B.Sc. ZBC-104

BOTANY PRACTICAL

DIRECTORATE OF DISTANCE EDUCATION

SWAMI VIVEKANAND
SUBHARTI UNIVERSITY
Meerut (National Capital Region Delhi)

BOTANY PRACTICAL

B.Sc. ZBC–104

Self Learning Material

SWAMI VIVEKANAND SUBHARTI UNIVERSITY
• 2008 •
उत्तिष्ठत जाग्रत प्राप्य वरान्निबोधत

Directorate of Distance Education

SWAMI VIVEKANAND SUBHARTI UNIVERSITY
MEERUT–250 005
UTTAR PRADESH

SLM module developed by :
Mr. Om Prakash

Reviewed by :
Dr. Anju Rani

Assessed by :
Study Material Assessment Committee, as per the SVSU ordinance No. VI (2)

Published by : Pragati Prakashan, 240 W.K. Road, Meerut – 250 001
Tel. 2640642, 2643636, 6544643, E-mail : pragatiprakashan@gmail.com

Typeset at : Pragati Laser Type Setters Pvt. Ltd., Meerut Printed at : Arihant Electric Press, M

EDITION : 2021

Syllabus

First Year : First Semester
Biodiversity (Microbes, Algae, Fungi and Archegoniate)
Course Code : B.Sc. ZBC–104

Objectives: To generate interest in the students about the different types of lower plants, which are economically very important.

List of Practicals:

1. Models of viruses, Types of Bacteria from temporary/permanent slides/photographs; EM bacterium; Binary Fission; Conjugation; Structure of root nodule, Gram staining.
2 Study of vegetative and reproductive structures of Nostoc, Chlamydomonas (electron micrographs), Oedogonium, Vaucheria, Fucuse and Polysiphonia through temporary preparations and permanent slides. (« Fucus Specimen and permanent slides).
3. Asexual stage from temporary mounts and sexual structures through permanent slides, Specimens/photographs and tease mounts: Alternaria, Rhizopus, Penicillium, Agaricus.
4. Puccinia: Herbarium specimens of Black Stem Rust of Wheat and infected Barberry leaves; section/tease mounts of spores on Wheat and permanent slides of both the hosts.
5. Lichens: Study of growth forms of lichens (crustose, foliose and fruticose), Mycorrhiza: ectomycorrhiza and endomycorrhiza (Photographs).
6. Marchantiamorphology of thallus, rhizoids and scales, v.s. thallus through gemma cup, w.m. gemmae (all temporary slides), V.S. antheridiophore, archegoniophore, LS. sporophyte (all permanent slides). Funariamorphology, leaf, rhizoids, operculum, peristome, annulus, spores (temporary slides); permanent slides showing antheridial and archegonial heads, L.S. capsule and protonema.
7. Equisetum, Selaginellaand Pteris morphology, leaf with ligule, T.S. stem, W.M. strobilus, W.M. microsporophyill and megasporophyll (temporary slides), L.S. strobilus (permanent slide).
8. Cycas- morphology (coralloid roots, bulbil, leaf), T.S. coralloid root, T.S. rachis, V.S. leaflet, V.S. microsporophyil, W.M. spores (temporary slides), L.S. ovule, T.S. root (permanent slide).Pinusmorphology (long and dwarf shoots, W.M. dwarf shoot, male and female), W.M. dwarf shoot, T.S. needle, T.S. stem, L.S./T.S. male cone, W.M. microsporophyll, W.M. microspores (temporary slides), L.S. female cone, T.L.S. & R.LS. stem (permanent slide).

CONTENTS

UNIT 1

LABORATORY REQUIREMENTS

INSTRUCTIONS

Observe the following when you are in the laboratory :

1. See that your seat is clean, contains all the apparatuses, chemicals and reagents required for today's practical, and all the chemicals and reagent bottles are well-labelled.
2. **Work** :
   (i) Independently;
   (ii) Strictly according to the instructions of your teacher and;
   (iii) Systematically, *i.e.*, first external morphology, then internal study and then the reproductive structures. (It is generally observed that students, just after getting the material, start the section cutting without going through its external study and then they think of the latter, which is now not possible because now they do not have the required material or those stages of material which are very much required for external study).
3. Also note strictly that you are not only required to do practical work (like section cutting or setting the experiment etc.), but equally important work is to :
   (i) Observe the details of your experiment;
   (ii) Note the observations and final results of the experiment;
   (iii) See whether all the informations, given to you in the instructions by your teacher, are present in your material or not, and;
   (iv) Draw the diagrams of different stages present in your material with the unaided eye or with the help of a magnifying lens, dissecting microscope, binocular microscope or compound microscope (Figs. 1, 3), there itself in the laboratory.

4. **Keep**
   - (i) Every thing clean;
   - (ii) Your desk and table in order;
   - (iii) Your hands clean and dry at the time of mounting the ribbons on slide in the process of microtomy;
   - (iv) Sightly corked the containers filled with anhydrous solutions;
   - (v) Every record and data up-to-date, and;
   - (vi) The bottle of Canada-balsam away from the light.

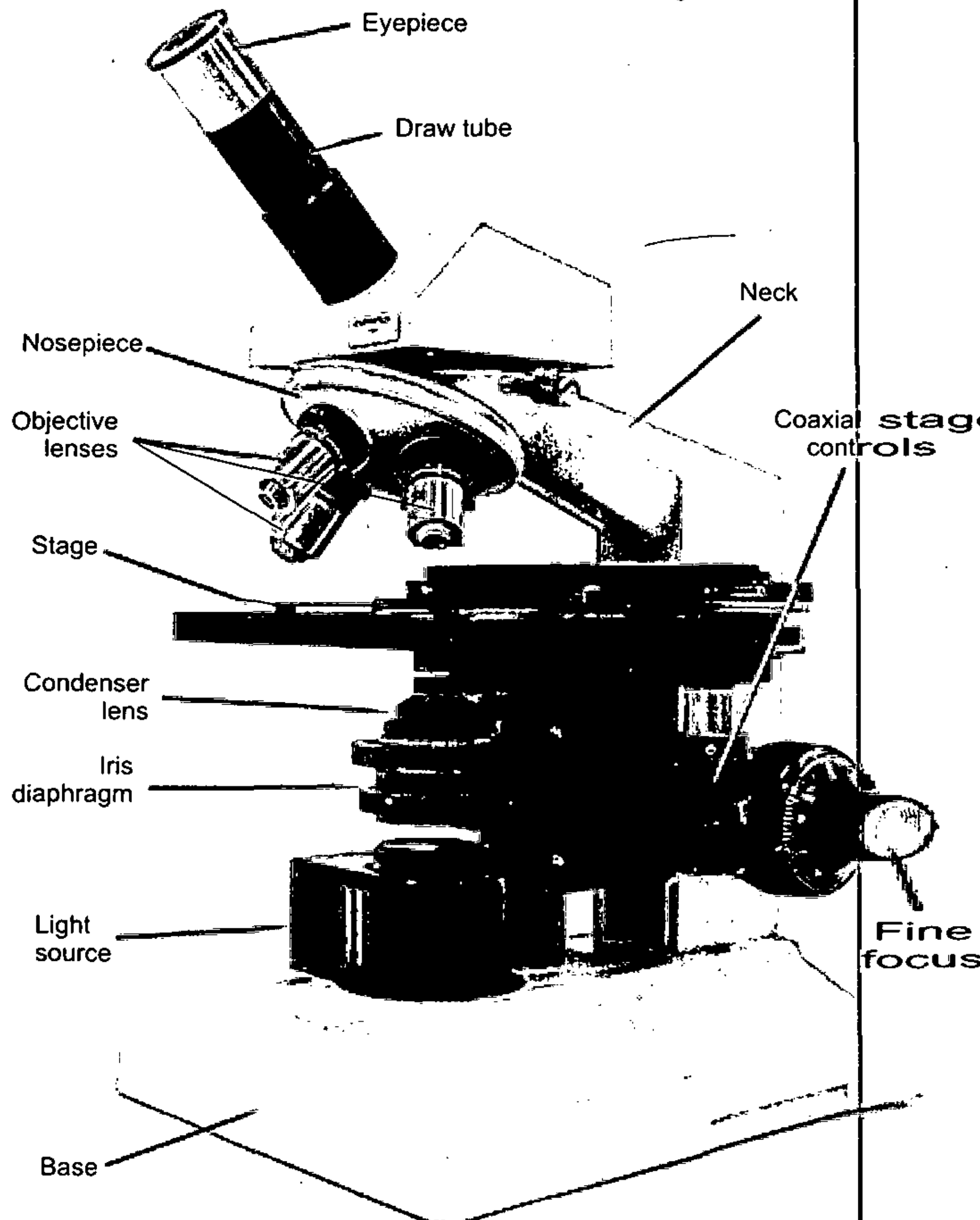


Fig. 1. ***A modern compound microscope.***

5. You will be provided by your teacher with a daily schedule, and you sho check your work for the day against the schedule to make sure that measurement or preparations have been overlooked.

6. Science students should have a note-book to record what they do day-by-day in the laboratory. Every thing pertaining to the given problem should be recorded in the note-book. *It should be kept up-to-date.* One cannot work efficiently in the laboratory periods unless the work of the preceding period is finished.
7. Read carefully the laboratory instructions before you report to the laboratory. A crowd of work may be there in the laboratory periods, and so you must arrive knowing what you are expected to do.
8. You will have to perform new experiments daily in the laboratory, and so a need to learn and use new technical words will arise. From *glossary*, you should see and note all these words.
9. You must do your best when you are performing an experiment with a team. In scientific investigations, both team-work and individual-work are very important.
10. *An extremely important part of a team-work experiment is the discussion.* Note the following at the time of discussion :
    (i) Treat the discussion time as the period of the practical class.
    (ii) Review the questions asked, procedures followed and results obtained from your investigations.
    (iii) Discuss clearly and frankly what you do not understand about the experiment you have performed.
    (iv) Try to suggest your own ideas in which the given experiment could be performed in a better way than the instructions given in the laboratory.
    (v) Try to develop the *"etch of curiosity"* about the ways and methods for the given experiment.

• INSTRUMENTS

Keep with you the following instruments while you are in the laboratory :

1.	Razor	1
2.	Forceps	2
3.	Needles	4
4.	Scissors	1
5.	Brushes	2
6.	Scalpel	1
7.	Blades	2
8.	Dropper	1
9.	Pencils (one each of HB, 2H and 4H)	3
10.	Rubber	1
11.	Scale	1
12.	Practical record note-book (one file with drawing and ruled papers)	

Following instruments, apparatuses, chemicals and stains will be provided to the students from the laboratory :

1.	Compound microscope	1
2.	Dissecting microscope	1
3.	Binocular microscope—one in between two students	
4.	Watch glasses	6
5.	Petri dishes	4
6.	Microscopic slides (3" × 1")	12

7. Coverslips (0.17 mm thickness)
8. Piece of soft cloth
9. Enamel tray
10. Polythene washing bottle
11. Spirit lamp
12. Glass-marking pencil
13. Ringing table
14. Microtome—one rocking and one rotary for entire class.
15. Dropping bottles for reagents
16. Staining bottles
17. A hone and a razor strip of leather for entire class
18. Necessary chemicals and stains.

Some laboratory instruments of common use are shown in Fig. 2.

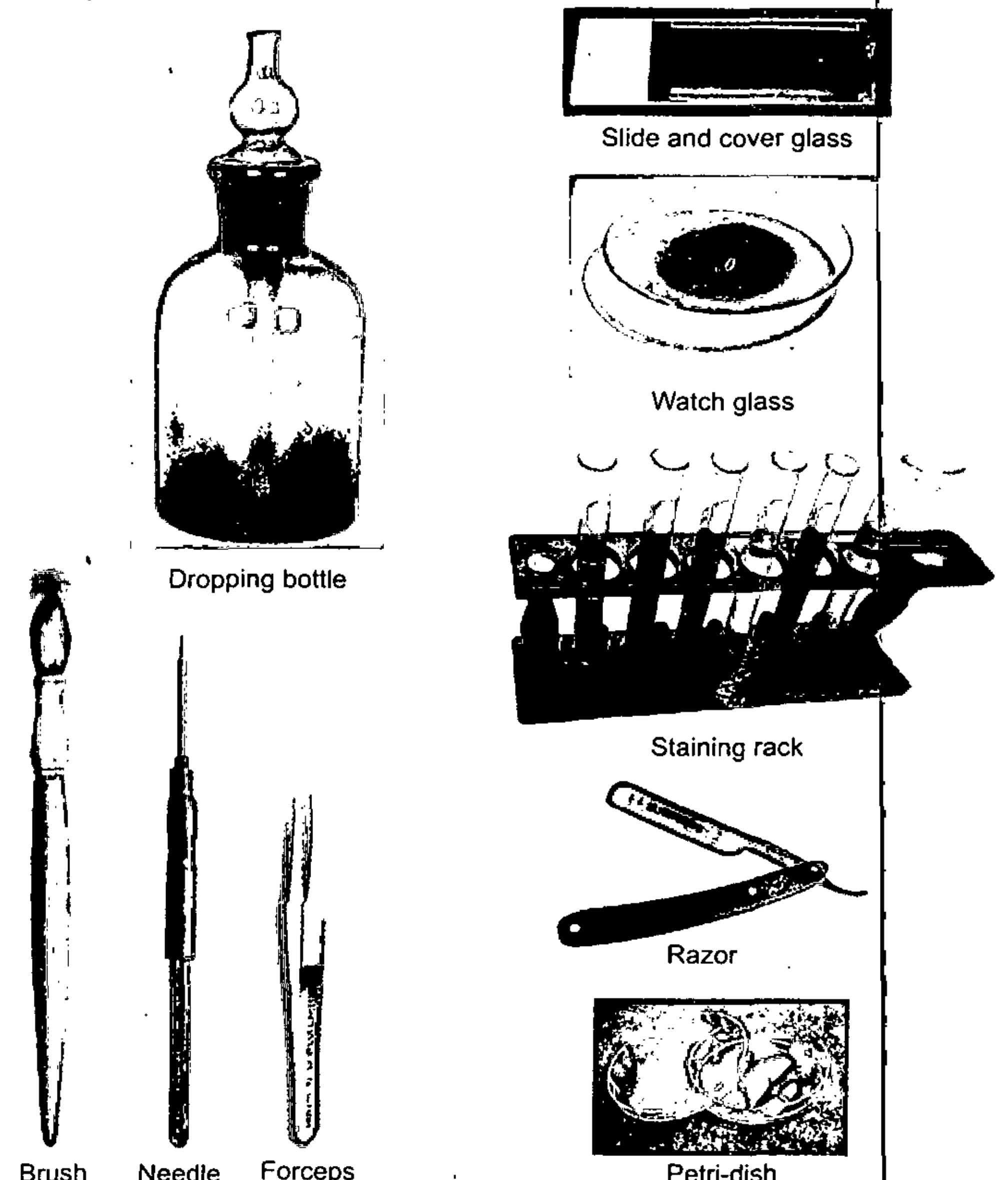


Fig. 2. ***Some laboratory instruments.***

• INSTRUMENTS FOR FIELD COLLECTION

While you are on field collection trip, you should have the following instruments with you :

1. One pocket lens
2. One good fixative (F.A.A.)
3. Collection bottles (three dozen)
4. Full-size blotting papers (three dozen)
5. Newspaper pieces (two dozen)
6. Polythene bags (four dozen)
7. Rubber bands (four dozen)
8. Plant press, made up of two flat plywood sheets and a thick, strong cord
9. Glass-marking pencil
10. Departmental labels.

• DISSECTING MICROSCOPE

It is actually a simple microscope that consists of only one lens unit. This lens unit may even be an ordinary magnifying glass. Dissecting microscope is used either for dissecting the material or for less magnifications, *i.e.*, only 6X,12X or rarely 20X. It is mainly used for embryo separation, taxonomic studies, etc.

A dissecting microscope (Fig. 3) consists of a basal foot and a limb. The 'stage', made up of a simple glass plate, is attached to the limb. For the light adjustment purposes, a mirror is attached to the limb under the stage. Mirror can be moved vertically with the help of an adjustment screw. At the tip of the limb is present a folded arm, on which a lens of definite magnification (6X, 12X, etc.) is fitted. Folded arm is moved to keep the lens in the desired position on the stage.

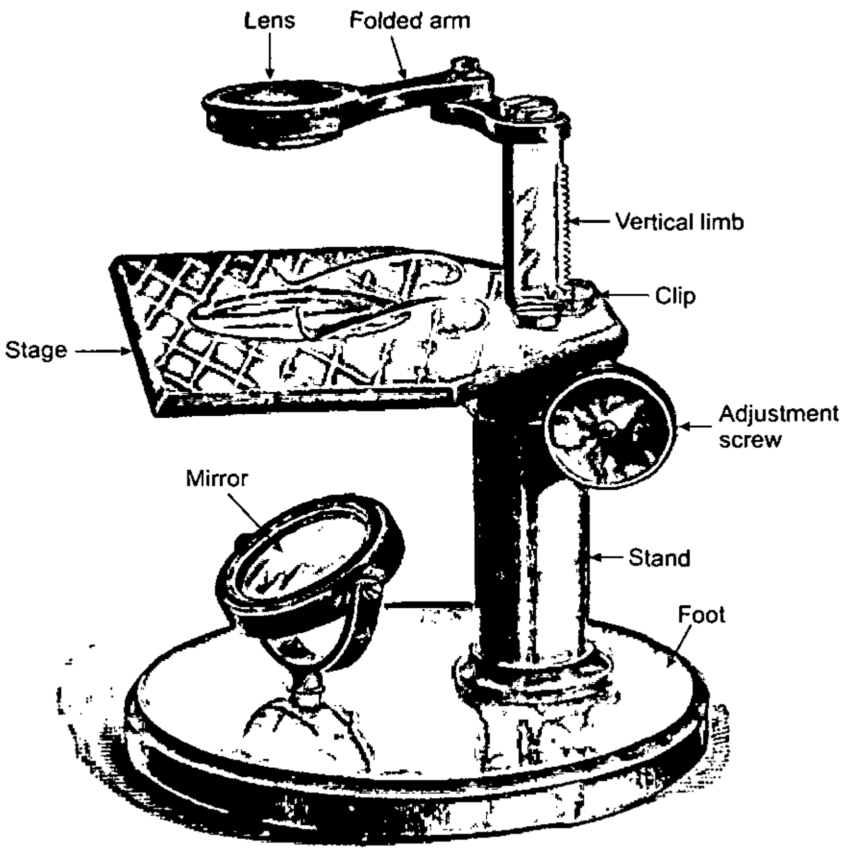


Fig. 3. *Dissecting microscope.*

The material, to be viewed or dissected, is placed on the stage. The eye is plac close to the lens. Folded arm is tilted to bring the lens over the material. Ligh adjusted by the movement of the mirror. Focussing is done with the help adjustment screw.

• COMPOUND MICROSCOPE

1. The instrument is so named because it consists of two or more lens syste (Figs. 1, 4).
2. At the top is present the *ocular lens*. It can be turned around or may removed. At the top of ocular lens is written 5X or 10X signifying the 5 times 10 times magnification, respectively.
3. Just below the ocular is a *body tube*, the bottom end of which contains a circu piece called *nose piece*. It contains three lenses called *objective lenses*. No piece can be rotated to change the position of objectives.

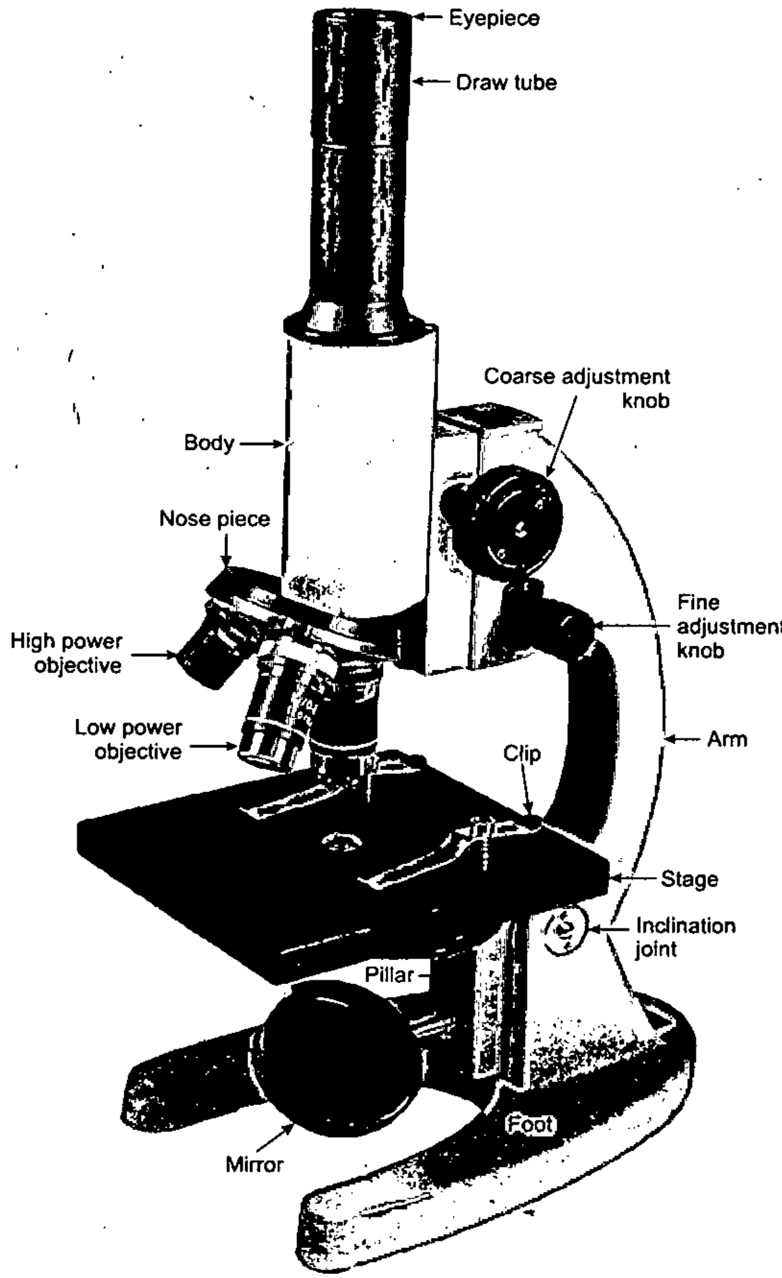


Fig. 4. ***Compound microscope.***

4. The flat platform present below the objectives is called *stage*.
5. On the arm of the microscope are present two knobs called *coarse adjustment knob* and *fine adjustment knob*.
6. Out of the three objectives, the shortest is the *low power objective*. It has the largest lens but its magnifying power is least of the objective lenses. On the objective may also be written 10X similiar to ocular lens. It means if a 10X ocular lens is used the magnification is

$$10 \times 10 = 100 \text{ times.}$$

7. The other objective is *high power objective*. Its magnification is equal to the number written on it multiplied by the power of ocular, *i.e.*, 5X or 10X (*i.e.*, objective × ocular).
8. The third objective is called *oil immersion* objective. Generally, it contains a black band around the lower end. Use a drop of oil on the slide at the time of studying with the oil immersion objective. Its magnification can be estimated as ocular × objective. The use of oil (*e.g.*, cedarwood oil) is essential in order to keep the light rays properly aligned with the small objective.
9. Just below the stage is the *condenser*. Its function is to gather light from the mirror and to direct it to the objective lens. Condenser may be lowered or raised by a knob present on one side of the microscope beneath the stage.
10. Condenser contains a shutter called *Iris diaphragm*.
11. Just below the condenser is present a *mirror* having its one surface flat and other concave. Use the concave surface in the day light. Flat surface of the mirror is used when electric lamp is used.

Precautions

1. Clean the ocular and objective lens with lens paper, and do not remove them.
2. While studying an object, learn to keep one hand on the fine focus knob, and focus continuously up and down.
3. While studying any kind of preparation, do not tilt the microscope.
4. Leave the low power objective in place after finishing all the observations.
5. To examine an object, always first use the low power and then the other objectives.
6. Never allow an objective lens to strike either the stage or a slide while focussing.
7. Use always the fine adjustment with high power objective.
8. All wet-mount preparations should be precovered by a cover slip.
9. Avoid the habit to remove the parts of the microscope.
10. Do not use oil immersion objective without oil.
11. Diaphragm should be wide open while using oil immersion objective.

• PREPARATION OF SOME FIXATIVES, STAINS AND REAGENTS

1. *Formalin–Aceto-Alcohol* (F.A.A.)

50% or 70% ethyl alcohol	90 c.c.
Glacial acetic acid	5 c.c.
Formalin	5 c.c.

It is the most commonly used preservative and is commonly called *'standard preservative'*.

2. *Safranin*	
Safranin stain	1 gm
Water (or 50% alcohol or 70% alcohol)	100 c.c.
3. *Fast green*	
Fast green stain	1 gm
90% alcohol	100 c.c.
4. *Cotton blue*	
Cotton blue stain	1 gm
Lactophenol	100 c.c.
5. *Acid alcohol*	
HC	10 to 20 drops
Alcohol	100 c.c.
6. *Acid water*	
HCl	10 to 20 drops
Water	100 c.c.
7. *Lactophenol*	
Phenol	100 gm
Lactic acid, glycerine and water	100 c.c. each
8. *Glycerine*	
Glycerine	10 to 15 c.c.
Water	100 c.c.
Formalin	one drop.

• STUDY OF PLANT MATERIAL

Take the fresh or preserved material and first study its external morpholog with unaided eye or with the help of a magnifying lens or dissecting microscope. the material is microscopic, stain it with a suitable stain, mount in glycerine ar then study under the low and high power of a compound microscope.

For observing the anatomical details, cut the sections of your material in variou planes like transverse section (T.S.), longitudinal sections (L.S.) like radi longitudinal section (R.L.S.) or tangential longitudinal section (T.L.S.) as under

Transverse Section (T.S.) : It is cut by passing the edge of the razor at righ angles to the longitudinal axis of the cylindrical organs, such as roots, stems, etc

However, in case of the dorsiventral organs (*e.g.*, leaf, thallus of bryophyte etc.), the transverse section is cut in vertical plane and hence it is called vertic transverse section or V.T.S.

Longitudinal Section (L.S.) : It is cut by passing the edge of the razor at righ angles to the transverse axis of the cylindrical organs, such as roots, stems etc.

If the longitudinal section is cut along one of the radii, it is called radi longitudinal section or R.L.S.

But if the L.S. is cut along the tangent, it is called tangential longitudina section or T.L.S. (Fig. 5).

• METHOD OF SECTION CUTTING

A good quality razor should be used for section cutting. Before section cuttin the razor should be sharpened on a hone (fine grit-stone). Sharpening is done b

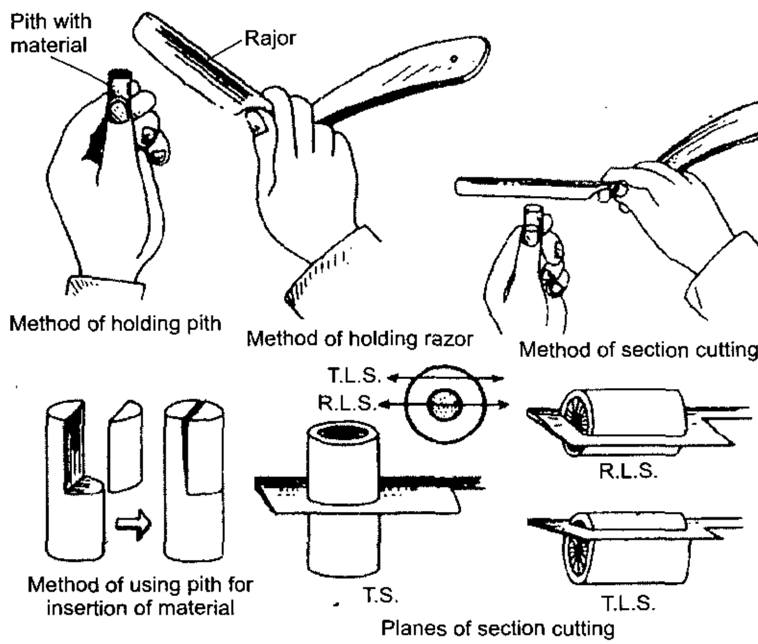


Fig. 5. *Methods and planes of section cutting.*

sliding the razor obliquely and uniformly over the wet hone. This process is called *honing.*

After the honing process, the razor is further sharpened by moving for some time over a leather strap. This process is called *strapping*. The leather strap should be first oiled before being used.

Pith (*i.e.*, pieces of carrot, potato, radish, etc.) is used for cutting sections of soft and delicate materials. At the time of using the pith, cut it longitudinally upto half of the length, and then remove the splitted piece by cutting one strip transversely. Insert the material in the centre and replace the piece of pith over it, as shown in Fig. 5.

For cutting the section, hold the pith (along with the material) in your left hand between the thumb and the fingers. Keep the forefinger in somewhat horizontal position and thumb at a little lower height. Hold the razor in your right hand in such a way in a horizontal position that its blade and handle form a right angle. Move the razor quickly over the pith (containing material) and complete the stroke in one action. Make 10 to 15 uniform strokes of the razor one after the other. You will get a few complete and thin sections. Immerse the sections in a watch glass containing water with the help of a fine brush. The material in pith and the razor blade should be kept flooded with water during the entire process.

Select thin and complete sections for staining. Thin sections usually keep on floating over the water surface. Only uniformly cut, very thin and complete sections should be stained.

• STAINING

Next step is *staining*. It is essential because different tissues and other structures can be easily differentiated by staining. The plant materials, in which there is no differentiation of tissues such as members of algae, fungi and bryophyta, are stained by a *single staining process*. But members of pteridophyta, gymnosperms and angiosperms are stained by the *double staining method* due to the presence of differentiation of tissues. Members of different groups can be stained in general as follows :

Algae : Algal members are generally stained with a few drops of *safranin* for a few minutes (time is variable for different members) and mounting is done in 10% glycerine. Aniline blue, fast-green, acetocarmine and haematoxylin are some of the other stains used for algal preparations.

Fungi : *Cotton blue* is supposed to be one of the good fungal stains. After staining with cotton blue, mount the material in lactophenol if the former is not prepared in the lactophenol. Cotton blue serves as a staining as well as mounting medium if prepared in lactophenol. Aniline blue or haematoxylin also give satisfactory staining results in certain cases.

Bryophyta : Members are stained usually with safranin and mounted in 10% glycerine. Other good stains, to be used for members of bryophyta, are Delafield's haematoxylin and fast-green.

Pteridophyta, Gymnosperms and angiosperms : Representatives of these groups are stained by a double staining method. Undermentioned are some of the commonly used methods of double staining.

• SOME DOUBLE STAINING METHODS

1. ***Safranin-Fast Green Method*** : Keep the material, to be stained, in *safranin* for three to five minutes and then wash it with water. See under the microscope that only thick-walled cells are stained. Excess of stain is destained by *acid alcohol*. Again wash the material very thoroughly with water so that even the traces of acid are removed. Now stain the material with few drops of *fast green* for few seconds. Time for keeping the material in fast green varies from few seconds to one minute for different materials. Wash the material with *glycerine* and mount in a drop of *glycerine.*

With this method, *all thick-walled cells get red stain and all thin-walled cells take the green stain*. Entire process can be tabulated as follows :

Select a thin section

↓

Stain with safranin (for 3 to 5 minutes)

↓

Wash with water

↓

Destain with acid alcohol (if need be)

↓

Wash thoroughly with water to remove the traces of acid

↓

Stain with fast green
(for a few seconds to one minute)

↓

Wash with glycerine

↓

Mount in glycerine

2. ***Safranin-Aniline Blue Method*** : Follow exactly the same procedure as mentioned above except that in place of fast green use aniline blue.
3. ***Haematoxylin-Safranin Method*** : Keep the sections in Delafield's haematoxylin for four to five minutes and remove the excess of stain with water. Wash with ammonia. Wash the material very thoroughly with water. Now stain with safranin for few minutes. Wash the sections with glycerine for removing excess of stain and mount in glycerine. The entire method can be tabulated as follows :

Select a thin section
↓
Stain with haematoxylin (4–5 minutes)
↓
Wash with water
↓
Wash with ammonia water till stain turns blue
↓
Wash with tap water
↓
Stain with safranin (2–3 minutes)
↓
Wash in glycerine
↓
Mount in glycerine.

• SOME METHODS OF PERMANENT SLIDE PREPARATION

1. Safranin-Fast Green Combination

Select a thin section
↓
Stain with aqueous safranin
(for 3 to 4 minutes)
↓
Wash thoroughly with water
(till water becomes colourless)
↓
Dehydrate with 15% alcohol
↓
30% alcohol
↓
50% alcohol
↓
70% alcohol
↓
90% alcohol
↓
Stain with fast green, prepared in 90% alcohol
↓
Destain with 90% alcohol
↓
Absolute alcohol
↓
Dealcoholize with 25% xylol
↓
50% xylol
↓
70% xylol
↓
90% xylol
↓
Pure xylol (two changes)
↓
Mount in Canada Balsam or DPX.

2. Crystal Violet-Erythrosin Combination

Use aqueous crystal violet stain in place of safranin and erythrosin in plac fast green in the method discussed above. Other details are same as safranin-fast green combination mentioned above.

3. Haematoxylin-Safranin Combination

It is also similar with above-mentioned safranin-fast green combination exc following differences :

(i) Use *haematoxylin* in place of safranin.

(ii) Wash with ammonia water and then thoroughly wash with tap wate

(iii) Use safranin stain after 70% alcohol and destain with 70% alcohol then use 90% alcohol and absolute alcohol.

Other details are same as in safranin- fast green combination met mentioned above.

• MOUNTING

Stained material is to be mounted on a slide in a suitable mounting medium *glycerine, canada balsam*, etc. It is then covered with a thin, round or square p of glass or plastic usually called a *cover glass* or *cover slip*. Hold clean microsc slide and cover glass always by their edges. Put a drop of mounting medium on material placed just in the centre of the slide. A medicine dropper may be used this purpose. Fix one edge of the cover glass on one side of the mounting medi and hold the cover glass approximately at an angle of 45° to the slide. Gently lo the cover slip with the help of dissecting needle, as shown in Figure 6. Under cover glass, there should be no air bubble.

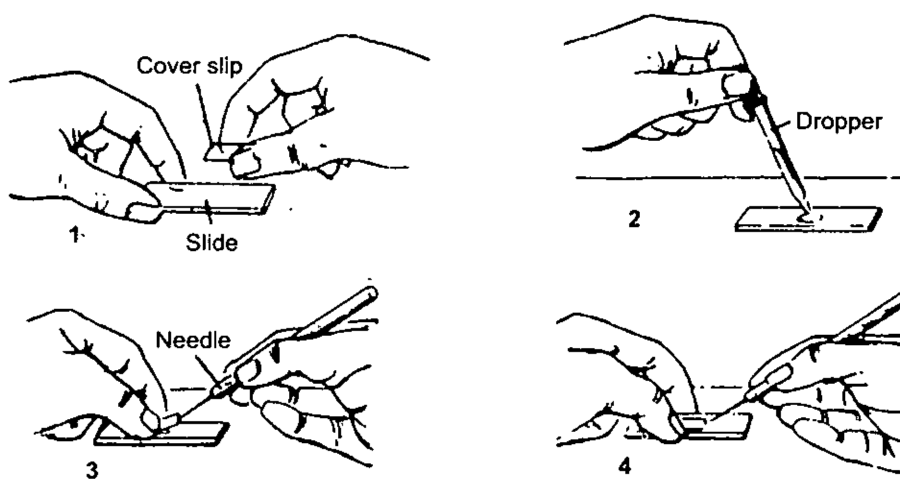


Fig. 6. ***Mounting of slide.***

• MICROMETRY

Micrometry is the science in which we have some measurement of dimensions of an object being observed under the microscope. The method empl some special types of measuring devices which are so oriented that these can v be attached to or put into the microscope and observed. The object to be measure calibrated against these scales.

Once we are observing an object under a microscope by the objective 10X and eye piece 10X we say that the image that we are able to perceive is 100 times of object. We get the magnified view no doubt and also that it is perfect coordinatio the dimensions, but to find out the absolute size of the object will need precision that is achieved through the application of *micrometers*. There are usually two types of micrometers, *i.e.*, *stage micrometer* and *ocular meter* (Figs. 7, 8).

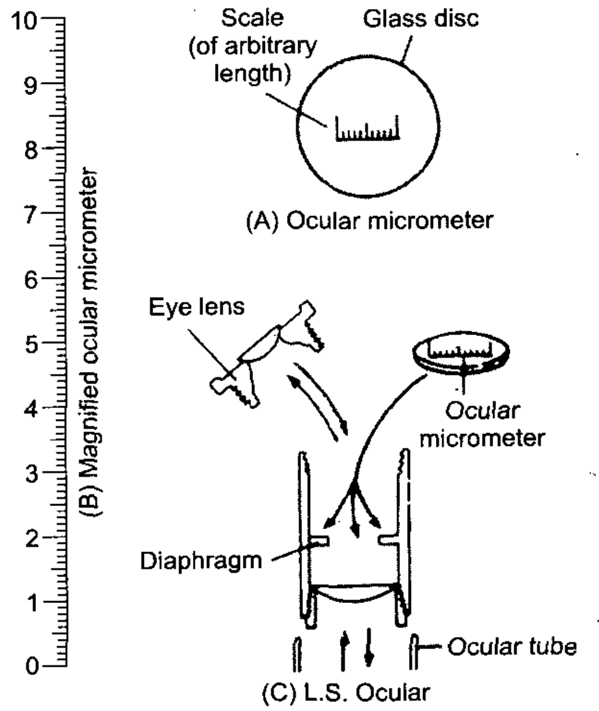


Fig. 7. A, An ocular micrometer; B, A magnified ocular micrometer scale; C, L.S. of ocular to show position of ocular micrometer in it.

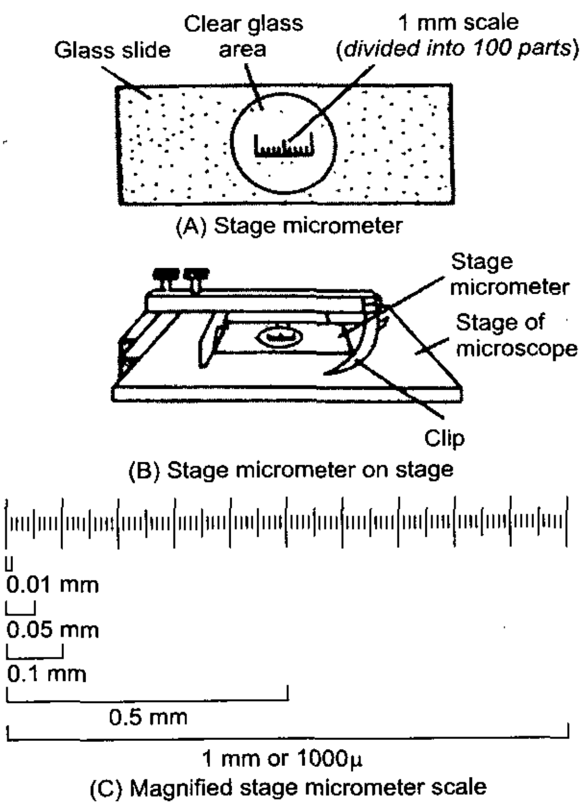


Fig. 8 A-C. ***A, A stage micrometer; B, Stage micrometer fixed on scale; C, Magnified stage micrometer scale.***

Stage Micrometer

As the name indicates it is for the measurement on the stage of the micros
where an object is to be kept. This micrometer is of slide's size and has a mou
very finely graduated scale (Fig. 8A). The scale measures only 1 millimetre and
a least count of 0.01 mm, *i.e.*, 1 mm region is divided into 100 divisions. As 1 mm
1000 μ, so one division of stage micrometer will be equivalent to 10 μ.

Ocular Meter

This is a micrometer which is used inside the eye piece. The upper eye le
unscrewed and the ocular meter is put into the tube of eye piece, and the eye le
again replaced. There are usually 50 or 100 divisions in the ocular meter which
engraved on the glass.

An Exercise of Micrometry

Exercise : *To measure the dimensions of common microorganisms*
calibration and standardization of microscope with the help of stage and oc
micrometers.

Requirements : Microscope, stage micrometer, ocular meter, slid
microorganisms (*e.g.*, bacteria or fungus) to be measured.

Procedure : To work out the measurements per ocular divisions, the s
micrometer is kept under low power of the microscope and is observed through
eye piece having ocular meter. Suppose, we have 10X objective and 5X eye pie
the microscope with a tube of 170 mm length. At this magnification the numb
ocular divisions coinciding the stage micrometer are observed and hence calcul
for microns per ocular divisions, *e.g.*, let us take that 6 ocular divisions coin
with 8 divisions of stage micrometer (Fig. 9).

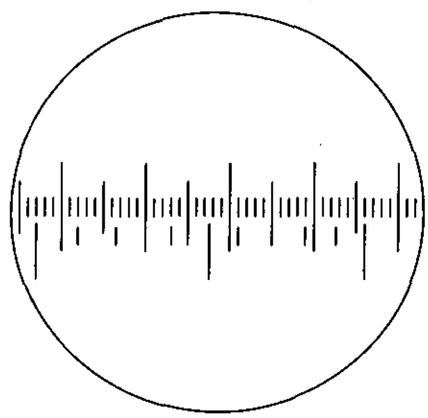


Fig. 9. ***Scales of stage and ocular meter, superimposed.***

Therefore, 6 ocular divisions = 8 stage micrometer divisions, or 6 oc
divisions = 0.08 mm (since 1 division = 0.01mm).

$$1 \text{ ocular division} = \frac{0.08}{6} \text{ mm}$$

$$= \frac{0.08 \times 1000}{6} \mu$$

$$= \frac{80}{6} \mu = 13.3\mu$$

Or, it can also be calculated by following formula :

$$\textit{One division of ocular} = \frac{\text{Number of stage micrometer divisions}}{\text{Number of ocular meter divisions}} \times 10$$

In the case mentioned above it will be :

$$= \frac{8 \times 10}{6} \mu = 13.3\mu$$

Thus, the microscope is caliberated for different combinations of eye pieces and objective lenses and is kept for record. It is to note hence that this caliberation will be just only of the tried lenses on a particular microscope.

In this way take three readings, and the mean value of these readings will be the actual value of one part of ocular meter. Record your data as in the following table :

Observations :

S. No.	Number of divisions of stage micrometer	Number of divisions of ocular meter	Value of one division of ocular meter $= \frac{\text{Stage}}{\text{Ocular}} \times 10\mu$	Mean/ Result
1.	8	6	$= \frac{8}{6} \times 10\mu = 13.3\mu$	
2.				
3.				

Result : The mean value (...... μ) of all the three readings of the above table is the actual value or calibrated value of one part of the ocular meter under this microscope.

Measurement of Spore or Any Other Object

Requirements : Microscope, calibrated ocular meter, permanent slide of spore/ bacteria/or of any other object to be measured.

Procedure : Insert the ocular meter in the eye piece of your calibrated microscope by unscrewing its upper eye lens. Now replace the eye lens again in its original position. Keep the spore or bacterial slide (or any other object), to be measured, on the stage of the microscope, and observe through the eye piece containing ocular meter. The object is measured in the particular magnification by ocular divisions and then is changed into microns by multiplying ocular divisions with calibrated value of one ocular division in that particular magnification.

Suppose, the width of the spore (or the object to be measured) is observed to be equal to 5 divisions of ocular meter (Fig. 10), so the width of this spore (or the object to be measured) in microns will be :

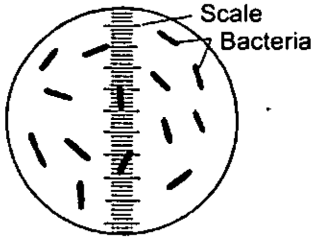


Fig. 10. ***Diagram showing an ocular micrometer used for measuring the dimensions of bacterial cells.***

$5 \times 13.3\,\mu$ (or any other calibrated value of one part of the ocular meter) $= 66.5\,\mu$.

Take at least four readings in this manner, and tabulate your observatic the form of following table :

S. No.	Number of divisions of ocular meter covering the spore/or any other object to be measured	Calibrated value of one division of ocular meter (in μ)	Measurement of the spore/or any other object to be measured (in μ)	Mean/Re (in μ
1.	5	13.3	5 × 13.3 = 66.5 μ	
2.		13.3		
3.		13.3		
4.		13.3		

Result : The mean value (...... μ) is the actual measurement of the leng width or diameter) of the given spore (or the object).

VIRUSES AND BACTERIA

• WHAT ARE VIRUSES ?

Viruses may be generalized to define as such very small sized etiological agents of disease that are capable of passing through filters that retain even bacteria, increase only in the presence of living cells, and give rise to new strains by mutation.

Bowden (1964) defined *viruses* as *'submicroscopic, infective entities that multiply only intracellularly and are potentially pathogenic.'*

Luria and Darnell (1968) defined viruses as *"entities, whole genome of which are element of nucleic acid that replicate inside the living cells using the cellular synthetic machinery and causing the synthesis of specialized elements that can transfer the viral genome to other cell."*

Gibbs and harrison (1976) defined viruses as *"a transmissible parasite whose nucleic acid genome is less than 3×10^8 Dalton in weight and that need ribosomes and other components of their host cells for multiplication."*

Mathews (1981) defined virus as *"a set of one or more nucleic acid template molecules, normally encased in a protective coat or coats or protein or lipoprotein, which is able to organise its own replication only within suitable host cells."*

Some define viruses as 'infectious nucleoproteins.'

The word *'virus'* is derived from the Latin language and means *'a poisonous liquid'* or *'poison'*.

• DISTINGUISHING FEATURES OF VIRUSES

1. Viruses do not fall into the category of unicellular microorganisms (bacteria, mycoplasmas etc.) because they do not possess a cellular organization.

2. Viruses contain only one type of nucleic acid, either DNA or RNA, but never both. However, all other microorganisms (bacteria, fungi, mycoplasmas etc.) contain both DNA as well as RNA.

3. They are obligate intracellular parasites and show their extreme dependence upon living cells for their existence and multiplication.

4. Viruses lack the enzymes necessary for protein and nucleic acid synthesis. For replication, they depend on the synthetic machinery of host cells.

5. The structural unit of a virus particle is called *virion.*

6. In size they range between 10 nm (foot-and-mouth-disease viruses) to 250 nm (poxviruses). They are, therefore, submicroscopic and can be seen only with the help of an electron microscope.

7. Viruses do not multiply by binary fission.

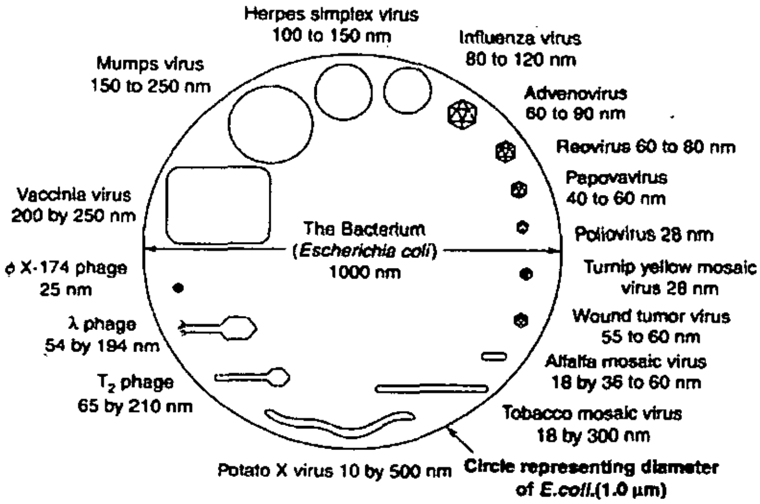
8. Antimicrobial antibiotics do not show any effect on viruses.

9. Viruses can be crystallized, like chemicals (Stanley, 1935).

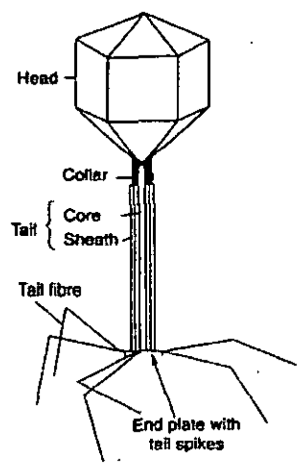
10. Viruses consist of high molecular weight nucleoproteins.

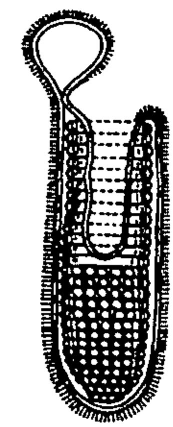
PRACTICAL No. 1

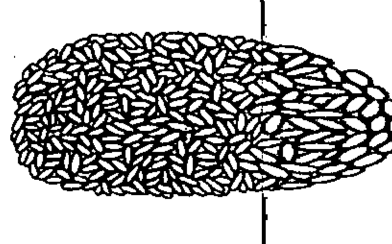
• (A) MODELS OF VIRU



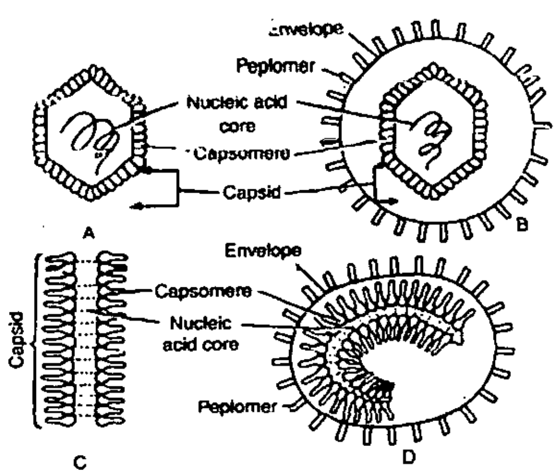
Model 1. Diagrammatic comparision of the sizes of some viruses. The largest circle enclosing the viruses represents *Escherichia coli* (a bacterium attaining a diameter of about 1.0 μm).



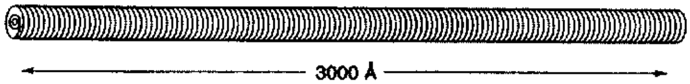




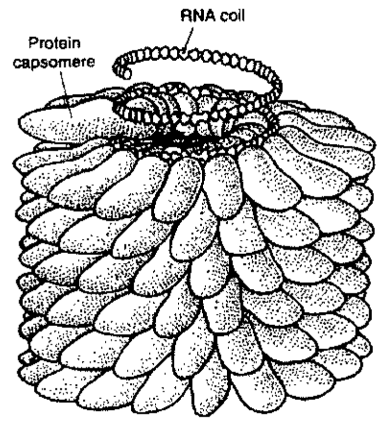
Model 2. Bacteriophage T4, a complex virus. Model 3. Rabies of Rhabdovirus, a bullet-shaped virus. Model 4. Poxvirus, a brick shaped virus.



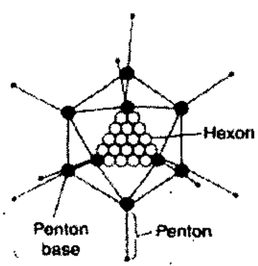
Model5. Diagrammatic representation of the arrangement of different components forming virion. A. Naked icosahedron; B. Enveloped icosahedron; C. Naked helical; D. Enveloped heli



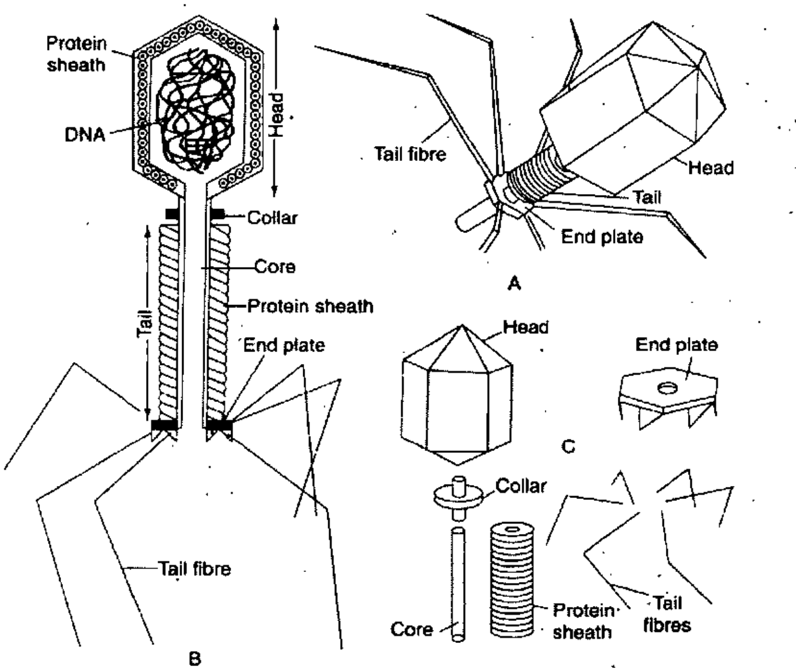
Model 6. A rood of Tabacco mosaic virus, showing arrangement of spirals forming the protein sheath.



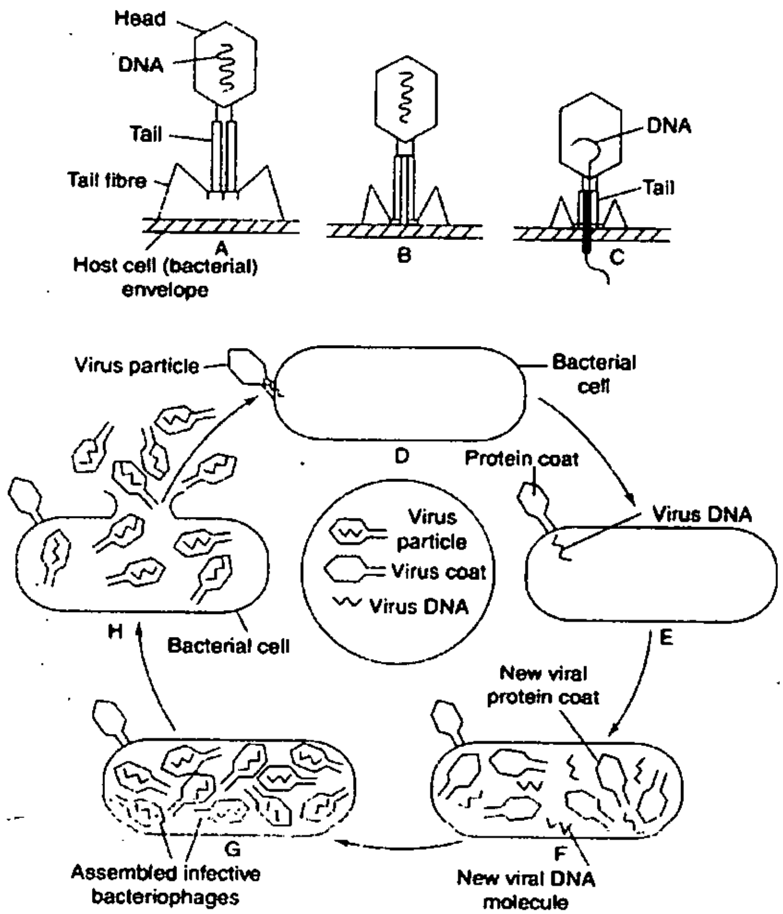
Model 7. Morphology of Tobacco mosaic virus-1 (TMV), a helical virus.



Model 8. Morphology of Adenovirus, a polyhedral virus.



Model 9. Morphology of T-even bacteriophage virus. A, External morphology; B. Diagrammatic representation of L.S. of a bacteriophage; C, Various components of a bacteriophage.



Model 10. Attachment, adsorption and penetration (A-C) of a bacterial host cell by a T-ph D-H, Stages of the cycle of infection of bacterial cells by bacteriophages

• (B) TYPES OF BACTERIA FROM SLIDES/PHOTOGRA

1. Azotobacter

Classification

Kingdom	:	Protista
Sub-kingdom	:	Prokaryota
Phylum	:	Schizophyta
Order	:	Eubacteriales
Family	:	Azotobacteraceae
Genus	:	*Azotobacter*
Species	:	*Chroococcum*

Character :

1. Widely distributed in the soil where they carry on nonsymbiotic nitrogen fixat
2. Large rod-shaped bacteria, sometimes coccoidal and yeast like.
3. They are motile and bear over 5 dozen flagella.
4. They are Gram negative.
5. They are obligate aerobes.
6. They are nonsymbiotic nitrogen fixing bacteria.

2. Bacillus subtilis

Classification :

Kingdom	:	Protista
Sub-kingdom	:	Prokaryota
Phylum	:	Schizophyta
Order	:	Eubacteriales
Family	:	Bacillaceae
Genus and Species	:	*Bacillus subtilis*

Fig. 1. Bacillus subtilis.

Characters :

1. It is rod shaped bacterium commonly known as bay bacillus.
2. Endoscope formation is very common.
3. It is commonly found in decomposing organic matters.
4. It produces as antibiotic called subtilin.
5. It is non-motile and Gram-positive.
6. It produces large quantity of extracellular hydrolytic enzyme "amylase".
7. Bacterial amylase is used for the production of dextrins from starch.
8. Cell organization is prokaryotic type.

3. Clostridium

Classification : Same as of *Bacillus.*

Characters :

1. The members are gram positive and rod shaped.

2. They from endospores, and the spores are more resistant to unfavourable conditions than vegetative cells.

3. Majority of the *Clostridium* species are nonpathogenic. However, *C. perfringens* causes fatal gas gangrene of wounds.

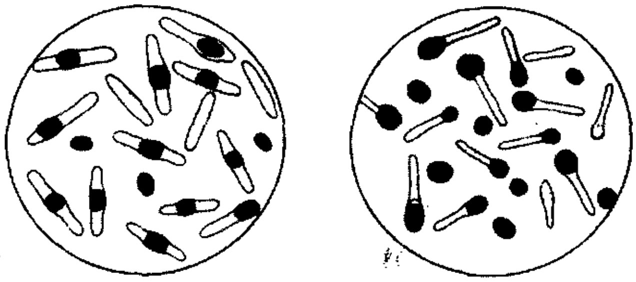


Fig. 2. *Clostridium botulinum and C. tetani.*

4. *C botulinum,* growing saprophytically in the absence of air, produces poisonous toxin of botulinal poisoning.

5. Tetanus is caused by *C. tetani* (Fig. 2).

6. Due to the presence of endospores, the cells are usually swollen.

4. Escherichia coli

Classification :

Kingdom	:	Protista
Sub-kingdom	:	Prokaryota
Phylum	:	Schizophyta
Order	:	Eubacteriales
Family	:	Enterobacteriaceae
Genus and Species	:	*Escberichia coli.*

Characters :

1. It is a common inhabitant of the intestine of man and many warm-blooded animals.

2. It is also found in the faecal materials of human being, and may be pathogenic producing enteritis and peritonitis.

3. Its rods are short. It is Gram negative.

4. The rods may be motile or non-motile, and are non-spore forming.

5. It ferments glucose and lactose, and its colonies produce a faecal odour.

6. It is of considerable significance in the sanitation of food, milk and water.

5. Lactobacillus

Classification :

Kingdom	:	Protista
Sub-kingdom	:	Prokaryota
Phylum	:	Schizophyta
Order	:	Eubacteriales
Family	:	Lactobacillaceae
Genus	:	*Lactobacillus*

Characters :

1. It is widely distributed in dairy products, silage or stored green cattle food, and manure.

2. *Lactobacillus casei* rod are generally long and slender (Fig. 3).

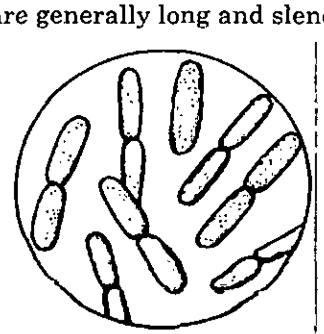


Fig. 3. ***Lactobacillus lactis.***

3. These are non-motile and Gram positive bacteria.

4. They are generally anaerobic and ferment carbohydrates to latic acid.

5. They bacteria are non-spore producing, and divide by binary fission.

6. They are very important in cheese ripening.

7. *L. acidophilus* is useful in making acidophilus buttermilk. It has been associated with dental caries.

6. Rhizobium

Classification :

Kingdom	:	Protista
Sub-kingdom	:	Prokaryota
Phylum	:	Schizophyta
Order	:	Eubacteriales
Family	:	Rhizobiaceae
Genus and Species	:	*Rhizobium leguminosarum*

Characters :

1. It is found in the root nodules of clover, alfalfa, lentils, beans, peas, soybeans and several other leguminous plants.

2. It is capable of fixing the atmospheric nitrogen.

3. It actually causes the formation of nodules on the roots of legumes where it lives symbiotically.

4. Its rods are small.

5. It is motile when young (Fig. 1 on P. 28 D).

6. It is aerobic and Gram negative.

7. Its cells utilize glucose and sometimes other sugars with little or no production of organic acids.

8. It cells are non-spore producing.

7. Streptococcus

(Gr. *streptos-twised*; *kokkus-a* seed, *i.e.*, a twisting chain of spherical cells).

Classification :

Kingdom	:	Protista
Sub-kingdom	:	Prokaryota
Phylum	:	Schizophyta
Order	:	Eubacteriales
Family	:	Lactobacillaceae
Genus	:	*Streptococcus*

Characters :

1. It is a chain shaped bacterium.

2. The cocci are round in shape.

3. These are non-motile and Gram+ve bacteria.

4. The cellular organization is prokaryotic type.

5. Species are saprophytic and pathogenic.

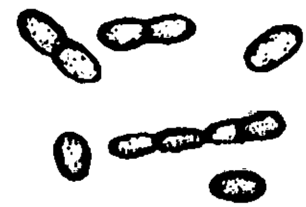


Fig. 4. *Streptococcus lactis.*

6. It is important in souring of milk (*S. lactis*).

7. *S. salivaris* is common in the mouth and nose of human beings.

8. *S. pyogenes* is pathogenic, causing wound infections, childbed fever, scarlet fev etc.

Fig. 5. Streptococcus pyogenes.

9. *S. agalactae* casues chronic mastitis in approximately 20% of the milk crows.

10. *S. cremoris* develops aroma and flavour in the creams.

11. *S. faecalis* occurs in human intestine and helps in food poisoning.

8. Staphylococcus

(Gr. *staphyla,* bunch of grapes; *kokkus*, a grain or seed).

Classification :

Kingdom	:	Protista
Sub-kingdom	:	Prokaryota
Phylum	:	Schizophyta
Order	:	Eubacteriales
Family	:	Micrococcaceae
Genus	:	*Staphylococcus*

Characters :

1. Cells are arranged in plates or irregular masses.

2. *S aureus* is a common inhabitant of human skin, mucous membrane, throat and postnasal discharges of persons recovering from cold.

3. It causes the food poisoning in man and may also be the cause of pimpl abscesses and infection of wound.

4. It is also the cause of *bovine mastitis,* which is an inflammatory disease of t udder.

5. The symptoms which are typical of *Staphylococcus* infection include naus vomiting, diarrhoea, acute prostration and abnormal cramps.

6. It also occurs commonly in bakeries and. kitchen products, if they are n immediately refrigerated at a temperature of 4°C to 10°C.

9. Spirillum

Classification :

Kingdom	:	Protista
Sub-kingdom	:	Prokaryota
Phylum	:	Schizophyta
Order	:	Eubacteriales
Family	:	Thiorhodaceae
Genus	:	*Spirillium*

Characters :

1. These are the spiral shaped bacteria commonly called spirilli.

2. These are coiled and found in the soil and water.

3. They contain bacteriopurpurin and sulphur granules.

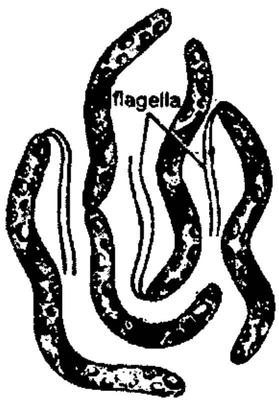


Fig. 6. ***Sulphur spirilla.***

4. They are motile and move with their flagella.

5. Prokaryotic type of cell organization is present.

10. Treponema

(Gr. *trepein* - turn; *nema* - thread)

Classification :

Kingdom	:	Protista
Sub-kingdom	:	Prokaryota
Phylum	:	Schizophyta
Order	:	Eubacteriales
Family	:	Treponemtaceae
Genus	:	*Tereponema*

Characters :

1. It is spiral shaped bacterium.

2. With the help of electron microscope flagella have been observed on two ends of the organism.

3. *T. pallidium* is the cause of veneral disease, syphilis.

4. It is transmitted from person to person usually through sexual contacts, although indirect transmission through contaminated objects may sometimes occurs.

5. Some species of *Treponema* occur in the human mouth and other animals.

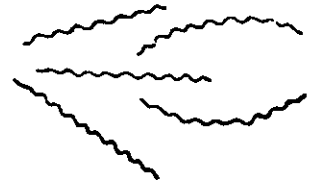


Fig. 7. Treponema.

• (C) ELECTRON MICROGRAPH OF A BACTERIAL CE

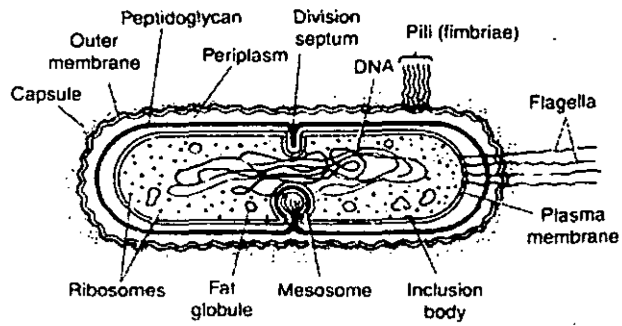


Fig. 1. A typical bacterial cell under electron microscope (diagrammatic)

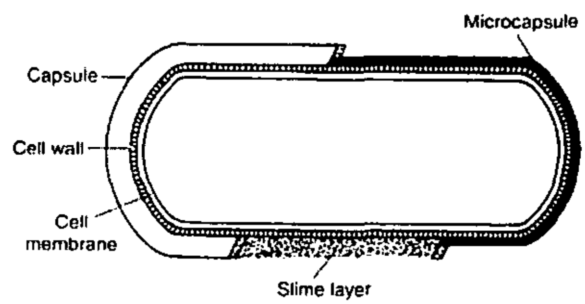


Fig. 2. Relative position of capsule, microcapsule and slime layer in a bacterial cell under electron microscope.

• (D) BINARY FISSION OR CELL DIVISIO

The three aspects that complete the cell division process in bacteria are *'DI duplication'*, *'DNA partitioning'* and *'cross-wall formation'*. All these three process are, however, equivalent to the mitosis of the eukaryotic organisms, but there is spindle formation and resolution of the chromatin material into chromosomes bacteria.

Electron microscopy has revealed following details of cell division :

The first step (*DNA duplication*) occurs by the separation of two strands of DN (nuclear body). Each separated DNA strand then replicates a new complimenta strand.

The second step (*DNA partitioning*) involves the partitioning of the doubled DN between the two daughter cells. It is a problematic aspect for the bacterial cell, mair because of the enormous length of DNA. How it actually happens is still not clear. It thought that DNA molecule is attached at some point to the plasma membrane (Klep *et al.*, 1979), and after first step (DNA duplication) each one of the double stran swings towards one of the cell halves, keeping itself in the same attached position. Bo the cell halves later on receive one double strand of DNA each.

The third step (cross-wall formation) starts immediately after the separation of daughter genomes into two separate cell halves. It starts by the inward growth of the cell wall, mid-way between the daughter genomes. The cell wall materials are deposited between the membranes and the cell is divided into two. There is evidence to indicate that mesosome plays some role in the synthesis of the cross-wall in bacterial cell. According to Peberdy (1980) the separation of daughter cells is brought about by the action of hydrolases in *Bacillus subtilis* and *Streptococcus faecalis*. In *Escherichia coli* the septation is brought about by the invagination of the cytoplasmic membrane and the peptidoglycan layer of the cell wall (Torti and Park, 1976).

The above-mentioned method of cell division is also called 'binary fission'. It is followed by almost all bacteria.

• (E) CONJUGATION INCLUDING GENETIC RECOMBINATION (SEXUAL REPRODUCTION)

During *recombination* in bacteria, usually a piece of donor DNA (called *exogenate*) must enter the recipient cell and become a stable part of the genome of the recipient cell and *indogenote*. In this way, the recombination in bacteria is a "one-way gene transfer from donor to recipient, whereas recombination in eukaryotes tends to be reciprocal."

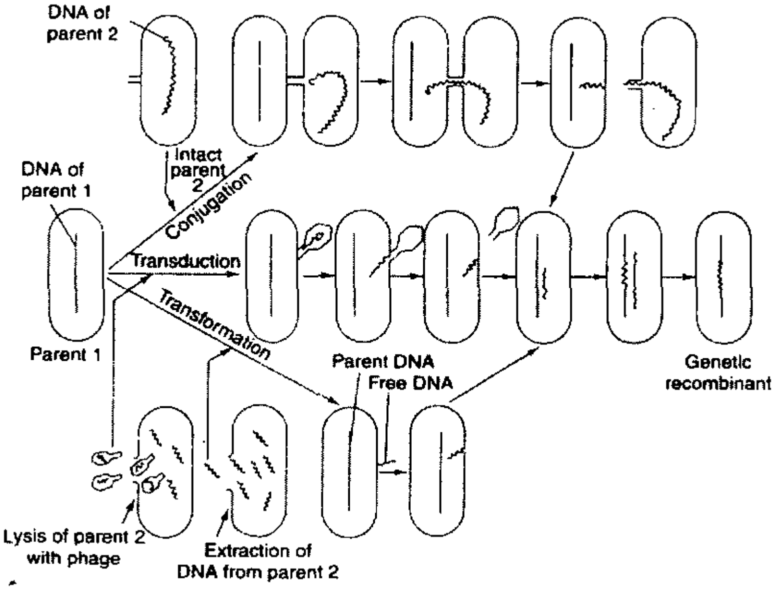


Fig. 1. Processes of transformation, transduction and conjugation (diagrammatic) in bacteria.

• (F) STRUCTURE OF ROOT NODULE

A large number of blue-green algae, specially the filamentous and heterocystous forms (*e.g., Anabaena, Nostoc, Tolypothrix and Aulosira*), have the ability to fix the atmospheric nitrogen. Several of such nitrogen-fixing organisms remain in symbiotic association with other organisms and are called *symbiotic nitrogen fixers*. These symbiotic nitrogen fixers are either blue-green algae or bacteria. They remain in association with several other organisms such as (i) blue-green algae in association with fungi to form lichens (*e.g., Peltigera*), (ii) blue-green algae (*e.g., Nostoc, Anabaena*) in association with bryophytes (*e.g., Anthoceros*), (iii) blue-green algae (*Anabaena* and *Nostoc*) in association with coralloid roots of *Cycas*, and (iv) bacteria (e.g., *Rhizobium*) in association with the roots of leguminous plants to form root nodules.

Rhizobium species have the ability to penetrate the tips of the root hairs leguminous plants (*e.g.*, pea, clover, alfalfa, beans and soybeans) and develop threads cells that grow into the roots. These infection threads swell, burst and release bacteria within the root hair cells of legumes. These free bacteria within the root hair cells assume a specific shape and called a *bacteroid*. The bacteroids may be T-shaped, γ-shaped, club-shaped or pear-shaped. Due to the presence of bacteria, the plant cells are stimulated and multiply several times, resulting in the development of a nodule.

The plant juices provide nourishment to bacteria, and plant gains nitrogen compounds by the bacteria which take nitrogen directly from the atmosphere. A symbiotic relationship, therefore, exists, and the end result is that the atmospheric nitrogen, which cannot be utilized directly by the plant, is converted by bacteria nitrogen compounds that remain in the soil for plant use.

Object : *To show the presence of bacteria in the root nodules of leguminous plants.*

Requirements : Roots of pea (or of other nodulated legumes such as beans, soybeans, clover and alfalfa), razor, microscope with oil immersion lens, needles, slides, burner, nigrosin negative stain.

Method :

1 . Take roots of any of the nodulated legumes such as pea, beans, soyabeans, clover, alfalfa, etc. and wash them thoroughly with water. Several nodules are clearly visible on the roots.

2. Remove a large nodule with the help of blade, cut a thin transverse section of the nodule with a razor and examine under low power of the microscope. Observe the shape of the nodule and bacteroidal tissue in the cortex (Fig. 1 A, B).

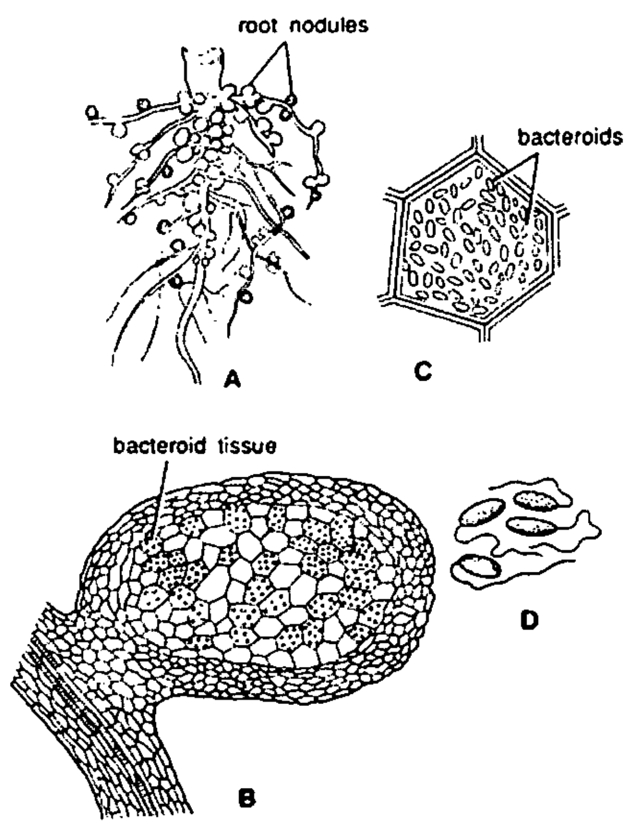


Fig. 1. A, Root nodules of a leguminous plant; B, T.S. of a nodule showing bacteroid tissue,- C, Bacteroids; D, Rhizobium leguminosarum.

3. *Preparation of Nigrosin Stain :* Add 10 gm water soluble nigrosin to 100 mL water ı a beaker and mark the top level of the contents with a glass-marking pencil. Put the eaker in a boiling water bath for about 30 minutes. The stain will be dissolved. .emove the beaker and add distilled water to regain the original volume up to the encil mark. Add a few drops of formalin, filter the stain at least two times through a lter paper, and use it.

4. Put a small nodule on a glass slide, add a drop of water over it can crush it with the elp of needles.

5. Prepare a heat-fixed smear by taking the slide very gently to the burner's flame or 3 or 4 times. But the slide should not be overheated. *(To avoid the slide to be verheated, always bold it several inches above the flame).*

6. Stain with nigrosin stain, and examine the bacteroids (Fig. 21) under oil nmersion lens.

Observations and Result : Small, rod shaped, Gram negative bacteria which are ıotile, when young, are visible. These belong to *Rhizobium leguminosarum* (order ubacteriales).

• (G) GRAM STAINING

It is a universally used bacterial stain. It is important because bacteria are eparated by this stain into two individual groups, *i.e.*, *Gram positive* and *Gram egative.* It is a differential stain because Gram positive bacteria stain blue by this ıethod while Gram negative bacteria take red stain.

Most of the species of cocci are Gram positive while most of the rod shaped bacteria re gram negative. There are, however, exceptions of this statement.

Object : *To prepare a bacterial slide using Gram stain.*

Requirements : Slides, linen cloth, coloured pencil, spirit lamp, loop bacterial ulture, gentian violet or crystal violet, Gram's iodine, alcohol, acetone, safranin, oil nmersion lens, microscope.

Method : There are three parts of the Gram staining process, *viz.* cleaning of the lides, making of the smear, staining.

(i) Cleaning of the slides : To about half a dozen of microscopic slides take to the sink nd wash thoroughly in some cleaning preparation and rinse well in warm water. Dry ıe slides with a thin linen cloth. Do not put your fingers on the cleaned slides. Do not lso use soap for cleaning the slide.

(ii) Making of the Smear : 1. Make a specific mark by the coloured pencil, say 'X', on ne end of each slide to have an idea of the top of the slide.

2. Flame the looped needle. Remove cotton plug from one of the slant cultures and ıke a small amount of growth with the help of loop and place it on the slide. Repeat the rocess once or twice. Now the smear is ready.

The loop should be flamed each time after using.

3. Dry the smear by taking the slides very gently to the burner's flame for 3-4 times. ut the slide should not be overheated.

(iii) Staining : Following is the procedure of Gram's staining :

Stain with gentian violet or crystal violet or methyl violet (all basic dyes) f seconds.

↓

Wash with tap water

↓

Flood with Gram's iodine (for 30 seconds).

↓

Wash with tap water

↓

Decolourize with alcohol or acetone.

↓

Wash with tap water

↓

Counterstain with safranin for 30 seconds

↓

Wash with tap water

↓

Gently blot dry with blotting paper or towel

↓

Observe under oil immersion lens.

Record the results in the following Table :

S.No.	Name of the microorganism	Morphology	Gram stain
1.	A		
2.	B		
3.	C		
4.	D		

ALGAE

• GENERAL CHARACTERISTICS

1. The world *algae* means 'sea weeds'. The science that deals with their study is called *'algology'*. The Greek word for algae is *'phykos'*, and, therefore, their study is also called *phycology* (Gr. *phykos,* sea weeds; *logos*, study or discourse).

2. Algae may be defined as "an assemblage of chlorophyll-bearing autotrophic Thallophytes, bounded by a cell wall, made up of pure or mixed carbohydrates".

3. *Out of a world total of 2475 genera* and 28,505 species of algae, 666 genera and 5136 *species are represented in India. This amounts to a proportion of 28.3% of global generic representation and 18.1% of world species.*

4. Algae are of universal occurrence because of their presence in nearly all types of habitats. They are found in freshwater (e.g. *Spirogyra, Oedogonium*), sea water (marine algae, *e.g. Sargassum,, Laminaria*), on soil (terrestrial algae, *e.g. Fritschiella, Vaucheria*), on rocks and stones (lithophytic algae, *e.g. Batrachospermum, Enteromorpha*), in highly concentrated salty water (halophytic algae, *e.g. Dunaliella*), on sand (psammophytic algae, *e.g. 'Phormidium, Vaucheria*), in very hot waters (thermal algae, *e.g. Onconema, Synechococcus*), on ice or snow (cryophytic algae, *e.g. Chlamydomonas nivalis, Raphidonema*), on other plants (epiphytic algae, *e.g. Oedogonium, Bulbochaete*), inside other plants (endophytic algae, *e.g. Nostoc* inside the thallus of *Anthoceros, Anabaena in the coralloid roots of Cycas*), on animals (epizoic algae, *e.g. Cladophora crispata on the shells of molluscs*), inside the animals (endozoic algae, *e g. Stigeoclonium in the nose of fishes*), as parasites (*e.g.* Cephaleuros on leaves of tea plants) and also in combination with fungi in the form of lichens.

5. *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. Eballocystis*), coccoid or non-motile (*e.g. Chlorella*), filamentous (*e.g. Oedogonium, Cladophora*), heterotrichous (*e.g. Ectocarpus*), siphon-like or siphonaceous (*e.g. Vaucheria*), uniaxial (*e.g. Batrachospermum*), multiaxial (*e.g. Polysipbonid*) or even parenchymatous (*e.g. Sargassum*).

6. The size of algal members is also highly variable. It varies between 0.5 μ (*e.g. Dunaliella, Chlamydomonas*) to as much as 30 metres or more (*e.g. Macrocystis*). There is an "unsubstantiated record of a plant of *Macrocystis pyrifera* reaching up to 700 feet (*i.e.* 213 metres) the longest plant in the world".

7. The *cell wall* is mostly made of cellulose but in some genera it also contains some other substances such as pectin, chitin, algin and fucoidin.

8. *Cytoplasm* contains structures such as contractile vacuoles, mitochondria, eyespot, chloroplast, pyrenoids, Golgi bodies, pigments and nucleus. However, in

7. Each cell is somewhat cylindrical or spherical in shape.

8. In filaments there are present some large, spherical or cylindrical, colou empty cells called *heterocysts*.

9. Heterocysts are generally intercalary but in the young condition, they ma terminal.

10. Two polar nodules are present in each heterocyst (Fig. 4).

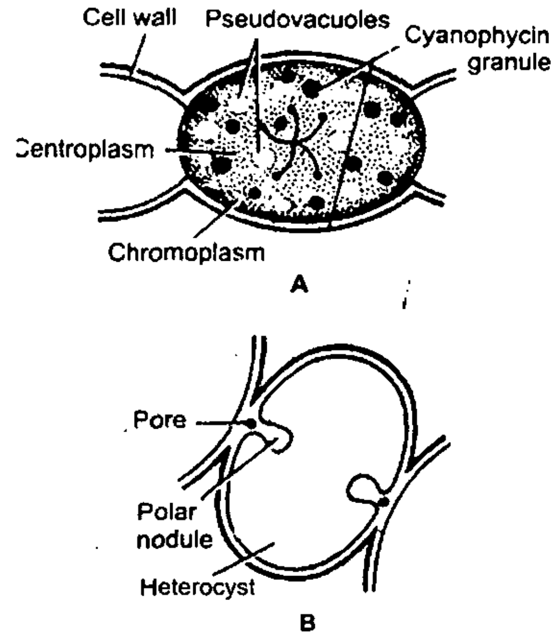


Fig. 4. Nostoc. A, A single vegetative cell; B, A heterocyst.

11. Some cells of the filament become enlarged and filled with the food mate These thick-walled cells are called *akinetes*. Akinetes are generally preser chain.

A. Single Cell

1. *Each cell* is surrounded by a cellulose cell wall (Fig. 4).

2. Protoplast shows typical Myxophycean structure, *i.e.*, inner colou *centroplasm* and outer pigmented *chromoplasm*. In the chromosplasm are pre pigments, proteinaceous cyanophycin granules and cyanophycin starch gran while in the centroplasm is present the incipient nucleus.

Reproductive Structure :

1. Sexual reproduction is totally absent. Plant reproduces only by vegeta means, *i.e.*, by hormogones and akinetes.

2. *Hormogones* are the small segments of trichome which separate generall the place of heterocyst.

3. On separation, hormogones develop into new trichomes.

4. *Akinetes* are thick-walled large cells, filled with reserve food material. Al cells between two herterocysts generally change into akinetes.

5. In some species, *e.g.*, *Nostoc commune*, heterocyst becomes functional germinates to form new trichome.

Identification :

(a) (i) *Simple thallus*

(ii) Cell wall consists of cellulose.

(iii) Presence of chlorophyll .... ***Algae***

(b) *(i) Protoplast divisible into centroplasm and chromoplasm.*

(ii) Pigments diffuse and blue-green in colour.

(iii) Presence of incipient nucleus.

(iv) Sexual reproduction is absent.

(v) Hormogones present .... ***Myxophyceae***

(c) *(i) Thallus with trichomes.*

(ii) Trichomes are unbranched ..... ***Nostocales***

(d) *(i) No differentiation of base or apex in trichomes.*

(ii) Filaments are surrounded by gelatinous matrix.

(iii) Presence of heterocyst.

(iv) Akinetes present .... ***Nostocaceae***

(e) *(i) Colony is in the form of bluish green balls.*

(ii) Reproduction by hormogones and akinetes.

(iii) Intercalary heterocysts present .... ***Nostoc.***

2. Chlamydomonas

(Chlamydo = Cloak; monos = single)

Common Indian Species : *Chlamydomonas grandistigma, C. eugametos, C. ehrenbergii.*

Systematic Position :

Sub-division	:	Algae
Class	:	Chlorophyceae
Order	:	Volvocales
Family	:	Chlamydomonadaceae
Genus	:	*Chlamydomonas*

Common Occurrence : Most of the species are fresh-water, found in ponds, ditches, pools, lakes and rain water. On the surface of water, it mostly forms a green scum.

Work to be done

To study (i) External morphology, (ii) Neuromotor apparatus, (iii) Electron micrograph, (iv) Palmella stage, and (v) To identify giving reasons.

External Morphology

Study the slide under oil-immersion lens :

1. The organism is an unicellular alga (Fig. 5).

2. The thallus is spherical to oblong in shape but some species are pyriform or ovoid.

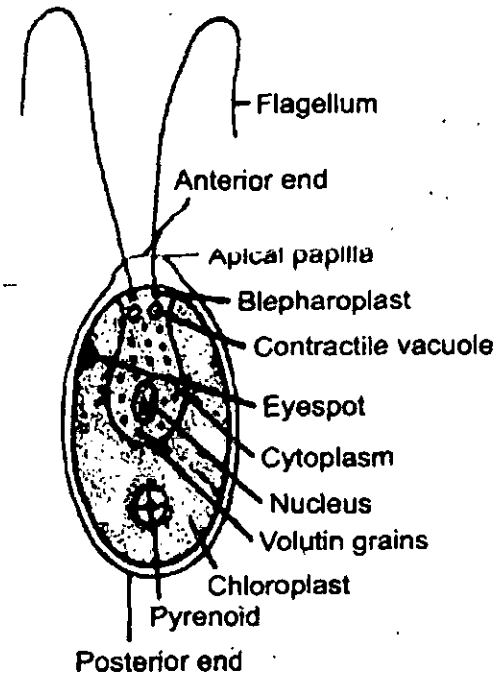


Fig. 5. ***Chlamydomonas. A vegetative cell.***

3. The cell is somewhat pointed towards the anterior side and compara broader towards the posterior side.

4. The cell is surrounded by a cellulose cell wall* which encloses the protopl

5. The protoplasm consists of cytoplasm and a centrally located nucleus.

6. Anterior pointed end consists of an apical papilla.

7. Two flagella are present on the anterior side of the cell. Both are of whi type.

8. At the base of each flagellum is present a basal granule or blepharoplas

9. Both the flagella are equal in size and bigger than the size of the cell.

10. Below the basal granules are present two contractile vacuoles whicl excretory in function.

11. Chloroplast is generally a cup-shaped structure in most of the species.

12. Each chloroplast encloses a single pyrenoid, which is the body of s formation.

13. More than one pyrenoids are present in some species.

14. Many volution granules are present in the cytoplasm.

15. An organe-coloured eyespot or stigma is situated in the protoplasm a anterior end of the cell.

16. The eyespot is supposed to be photo receptive in function.

Neuromotor Apparatus

1. It is also known as *flagellar apparatus.*

2. It consists of following :

(i) Two *blepharoplasts* connected by a fibre called *paradesmose* (Fig. 6).

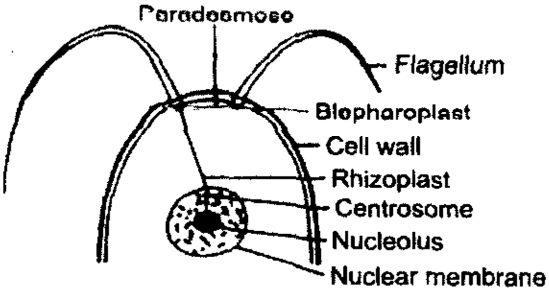


Fig. 6. ***Chlamydomonas. Neuromotor apparatus.***

(ii) One of the blepharoplasts remains connected to the centrosome of the nucleus by a descending thread called *rhizoplast**;

(iii) Intranuclear or extranuclear centrosome is also connected with the nucleolus by a fibril.

Electron Micrograph :

Study the microphotography or its line diagram from the book :

1. This is the electron micrograph of *Chlamydomonas* (Fig. 7).

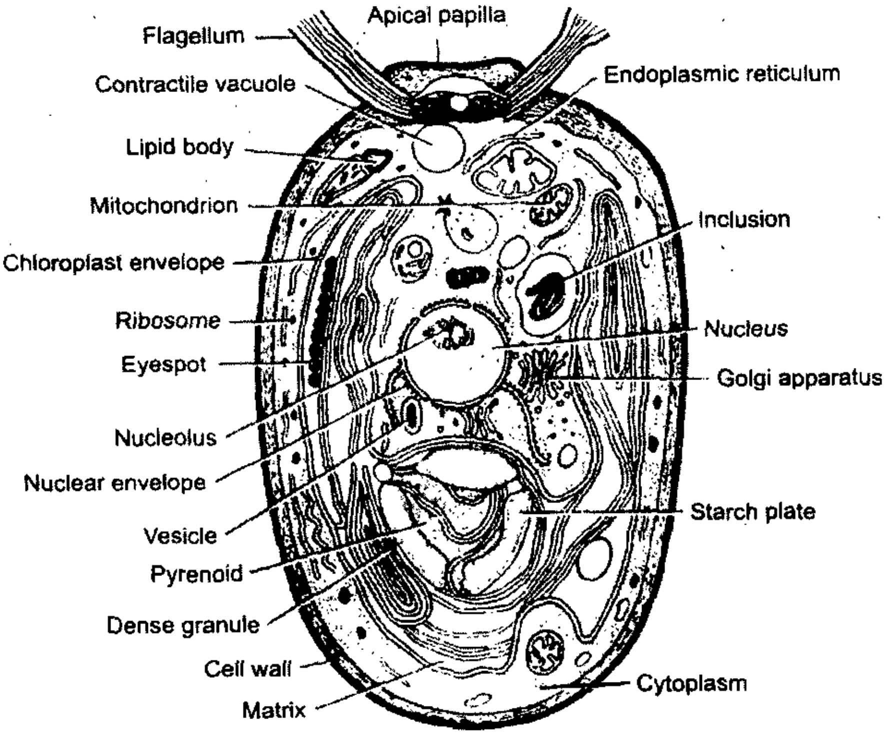


Fig. 7. ***Electron micrograph (diagrammatic) of Chlamydomonas.***

2. The cytoplasm is bounded by a cytoplasmic membrane.

3. Cytoplasmic membrane consists of two electron opaque layers.

4. A few strands of granular endoplasmic reticulum, free ribosomes and mitochondria and visible near the periphery in the cytoplasm.

5. The chloroplast is cup-shaped. Inside the chloroplast lie the volution granules, some strands of the granular reticulum tubules, Golgi bodies, free ribosomes, etc.

6. Plate-like cristae are present in the mitochondria.

7. Single nucleus contains a large nucleolus.

8. Nuclear membrane is two-layered.

9. Granular matrix of the cup-shaped chloroplast remains enclosed by a dou membraned envelope.

10. In the granular matrix or stroma are present many tube-like thylakoid

11. Synthesized starch plates are present around the edge of the pyrenoid.

12. Pyrenoid lacks ribosomes.

13. Eyespot consists of 2-3 rows of globules, which are arranged concentric near the anterior end of the cell inside the chloroplast.

14. Two contractile vacuoles are present.

15. Two long, whiplash type of flagella are present.

Palmella-stage

1. It is a temporary phase in the life-cycle resembling to the alga *Palmella* (8).

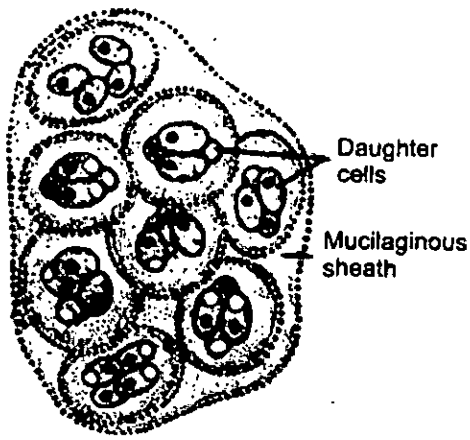


Fig. 8. ***Chlamydomonas. Palmella-stage.***

2. Under unfaourable conditions, the cells become non-motile by loosing th flagella.

3. Divided parts of the protoplast of each cell remain surrounded by mucilaginous matrix formed by the gelatinization of the cell walls.

4. Many such cells come together in the form of a colony surrounded by common mucilaginous sheath, and the whole structure resembles the a *Palmella*.

Identification :

(a) *(i) Thallus simple.*

(ii) Cell wall consists of cellulose.

(iii) Chlorophyll present in the plastids.

*(iii) Autotrophic mode of nutrition .... **Algae***

(b) *(i) Photosynthetic pigments present in chromatophores.*

(ii) Presence of pyrenoids.

(iii) Starch is the reserve food material.

(iv) Reproductive units are motile.

(v) Flagella of equal length .... ***Chlorophyceae.***

(c) (i) Motile vegetative cells.

(ii) Simple and unspecialized structure of thallus.

(iii) Contractile vacuoles present ..... ***Volvocales***

(d) (i) Flagella are of equal length.

(ii) Unicellular plant body.

(iii) Presence of Plamella stage. .... ***Chlamydomonadaceae***

(e) (i) Presence of eyespot.

(ii) Presence of contractile vacuole and cup-shaped chloroplast.

.... Chlamydomonas.

3. Oedogonium

(*Oedos*, swelling; *gonas* = fruit)

Common Indian Species : *Oedogonium sociale, O. intermedium, O. vaucheruii, O. perfectum.*

Systematic Position

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

Common Occurrence : This fresh water alga occurs generally as epiphyte on many hydrophytic plants. It is not commonly found in running water. Generally, young filaments are attached and the mature filaments are free-floating.

Work to be done

Study external features of filaments, detailed structure of a young and a mature cell, antheridia, oogonia, dwarf male and zygote.

External Morphology

1. Plant body consists of green narrow, unbranched (fig. 9) and multicellular filaments.

2. Cells are cylindrical and arranged end to end in each filament.

3. All the cells of the filaments are similar in shape except lowermost holdfast and uppermost apical cell.

4. Uppermost apical cell is somewhat broad and round at its apex and green in colour.

5. Lowermost basal cell is generally colourless but may contain ill-developed chloroplast. It produces certain outgrowths, which help in attachment of the filament to the substratum. It is known as *holdfast*.

6. Other cells of the filament are similar in structure and green in colour.

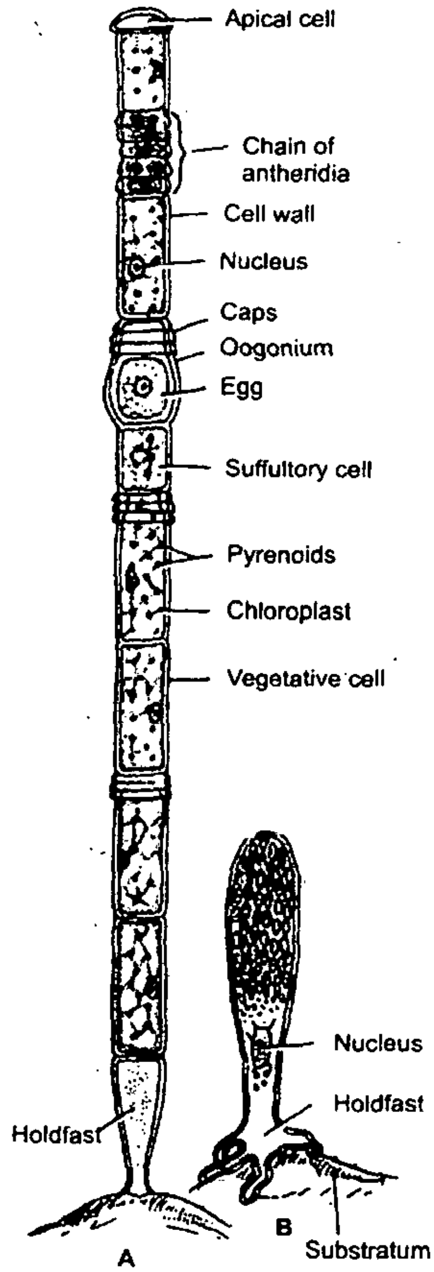


Fig. 9. ***Oedogonium. A, A. filament of a monoecious species; B, A germinating zoospore showing formation of holdfast.***

7. Some cells of the filaments contain ring-like structures at their upper end. These are known as *caps*.

8. The number of caps on a cell indicates the number of times the cell has divided.

9. *Each cell* (fig. 10) is surrounded by a wall made up of three layers, the outermost of which consists of chitin, middle of pectose and innermost of cellulose.

10. Cells are uninucleate, and the nucleus is generally present in the middle of the cell but sometimes it is eccentric.

11. A single large reticulate chloroplast is present in each cell. It contains many pyrenoids (Fig. 10).

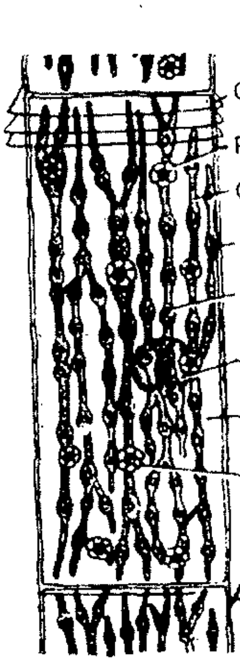


Fig. 10. ***Oedogonium. A young single cell.***

Fig. 11. ***Oedogonium. A mature single cell.***

12. Each cell contains a large central vacuole, which is filled up by cell sap.

Asexual Reproduction

1. Zoospores and akinetes are the common means of asexual reproduction in *Oedogonium*.

2. Zoospores are formed in the cap cells singly, when the cell starts to function as zoosporangium.

3. In each zoosporangium develops a single, spherical to ovoid, uninucleate and multiflagellate zoospore.

4. Zoospore comes out in the membranous vesicle formed by rupturing of the outer cell wall.

5. On coming out of vesicle, zoospore settles on some substratum through its flagellar end. Its flagella withdraw and it germinates into new plant.

6. *Akinetes* are also formed in chain in some species of *Oedogonium*. These are the centres of reserve food.

Sexual Reproduction

1. It is of oogamous type.

2. Some species are monoecious or homothallic while others are dioecious or heterothallic.

3. Dioecious species are again of two types :

(a) In some, the male and female filaments are of normal size. These are known as *macrandrous species.*

(b) In others, the male filament is smaller in size, *i.e., dwarf male* or *nannandrium*. These are known as *nannandrous species.*

Antheridia

1. *Antheridia* may develop in any cell possessing caps.

2. The cell divides into many small segments or chambers and each segmen known as antheridium (Fig. 12A).

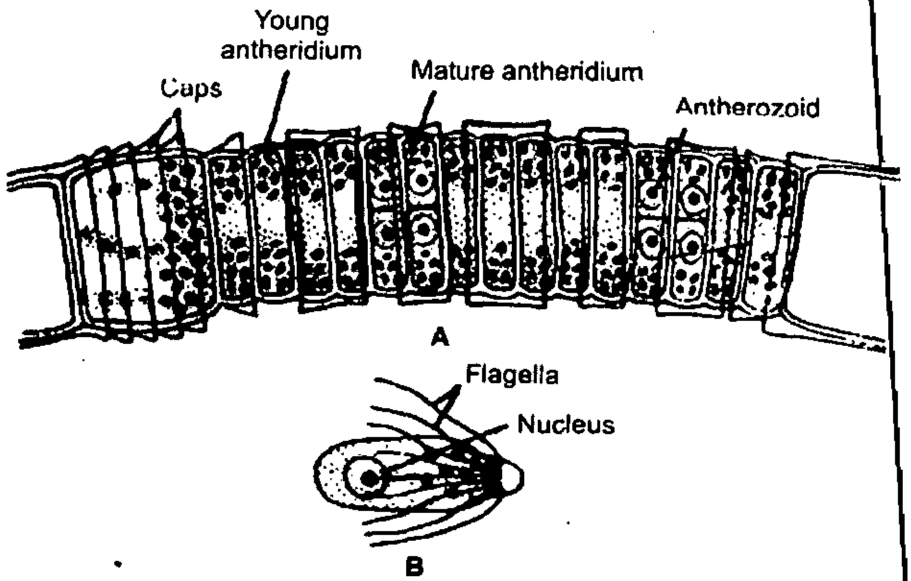


Fig. 12. Oedogonium. A, Chain of antheridia; B, A multiflagellate antherozoid.

3. In each antheridium, two small, uninucleate and multiflagellate structures are formed. These are known as *antherozoids* (Fig. 12 B).

Oogonium :

1. *Oogonium* also develops in cap cells.

2. A single filament may contain many oogonia.

3. An oogonium is a spherical, uninucleate structure, which contains and eggs (Fig. 13).

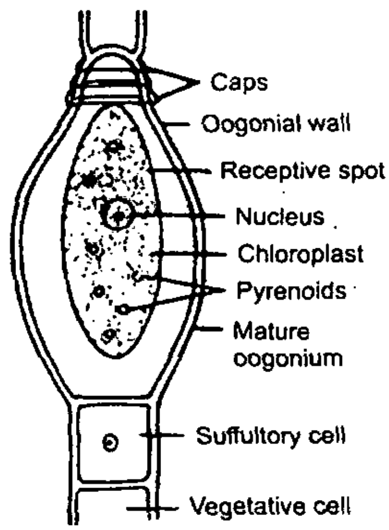


Fig. 13. ***Oedogonium. A mature oogonium with suffultory cell.***

4. Below each oogonium, a slightly elongated or cylindrical cell is present. It is known as supporting cell or suffultory cell.

5. At the time of fertilization a hyaline spot develops on the side of one egg. It is called *receptive spot.*

6. At the place of receptive spot, wall of the oogonium breaks up, antherozoids get in and perform the process of fertilization.

7. A thickwalled, reddish zygote develops after fertilization.

Dwarf male

1. Male cells in non-nandrous species are called *androsporangia.*

2. Androsporangia are formed in the same way as antheridia in case of macrandrous species, *i.e.*, by the division of a cap cell into many segments.

3. In each segment only one uninucleate and multiflagellate structure is present. It is known as *androspre.*

4. Species are known as *gynandrosporous,* if androspore and oogonium develop on the same filament.

5. If the androspore and oogonium develop on different filaments, the species is known as *idioandrosporous.*

6. Androspores develop into male filaments, which are smaller in size and are known as *dwarf males* (fig. 6) or *nannandria.*

7. Androspore settles either on the oogonium or on the supporting cell or suffultory cell (fig. 14).

8. At the tip of the androspore develops antherdia, each having two antherozoids.

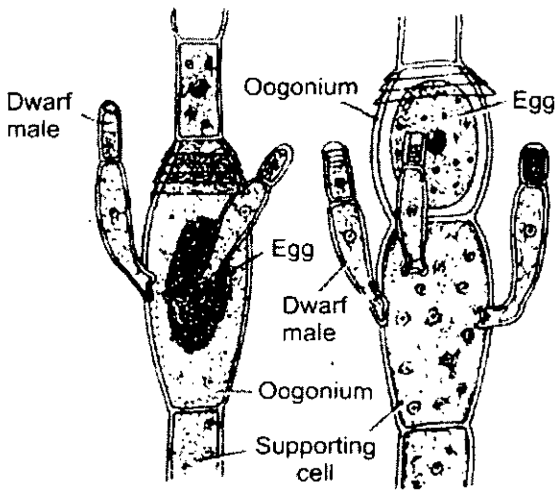


Fig. 14. ***Oedogonium. Oogonia with dwarf males.***

9. Antherozoids are uninuclate, multiflagellate and very small structures.

Points of Interest in the Genus Oedogonium :

(i) Two types of male filaments are present, *i.e.*, normal and dwarf.

(ii) Unbranched thallus contains caps.

(iii) In case of nannandrous species, antherozoids do not develop in the main filament but in the androspore or dwarf male.

(iv) Egg contains a receptive spot.

Identification :

(a) (i) Thallus simple.

(ii) Cell wall is made up of cellulose.

(iii) Presence of chlorophyll .... ***Algae***

(b) (i) Reserve food material is starch.

(ii) Pyrenoids are present.

(iii) Chromatophores are green in colour.

(iv) Motile bodies flagellate .... ***Chlorophyceae***

(c) (i) *Reticulate chloroplast.*

(ii) *Multiflagellate zoospores, androspores and antherozoids are present.*

(iii) *Sexual reproduction is by a process of advance type of oogamy.*

(iv) *Caps are formed during cell division*

.... ***Oedogoniales and Oedogoniace***

(d) (i) *Presence of caps.*

(ii) *Presence of holdfast.*

(iii) *Filamentous plant body is unbranched.*

(iv) *Dwarf male present* ..... ***Oedogonium.***

4. Vaucheria

*(Named after **J.P. Vaucher**)*

Common Indian Species : *Vaucheria geminata, V. sessilis, V. uncinata, terrestris and V. walzi.*

Systematic position :

Sub-division	:	Algae
Class	:	Xanthophyceae
Order	:	Heterosiphonales
Family	:	Vaucheriaceae
Genus	:	*Vaucheria.*

Common Occurrence : It occurs in fresh water, on soil (terrestrial) and also in sea water. During winters it occurs as green matty structure on damp soil.

Work to be done

Study the external morphology of the thallus from the given material and also the zoospores, aplanospores, Gongrosira-stage and sex organs (antheridia and oogonia) using fresh material and/or permanent slides.

External Morphology :

Stain the thallus in safranin, mount in glycerine and study :

1. Plant body is tubular or cylindrical and irregularly branched (Fig. 15).

2. Terrestrial species are attached to the substratum by rhizoids.

3. Plant body is aseptate and multinucleate (*coenocytic*). However, some septa formation is observed at the base of reproductive organs.

4. Wall of the filament is thin, elastic and consists of an outer layer of pectose and an inner layer of cellulose.

5. In the cytoplasm are present many chloroplasts and nuclei.

6. Chloroplasts are oval or discoid in shape and present in the form of a layer.

7. Next to the chloroplasts is a layer of nuclei.

8. Pyrenoids are absent.

9. Large number of oil droplets make the reserve food material of the thallus.

10. A large central *vacuole* is present in the centre of the thallus, which is the characteristics feature of the order.

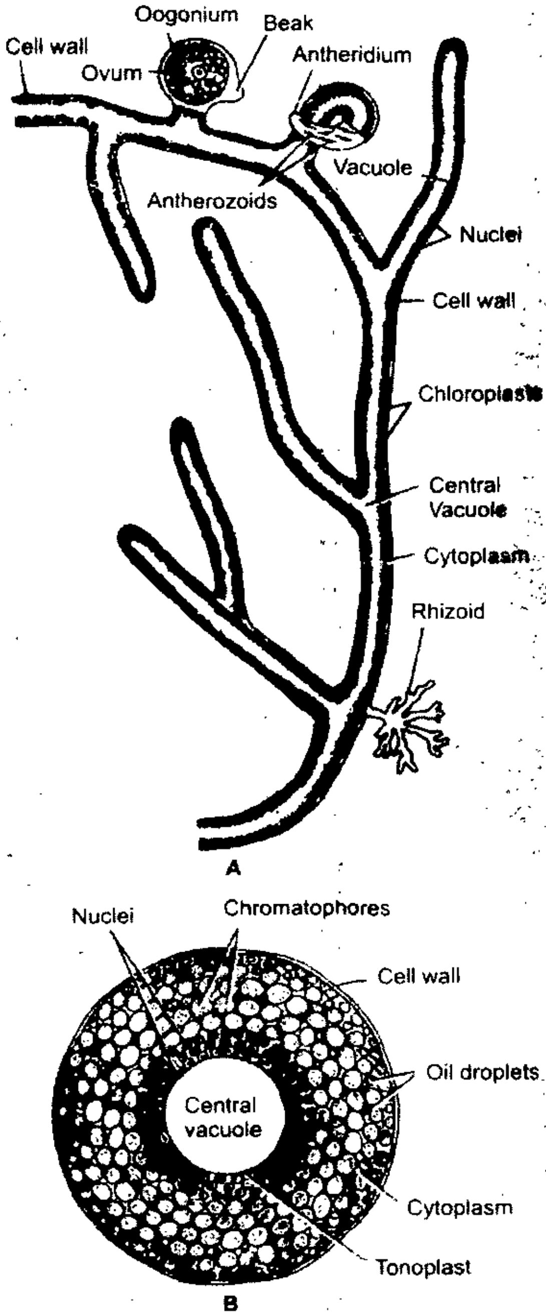


Fig. 15. Vaucheria. A, Thallus of a monoecious species; B, T.S. vegetative thallus (diagrammatic).

Reproductive Structures :

Various means of asexual reproduction are :

(a) Zospores,

(b) Aplanospores

(b) Hypnospores,

(c) Cyst formation.

Zoospore

1. *Zoospore* is formed singly in the zoosporangium, formed in the terminal part of the thallus (Fig. 16).

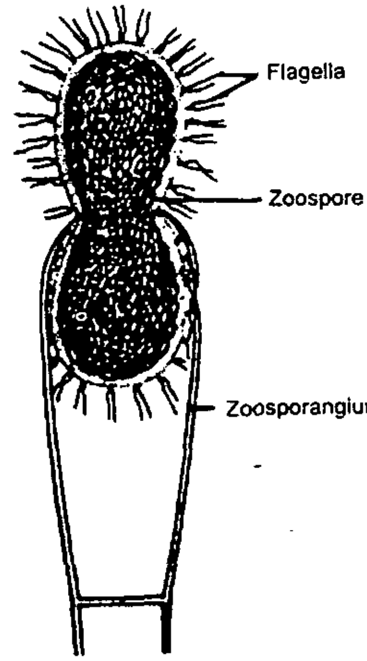


Fig. 16. ***Vaucheria. A zoosporangium with a multiflagellate zoospore.***

2. Zoosporangium is a club-shaped swollen structure separated from rest of thallus by a septum.

3. The protoplasm of the zoosporangium is accumulated, the nuclei co towards outer side of the chloroplasts and the central vacuole disappears. T complete structure becomes rounded. Two flagella are formed opposite to ea nucleus.

4. This multinucleate and multiflagellated structure is known as compou *zoospore* or *synzoospore*.

5. On liberation, these synzoospores loose their flagella and germinate.

Aplanospores

1. Aplanospores are produced at the ends of the terminal branches known a aplanosporangia (Fig. 17).

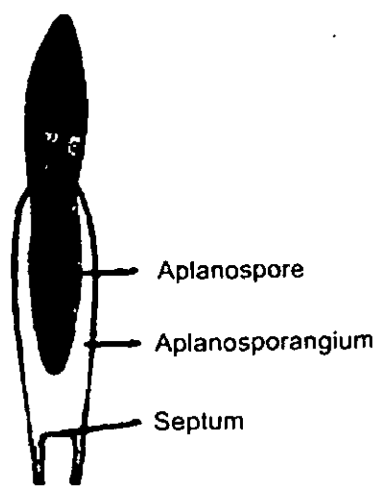


Fig. 17. Vaucheria. Aplanosporagium with an aplanospore.

2. Aplansopores are normally produced in the terrestrial species.

3. These are non-motile and thin-walled spores.

4. They may be rounded or elongated in shape.

5. A single aplanospore is formed in each aplanosporangium.

Gongrosira Stage*

1. *Hypnospores* are formed in the filament by its division into many compartments or chambers.

2. These are thick-walled spores.

3. A large amount of food material is present in these spores. Each of them germinates into new plant.

4. In some species hypnospores divide into many small structures known as *cysts* (Fig. 18).

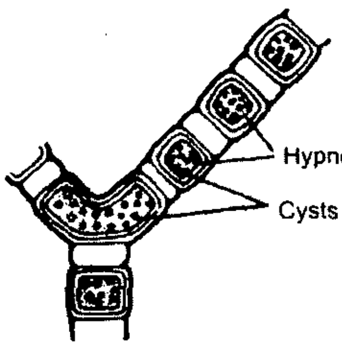


Fig. 18. ***Vaucheria. Gongrosia-stage.***

5. At this stage, when hypnospores in the filament of *Vaucheria* have divided into cysts, the filament resembles to another algae *Gongrosira*, and so this stage has been termed as *Gongrosira stage*.

6. On liberation, these cysts germinate into new filaments.

Sexual Reproduction

1. It is of oogamous type.

2. Most of the species are monoecious but a few are dioecious.

3. In homothallic species, antheridia and oogonia develop side by side. More than one oogonia may also develop alongwith an antheridium (Fig. 19).

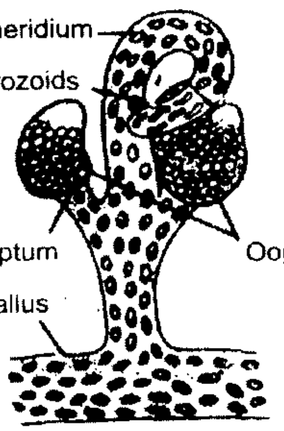


Fig. 19. ***Vaucheria. An antheridium with two oogonia.***

Antheridium

1. It is aubular, straight or curved and hook-like structure (Fig. 20 AD).

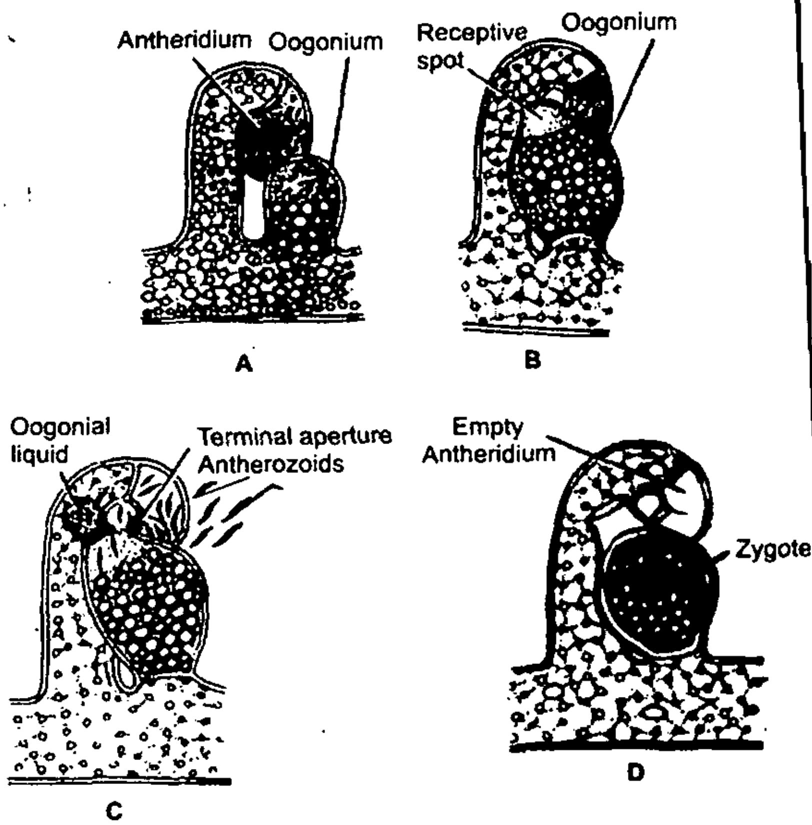


Fig. 20. Vaucheria. A, Young sex organs; B-D, Mature sex organs.

2. It may be green or a colourless structure.

3. At maturity it is separated from rest of the thallus by a septum (Fig. 20 C).

4. Many nuclei and chloroplasts are present in the antheridium when young, b at maturity it is filled up with many antherozoids.

5. Uninucleate and biflagellate antherozoids are liberated through an openi formed at the tip of antheridium.

Oogonium

1. It is ovoid or spherical (Fig. 20 AD) and sessile structure.

2. It is a uninucleate body with many chloroplasts. Oil is the reserve foo material.

3. At maturity it is also separated from rest of the portion of the thallus by septum.

4. All the contents of oogonium become rounded and form a single uninucleat egg.

5. Mature oogonium contains a short, rounded beak, through which it receive antherozoids.

6. On fertilization, zygote is formed. It is a multilayered structure.

7. Zygote germinates by forming many tubular branches.

Points of interest in the Genus Vavcheria

1. Coenocytic thallus.
2. Thallus contains a large central siphon-like vacuole.
3. Instead of starch, oil is the food material.
4. Synzoospores are found.

Identification :

(a) (i) Thallus simple.
(ii) Cell wall consists of cellulose.
(iii) Presence of chlorophyll. .... ***Algae***

(b) (i) Reserve food material is oil.
(ii) Motile bodies contain unequal flagella.
(iii) Chromatophores are yellowish-green. .... ***Xanthophyceae***

(c) (i) Coenocytic plant body.
(ii) Central siphon-like vacuole present. .... ***Heterosiphonales***

(d) (i) Absence of pyrenoids.
(ii) Absence of siphonein and siphonoxanthin.
(iii) Tubular, irregularly branched thallus.
(iv) Multiflagellate zoospores. .... ***Vaucheriaceae***

(e) (i) Coenocytic thallus.
(ii) Thallus is irregularly branched.
(iii) Sex organs are septate .... ***Vaucheria***

5. Fucus (*Phykos*-a sea weed)

Common Species : *Fucus serratus, F. vesiculosus, F. identatus.*

Systematic Position

Sub-division	:	Algae
Class	:	Phaeophyceae
Order	:	Fucales
Family	:	Fucaceae
Genus	:	*Fucus*

Common Occurrence : This marine alga is common in water of cold sea in intertidal zones. it is found attached to rocks.

What is to be done ?

To study (i) External morphology of plant, (ii) Anatomy of frond, (iii) Male conceptacle, and (iv) Female conceptacle, with the help of fresh or preserved material and permanent slides.

External Morphology :

It is branched, ribbon-like, leathery and dark brown thallus reaching up to 30 cm in length.

2. Plant body (Fig. 21) consists of a holdfast, stipe and frond.

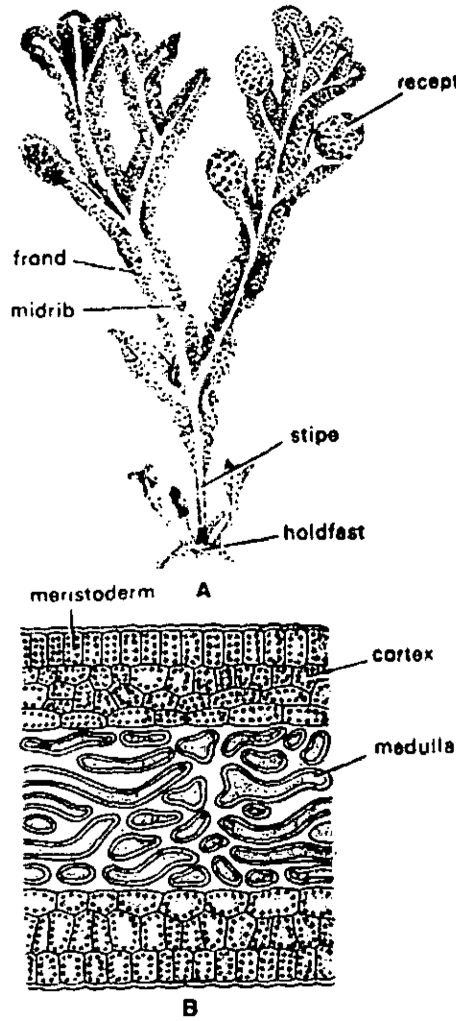


Fig. 21. ***Fucus. A, A mature plant; B, T.S. thallus.***

3. *Holdfast* (Fig. 21 A) is a broad, disc-shaped structure, and functions as a organ of attachment.

4. From the holdfast arises a short steam-like stipe which continues as midrib i upper portion.

5. Leafy and flattened portion above the stipe, which constitutes the main pa of the thallus, is known as *frond* (Fig. 21 A).

6. Frond is dichotomously branched, leathery structure which is slipary i touch.

7. Frond is traversed by a clear midrib.

8. The margin of a frond may be smooth, serrate or entire.

9. Each branch of frond is bifurcated at the tip, and in the groove of this bifurcation is present an apical cell.

10. Many spherical, swollen and hollow structures are present in the upper part of the frond. These are known as air bladders.

11. Air bladders are filled with the air and help in oxygen supply and floatation to the plant.

12. Many cavities are present on the frond bearing sterile hairs. These are known as *cryptoblasts, cryptostomata* or *sterile conceptacles.*

13. At the time of reproduction, the ends of the branches become swollen and contain fertile conceptacles bearing sex organs. These are flask-shaped cavities which lack midrib. These swollen ends are termed as receptacles.

Anatomy of Frond

Cut T.S. of the frond by putting it in pith, stain in safranin, mount in glycerine and study:

1. The outermost layer is made up of closely-packed small rectangular cells which are meristematic in nature and represent *meristoderm* (Fig. 21 B).

2. Cells of the meristoderm are filled with chromatophores, and remain surrounded by a layer of cuticle.

3. Below the meristoderm is the cortex, which is made up of thin-walled, packed and comparatively large cells. Cells of the cortex are parenchymatous in nature and contain less number of chromatophores.

4. Cortex is sometimes differentiated into outer and inner cortex (Fig. 22D).

5. In the centre of frond is present the *medulla.* Cells of this region are long and filament like, and leave large mucilage-filled spaces. The function of medulla is conduction.

Sexual Reproduction

1. It is of oogamous type. Species are monoecious as well as dioecious.

2. Sex organs develop in the fertile conceptacles which are limited only in the region of receptacle.

Male Conceptacle

1. Conceptacles containing antheridia are known as male conceptacles and those with oogonia as female conceptacles. But in monoecious species, both the sex organs are borne in the same conceptacle (fig. 22 D).

2. *A mature conceptacle is a flask* - shaped structure having a basal layer of fertile cells. It opens by an opening or ostiole. Many sterile hairs or paraphyses are present in a conceptacle. Paraphyses are multicellular and branched or unbranched.

3. Male conceptacle bears many antheridia (Fig. 22A).

4. Antheridia are stalked structures and develop either on the fertile layer of conceptacle or at the base of paraphyses.

5. *A mature antheridium* is surrounded by an outer exochite and an inner endochite. It bears many haploid antherozoids formed by the meiotic division of its diploid nucleus.

6. Each antherozoid is a pear-shaped, uninucleate structure with two laterally attached unequal flagella.

Female Conceptacle

1. Female conceptacle is filled with many oogonia

2. Each oogonium is spherical and stalked structure.

3. Oogonium is surrounded by an outer exochite, middle mesochiie and endochite layer.

4. Female conceptale is a flask-shaped structure opening outside by an os Inside the cavity are present many unbranched, multicellular paraphyses oogonia.

5. Each oogonium contains eight eggs (Fig. 22 C).

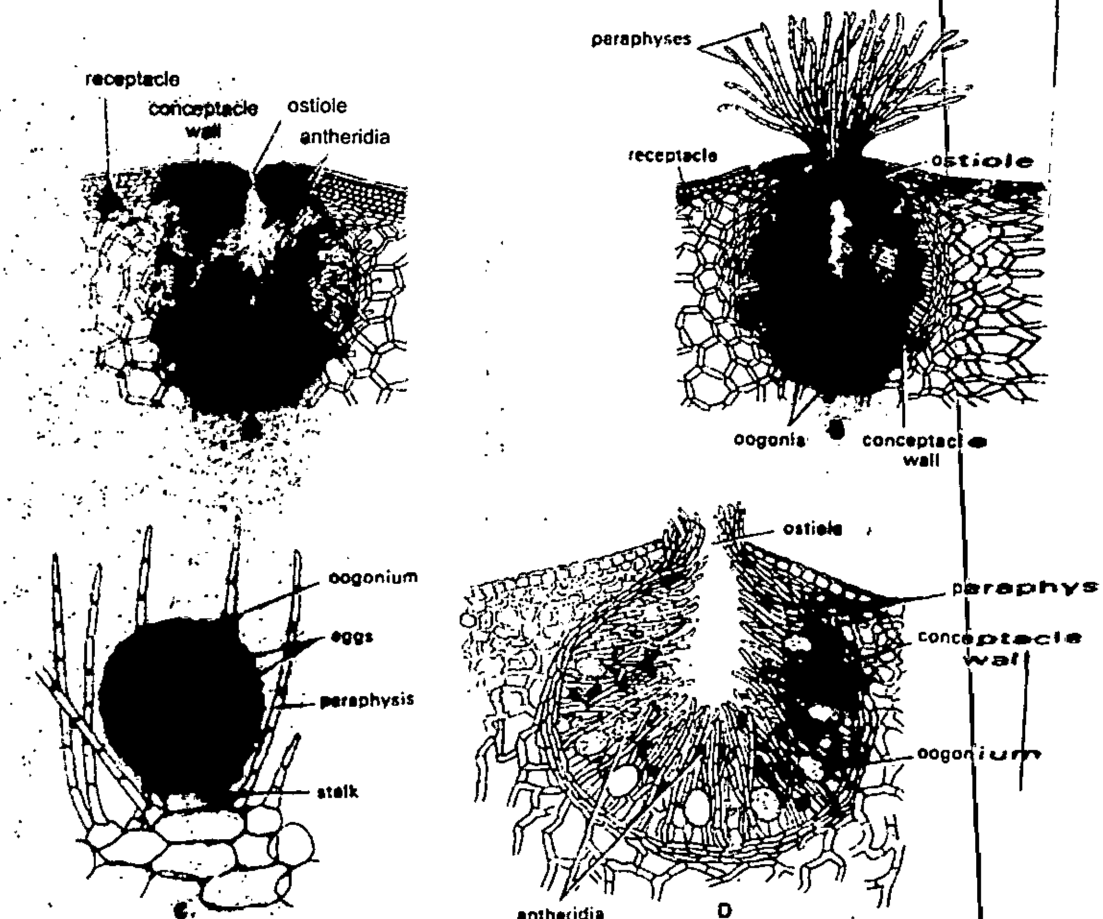


Fig. 22. Fucus. A, A male conceptacile; B, A female conceptacle; C, An oogonium bearing eight eggs, D. A monoecious conceptable.

Identification

(a) (i) *Simple thallus.*

(ii) *Cell wall is made up of cellulose.*

(iii) *Presence of chlorophyll.* ..... ***Algae***

(b) (i) *Brown-coloured chromatophores.*

(ii) *Reserve food materials are mannitol and laminarin.*

(iii) *Flagella unequal and laterally attached.* ...... ***Phaeophyceae***

(c) (i) *Plant body parenchymatous.*

(ii) *Antheridia and oogonia in conceptacles.*

(iii) *Eggs are eight in number.*

(iv) *Asexual reproduction absent.* .... ***Fucales***

(d) (i) *Plant body flat and ribbon-like.*

(ii) Asexual reproduction is absent.

(iii) Oogonium with 8 eggs. ..... ***Fucaceae***

(e) *(i) Fronds are dichotomously branched.*

(ii) A clear midrib is present. ..... ***Fucus***

6. Polysiphonia (*Poly* = many; *Siphonia* = siphons)

Common Indian Species : *Polysiphonia platycarpa, P. variegata, P. sertularioides and P. unguiformis.*

Systematic Position

Sub-division	:	Algae
Class	:	Rhodophyceae
Sub-class	:	Florideae
Order	:	Ceramiales
Family	:	Rhodomelaceae
Genus	:	*Polysiphonia*

Common Occurrence : It occurs in the sublittoral as well as in tidal marshes, brackish estuaries and tidal pools.

Work to be done

To study (i) External features, (ii) T.S. of a branch, (Hi) Spermatangia, (iv) Carpogonia, (v) Carposporophyte, and (vi) Tetrasporophyte, with the help of provided material and permanent slides.

External Morphology

1. Plant body is bush-like (Figs. 23, 24) and red or dark blue-coloured.

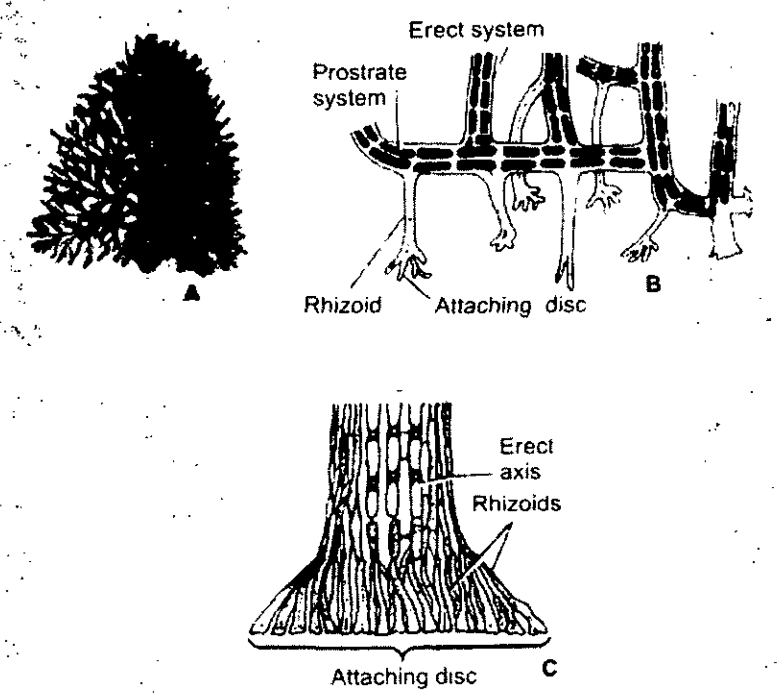


Fig. 23. ***Polysiphonia. A, Thallus showing habit; B, Part of a prostrate system bearing rhizoids, attachment discs and erect branches; C, Attachment system.***

2. It is multiaxial in habit and few centimeters to several inches in length.

3. Prostrate part of the thallus is attached to the substratum with the help many elongated and aseptate rhizoids. These rhizoids sometimes form attachment disc (Fig. 24).

4. Upright filaments are laterally or dichotomously branched.

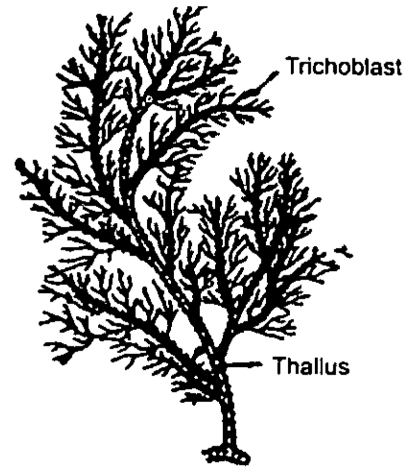


Fig. 24. Polysiphonia. External features.

5. Upright or erect system consists of a main axis having long and shor branches.

6. Plant body is made up of many siphons.

7. Each long branch and main axis consists of a central siphon made up of many elongated cells arranged one upon the other.

8. Central siphon is surrounded by many (4 to 20) pericentral siphons (fig. 25).

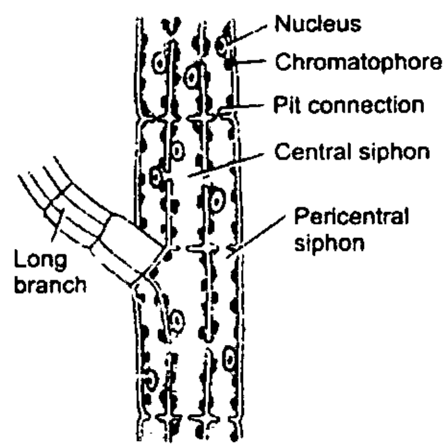


Fig. 25. ***Polysiphonia. V.S. main axis showing central and pericentral siphons and some details of cell structure.***

9. Cells of the central and pericentral siphons are connected with each other by cytoplasmic connections of pit connections.

10. Short branches consist of cells arranged in a single row and so are unisiphonous. These are called trichoblasts.

11. Trichoblasts are present on main axis and long branches, and are dichotomously branched.

12. *Each cell* is uninucleate with many discoid chromatophores arranged peripherally in the cytoplasm. Cell was is thick and each cell contains a large central vacuole. Cells lack pyrenoids (Fig. 26).

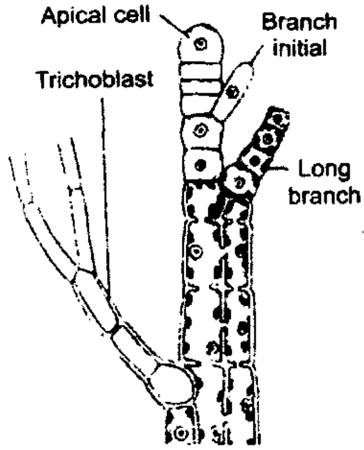


Fig. 26. ***Polysiphonia. Surface view of a growing apex.***

T.S. of a Branch

1. In the centre is present the central siphon.

2. Central siphon is surrounded by many pericentral siphons (Fig. 27).

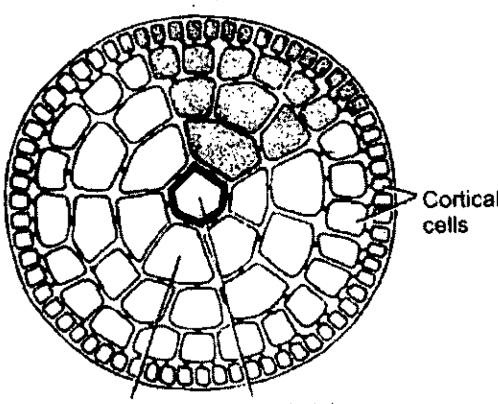


Fig. 27. ***Polysiphonia T.S. of a branch.***

3. From the pericentral siphons arise many cortical cells.

4. All the cells are connected with each other by the cytoplasmic connections.

Reproduction

1. Following three kinds of plants complete the life-cycle :

(i) *Gametophyte :* Male and female plants. Both these plants are haploid and independent.

(ii) *Carposporophyte :* It is diploid and dependent on gametophyte.

(iii) *Tetrasporophyte :* It is diploid but independent and produces haploid tetraspores, which germinate into male and female gametophytes.

2. Sex organs are spermatangia and carpogonia.

Antheridia or Spermatangia

1. Branched, filamentous and red-coloured plant body bears many club-shaped clusters of antheridia or spermatangia.

2. A spermatangium is present on a branch of dichotomously branched m trichoblast (Fig. 28).

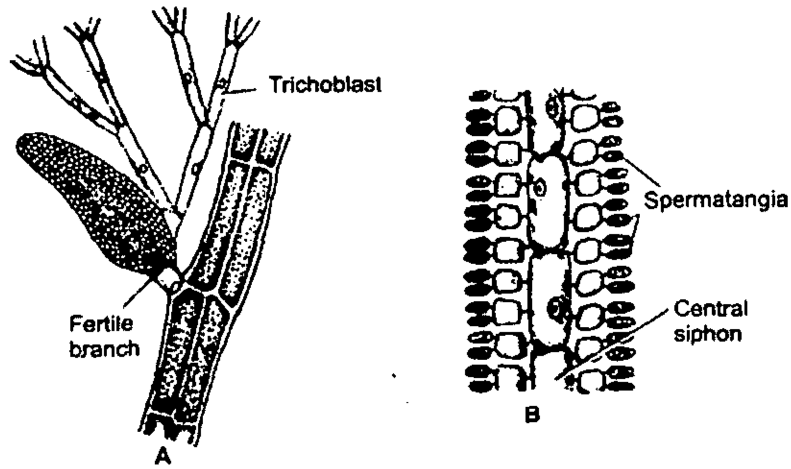


Fig. 28. Polysiphonia. A, Fertile spermatangial trichoblast; B, L.S. of an antheridial branch.

3. Unisiphonous male trichoblast bears many spermatangial cells, each of whic forms many spermatangia.

4. In each spermatangium is present a single non-motile male gamete (spermatium.

Carpogonia :

1. Carpogonia are present on female trichoblast.

2. Each carpogonium develops on a supporting cell, present on carpogonia filament.

3. Terminal cell of the carpogonial filament contains a globular or swollen basa carpogonium with a long neck-like trichogyne (Fig. 29).

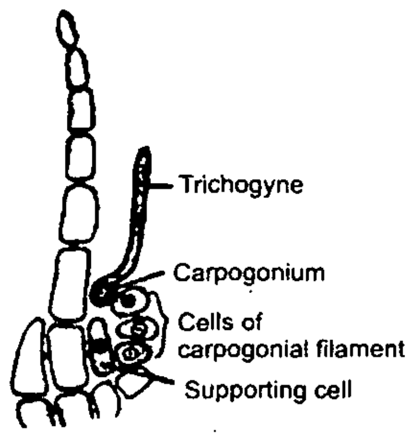


Fig. 29. Polysiphonia. A carpogonial filament bearing carpogonium and trichogyne.

Carposporophyte or Cystocarp

1. Carposporophyte is an urn-shaped (Fig. 30) or spherical structure opening outside by a pore or ostiole.

2. It contains a placental cell or fusion cell at its base.

3. From the placental cell arise many filaments called *gonimoblast filaments.*

4. Tip or terminal cell of each gonimoblast filament bears a *carposporangium.*

5. Each carposporangium contains of diploid *carpospore* (Fig. 30).

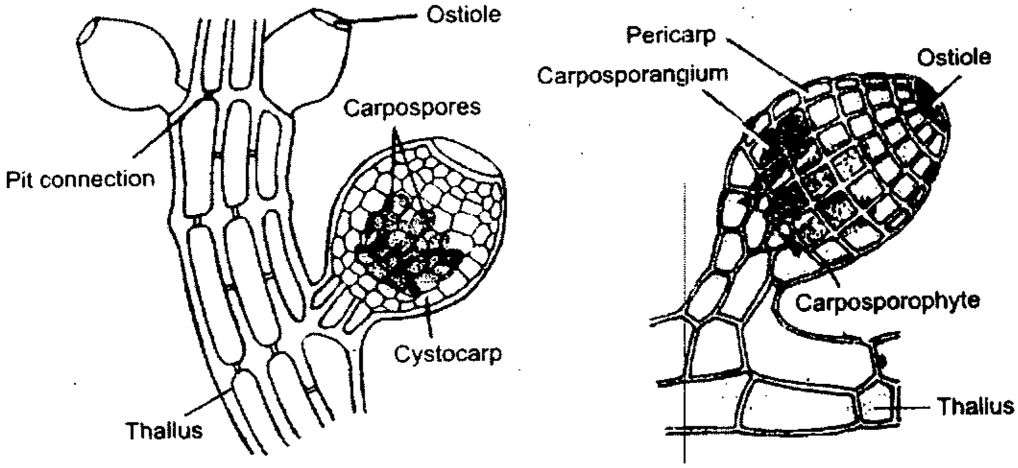


Fig. 30. Polysiphonia. Two thalli bearing cystocarps.

6. Wall of the carposporophyte is known as pericarp. It is made up of a single layer of cells.

Tetrasporophyte

1. Plant body is diploid and exactly similar in structure to that of gametophytic plant.

2. Many small, spherical and stalked sporangia are present. These are called tetrasporangia.

3. Each tetrasporangium contains four tetraspores arranged tetrahedrally (Fig. 31).

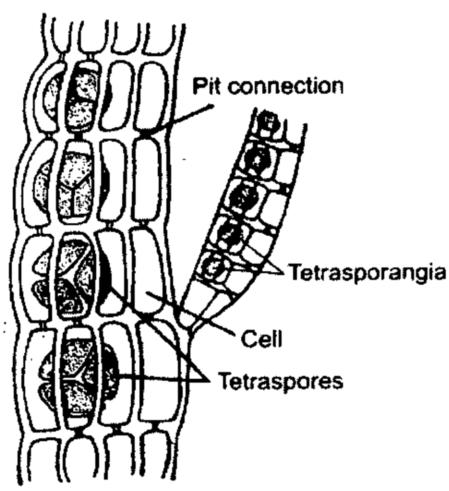


Fig. 31. ***Polysiphonia. Tetrasporophytic plant bearing tetrasporangia and tetraspores.***

Identification :

(a) *(i) Simple thallus.*

(ii) Cell wall is made up of cellulose.

(iii) presence of chlorophyll. .... ***Algae***

(b) *(i) Red-coloured chromatophores.*

(ii) *Reserve food is floridean starch.*

(iii) *Male gametes are non-motile.*

(iv) *Spermatangia and carpogonia are present.*

(v) *Presence of carposporophyte.* .... ***Rhodophyceae***

(c) (i) *Pseudoparenchymatous thalli.*

(ii) *Presence of pit connections.*

(iii) *Highly differentiated sex organs.* .... ***Florideae***

(d) (i) *Multiaxial in construction.*

(ii) *Carpogonial branch four-celled.*

(iii) *Trichoblasts present.*

(iv) *Auxiliary cell present.*

(v) *Triphasic or diplobiontic life-cycle.* ..... ***Ceramiales***

(e) (i) *Uninucleate cells with many discoid chromatophores.*

(ii) *Thallus polysiphonous.*

(iii) *Pericentral cells present.*

(iv) *Male trichoblast dichotomously branched.*

(v) *Female trichoblst unbranched.* ..... ***Rhodomelaceae***

(f) (i) *Many shiphons are present.*

(ii) *Tetransporangia borne single.* .... ***Polysiphonia***

FUNGI

• GENERAL CHARACTERISTICS

1. Fungi (*L. fungus,* mushroom) are heterotrophic, chlorophyllous, eukaryotic and spore-bearing organism surrounded by a well-defined cell wall, chiefly made of *chitin* alongwith many other complex organic substances. Study of fungi is also called *mycology* (Gr. *mykes,* mushroom; *logos*, discourse).

2. Fungi are represented by about 5100 genera and over 100,000 recognized species. They comprise molds, mildews, yeasts, smuts, rusts, mushrooms, puffballs, morels, *etc.*

3. Fungi reproduce by several types of spores. The spore germinates by producing one or more germ tubes. The germ tube elongates to form fine, branched filaments called *hyphae.* A mass of more or less loosely interwoven hyphae constituting the vegetative body of majority of fungi is called *mycelium.*

4. The mycelium may be intercellular (growing in between the host cells) or intracellular (penetrating into the host cells).

5. If the vegetative mycelium is absent the fungus is called *holocarpic* (*e.g., Synchytrium*), but if vegetative mycelium is present, it is called *eucarpic* (as in majority of fungi).

6. From the mycelium develop some special knob-like modifications called *haustoria,* through which it seeks nutrition from the host.

7. Fungi, being heterotrophic, exist either as parasites or as saprophytes. *Parasitic fungi* take all their nutrients from the tissues of another organisms whereas *saprophytic fungi* obtain all their nutrients from dead organic matters.

8. Asexual reproduction takes place by several kinds of *spores, e.g., conidia, zoospores, chlamydospores, basidiospores and aplanospores.*

9. Sexual reproduction is highly reduced and is totally absent in members of Deuteromycotina. After fertilization, the characteristic fruiting bodies (*ascocarp* in Ascomycotina and *basidiocarp* in Basidiomycotina) develops.

PRACTICAL No. 3

• ASEXUAL STAGES OF SOME FUNGI

1. Alternaria

Common Name : Early-blight.

Systematic Position :

Kingdom	:	Mycota
Division	:	Eumycota
Sub-division	:	Deuteromycotina
Class	:	Hyphomycetes
Order	:	Moniliales
Family	:	Dematiaceae
Genus	:	*Alternaria*

Occurrence : In nature, the fungus occurs most commonly as saprophyte several species are parasitic on plants. Most common species is *Alternaria so* causing early-blight of potato (*Solanum tuberosum*). Other hosts are chillies, *Bras* wheat, *Rumex*, etc. It is the most common laboratory contaminant of cultures.

Work to be done : *To study the (i) Symptoms of the disease "early-blight of pota (ii) Vegetative structures, and (iii) Conidia of the fungus.*

Symptoms of "Early Blight of Potato"

1. Small, yellowish-brown spots appear on the leaves of potato when the plants three to four weeks old.

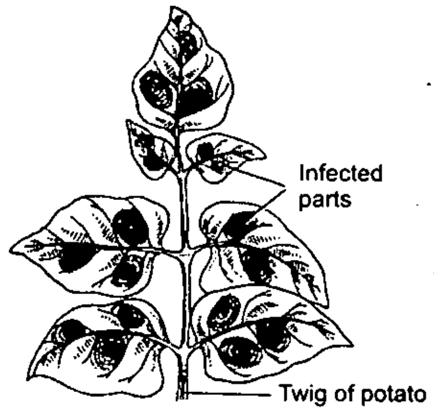


Fig. 1. *Alternaria solani* causing early blight disease of potato (*Solanum tuberosum*).

2. Spots enlarge in size and become rounded to form concentric rings (Fig. 1). Colo of the infected party, later on, changes to black.

3. At the time of severe infection, complete lamina of leaf turns black, and spots a observed on petiole, stem and even on tubers.

4. Inner edible part of infected tuber turns brown.

Fungal Morphology : *Cut thin transverse sections of the host through the infect portion, stain in cotton blue, mount in lactophenol and study* :

Vegetative Structures :

1. Mycelium of the fungus is intercellular or intracellular.

2. Light-brown coloured hyphae are well-branched, septate, and each cell multinucleate (Fig. 2).

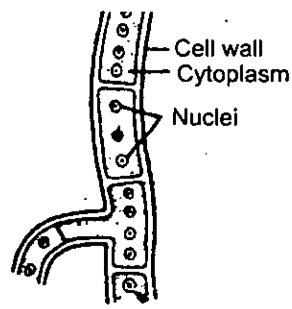


Fig. 2. *Alternaria*. A part of *mycelium*.

3. Haustoria are absent.

Conidia : Fungus reproduces only be asexual reproductive bodies called *conidia* which show following characters :

1. These are present terminally on conidiophores (Fig. 3).

2. Each conidiophore is a multicellular, short or elongated, dark-coloured structure.
3. Each cell of the conidiophore is multinucleate.
4. Each conidium is multicellular, obovoid, elliptical or spindle-shaped structure.

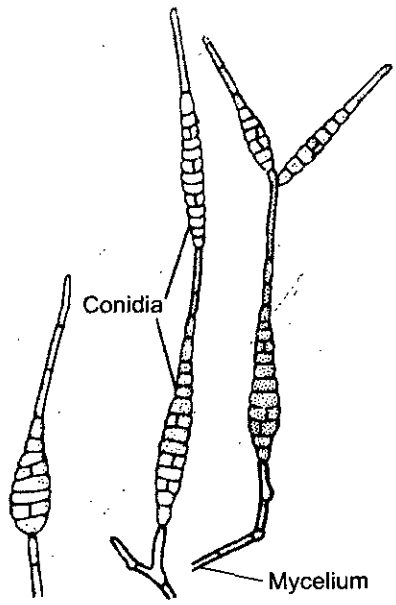


Fig. 3. *Alternaria*. Hyphae bearing conidia

5. Some 5-15 transverse and longitudinal septa are present in each conidium.
6. The colour of the conidia is yellowish brown or dark brown.
7. Conidia may also be present in chain, as in *Alternaria tenuis*.
8. In moist conditions, conidia germinate (Fig. 4) with the help of 5 to 10 germ tubes.

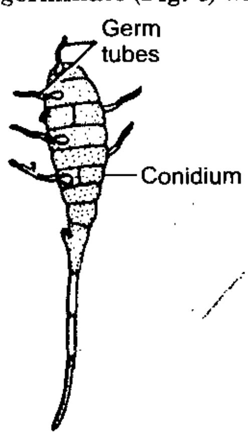


Fig. 4. *Alternaria*. A germinating conidium.

Identification

(a) (i) Chlorophyll absent.

(ii) Glycogen is reserve food.

(iii) Cell wall of fungal cellulose. ....***Mycota***

(b) (i) Plasmodium absent.

(ii) True mycelium present... ......***Eumycota***

(c) (i) Zygospores, zoospores and basidiospores absent.

(ii) Reproduction only by asexual means. ......***Deuteromycotina***

(d) (i) Pycnidia or acervuli absent. ....*Hyphomy*

(e) (i) Conidia develop at the tip of conidiophores.

(ii) Conidia of varying shape. ......*Monili*

(f) (i) Mycelium and conidia dark coloured.

(ii) Absence of fruiting body. ......*Dematia*

(g) (i) Conidia are transversely as well as longitudinally septate.

(ii) Conidiophores are erect bodies. ....*Alterna*

2. Rhizopus

Common Name : *'Bread mold'.*

Systematic Position

Kingdom	:	Mycota
Division	:	Eumycota
Sub-division	:	Zygomycotina
Class	:	Zygomycetes
Order	:	Mucorales
Family	:	Mucoraceae
Genus	:	*Rhizopus*

Occurrence : Majority of the species are saprobes growing on dead and deca plant or animal matter. It occurs very commonly on rotten or stale bread and is c *bread-mold.* It causes the common fruit-rot disease of many fruits like *Artoca* *Pyrus malus*, etc.

Work to be done : *To (i) Culture Rhizopus in laboratory, and to study (ii) Veget structures, (iii) Asexual reproductive structures and (iv) Sexual reproductive struc of the fungus.*

To Culture Rhizopus in Laboratory : It can be cultured easily by placi moistened piece of bread in a petridish (Fig. 1) for a few days at room temperat *Rhizopus* first appears as small white patches, which later surface increase in size cover the complete bread as a white tuft.

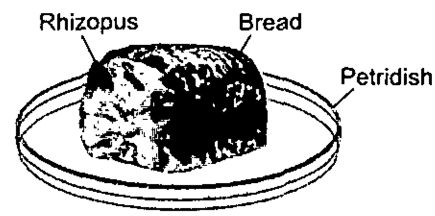


Fig. 1. Rhizopus culture on moistened bread.

Fungal Morphology : *Pick up a small part of the hyphae growing on moiste bread, stain in cotton blue, mount in lactophenol and study :*

Vegetative Structures :

1. Mycelium consists of many branched, white, filamentous and tubular hyp giving a cottony appearance.

2. Mycelium is aseptate and multinucleate.

3. Hyphae are surrounded by a wall made up of chitin. It encloses the cytoplasm

4. Cytoplasm is granular and multinucleate, and contains oil and glycogen as reserve food material.

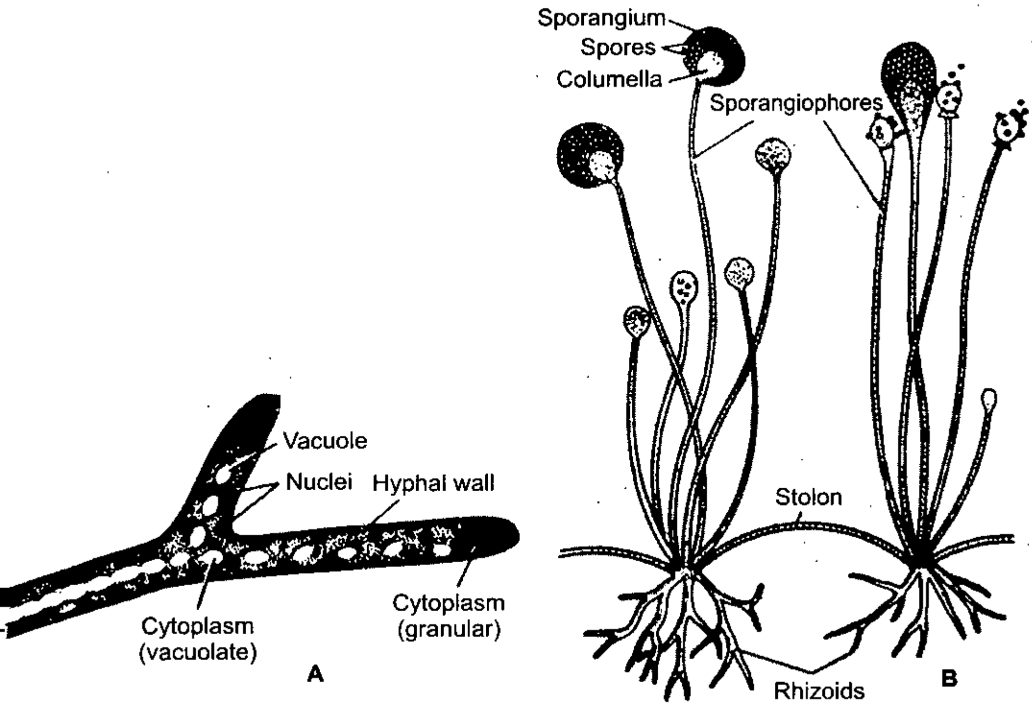


Fig. 2. Rhizopus. A, A part of mycelium; B, Mycelium with sporangia and sporangiophores.

5. Mature mycelium contains following three kinds of hyphae (Fig. 2) :

(a) Stolons : These are aerial, unbranched hyphae usually running horizontally over the surface of the substratum. Stolon is divisible into nodes and internodes.

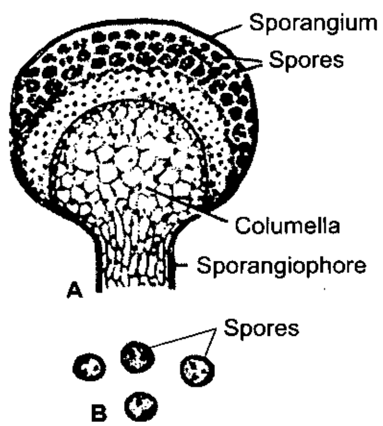


Fig. 3. Rhizopus. A, A mature sporangium, B, Spores.

(b) Rhizoids or Holdfast : From the node of stolon many branched rhizoid-like hyphae arise towards lower side. These are called holdfast or rhizoids. These absorb water and food.

(c) Sporangiophores : From the node of stolon many erect sporangia-bearing hyphae arise towards upper side. These are called sporangiophores.

Asexual Reproductive Structures

1. Asexual reproduction takes place by spores formed in the sporangium.

2. A single sporangium develops at the tip of long, erect sporangiophore.

3. A dome-shaped columella (Fig. 3 A) is present in each sporangium. The pro of columella is in continuation with that of sporangiophore.

4. The space between columella and wall of sporangium is known as spore remains filled with sporangiospores or aplanospores.

5. Each sporangiospore is ovoid, non-motile, unicellular and multinu structure. There is no flagella on the spores.

6. Spores come out on dehiscence of sporangium and germinate immediat forming new mycelium.

7. Another means of asexual reproduction is the formation of *chlamydospore*

Sexual Reproductive Structures

1. It takes place by the fusion of multinucleate gametangia. Species are dioeci heterothallic.

2. Two fusing gametangia (male and famale, or + and –) are morphologically s but physiologically different.

3. Developing gametangia are known as *progametangia.* These are filled cytoplasm and nuclei in their swollen tip.

4. At the time of their fusion each gametangium is separated from the 'susp with the help of a septum.

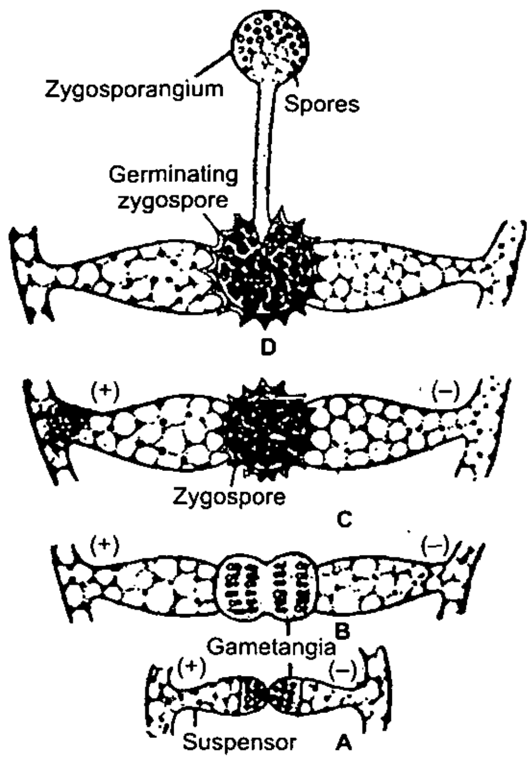


Fig. 4. Rhizopus. Stages of sexual reproduction.

5. The multinucleate protoplasm of each gametangium is known as coenogar (Fig. 4).

6. Fusion of two gametangia takes place. The nuclei of + gametangium fuse those of –, and thus many diploid nuclei are formed. Around this fusion produc thick, spiny wall develops, and now it is called *zygospore.*

7. Zygospore germinates meiotically by producing a long sporangiophore bearin sporangium at the tip (Fig. 4D).

Identification

(a) (i) Chlorophyll absent.

(ii) Glycogen is the reserve food.

(iii) Cell wall of fungal cellulose. ......***Mycota***

(b) (i) Plasmodium absent.

(ii) True mycelium present. ......***Eumycota***

(c) (i) Zoospores absent.

(ii) Perfect-state spores are zygospores. ......***Zygomycotina***

(d) (i) Mostly saprobic. ....***Zygomycetes***

(e) (i) Presence of stolon.

(ii) Presence of rhizoids or holdfast. ......***Rhizopus***

. Penicillium
(*penicilli* = small brush)

Common Name : Blue-mold

Systematic Position

Kingdom	:	Mycota
Division	:	Eumycota
Sub-division	:	Ascomycotina
Class	:	Plectomycetes
Order	:	Eurotiales (Aspergillales)
Family	:	Aspergillaceae
Genus	:	*Penicillium*

Occurrence : Common blue or green-mold occurs saprophytically on decaying organic substances like lemons, oranges and other fruits and vegetables.

What is to be done?

(i) To culture Penicillium in laboratory, and to study (ii) Vegetative structures, (iii) Asexual reproductive structures, and (iv) Fruiting body of the fungus.

Laboratory Culture : If a moist Citrus fruit or cheese piece is placed in damp warm place covered with a hollow large basin or cup, some bluish-green patches of *Penicillium* appear within a few days.

Fungal Morphology

Pick up a few hyphae, stain in cotton blue, mount in lactophenol and study.

Vegetative Structures

1. Mycellium consists of many branched, septate and hyaline hyphae (Fig. 1).

2. Some of the hyphae enter into the substratum and absorb the food material while others remain on the surface and produce the sex organs.

3. Each cell is generally uninucleate but sometimes more than one nuclei are also seen.

4. In the cytoplasm are present many oil globules in the form of reserve food.

5. Sometimes, many hyphae combine and form a solid structure called *sclerotium*.

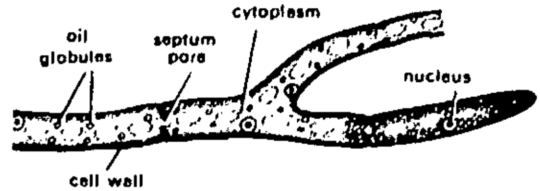


Fig. 1. Penicillium, a part of mycelium

Asexual Reproductive Structures

1. Asexual reproduction takes place by conidia.

2. Conidia develop on branched, septate and multinucleate conidiophores.

3. A conidiophore is an erect body developed from any cell of the mycelium.

4. Brush-like apical portion of the conidiophore is known as penicillus, which h many bottle-shaped *phialides* (Fig. 2). Sometimes, it bears primary, seconda tertiary branches, and the ultimate branches are called *metulae*, which bear sterigmata of phialides.

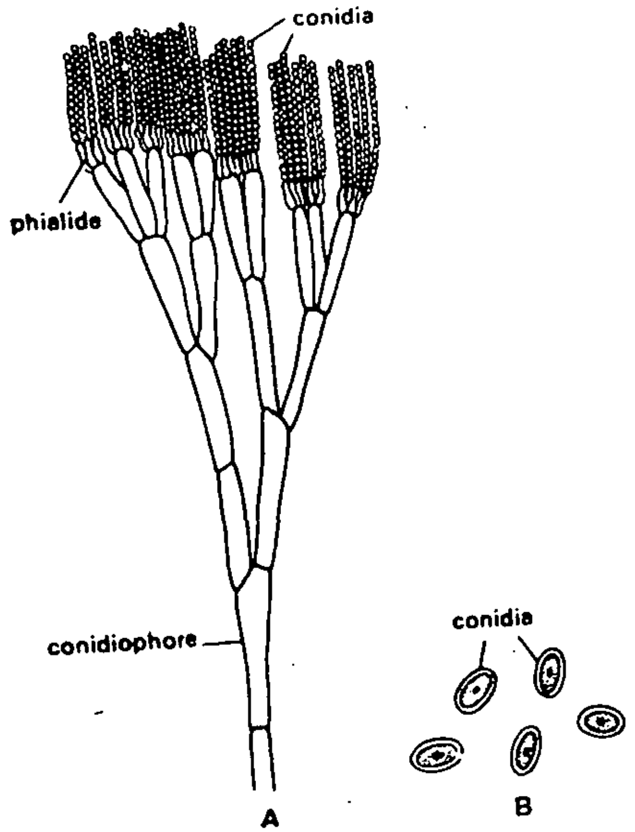


Fig. 2. Penicillium. A, A conidiophore with conidia, B, Some conidia.

5. Sterigmata are uninucleate structures, and bear conidia, arranged basipetalous chains.

6. Each conidium is a small, ovoid or globose, uninucleate but sometim multinucleate, bluish-green or pale coloured structure with a smooth or sometim rough wall.

7. Conidia are dispersed by the wind, and on getting the suitable conditions th germinate into a new mycelium.

Fruiting Body

1. Sexual reproduction takes place by ascospores formed in ascus (Fig. 3).
2. Fruiting body is of cleistothecial type.
3. In each cleistothecium are present many asci.

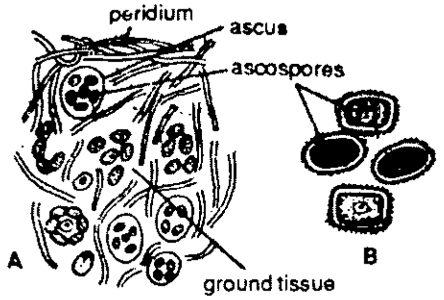


Fig. 3. Penicillium. A, A part of fruiting body, B, A few ascospores.

4. Each ascus contains eight ascospores.
5. Each ascospore germinates and form new mycelium.

. Agaricus

Common Name : *'Mushroom'.*

Systematic Position

Kingdom	:	Mycota
Division	:	Eumycota
Sub-division	:	Basidiomycotina
Class	:	Hymenomycetes
Order	:	Agaricales
Family	:	Agaricaceae
Genus	:	*Agaricus*

Occurrence : It is a saprophytic, edible fungus occurring commonly in rainy season ın humus soil, rotten woods, tree trunks and other organic substances. The most common species is *Agaricus campestris.*

Work to be done : *To study (i) Vegetative structures, (ii) Buttons tage, (iii) Mature fruiting body, (iv) T.S. through gills of the fungus.*

Fungal Morphology

Stain mycelium and various stages of button stage and gills with cotton blue, mount in lactophenol and study:

The body of the mature fungus is differentiated into

(a) Somatic parts, made of vegetative mycelium and

(b) Reproductive parts or fructification or sporophore.

Vegetative Structures

1. Vegetative mycelium grows within the soil.

2. The primary mycelium is septate, haploid, short-lived, and each cell contains oil globules, vacuoles and one nucleus.

3. The secondary mycelium is dikaryotic and long-lived.

4. The hyphae of the secondary mycelium are long, branched and remain twisted to form a thick hyphal cord, called the *mushroom's body* or *basidiocarp.*

Button Stage and its Longitudinal Section

1. The fruiting-bodies arise as small, white, globular, apical swellings (Fig. 1) – branches of the subterranean mycelial strands.

2. These small tiny knots represent the common "*Button-Stage*" of the fungus

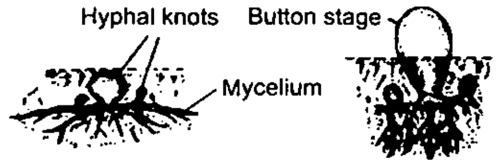


Fig. 1. Agaricus. Some stages of the formation of young fruiting bodies.

3. The dome-shaped upper portion is known as pileus (Fig.2).

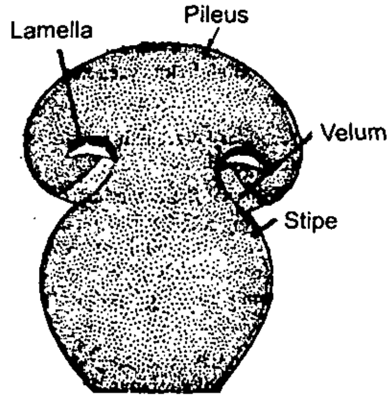


Fig. 2. Agaricus. L.S. button stage

4. The lower hyphae constitute the stalk or stripe.

5. The margins of the pileus are connected with the stipe with the help membrane called inner veil or velum.

6. There is present a constriction between stripe and pileus.

7. Two gill-chamber cavities are present, one on either side of the pileus.

8. From the roof of these cavities arise many gills or lamellae.

9. Button stage is a developmental stage of the fruiting body of *Agaricus.*

Mature Fruiting Body

1. The basal underground mycelial portion is known as rhizomorph, from wh develops the basidiocarp or fruiting body (Fig. 3).

2. The basidiocarp is differentiated into a long stalk-like stipe and an upper cap-l pileus (Fig. 3).

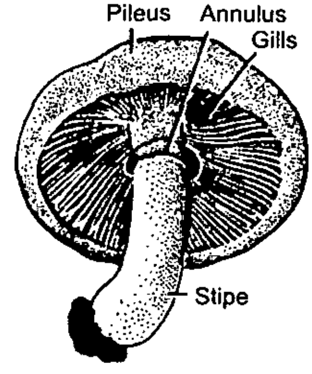


Fig. 3. Agaricus. A mature fruiting body (basidiocarp).

3. The stipe is fleshy, and made up of pseudoparenchymatous mass of hyphae. It gives support to the pileus.

4. Pileus is an umbrella-shaped structure, the underside of which is lined by many gills.

5. On the stipe is present a membranous ring of velum or annulus, which, in the early stages, remains in contact with the pileus.

T.S. Through Gills

1. Three types of gills are present, which vary only in their size. These are known as long gills, half-length gills and quarter-length gills (Fig. 4B).

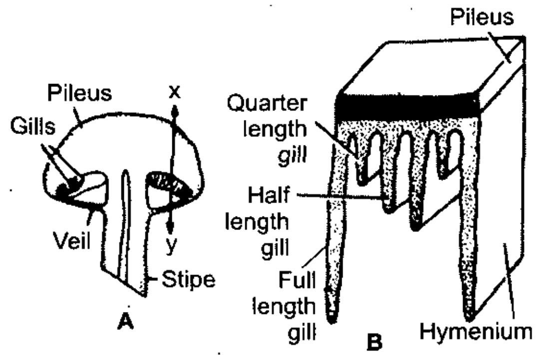


Fig. 4. Agaricus. A, V.S. of basidiocarp, B, V.S. of pileus along the axis 'x' – 'y' in 'A'.

2. In each gill, three different layers are present, *i.e.*, trama, sub-hymenium and hymenium (Fig. 5).

3. The *trama* consists of many anastomosing, interwoven sterile hyphae. It is central in position.

4. The hyphae of trama region develop into a compact hypodermal layer on its either side. This is known as *sub-hymenium.*

5. The hyphae of the sub-hymenial layer terminate into the superficial layer of the gills called hymenium.

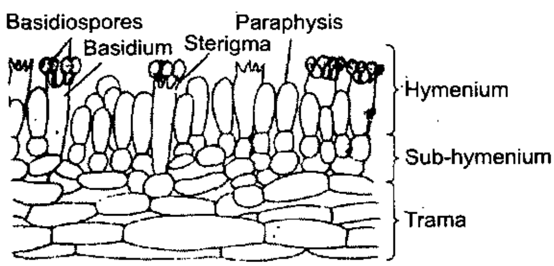


Fig. 5. Agaricus. T.S. gill (a part).

6. The *hymenium* (Fig. 5, 6) consists of many club-shaped cells of two types, of which some are fertile cells and called *basidia,* and others, are sterile cells and called *paraphyses.*

7. From each basidium develop four basidiospores.

8. Basidiospores remains attached with the basidium with the help of *sterigmata* (sing. *sterigma*).

9. Each basidiospore is a purple coloured, oval and uninucleate structure.

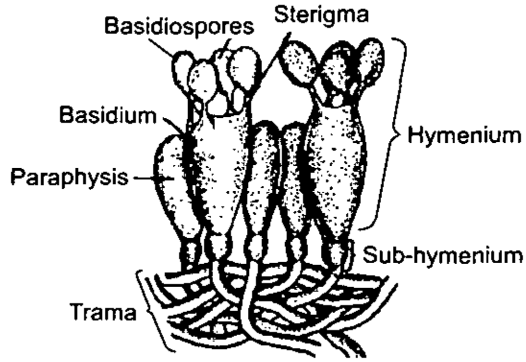


Fig. 6. Agaricus. A few basidia and paraphyses.

Identification

(a) (i) Chlorophyll absent.
(ii) Glycogen is reserve food.
(iii) Cell wall of fungal cellulose. ......***Mycota***
(b) (i) Plasmodium absent.
(ii) True mycelium present. ......***Eumycota***
(c) (i) Zoospores and zygospores absent.
(ii) Basidiospores present. ....***Basidiomycotina***
(d) (i) Mostly saprophytic.
(ii) Basidiocarp present.
(iii) Basidiospores ballistospores. ......***Hymenomycetes***
(e) (i) Fructification is present above the ground.
(ii) Hymenium consists of basidia and paraphyses. ......***Agaricales***
(f) (i) Edible, fleshy fungus.
(ii) Fruiting body is differentiated into a stipe and pileus.
(iii) Undersurface of pileus contains gills. ......***Agaricaceae***
(g) (i) Presence of annulus.
(ii) Gills are of three different sizes. ......***Agaricus***

PRACTICAL NO. 4

Puccinia

Common Name : *Rust*

Systematic Position

Kingdom	:	Mycota
Division	:	Eumycota
Sub-division	:	Basidiomycotina
Class	:	Teliomycetes
Order	:	Uredinales
Family	:	Pucciniaceae
Genus	:	*Puccinia*

Occurrence : It occurs as an obligate parasite on many cereals, millets and other important crops, and causes the rust disease. Important hosts are wheat, oat, jwar, bajra, maize, *Asparagus, Malva, Berberis, Thallictrum, Antirrhinum,* etc.

Heteroecious and Autoecious Rusts

Some species of *Puccinia* complete their life-cycle on two different hosts, and are called heteroecious, *e.g.*, *P. graminis.* It completes some stages of its life-cycle on wheat (*primary host*) and the remaining stages on *Berberis vulgaris (secondary host). Puccinia. butleri* is an example of *autoecious rust* because it completes all the stages of its life cycle on one and the same host, *i.e.*, *Launea.*

Macrocyclic and Microcyclic Rusts

It the life-cycle of rusts, following stages are generally observed, *i.e.*, pycnidiospores, aeciospores, uredospores, teleutospores and basidiospores. If all these five stages are present in the life-cycle of a rust, it is known as *macrocyclic rust, long-cyclic rust* or *euform rust.* But in some rusts uredospores and aecidiospores (or aeciospores) are not present. These are called *microcyclic* or *short-cyclic rusts.*

Black, Yellow and Brown Rusts

Wheat (*Triticum vulgare* Vern. Gehun, Fam. Poaceae) is commonly attacked by three species of *Puccinia, i.e., P. graminis, P. recondita* and *P. striformis* which cause black rust, brown rust and yellow rust, respectively.

Work to be done

(i) To study general symptoms of Puccinia graminis tritici on wheat and berberis plant, (ii) Vegetative structure of the fungus, (iii) T.S. uredosorus, (iv) Teleutosorus, (v) Pycnidial cup, (vi) Aecial cup, (vii) Puccinia butleri on Launea, and (viii) Symptoms of rust on Berberis plant.

Symptoms of Puccinia graminis tritici

(a) On Wheat

1. Large, elongated and brown pustules appear on the stem, leaf sheath and leaf. These are the pustules of uredosori.

2. Brown pustules change into black-coloured large pustules. These are due to teleutosori (Fig. 1B).

3. These pustules, on the infected region, give rusty appearance.

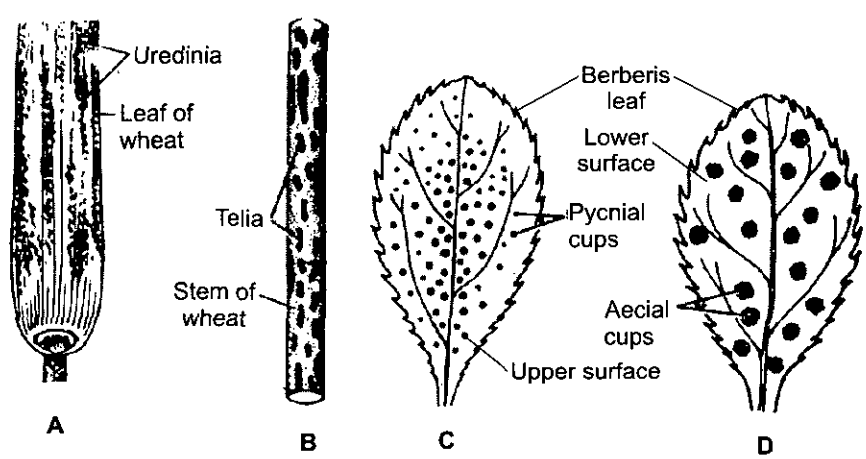


Fig. 1. Infection of Puccinia graminis. A, On leaf of wheat showing uredosori; B, Teleutopustules on stem of wheat; C, Pycnidial cups on upper surface of berberis leaf; D, Aecial cups on lower surface of Berberis leaf

4. Grains of the infected plants are shrivelled and thus reduce the yield.

(b) On *Berberis vulgaris*

5. Infection first starts on the dorsal surface of the leaf, and pycnidial cups formed which appear as yellow spots (Fig. 1C).

6. Ventral surface of the leaves is affected at the time of the formation of aecial cu (Fig. 1D).

Fungal Morphology

It is a macrocyclic, heteroecious fungus, of which first two stages, *i.e.*, uredo a teleutospores develop on wheat (*primary host*), and last two stages *i.e.*, pycnidiospor and aecidiospores on *Berberis* or *Thallictrum* (*secondary host*). Teleutospores, germination, produce basidiospores which infect the secondary host.

Vegetative Structures

1. The mycelium is dikaryotic in wheat while monokaryotic in *Berberis*.
2. The mycelium is well-branched, septate and intercellular.
3. The wall of hyphae consists of fungal cellulose.
4. Each cell contains either one or two nuclei and many oil globules and glycoge bodies in the form of the reserve food.
5. Sometimes, branched or knob-like haustoria are also developed.

Reproductive Parts

For uredo-, teleuto- and basidiospore stages, cut the transverse sections of the infectec leaf of wheat, stain them in cotton blue, mount in lactophenol and study unde microscope :

T.S. Uredopustule or Uredosorus

1. The mycelium is intercellular, branched, septate, and each cell contains twc nuclei.

2. The mycelium aggregates below the epidermis at certain places and produces many stalked, unicellular spores called *uredospores* (Fig. 2).

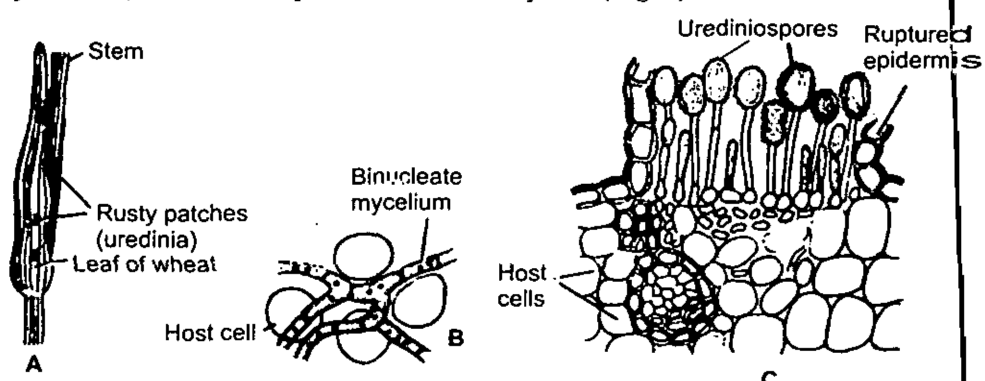


Fig. 2. Puccinia graminis. A, Infected wheat leaf, B, Mycelium in between wheat cells, C, T.S. of host leaf through a uredosorus.

3. Due to the pressure of uredospores, the epidermis is ruptured (Fig.2)

4. Each uredospore is a stalked structure and bears swollen rounded or oblong body.

5. Each uredospore contains two nuclei, and remains surrounded by a thick warty or spiny wall called exine and an inner smooth wall called intine (Fig. 3).

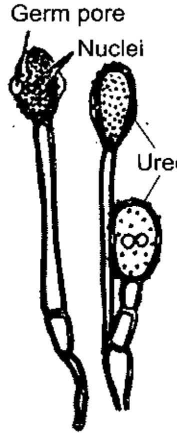


Fig. 3. Puccinia graminis. A few uredospores.

6. Thick outer wall contains 5 to 6 thin areas called germ pores.

7. Many uredospores are present in a group called uredosorus, and they all provide rusty appearance.

8. Uredospores can reinfect fresh plants of wheat by producing new mycelium.

9. The tip of the germ tube, formed by the spore, swells up to form an appresorium.

T.S. Teleutopustule or Teleutosorus

1. In teleutopustules many teleutosori are present, each containing many teleutospores. Teleutosori appear black on the host.

2. The mycelium is intercellular, branched, septate, and each cell contains two unclei.

3. The mycelium aggregates below the epidermis, and produces many stalked, bicelled *teleutospores* (Fig. 4).

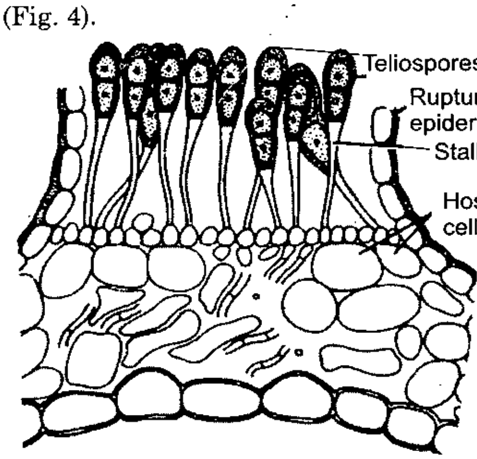


Fig. 4. Puccinia graminis. T.S. leaf through a teleutosorus.

4. Teleutospores develop from the uredia in the uredospores, in the late growing season.

5. Each teleutospore contains a long stalk and a spindle-shaped bicelled structure.

6. The wall of the bicelled spore consists of smooth, black and very thick exine and a thin intine (Fig. 5).

7. Each cell contains a single germ pore.

8. In the young stages each cell contains two nuclei, which fuse at maturity and form a diploid nucleus or synkaryon.

9. The teleutospores cannot reinfect the wheat plant.

10. Each teleutospore germinates and produces the basidiospores.

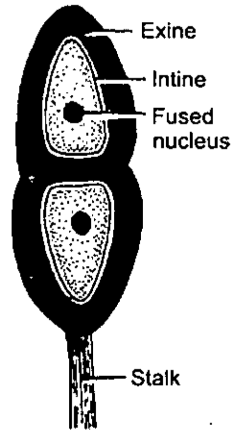


Fig. 5. Puccinia graminis. A single teleutospore.

Basidial Stage

1. Each cell of the teleutospore germinates and produces an epibasidium promycelium (Fig. 6).

2. The epibasidium is a four-celled structure, each cell of which is uninucleate. The nuclei in these cells are the product of the meiotic division of the diploid nucleus of each cell of teleutospore.

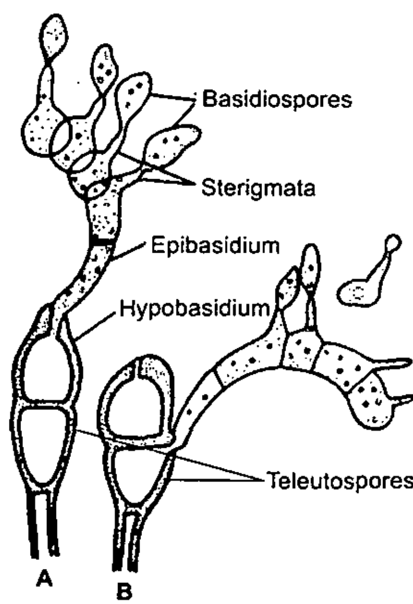


Fig. 6. Puccinia. Two germinating teleutospores

3. Each cell produces a tube-like sterigma, the free tip of which swells and produces a basidiospore (Fig. 6).

4. Each basidiospore is a haploid, uninucleate, unicellular and small structure.

5. Basidiospores are taken away to the hills by the wind where they infect the alternate host, *i.e.*, *Berberis* or *Thallictrum*.

T.S. Pycnidial Cup

Cut the transverse section through the infected portion of Berberis leaf, stain in cotton blue, mount in lactophenol and observe pycnidial cups on the upper surface.

1. The mycelium is monokaryotic.

2. Below the upper epidermis the mycelium collects and forms a flask-shaped cavity called the *pycnidial cup, pycnidium* or *spermogonium*.

3. Pycnidium opens outside with an opening or ostiole.

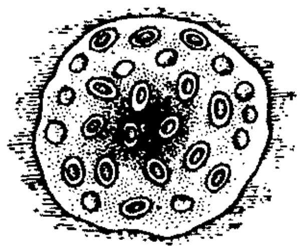


Fig. 7. Puccinia graminis. A magnified aecial cluster.

4. From the monokaryotic mycelium present at the base of the pycnidium, arise many erect pycniophores (Fig. 8).

5. At the base of each pycniophore is present a basal cell.

6. From the tip of pycniophore develops many pycniospores.

7. Each pycniospore or pycnidiospore is an oval, thin-walled, small structure containing one nucleus.

8. Some of the erect hyphae do not produce the pycnidiospores and project out of the cup. These are known as receptive hyphae or flexuous hyphae.

9. Pycniospores cannot infect any of the hosts.

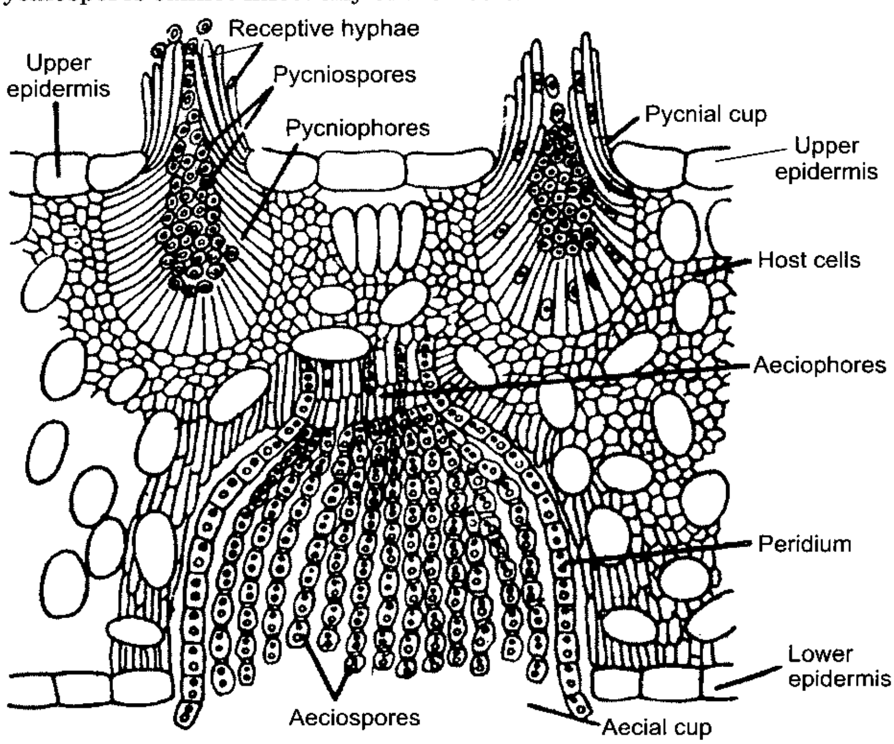


Fig. 8. Puccinia. V.S. through a Berberis leaf showing pycnidia on upper surface and aecidia on lower surface.

10. Pycniospores and flexuous hyphae of different strains unite and form the dikaryotic mycelium, which gives rise to the *aecidial stage* on the lower surface of the leaf.

T.S. Aecial Cup or Aecidial Cup

Cut T.S. of Berberis leaf through the infected part, stain in cotton blue, moun lactophenol and observe aecial cups on the lower surface of the leaf :

1. Aecial cups are present on the lower surface of the leaves of *Berberis* plant.

2. The mycelium is dikaryotic, from which develops many erect hyphae ca sporophores.

3. From the tip of the sporophores are cut many aecidiospores.

4. Aecidiospores are arranged in a basipetalous manner (Fig. 8, 9).

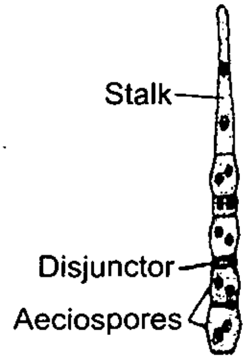


Fig. 9. Puccinia. Aecidiospores or aeciospores on a stalk.

5. Between the two aeciospores is present a sterile separation disc called disjunctu or intercalary disc.

6. The wall of the aecial cup is made up of sterile layer called peridium.

7. Each aecidiospore is a polyhedral, binucleate structure, having an outer thic smooth exine and inner thin intine.

8. The aecidiospore can only infect the wheat plant.

Puccinia butleri on Launea

1. *Puccinia butleri* is an *autoecious rust, i.e.,* all the stages of the life-cycle a completed only on one host.

2. The host *Launea* (Fig. 10) is a member of family *Compositae or Asteraceae*.

3. On the leaves, various stages of life-cycle are present in the form of pustules.

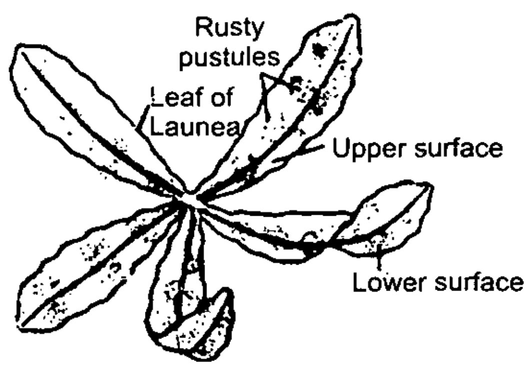


Fig. 10. Puccinia butleri, an autoecious rust on Launea.

Symptoms on Berberis Plant

1. *Berberis* plant is an alternate host of *Puccinia graminis.*

2. It is a member of family Berberidaceae.

3. The primary host is wheat.

4. *Berberis* plants are found only in the hilly regions.

5. Pycnidial and aecial stages of life-cycle are completed on this host.

6. On the upper surface of the leaf are present many circular spots of yellow colour, which are pycnidial cups (Fig. 11).

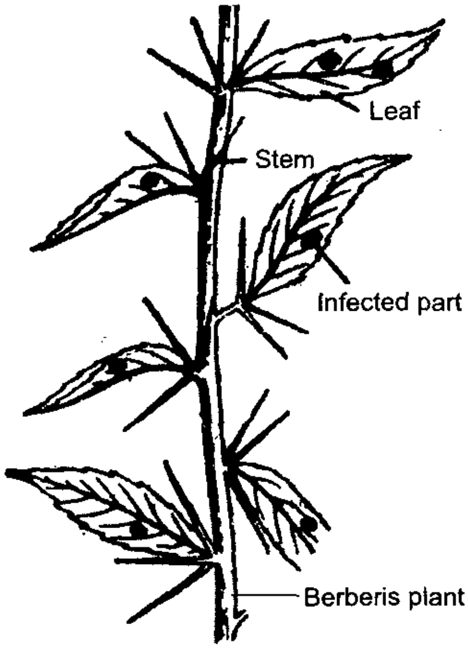


Fig. 11. A branch of Berberis plant infected by Puccinia graminis.

7. On the lower surface of the leaf are present numerous purplish or red, circular spots, which are the aecial stages of the fungus.

Identification

(a) (i) Chlorophyll absent.

(ii) Glycogen is reserve food.

(iii) Cell wall of fungal cellulose. ....***Mycota***

(b) (i) Plasmodium absent.

(ii) True mycelium present. ....***Eumycota***

(c) (i) Zoospores and zygospores absent.

(ii) Basidiospores present. ......***Basidiomycotina***

(d) (i) Parasitic on vascular plants.

(ii) Basidiocarp absent.

(iii) Teliospores present. ....***Teliomycetes***

(e) (i) Obligate parasite giving rusty appearance.

(ii) Heteroecious and polymorphic rust.

(iii) Basidiospores develop on sterigmata.

(iv) Basidium is transverse septate. ....***Uredinales***

(f) (i) Four basidiospores are formed laterally.

(ii) Basidium is external.

(iii) Stalked, more than one-celled teleutospores. ......***Puccinia***

(g) (i) Bicelled teleutospores.

(ii) Fungus completes the life-cycle on wheat and Berberis.

(iii) Exhibits rusty appearance. .....***Puccinia grami***

LICHENS AND MYCORRHIZAE

PRACTICAL NO. 5

WHAT IS A LICHEN?

Lichen is a combination of two different members belonging to two different groups, *i.e.*, one is a fungal component while the another is an algal component. So, it is a dual organism. Algal component is called *phycobiont* while the fungal component is known as *mycobiont.*

About 400 genera and more than 15000 species of lichens have been reported so far, and now it has become a separate branch of Botany with the name *Lichenology*. Some common lichens are shown in Fig. 1.

Systematic position of lichens has always been a controversial point only due to their dual nature. Bessey (1950) has placed them in order Lecanorales of Ascomycetes while Bold (1959) has proposed a new name *Mycophycophyta* (*mykes-fungi, phykos-algae, phyta-plants*) due to the dual nature of these organisms.

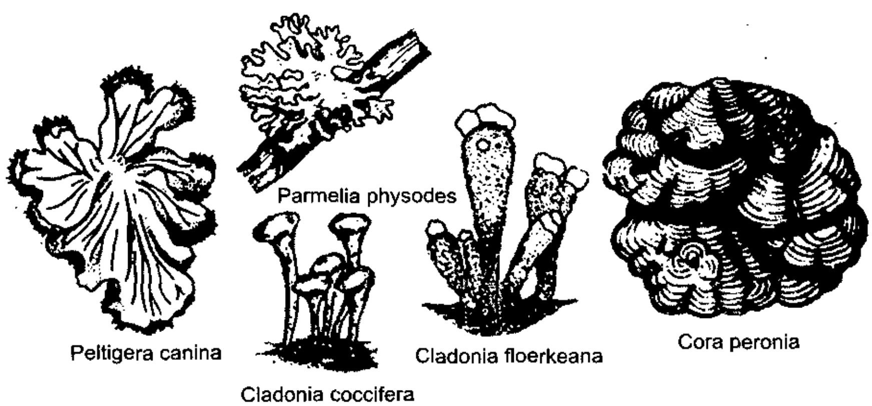


Fig. 1. Some common lichens.

Regarding the nature of the lichens, there are various schools of thought. Some are of the opinion that fungus lives as parasite on algal partner and so alga is simply a victim of the fungus, while others opine that the two partners of the combination remain in symbiotic relationship helping each other in some or the other way. A few lichenologists are of the opinion that, of course, there is the symbiotic relationship between two partners but the fungal partner has its upper hand in the partnership, and thus alga lives as prisoner, and this phenomenon has been termed as *helotism*.

Most of the fungal members in the lichens belong to class Ascomycetes, except a few which belong to Basidiomycetes. Algal members belonging to lichens are mostly the members of class Cyanophyceae, except a few of Chlorophyceae.

Classification of Lichens

Lichens have been classified on the basis of their fungal members and nature of their fruiting bodies into following two groups :

(a) **Ascolichens,**

(b) **Basidiolichens.**

Ascolichens are further divided into :

(i) *Gymnocarpae :* (fruiting body of apothecium type)

(ii) *Pyrenocarpae :* (fruiting body of perithecium type)

Occurrence : Lichens occur commonly on tree trunks or bare rocks, old w roofs of houses in hilly regions, and on the basis of their habitat they have classified into *saxicoles* and *corticoles*. Saxicole lichens occur on stones or rocks the corticoles occur on the bark of the trees.

Plant Body : Lichens are thallus-like bodies, variously lobed and flat or some cylindrical or erect structures, of various colours. On the basis of their form, foll five categories *of thallus organization* of lichens have been recognized by **Hawksw** and **Hill** (1984) :

1. **Leprose Lichens :** In this simplest form of thallus organization of lichens fungal hyphae envelope either one or only a very small numberr of algal cells. A dis fungal layer does not surround the algal cells all over. The so-formed simple li thallus develops superficially over the substratum, provides a powdery appear and is called leprose lichen, e.g., Lepraria incana.

2. **Crustose Lichens :** These are very closely adhered to the substratum (Fig. 2 which they are present, and give a crust-like appearance. It is very difficult to sepa them from their substratum. Fruiting bodies are present on the upper surface, *Graphis scripta, Lacidia, Verrucaria,* etc.

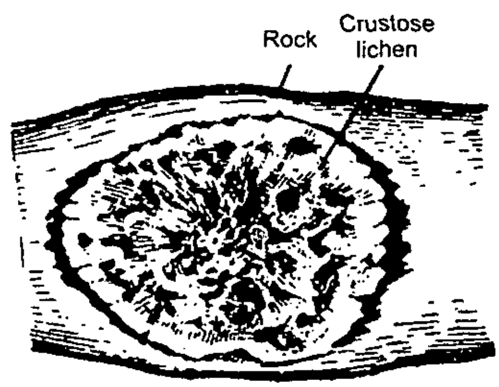


Fig. 2. A crustose lichen attached on a rock.

3. **Foliose Lichens :** Thallus in these lichens is flat, leaf-like, well-branched a attached to the substratum with the help of rhizines, *e.g.*, *Physcia, Parmelia, Peltide* etc. (Fig.3).

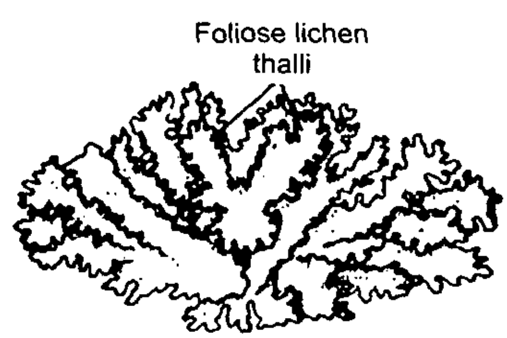


Fig. 3. A foliose lichen.

4. **Fruticose Lichens :** Lichens of this category are well-branched structures (Fig. 4, 5), which are generally erect or sometimes prostrate, and give shrub like appearance, *e.g.*, *Usnea, Cladonia, Everinea,* etc.,

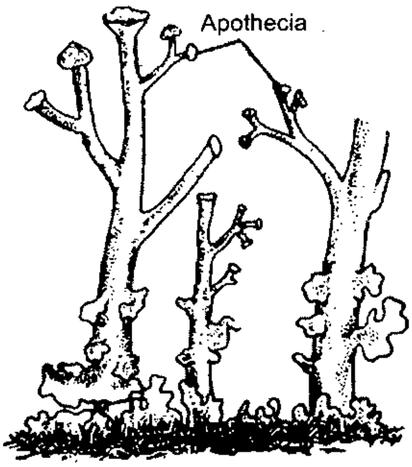


Fig. 4. Cladoniam a fruticose lichen.

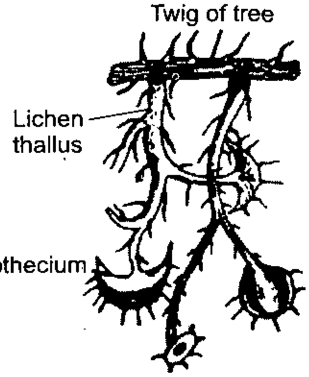


Fig. 5. A fruticose lichen.

5. ***Filamentous Lichens :*** It some lichens, instead of fungal, the algal partner is nore developed. Such algal partners of the lichens are filamentous and remain ensheathed or covered by only a few fungal hyphae. Such lichens are filamentous in appearance, have are dominance of algal partner, and are named *filamentous lichens* by Hawksworth and Hill (1984).

Examples : Ephebe, Coenogonium, Cystocoleus and *Racodium.*

Internal Structure

Internally, a *foliose lichen* is divisible into four different regions, *i.e.*, upper cortex, algal zone, medulla and lower cortex (Fig. 6).

Upper cortex is a thick, protective layer made up of dense, closely woven pseudoparenchymatous mass of fungal hyphae. Outer to this, an epidernal layer may or may not be present.

Algal zone consists of mostly blue-green, or green algal filaments of Cyanophyceae or Chlorophyceae. Some fungal hyphae also remain embedded in this layer. Common algal members are *Nostoc, Gloeocapsa, Rivularia* and *Chlorella.* This is also known as *gonidial layer.*

Medulla forms the central region of the lichen. It consists of loose mass of fungal hyphae.

Lower cortex forms the lower surface of lichen and from this arises rhizines. It is composed of densely packed hyphae.

A crustose lichen (Fig. 7.) is internally differentiated into cortex, algal zone and medulla. Some hyphae o3f medulla form pointed rhizoids.

Fig. 6. Structural organisation of a foliose lichen.

Fig. 7. Structural organisation of a crustose lichen.

V.S. Apothecium

1. Apothecium is a cup-shaped (Fig. 8) body.

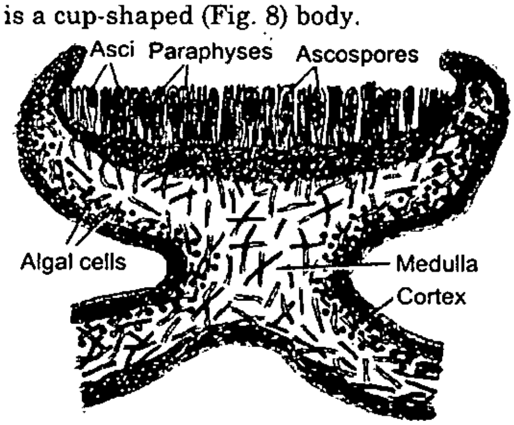


Fig. 8. V.S. apothecium of an ascolichen.

2. In the cavity of the cup are present many asci and sterile paraphyses.

3. In each ascus are present generally eight uninucleate ascospores.

4. Other structures of the internal organisation are same as those of vegetative ıallus.

• MYCORRHIZAE

The roots of many plants enter into a mutualistic symbiotic association with fungi. 'he existence of such a relationship was first noted in 1885 by A.B. Frank, a German otanist, who coined the term "*mycorrhiza*" (Gr. *mykes* = fungus + *rhiza* = root).

'ypes of Mycorrhizae

There are six major types of mycorrhizae, which can be categorised as under :

(A) *Ectomycorrhizae* (or *Ectotrophic mycorrhizae*)

(B) *Endomycorrhizae* (includes 3 major types *viz. Arbuscular mycorrhizae* or AM, *Ericoid mycorrhizae* and *Orchidoid mycorrhizae*).

(C) Ectendomycorrhizae (includes 2 major types *viz.*, Arbutoid mycorrhizae and Ionotropoid mycorrhizae)

Some of these mycorrhizae are shown in Fig. 9.

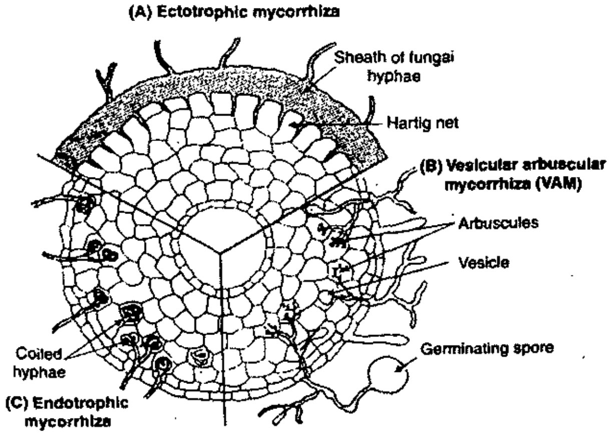


Fig. 9. Three types of mycorrhizae shown diagrammatically in transverse section of root. A, Ectotrophic mycorrhiza; B, Vesicular-arbuscular mycorrhiza; C, Endotrophic mycorrhiza of an orchid.

Ectomycorrhizae

The ectomycorrhizae are characterized by the presence of an external pseudoparenchymatous sheath, called *mantle*, on the terminal nutrient absorbing rootlets. The sheath may be more than 40 μm thick, and may constitute up to 40% of the dry weight of combined (root+fungus) structure. Beneath the sheath, the fungal hyphae penetrate the intercellular spaces of epidermis and cortex to form an intercellular network of hyphae called *Hartig net.* Though the hyphae of the Hartig net are in close contact with the root cells in the region, there is no penetration of host cells.

Endomycorrhizae

In case of endomycorrhizae, the fungal partner grows mainly inside the roots, penetrating the outer cortical cells of the plant root. Only a small portion of the fungal component lies externaly as a loose mass of hyphae in soil. Three major types of endomycorrhizae are : (a) arbuscular mycorrhizae (AM); (b) ericoid mycorrhizae, and (c) orchidoid mycorrhizae.

BRYOPHYTA

• GENERAL CHARACTERSTICS OF BRYOPH

1. ***Occurrence :*** Majority of the bryophytes are terrestrial, except a few, which in aquatic surroundings like *Riccia fluitans, Ricciocarpus natans, Riella Sphagnum.*

They like to grow in moist shady places. Some are epiphytic, *e.g.*, membe Jungermanniales, and even some members are saprophytes, *e.g. Buxbaumia*.

2. ***Plant body :*** Either thalloid, *e.g.* members of Hepaticae, or foliose, *e.g.* men of Musci. Plant body is gametophytic and green in colour.

3. ***Size :*** It is variable from microscopic, as in *Zoopsis* or order (Jungermanniale gigantic size up to 40 cm. or even more as in *Dawsonia*.

4. ***Absorptive organs :*** These are fine thread like structures called *rhiz* Rhizoids are present in thalloid plants on their ventral surface, and in foliose plan their lower side.

5. ***"Leaves" :*** In foliose plants, the leaves are present. In Jungermanniales, le are arranged in 2 to 3 rows and there is no midrib, but in mosses leaves are arrar spirally and a distinct mid rib is present.

6. ***"Axis" :*** Axis is present in Jungermanniales as well as in mosses. Jungermanniales, it is homogeneous structure but a conducting strand is presen mosses.

7. ***Vegetative reproduction :*** Various means of vegetative propagation are progressive death and decay of older parts of the plants, or by adventitious branch gemmae, tubers, bulbils, and sometimes also by protonema.

8. ***Sexual reproduction :*** It is oogamous type, and sex organs are antheridia a archegonia. Antheridia are club-shaped structures, and produce many unicellul uninucleate and biciliate antherozoids. Archegonia are flask-shaped structures, a each encloses a single non-motile egg.

9. ***Fertilization :*** It takes place in the presence of water and its ultimate product an oospore or zygote. Oospore is the first cell of the sporophyte.

10. ***Sporophyte :*** Sporophyte is always dependent on gametophyte. It differentiated generally into foot, seta and capsule. There may also be variations (eith foot or seta is absent, or both are absent as in *Riccia*).

Foot : Function of foot is absorption. It absorbs food material from gametophyte.

Seta : Function of seta is elongation.

Capsule : Function of capsule is spore-production.

In capsule, spore mother cells and sometimes elaters are also present, *e.g. Marchantia*.

11. ***Spores :*** After reduction division in spore mother cells four spores are formed which remain arranged tetrahedrally. All the spores are alike (*homosporous*). The germinate into gametophytic plants.

12. ***Alternation of generations :*** Bryophytes exhibit best example c heteromorphic alternation of generations.

PRACTICAL NO. 6

• MARCHANTIA

Common Indian Species : *Marchantia polymorpha, M. simalana, M. nepalensis and M. palmata.*

Systematic Position

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

Occurrence : It is cosmopolitan in distribution, and is found commonly in moist and cool surroundings, banks of streams, damp shady places and on the rocks of hills.

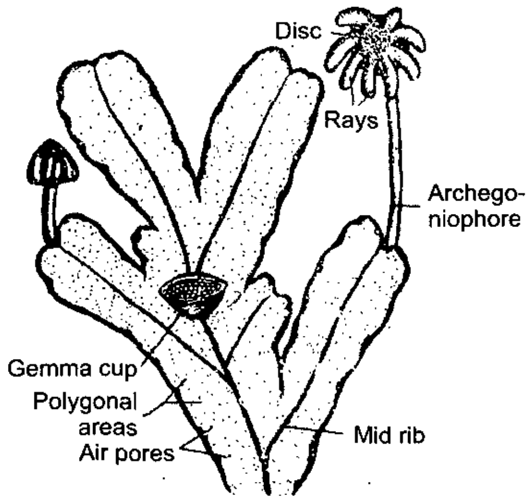


Fig. 1. Marchantia. Dorsal surface cf thallus with two archegoniophores and gemma cup

Work to be done

To study (i) External: features of thallus; (ii) Anatomy of thallus; (iii) V.T.S. through gemma cup; (iv) A single gemma, (v) T.S. stalk, (vi) L.S. antheridiophore, (vii) L.S. archegoniophore (young and mature), (viii) Mature sporogonium, and (ix) To identify the genus.

External Features of Gametophyte

Study the dorsal and ventral surfaces of thallus through a hand lens; separate two types of scales and two types of rhizoids from the ventral surface, stain them in safranin, mount in glycerine and study :

1. The plant body is thalloid, prostrate, green in colour, and larger than *Riccia.*
2. It is dichotomously branched and is differentiated dorsiventrally (Fig. 1).
3. The thalli attain a length of 2 to 10 cm or more.
4. A growing pint is situated at the notch of the apex of each lobe.

Dorsal Surface

5. A clear mid-dorsal groove is present.

6. Many diamond-shaped rhomboidal or polygonal areas are present, if obser under a magnifying lens (Fig. 2).

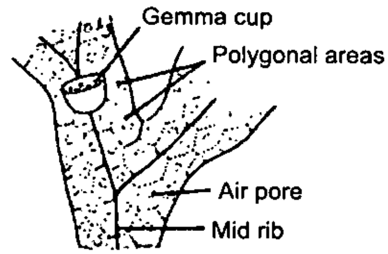


Fig. 2. Marchantia, A part of dorsal surface of thallus showing polygonal areas.

7. These polygonal areas indicate the outline of the air chambers, and contain dot-like structure in their centre. This dot represents the *air pore.*

8. Sometimes many cup-shaped, sub-sessile structures are present on tl mid-dorsal groove. These are *gemma cups* (Fig. 1, 2).

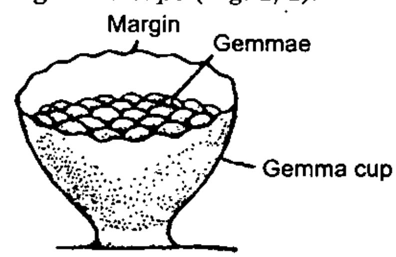


Fig. 3. Marchantia. A single gemma cup.

9. Each gemma cup contains many gemmae which are the means of vegetative reproduction.

Ventral Surface

10. Mid dorsal groove appears in the form of a clear midrib on the ventral surface.

11. On the midrib are present many scales and rhizoids (Fig. 4).

Scales

1. These are purplish to violet coloured, multicellular but one-celled thick structures, arranged in 2 to 3 rows on either side of the midrib.

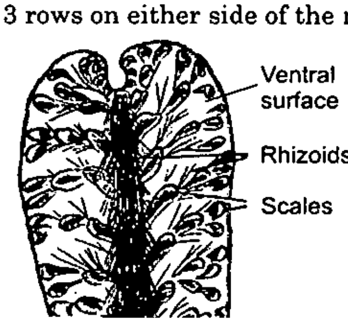


Fig. 4. Marchantia. Ventral surface of thallus.

2. Scales are of two types, *i.e.*, *appendiculate and ligulate* (Fig. 4).

3. *Appendiculate scale* contains an appendage at its apical side (Fig. 5). They remain situated in one row just on both the sides of midrib. These are bigger in size than ligulate scales.

4. *Ligulate scales* are ligule like (Fig. 6) and devoid of any type of appendage. These are present in one or two rows on either side of midrib.

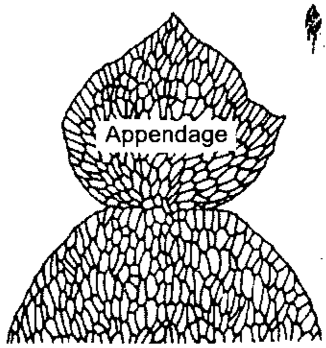


Fig. 5. Marchantia. An appendiculate scale.

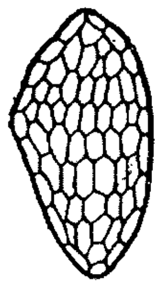


Fig. 6. Marchantia. A ligulate scale.

5. Functions of the scales are to retain the moisture and protect the growing apex.

Rhizoids

1. These are elongated, unicellular, hair-like outgrowths of two types, *i.e.*, smoothwalled and tuberculate (Fig. 7E).

2. *Smoothwalled rhizoids* are broad, thin-walled, unicellular, and filled with colourless contents.

3. *Tuberculate rhizoids* are narrow, comparatively thick-walled, and contain many peg-like in growths (Fig. 7B).

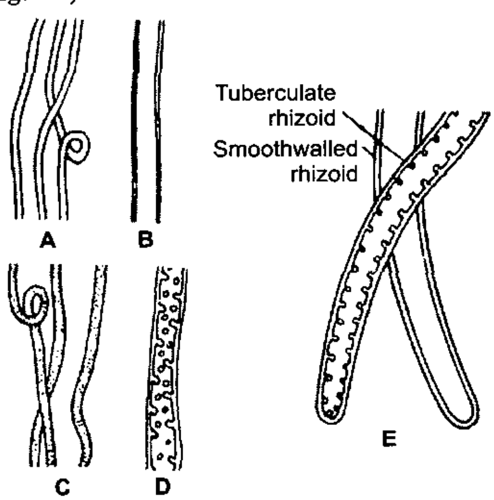


Fig. 7. *Marchantia rhizoids*. Smoothwalled rhizodis in surface view (A) and optical section (B); Tuberculate rhizoids in surface view (C) and optical section (D); Both types of rhizoids in optical section (E).

4. The functions of the rhizoids are absorption and fixation.

Anatomy of Gametophyte

Cut V.T.S. thallus, stain in safranin, mount in glycerine and study. Also cut the section of the thallus through gemma cup.

Anatomically, the thallus is divisible into two clear regions, *i.e.*, photosynthetic region and storage region (Fig. 8).

Photosynthetic Region :

Upper, dorsal, green photosynthetic region consists of upper epidermis, air pores, air chambers and photosynthetic filaments.

Upper Epidermis : 1. It is the outermost layer made up of thin-walled cells, having a slightly thicker outer wall.

2. Cells contain chloroplasts.

3. Function of the epidermis is protection.

4. Continuity of epidermis is broken by many air pores (Fig. 8).

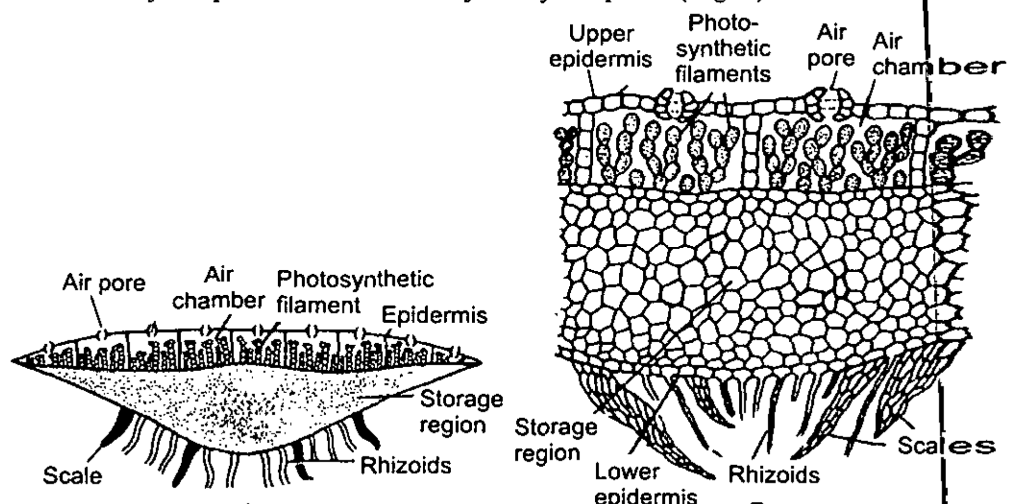


Fig. 8. Marchantia. A, V.T.S. thallus (diagrammatic); B, V.T.S. thallus (a part cellular).

5. ***Air Pores :*** These are barrel-shaped structures.

6. Each pore is surrounded by four to eight tiers of cells (Fig. 9).

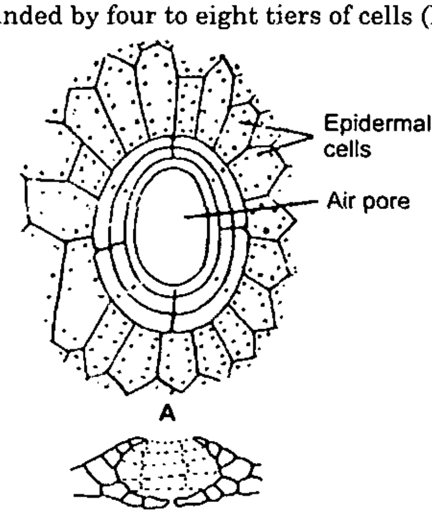


Fig. 9. Marchantia. An air pore in surface view (A) and in cross-section (B).

7. Each tier consists of 4 to 5 cells.

8. Tiers are present in the form of rings one above the other, but in sections they are seen to be arranged in the form of a chimney.

9. Wall of the pore lies half above and half below the epidermis.

10. ***Air Chambers :*** These are arranged in a single, horizontal layer below the upper epidermis.

11. They are separated from each other by 2 to 6-celled partition walls.

12. Cells of partition wall also contain chloroplast.

13. An air chamber opens outside by a barrel-shaped air pore.

14. ***Photosynthetic Filaments :*** These are green, chlorophyll-containing filaments present in air chambers.

15. They may be branched or unbranched, and consist of 2 to 6 or more cells.

Storage Region :

It consists of many types of cells, conducting strand, lower epidermis, scales and rhizoids.

16. In the centre, it is composed of many thin-walled parenchymatous cells, but on the margins only 2 to 3 layers of cells are present.

17. Most of the cells contain starch and are devoid of chloroplast.

18. In the midrib region, some cells may bear reticulate thickenings.

19. Cells of the lower epidermis contain both the kinds of scales and rhizoids.

V.T.S. Thallus through Gemma Cup :

1. On the dorsal surface of the thallus is present a large cup-shaped cavity called gemma cup (Fig. 10).

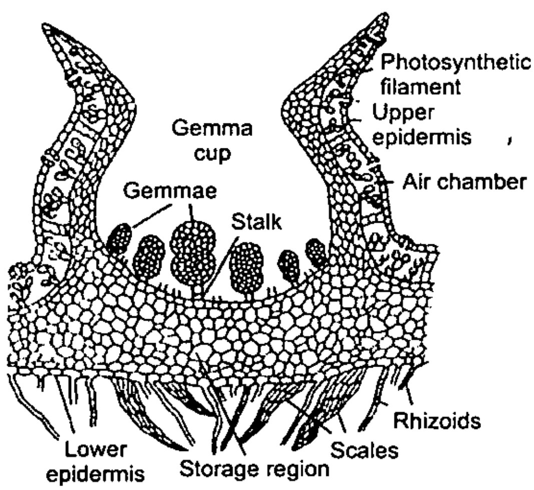


Fig. 10. Marchantia. V.T.S. thallus through gemma cup (diagrammatic).

2. On the floor of the gemma cup are present many multicellular discoid bodies known as *gemmae.*

3. Many club-shaped, mucilaginous hairs are also present in gemma cup.

4. Gemmae are the means of vegetative reproduction.

5. Other details of the thallus are same as described above, *i.e., photosynthetic region* consisting of air pores, air chambers and photosynthetic filaments, and *storage region* consisting of parenchymatous cells with lower epidermis having scales and rhizoids.

A Single Gemma :

1. It is a multicellular, discoid, green structure attached with the help of a unicellular stalk (Fig. 11).

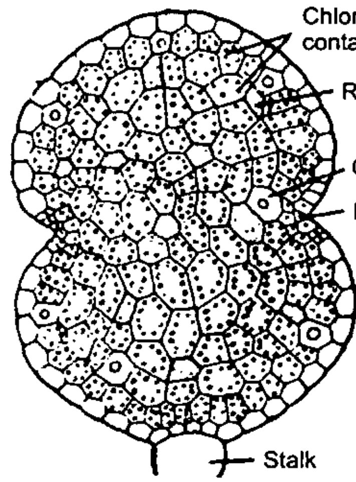


Fig. 11. Marchantia. A single gemma

2. It is many cells thick in the middle but only one cell thick on the margins.
3. On each lateral side is present a deep notch.
4. Each notch contains many apical cells at the base.
5. Most of the cells of the body of gemma are green and contain chloroplast.
6. Some isolated cells contain oil bodies and called *oil cells*, while a few cells near the margins are *rhizoidal cells.*
7. Gemmae germinate by falling on the ground.
8. From each notch develops a single individual plant,

Sex Organs

Study of the arrangement of antheridia on antheridiophore and archegonia on archegoniophore; cut T.S. of receptacular stalk and L.S. of antheridiophore and archegoniophore, stain in safranin, mount in glycerine and study :

1. Sexual reproduction is oogamous.
2. All species are dioecious.
3. Sex organs develop on highly specialized, long branches borne terminally.
4. At the tip of each gametophore is present a disc or receptacle.
5. Gametophore, which bears male sex organs, is termed as antheridiophore. It contains many antheridia.
6. Gametophore, which bears female sex organs, is termed as archegoniophore. It contains archegonia.

T.S. Stalk

1. Structurally, the stalks of male and female receptacles are simply the elongations of thallus and contain the same structure.
2. There are present air pores, air chambers and. photosynthetic filaments on posterior side. The posterior side represents the dorsal surface of thallus (Fig. 12).
3. On the anterior side are present two longitudinally running grooves.
4. Many rhizoids and sometimes scales are present in the groove. The groove thus represents the ventral surface of the thallus.
5. The structure is dorsiventrally symmetrical, and resembles with that of thallus.

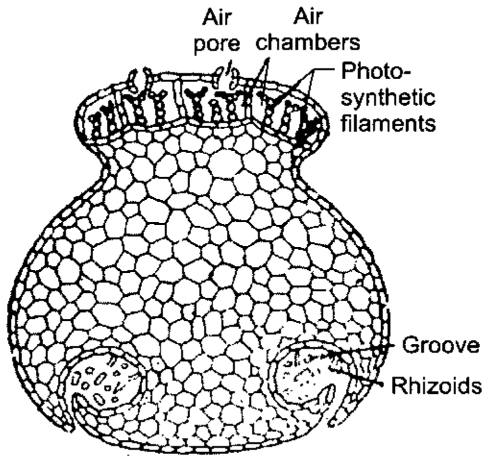


Fig. 12. Marchantia. T.S. stalk of receptacle.

Antheridiophore or Male Receptacle :

1. It is a stalked structure ranging from 2 to 3 cm. in length.

2. At its tip is present an eight-lobed disc (rarely four lubed).

A vertical longitudinal section (V.L.S.) through the antheridiophore and adjoining portion of the thallus reveals the following details (Fig. 13) :

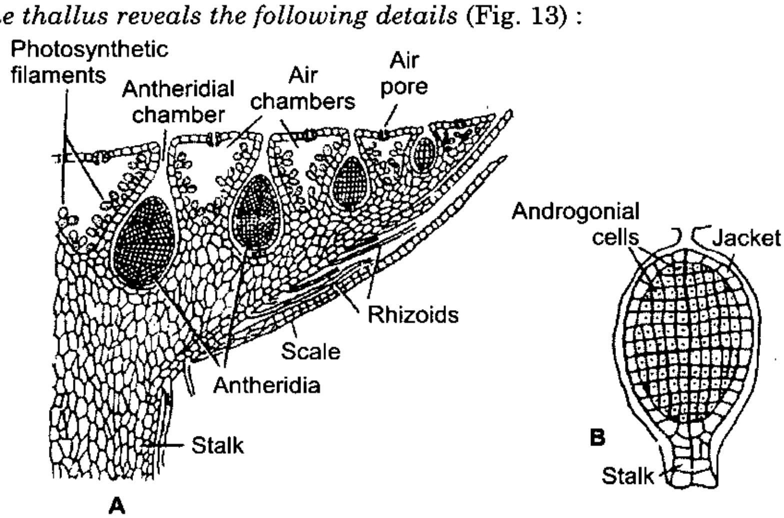


Fig. 13. Marchantia. L.S. antheridiophore (A) and a single antheridium (B).

3. Disc is bounded by a layer of epidermis, the continuity of which is broken by barrel-shaped air pores.

4. Each air pore opens in an air chamber containing photosynthetic filaments.

5. Each lobe on the disc contains a growing region at its apex.

6. Besides the air chambers, there are also present certain flask-shaped cavities which open outside by a pore or ostiole. These are antheridial chambers.

7. In each cavity is present a single antheridium.

8. These antheridia remain arranged acropetally *i.e.*, the oldest antheridium is present in the centre and the youngest near the apex of the lobe.

9. A *mature antheridum* is a stalked, Club-shaped structure situated at the base of the antheridial chamber.

10. A sterile layer of jacket encloses the androcyte mother cells, each of which divides into two androcytes. Androcyte metamorphoses into a biflagellate antherozoid.

11. The disc is held high by a long stalk, which is the continuation of the thallu.

12. Thallus is divided into same photosynthetic and storage regions having all same normal structures.

Archegoniophore or Female Receptacle

1. It is also a stalked structure but the stalk is comparatively shorter th antheridiophore (Fig. 1).

2. An eight-lobed disc is also present at the apex but the lobes are much dissec here. In *Marchantia polymorpha* nine-rayed disc is present.

3. The disc is much convex than that of the disc of antheridiophore.

V.L.S. Young Female Receptacle *(Before Fertilization)*

1. Each lobe of the disc contains its own growing point.

2. On each lobe is present a group of archegonia arranged acropetally.

3. Neck of each archegonium faces upward (Fig. 14).

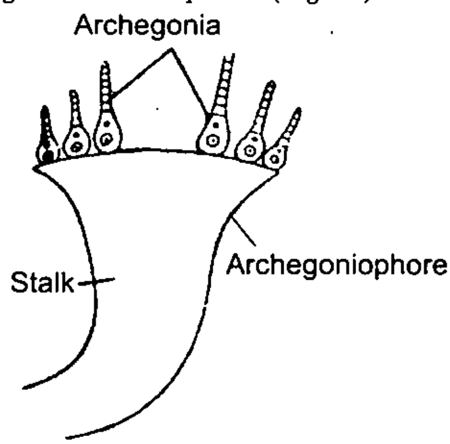


Fig. 14. Marchantia. L.S. archegoniophore before fertilization (diagrammatic).

4. Disc resembles in structure with the thallus.

5. A *single archegonium* is a flask-shaped (Fig. 14). body having a long, slender ne and a globular venter.

6. Venter remains surrounded by a layer of sterile cells, and encloses a venter car cell and an egg.

7. Neck is surrounded by six longitudinal rows of neck cells enclosing 6 to 8 ne canal cells. It opens by four cover cells.

V.L.S. Mature Female Receptacle (After Fertilization)

1. Each lobe of the disc contains a group of archegonia (Fig. 14) in the inverte position, and the youngest archegonium is near the stalk and the oldest away from th stalk.

2. From the periphery of the disc arise long, green, cylindrical elongations calle *rays*.

3. A bilipped, pendant, one-celled thick sheath with fringed margins arises fro both the sides of the group of archegonia. It is known as *perichaetium* (Fig. 15)

4. At the base of the venter of each archegonium develops a ring of cells in the form a collar-like structure called *perigynium* or *pseudoparianth*.

5. Structure of thallus is same as discussed earlier on p. 72.

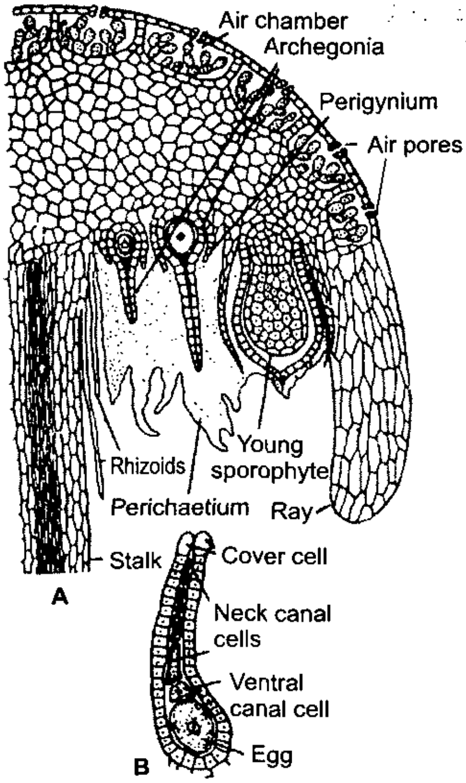


Fig. 15. Marchantia. A part of L.S. archegoniophore after fertilization (A) and a mature archegonium (B).

Mature Sporogonium

Cut L.S. through the disc of mature archegoniophore. Sporogonia are present on the under surface. Stain the section in safranin, mount in glycerine and study :

1. A mature sporogonium is clearly differentiated into foot, seta and capsule (Fig. 16).

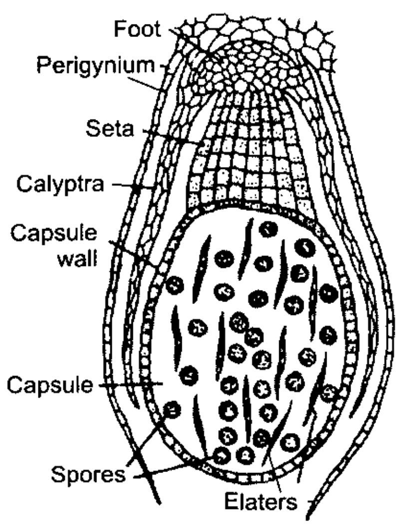


Fig. 16. Marchantia. A mature sporogonium.

2. *Foot* is bulbous, multicellular, and helps in attachment and absorption of food.

3. *Seta* consists of vertical rows of cells. Its cells are cubical and its function is elongation.

4. *Capsule* is surrounded by an outer layer of amphithecium and inner archesporial endothecium.

5. Endothecial region is in the form of sterile elaters and fertile spore mothe

6. Elaters (Fig 17) are long, pointed at both the ends and spirally thi structures. These are hygroscopic in nature and help in dispersal of spores.

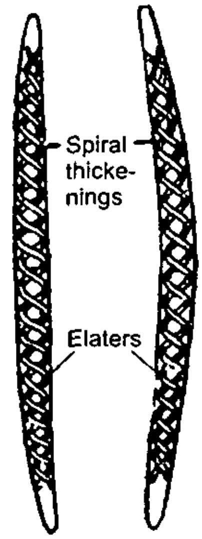


Fig. 17. Marchantia. Two elaters.

7. Spore mother cells divide reductionally, and change first into spore tetrac then into haploid spores.

8. Young sporogonia remain surrounded by three types of coverings, *i.e.*, cal perigynium and pseudoparianth or perichaetium. But at maturity, due to the acti seta, sporogonium elongates and comes out on rupturing all these three covering

9. In the earlier stages the cells of foot, seta, capsule and spores contain chloroplast.

10. The spores (Fig. 18) are surrounded by two layers, *i.e.*, an outer, thick which may be smooth or ornamented an inner thin *intine* which remains smooth spore wall surrounds the granular cytoplasm and a nucleus.

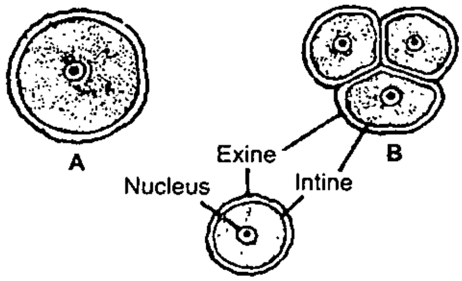


Fig. 18. Marchantia. A, A spore mother cell; B, A spore tetrad; C, A single spore.

Identification

(a) (i) Plant body gametophytic and independent.

(ii) Amphibious in nature.

(iii) True roots and vascular strands absent.

(vi) Presence of a layer of sterile jacket around antheridium.

*(v) Presence of heteromorphic alternation of generations......**Bryophyta***

(b) (i) Plant body thalloid.

(ii) Pyrenoids absent.

(iii) *Scales and two types of rhizoids present.*

(iv) *Columella absent.*

(v) *Absence of stomata and chlorophyllous tissue in sporogonium*

.... ***Hepaticopsida***

(c) (i) *Thallus is flat and dichotomously branched.*

(ii) *Presence of scales on the ventral surface.*

(iii) *Photosynthetic and storage regions are present.*

(iv) *Presence of air chambers.* ....***Marchantiales***

(d) (i) *Air pores barrel-shaped.*

(ii) *Presence of gemma cups on dorsal surface.*

(iii) *Sex organs on antheridiophore and archegoniophore.*

(iv) *Foot and seta present.* ......***Marchantiaceae***

(e) (i) *Presence of ligulate and appendiculate scales.*

(ii) *Presence of photosynthetic filaments.*

(iii) *Presence of elaters.* ....***Marchantia***

PTERIDOPHYTA

• DISTINGUISHING FEATURES OF PTERIDOP

The Pteridophyta are commonly called *vascular cryptogams*. They are repr by more than about 10,000 species, and are characterized by the following peculi

1. Members of this group are most primitive living vascular plants, s *Selaginella, Lycopodium, Equisetum* etc., and also fossil vascular plants, *Rhynia, Horneophyton, Asteroxylon,* etc.

2. Most of the plants prefer to grow in cool and shady places while so xerophytic, *e.g.*, *Selaginella rupestris*, and many occur in aquatic conditio *Marsilea, Salvinia, Azolla,* etc.

3. Plant body is sporophytic and differentiated into roots, stem and leaves. I plants an intermediate stage between root and stem, *i.e.*, *rhizophore*, is prese *Selaginella*.

4. Young sporophyte is partially or completely dependent on gametoph sometime, but at maturity it becomes independent.

5. The fibro-vascular cylinder, consisting of xylem, phlorem, etc., is presen stem of these plants.

6. In lower members, the stele is protostele, as in *Lycopodium, Selaginella,* e it is siphonostele in *Marsilea* (amphiphloic), *Equisetum* (ectophloic), and dictyo in *Pteris, Aspidium, Polypodium, Dryopteris,* etc.

7. Vascular supply to the leaves takes place by leaf traces through leaf gap vascular cylinder of stem.

8. Leaves may be simple, small and sessile, as in microphyllous types s *Selaginella, Lycopodium,* etc., or very large, petiolate and megaphyllous types, a members of Filicinae.

9. Plants reproduce by the spores formed in sporangia. Sporangia develop ei the ventral surface or in the axil of leaves.

10. Plants may be *homosporous, i.e.*, all the spores are of one type as in *Equise* *heterosporous, i.e.*, two types of spores are present (microspores and megaspores *Selaginella*.

11. On the basis of the development of sporangia, the plants can be classifi following two categories ;

(a) *Eusporangiate type*, in which the sporangium develops from a group of sup initials, *e.g., Selaginella, Equisetum.*

(b) *Leptosporangiate type*, in which the sporangium develops from a single *e.g., Marsilea.*

12. Spores develop into a multicellular, gametophytic body called prothallus

13. Two sex organs, *i.e.*, antheridia and archegonia develop on the prothallu

14. Fertilization takes place with the help of water, and results into the forma a diploid zygote. It develops into a structure bearing roots, stem and leave structure is called sporophyte.

15. Plants show clear *alternation of generations.*

PRACTICAL NO. 7

EQUISETUM

(L. *equs*-horse; *saeto*-bristle)

Common Name : "Horse tail."

Common Indian Species : *Equisetum debile, E. arvense, E. ramosissimum.*

Systematic Position

Division	:	Pteridophyta
Sub-division	:	Sphenopsida
Order	:	Equisetales
Family	:	Equisetaceae
Genus	:	*Equisetum*

Occurrence : Most of the species grow in damp shady places. Some are found in ıds and marshes while some prefer to grow in exposed and relatively dry habitats.

ɔrk to be done

To study (i) External features of plant, (ii) Anatomy of internode of aerial sterile shoot d fertile shoot, (iii) Anatomy of internode of rhizome, (iv) Anatomy of node of aerial rile shoot, (v) Anatomy of root, (iv) Strobilus or cone anatomy, (vii) Spores and ters, (viii) Gametophyte, and (ix) To identify the plant giving reasons.

ternal Features of Plant Body or Sporophyte

1. Plant body is sporophytic and the sporophyte is a well-branched perennial herb.

2. Size of the plant body ranges from a few centimeter as in *Equisetum scirpoides* to ʼeral metre as in *E. giganteum* (upto 13 metre). Most of the species are less than a ter in height.

3. Plant body consists of a long, horizontal, underground rhizome, from which arise ıny roots towards the lower side and many erect aerial shoots towards upper side gs. 1,2).

4. *Rhizome* is long, creeping and well-branched. It is divisible into nodes and ernodes.

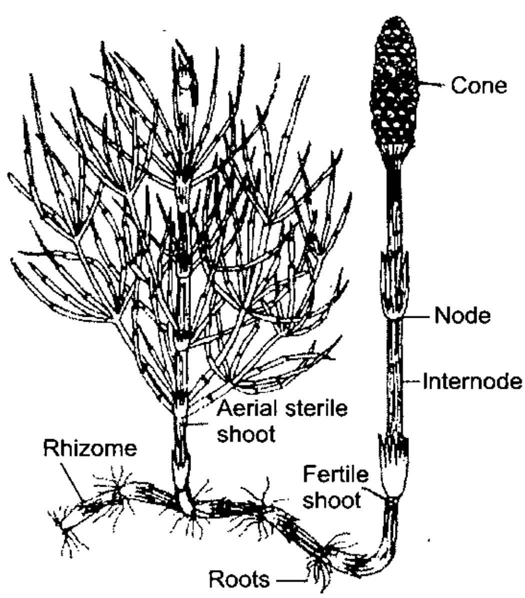


Fig. 1. Equisetum. External features of plant body

5. *Roots,* which develop from the node of rhizome, are long, slender, well-bra and adventitious.

6. *Aerial shoots*, which arise from the rhizome towards upper side, are of two *i.e.*, sterile or vegetative shoots and fertile or reproductive shoots.

7. Both the sterile and fertile aerial shoots are ribbed and divisible into node internodes, but the former is well-branched and long-lived while the latter (shoots) are generally unbranched and short-lived structures.

8. Aerial shoots as well as rhizome are *articulated i.e.*, jointed.

9. From the nodes of aerial sterile shoots arise two types of branches in whorls. are long, unlimited in growth, well-branched and contain the same structure a main axis of aerial sterile shoot. Others are short, also bear nodes and inter-node are limited in growth and unbranched (Figs. 1, 2).

10. *Fertile shoots* are unbranched, colourless or pale-yellow colour branches, e which bears a strobilus at the tip.

11. On the nodes of rhizome, sterile shoot and fertile shoots are present many leaves.

12. *Scaly leaves* are minute, thin, uninerved, present in the form of a whorl and in number from 3 to 40 in different species.

13. These leaves are green when young but become yellow or red-colour maturity.

14. The upper end of each leaf is free and pointed but all of them unite below a base to form a sheath on the node.

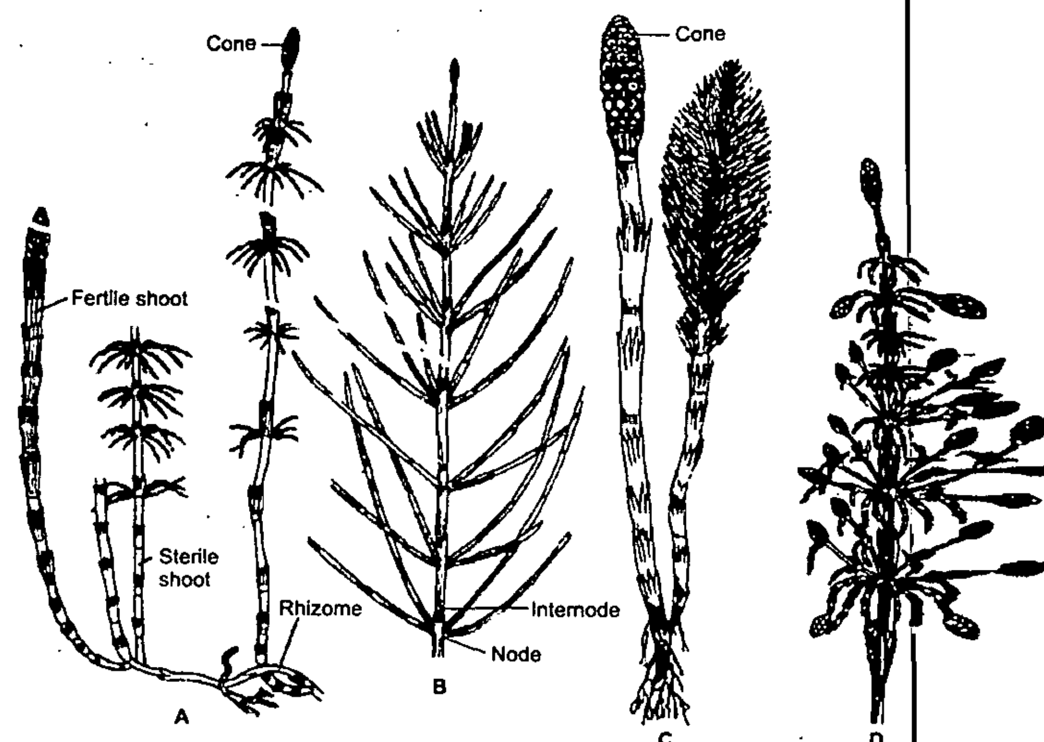


Fig. 2. Equisetum. External features of plant body of some species. (A) E. pratense; (B) E. palustre; (C) E. telmateia, (D) E. sylvaticum.

15. The number of leaves represents the number of ridges on the internode.

16. Many round or irregular bodies are present on the rhizome. These are ickwalled and meant for vegetative reproduction. These are called *tubers* (Fig. 1)

17. Functions of various parts of the sporophyte are as follows :

(a) *Roots :* Absorption and fixation;

(b) *Rhizome :* Storage;

(c) *Sterile shoot :* Photosynthetic;

(d) *Fertile shoot :* Reproductive.

Thus, *Equisetum* shows an example of physiological division of labour.

INATOMY

Cut thin transverse sections of different plant parts, stain in safranin-fast green mbination, mount in glycerine and observe the anatomical details :

. S. Internode of Aerial Sterile Shoot

In a cross-section following structures are visible (Figs. 3, 4):

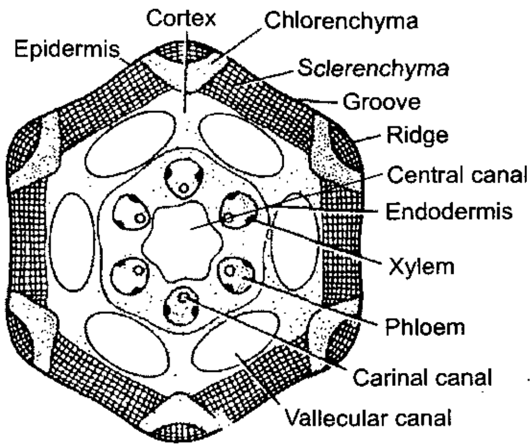


Fig. 3. Equisetum. T.S. internode of aerial sterile shoot (diagrammatic).

1. It is wavy in outline because of the presence of ridges and grooves.

2. Outermost layer is the epidermis, cells of which have a deposit of silica in their uter and lateral walls.

3. Due to the presence of silica, the stem appears hard and rough to touch.

4. The continuity of epidermis is broken by sunken stomata present in each groove. n each sunken stoma, the guard cells are covered completely by subsidiary cells, thus iving the appearance of two sets of guard cells.

5. Below the epidermis is present a well-developed cortex.

6. Just below each ridge is present a large patch of sclerenchyma, which is iechanical in function. Sclerenchyma is also present below the grooves in between hlorenchyma (Figs. 3, 4).

7. Inner to be sclerenchyma is present chloren-chymatous tissue below each ridge. It s photo-synthetic in function. It extends upto the epidermis in each groove, where the tomata lie.

8. Rest of the cortex is parenchymatous and many layered.

9. Just below each groove is present a large air canal in the parenchymatous cortex. t is known as *vallecular canal.*

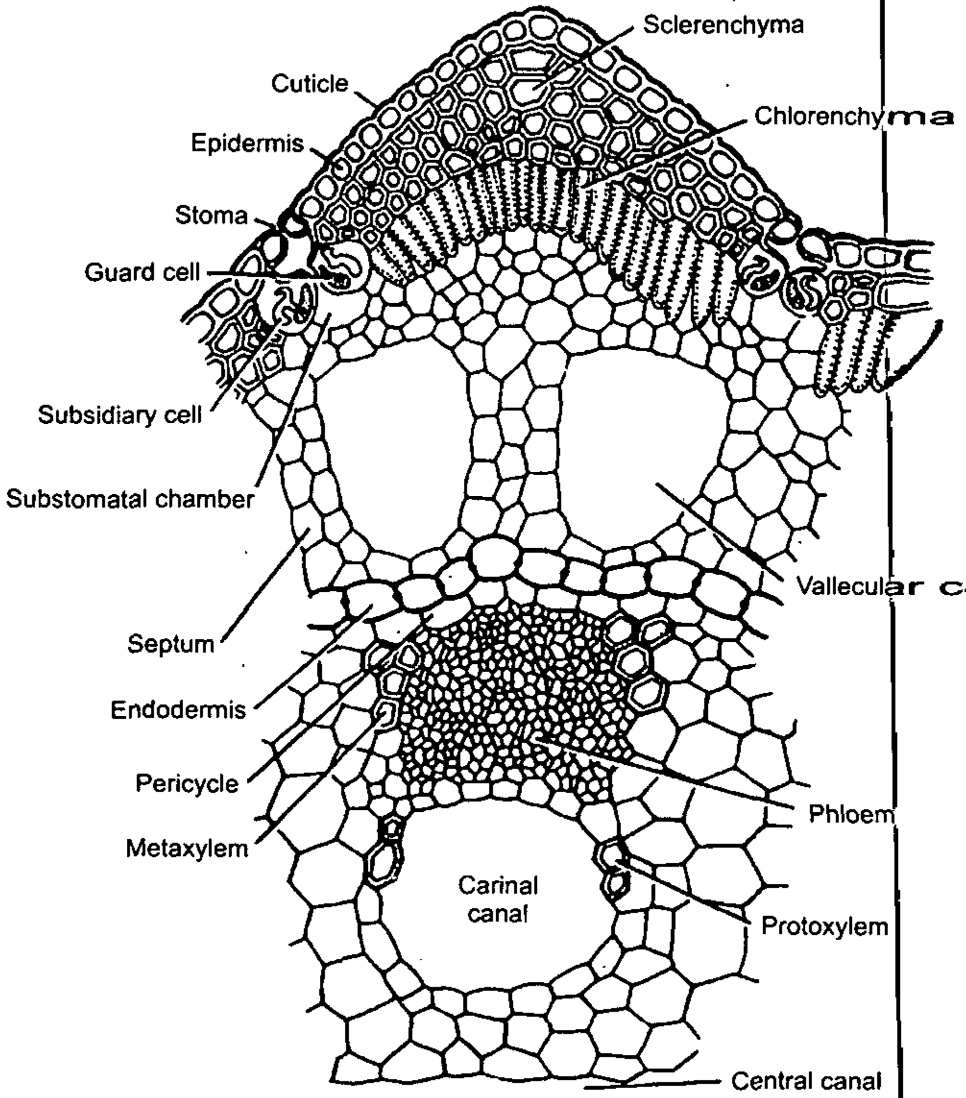


Fig. 4. Equisetum. T.S. internode of aerial shoot (a part).

10. Innermost layer of cortex is the endodermis, the cells of which contain caspa strips. But in species like *E. sylvaticum*, a layer of inner endodermis is also pres (Fig. 5). In *E. litorale*, each vascular bundle contains its individual endodermis (Fi on next page.

11. Below the endodermis is present a single-layered pericycle.

12. Vascular bundles are present below the ridges, *i.e.*, alternate to the vallec canals of the cortex. They are present in the ring.

13. The number of vascular bundles and vallecular canals is equal to the numbe ridges and grooves, respectively (Fig. 3).

14. Stele is of *ectophloic siphonostelic* type.

15. Each vascular bundle is conjoint, collateral, closed, and consists of xyl phloem and some parenchyma.

16. In each vascular bundle is present a water-containing cavity or canal cal *carinal canal* (Fig. 4).

17. Xylem is 'V' shaped.

18. Protoxylem is endarch lying opposite to carinal cavity. It consists of annular a spiral tracheids.

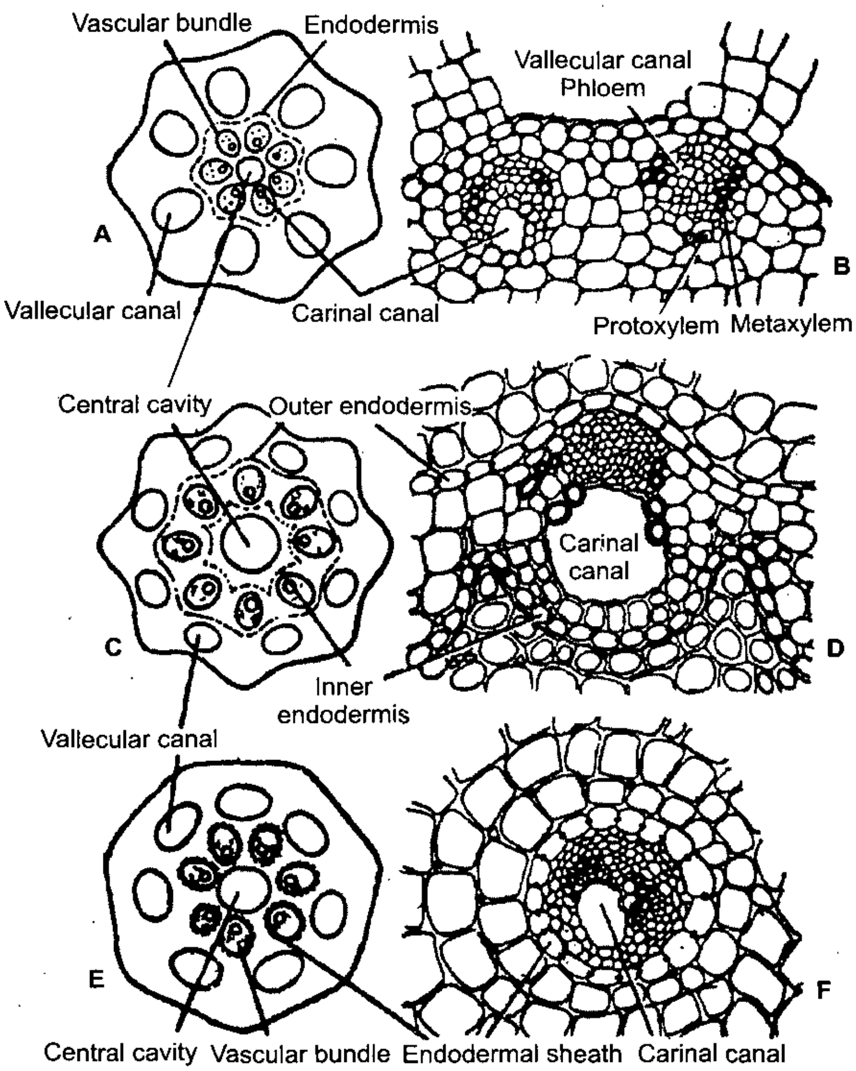


Fig. 5. Equisetum. Diagrammatic and a part cellular diagrams of different species. (A-B) T.S. stem of E. palustre showing a common endodermis of vascular bundles; (C-D) T.S. rhizome of E. sylvaticum showing outer and inner endodermal layers; (E-F) T.S. rhizome of E. litorale showing individual endodermal sheath of each vascular bundle.

19. Two strands of metaxylem are present.

20. Phloem is present in between two strands of metaxylem and is made up of phloem parenchyma and sieve tubes.

21. Pith is present in the form of pith cavity, located in the centre of the aerial shoot (Figs. 3, 4).

T.S. Internode of Aerial Fertile Shoot

The structure is exactly similar with that of aerial sterile shoot, except a few following minor differences :

1. Absence of stomata.

2. Less developed chlorenchyma and sclerenchyma regions.

T.S. Internode of Rhizome

It also resembles in structure with the aerial stertile shoot except a few following dissimilarities :

1. Ridges and grooves are not so much well-marked as in sterile shoot.

2. Absence of stomata (Fig. 6).

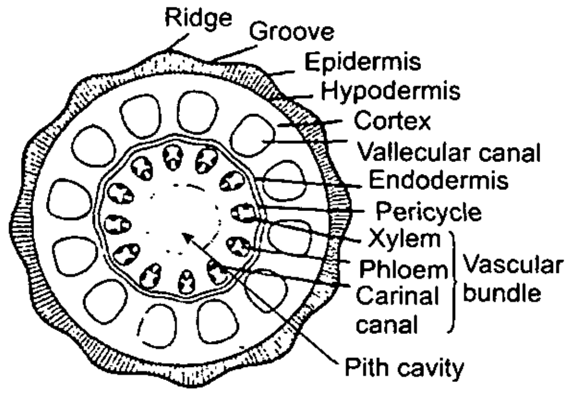


Fig. 6. Equisetum. T.S. internode of rhizome (diagrammatic).

3. Absence of chlorenchymatous region.
4. Sclerenchyma is poorly developed.
5. Hollow pith cavity is not well-developed and sometimes it becomes solid,

T. S. Node of Aerial Sterile Shoot

It also resembles the internode of aerial sterile shoot except following differences :

1. Absence of all three types of canal, *i.e.*, vallecular canal, carinal canal and central canal.
2. Instead of a central pith cavity, a nodal diaphragm is present (Fig. 7).
3. Vascular bundles are present in the form of a ring after getting fused with each other.
4. Leaf traces and branch traces arise below the ridges and grooves, respectively.

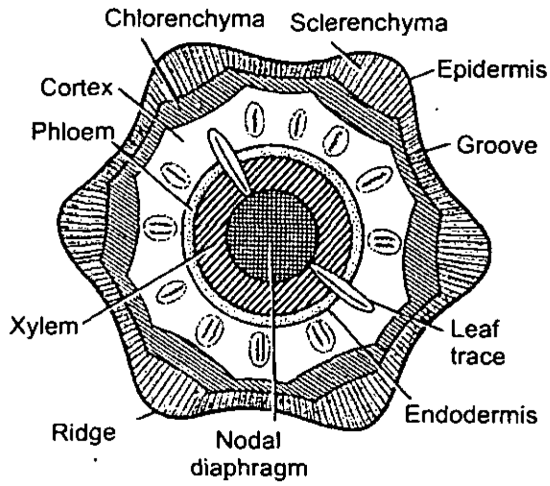


Fig. 7. Equisetum. T.S. node of aerial sterile shoot (diagrammatic).

T. S. Adventitious Root

1. Outermost layer is epidermis, from which arise many root hair.
2. Cortex is thick and multilayered.
3. Outer zone of cortex consists of 3 to 4-celled thick exodermis.
4. Inner zone is parenchymatous with many intercellular spaces.
5. Endodermis is two-layered (Fig. 8).

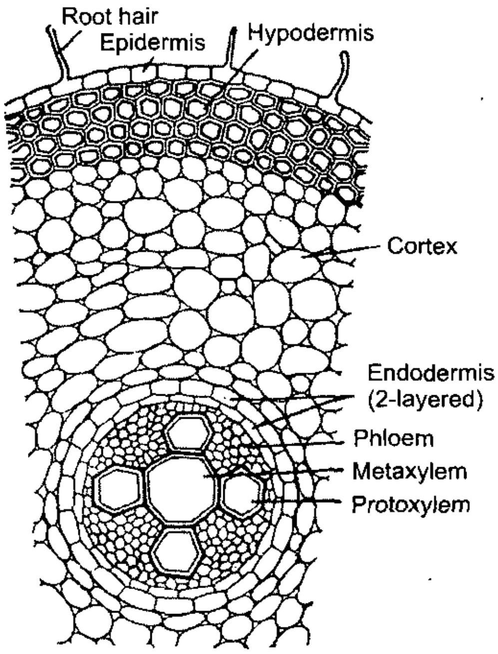


Fig. 8. Equisetum. T.S. root (a part cellular).

6. Pericycle is absent.

7. Stele is a protostele, which is triarch or tetrarch.

8. In the centre is present a large metaxylem tracheid having many protoxylem groups towards the periphery.

9. Phloem is present in between the angles of protoxylem.

SPORE-PRODUCING ORGANS

Study the external features of cone, cut its transverse and longitudinal sections, stain in safranin, mount in glycerine and study. Also prepare slides of spores and elaters :

1. Fertile aerial, unbranched shoots bear at their apices (Fig. 9) the spore-bearing compact organs known as *strobili* (sing. *strobilus*) or *cones*. In some rare cases branched fertile axis is also present (Fig. 10).

2. Each cone or strobilus has a thick central axix known as *strobilus* axis or *cone axis* (Figs. 11, 12).

3. On the strobilus axis are attached many umbrella like sporangiophores in whorl.

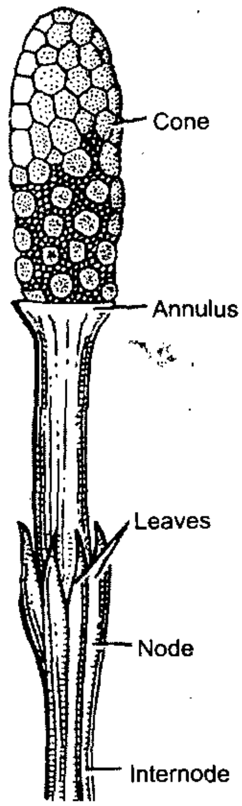


Fig. 9. Equisetum arvense. A fertile shoot bearing cone.

4. Each sporangiophore is a stalked structure, the free end of which becomes flattened to form a *peltate disc* (Fig. 11).

5. The disc is a hexagonal structure and present at right angle to the stalk.

6. On the undersurface of disc are present many sporangia, horizontally towards the axis of strobilus.

7. Each sporangium is elongated and pendant, and contains a rounded apex (Fig. 13)

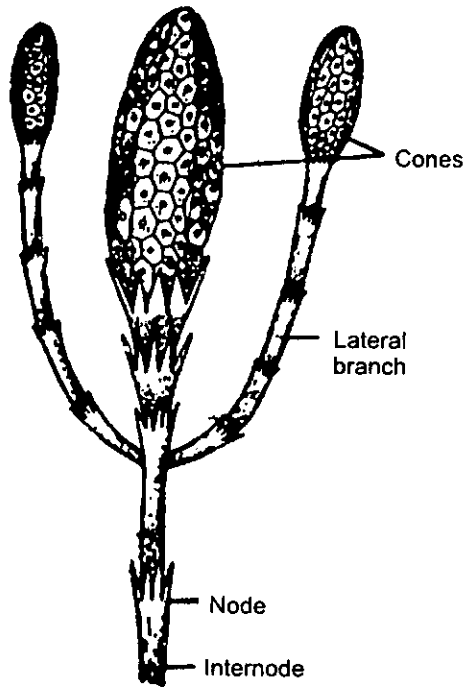


Fig. 10. Equisetum debile. Branched fertile axis bearing cones.

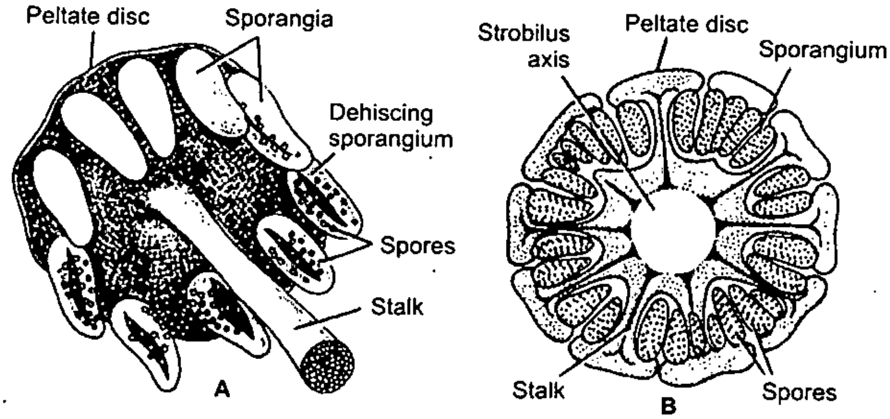


Fig. 11. Equisetum. A, Ventral view of a single sporangiophore; B, T.S. strobilus.

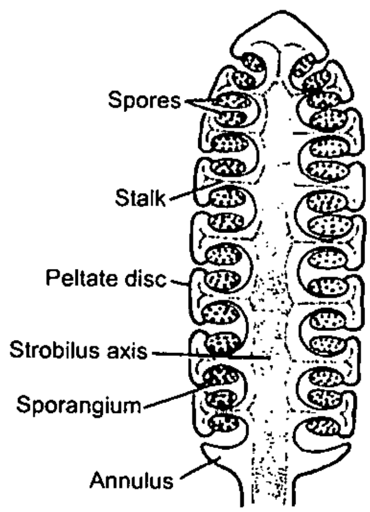


Fig. 12. Equisetum. L.S. cone.

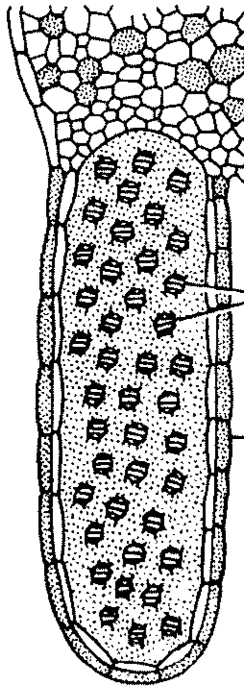


Fig. 13. Equisetum telmateia. A sporangium.

8. The sporangium is surrounded by jacket and tapetal layers which enclose many spores. *Equisetum is homosporous* (Fig.13).

9. A ring-like outgrowth is present below each strobilus. This is called *annulus.* Morphological nature of annulus is controversial. According to some it is the uppermost whorl of sterile leaves while others are of the opinion that it is the lowermost sterile sporangiophore (Fig. 12).

Spores and Elaters

1. Each spore is uninucleate, globular or spherical in shape and contains many chloroplasts.

2. The wall of each spore consists of four layers. Innermost cellulose layer is called endospore, which is enclosed by exospore, Outside the exospore is a middle layer (cuticular), which is surrounded by the outermost epispore (Fig.14).

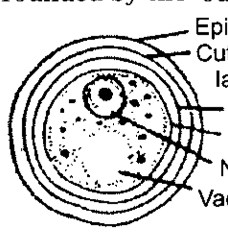


Fig. 14. Equisetum telmateia. A spore.

3. The middle layer and the outermost layer of epispore are the derivatives of periplasmodial liquid formed by the degeneration of some spore mother cells and tapetal cells.

4. The epispore splits and thus forms four distinct appendages, which remain attached on a common point on the spore wall. These four appendages are known as elaters (Fig. 15).

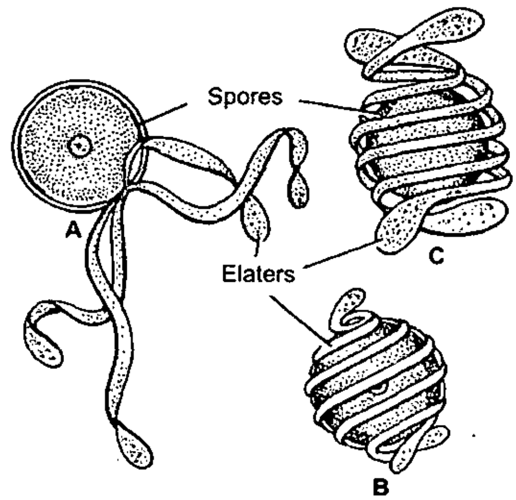


Fig. 15. Equisetum. Mature spores. A, A spore with uncoiled elaters of E. telmateia; B, With coiled elaters of E. telmateia, C, A mature spore of E. arvense with coiled elaters.

5. The tip of these spiral band-like elaters is flattened and spoon like.

6. The elaters are hygroscopic in nature.

7. In moist conditions, the elaters remain coiled around the spores, while in dry conditions, they separate and become free (Fig. 15).

8. Elaters help in the dehiscence of sporangium and dispersal of spores.

Identification

(a) (i) Roots, stem and leaves constitute the plant body.

(ii) True vascular strand present.

(iii) Plant body sporophytic.

(iv) Gametophyte is small and independent. ....*Pteridophyta*

(b) (i) Plant body articulated.

(ii) Stem with distinct nodes and internodes.

(iii) Ridges and grooves present.

(iv) Leaves small and scaly. ....*Sphenopsida*

(c) (i) Plants herbaceous and not tree-like.

(ii) Secondary thickenings absent.

(iii) Vascular cylinder siphonostelic.

(iv) Compact cones present. ....*Equisetales*

with a single family. ....*Equisetaceae*

and the single genus. ....*Equisetum*

GYMNOSPERMS

• WHAT ARE GYMNOSPERMS?

"Phanerogams without an ovary" have been referred as *Gymnosperms* (*gymnos*, naked; *sperma*, seed) or *naked seeded* plants by Goebel. The lower Gymnosperms, such as members of Cycadales, resemble with the *higher Cryptogams* (*Pteridophytes*), while the higher Gymnosperms, such as Gnetales and Coniferales, resemble with members of Angiosperms. This sub-division of *Phanerogams (flowering plants), i.e.*, Gymnosperms, is represented by over 60 genera and 700 speices, of which about 16 genera and 53 species have been reported from India.

DISTINGUISHING FEATURES

1. Plants are woody perennials with shruby or tree like habit and show xerophytic characters.
2. Some of the members attain a height of more than 100 meters, *e.g.*, *Sequoiadendron giganteum.*
3. Plant body is differentiated into roots, stem and leaves.
4. Tap root system is exarch and diarch to polyarch.
5. Leaves are of two types foliage leaves and scaly leaves. They are pinnately compound as in *Cycas*, or needle-like as in *Pinus*.
6. The vascular bundles in stem are conjoint, collateral, open and endarch.
7. Due to the presences and activity of cambium, secondary growth is present.
8. The wood becomes closely packed or pycnoxylic in conifers due to the much reduced nature of pith and cortex. It is *manoxylic* or loose in *Cycas* due to the presence of well-developed pith and cortex.
9. The xylem is composed of tracheids with bordered pits.
10. In phloem, companion cells are absent.
11. The reproductive parts are generally arranged in the form of compact and hard *cones or strobili.* The cones are generally unisexual.
12. In male cones, many microsporophylls are arranged on the central axis, each having many microsporangia containing microspores or pollen grains.
13. The ovules are covered by a single integument, and are orthotropous. The integument consists of an outer fleshy, a middle stony and inner fleshy layer. It surrounds the nucellus.
14. Each ovule opens with the help of a mouth opening or micropyle.
15. Formation of embryo is meroblastic, *i.e.*, develops from a small part of zygote.
16. *Polyembryony* is present in many members, *e.g., Pinus.*
17. True fruits are lacking.
18. Plants show alternation of generations.
19. Members of most of the orders (Cycadales, Coniferales and Ginkgoales) are living while that of the other orders like Cordaitales and Cycadeoideales are represented by fossil genera.

Indian Genera : Sixteen genera of gymnosperms reported from India are *Cycas, Pinus, Gnetum, Ephedra, Abies, Cedrus, Picea, Larix, Tsuga, Cupressus, Cephalotaxus, Juniperus, Podocarpus, Libocedrus, Taiwania and Taxus.*

PRACTICAL NO. 8

Some Indian Species : *Cycas revoluta, C. circinalis, C. rumphii, C. pectinata, C. siamensis, C. beddomei, etc.*

Systematic Position

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

What is to do done?

To study (i) External morphology of plant, (ii) Anatomy of normal young root, normal old root, coralloid root, stem (young), stem (old), rachis, leaflets of Cycas revoluta and circinalis, (iii) External morphology of male cone, (iv) Microsporophyll, microsporang and microspores, (v) T.S. microsporophyll, (vi) Megasporophylls of different species, (v L.S. mature ovule, (viii) L.S. seed, and (ix) To identify the plant giving reasons.

External Features

1. Sporophytic plant body attains a height of 8 to 15 feet or more and appears like small palm.

2. The plant body is differentiated into roots, stem and leaves (Fig. 1).

3. Roots are of two types : normal roots and coralloid roots.

4. *Normal roots* grow deep into the soil and are well-branched and positive geotropic.

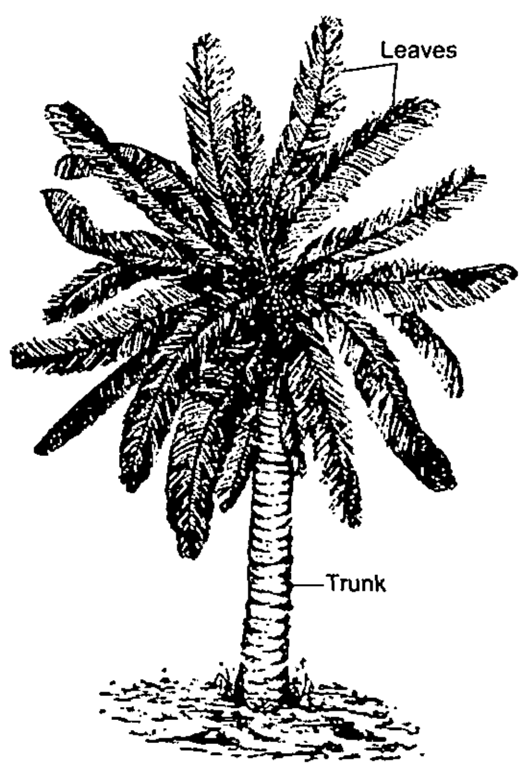


Fig. 1. Cycas circinalis. View of an entire plant.

5. *Coralloid roots* (Fig. 2.) are coral-like, dichotomously branched, fleshy, negatively -eotropic and arise from the lateral branches of normal roots.

6. Coralloid roots are green in colour because of the presence of an algal zone. In this ɔne are present members of Myxophyceae, such as *Nostoc* and *Anabaena*, and some acteria.

7. Due to the presence of endophytic algae, these roots become swollen, appear like a ɔral and hence named coralloid.

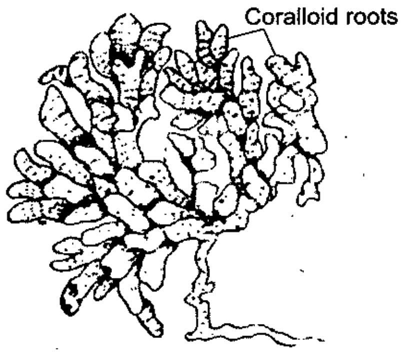


Fig. 2. Cycas. A bunch of coralloid roots.

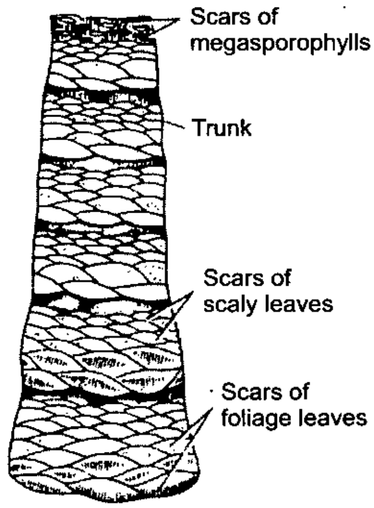


Fig. 3. Cyas. A part of stem showing leaf scars.

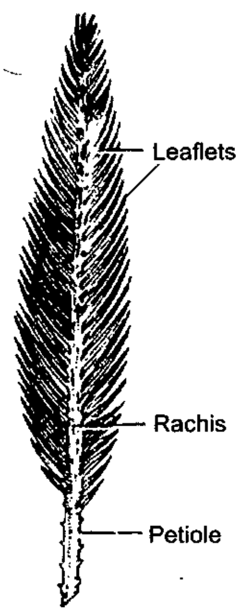


Fig. 4. Cycas. A single foliage leaf.

8. The *stem* is erect, stout and unbranched and remains covered with hard arm (Fig.3). of woody leaves and sporophyll bases.

9. At the apex of stem is present a crown of leaves arranged spirally.

10. Leaves are of two types ; foliage leaves and scaly leaves.

11. *Foliage leaves* (Fig. 4) are green, large, pinnately compound and may reach 3 to 5 feet or more in length.

12. A foliage leaf consists of a hard *rachis* having many pinnae or *leaflets* arrange two rows on both sides (Fig. 4).

13. Each leaflet is sub-sessile and lanceolate in shape having an acute ape: midrib is present in each leaflet.

14. The margin of leaflets is revoluted or curved downward in *C. revoluta*, while flat in *C. circinalis* and *C. rumphii*.

15. Leaves are circinately coiled when young (Fig. 5).

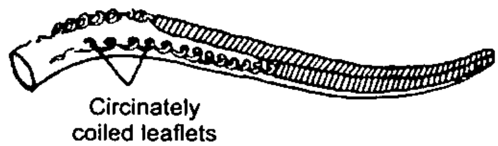


Fig. 5. Cycas. A young leaf showing circinate vernation.

16. *Scaly leaves* are dry, brown-coloured, covered with many hair and present at apex of stem.

17. Sometimes, many "*bulbils*" arise in between the leaf bases of the stem. They covered with scaly leaves at the base and germinate into new plants in favoura conditions (Fig. 6).

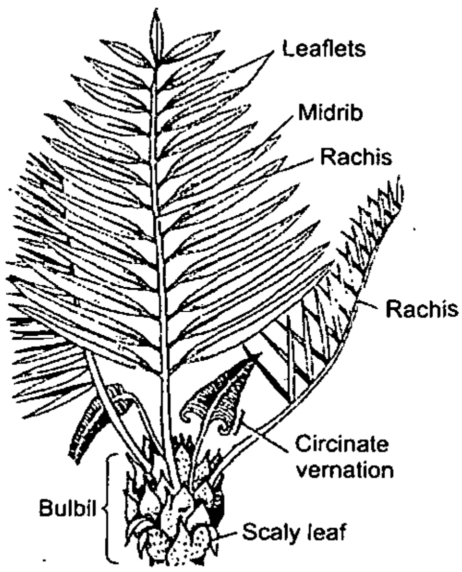


Fig. 6. Cycas. A single bulbil.

Anatomy of different parts

Cut thin transverse sections of different parts (young normal root, old normal ro coralloid root, young stem, old stem, rachis and leaflets of Cycas revoluta and circinalis), stain them separately in safranin-fast green combination, mount in glyceri and observe the anatomical details.

T.S. Normal Root (Young)

1. Outermost layer of the root, which is circular in outline, is called epiblema. It consists of many tangentially elongated cells. From some cells arise the root hair (Fig. 7).

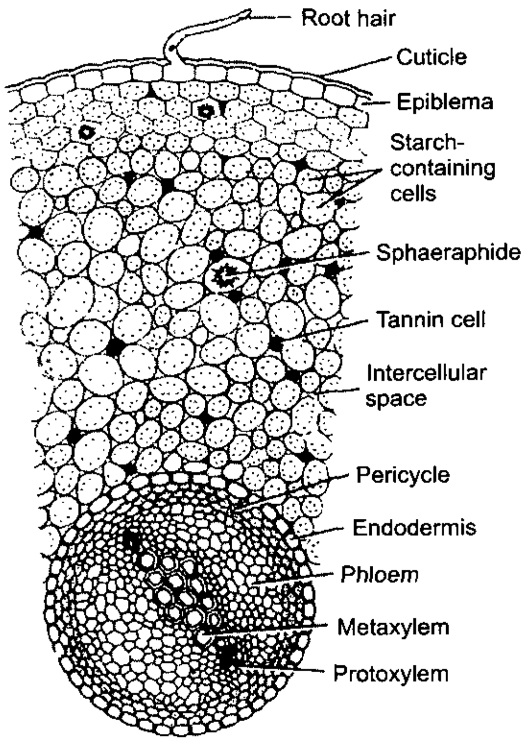


Fig. 7. Cycas revoluta. T.S. normal root (*Young*).

2. Inner to the epiblema is the parenchymatous cortex with many intercellular spaces. The cells are filled with starch.

3. Tannin cells and sometimes mucilage canals are also present in cortex.

4. Inner to the cortex is a single-layered endodermis having casparian strips and multilayered pericycle.

5. Vascular bundles are radial, *i.e.*, xylem and phloem are present on different radii. Xylem is exarch and generally diarch, but sometimes protoxylem strands range from 3 to 8 in number.

6. Exarch protoxylem contains spiral thickenings, while metaxylem has scalariform thickenings.

7. The phloem consists of sieve tubes and phloem parenchyma and present alternate to the xylem groups.

8. Pith is generally absent.

T.S. Normal Root (*Old*)

1. Epiblema is ruptured and lacks root hair.

2. Below the epiblema is present multilayered cork followed by cork cambium and many-layered parenchymatous cortex (Fig. 8).

3. Endodermis and pericycle are same as in young normal root.

4. Cambium cuts secondary phloem towards outer side and secondary xylem towards inner side. Vascular rays are also clear.

5. Outside the secondary phloem may also present a layer of crushed primary phloem.

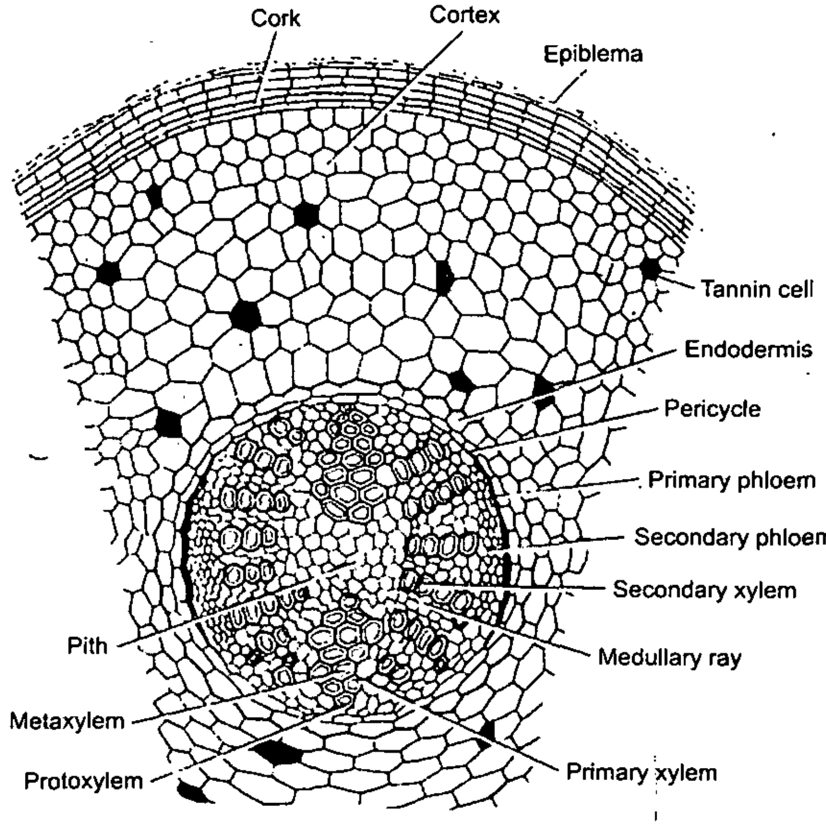


Fig. 8. Cycas. T.S. old normal root (a part cellular).

T.S. Coralloid Root

It is similar in many aspects to the normal root except a few variations as giv below :

1. It is circular in outline and the outermost layer is epiblema. But at maturity c as well as cork cambium develop. Root hair are normally absent.

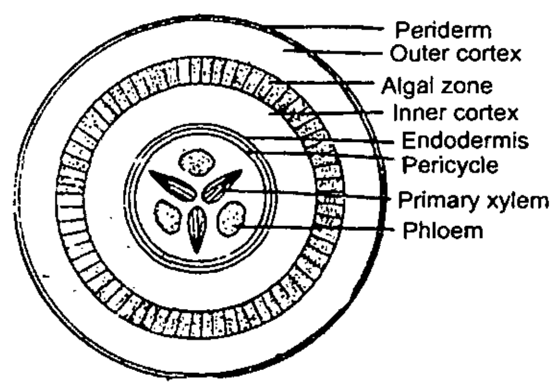


Fig. 9. Cycas. T.S. coralloid root (diagrammatic).

2. Cortex is parenchymatous and divisible into outer cortex and inner cortex havi a middle algal zone (Fig. 9, 10).

Following algae and bacteria have been reported from algal zone ;

(i) Nostoc punctiforme,

(ii) Anabaena cycadeae,

(iii) Oscillctoria,

(iv) Members of Bacillariophyceae (diatoms),

(v) *Bacteria*, such as *Pseudomonas* and *Azotobacter*.

3. Details of endodermis, pericycle and vascular bundles are same as in normal root. Xylem is exarch and triarch.

4. Normally, secondary growth is absent (fig. 9, 10).

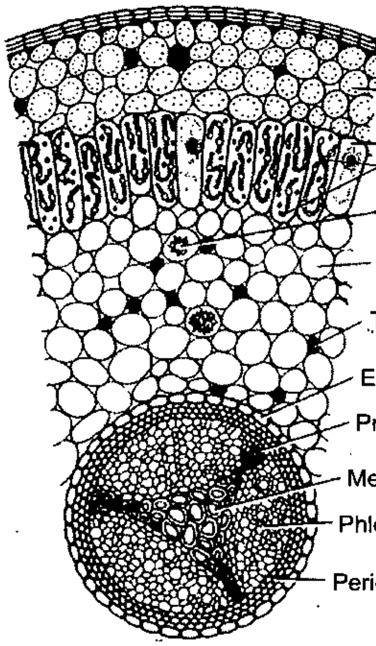


Fig. 10. Cycas revoluta. A part of T.S. coralloid root.

T.S. Stem (*Young*)

1. It is wavy or irregular in outline.

2. Outermost layer is the epidermis, which consists of compactly arranged thickwalled cells. Due to the presence of persistent leaf bases and woody scales, the epidermis is irregular and sometimes not very clear (Fig. 11).

3. Cortex is very large, parenchymatous and contains many girdle traces and mucilaginous ducts.

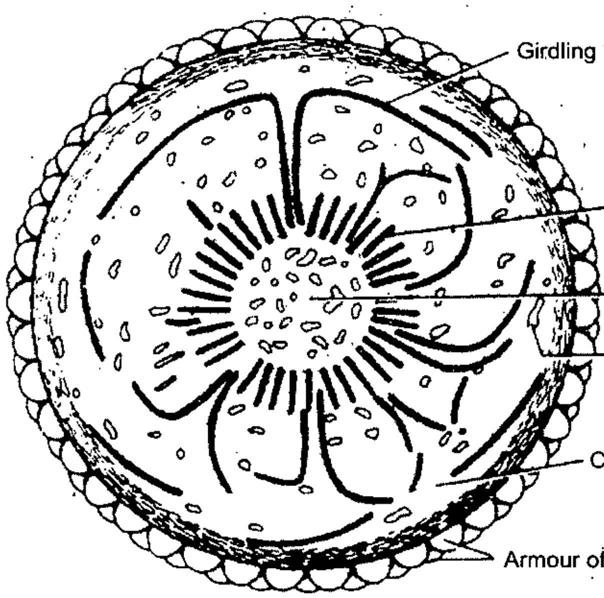


Fig. 11. Cycas. T.S. stem (young).

4. Each mucilaginous duct remains bounded by many epithelial cells or secr cells.

5. Endodermis and pericycle are not very clear.

6. The vascular cylinder is *ectophloic siphonostele* and many vascular bundle arranged in a ring. Each vascular bundle is conjoint, collateral, open and endarch

7. Xylem consists of tracheids and xylem parenchyma. There is no vessel.

8. Outside the xylem is the phloem which consists of sieve tubes and ph parenchyma.

9. Companion cells are absent.

10. In the centre of the stem is present pith.

11. Many mucilage ducts are present in the pith.

T.S. Stem (*Old*)

1. Secondary growth is present.

2. A thick outer periderm followed by large parenchymatous cortex, having r leaf traces and mucilaginous ducts, are present in the old stem.

3. Vascular strands are present in the form of rings (Fig. 12) Medullary rays are common.

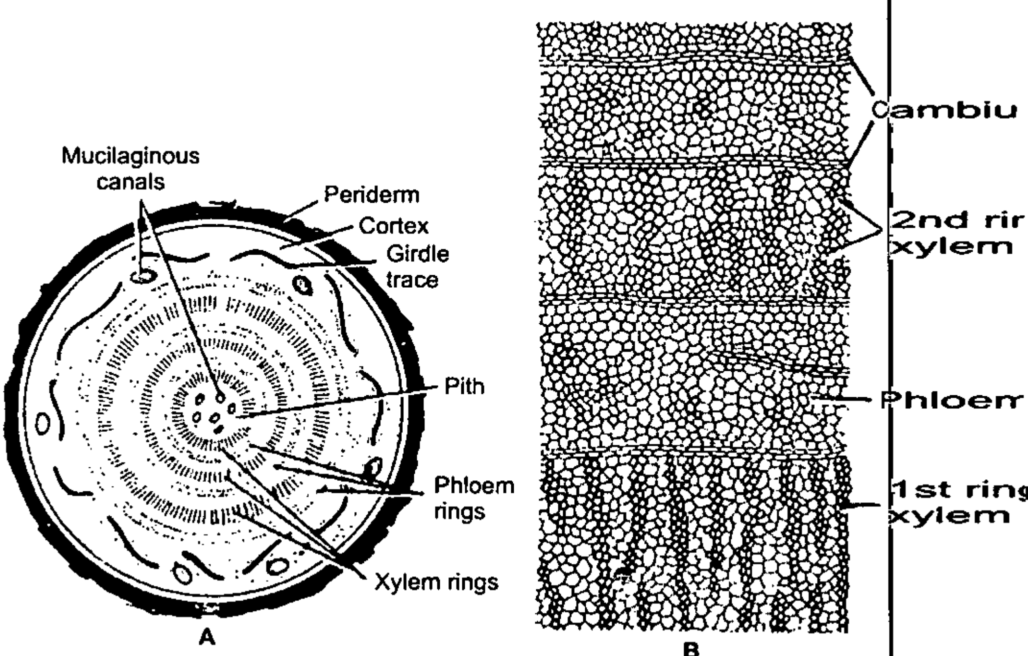


Fig. 12. Cycas. (A) T.S. old stem (diagrammatic); (B) T.S. old stem (a part cellular).

4. The number of vascular rings is variable from 2 to 14 in different species, showing polyxylic condition.

Other details are similar to those of young stem, discussed earlier.

T.S. Rachis

1. It is rhomboidal, biconvex or roughly cylindrical in outline, if the section pa through the base, middle or apex of the rachis, respectively.

2. Two arms are present on rachis, one on each side. These are the bases of leaflets, which arise from the rachis (Fig. 13).

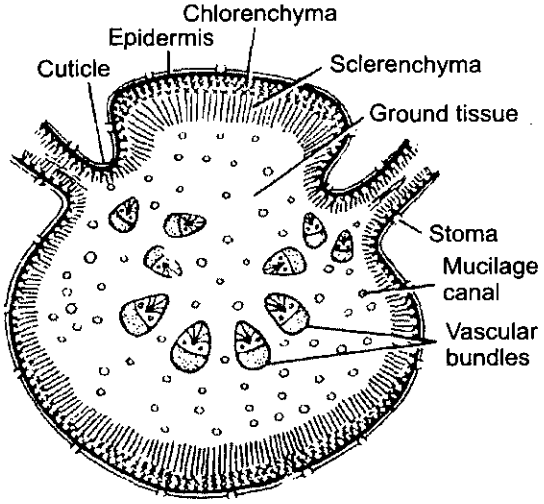


Fig. 13 Cycas. T.S. rachis (diagrammatic).

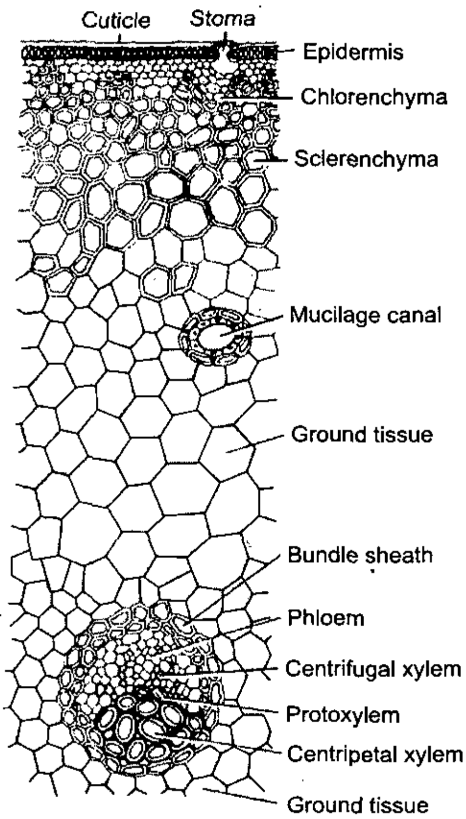


Fig. 14. Cycas revoluta. A part of T.S. rachis.

3. The outermost layer consists of thick walled epidermis which is heavily cuticularized.

4. The continuity of epidermis is broken by many sunken stomata present on upper as well as lower sides of rachis.

5. Below the epidermis are present chlorophyll-containing cells of chlorenchyma, followed by thick-walled sclerenchymatous region (Fig. 14).

6. Sclerenchyma is four to six-layered.

7. Below the sclerenchyma is present a large region of ground tissue consisting of thin-walled parenchymatous cells. In this region are present many mucilaginous canals and vascular bundles.

8. Each mucilaginous canal is a double-layered structure consisting of an inner of epithelial cells surrounded by an outer layer.

9. *Vascular bundles* are arranged in omega (Ω)-shaped manner (Fig. 13).

10. Each vascular bundle is conjoint, collateral and open, and remains surrou by a bundle sheath.

11. In vascular bundles, the xylem is present towards inner side consisti tracheids and xylem parenchyma with no vessels. It is separated from the phloe the cambium. The xylem is *diploxylic, i.e.,* consisting of centripetal and centri xylem (Fig. 14).

12. Phloem is present on outer side and consists of sieve tubes and ph parenchyma with no companion cells.

Vascular bundles show different structures at different levels of rachis starting the base to the apex with regard to their diploxylic nature as under :

(a) *Vascular bundles at the Base of Rachis*

1. Only centrifugal xylem is well developed (Fig. 15A).

2. Its protoxylem faces towards the centre showing endarch condition.

3. Centripetal xylem is not developed.

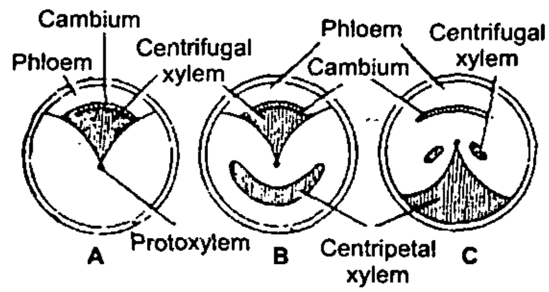


Fig. 15. Cycas. Diagrammatic representation of vascular bundles of rachis at different levels. (A) At the base; (B) In the middle; (C) At the apex.

(b) *Vascular Bundles in the Middle of Rachis*

1. Centripetal xylem as well as centrifugal xylem are present showing diplox condition (Fig. 15B).

2. Centripetal xylem is present just opposite to the protoxylem of the centrifu xylem.

(c) *Vascular Bundles at the Apex of Rachis*

1. Centripetal xylem is well-developed, triangular and exarch (Fig. 15C).

2. Centrifugal xylem is much reduced and present in the form of two patches ly one on each side of the protoxylem elements of centripetal xylem.

3. At the extreme tip, the centrifugal xylem is totally absent.

T.S. Leaflet of Cycas revoluta

1. It can be differentiated into a swollen midrib portion and two lateral wings.

2. The wings are curved downward or revoluted at the margins (Fig. 16).

3. Outermost layer consists of thick-walled epidermis surrounded by a layer cuticle.

4. Upper epidermis is a continuous layer while the continuity of lower epidermi broken by many *sunken stomata.*

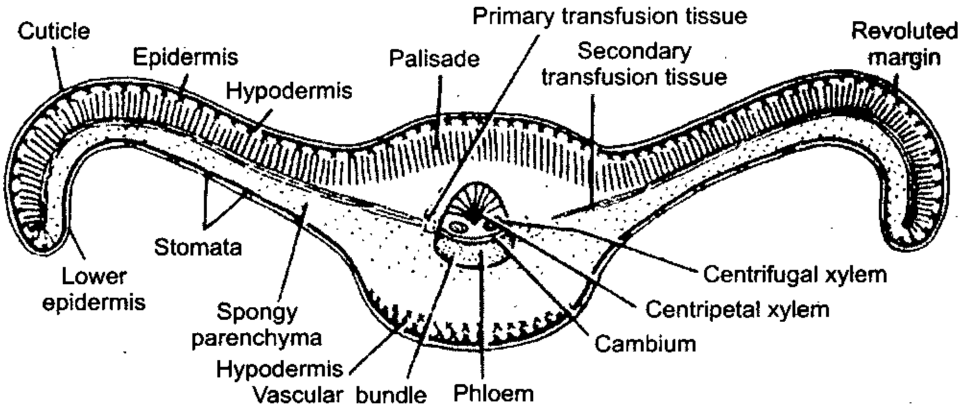


Fig. 16. Cycas revoluta. T.S. leaflet (diagrammatic).

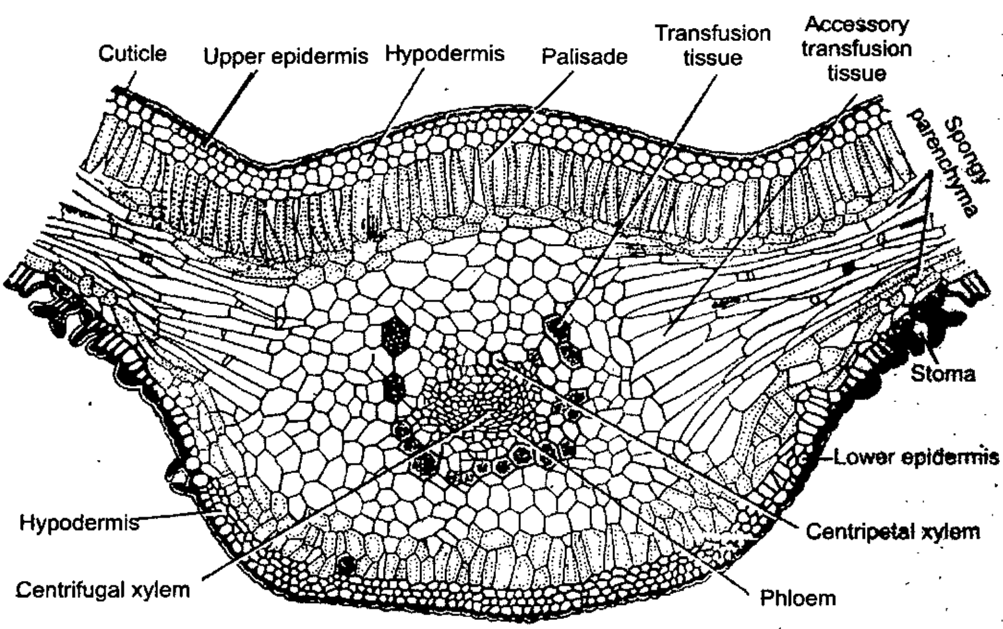


Fig. 17. Cycas revoluta. T.S. leaflet (a part cellular).

5. Below the upper epidermis is present the sclerenchymatous hypodermis which is more cells thick in the midrib region.

6. Hypodermis is absent below the lower epidermis, except in the midrib region.

7. Mesophyll is differentiated into *palisade* and *spongy parenchyma* (Fig. 17).

8. *Palisade* is present in the form of a continuous layer below the scleren chymatous hypodermis. Cells of the palisade are radially elongated and filled with chloroplast.

9. *Spongy parenchyma* is present only in the wings directly above the lower epidermis. The cells are oval, filled with chloroplast and are loosely arranged having many intercellular spaces, filled with air.

10. Few layers of transversely elongated cells are present in both the wings (blades) just in between the palisade and spongy parenchyma. This is *secondary transfusion tissue.*

11. *Primary transfusion tissue* present just on either side of the centrally located vascular bundle.

12. A single vascular bundle is present in the midrib portion of the leaflet.

13. The *vascular bundle* is conjoint, collateral and open. It is *diploxylic.* The triangular centripetal xylem is well-developed with endarch protoxylem. Centrifugal

xylem is represented by two small groups on either side of protoxylem. Phloem arc-shaped and remains separated from the cambium. Each vascular bundle surrounded by a bundle sheath.

14. The portion of the midrib in between the palisade layer and lower hypoderm region is filled with parenchymatous cells, of which some cells contain calcium oxal crystals.

T.S. Leaflet of Cycas circinalis

It resembles very much with the *Cycas revoluta,* discussed above, except followi distinctions :

1. The margins of wings are straight and not revoluted.
2. The upper side of the midrib is much ejected out.
3. Stomata, which are present on lower epidermis, are not much sunken.
4. Hypodermis in wings is present only at the corners.
5. Palisade is present only in the wings and not in the midrib region.

Structures of vascular bundle, spongy parenchyma and other details are similar *Cycas revoluta.*

REPRODUCTIVE STRUCTURES OF CYCAS

1. Plants are strictly dioecious.

2. Male structures are in the form of a compact conical body called *male strobilus male cone* (Fig. 18).

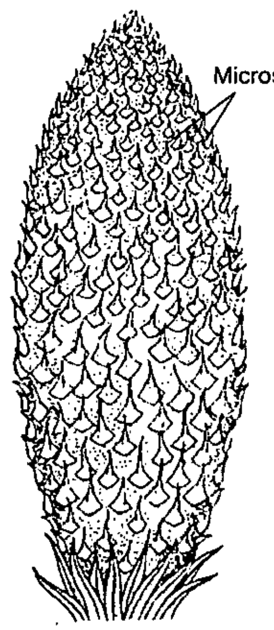


Fig. 18. Cycas. A male cone.

3. Female structures are not present in the form of compact cones but they ar loosely arranged and called megasporophylls.

Male Cone

1. It is very large (Fig. 18), conical or ovoid structure, reaching sometimes upto O. metre in length.

2. In the centre of each male cone is present a cone axis, which is clearly seen in L.S (Fig. 19).

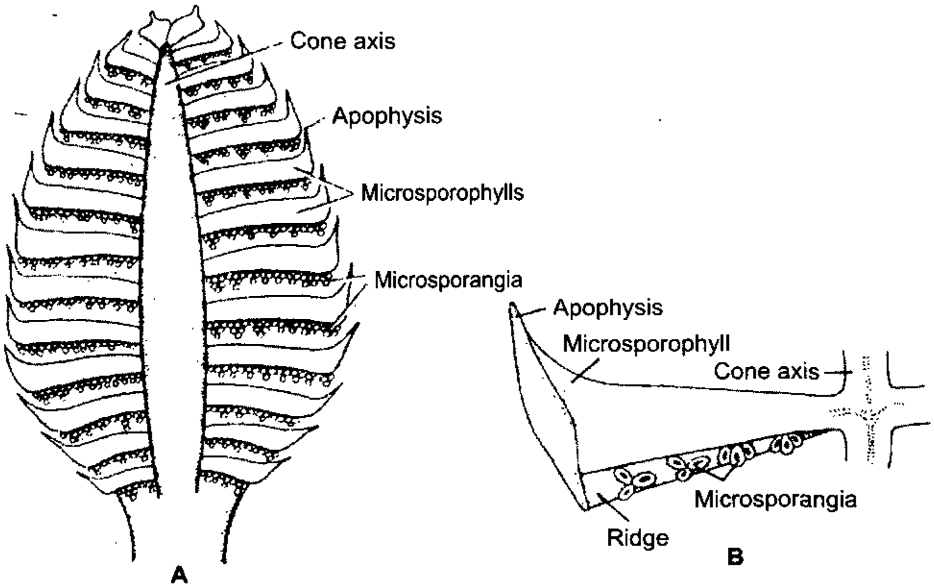


Fig. 19. Cycas. (A) L.S. male cone; (B) L.S. through a single microsporophyll with cone axis.

3. On the cone axis are attached many leafy structures at right angle. These are called microsporophylls.

4. At the base of the male cone are present many young leaves.

5. All the microsporophylls in the male cone are fertile, except a few at the base and a few at the apex.

Microsporophylls, Microsporangia and Microspores

Separate a microsporophyll from the male cone and observe the shape and arrangement of microsporangia on its lower surface.

1. Microsporophylls are flat, leaf-like, woody and brown-coloured structures with narrow base and expanded upper portion.

2. Upper expanded portion becomes pointed and is called apophysis.

3. Narrow base is attached to the cone axis with a short stalk.

Each microsporophyll has two surfaces : an adaxial or upper surface and an abaxial or lower surface.

4. *On the adaxial surface* is present a ridge-like projection in the middle and an apophysis at the apex (Fig. 20).

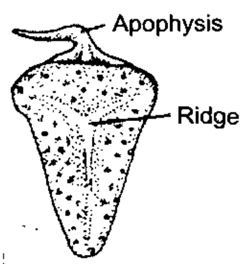


Fig. 20. Cycas. A single microsporophyll (adaxial surface)

5. *On the abaxial surface* are present thousands of microsporangia in the middle region in groups of 3 to 5. Each such group is called a *sorus* (Fig. 21A).

6. In between these groups are present many hair-like structures (Fig. 21B).

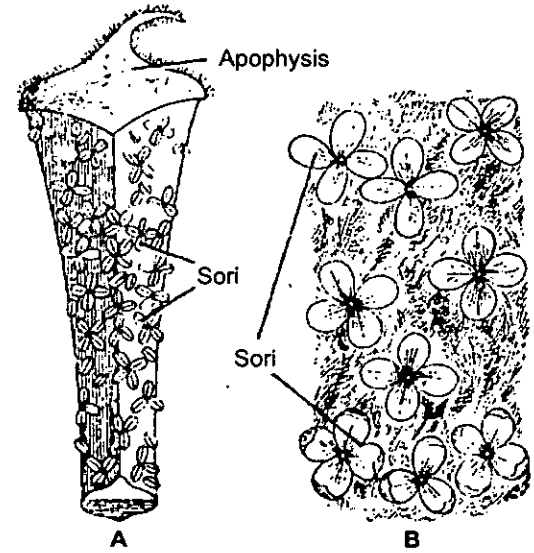


Fig. 21. Cycas. (A) Abaxial surface of a microsporophyll; (B) Groups of sori enlarged.

7. Each microsporangium is an oval or sac-like structure with a short stalk encloses many *microspores* or *pollen grains*.

8. Each pollen grain is a rounded, uninucleate structure, surrounded by an ou thick exine and inner thin (or thick on lateral sides) intine.

T.S. Microsporophyll

Cut transverse section of microsporophyll, stain in safranin-fast green combinati mount in glycerine and study :

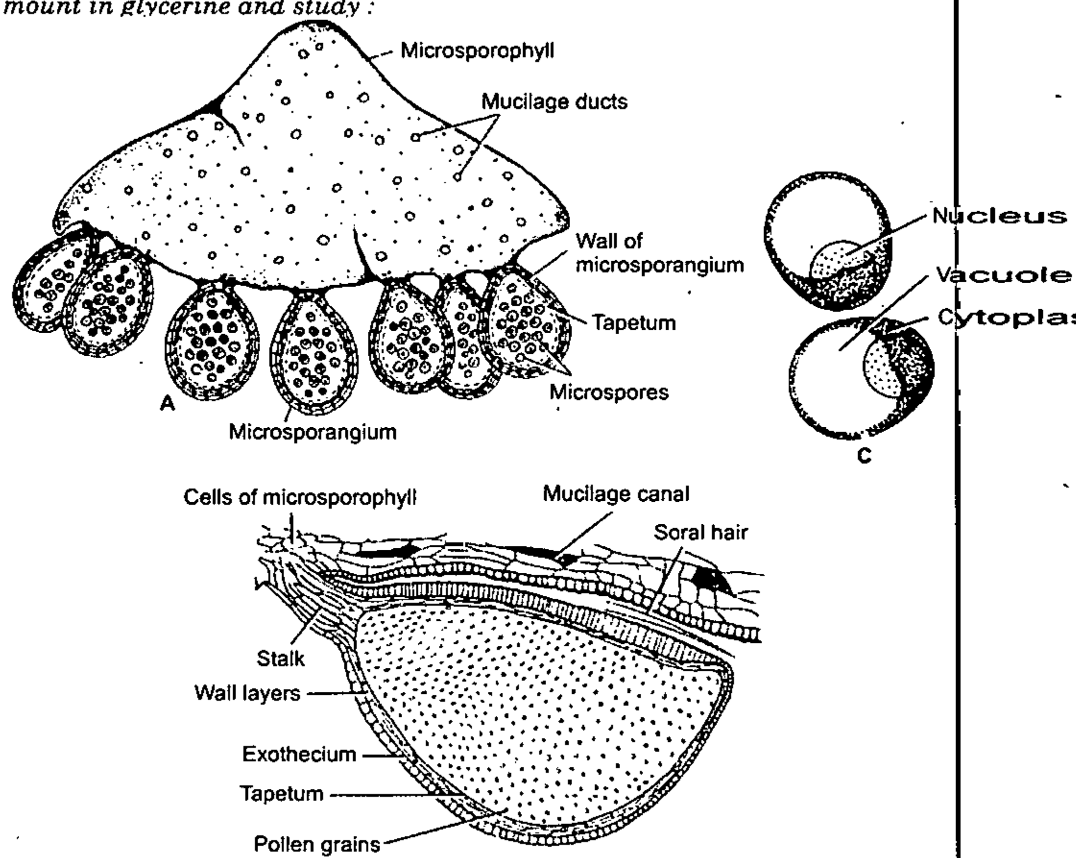


Fig. 22. Cycas. (A) T.S. microsporophyll; (B) An enlarged mature microsporangium cut longitudinally; (C) Two ungerminated pollen grains.

1. Many microsporangia (Fig. 22) are present on abaxial side.

2. Each shortly-stalked sporangium is surrounded by many layers with the innermost layer of tapetum. Many pollen grains are present in each sporangium.

3. Many mucilaginous canals and vascular bundles are present in the microsporophyll.

Female Reproductive Organs

There is no true female cone or strobilus. Female reproductive organs are present in the form of *megasporophylls* (Fig. 23).

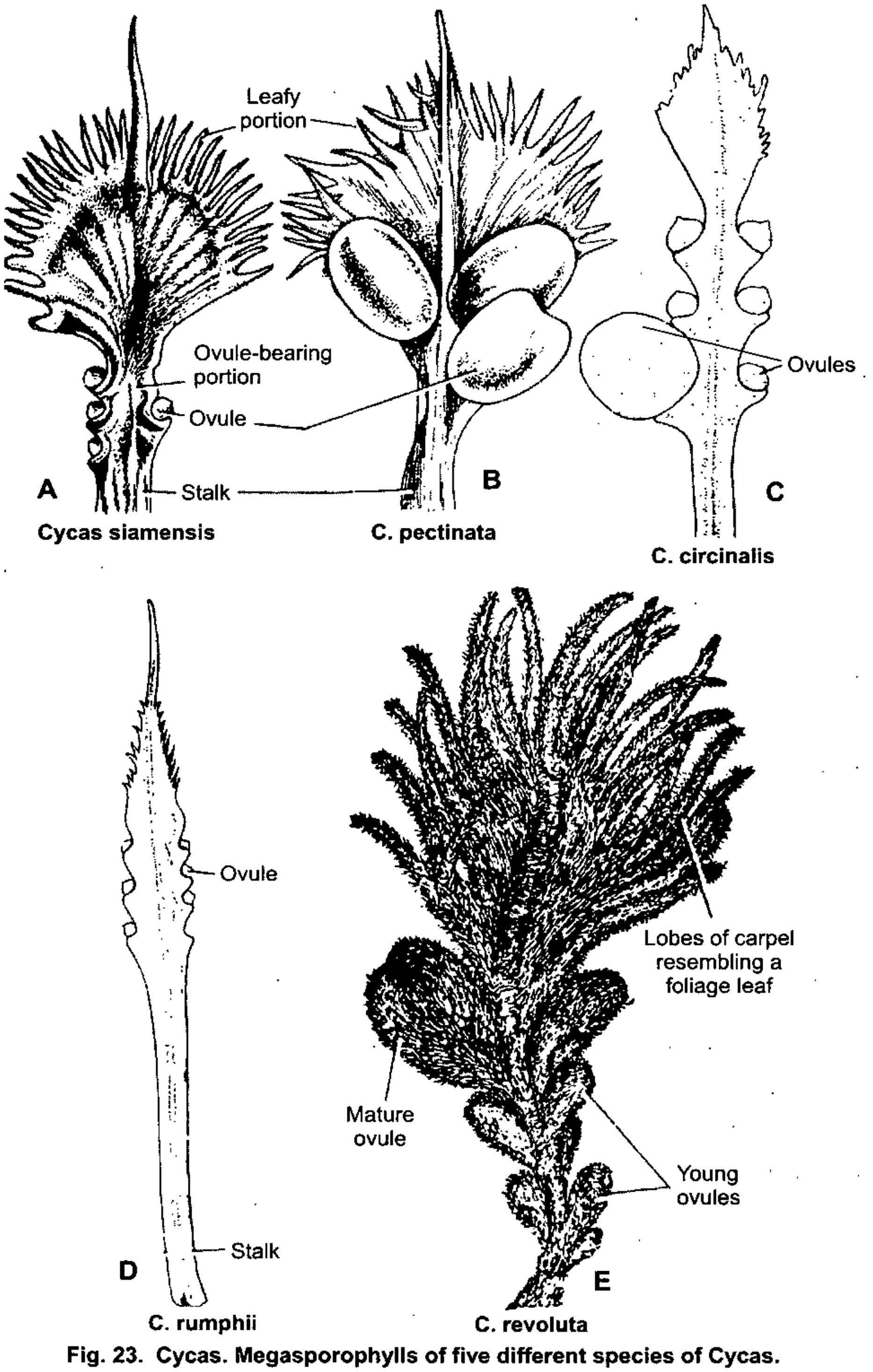


Fig. 23. Cycas. Megasporophylls of five different species of Cycas.

Megasporophylls

Observe the specimens of megasporophylls of different species and note the followi features :

1. Like foliage leaves, megasporophylls are spirally arranged at the apex of stem, very large number, and thus appear like a rosette.

2. They are loosely arranged in acropetal succession without showing any effect apical meristem.

3. They are formed once in a year in the mature plant.

4. *Each megasporophyll* is considered as a modification of foliage leaf and reaches to 20 cm or more in length.

5. Each megasporophyll is a flat body consisting of an upper dissected or pinna leafy portion and a lower stalk. On stalk, the ovules are arranged in two rows.

6. Megasporophylls are covered by many yellow or brown-coloured hair.

7. The ovules are green when young but at maturity they are fleshy and brig orange or red-coloured structures.

8. The ovule of *C. circinalis* is the **largest amongst the living gymnosperm** measuring about 6 cm in length.

External morphology of megasporophyll is different in different species of Cycas wi regard to the number of ovules and the dissected nature of the upper portion.

(A) Megasporophyll of *Cycas rumphii*

1. The pinnae are reduced in size (Fig. 23D).
2. The number of ovules is 4 to 6.
3. The base of the megasporophyll is covered by scaly leaves.

(B) Megasporophyll of *Cycas revoluta*

1. Upper part is much dissected and pinnate (Fig. 23E).
2. Tip of each pinna is generally acute.
3. The size of megasporophyll ranges between 15 to 20 cm.
4. The number of ovules is 2 to 12.

(C) Megasporophyll of *Cycas circinalis*

1. The upper part is not much dissected.
2. The margin of the upper part is serrate (Fig. 23C).
3. The *ovule is largest amongst the living gymnosperms.*

(D) Megasporophyll of *Cycas siamensis*

1. The leafy portion is much dissected.
2. Few upper pinnules unite to form a solid structure (Fig. 23A).
3. The number of ovules is only two.

L.S. Mature Ovule

1. The ovule (Fig. 24) is *orthotropous and unitegmic.*

2. The single integument is very thick and covers the ovule from all the sides excep at mouth-like opening called micropyle.

3. Single integument consists of following three layers (Fig. 24 A) :

(a) *Outer,* green or orange, fleshy layer called *sarcotesta;*

(b) *Middle,* yellow, stony layer called *sclerotesta* and;

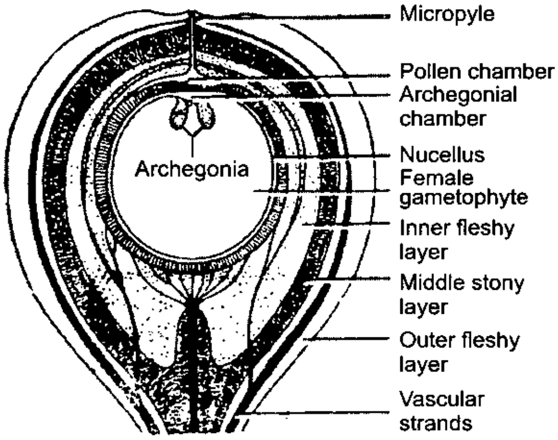


Fig. 24. Cycas. L.S. ovule showing two archegonia and female gametophyte.

(c) *Inner,* fleshy layer.

4. Integument remains in close association with the nucellus.

5. The nucellus develops the nucellar beak in the micropylar region.

6. In the nucellar beak is present a hollow small cavity or chamber called *pollen chamber.*

7. In the centre of the ovule is present a female gametophyte, in which an *archegonial chamber* develops just below the pollen chamber.

8. Two *archegonia* are present in the female gametophyte near the archegonial chamber.

9. Ovule gets vascular supply by the vascular strand in the outer and inner fleshy layers. There is no vascular supply for middle stony layer.

L.S. Seed

1. Ovule, as a whole, develops into the seed after fertilization.
2. It is very large, ovoid or globose in shape and attains a size of 2.5 to 5 cm.
3. Seed is red to orange-coloured structure.
4. Only one embryo is present in each mature seed.
5. Two cotyledons (Fig. 25) are present in the embryo.

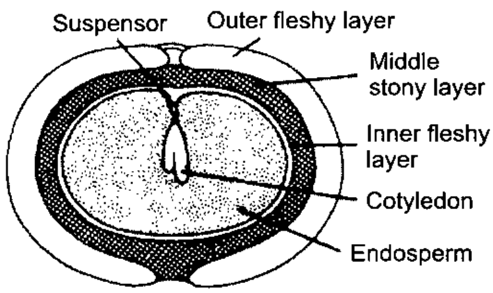


Fig. 25. Cycas. L.S. seed.

6. Embryo remains surrounded by the endosperm.

7. Endosperm stores a considerable quantity of food material for the growth of embryo.

8. Seed remains covered by an outer thick fleshy layer, middle stony layer and the innermost papery layer.

Identification

(a) (i) Sporophytic plant body is differentiated into roots, stem and leaves.

(ii) Ovules are naked.

(iii) Xylem lacks vessels.

(iv) Phloem lacks companion cells.

(v) Plant unisexual. .... Gymnosperm

(b) (i) Leaves large, frond-like.

(ii) Wood manoxylic.

(iii) Dioecious plants with motile male gametes.

(iv) Seeds show radial symmetry. .... Cycadopsida

(c) (i) Plants are not large trees.

(ii) Stem unbranched and covered by leaf bases.

(iii) Plant is palm like.

(iv) Young leaves are circinately coiled.

(v) Mucilage canals are present both in pith as well as cortex.

(vi) Ovule is orthotropous and unitegmic. ....Cycadales

(d) (i) Coralloid roots are present.

(ii) Leaves large, pinnately compound and circinately coiled when young.

(iii) Leaf-like megasporophylls. ....Cycadaceae

(e) (i) Roots are of two types-normal and coralloid.

(ii) Leaves are of two types-foliage and scaly.

(iii) Foliage leaves are circinately coiled, when young.

(iv) Male cones are very large, occur rarely and singly.

(v) Diploxylic vascular bundle in leaflets.

(vi) Leaf-like megasporophyll contains orthotropous and unitegmic ovules.

(vii) Embryo contains two cotyledons. .... Cycas

B.Sc. ZBC-104

ॐ सर्वे भवन्तु सुखिनः सर्वे सन्तु निरामयाः ।
सर्वे भद्राणि पश्यन्तु मा कश्चिद् दुःख भाग्भवेत् ।

DIRECTORATE OF DISTANCE EDUCATION

Swami Vivekanand

SUBHARTI UNIVERSITY

Subhartipuram, NH-58, Delhi-Haridwar Bypass Road,
Meerut, Uttar Pradesh 250005
Phone : 0121-243 9043
Website : www.subhartidde.com, E-mail : ddesvsu@gmail.com