Brown recognized the gymnosperms as a separate group due to the prescence of naked ovules. Bentham and Hooker considered them equivalent to monocotyledons and dicotyledons. Many classifications were proposed by time to time on the basis of different characters. Sporne (1965) divided the gymnosperms into three classes and nine orders.

Gymnosperm - 3 classes and 9 orders

Cycadopsida	**Coniferopsida**	**Gnetopsida**
Pteridospermales	**Cordaitales**	**Gnetales**
Bennettitales	Coniferales (Pinus)	(Ephedra)
Pentoxylales	Taxales	
Cycadales(Cycas)	Ginkogales	

Cycas (Sago palm or Ever-green living fossil)

Classification

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

It is the only genus of family cycadaceae which is represented in India. The genus is represented by 20 species which are widely distributed in states of Japan, China, Australia, Africa, India and Nepal etc. In India the genus is represented by several species in which *C. revoluta, C. circinalis, C. rumphii, C. pectinata, C. beddomei and C. siamensis* etc are mainly include.

Exercise 1

Study of external features of the plant

Work procedure

Study the external features of the plant. Observe the armour of leaf bases on the stem, absence of branching, crown of leaves and two types of roots etc.

Comments

1. Plant body is differentiated into an underground root system and an aerial root system.
2. Roots are of two types: (i) Primary or normal root and (ii) secondary of **coralloid root**.
3. Normal root is a tap root, growing deep into the soil (positively geotropic). It is less branched and sometimes grows as thick as aerial stem.

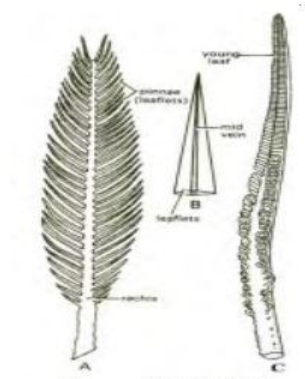


Fig. Cycas Leaf-let
A, Compound leaf. B.Upper portion of a leaf-let
C. Young leaf showing circinate vernation of leaf-lets

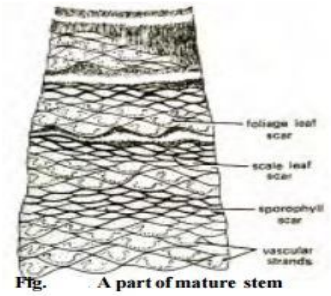


Fig. A part of mature stem

Fig. 1.57. Coralloid roots

4. Secondary roots are negatively geotropic projecting above the soil surface, repeatedly dichotomously branched and appear as coralloid clusters.
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 to 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.
10. Cycas is a dioccious and as such bears terminally, either male cone or female reproductive structures.
11. The male cone is borne terminally at the apex of the stem and the further growth of the stem continues by axillary bud (developed at the base of the cone) which pushes the male cone on one side. The branching in Cycas stem is thus referred to as sympodial.
12. The female reproductive structures are the sporophylls developing in place of foliage leaves. The vegetative apex continues to grow as usual.
13. The sporophylls are smaller than the foliage leaves. They are brown or light brown in colour and are densely covered with wooly hairs.

Exercise 2

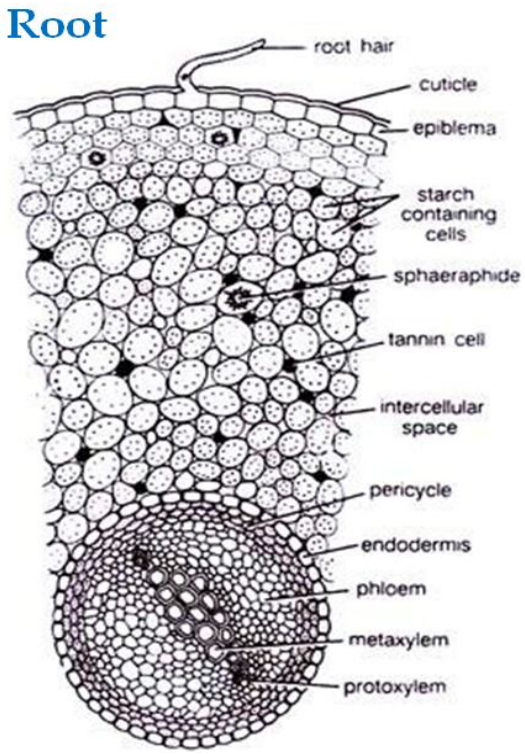
Study of anatomy of normal young root

Work Procedure

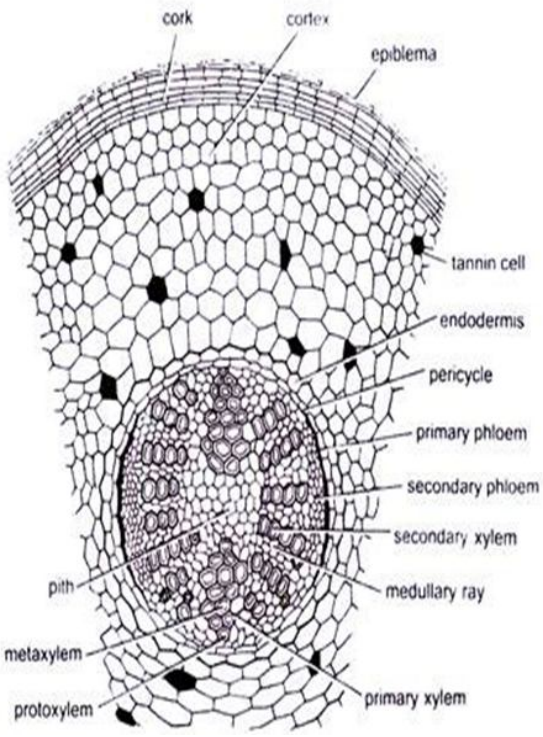
Cut a T.s. of the youngpart of primary root, stain in safranin-fast green combination, mount in glycerine and study.

Comments

1. The section is circular in outline. It shows an outer layer or epiblema, cortex and centrally located stele.
2. Epiblema is made up of single layer of thin walled cells. Some of these cells bear unicellular root hairs.
3. Cortex is multilayered and parenchymatous and filled with starch. A few tannin filled cells are also scattered in this region.
4. Endodermis is single layered indistinguishable. Multilayered 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.



Young root



Old root

Exercise 3

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.

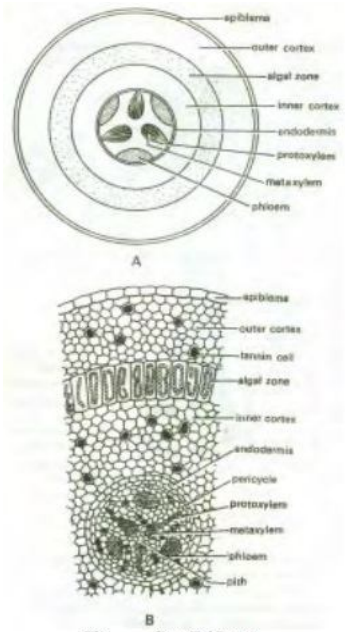


Fig. Coralloid root
A. Ground plan TS B. A portion enlarged

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 thick. 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 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 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 there is small parenchymatous pith.

Exercise 4

Study of anatomy of coralloid root
Work procedure
Cut a T.s. of the root, stain in safranin- fast green combination, mount in glycerine and study.

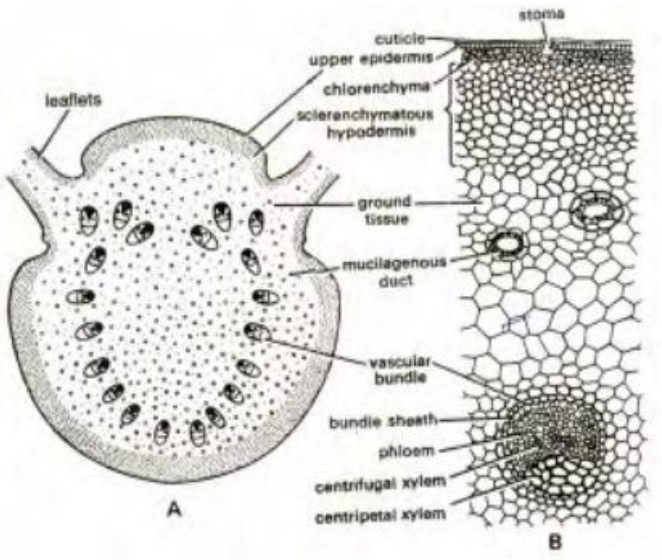


Fig. Rachis A. Ground plan B. A portion enlarged

Comments

1. The structure is almost similar to that of a normal root. It consists of epiblema, 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 and composed of parenchymatous cells.
4. The middle cortex is also called 'Algal zone'. A blue-green alga *Anabaenacycadae* occurs endophytically in these cells and it is believed that it helps in nitrogen fixation.

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

Exercise 7

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. It is cylindrical and 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. This condition is known as amphistomatic.
4. Hypodermis is mainly composed of thick-walled sclerenchymatous cells. Intermixed with these cells are a few cells having chloroplasts-chlorenchyma.
5. This sclerenchymatous hypodermis is 2-3 layered toward adaxial side and many layered toward abaxial side.
6. 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 (Ω) 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 basecentral and upper region of the rachis.

a. 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.

b. At the very base of the rachis, vascular bundles show only centrifugal xylem which is endarch. Phloem occupies the abaxial side of the rachis.

c. 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.

Features of special interest

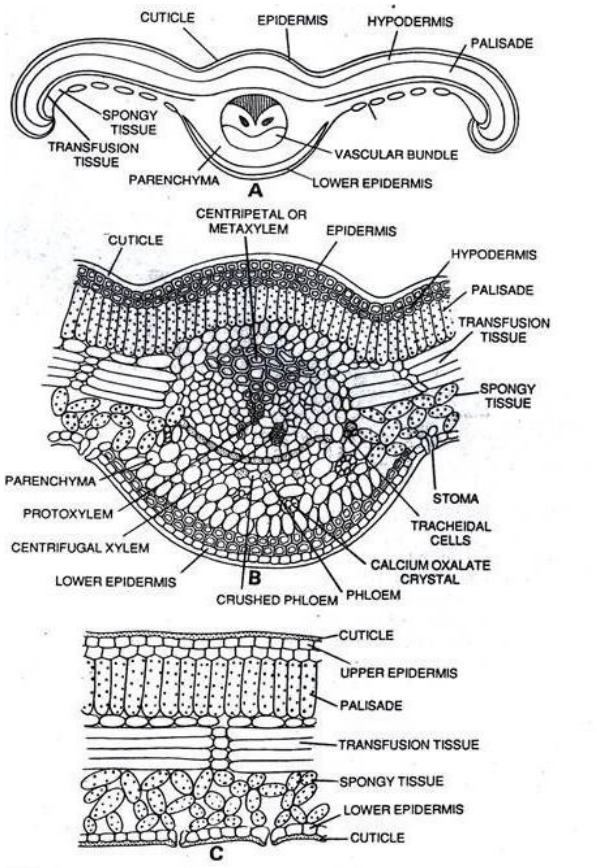
1. Presence of chlorenchyma, dispersed among the thick walled sclerenchymatous hypodermis.
2. Presence of sunken stomata all over the surface (xerophytic characters).
3. Vascular bundles arranged in inverted omega (Ω) shaped arc. Diploxylic nature of the vascular bundles. Mucilage ducts scattered throughout.

Exercise 8

Study of anatomy of leaflet (pinna)

Work procedure

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



2. *Cycas* sp. Anatomy of leaflet. A, T.S. of leaflet (diagrammatic structure); B, the detailed structure of mid-rib portion of the leaflet; C, T.S. of a portion of the wing of leaflet.

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.

a. In *C. revoluta* midrib is less projected than in *C. circinalis*, where it is much projected on the upper side.

b. 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.

a. In *C. revoluta*, hypodermis is present in the midrib (near both upper and lower epidermis) and wings (below the upper epidermis).

b. 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 corners, 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 layers of spongy parenchyma.

a. In *C. revoluta* palisade is present beneath the hypodermis, both in the midrib and the wings.

b. In *C. circinalis* palisade is absent from the midrib region.

6. Spongy parenchyma with many intercellular spaces lies immediately above the lower epidermis.
7. On either side of the centripetal metaxylem of midrib bundle and somewhat connected with it are present two small groups of tracheid-like cells the transfusion tissue.
8. Between the palisade and spongy parenchyma cells, there are 3 or 4 layers of tracheid-like, long colorless 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. 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 bundles are similar in all respects to that found in the upper region of the rachis. They are conjoint, collateral, open and diploxylic.
13. Phloem lies towards the abaxial (lower) side. In between xylem and phloem group, cambium is present.
14. It shows a large triangular patch of centripetal xylem and two small groups of centripetal protoxylem.

Features of special interest

1. Lateral veins are absent.
2. Thickly cuticularized upper and lower epidermis.
3. Sunken stomata in the lower epidermis.
4. Presence of transfusion tissue.
5. Diploxylic nature of vascular bundle.

Exercise 9

Study of Bulbils

Work procedure

Study the position on the lower part of the stem and observe external characters

Comments

1. Bulbils are produced adventitiously, on basal part of the plant, in the crevices between the persistent leaf bases.
2. The decurrent base of the bulbil remains covered with scale leaves.
3. A few foliage leaves are given out from the central part.
4. It germinates under favorable conditions and produces new plant or else develops into a branch (rarely), giving an appearance of dichotomous branching.
5. This is the commonest method of reproduction in *C. revoluta* in north India, male plants being rare in this region.

Exercise 10

Study of external features of male cone

Work procedure

Study the male cone attached to the plant if possible

Comments

1. 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.(Since the male cone terminates the growth of the apex of male plant, a lateral bud later grows and takes over the continuation of growth of apex. The male plant thus shows sympodial growth).
2. The outer covering of the male cone is formed by closely set sterile ends of the microsporophylls usually possessing up curved apices apophysis.

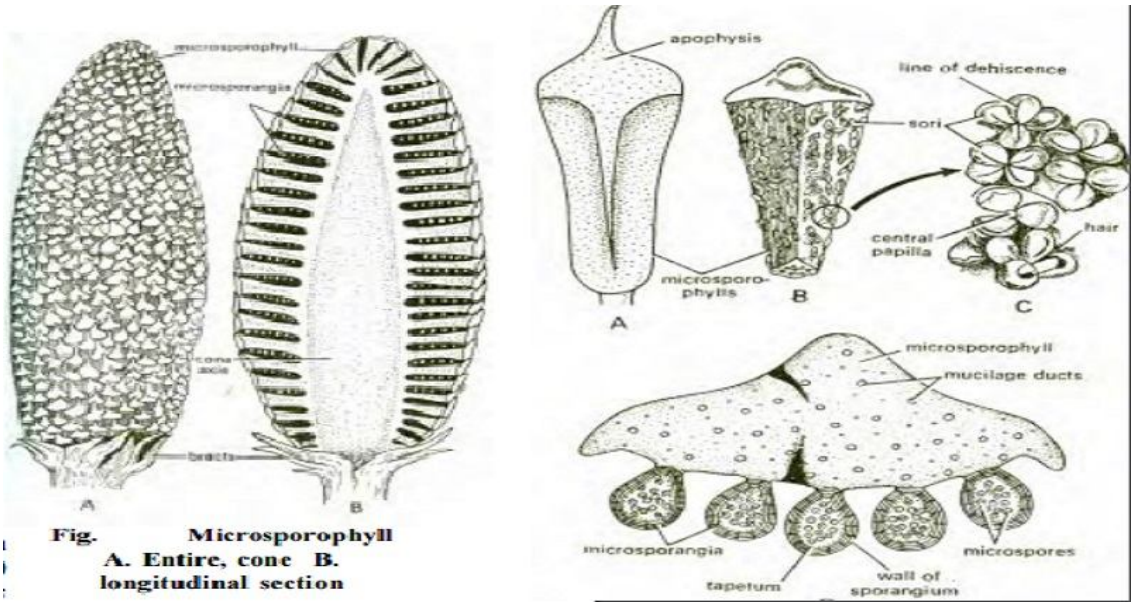


Fig. Microsporophyll
A. Entire, cone B. longitudinal section

Exercise 11

Study of L.S. of the male cone
Work procedure:
Since the male cone is about 60-80 cm in height, it can be split into two and specimen is studied.

Comments
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.

Exercise 12

Study of microsporophyll and sporangia
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 structure and triangular in shape.
2. It is differentiated into 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.

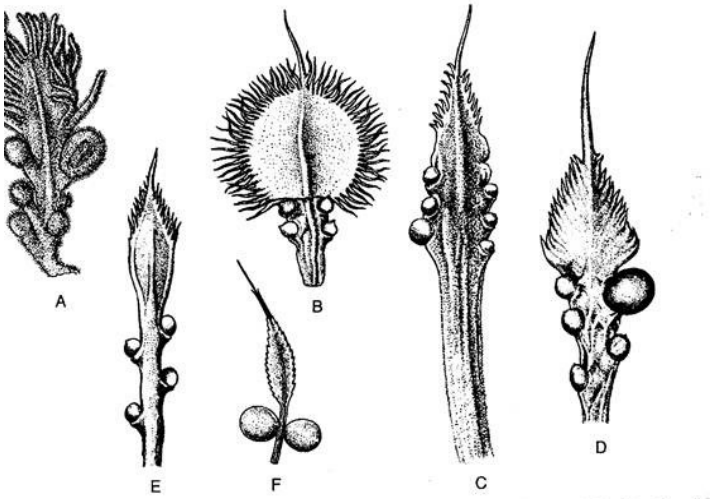
Exercise 13

Study of T.S. of microsporophyll
Work procedure:
Study a double stained slide of T.S. of microsporophyll

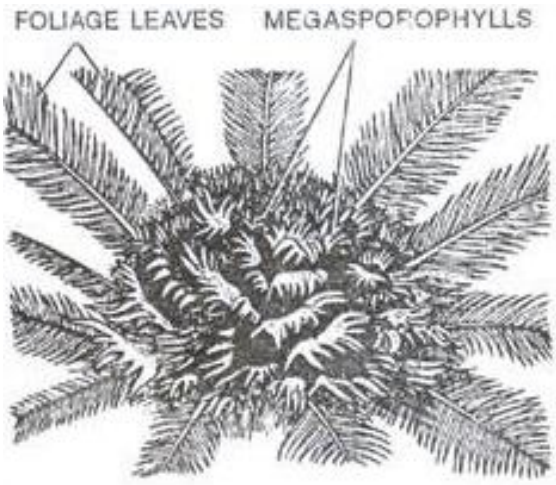
Comments
1. The section shows microsporangia attached to the abaxial (lower) surface by their short stalks.
2. A mature microsporangium has three layered wall. The outermost layer is thick and cutinized, termed as exothecium. The remaining inner layers are thin and are collectively known as endothecium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

Exercise 14

Study of megasporophyll
Work procedure
Since there is no female cone, megasporophyll form a crown at the apex like foliage leaves. Only a megasporophyll can be studied as a specimen.



: Megasporophylls : A. *Cycas* revotuta, B. *C. pectinata*, C. *C. circinalis*, D. *C. rumphii*, E. *C. beddomei*, F. *C. normanbyana*



Comments
1. Female reproductive body consists of megasporophyll 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 nature of upper sterile part varies with species.

a. In *C. revaluta,* the upper part is very much dissected, forming many pinnae.

b. In *C. rumphii*, the upper part bears only short spines which represent reduced pinnae.

c. In *C. circinalis*, the pinnate character is altogether absent and upper part shows only dentate or serrate margins.

7. 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.

8. 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.

a. In *C. revaluta*, ovules are many and orange coloured.

b. In *C. circinalis*, ovules are numerous but these are dark green and attain a large size.

c. In *C. siamensis*, the number of ovules is reduced to only two.

d. In *C. thouarsii*, the ovules become still larger and may be that they are the largest ovules in the plant kingdom.

9. All the ovules do not develop fully. Some of those which remain unpollinated and small finally abort.

Exercise 15

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 orthotropaus.

2. It is unitegmic (possesses a single integument). The integument is very thick and 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 fishy layers are supplied with vascular strands but the middle stony layer receives no vascular supply.

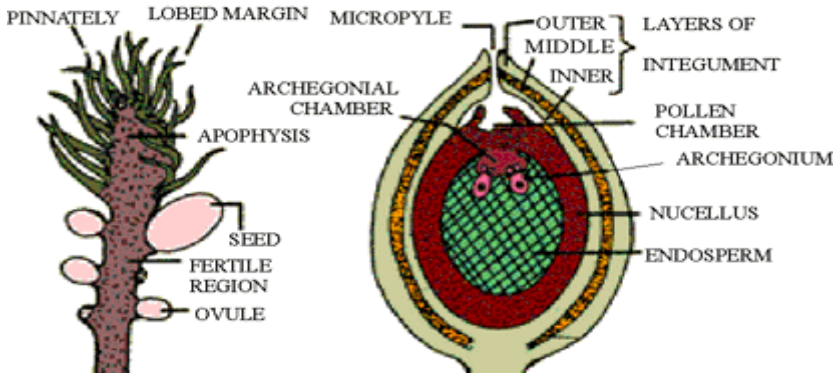
4. The nucellus lies just below the integument and forms a nucellar beak in the region of the micropyle.

5. A few cells of this nucellar beak dissolve themselves and form a pollen chamber that lies in the central region of the beak.

6. The innermost region of the ovule is filled with the tissue of female gametophyte, where 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.



Exercise 16

Study of L.s. of seed
Work procedure
Study a double-stained preparation of the L.S. of seed.

Comments
1. It shows seed coat nucellus embryo and the female gametophyte.
2. Seed coat consists of sarcotesta (from outer fleshy layer of integument), and middle sclerotesta (from middle stony layer). The inner fleshy layer of the integument appears as thin and papery structure in the seed.
3. Nucellus is papery and is situated inside the seed coat.
4. Endosperm and female gametophyte form the inner part of seed.
5. A straight embryo remains embedded in the endosperm. It has two unequal cotyledons.

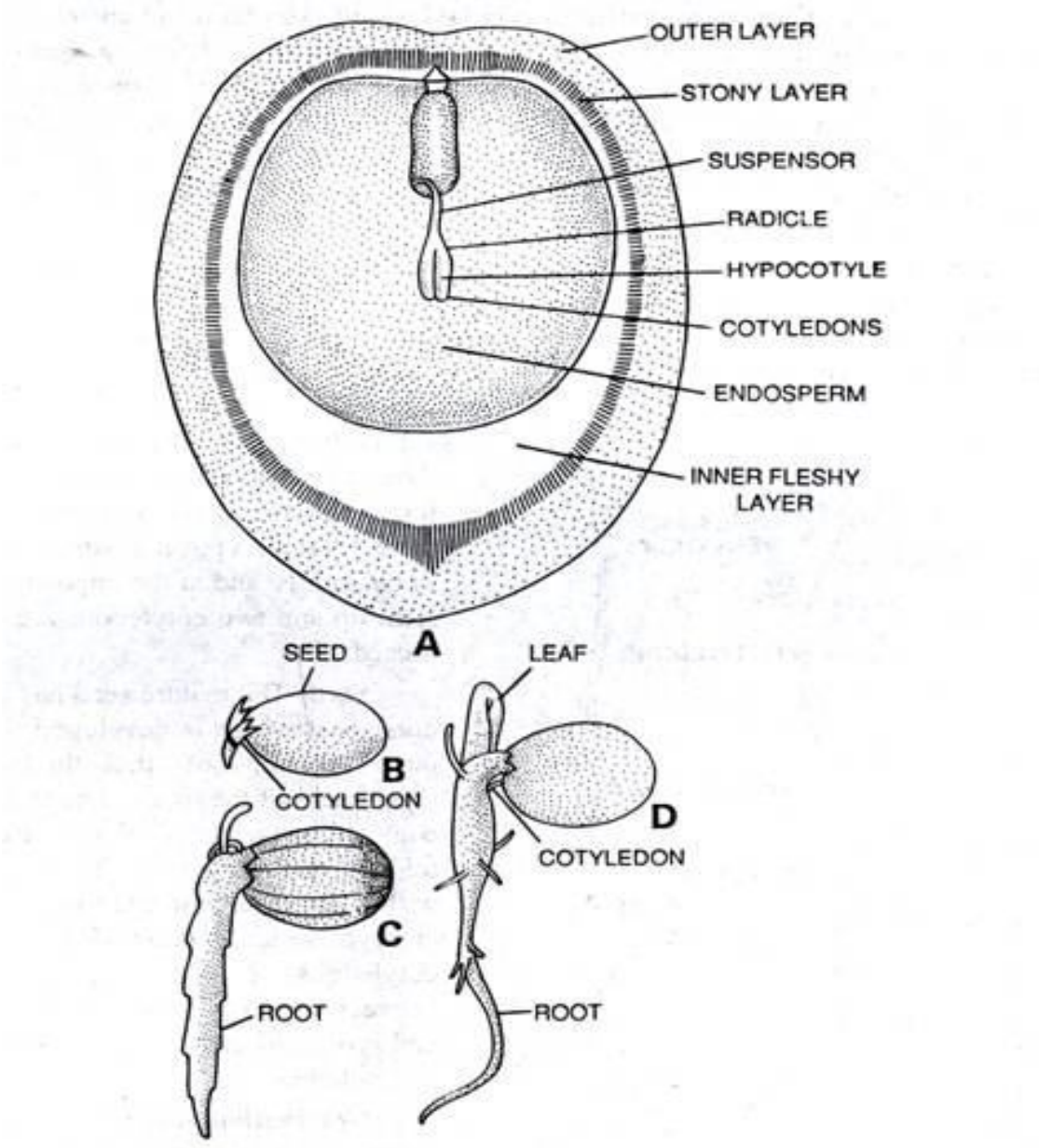
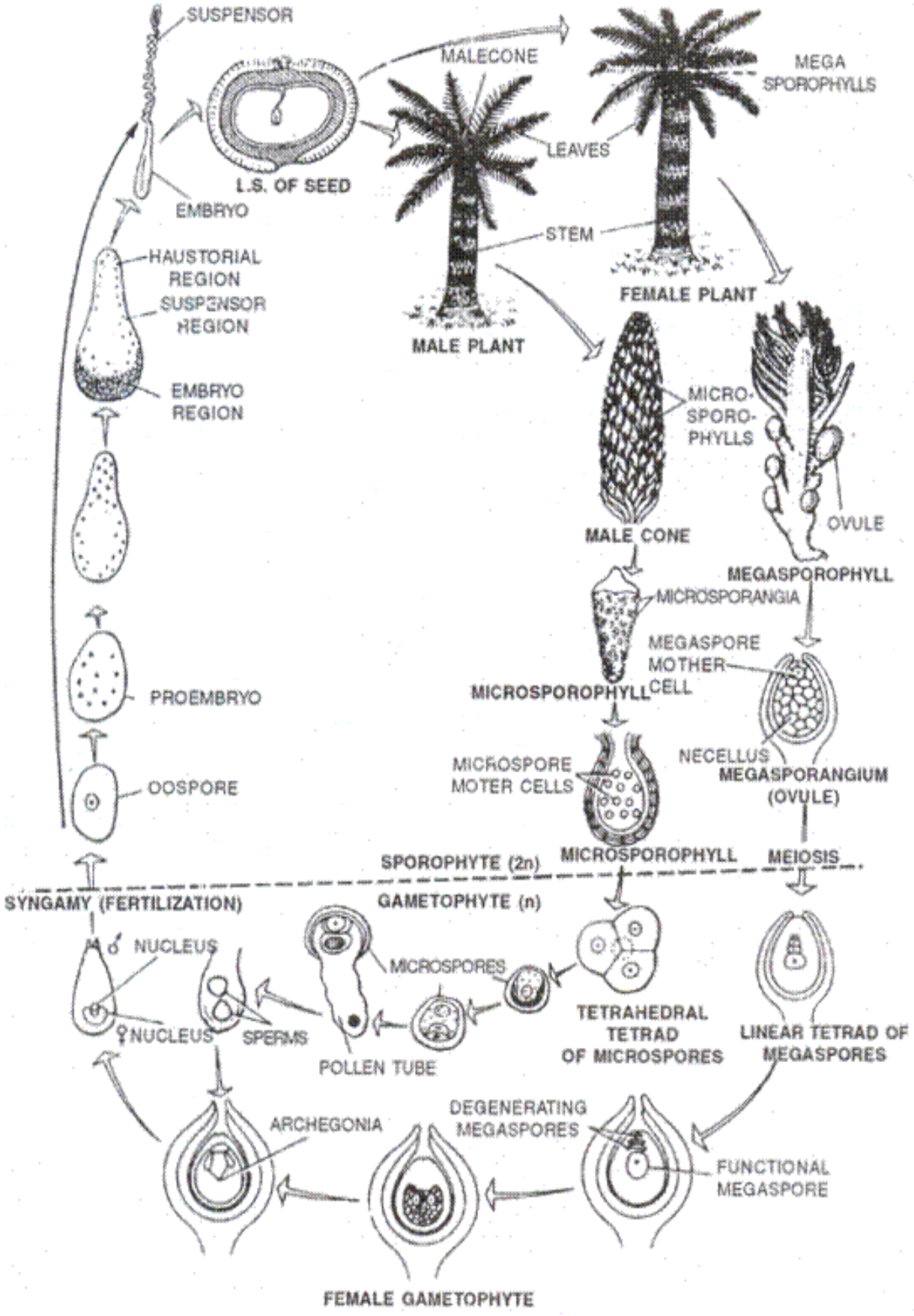


Fig. 3.50. *Cycas* sp. A, L. S of seed; B-D, successive stages in seed germination.



Life cycle of cycas

Pinus (Pine/ Chir pine)

Classification

Division	Gymnosperms
Class	Coniferopsida
Order	Coniferales
Family	Pinnaceae
Genus	*Pinus*

Some very common species of *Pinus* in India are- *P. gerardiana*, *P. roxburghii*, *P. wallichiana* and *P. insularis* etc.

Exercise 1

Study of external morphology

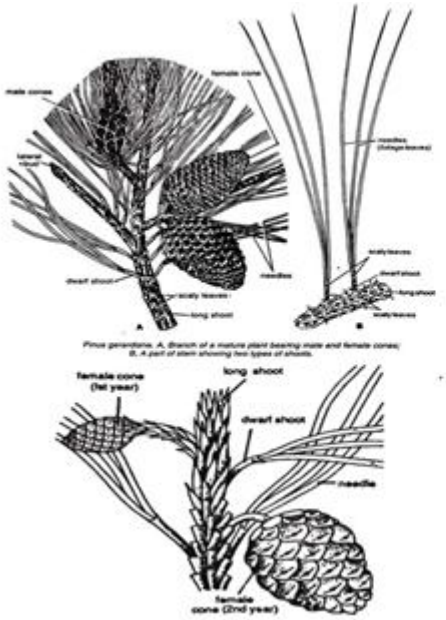
Work procedure

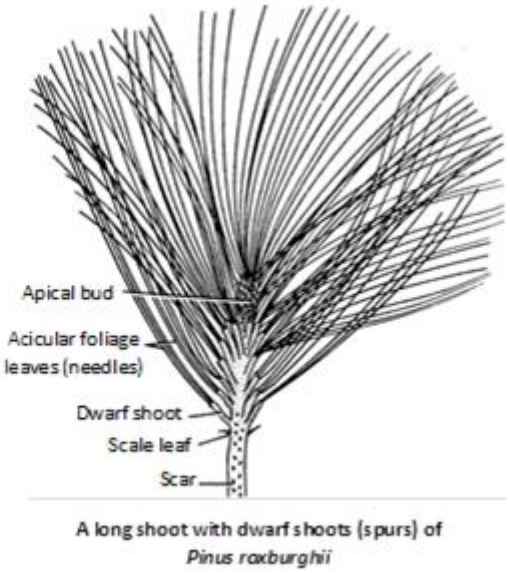
Note the pattern of branching, the two types of branches, two types of leaves, and male and female cones.

Comments

1. It is a tall conical tree and therefore, commonly grouped under conifers.
2. The plant body is differentiated into root, stem and leaves.
3. Underground root system is formed by tap roots which disappear early and only lateral roots persist later on.
4. The younger roots are generally surrounded by fungal hyphae-the ectotrophic mycorrhizae.
5. Aerial branch system consists of cylindrical rough (being covered with scaly bark) and branched stem.
6. The branching is monopodial and the branches are arranged in whorls.
7. The branches are dimorphic (of two types) branches of unlimited growth or long shoots and branches of limited growth or dwarf shoots.
8. Branches of unlimited growth or long shoots are present on the main trunk. These are produced at regular intervals.
9. Branches of limited growth or dwarf shoots are borne on the main stem and on long shoots, in the axils of scale leaves. Dwarf shoots also possess many scale leaves and bear group of foliage leaves at the apex.
10. The leaves are also dimorphic (of two types) scale leaves and foliage leaves.
11. Scale leaves are brown membranous and small. They are present on both the types of branches (i.e. long and dwarf shoots).
12. Foliage leaves are green acicular and needle-like. They are borne only by the dwarf shoots.
13. A dwarf shoot with a group of needle-like foliage leaves is known as a foliar spur. The number of needles in a group varies from species to species. *P. monophylla* has a single leaf and spur is known as monofoilar, while in *P. sylvestris*, two leaves are present and spur is called as bifoliar. In *P. longifolia* and *P. gerardiana*, have three in number, the spur being called as trifoliar. Quadrifoilar spur occurs in *P. quadrifolia* and pentafoliar in *P. excelsa.*
14. The shape of the needle varies, according to their number in a spur. In *P. sylvestris* (with bifoliar spur) single needle is semi-circular in T.s while in *P. longifolia*(with trifoliar spur) single needle is almost triangular in shape.
15. *Pinus* is monoecious. Plant bears male and female reproductive parts in cones on the same plant.

16. The male cones are borne on lateral branches of unlimited growth. They are produced in clusters and replace the dwarf shoots. They are formed earlier in the season than the female cones.
17. The female cones are borne terminally on branches of unlimited growth. They are produced singly and replace the long shoot. The female cone appears after every three years.
18. Generally male and female cones are not formed on one and same branch.





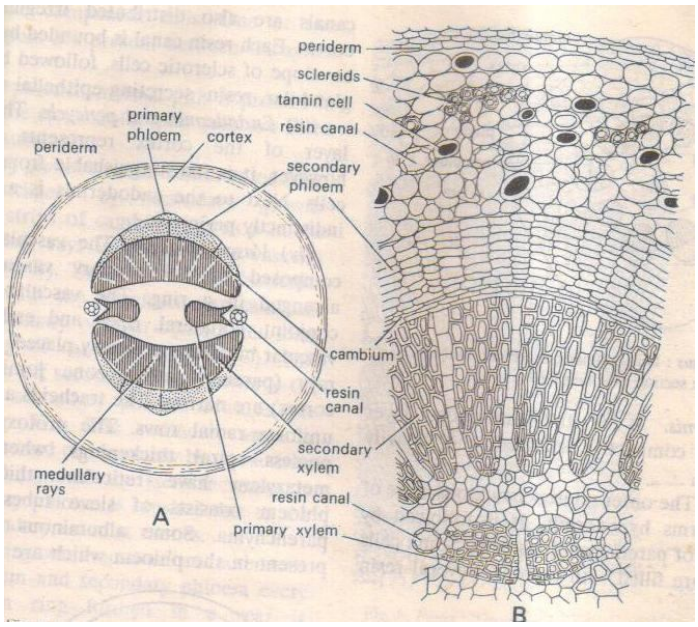
A long shoot with dwarf shoots (spurs) of *Pinus roxburghii*

Exercise 2

Study of anatomy of young root

Work procedure

Study a double stained prepared slide of T.s of young part of root.

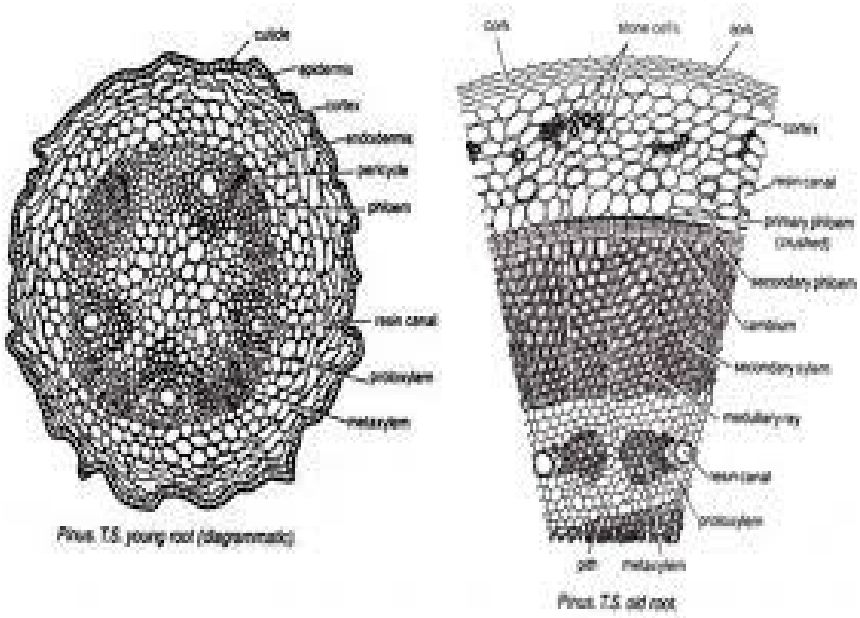


Comments

1. The section is almost circular in outline.
2. The tissues arc differentiated into epiblema, cortex and vascular tissues.
3. Epiblema is outermost single layered and it gives out many thin and unicellular root hairs.
4. Cortex is multilayered and parenchymatous.
5. Endodermis separates outer cortex and central vascular cylinder. It is single layered and cells are radially thickened.
6. Pericycle follows endodermis and it is multilayered.
7. Vascular bundles are radial, exarch and diarch to hexarch.
8. Protoxylem is generally Y-shaped and a resin canal is present in between the arms of Y.
9. Pith is very small and lies between the groups of xylem.

Exercise 3

Anatomy of the old root
Work procedure
Study a double stained prepared slide of T.S. of old parts of root.



Comments

1. The section shows cork, cortex, primary and secondary vascular tissues and small pith.
2. Cork forms the outermost several layers (developed from pericycle and hence primary cortex is completely peeled off).
3. Stone cells occur in many groups scattered just below the zone of cork.
4. Secondary cortex follows cork and it is parenchymatous and a few layered deep.
5. Many resin canals are found in the secondary cortex.
6. Primary phloem occurs in two patches. The tissues are mostly crushed and obliterated.
7. Secondary phloem that follows is a few layered deepring. It consists of sieve tubes, sieve plates, phloem parenchyma and albuminous cells.
8. Secondary phloem and secondary xylem are separated by a cambium.
9. Secondary xylem is composed of tracheids arranged in regular rows. It is traversed by uniseriate medullary rays.
10. Pith is small and parenchymatous. Two groups of primary xylem are situated on opposite radii.
11. The characteristic of the Pine root is the presence of large resin canal between the divided arms of Y, close to each primary protoxylem group.

Exercise 4

Study of anatomy of the young long shoot
Work procedure
Cut a T.S. of younger part of the long shoot towards the apex, stain in safranin-fast green combination, mount in glycerine and study.

Comments

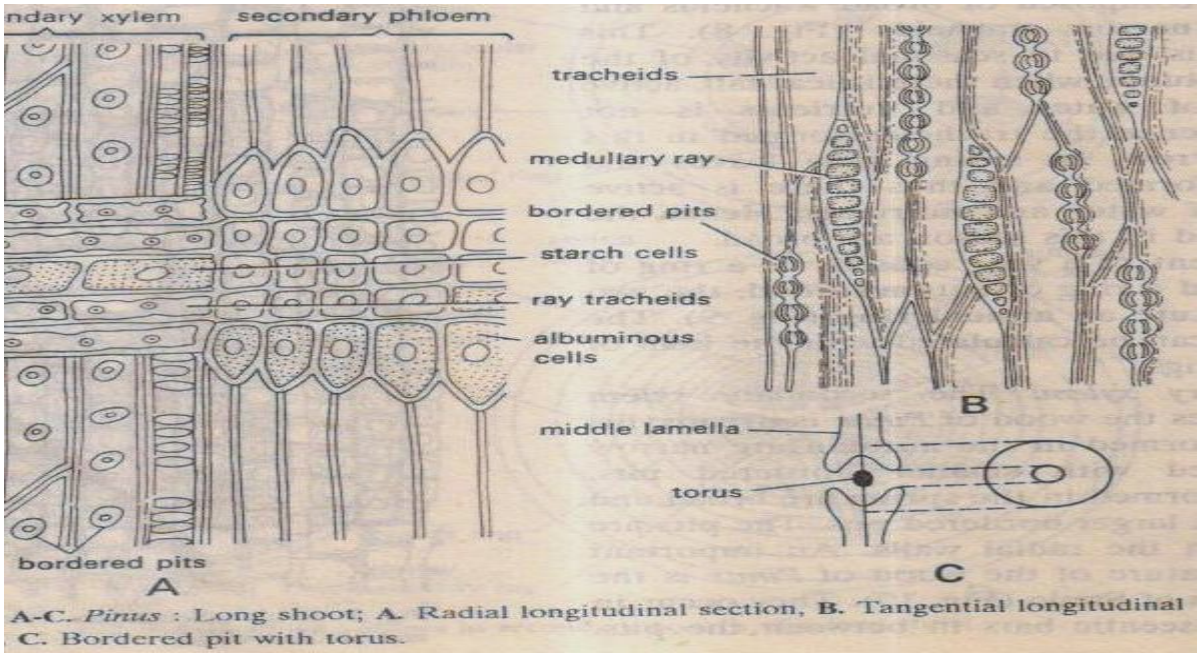
1. Outline is wavy due to the presence of scaly leaves.
2. The stem is differentiated into epidermis, cortex and stele.
3. Epidermis is the outermost single layer and it is thickly cuticularized.
4. Cortex is multilayered and lies below the epidermis. The outer few layers forming hypodermis are sclerenchymatous. Inner layers are thin walled and parenchymatous in which large number of resin canals and leaf traces are distributed irregularly.

5. The cavity of resin canal is bounded by a glandular, resin secreting epithelial layer. Outer to this layer are one or two layers of sclerotic cells.
6. In *Pinus,* resin canals are present in the cortex and secondary wood of both stem and root and on margins of the primary xylem in the root.
7. The stele is ectophloic siphonostele.
8. Endodermis is present but is undistinguishable and so also a few layered pericycle located inner to it.
9. Vascular cylinder is composed of 5-8 vascular bundles, separated by medullary rays. Vascular bundles are arranged in a ring.
10. Each vascular bundle is conjoint, collateral endarch and open.
11. Xylem is composed of tracheids and xylem parenchyma only but vessels are absent.
12. The phloem is made up of sieve tubes, sieve plates and phloem parenchyma and albuminous cells are also present.
13. Pith lies in the Centre and is parenchymatous. It is connected with the cortex but narrow medullary rays separate the vascular bundles.

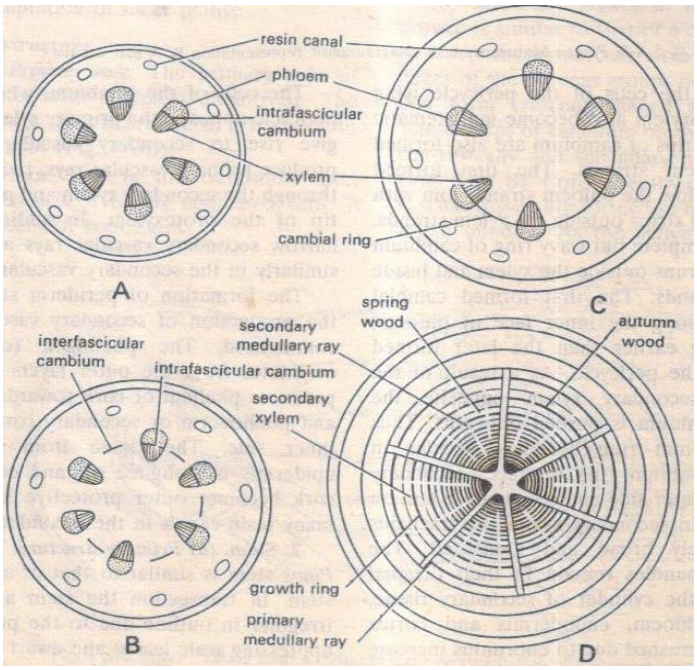
Exercise 5

Study the anatomy of the old long shoot
Work procedure
Cut a T.S. of the old part of the stem, stain with safranin-fast green combination, mount in glycerine and study.

Comments
1. The section shows cork, cortex, primary and secondary vascular tissues and pith.
2. The outmost region is formed by the successive layers of cork. It consists of thick and suberized cells.
3. Cork cambium follows cork. It is made of a few layers of regularly arranged cells.
4. Secondary cortex present below is parenchymatous.
5. Primary cortex is parenchymatous and many layered. The resin canals occur irregularly distributed in this region.



A-C. *Pinus* : Long shoot; A. Radial longitudinal section, B. Tangential longitudinal C. Bordered pit with torus.



6. Primary phloem that lies inner to primary cortex occurs as small patches of crushed tissues.
7. Secondary phloem occurs as a well distinguished ring. Phloem is composed of sieve tubes and phloem parenchyma.
8. Cambium separates the secondary phloem on its outer side and secondary xylem on its inner side.
9. Secondary xylem shows distinct and sharp annual rings. Thin walled and large xylem elements form a ring of spring wood. Thick walled and small xylem elements form a ring of autumn wood. The wood is pyconoxylic (compact).
10. Rings of secondary xylem — autumn and spring wood alternate one another and together form annual ring.
11. Secondary xylem (wood) is composed of tracheids and xylem parenchyma. Vessels are completely absent. Hence it is called **non-porous wood**.
12. Medullary rays traverse xylem and phloem. Primary medullary rays run from primary xylem to secondary phloem while secondary medulary rays run from secondary xylem to secondary phloem.
13. Primary xylem groups are endarch and lie just near the pith.
14. Resin canals are scattered in the primary and secondary xylem as in the cortex.
16. Pith is small, parenchymatous and its many cells are filled with tannin.

Exercise 6

Study of T.s. of dwarf shoot at the base (before secondary growth)
Work procedure
Take out a spur invert it with needles downwards, the base is now outermost and cut a T.s. at the base, stain in safranin-fast green combination, mount in glycerine and study.

Comments
1. The section almost resembles with that of the main stern.
2. The outline is wavy, due to ensheathing scaly leaves.
3. The tissues are differentiated into epidermis, cortex and stele.
4. Epidermis is made of single layer of thick walled cells.
5. Cortex follows epidermis. Outer few layers, close to epidermis are thick walled, while the inner layers are thin walled and parenchymatous.
6. Resin canals are present in the cortex. These are about six in number. Tannin cells are also irregularly scattered in this region.
7. Stele is an ectophloic siphonostele.
8. Endodermis is single layered and is followed by pericycle. Both the layers are indistinguishable.
9. Vascular bundles vary in number. They are generally six. Each vascular bundle is conjoint, collateral, endarch and open.

10. Pith is small. The cells are thick walled.
11. Medullary rays connect the pith and cortex and separate vascular bundles from one another.

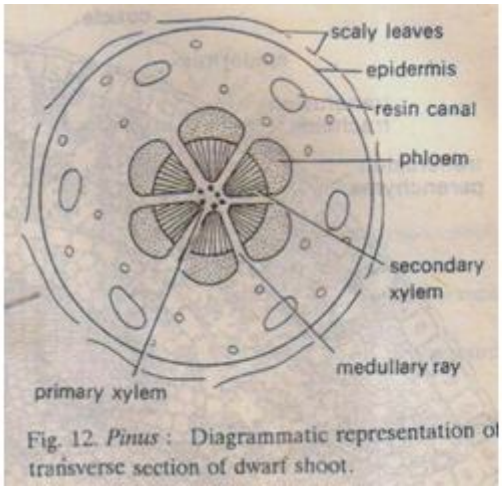
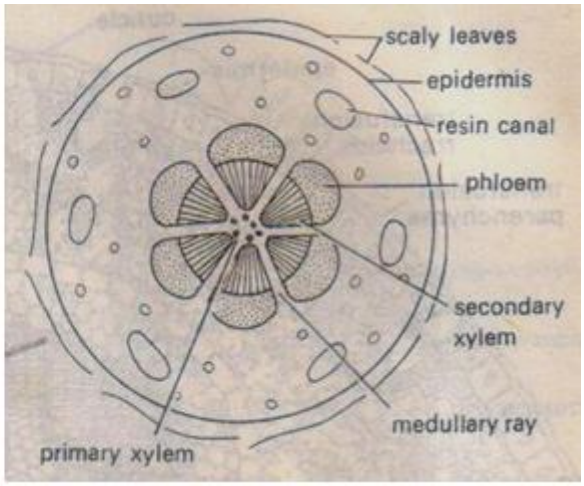


Fig. 12. *Pinus* : Diagrammatic representation of transverse section of dwarf shoot.



Exercise 7

Study of T.s. of dwarf shoot at base (showing secondary growth)
Work procedure
Cut a T.s of dwarf shoot above the base, stain in safranin-fast green combination, mount in glycerine and study.

Comments
1. Dwarf shoot also shows a little amount of secondary growth.
2. The section shows scale leaves, single-layered thick walled epidermis, few layers of cork cells and tannin-filled cells.
3. Primary phloem is crushed and form patches. Secondary phloem underlies it and forms a complete ring.
4. Secondary xylem is small and is separated by a thin ring of cambium from the phloem region. Medullary rays traverse the secondary xylem.
5. Protoxylem group lies just near the pith. It is small and consists of thick-walled cells. Few cells are tannin-filled.

Exercise 8

Study of T.s. of needle (leaf)
Work procedure
Cut a thin T.s. of a needle, stain with safranin and fast green combination, mount in glycerine and study.

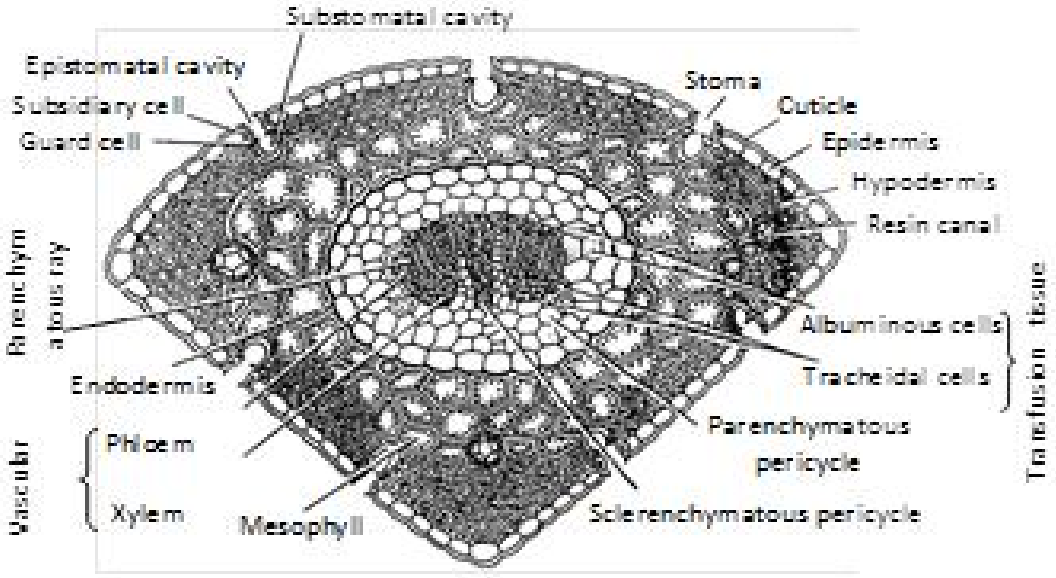
Comments
1. The outline of the section varies according to the species. (Triangular if spur is trifoliar, semi-circular if spur is bifoliar)
2. The needle is differentiated into epidermis, mesophyll and stele.
3. Epidermis is single with tangentially elongated and thickly cuticularized cells.
4. Stomata 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 barrel-shaped 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.

Features of special interest

It shows the following xerophytic characters —

1. Narrow acicular form of the leaf.
2. Presence of thick cuticle.
3. Aphistomatic nature.
4. Sunken stomata.
5. Thick and sclerenchymatous hypodermis.
6. Infolded peg-like structures in mesophyll cells.
7. Presence of transfusion tissue.
8. Simple vascular system.



T.S. needle of *Pinus roxburghii*

Exercise 9

Study of male cone, microsporophyll and microsporangia
Work procedure
Dissect out the male cone, separate the microsporophylls, study the shape, size structure of microspores.

Comments

1. Male cones replace the dwarf shoots. Each male cone arises in the axile of a scale leaf. The main shoot, on which these are produced, continues to grow further.
2. Male cones are grouped in clusters on the shoots of the same year only.
3. Each male cone has single, centrally located cone axis around which many scaly microsporophylls are spirally arranged.
4. Each microsporophyll has an expanded triangular central part and stalk-like base. Terminal part projects into a tip.
5. Few lowermost sporophylls are sterile and do not bear any male reproductive structures.
6. On the abaxial side, each microsporophyll bears two ovoid microsporangia or pollen sacs on its lateral sides.
7. Each microsporangium has its own wall which encloses many microspores.
8. The young microspore is globular or spherical in shape and is uninucleate.
9. A mature microspore or pollen grain shows two layered thick wall- exine and intine, 2 prothallial cells and an antheridial cell.
10. Pollen grain has a thick expanded exine in the form of wings on the sides, followed by a smooth intine.

Exercise 10

Study of L.s of male cone
Work procedure
Study a slide showing L.s. of male cone.

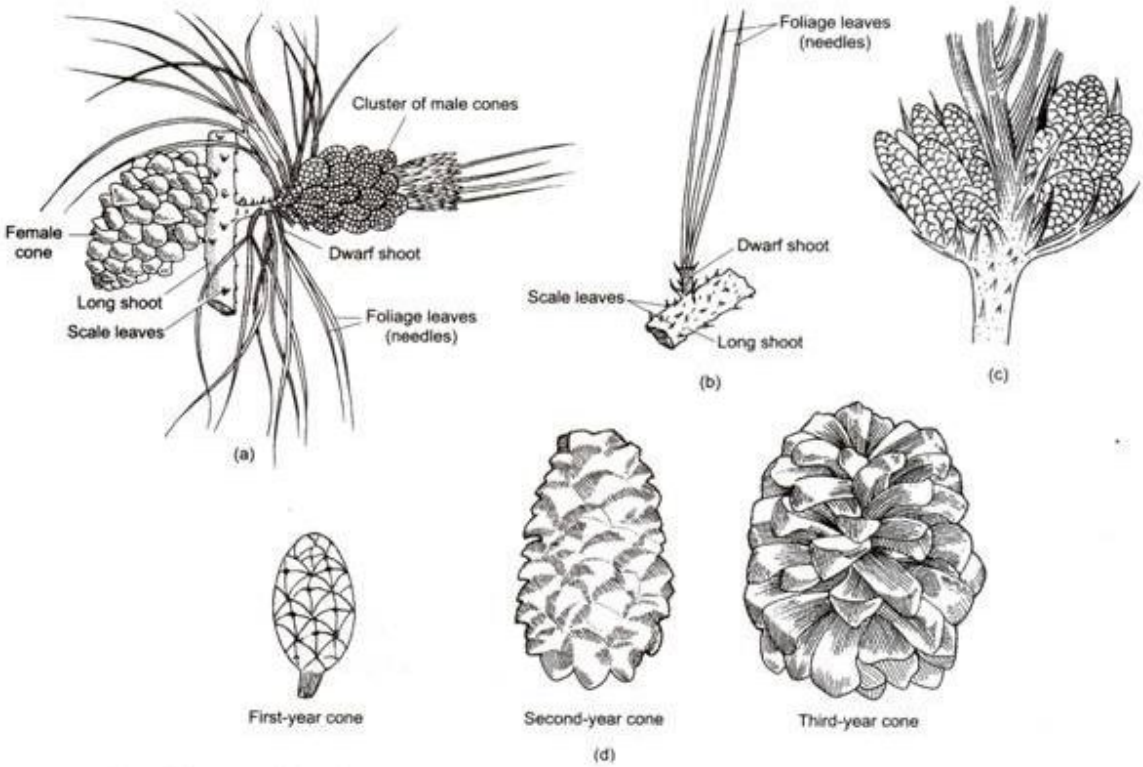
Comments

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-like base.
5. The outer expanded part is sterile and is known as apophysis.
6. Microsporangia are present on the lower or abaxial surface.
7. Each microsporangium 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.

Exercise 11

Study of morphology of the female cone
Work procedure
Study the external features of 1st, 2nd and 3rd year female cones. Note the position, arrangement and structure of megasporophylls.

Comments
1. Female cones are larger than the male cones. They are borne at the apices of the young elongated shoots, replacing the shoot of unlimited growth (long shoots).
2. Single shoot may bear one to four female cones which are reddish green in colour and mature in three years.
3. In the first year, cones are compact and sporophylls are closely arranged.
4. The second year, cones are larger in size and woody in nature but sporophylls are compactly arranged.
5. In the third year, cone becomes loose, sporophylls separate from one another due to elongation of the cone axis.
6. Each female cone consists of many megasporophylls, arranged spirally around the cone axis.



Pinus (a) Long and dwarf shoots with male and female cones (b) A part of stem showing two types of leaves and branches (c) Cluster of male cones (d) Female cone in different stages

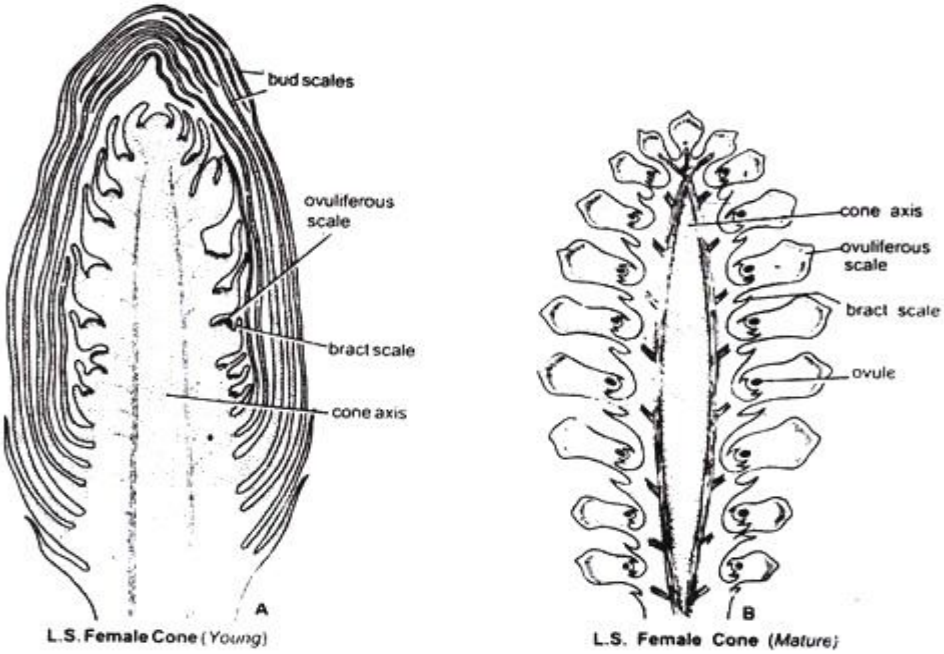
Exercise 12

Study of L.s. of female cone
Work procedure:
Study a prepared slide of L.s. of female cone.

Comments
Female cone is made of centrally located cone axis and spirally arranged megasporophylls.

1. Each sporophyll consists of two kinds of paired scales: (i) bract scale or cone scale and (ii) ovuliferous scale or seminiferous scale.

2. 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.
3. On the adaxial (upper) side of the bract scale, a thick, large, woody and triangular ovuliferous scale is present.
4. 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 micropyles directed towards cone axis.
9. The terminal part of the ovuliferous scale is broad and sterile and is known as apophysis.



Exercise 13

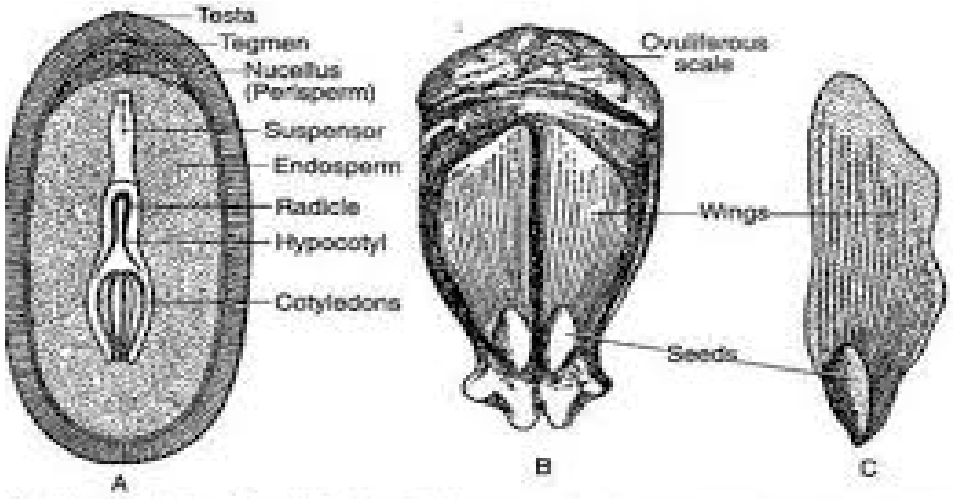
Study of L.s. of ovule
Work procedure
Study a prepared slide of L.s. of ovule. Note- integuments, nucellus, female gametophyte and archegonia.

Comments
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 (endosperm) is differentiated from nucellus. About 2-5 archegonia are situated in this region at the micropylar end near the base of the archegonial chamber.

Exercise 14

Study of seed
Work procedure
Study the position and arrangement of seeds on ovuliferous scale, also study a prepared slide of L.s. of seed.

Comments
1. Fertilized ovules get transformed into seeds which are situated on the adaxial side of the ovuliferous scale at its base near the cone axis.
2. Seeds are small, elongated and winged. The wing is a thin layer of tissue which splits off from the adaxial face of the ovuliferous scale. (Seed can be best studied by cutting longitudinal section of the seed of *P. gerardiana*; vern. chilgoza).
3. The seed is covered with red and brown testa.
4. Inner fleshy layer of the integument still persists. It is membranous, thin and papery, termed as tegmen.
5. The nucellus is present as a thin layer and forms a nucellar cap at the micropylar end.



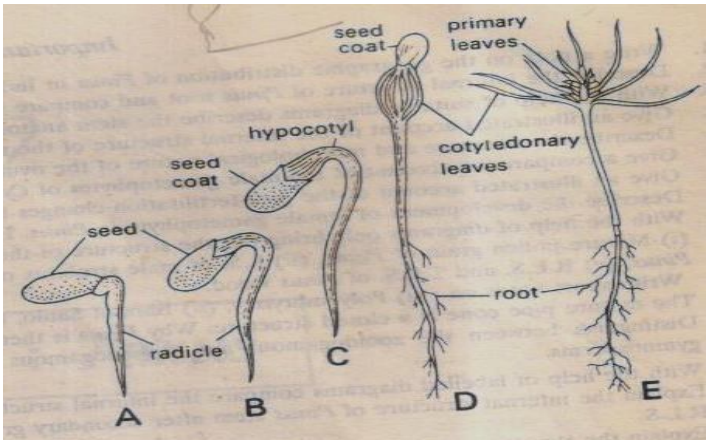
i8 : *Pinus* : A. L.S. of seed. B. An ovuliferous scale bearing seeds, C. A seed with wing

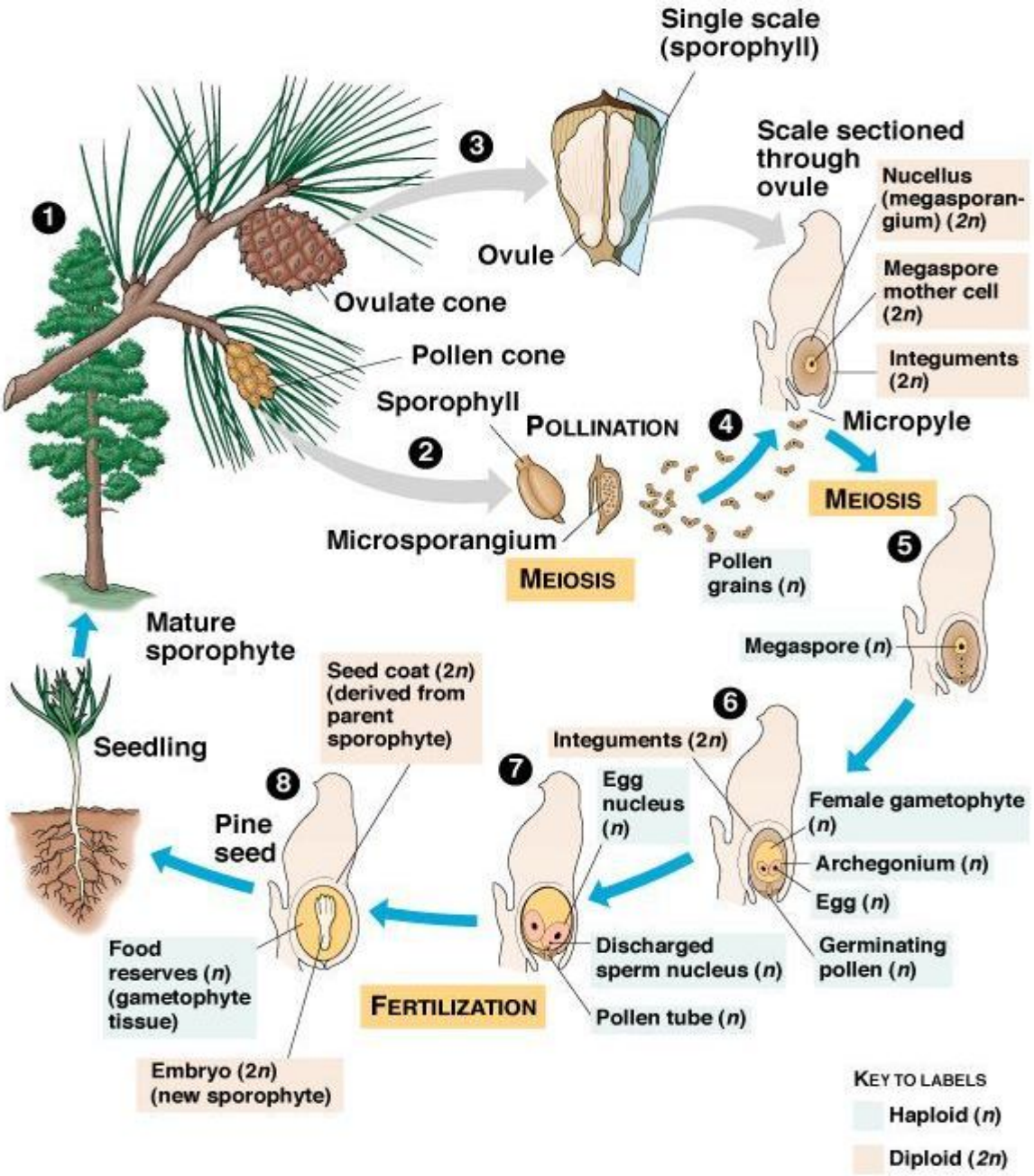
6. The larger part of the seed consists of oily endosperm.
7. The suspensor is long and becomes coiled. Embryo is differentiated into radicle, plumule and cotyledons (3-8 in number).
8. In between the radicle and plumule, is present a well-developed hypocotyl.

Exercise 15

Study of seedling
Work procedure
Study a newly germinated seed. Observe various organs.

Comments
1. Seedling shows three parts — (i) roots (ii) hypocotyle (iii) leaves.
2. The roots are well branched and arise from the radicle.
3. Hypocotyl gives rise to unbranched slender and thin primary shoot.
4. Leaves are green and needle-like which are borne in whorls on the primary shoot. These are cotyledonary leaves.
5. The primary leaves or first spur shoots arise in the axils of some of these juvenile (cotyledonary) leaves and are borne in spiral series on the primary shoot



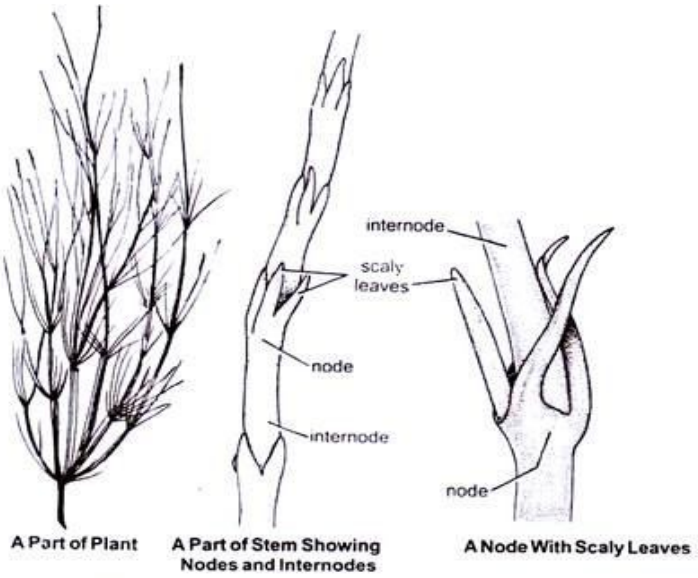


Life cycle of pinus

Ephedra (Jointed Fire)

Classification

Division	Gymnosperm
Class	Gnetopsida
Order	Gnetales
Family	Ephedraceae
Genus	*Ephedra*



It is represented by 50 species and most of them grow in dry habitat over a wide area of northern hemisphere including north-America, Europe, North-Africa, South-West Central Asia. Eight species of *Ephedra* have been reported from India. In most common Indian species, *E. intermedia, E. gerardiana, E. saxatilis* and *E. foliata* etc. are included. These species are very common in dry regions of Punjab, Haryana, Rajasthan and some parts of U.P etc.

Exercise 1

Study of external morphology

Work procedure

Study the external features of the plant, note the jointed nature of stem, scale leaves and underground tap root and observe the positions of male and female strobili.

Comments

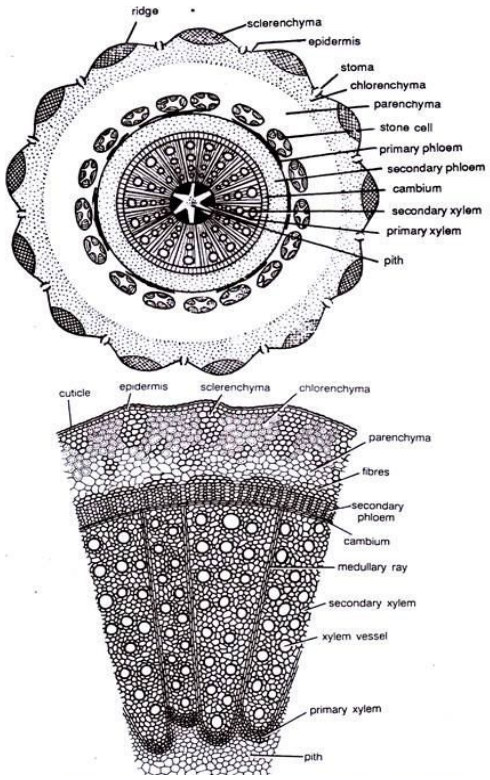
1. Plants are small, bushy, trailing or climbing shrubs attaining a height of not more than 2 meters. However, *E. antisiphilitica* is a small tree, reaching a height of 3-5 meters.
2. The plant body is branched and possesses only minute leaves at the nodes. It therefore, resembles superficially with the species of *Psilotum* and *Equisetum*.
3. It is differentiated into stem, leaves and underground roots.
4. The stem remains anchored by a deep tap root and many adventitious roots.
5. The stem is delicate, slender and green when young. It is ribbed irregularly and is differentiated into short nodes and long internodes.
6. Two or three branches arranged in whorls arise from the nodes in the axils of leaves. The branches are shed off during dry season.
7. Older part of the stem may bear many branches. It becomes hard and woody due to secondary growth.
8. Leaves are borne in a whorl of 2-4 at each node.
9. Leaves that are small and scale-like are connate at the base and thus form a sheath around the node.
10. Each scale leaf is traversed by two parallel and unbranched veins.
11. Foliage leaves are completely absent.
12. The male and female reproductive organs are borne in small strobili..
13. The plants are mostly dioecious and bear only one type of reproductive organs. They may also be monoecious when they bear both kinds of strobili.

Exercise 2

Study of T.s. of young stem

Work procedure

Cut a T.s. of the younger part of the stem, stain in safranin-fast green combination, mount in glycerine and study.



Comments

1. The outline of the section shows ribbed nature of the stem.
2. The tissues are differentiated into epidermis, cortex and stele.
3. Epidermis is the outermost layer. It is very thick and heavily cuticularized.
4. The stomata are sunken. These interrupt epidermis frequently and occupy a position just on the slopes of the ridges.
5. Hypodermis is sclerenchymatous and occurs in small groups below the ridges.
6. Rests of the cortical tissues are chlorenchymatous. The cells are often radially elongated and contain abundant chloroplasts. Large intercellular spaces are present between these cells.
7. A few patches of sclerenchyma occur dispersed in the cortex (specially in young axis rendering hardness and resistance).
8. Stele is ectophloic siphonostele. It is composed of many vascular bundles, their number being variable.
9. Endodermis is single layered and is followed by a pericycle.
10. A few vascular bundles are arranged in a ring. Each is conjoint, collateral, endarch and open.
11. External phloem group is separated from internal xylem group by a narrow layer of cambium.
12. Pith is parenchymatous and occurs in the central region.
13. The characteristic anatomical feature is the presence of diaphragm-like plate of cells at the base of each internode or at the nodes.

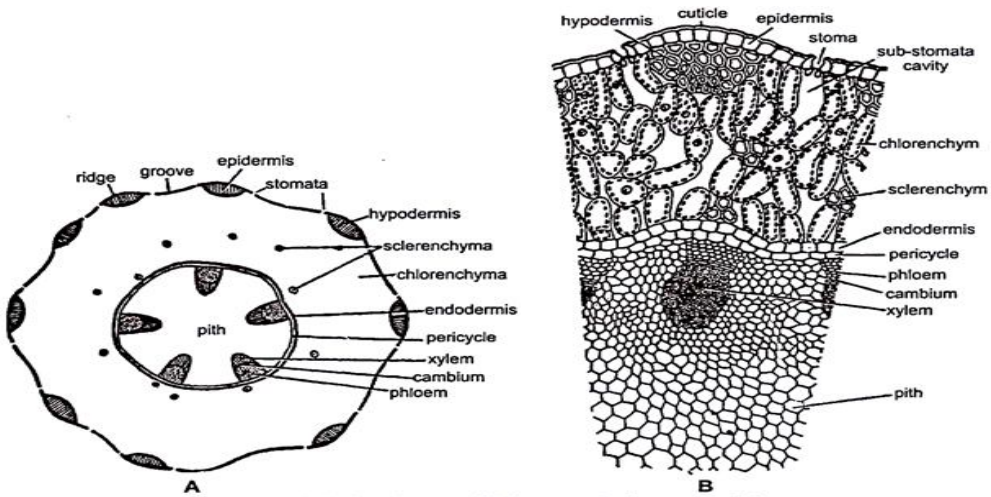


Fig. 2 (A, B) *Ephedra*. T.S. of stem, (A) diagrammatic; (B) a part of cellular.

Exercise 3

Study of T.s. of stem showing secondary growth

Work procedure

Cut a T.s. of the older part of the stem, stain in safranin-fast green combination, mount in glycerine and study.

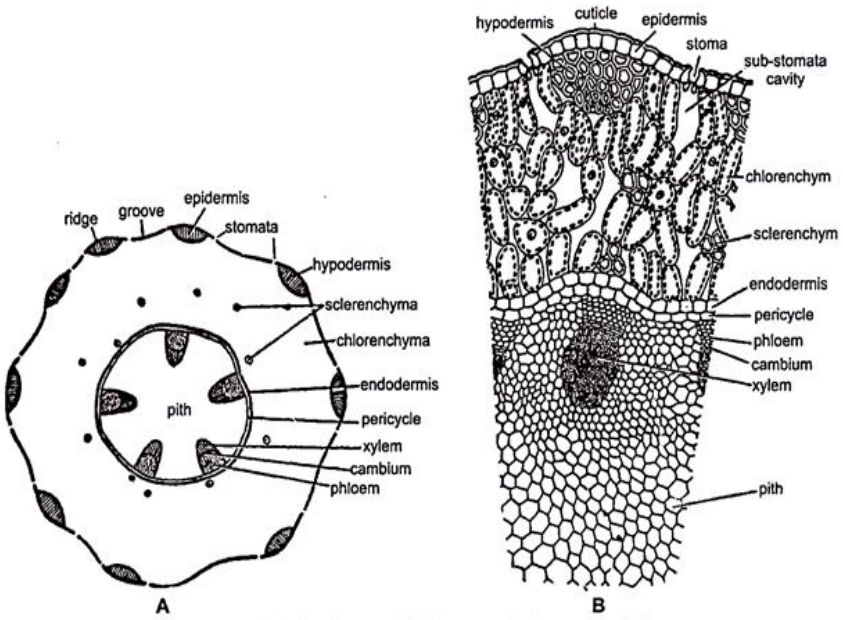


Fig. 2 (A, B) *Ephedra*. T.S. of stem, (A) diagrammatic; (B) a part of cellular.

Comments

1. The section shows epidermis, cortex, primary and secondary vascular tissues.
2. The epidermis and cortex remain unchanged. However, after (3-4 Years) of growth, cork develops just outside the phloem and outer tissues (epidermis, cortex, etc.) are, therefore, cast off.
3. Sclerotic cells (stone cells) develop just above the zone of secondary tissue.
4. Primary phloem occurs as obliterated patches.
5. Secondary phloem forms a zone below. Phloem is composed of sieve tubes and phloem parenchyma.
6. Annual rings are distinct comprising autumn and spring wood each. These are formed in the secondary xylem (wood).
7. The secondary xylem shows a thin walled spring wood and thick walled autumn wood, successively formed in alternating zones.
8. Autumn wood is made of smaller cells, while those of spring wood are bigger in size.
9. The tracheidal cells of the secondary wood are associated with broad vessels. Though absence of vessels is characteristic of the Gymnosperm. *Ephedra* (i.e. order Gnetales) itself, is an exception.
10. Vessels are most abundant in the spring wood and a few or none at all, in the autumn wood. Spring wood is often ring porous.
11. Tracheids and vessels have uniseriately or irregularly distributed bordered pits. Protoxylem elements of primary xylem show spiral, annular or reticulate tracheids.
12. Medullary rays traverse the wood. Primary medullary rays run from primary xylem to primary phloem while secondary medullary rays run from secondary xylem to secondary phloem.
13. Medullary rays are uniseriate in the young stem but are very broad and long (multiseriate) in the old stem.
14. Primary xylem groups are present at the end of the secondary wood near the pith. These are endarch.
15. Pith is large and parenchymatous. It occupies the centre.

Features of special interest

It shows the following xerophytic characters.

1. Thickly cuticularized epidermis.

2. Sunken stomata.
3. Palisade and spongy parenchyma in the cortex.
4. Patches of sclerenchyma.
5. Shedding of branches.
6. Presence of nodal diaphragm.
7. Vessel in the secondary wood.

Exercise 4

Study of male strobilus

Work procedure

Study the position, structure and organization of male strobilus. This can be done by studying external morphology of the strobilus and a slide of its longitudinal section.

Reproductive parts are borne in strobili. One of the following conditions may be found

(i) Usually male and female strobili are different: in such a case, the strobilus may be termed as monosporangiate. These strobili may be borne on two different plants (dioecious sps.).

(ii) Sometimes one plant may bear both the strobili (monoecious sps.).

(iii) A few plants, sometimes, bear both the reproductive parts in one strobilus only (bisporarangiate strobilus), e.g. *E. foliata* and *E. intermedia*. In such cases, male flowers are situated below the female flowers which occur at the higher level in the same strobilus.

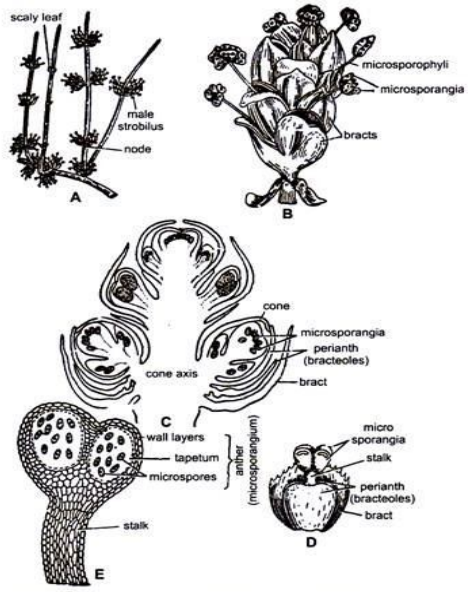


Fig. 8 (A – E). *Ephedra*. Male strobilus. (A) Staminate shoot, (B) Male strobilus; (C) L.S. male strobilus; (D) A single male flower, (E) L.S. microsporangium.

Comments

1. The strobilus resembles on inflorescence of spike type.
2. Each stobilus consists of an axis which bears decussately arranged sterile scales and stamens.
3. Male spike (male strobilus or staminate strobilus) arises in the axil of scale leaf.
4. Each spike is generally round in shape but may be ovoid or spherical.
5. A spike has a short axis with many scaly bracts. The bracts are arranged in decussate pairs. The number of pairs varies from 2-12.
6. In the axil of each bract arises a single male flower.

Exercise 5

Study of male flower

Work procedure

Place a male strobilus under the dissecting microscope, separate the sterile scale to isolate single male flower, stain in safranin, mount in glycerine and study. Also study slide of L.s. of a stamen.

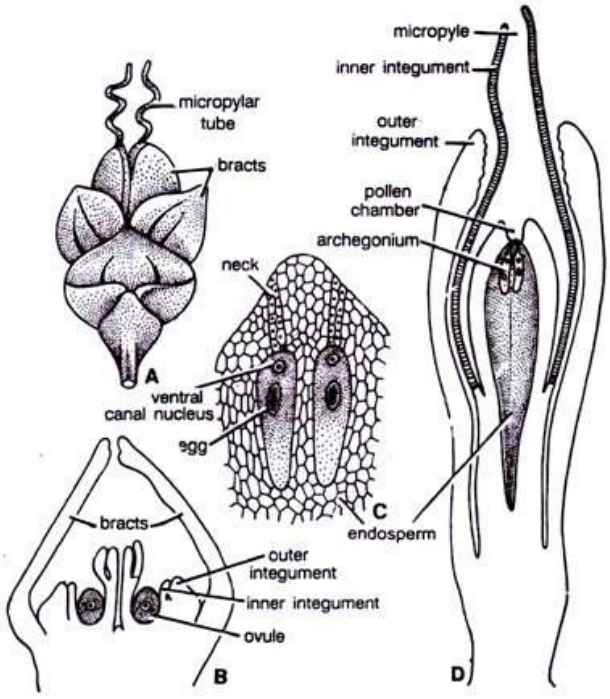
Comments

1. A male flower has a perianth of bract scale which encloses a stamen.
2. Stamen consists of a stalk (variously termed as column or antherophore). It bears 2-5 microsporangia or anthers at its tip.

3. Each microsporangium is a bilocular structure. It has two wall layers and a prominent tapetal layer which encloses pollen grains or microspores.
4. Each microspore is elliptical and has an outer thick and ribbed exine and a thin intine.
5. A microsporangium opens by apical part (apical dehiscence).

Exercise 6

Study of female strobilus
Work procedure
Study the position, structure and organization of the female strobilus. This is done by studying the external features and a slide of its longitudinal section.



Comments
1. The strobilus resembles spike inflorescence.
2. Each strobilus consists of an axis bearing decussately arranged sterile bracts (scales) and ovules.
3. The female or ovulate strobili arise in the axil of scale leaves. The female strobilus is also sessile and not so richly branched as male.
4. The apex of ovulate strobilus is mostly acute.
5. The spike has a short axis on which about 4-7 pairs of bracts are arranged decussately.
6. Lower most I or 2 pairs are sterile while terminal pairs bear short stalked ovules. The bracts are generally dry, winged and may be variously coloured.
7. Each bract mostly encloses two ovules out of which one may be abortive.

Exercise 7

Study of L.s. ovule
Work procedure
Study a prepared slide of L.s. of ovule.

Comments
1. Ovule is covered by two integuments, which consist of two envelops.
2. Outer integument (involucre or perianth) is a cup-like structure, attached at the base of the ovule and free above.
3. Inner Integument is delicate, composed of two segments. It prolongs into a tubular process and comes out beyond the bracts and involucre at the time of pollination.
4. Micropyle is an opening in between the integuments, in the upper region of the ovule.
5. Nucellus lies below the integuments. A small pollen chamber is present just below the micropyle in the tissue of nucellus.
6. Female gametophyte is a tissue situated below the pollen chamber. Two archegonia are present, just below the pollen chamber, in the female gametophyte.

7. Haustorial region lies opposite the micropylar end. It is occupied by tissue filled with stored food material. It also gives out haustorial processes for the absorption of food and is known as haustorial region.

Exercise 8

Study of L.s. of seed
Work procedure
Study a double stained prepared slide of L.s. of seed. (A seed is formed as a result of fertilization).

Comments
1. Outer integument encloses entire seed. It is thick walled.
2. The inner integument ('true integument') persists at the micropylar end only.
3. Nucellus forms shrivelled layer in the form of a disorganized sheath of cells. It is located inside the inner integument.
4. Female gametophyte (endosperm) surrounds a big embryo which has two large cotyledons.
5. Bracts adjacent to strobilus are fleshy and thick in a completely mature seed. These form an additional envelope.

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