Indira Gandhi National
Open University
School of Sciences

BBYCL-132
BIODIVERSITY
(MICROBES, ALGAE, FUNGI AND ARCHEGONIATES)

Course Design Committee

Dr. A.K. Kavathekar (Retd.)
Department of Botany
Sri Venkateswara College,
University of Delhi, New Delhi

Dr. Sneh Chopra (Retd.)
Department of Botany
Kalindi College, University of Delhi,
New Delhi

Prof. Jaswant Sokhi
School of Sciences
IGNOU, Maidan Garhi
New Delhi

Prof. M.S. Nathawat
Director, (Ex.)
School of Sciences

Prof. Vijayshri
Director, (Ex.)
School of Sciences

Prof. Amrita Nigam
School of Sciences
IGNOU, Maidan Garhi
New Delhi

Block Preparation Team

Prof. Amrita Nigam
School of Sciences
IGNOU, Maidan Garhi
New Delhi

Prof. Jaswant Sokhi
School of Sciences
IGNOU, Maidan Garhi
New Delhi

Dr. Swati Omanwar
Consultant
School of Sciences
IGNOU, New Delhi

Dr. Pooja Sinha Gokhale
Department of Botany
Sri Venkateswara College
University of Delhi
New Delhi

Dr. A.K. Kavathekar
Sr. Consultant
School of Sciences
IGNOU, New Delhi

Course Coordinators: Prof. Jaswant Sokhi and Prof. Amrita Nigam

Print Production

Sh. Sunil Kumar
Assistant Registrar (P)

Acknowledgements: The following members of IGNOU, School of Sciences, Maidan Garhi, New Delhi:
Dr. Swadesh Teneja (Retd.), Reader, for forming the base materials for Algae, Fungi and Bryophytes
Dr. Bhupinder Dhir, Consultant, for Proof Reading
Dr. Eklavya Chauhan, Consultant, for Proof Reading
Mr. Manoj Kumar, Assistant, for Word Processing.

September, 2019

ISBN:

Further information on the Indira Gandhi National Open University courses may be obtained from the University's office at Maidan Garhi, New Delhi-110 068 or the official website of IGNOU at www.ignou.ac.in.

Printed and published on behalf of Indira Gandhi National Open University, New Delhi by Prof. Poornima Mital, Director, SOS, IGNOU.

Laser Typeset by : Rajshree Computers, V-166A, Bhagwati Vihar, (Near Sec. 2, Dwarka), Uttam Nagar, New Delhi-110059

Printed by :

Biodiversity (Microbes, Algae, Fungi and Archegoniates) : Introduction

This course is designed to give you hands-on experience of biodiversity – microbes, algae, fungi, bryophytes, pteridophytes and gymnosperms that you studied in the theory course. You will study their cellular organization, morphology, anatomy and reproductive structures in this course that is of 2 credits. Many of these biodiversity forms are microscopic in nature and you will probably see them for the first time. This study is proposed to be undertaken in 17 exercises conducted in 12 sessions of 4 hours each.

To ensure the successful conduct of practicals within the prescribed time we have provided in every exercise a brief theoretical background, list of materials to be used, procedure to be followed and the observations to be made. At the end of each exercise you should be able to identify the given plant, its part(s) in vegetative and/or reproductive phase(s) giving scientific justification. You are expected to provide a taxonomic classification of the given biodiversity form giving reasons. Through this course it is also aimed that you develop the ability and skills to prepare specimens for examination, study the mentioned biodiversity forms, record observations, describe and identify the plant under investigation in view of what you have learnt in the theory course.

To begin the study of this course it is pertinent to realize the importance of laboratory work. One must understand what one is doing while performing these exercises, make ones observations and prepare a meticulous record of the same. This pursuit will make the exploration of the biodiversity forms interesting and help in the development of the spirit of scientific enquiry.

During hands-on laboratory work one needs to be mentally alert for the best outcomes. By the end of this course it is expected that you get trained, develop the ability to work independently and be confident about your own observations. Initially, it is quite likely that you may not see in a preparation the structures described in the theory text. You must not make conclusions after the first glance of the material preparation. It is necessary to explore a few preparations of the material and study them for some time. In scientific investigations repetitions are necessary to confirm the results. The results are not valid if they are different when repeated. Therefore, if you are not sure about your investigation you should repeat it one or more times till the outcome is satisfactory. Besides, you must cultivate intelligent curiosity and sharpen your faculty of exploration. Problems may occasionally arise and when they do you may seek the help of your counsellor. Once you have finished with the exercise it is often valuable to discuss the findings and conclusions with your fellow students. These exercises are also expected to enhance your observational skill. You should follow the three principles while making an observation: observe what you are expected to observe; do not be perturbed if a feature for observation is not present in the specimen or the preparations; and observe anything new and interesting which is present in your specimen or the preparation you made even if you were initially not expected to observe the same.

You must realize conducting laboratory work is a very expensive affair. It also requires a lot of effort on the part of the counsellor and technical staff of the laboratory. Therefore, it must be taken seriously from a learning standpoint and avail this opportunity to inculcate the scientific method and apply it in real life situations.

Laboratory exercises are interesting and enjoyable. To make the best use of the given time one needs to come prepared with background knowledge on the topic. For this the text pertaining to the topics as mentioned under *Study Guide/Prior Reading* in each exercise be

read beforehand. One need not bring the blocks of the theory course or any other botany book to the laboratory as the requisite information regarding the exercise is provided in this block.

Objectives

After doing the exercises of this course, you should be able to:

- follow a suitable procedure for the study of morphological, anatomical and reproductive features of microbes, algae, fungi, bryophytes, pteridophytes and gymnosperms;
- prepare whole mounts, peel mounts, smears to study them;
- dissect and cut sections of the above-mentioned biodiversity forms or their parts;
- understand host-parasite and symbiotic relationships;
- classify the microbes, algae, fungi, bryophytes, pteridophytes and gymnosperms into their respective sub-groups on the basis of their distinguishing features;
- display the characteristic features of the these organisms by selecting suitable techniques;
- use specific stains required for resolving the cellular and anatomical features of the organisms; and
- conduct yourself in a disciplined way, following all the safety guidelines of laboratory and accomplish the outlined objective of the course.

Instruments and Other Requirements

Before your laboratory course gets underway, we suggest that you prepare a small *kit — Biology Laboratory Student's Kit,* containing the following items. C*arry this kit everyday with you for the one-week lab course*.

- **A** pair of fine tipped forceps
- **Two** fine, long-handle dissecting needles
- **A** sharp razor or a fresh unused blade
- **A** pair of fine-hair brushes
- **A** pair of scissors
- **Two** sharpened pencils, one each of HB and H grade
- **Six** coloured pencils for highlighting the important points and details
- **A** pencil eraser
- **A** sharpener
- **A** small (6" or 15 cm) scale
- **A** clean, soft, handkerchief-sized piece of cloth
- **A** lab-coat to be worn throughout the session
- **A** Practical Notebook.

Along with the above kit, carry a small note-book (80-100 pages), and this block that you are holding everyday to the laboratory.

Instruments and items such as the dissecting and compound microscopes staining racks and bottles, microslides (= slides) and micro-coverslips (= coverslips) and other requirements for your practical work would be made available to you in the laboratory itself.

Laboratory Ettiquettes

To get the most out of the lab. work, one needs to inculcate or develop certain qualities such as curiosity to learn, sincerity, honesty, and an unbiased, analytical frame of mind. This laboratory course besides helping you to increase your knowledge on the subject matter, would also give you an opportunity to further develop the qualities mentioned above. To utilize any opportunity fully, it is essential to respect and follow certain rules or observe the etiquettes for the given situation. The six points given below may be helpful to you in this regard:

i) Read the laboratory exercise in advance along with related theory portions given in the course before coming to the laboratory.

ii) Complete the assigned work within the stipulated time, prior planning and proper use of time would help in accomplishing your targets.

iii) Judicious and optimum utilization of the facilities provided is the key to success.

iv) Follow the instructions written in this block as well as those given by your Counsellor and get your work checked immediately after completing the given exercise.

v) Do not underestimate your observational skills. If your observations are at variance with the expected ones, do not hesitate to explore its reasons. Also, freely discuss with your Counsellor whenever in doubt.

vi) Handle laboratory provisions with utmost care and leave your place clean and in order at the end of the class each day.

By following these points, both success and satisfaction will be yours! We wish you all the best for the course.

Practicals
Session Plan

Day	Session	Practicals	Hrs.	Total Hrs.
Day 1	Session 0	Introduction	1	1
	Session I	Exercise 1 - E.M. phage, E.M. TMV, E.M. lytic, and E.M. lysogenic cycle	1½1½	3
	Session II	Exercise 2 - Types of bacteria, E.M. bacterial cell, E.M. binary fission, E.M. conjugation		
		Exercise 3 - Gram-staining	31	4
Day 2	Session III	Exercise 4 - *Nostoc*, *Chlamydomonas*, *Oedogonium*, *Vaucheria*	½+½1½+1½	4
	Session IV	Exercise 4 - *Fucus*, *Polysiphonia*		
		Exercise 5 - *Rhizopus*, *Penicillium*	1+11+1	4
Day 3	Session V	Exercise 6 - *Alternaria*		
		Exercise 7 - *Puccinia*	13	4
	Session VI	Exercise 8 - *Agaricus*		
		Exercises 9 & 10 - Lichens, Mycorrhiza	2½1½	4
Day 4	Session VII	Exercise 11 - *Marchantia*		
		Exercise 12 - *Funaria*	22	4
	Session VIII	Exercise 13 - *Selaginella*	4	4
Day 5	Session IX	Exercise 14 - *Equisetum*	4	4
	Session X	Exercise 15 - *Pteris*	4	4
Day 6	Session XI	Exercise 16 - *Cycas*	4	4
	Session XII	Exercise 17 - *Pinus*	4	4
Day 7	Session XIII	Revision	4	4
	Session XIV	Practical Examination	4	4

Scheme of Practical Examination

Time: 4 Hours **Maximum Marks: 30**

Q 1.	Gram-staining of given bacteria·	1	
	• Preparation		
	• Identification (gram-positive/negative)	1	(2)
Q.2	Study of vegetative/reproductive parts of an alga		
	• Temporary-stained preparation	1	
	• Labelled diagram	1	
	• Salient characteristics	1	
	• Identification and classification	½ + ½	(4)
Q.3	Study of vegetative/reproductive parts of a fungus		
	• Temporary-stained preparation	1	
	• Labelled diagram	1	
	• Salient characteristics	1	
	• Identification and classification	½ + ½	(4)
Q.4	Study of vegetative/reproductive parts of a bryophyte		
	• Temporary-stained preparation	1	
	• Labelled diagram	1	
	• Salient characteristics	1	
	• Identification and classification	½ + ½	(4)
Q.5	Study of vegetative/reproductive parts of a pteridophyte		
	• Temporary-stained preparation	1	
	• Labelled diagram	1	
	• Salient characteristics	1	
	• Identification and classification	½ + ½	(4)
Q.6	Study of vegetative/reproductive parts of a gymnosperm		
	• Temporary-stained preparation	1	
	• Labelled diagram	1	
	• Salient characteristics	1	
	• Identification and classification	½ + ½	(4)
Q.7	Spots, any four		
	• Viruses	1	
	• Bacteria	1	
	• Algae	1	
	• Fungi/lichen/mycorrhiza	1	
	• Bryophytes	1	
	• Pteridophytes	1	
	• Gymnosperms	1x4	(4)
Q. 8	Records		(2)
Q. 9	Viva-voce		(2)

EXERCISE 1

MODELS OF VIRUSES - T PHAGE AND TMV

Structure

1.1 INTRODUCTION

Viruses are small obligate, intracellular particles seen only by electron microscope that infect and take over the host cell to replicate. Viruses do not have the ability to grow in any artificial media as they are metabolically inert (inactive) and can be metabolically active only when they are inside a living host cell. All viruses are **acellular entities** and are made of genetic material (DNA or RNA) inside a protein coat.

The viruses are made up of only two components: a) protein (capsid) and b) nucleic acid (viral genome). **Capsid** is a protein coat that protects the viral genome against chemical and physical damage. It also helps the virus to enter into the host cell.

Some viruses have an outer membranous layer that surrounds the nucleocapsid and are called **enveloped viruses**. Viruses which do not have an envelope are called **naked viruses or non-enveloped virus**.

The viral nucleic acid or genome is made of only one type of nucleic acid either deoxy ribonucleic acid (DNA) or ribonucleic acid (RNA). The viruses are usually classified into two groups on the basis of their nucleic acid/genome (DNA or RNA) i) **DNA viruses** and ii) **RNA viruses**.

In these exercises you will examine the morphological, anatomical and reproductive (lytic and lysogenic) features of a couple of viruses: T-Phage (DNA virus) and TMV (RNA virus) with help of models, E.M photographs and line drawings.

Objectives

After doing these exercises you will be able to :

- ❖ study the anatomical structure of virus;
- ❖ identify various parts of virus;
- ❖ differentiate the two principal groups of virsus: DNA virus (T-phage) and RNA virus (TMV); and
- ❖ understand lytic and lysogenic cycle.

Study Guide/Prior Knowledge

For doing satisfactory work you must read the following before coming to the laboratory.

The B.Sc. Course (CBCS), Core Botany, Paper-I Biodiversity (Microbes, Algae, Fungi and Archegoniates), Block 1: Microbes; Unit 1 : Viruses : General Account and Economic Importance and Unit 2 : Virus : Replication.

1.2 METHOD OF STUDY

You will study the salient morphological features of Viruses : T-phage (DNA virus) and TMV (RNA virus) by observing virus models, line drawings and EM photographs.

You will study the different steps in viral replication (lytic and lysogenic cycle) by observing virus models, line drawings and EM photographs.

1.3 OBSERVATIONS

(A) Study of T-Phage (DNA virus)

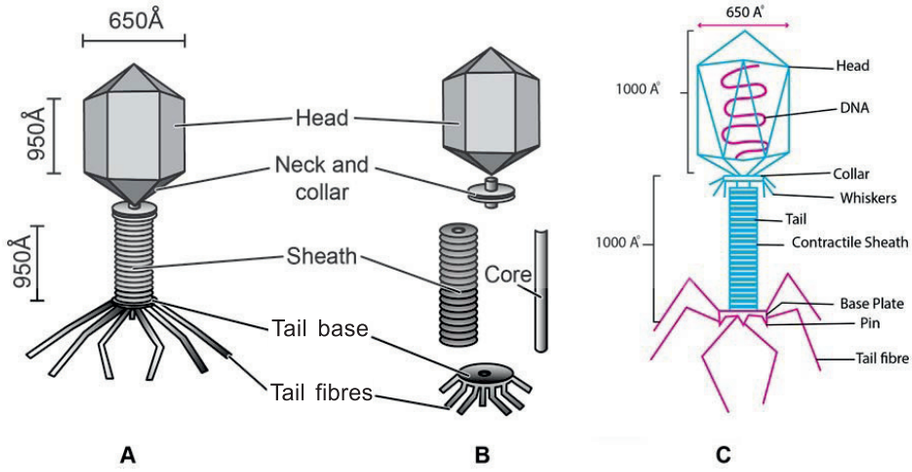


Fig. 1.1: A) T4 Bacteriophage; B) Parts; C) LS of a virion.

Observe the Fig. 1.1 of T-Phage virus

- Fig. 1.1A shows that T-even phage has a tadpole like structure with five important sub-structures such as the head, head-tail connector, tail base plate and fibres.

- The viral particle is naked icosahedral and tailed (Fig. 1.1B).
- The head is an elongated, bi-pyramidal, hexagonal like prism composed of about 2000 capsomeres and encloses a tightly packed dsDNA (50 nm long) (Fig. 1.1C).
- It has a long helical tail which is connected to the head with a connector having a collar with attached whisker (Fig. 1.1 B and 1.1.C).
- The tail consists of an inner hollow tube called core, surrounded by a contractile sheath which consists of 24 annular rings (Fig. 1.1B).
- The distal end of the tail is connected to a hexagonal basal plate with spike or tail spin at each corner. Six long, flexible tail fibers also arise from the basal plate which helps in adsorption to bacteria

(B) Study of TMV (RNA Virus)

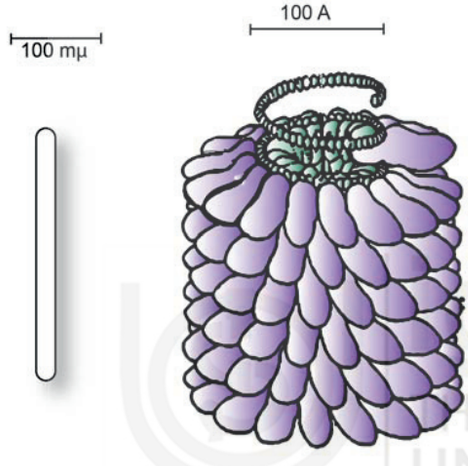


Fig. 1.2: Tobacco mosaic virus (TMV) showing protein subunits tagged on the RNA helix.

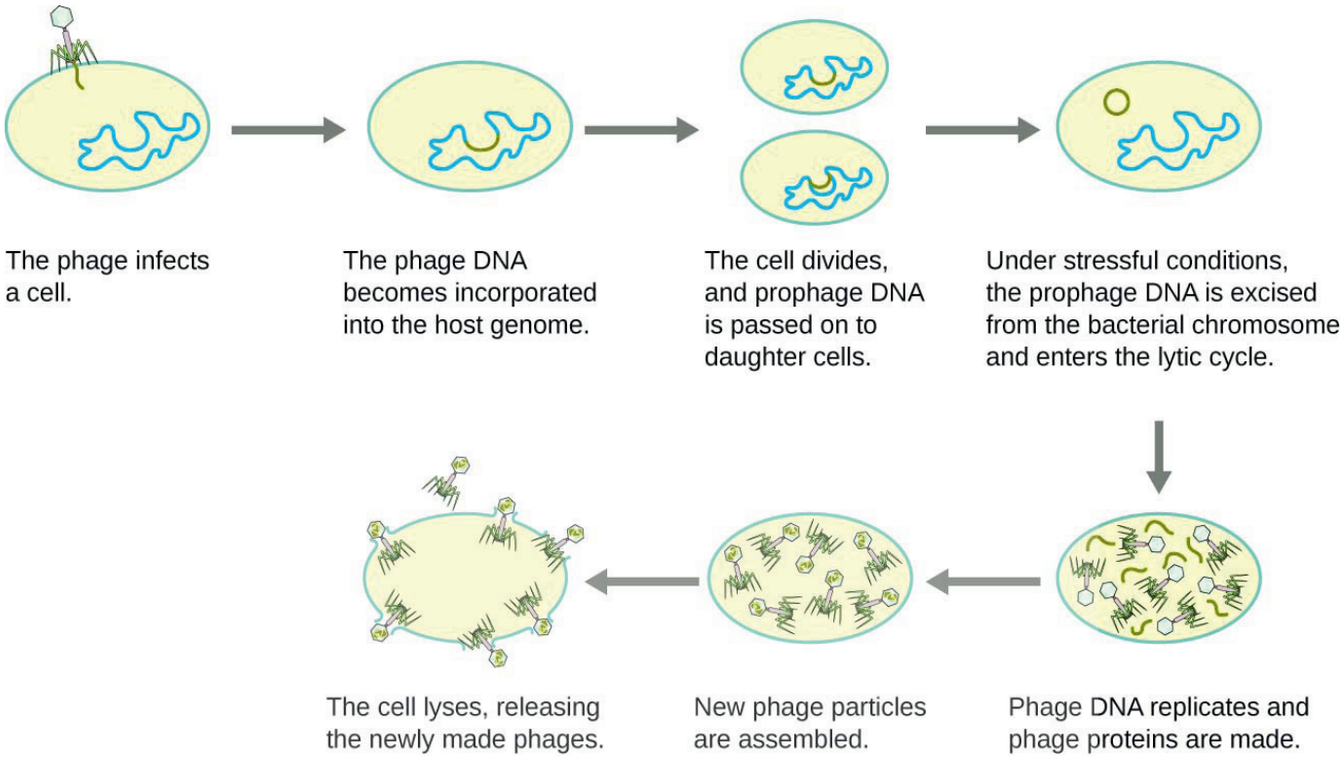
Observe the Fig.1.2 Tobacco Mosaic virus (TMV)

- Fig. 1.2 shows TMV as a simple rod-shaped helical virus consisting of centrally located single-stranded RNA enveloped by a protein coat.
- The rod is about 300 nm in length and 15 to 17 nm in diameter.
- The tube is hollow and composed of protein capsomeres arranged helically around the central core. There are 16 capsomeres in each helical turn and about 130 turns per rod of TMV.
- The central core is 4 nm in diameter and runs through the entire length of the virus.
- The positive sense (+) ssRNA is helically coiled. Its genome is a single copy of positive strand RNA and the RNA molecule folds into tRNA like structure.

(C) Study of Lytic Cycle

Lytic cycle: As in the lytic cycle, many virions are produced, it is called a productive infection.

Observe the Fig. 1.3 to study lytic cycle.



bacterial cells, the tail fibres of phage base plate gets attached to its specific complementary receptor site on the surface of bacterial cells.

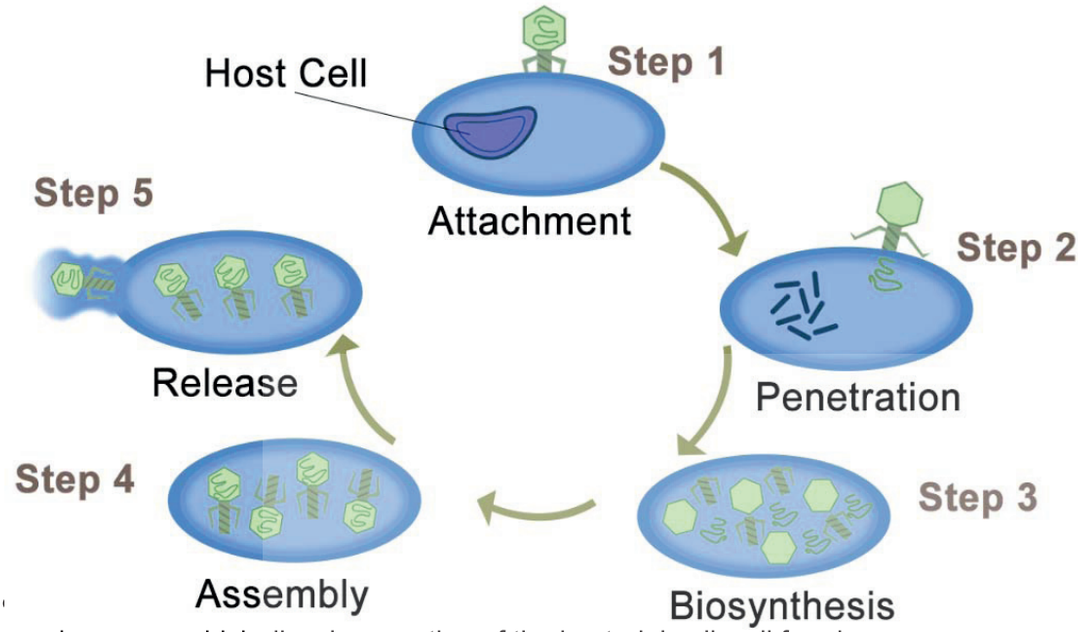
- **Step 2: Penetration:** On attachment the tail of phage releases lysozyme which dissolves portion of the bacterial cell wall forming a pore. The tail core is pushed through the cell wall and DNA is ejected through the core into the cytoplasm and the capsid remains outside the bacterial wall.
- **Step 3: Biosynthesis:** The viral genome after entering the host cytoplasm, uses the cells' ribosomes and translation machinery to synthesize new phage proteins (head, tails and tail fibers). It also uses the bacterial cells enzymes to make more copies of the viral genome.
- **Step 4: Maturation and Assembly:** The phage proteins and the copies of viral genome spontaneously self assemble to form virus particle.
- **Step 5: Release: T**he lysozyme, encoded by phage genome, causes the lysis of the wall of the host bacterium and the phages are released from the ruptured bacterial shell.

(D) Study of Lysogenic Cycle

THE LYSOGENIC CYCLE : Lambda (λ) phage of the bacteria *E. coli* is the most widely studied example of lysogeny. The phages which replicate by lysogenic cycle are called lysogenic phage (temperate phage) and the bacteria in which it occurs is called a lysogenic cell.

Observe the Fig. 1.4 to study lysogenic cycle.

- **Step 1: Attachment/Adsorption:** On random collision of phage and bacterial cells, the tail fibres of phage base plate gets attached to its specific complementary receptor site on the surface of bacterial cells.



lysozyme which dissolves portion of the bacterial cell wall forming a pore. The tail core is pushed through the cell wall and DNA is ejected through the core into the cytoplasm and the capsid remains outside the bacterial wall.

- **Step 1 and 2 are similar as in case of lytic cycle.**
- **Step 3: Latent infection**: In the host cell cytoplasm, the phage DNA inserts into the circular DNA (chromosome of bacterium) and is called prophage. The prophage replicates along with the bacterial chromosomes during reproduction and remains latent in the bacterial progenies. As the prophage does not lyse the bacterial cell, the infection is referred to as **latent infection**.
- **Step 4**: **Induction**: When the bacterial cell becomes "stressed" due to UV radiations, presence of chemicals, the prophage excises itself from the bacterial chromosome and is released in the cytoplasm. This disassociation is called **induction**. After induction lytic cycle is initiated, lyses of the bacterial cell occurs and new phages are released.

EXERCISE 2

STUDY OF TYPES OF BACTERIA

Structure

2.1 INTRODUCTION

Bacteria are an extremely diverse group of microorganisms ubiquitous by present on Earth and exhibit different morphology and physiology.

Bacterial cell is a prokaryotic cell and has simple structure. The cell wall is made up of mucopeptide, amount of which varies in the cell wall of various bacterial species. On this basis bacteria have been divided into Gram+ve and Gram-ve bacteria. The cell membrane is surrounded by the cell wall and encloses the cytoplasm. In some bacteria the cell wall is surrounded by a definite polysaccharide layer called capsule. The bacterial cell lacks an organised nucleus and other organelles-chloroplast, mitochondria, golgi body etc. Some bacteria have flagella which help in movement. Depending on their shape bacteria are classified into cocci, bacilli, vibrous etc.

Bacteria can reproduce by asexual reproduction through binary fission, endospore or cyst formation. Binary fission is unique to bacteria. Sexual reproduction in bacteria is different from that of eukaryotes and genetic transfer occurs via mechanisms of conjugation, transformation and transduction.

Objectives

After doing these exercises you will be able to :

- ❖ list the types of bacteria;
- ❖ identify various parts of bacteria (EM);
- ❖ study binary fission and conjugation; and
- ❖ describe the structure of a root nodule.

Study Guide/Prior Knowledge

For doing satisfactory work you must read the following before coming to laboratory.

- The B.Sc. Course (CBCS), Core Botany, Paper-I Biodiversity (Microbes, Algae, Fungi and Archegoniates), Block 1: Microbes; Unit 3 Bacteria : General Account and Economic importance.

2.2 METHOD OF STUDY

You will study the salient morphological features of Bacteria by observing the line drawings, permanent/temporary slides, EM photographs.

2.3 OBSERVATIONS

(A) Types of Bacteria

Depending on their shape, bacteria are classified into several varieties. Study and observe the table given below.

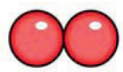
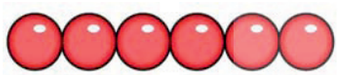
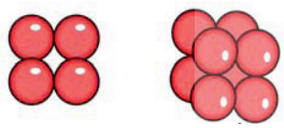
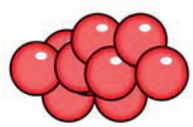
 **Fig. 2.1: Coccus.**	Fig. 2.1 shows spherical or oval shaped bacteria which are known as Coccus (from *kokkos* meaning berry).
 **Fig. 2.2: Diplococcus.**	Fig. 2.2 shows cocci arranged in pairs. Such cocci are known as diplococci.
 **Fig. 2.3: Streptococcus.**	Fig. 2.3 shows cocci arranged in chains. Such cocci are known as streptococci.
 **(a) (b)** **Fig. 2.4: (a) Tetrad, (b) Sarcina.**	Fig.2.4(a) shows cocci arranged in groups of four. Such cocci are known as tetrads. Fig. 2.4(b) shows cocci arranged in groups of eight. Such cocci are known as sarcina.
 **Fig. 2.5: Staphylococcus.**	Fig. 2.5 shows cocci arranged in grape like clusters. Such cocci are known as staphylococci.
 **Fig. 2.6: Bacillus.**	Fig. 2.6 shows rod shaped bacteria which are known as Bacilli (from *baculus* meaning rod).

 **Fig. 2.7: Vibrio.**	Fig. 2.7 shows comma shaped, curved rod shaped bacteria which are known as Vibrios. They derive their name from their characteristics vibratory motility.
 **Fig.2.8: Spirillia.**	Fig. 2.8 shows rigid spiral bacteria which are known as Spirilla.
 **Fig. 2.9: Spirochete.**	Fig. 2.9 shows flexuous spiral bacteria which are known as Spirochetes (from *speira* meaning coil and *chaite* meaning hair).
 **Fig. 2.10: Actinomycetes.**	Fig. 2.10 shows branching filamentous bacteria, which resembles the radiating rays of the sun when seen in tissue lesions are known as Actinomycetes (from *actis* meaning ray and *mykes* meaning fungus).

(B) Structure of Bacteria

Observe the Fig. 2.11(a) and Fig. 2.11(b). Fig. 2.11(a) is a line drawing of rod shaped (Bacillus) bacteria and Fig. 2.11(b) is its corresponding electron microscopic photograph.

You will observe :

- Cell Wall: Cell wall of bacteria is rigid and gives shape to the cell and protects the bacteria. It is made of mucopeptide and is unique to bacteria and is not found in any other group.
- Outer Membrane: Outer membrane is found only in Gram-negative bacteria, it functions as an initial barrier to the environment and is composed of lipopolysaccharide (LPS) and phospholipids.
- Cytoplasmic membrane: Cytoplasmic membrane is present immediately beneath the cell wall, found in both Gram positive and negative bacteria. It is semipermeable membrane and controls the movement of metabolites.
- Cytoplasm: The cytoplasm is a colloidal system and is rich in ribosomes, DNA and fluid.

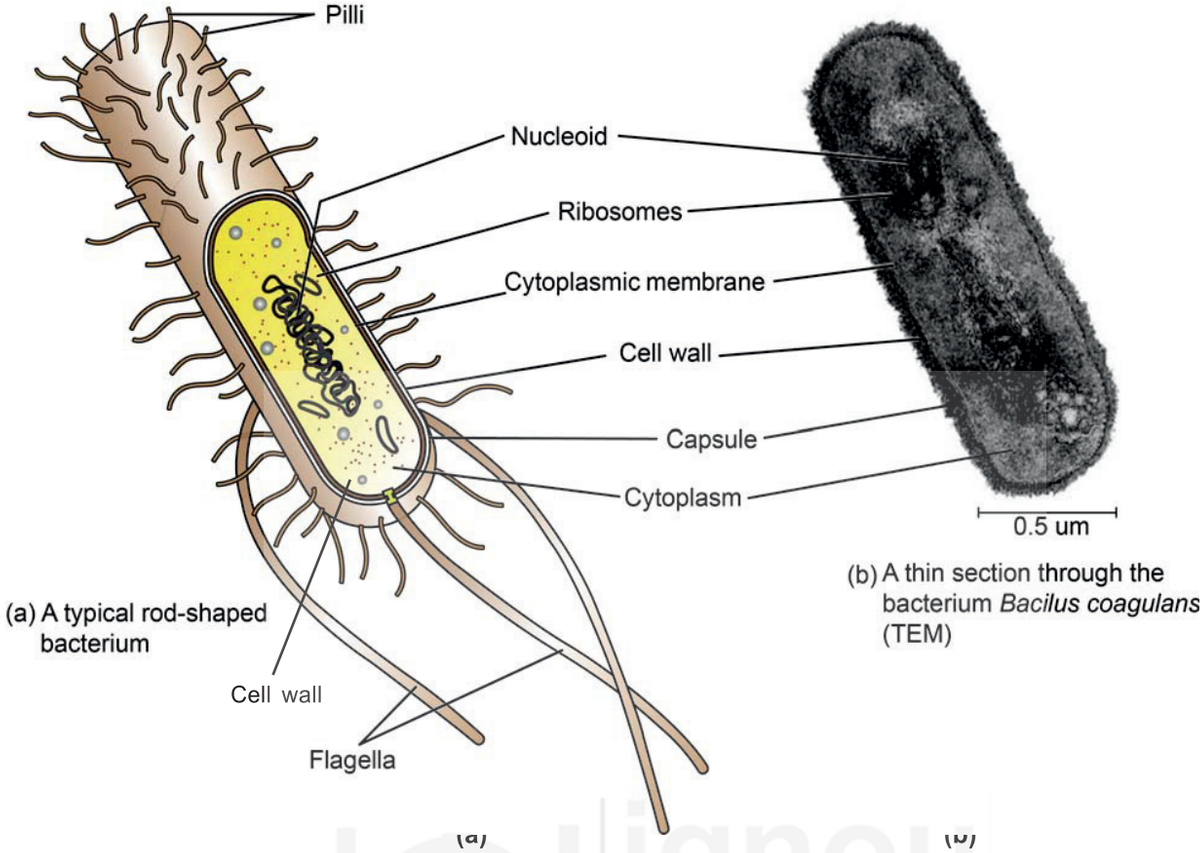


Fig. 2.11: (a) Bacterial cell is a prokaryotic cell; (b) TEM of Bacteria.

- Ribosomes :They are slightly smaller than the ribosomes of eukaryotic cells.
- Nucleoid: The Nucleoid does not have a distinct nuclear membrane or nucleolus and DNA is the genetic material.
- Plasmids: Plasmids are extra nuclear circular DNA. The cytoplasmic carriers of genetic information are termed plasmids or episomes.
- Capsule: Capsule is the outer most layer of the bacteria. The Capsule protects against complement and is antiphagocytic.
- Flagella: Flagella are long hair like helical filaments extending from cytoplasmic membrane to exterior of the cell.
- Pili / Fimbriae: Hair-like proteinaceous structures that extend from the cell membrane to external environment are pili which are otherwise known as fimbriae. The sex pili helps in conjugation.

(C) Binary Fission and Conjugation in Bacteria

Bacteria can reproduce by asexual reproduction through binary fission, endospore or cyst formation and sexual reproduction via conjugation, transformation and transduction.

You will study the salient features of Binary fission and conjugation in bacteria by observing line drawings given in Fig. 2.12 and 2.13.

Binary fission:

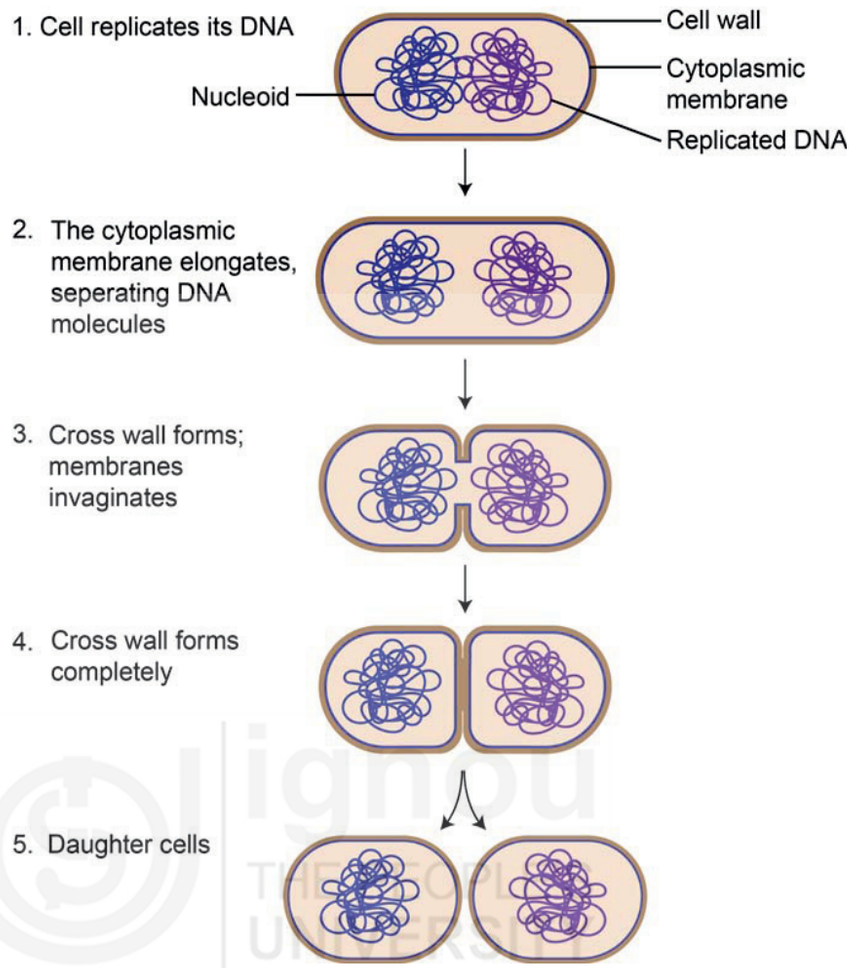


Fig. 2.12 : Binary fission.

In **Step 1** replication of DNA takes place, so that all the genetic information is carried to the new cell. The bacteria makes a duplicate copy of its DNA.

In Step 2 growth of the bacteria takes place. The parent cell becomes considerably larger in size and the two DNA copies begin moving away from each other.

In Step 3 segregation of bacteria starts. The cell becomes longer and the cell membrane pinches inwards from the middle, with the two DNA at opposite poles of the cell.

In Step 4 division of bacterial cell takes place. A cleft is formed in the middle and the parent bacterial cell splits into two identical daughter cells (step 5), each with its own DNA.

Conjugation - Pilus transfers plasmid from one bacterium to another.

The donor bacterial cell has a fertility factor and sex factor (F factor) plasmid DNA and the recipient bacterial cell does not have a F factor plasmid DNA.

In **Step 1** the donor bacterial cell (F+) attaches to recipient bacterial cell (F-) with help of its pili.

In Step 2 the cells F+ and F- contact one another.

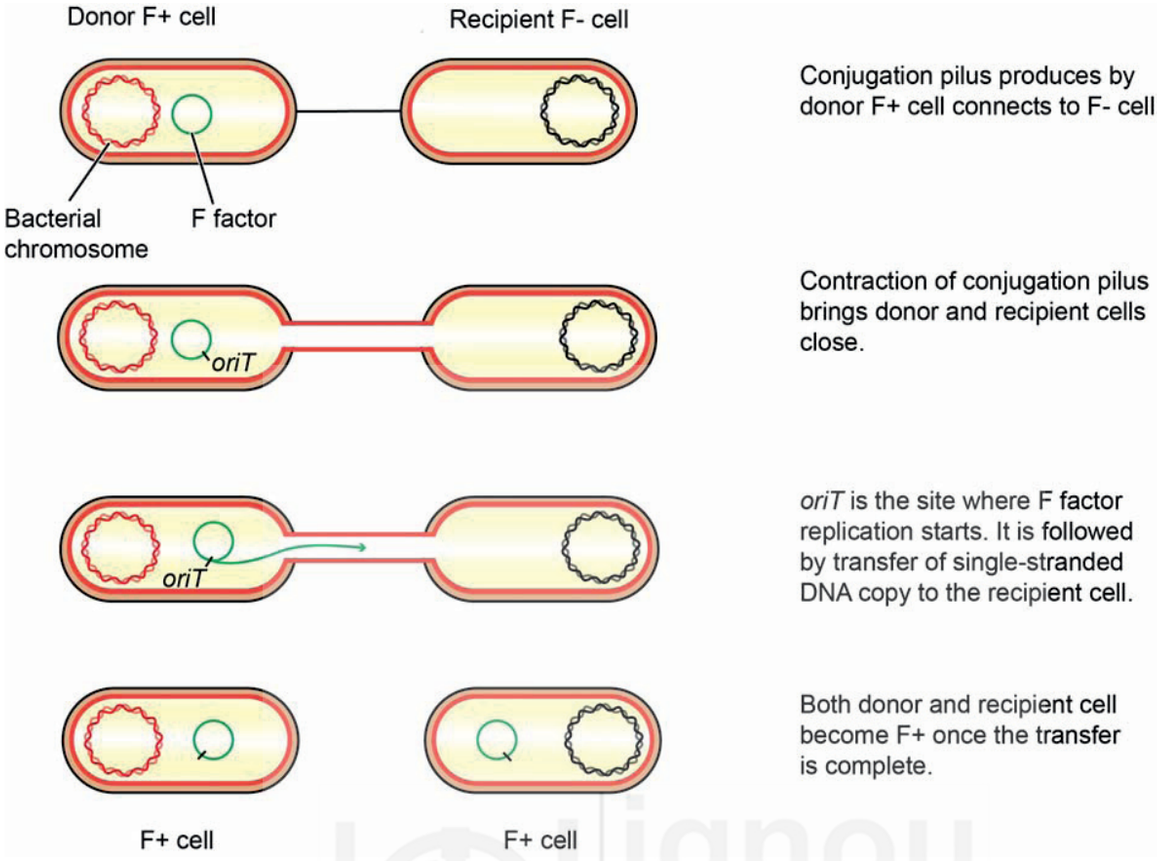


Fig. 2.13: Conjugation.

In Step 3 the plasmid DNA of donor cell, F+ replicates to form a copy of the plasmid DNA and transfers the copy to the recipient F-cell.

In Step 4 the F- cell synthesizes a complimentary strand of plasmid DNA and becomes a F+ cell.

In some bacterial cells the F factor gets integrated into the recipient bacterial chromosome. Therefore, strains with an integrated F factor are termed high frequency of recombination (Hfr) strains. The integrated F factor occasionally leaves the chromosome of an Hfr cell and moves back to the cytoplasm, in some rare cases carrying a few host chromosomal genes along with it. This modified F, called **F**2 (pronounced "F prime"), can now transfer these specific host genes to a recipient (F") cell in an infectious manner, in the same way that F is spread.

(D) Structure of Root Nodule

You will study the morphological features of root nodule by observing line drawing in Fig. 2.14.

Many legumes have root nodules that provide a home for symbiotic nitrogen-fixing bacteria called rhizobia. The Rhizobia convert nitrogen gas from the atmosphere into ammonia, which is then used in the formation of amino acids and nucleotides.

In step 1 the root emits chemical which attract the rhizobium bacteria. The rhizobioum bacteria emit signals that stimulate the root hair to elongate and form infection thread by invagination of plasma membrane.

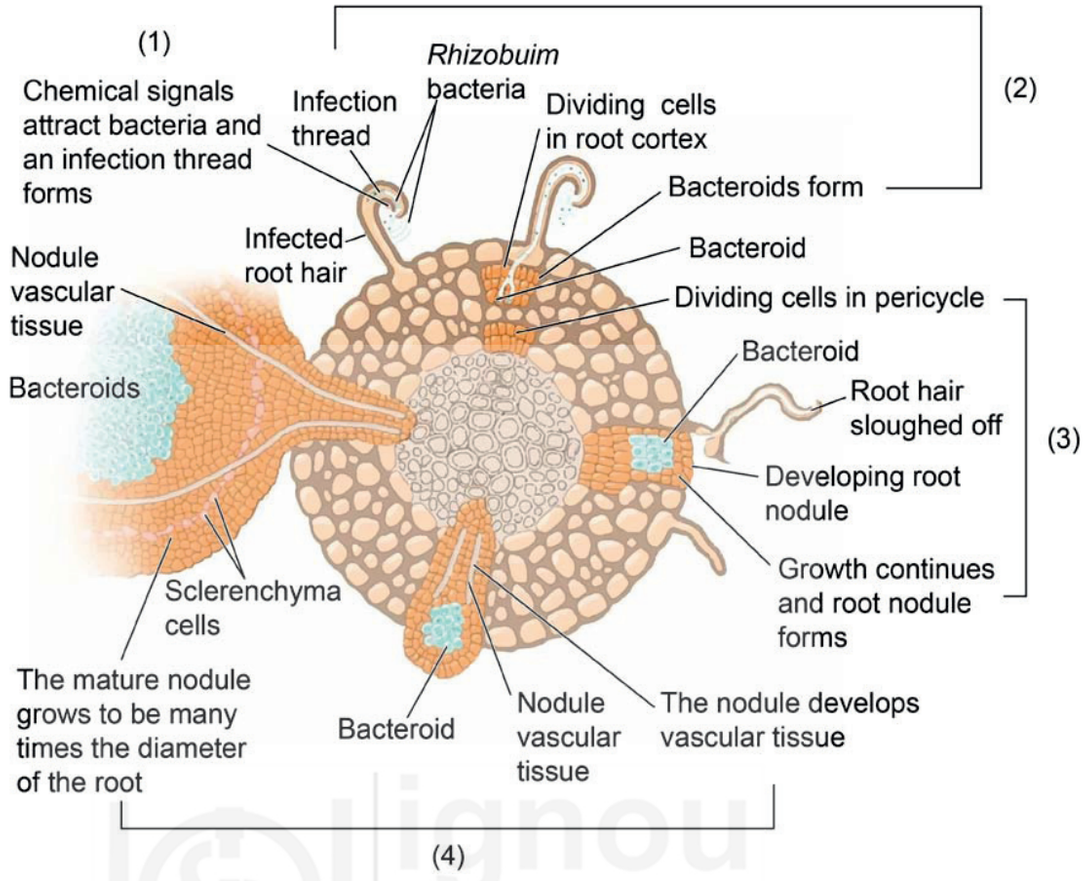


Fig. 2.14: Root nodule.

In step 2 the bacteria penetrates the root cortex within the infectious thread and bacteria bud/bacteroids are formed in the cortical cells.

In step 3 growth continues in the affected regions of the cortex and cortex and pericycle fuses to form the nodule.

In step 4 the nodule, develops to the vascular tissue which connects the nodule to the xylem and phloem. The vascular tissue supplies nutrients to the nodule and carries nitrogenous compound from the nodule to the plant.

EXERCISE 3

GRAM STAINING TECHNIQUES

Structure

3.1 INTRODUCTION

Most bacteria possess a cell wall that contains either a thick peptidoglycan layer or a thin peptidoglycan layer with an additional outer membrane composed of lipopolysaccharide (LPS). The chemical difference in bacterial cell wall is identified with the Gram stain. The Gram stain is the stain most frequently used; to identify unknown bacterial cultures, because it can provide information on gram reaction, cell size, cell shape, and cell arrangement.

During the Gram-staining procedure, all bacteria are stained purple by crystal violet, the primary stain. Bacterial cells that have a thick peptidoglycan layer retain the crystal violet during subsequent decolourisation and counter stain steps. These bacteria appear purple when viewed under the microscope and are referred to as gram-positive. Bacterial cells that have a thin peptidoglycan layer lose the crystal violet colour during the decolourisation step, and take up the colour of counter stain safranin. These bacteria appear red when viewed under the microscope and are referred to as gram-negative.

Objectives

After doing this experiment you will be able to :

- learn about chemical and theoretical basis of differential staining procedures and the chemical basis of the gram stain;
- differentiate between the two principal groups of bacteria-gram-positive and gram-negative; and
- In this exercise, you will use the Gram stain on selected 18-24 hour bacterial cultures.

Study Guide/Prior Knowledge

For doing satisfactory work you must read the following before coming to laboratory.

- The B.Sc. Course (CBCS), Core Botany, Paper-I Biodiversity (Microbes, Algae, Fungi and Archegoniates), Block 1: Microbes; Unit 3 Bacteria: General Account and Economic importance.

3.2 METHOD OF STUDY

You will conduct the exercise by using Gram stain on bacterial cultures.

3.3 MATERIALS REQUIRED

Materials

Crystal violet stain,

Gram's iodine,

Safranin stain,

95% Ethyl alcohol

Blotting paper,

Glass slides/microslides,

Distilled water,

Wash bottles

Pasteur pipettes,

Inoculating loop (metal loop),

Bunsen burner/spirit lamp,

Cultures to select from (18-24 hour broth or agar)

*Bacillus cereus (*Gram-positive rod)

Enterobacter aerogenes (Gram-negative rod)

Enterococcus faecalis (Gram-positive coccus)

Escherichia coli (Gram-negative rod)

Neisseria sicca (Gram-negative coccus)

Proteus vulgaris (Gram-negative rod)

Pseudomonas aeruginosa (Gram-negative rod)

Staphylococcus epidermidis (Gram-positive coccus)

Or smear of root nodule, curd/soil water

3.4 METHOD

Sterilise the inoculating loop before using it, by heating in the flame of a Bunsen burner lamp and then cooling.

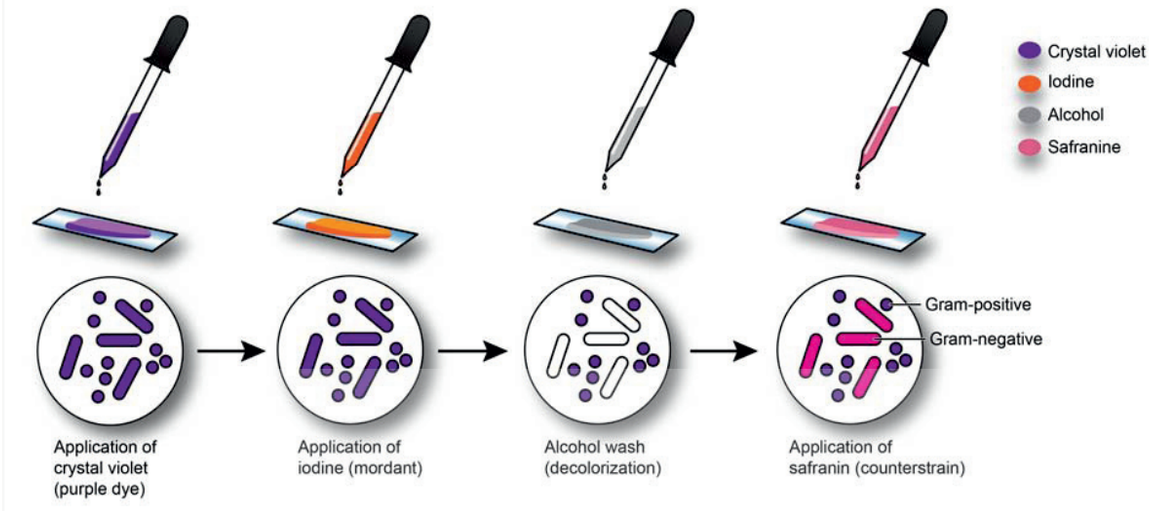


Fig. 3.1: Gram stain procedure.

1. Transfer a loopful of bacterial culture provided to you by your counsellor on a clean glass slide. After transfer of the culture on the slide again re-sterilise the loop before setting it down on the working table.
2. Make a bacterial culture smear by spreading the bacterial culture on the slide with the help of another slide. Allow the smear to air-dry. Pass this air dried smear through the Bunsen burner flame, 4 to 6 times in order to heat fix the bacteria. Because of heat fixing the bacterial enzymes are denatured and so autolysis is prevented. Also the bacterial cells adhere to the slide because of heat exposure.
3. With the help of a Pasteur pipette add a drop of crystal violet stain solution (called primary stain) on the fixed bacteria smear. After one minute, wash off the excess stain with tap water. You will observe that the bacteria are stained purple.
4. Next apply by means of a dropper, a drop of iodine solution (Gram's iodine also called mordant) to the fixed bacterial smear that has earlier been stained with crystal violet stain. Iodine solution intensifies the ionic bond between the crystal violet stain and the bacteria. After one minute wash off the excess stain with tap water.
5. After this with the help of a dropper apply a drop of 95 % ethyl alcohol which acts as decolorising agent for the primary stain i.e. crystal violet. After one minute wash off the excess alcohol with tap water.
6. The decolorising agent may wash out the crystal violet stain from bacterial smear (decolorizing it). If decolorisation occurs then this indicates that the smear contains gram negative bacteria. However if decolorisation does not occur and the bacterial smear retains the deep violet colour due to crystal violet stain then this indicates that the smear is unaffected by the decolouring agent and contains gram positive bacteria.
7. The decolorised smear consisting of gram negative bacteria is further stained. Apply a drop or two of the safranin stain with help of a dropper which is called a secondary stain or counter stain. After forty five seconds wash off the excess stain with tap water. Blot dry the slide with blotting

paper. The decolorised bacterial smear consisting of gram negative bacteria will be stained red due to counter staining.

3.5 OBSERVATIONS

8. You can observe your slides under a compound microscope under oil immersion to see the difference in the staining of bacteria.
9. Classify the given bacteria under study as Gram (+) or Gram (-)

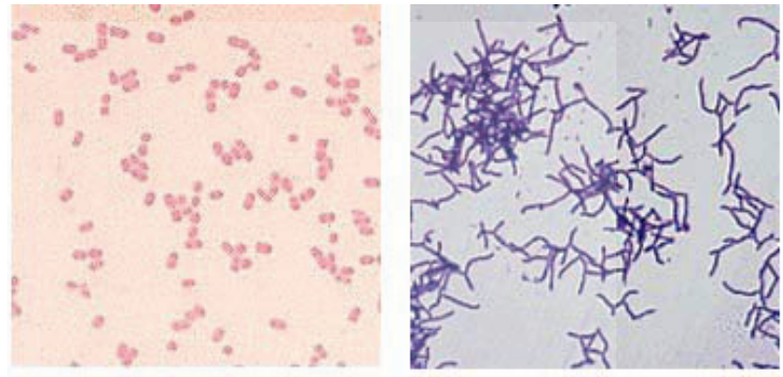


Fig 3.2: Showing: a) gram negative and b) gram positive bacteria after staining. (https://www.tumblr.com/search/gram%20%20stain).

EXERCISE 4

NOSTOC, CHLAMYDOMONAS, OEDOGONIUM VAUCHERIA, FUCUS AND *POLYSIPHONIA*

Structure

4.1 INTRODUCTION

Algae represent a heterogenous group of organisms that share some common characteristics. Traditionally, along with fungi and lichens, algae form a division termed **Thallophyta**. Along with bryophytes and pteridophytes they are collectively called cryptogams (flowerless or seedless plants). Under the modern system of classification, they are placed in the kingdom Protista along with protozoa, and not classified as plants. The organisms placed in the group algae differ in their form; photosynthetic pigments; and food reserves. However, the photosynthetic pigment chlorophyll a occurs universally in all kinds of algae. Sex organs in algae are unicellular and even if multicellular, they

are without any sterile cover. The bodies of algal forms are not differentiated into roots, leaves and stems. Algae are both prokaryotic and eukaryotic. The prokaryotic group is placed along with bacteria in separate kingdom **Monera**. Such algae are also designated as cyanobacteria - the blue-green algae. As a group algal forms are widely distributed and they inhabit almost all kinds of habitats such as: freshwater, marine water, brackish water, snow, deserts, hot springs, soil, and as symbionts. They exhibit diverse patterns of alternation of generations. Planktonic algae, probably, constitute the largest reservoir of carbon and are also very important, essential, and fundamental component of all kinds of food-chains. For the classification followed in these exercises, you are advised to consult the Section 6.4; Unit 6 of your theory course.

Objectives

After doing these exercises you should be able to:

- appreciate the range of morphological and reproductive features exhibited by the different forms of algae;
- examine a specimen of an alga with a hand lens/ dissection microscope/compound microscope and describe its morphological and cellular features;
- know and use the appropriate staining and mounting media for making temporary preparations to study algal forms;
- develop skills to distinguish unicellular, tubular, filamentous and polysiphonous forms of algae;
- diagrammatically represent the vegetative and reproductive characteristics as viewed under the compound microscope through photographs or electron photographs and label the structures appropriately; and
- identify the genera of algae under investigation based on the characters studied in theory course.

Study Guide/Prior Reading

For doing satisfactory work, we advise you to read the following before coming to the laboratory:

B.Sc. UGC CBCS, Core Course Botany Paper – I Biodiversity (Microbes, Algae, Fungi and Archegoniates).

Units 5, 6 and 7, and Sections 6.1 Introduction, 6.2 Range of organization, 6.3 Reproduction, 6.4 Classification.

You are also advised to read Section 7.2 for life cycles of different algal forms.

4.2 *NOSTOC*

4.2.1 Introduction

Nostoc is a very common terrestrial and aquatic blue-green alga. It occurs abundantly after the first few rains. It can be collected from water pools, paddy fields, water-logged soil, stagnant water bodies, and moist rocks. It grows as

an epiphyte on aquatic weeds. It lives as an endophyte inside the bryophytes (*Blasia* and *Anthoceros*), a fern (*Azolla*) and a gymnosperm (coralloid roots of *Cycas*). It also forms a symbiotic association with fungi within lichens. The mucilage surrounding *Nostoc* filaments form ball-like structures. Structurally *Nostoc* represents a typical photosynthetic prokaryotic cell.

Objectives

After doing this exercise you should be able to:

- observe a mucilaginous *Nostoc* colony;
- prepare a temporary stained preparation of *Nostoc* filaments;
- study and draw a contorted, beaded, unbranched, uniseriate filament/ trichome of *Nostoc* as observed in the preparation you make and/ or through a permanent slide;
- diagrammatically represent its prokaryotic characteristics with the help of an electron micrograph / a representative labelled sketch of a vegetative cell of *Nostoc*;
- locate, observe, study and draw a heterocyst with special reference to its position in a *Nostoc* filament;
- study the structure of an akinete and differentiate it from a vegetative cell and a heterocyst;
- compare the structures of a vegetative cell, a heterocyst and an akinete of *Nostoc*; and
- record your observations and write its taxonomic classification with identification points.

Study Guide/Prior Reading

For doing satisfactory work you must read the following before coming to the laboratory:

B.Sc. UGC CBCS, Core Course Botany Paper – I Biodiversity (Microbes, Algae, Fungi and Archegoniates)

Unit 6 Algae: Organization, Reproduction and Classification; Section 6.2 Range of Organisation, Sub-section 6.2.1 Structure of an algal cell: Prokaryotic and Eukaryotic forms; Section 6.4 Classification, Sub-section 6.4.1 Division Cyanophyta, and Unit 7 Algae: Morphology and Life Cycles, Sub-section 7.2.1 *Nostoc.*

4.2.2 Method of Study

You shall conduct the exercise by observing a stained temporary preparation. Carefully study, draw and describe the structure of a *Nostoc* filament, vegetative cell, heterocyst, and akinete. You may use safranin - glycerine or cotton blue - lactophenol as staining - mounting medium combinations for preparation of temporary slides. You will observe the electron micrograph / an illustration of prokaryotic cell structure of a photosynthetic blue green alga the *Nostoc*, make suitable labelled sketches of the observations made, you will make a written report of your observations and assign *Nostoc* to various ranks of taxa of classification.

4.2.3 Materials Required

In addition to *Biology Laboratory Student's Kit* you require the following:

Plant Material:

(A) Fresh/Fixed materials of *Nostoc* balls/colonies.

(B) Permanent slides of *Nostoc* trichomes showing heterocyst and akinetes.

(C) An electron micrograph of *Nostoc* vegetative cell and/or a well-labelled sketch of electron micrograph of a *Nostoc* cell.

Chemicals: Cotton blue, lactophenol, safranin (0.5%), and glycerine (1.0%).

Glassware and Apparatus: Dissection microscope, compound microscope, macroslides, microcover slips, petridishes, and watch glasses.

4.2.4 Procedure

Preparation of temporary-stained slides

Nostoc filaments should be spread out evenly in thin layers on a slide with the help of a needle and forceps. With a fine forceps transfer a few strands to a drop of water on a clean slide. Stain with cotton blue/safranin. Drain-out the excess stain and put a drop of lactophenol/glycerine respectively. Spread-out material to get a thin layer of filaments. Put a microcover slip and observe under a compound microscope. You may use a magnifying glass/dissection microscope to help you to prepare the slide. Observe, study, draw, label the structures you observe and write a report.

1. Observe the permanent slide of *Nostoc* filaments (w.m.) and/or electron micrograph/diagrammatic sketch of the same. Draw, label and write a report.
2. Prepare a comparative chart/table of the observations made by you of the structure of a vegetative and a heterocyst cell and an akinete.
3. Provide a classification of *Nostoc* by assigning it to various ranks of taxa of classification and mention points for its identification.

4.2.5 Observations

Refer to Fig. 4.1.

(A) Study of Vegetative Thallus

i) Colony

Observe the following characteristics:

(1) Mucilaginous structures in the form of a smooth, round ball. The form could be warty, lobed or leaf-like.

(2) Spread within a gelatinous mass lie a large number of contorted, beaded, entangled filaments of *Nostoc*.

ii) Trichome (filament)

Observe the temporary/permanent slide for the following characteristics:

(1) A trichome (filament) is uniseriate rows of cells.

(2) It is unbranched.

(3) It consists of a large number of cells.

(4) The individual cells are spherical, constricted at the cross walls providing the trichome an appearance of a string of beads.

(5) The cells are prokaryotic in organization. The nucleus and membrane-bound cell-organelles are not observed.

(6) The peripheral region of the cell is coloured.

(7) The central part of cell is relatively hyaline.

(8) A few shinning cyanophycean granules are observed in the peripheral part of the cell.

(9) All the trichome cells are similar in size and structure.

(10) The trichome may possess one or a few heterocysts in intercalary position. It may also possess akinetes.

iii) Vegetative Cell (Electron Micrograph)

Observe the electron micrograph/illustration of a vegetative *Nostoc* cell and observe its following aspects:

(1) The shape of the cell is spherical.

(2) It possesses a peptidoglycan-rich thick cell wall (made-up of polymer of N-acetylmuramic acid and N-acetyl glucosamine cross-linked by peptide and other compounds).

(3) A periplasmic space surrounds the peptidoglycan cell wall.

(4) A plasma-membrane surrounds the non-membranous organelles beneath the cell-wall.

(5) The cyloplasm is granular with several kinds of granules.

(a) Between thylakoids are present *glycogen granules* of different sizes;

(b) Protein-like polymers are located in non-membranous region of cytoplasm. These are called cyanophycean granules. They represent localized sites of N-storage.

(c) Granules rich in polyphosphate also occur in cytoplasm.

(d) Polyglucan granules, polymer of glucose, occupy the spaces between the thylakoids.

(e) Big crystals made up of polyβ hydroxybutyrate may also be observed.

(f) Polyhedral crystalline bodies-called carboxysomes are abundantly present. They possess the enzyme RuBisCO.

(6) *Pigmented membranes* (no chloroplasts) occupy peripheral region of the cell. It is called chroniatoplasm.

(7) Within the double membranes of chroniatoplasm are located photosynthetic pigments *chlorophyll a* and *phycobiliproteins*.

(8) On the surface of thylakoids are rows of *granules rich in phycobilisomes containing phycoerythrin and phycocyanins*.

(9) *70s ribosomes* are scattered in the cytoplasm in large numbers.

(10) *Gas-vesicles* are dispersed in cytoplasm. These are bounded by single proteinaceous layer impermeable to gases but permeable to water.

(11) The central region of cell is occupied by *nucleoplasm*. It possesses a network of DNA fibrils. It constitutes the ring of circular chromosome. There are a number of copies of DNA fibrils.

Study of Reproductive Structures

i) Akinetes

Observe the permanent slide of *Nostoc* colony showing akinetes. Look for the following characteristics:

(1) Akinetes are developed in a mature colony.

(2) They occur in large numbers, in series between two heterocysts of a trichome.

(3) Usually all the vegetative cells between two successive heterocysts in a trichome develop into akinetes. They are larger than the vegetative cells in size.

(4) The cells are thick-walled. They could be possessing ornamented cells-walls.

(5) They are rich in food reserves and cyanophycean granules.

(6) They help to form new trichomes under favourable conditions.

(7) They are also called resting spores of *Nostoc*.

ii) Heterocyst

Observe the heterocyst under a compound microscope and/or in an electron micrograph for the following characteristics:

(1) These are cells larger than vegetative cells in size.

(2) They occupy intercalary position.

(3) Cells are double-walled, pale yellow.

(4) The cell wall is multilayered. The innermost layer is made up of glycol-lipids and is impermeable to oxygen.

(5) Very fine plasmodesmatal connections are present at two poles of the cells where they join the vegetative cells.

(6) Large shiny polar granules, at both the ends.

(7) Many photosynthetic lamellae occupy the space of the cells but are less dense than in vegetative cell.

(8) Polyphosphate granules and glycogen granules, so characteristic of vegetative cells are absent.

4.2.6 Classification and Identification

Kingdom - Monera	:	1) Nucleus absent. 2) Membrane-bound cell organelles absent.
Division - Cyanophyta	:	1) Photosynthetic prokaryotes. 2) Release O_2 during photosynthesis. 3) Photosynthetic pigments diffused, blue-green.
Class - Cyanophyceae (Myxophyceae)	:	1) Photosynthetic reserve cyanophycean starch. 2) No sexual reproduction.
Order - Nostocales	:	1) Thallus with trichomes. 2) Trichomes unbranched or branching false. 3) Hormogones, heterocysts, expospores, and endospores generally present.
Family - Nostocaeae	:	1) Trichomes simple, unbranched, uniseriate and approximately of same diameter throughout. 2) Heterocyst present. 3) Akinetes present. 4) Trichomes undifferentiated.
Genus - *Nostoc*	:	1) Curved or bent trichomes. 2) Trichomes curved/constricted at cross walls, appear as strings of beads. 3) Trichomes embedded in a stiff mucilage of definite form. 4) Mucilage forms could be round, lobed, leafy, warty. 5) Heterocyst intercalary and single.

4.3 *CHLAMYDOMONAS*

4.3.1 Introduction

The genus *Chlamydomonas*, an unicellular freshwater, motile, microscopic, eukaryotic alga. It is one of the simplest life forms. More than 500 species of *Chlamydomonas* are found in ponds, pools, lakes and water bodies rich in nitrogenous substances. Some species inhabit moist soils.

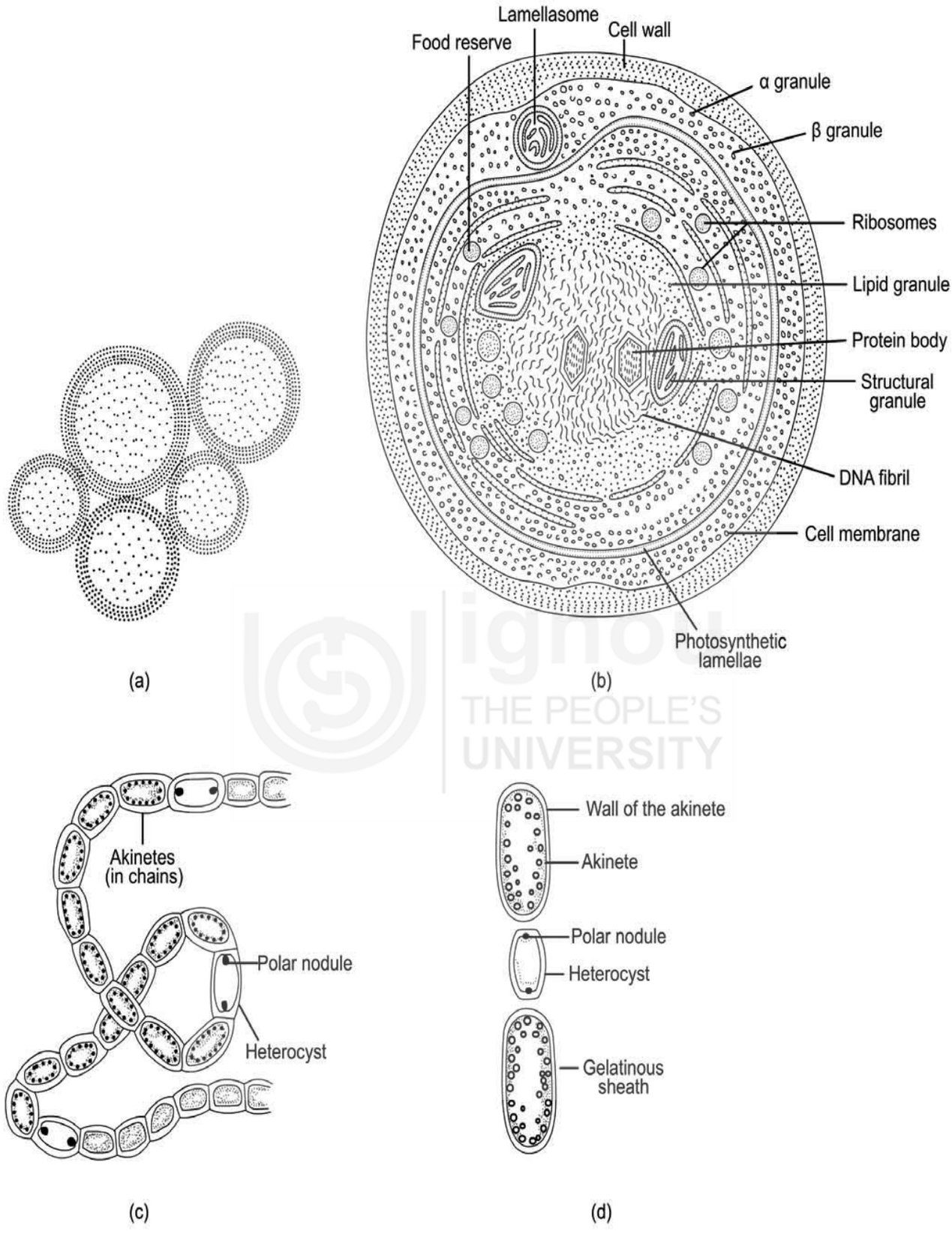


Fig. 4.1 : *Nostoc* : (a) *Nostoc* colony; (b) Diagrammatic representation of ultrastructure of a vegetative cell; (c) A trichome with heterocyst, akinete and vegetative cells; (d) Heterocyst and Akinete enlarged.

Chlamydomonas reproduces asexually by the formation of zoospores, aplanospores and has a palmella stage. Sexual reproduction in *Chlamydomonas* could be isogamous, anisogamous or oogamous.

Objectives

After doing this exercise, you should be able to:

- describe the ultrastructure of the thallus of *Chlamydomonas*;
- draw the morphological characteristics of a vegetative *Chlamydomonas* thallus;
- identify, study and differentiate various kinds of cell organelles in a *Chlamydomonas* cell;
- explain and list the unique characteristics of a *Chlamydomonas* cell; and
- record your observations, write its salient taxonomic characteristics and write its taxonomic classification with identification points.

Study Guide/Prior Reading

You are advised to read the following before coming to the laboratory:

B.Sc. UGC CBCS, Core Course Botany Paper – I Biodiversity (Microbes, Algae, Fungi and Archegoniates).

Unit 6 Algae: Organization, Reproduction and Classification, Section 6.2 Range of Organization, Sub-section 6.2.1 Structure of an algal cell: Prokaryotic and Eukaryotic forms, Section 6.4 Classification, Sub-section 6.4.4 Division Chlorophyta; and Unit-7 Algae: Morphology and Life Cycles, Section 7.2.2. *Chlamydomonas.*

4.3.2 Method of Study

- You shall study the ultrastructure of vegetative *Chlamydomonas* thallus by observing electron micrograph(s) of the thallus and its organelles. You may also take the help of diagrammatic labelled sketches of the *Chlamydomonas* vegetative cell and its organelles.
- Draw, label, describe and comment on the morphological details as observed by you and write the classification of *Chlamydomonas*.

4.3.3 Materials Required

In addition to *Biology Laboratory Student's Kit* you would require:

Plant Materials

i) Electron micrograph of vegetative thallus of *Chlamydomonas*.

ii) Electron micrograph of cell organelles of *Chlamydomonas* especially chloroplast with pyrenoid.

iii) Labelled sketches of the ultrastructure of vegetative thallus and/or cell organelles of *Chlamydomonas*.

4.3.4 Procedure

Study structure of the vegetative cell through Electron Micrographs/ diagrammatic sketches.

4.3.5 Observations

Refer to Fig. 4.2.

Study of vegetative cell

Observe and look for the following characteristics in an electron micrograph/or diagrammatic sketch.

(1) The thallus is unicellular.

(2) The thallus body is pyriform, globose or oval in shape.

(3) The anterior-end is papillate and the posterior end is broader.

(4) A distinct cell-wall surrounds the protoplast. The cell wall is multilayered and cellulosic and is made up of fibrils.

(5) The protoplasm is encircled by plasma membrane - the plasmalemma.

(6) A large, cup-shaped chloroplast is situated at its posterior end.

(7) One or two proteinaceous pyrenoid(s) are found within chloroplast.

(8) The pyrenoid is surrounded by starch/starch grains, i.e., food reserves stored within the chloroplast.

(9) Pyrenoid(s) may possess photosynthetic thylakoids traversing the matrix of pyrenoid or such thylakoids are closely associated with pyrenoids.

(10) Two or three rows of fatty red granules lie at the anterior side of the chloroplast. It is called stigma or eye-spot. The eye spot is photo-sensitive.

(11) Two anteriorly placed whiplash-type (smooth surfaced) flagellae are present on either side of the papilla.

(12) Each of the flagellae has a granular structure, with blepharoplast at the base.

(13) A thin and soft thread connects blepharoplast to the centrosome of the nucleus. This is termed as rhizoplast.

(14) A transverse filament connects the two blepharoplasts. This is called paradesmos.

(15) The two blepharoplasts, the paradesmos, the rhizoplast and the centrosome together constitutes the neuromotor apparatus associated with flagella.

(16) The cell possesses numerous mitochondria, ribosomes and endoplasmic reticulum network.

(17) Two contractile vacuoles, one each at the base of a flagellum, are located in the cell.

4.3.6 Classification and Identification

Kingdom: Protista (Protoctista)	:	1) Eukaryotic. 2) May be uni-/multi-cellular.
Division: Chlorophyta	:	1) Chlorophyll *a* and *b* present, is grass- green. 2) Food stored as starch. 3) Starch stored within chloroplasts. 4) Chloroplast usually possesses pyrenoid(s). 5) Locomotion through flagella/by secretion of mucilage. 6) Photoactive movement through eyespot/stigma present in chloroplast. 7) Stigma red/orange-red due to presence of carotenoid.
Class: Chlorophyceae	:	1) Flagella-anterior, equal in length. 2) Rhizoplast present. 3) Possess enzymes: glycolate dehydrogenase and urea amidolyase.
Order: Volvocales	:	1) Vegetative cells flagellated and motile 2) Uni-/multi-cellular 3) If multi-cellular, the number of cells in multiple of 2. 4) Protoplast with contractile vacuoles.
Family: Chlamydomonaceae	:	1) Unicellular. 2) Chloroplast cup-shaped.
Genus: *Chlamydomonas*	:	1) Oval or pyriform thallus. 2) Thallus unicellular, biflagellate and uninucleate. 3) Chloroplast cup-shaped. 4) With or without prominent anterior eye-spot in chloroplast. 5) Formation of palmella-stage. 6) Cellulosic cell wall surrounds the protoplast. 7) A central pyrenoid.

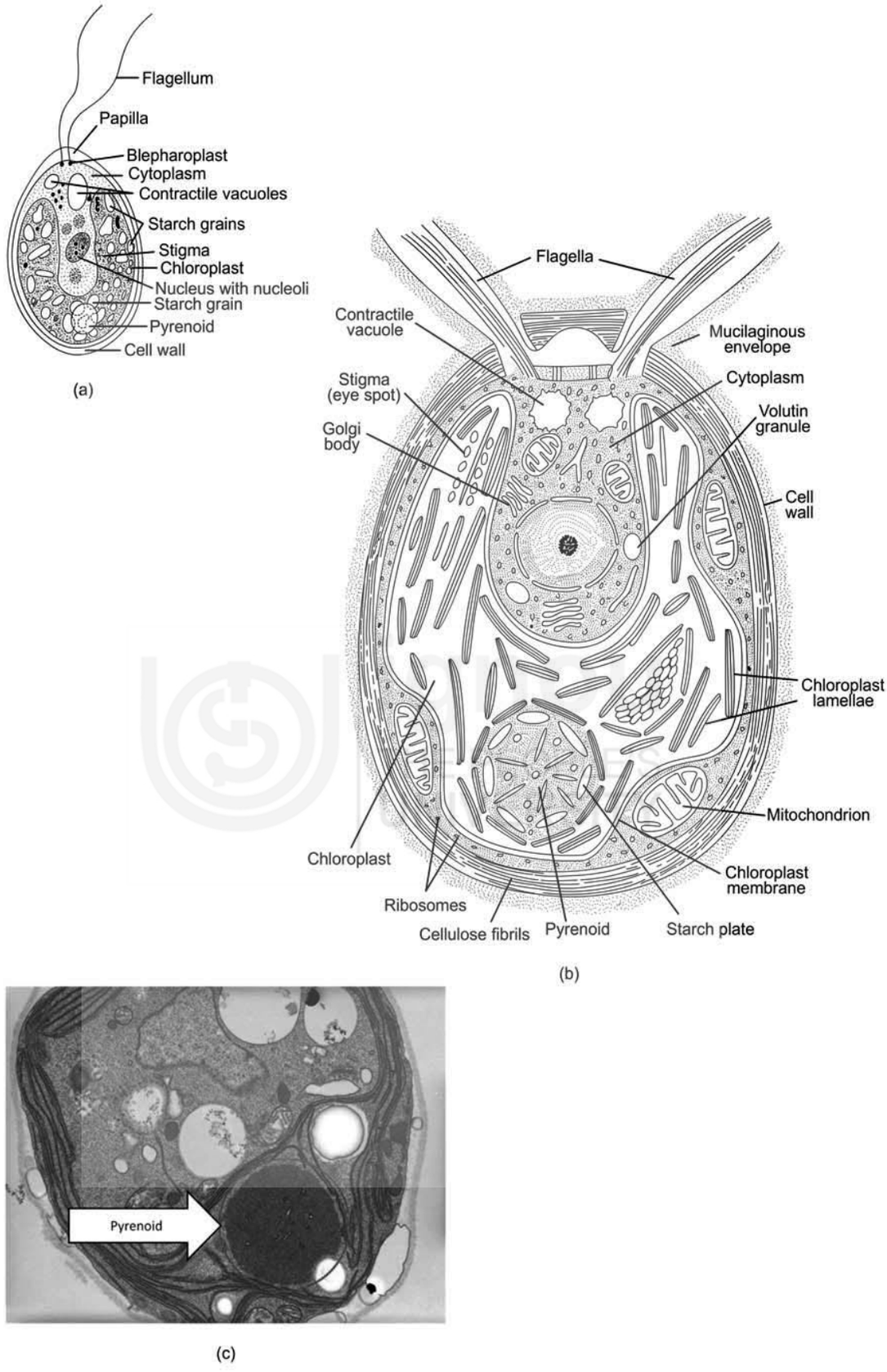


Fig. 4.2 : *Chlamydomonas*. (a) Vegetative cell structure (diagrammatic). (b) Diagrammatic representation of ultrastructure of a vegetative cell. (c) structure of a pyrenoid. Source: (c) https://cambridgecapp.wordpress.com/improving-photosynthesis/pyrenoid/

4.4 *OEDOGONIUM*

4.4.1 Introduction

Oedogonium, a green alga is mostly aquatic and occurs abundantly in small permanent and semi-permanent bodies of water such as pools and ponds. The filaments of *Oedogonium* may form a free-floating mass or are attached to stones. Species also occur widely as epiphytes on water plants or even on other larger algal forms. This genus is characterized by the presence of caps in the cells of filaments, and by homo- or heterothallic oogamous mode of sexual reproduction.

Objectives

After doing this exercise you should be able to:

- prepare stained-temporary preparations of vegetative and reproductive filaments of *Oedogonium*;
- study the morphology of the vegetative thallus of *Oedogonium*;
- differentiate between apical cell, intercalary cells, cap cells and holdfast cell of an *Oedogonium* somatic filament;
- observe and identify the reproductive structures, antheridium and oogonium in *Oedogonium*;
- identify whether the given reproductive *Oedogonium* filaments are macrandrous or nannandrous; and
- write your observations and list the unique characteristics of the genus *Oedogonium*, write its classification and list its identification points.

Study Guide/Prior Reading

For doing satisfactory work, you must read the following before coming to the laboratory:

B.Sc. UGC CBCS, Core Course Botany Paper – I Biodiversity (Microbes, Algae, Fungi and Archegoniates).

Unit 6 Algae: Organization, Reproduction and Classification, Section 6.2 - Range of Organization, Sub-section 6.2.1 Structure of an algal cell: Prokaryotic and eukaryotic forms, Section 6.4 Classification, Subsection 6.4.4 Division Chlorophyta. Unit 7 Algae: Morphology and Life Cycles, Subsection 7.2.3 *Oedogonium*.

4.4.2 Method of Study

You shall conduct this exercise by the following methods:

- study of temporary-stained preparations of vegetative and reproductive filaments of *Oedogonium*;
- study of permanent slides of vegetative and reproductive filaments of *Oedogonium*;
- make neat, labelled sketches of its various features observed under a compound microscope;

Structure

- write explanatory notes on the observations made; and
- mention the identification points of *Oedogonium* and assign this genus to various ranks of taxa of classification.

4.4.3 Materials Required

In addition to *Biology Laboratory Student's Kit* you would require:

1. Plant Materials

(A) Fresh Material – *Oedogonium* filaments: vegetative and sexual.

(B) Fixed Material – Oedogonium filaments: vegetative, macrandrous, nannandrous, and oogonial.

(C) Permanent Slides – *Oedogonium*: vegetative, macrandrous, nannandrous, and oogonia.

2. Stains and chemicals – safranin (0.5%), glycerine 1.0(%), I_2KI 1.0(%), tap/ distilled water.

3. Glassware and Apparatus – dissection microscope, compound microscope, wash bottle, petridishes, watch glasses, macroslides, and microcoverslips.

4.4.4 Procedure

(1) Preparation of temporary-stained slides.

Pick-up strands of *Oedogonium* filaments. Spread them evenly on a clean macroslide. You may use dissection microscope. Soak out fixative or water with the help of a blotting paper. Put a drop of I_2KI/Safranin (1.0%) on the filaments. Soak out the staining material using a blotting paper. Cleanse with distilled water. Place a drop of glycerine over the filament. Put a micro-coverslip over it, avoid any air-bubbles. Remove/soak excess peripheral mounting medium.

(2) Observe under a compound microscope.

(3) Draw its structural details, label the parts and write explanatory notes.

(4) Write the classification of *Oedogonium* by assigning it to various ranks of taxa and mention its identification points.

4.4.5 Observations

Refer to Fig. 4.3.

(A) Study of vegetative thallus

Filament(s)

(1) Thallus is multi-cellular, unbranched filament.

(2) A filament has 3 distinct parts: apical, intercalary and basal.

Apical Cell

(1) A cell at the tip of the filament is termed apical cell.

(2) This cell is rounded at its distal, free surface.

Basal cell

(1) The basal cell functions as holdfast.

(2) The lower or proximal part of the holdfast is either finger-shaped or disc-like.

(3) The upper or distal part is mostly broad and rounded. The holdfast cell does not possess green-pigment and is colourless.

Intercalary cells

(1) The majority of the cells of the filamentous thallus that lie between apical and basal cells are called intercalary cells. They possess the features that are characteristic of the genus *Oedogonium*.

(2) The cells are cylindrical in shape.

(3) They possess 3 layered cellulosic cell walls.

(4) Inner to the cell-wall is protoplast characterized by the presence of a large, reticulate chloroplast which occupies the whole cell.

(5) A number of pyrenoids are present in a chloroplast each at the intersection of reticulum.

(6) The cell is uni-nucleate. The nucleus is held by thin, delicate cytoplasmic strands.

(7) Intercalary cells possess plasmodesmata in cross-walls separating them.

Cap Cells

(1) The mature and old intercalary cells of the filament show 'caps' at their distal ends.

(2) A number of caps may be observed in close proximity (for details of cap formation read Unit 7 Section 7.2.3.)

(B) Study of Sexual Reproductive Structures

The genus *Oedogonium* may be monoecious or dioecious. The sexual reproduction is oogamous. The monoecious or homothallic species produce male reproductive structures - antheridia and female reproductive structures - oogonia on the same filament, and they are macrandrous forms.

The dioecious or heterothallic species produce male sex organs - antheridia on a separate, smaller filament called dwarf male or nannandrium. The female sex-organ - oogonium is formed on normal filaments. The dioecious *Oedogonium* are called nannandrous.

i) Oogonium

(1) Oogonium is always present on larger, normal filament irrespective of the species being macrandrous or nannandrous.

(2) It is generally intercalary or terminal in position in a filament.

(3) It may be solitary or may occur in a row of 2-3 or rarely more.

(4) An oogonium generally shows one or more cap cells at its upper (distal end). It suggests that a mature, older, vegetative cell matures into an oogonium.

(5) The shape of an oogonium is spherical or oval.

(6) The oogonial cell is relatively larger than the vegetative cells.

(7) At the base of every oogonium lies a small and flat daughter cell. This is termed supporting cell or suffultory cell.

(8) An oogonium bears a large ovum.

(9) Every oogonium has one small pore on one side - the receptive pore.

(10) Just opposite the receptive pore, the protoplast of the oogonium has a hyaline area. It is called receptive spot.

(11) The protoplast of an ovum is rich in reserve food.

ii) Antheridium (Macrandrous form)

(1) A large number of antheridia are formed together, in a chain as if in a series.

(2) These antheridia are intercalary in position.

(3) Each antheridium is a small, flat cell.

(4) At maturity within each antheridium there are 2 nuclei.

(5) Dense cytoplasmic contents surround each of these 2 nuclei.

(6) Each of such units ultimately metamorphose into a multi-flagellate antherozoid.

(7) These flagella occur in a whorl at one end, distal, of the antherozoid. They are called stephenokonts.

iii) Nannandrium (Dwarf-male)

(1) A dwarf-male is produced by the germination of androspore.

(2) Androspores are formed inside the androsporangia.

(3) An androsporangium produces one androspore. The androsporangia are formed on a larger filament that bears oogonia.

(4) Androsporangia form a long chain of small and flat cells, intercalary in position in a filament.

(5) One multi-flagellate androspore is produced per androsporangium.

(6) An androspore after germination produces a dwarf male or nannandrium.

(7) A dwarf-male remains attached to an oogonium or the suffultory cell of larger normal filament.

(8) Every dwarf-male has a sterile stalk cell and terminal row of 2-3 cells. Stalk cell helps in attaching to the oogonium or suffultory cell. Stalk cell's proximal end is disc-shaped or finger-like.

(9) Terminal 2-3 cells develop into antheridia.

(10) Each of these antheridia produces 2 multi-flagellate antherozoids which are stephenokonts.

iv) Zygote

(1) Zygote is a thick-walled post-fertilization structure. The cell wall is three-layered.

(2) The zygote may appear smooth, ornamented or verrucose.

(3) Zygote accumulates reddish oil drops as reserve food.

4.4.6 Classification and Identification

Kingdom: Protista (Protoctista)	:	1) Eukaryotic. 2) May be uni-/multi-cellular.
Division: Chlorophyta	:	1) Chlorophyll a and b present, is grass-green. 2) Food stored as starch. 3) Starch stored within chloroplasts. 4) Chloroplast usually possesses pyrenoid(s). 5) Locomotion through flagella/by secretion of mucilage. 6) Photoactive movement through eyespot/stigma present in chloroplast. 7) Stigma red/orange-red due to presence of carotenoid.
Class: Chlorophyceae	:	1) Flagella-anterior, equal in length. 2) Rhizoplast present. 3) Possess enzymes: glycolate dehydrogenase and urea amidolyase.
Order: Oedogoniales	:	1) Filamentous thallus, branched/ unbranched. 2) Cells uninucleate. 3) Unique kind of cell division resulting in the formation of caps. 4) Oogamous sexual reproduction. 5) Zoospores, antherozoids with whorls of flagella. 6) Produce dwarf males.

Family: Oedogoniaceae	:	1) Chloroplast reticulate. 2) Many pyrenoids per chloroplast. 3) Pyrenoids at intersection of reticulum. 4) Plasmodesmata in between cells. 5) Cell division involves break-down of parental cell wall resulting in cap-cell formation.
Genus: *Oedogonium*	:	1) Unbranched filamentous. 2) Cell division initiated by formation of a ring in the upper part of the cell. 3) Asexual reproduction through multi-flagellate zoospores. 4) Formation of solitary zoospore in a cell. Zoospore formed usually in a cell that possesses cap(s). 5) Sexual reproduction oogamous. 6) Oogonium with a suffultory sterile cell. 7) Could be macrandrous or nannandrous. 8) Antheridia in macrandrous filaments could be terminal or intercalary. 9) Zygote is separated from oogonial wall by a space.

Structure

4.5 *VAUCHERIA*

4.5.1 Introduction

Vaucheria, a yellow-green alga occurs in both aquatic and terrestrial habitats. The terrestrial species occur on damp soils of gardens, lake sides, ploughed fields, as an extensive green belt on soil surface especially when winter sets in. Large floating green mats are characteristic of aquatic species. Some characteristics of *Vaucheria* include: coenocytic tubular thallus, usually homothallic, curved antheridia, and oogonia with beaks. Similar to diatoms and brown algal forms they produce two unequal laterally inserted flagellae of different kinds. *Vaucheria* possesses chlorophyll a and c as principal photosynthetic pigments. The reserve food is deposited as lipid droplets.

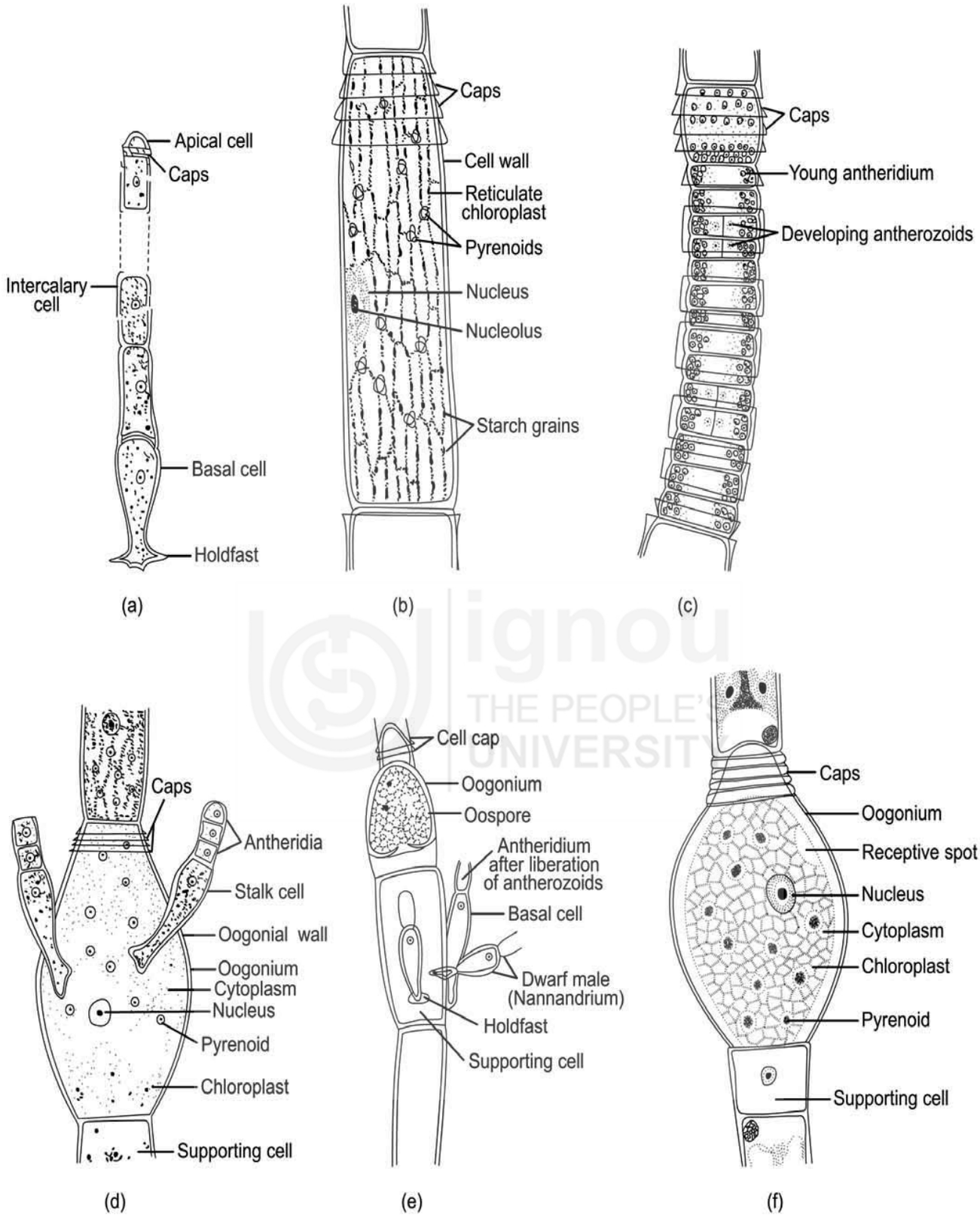


Fig. 4.3 : *Oedogonium*. a) A vegetative filament showing holdfast, intercalary cells, cap cells and apical cell; b) An intercalary cell with caps' c) A macrandrous male thallus; d) An oogonium with a few nannandrous filaments; e) Nannandrium filaments on a supporting cell; f) An oogonium.

Objectives

After doing this exercise you should be able to:

- prepare stained-temporary preparations of vegetative and reproductive thalli of *Vaucheria*;
- study the morphology of the vegetative thallus of *Vaucheria*;
- observe and identify the reproductive structures - antheridium and oogonium in *Vaucheria*;
- write your observations, prepare a list of the unique characteristics of the genus *Vaucheria* and write its classification by listing the identification points.

Study Guide/Prior Reading

For doing satisfactory work, you must read the following before coming to the laboratory:

B.Sc. UGC CBCS, Core Course Botany Paper – I Biodiversity (Microbes, Algae, Fungi and Archegoniates).

Unit 6 Algae: Organization, Reproduction and Classification, Section 6.2 Range of Organization, Sub-section 6.2.1 Structure of an Algal Cell: Prokaryotic and Eukaryotic forms, Section 6.4 Classification, Sub-section 6.4.9 Heterokontophyta, Class Xanthophyceae and Unit 7 Section 7.2 Algae: Morphology and Life Cycles, Sub-section 7.2.4 *Vaucheria.*

4.5.2 Method of Study

You shall conduct this exercise by the following methods:

- study the temporary-stained preparations of vegetative and reproductive thalli of *Vaucheria*;
- study of permanent slides of vegetative and reproductive thalli of *Vaucheria*;
- draw neat, and labelled sketches of the various features of vegetative and reproductive structures of *Vaucheria* observed under a compound microscope;
- write observations as explanatory notes; and
- list the identification points for *Vaucheria* and write its classification.

4.5.3 Materials Required

In addition to *Biology Laboratory Student's Kit* you would require:

1. **Plant Materials:**
   (A) Fresh/fixed thalli of *Vaucheria:* vegetative, sex organs, and zygote.
   (B) Permanent slides: *Vaucheria* thalli – vegetative, antheridium, oogonium, and zygote.
2. **Stains and Chemicals:** Safranin (0.5%), glycerine (1.0%), distilled water.

3. **Glassware and Apparatus:** Dissection microscope, compound microscope, micro slides, microcoverslips, watch glasses, and petridishes.

4.5.4 Procedure

(1) Preparation of temporary stained slides:

Pickup the thalli of *Vaucheria*, spread them evenly on a clean micro slide, you may use a dissection microscope. Soak out fixative or water with the help of a blotting paper. Put a drop of safranin on the thallus. Soak out the stain. Cleanse the thallus with distilled water. Place a drop of glycerine over the thallus. Put a microcoverslip, avoid any air bubble. Remove or soak the excess peripheral mounting medium.

(2) Observe under compound microscope.

(3) Draw labelled figure/s and write explanatory notes on the observations made.

(4) Provide the classification of *Vaucheria* and mention its identification points.

4.5.5 Observations

Refer to Fig. 4.4.

Observe the temporary, stained / permanent slide of vegetative thallus of *Vaucheria* under a compound microscope and look for the following features:

(A) Study of vegetative thallus

Thallus

(1) Thallus is unicellular, aseptate, multinucleate (coenocytic), filamentous and profusely branched.

(2) The branching is lateral but appears dichotomous.

(3) If the thallus is terrestrial, then a few colourless rhizoidal branches can be observed. Rhizoids penetrate the soil.

(4) The cell wall is two-layered. The outer layer is made up of pectose while the inner layer is cellulosic.

(5) A very large vacuole is present throughout the length of filament.

(6) The other cytoplasmic contents are peripheral to the vacuole.

(7) A large number of nuclei are found scattered in cytoplasm near the vacuolar membrane.

(8) A very large number of coloured chromatophores are also found scattered in cytoplasm beneath the cell wall.

(9) Chromatophores are circular or elliptical in shape and are not associated with pyrenoids.

(10) The reserve food material is in the form of small oil droplets.

B) Study of sexual reproductive structures

i) Antheridium

(1) Thalli are mostly monoecious. Some species could be dioecious. Draw only what you observe.

(2) When monoecious, an antheridium/antheridia is/are borne in close proximity of oogonium on the same filament.

(3) Antheridium is sessile, rarely it may possess a stalk.

(4) It is terminal, curved, hook-like and cylindrical.

(5) A distinct transverse septum is present in between an antheridium and the vegetative filament of the thallus.

(6) The multinucleate protoplast within the antheridium metamorphoses into a large number of biflagellate, uninucleate antherozoids.

(7) Antherozoids are liberated from the antheridium through a small-pore at its tip. You may observe an empty antheridium, if the antherozoids have already been liberated.

(8) Each antherozoid possesses two lateral, unequal flagellae. The anterior flagellum is longer and is of tinsel type. The posterior flagellum is shorter and is of whiplash type.

ii) Oogonium

(1) One-or more oogonium/oogonia is/are present at the tip of a small filament that emerges as a branch. Such filaments could be forked, bearing an oogonium each at the tip.

(2) The oogonium is oval or spherical in shape and possesses a small beak.

(3) The entire protoplast forms a single oosphere.

(4) Younger oosphere is multi-nucleate but at maturity it is always uni-nucleate.

(5) A small, colourless area known as receptive spot is produced near the beak. This spot is formed due to withdrawal of protoplasm from this area.

(6) Protoplast is rich in food reserves in the form of oil droplets.

iii) Zygote

(1) Zygote is a post-fertilization structure.

(2) It is present inside the oogonium.

(3) It is a thick-walled structure. The wall is 3-7 layered.

(4) The protoplast is very dense.

(5) It has food stored in form of oil droplets.

(6) The nucleus is diploid.

4.5.6 Classification and Identification

Kingdom: Protista (Protoctista)	:	1) Eukaryotic. 2) Uni-/multi-cellular organisms.
Division: Heterokontophyta	:	1) Motile swarmers with two unequal flagella - anterior one is long, tinsel-type while posterior one is smaller, whiplash type.
Class: Xanthophyceae	:	1) Chlorophyll a and c, and xanthophylls are present in the chromatophores. 2) Cell walls are cellulosic. 3) Mannitols and glucose accumulate in plastids. 4) Principal storage substances are β-1, 3,-linked glucan-Paramylon. Lipids in the form of oil droplets may also be present. 5) Thallus is uni-cellular, motile with two unequal flagella or is multi-nucleate, coenocytic and is branched or unbranched. 6) Zoospores are bilagellate. 7) Sexual reproduction is oogamous with biflagellate antherozoids.
Order: Vaucheriales	:	1) Thallus is multi-nucleate and coenocytic. Septae to demarcate sex-organs / zoosporangium.
Family: Vaucheriaceae	:	1) Chlorophyll a and c present. 2) Thallus is homothallic. 3) Oogamous sexual reproduction. 4) Spermatozoid with proboscis.
Genus: *Vaucheria*	:	1) Coenocytic, aqueous or terrestrial thallus. 2) 90 per cent of cell wall cellulosic. 3) Thallus with a large central vacuole. 4) A large number of elliptical chloroplasts lie at the periphery of protoplasm. 5) A very large number of nuclei present beneath the chloroplasts.

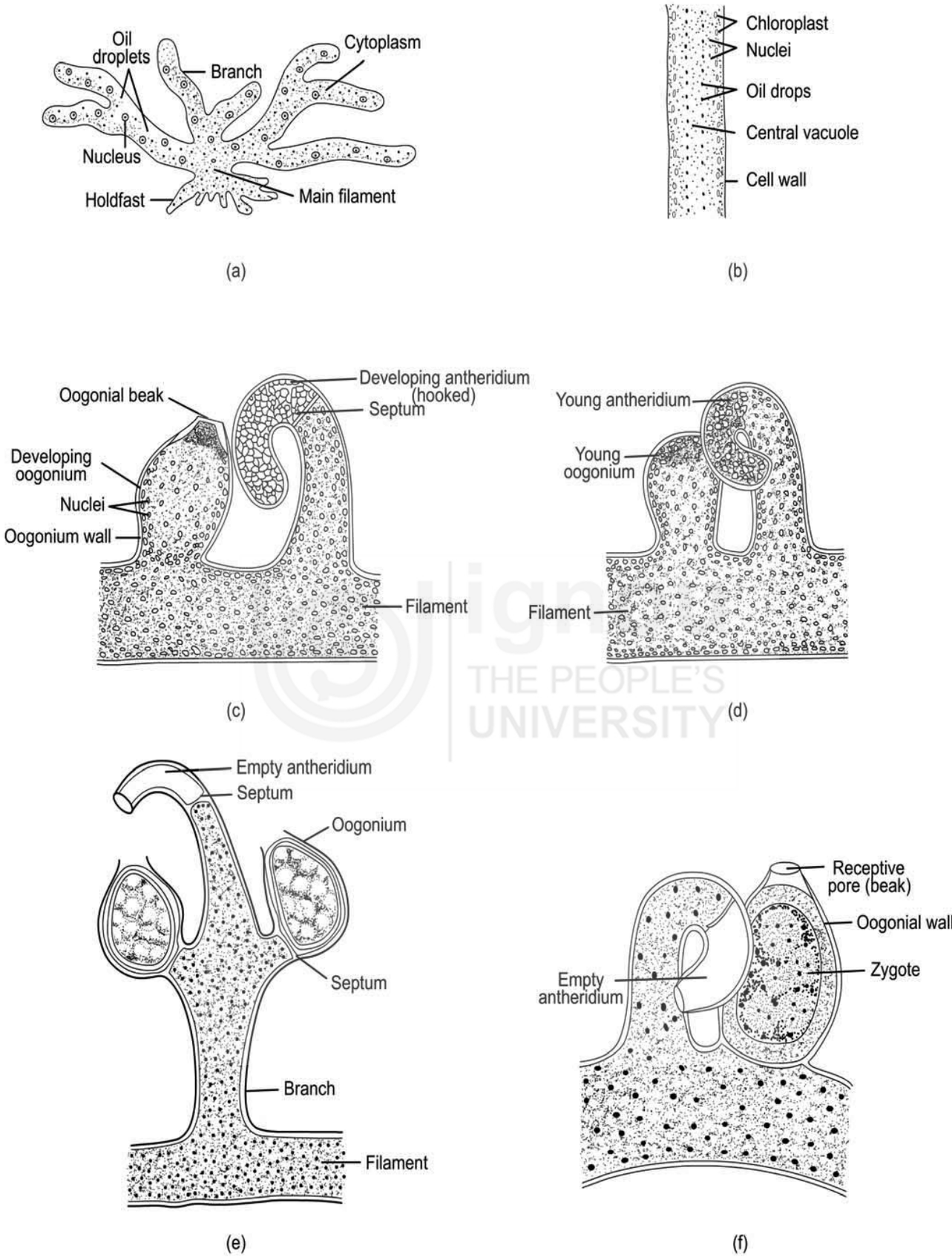


Fig. 4.4: *Vaucheria*. (a) A coenocytic, tubular filament with holdfast. (b) A part of the filament. (c-e) Antheridium and oogonium. (f) Empty antheridial cell and zygote.

6) Tip of the thallus is without chloroplasts.

7) Sexual reproduction is oogamous.

8) A large oil-rich egg cell is present in a beaked oogonium. Oogonium is cut off from thallus by a septum.

9) Antheridium is formed singly. It is a curved, cylindrical tube-like structure separated from thallus by a septum.

10) Antherozoid with two unequal, dissimilar flagella - anterior one is long, tinsel type and the posterior one is smaller, whiplash type.

4.6 *FUCUS*

4.6.1 Introduction

Structure

The genus *Fucus*, a brown alga belongs to division Heterokontophyta. It occurs attached to the rocks in the intertidal rocky coasts. The thallus of *Fucus* is multi-celled with distinct pseudo-parenchymatous tissue differentiation. Sex organs - antheridia and oogonia are located in very specialized structures. The life cycle is diplontic. Motility is restricted only to the male reproductive unit - the antherozoid. The two dissimilar, unequal flagella are laterally placed. The cells of the thallus possess algin and fucoidin in addition to cellulose in their cells walls. Chlorophyll a, c and carotenoids constitute the photosynthetic pigments. The ova of *Fucus* have been used extensively for research on polarity in plant physiological studies.

Objectives

After doing this exercise you should be able to:

- study a specimen of fresh / fixed, flat, dichotomous, vegetative thallus of *Fucus*, or from a herbarium sheet or a museum specimen;
- locate stipe, mid-rib, air bladders, cryptostomata, and holdfast / disc in a vegetative thallus of *Fucus*;
- observe, draw, and differentiate the various kinds to tissues that make up the thallus body with the help of permanent slides;
- observe and identify reproductive structures - receptacles in *Fucus*; and
- Record your observations and write its classification mentioning the identification points.

Study Guide/Prior Reading

For doing satisfactory work, you must read the following before coming to the laboratory:

B.Sc. UGC CBCS, Core Course Botany Paper–I Biodiversity (Microbes, Algae, Fungi and Archegoniates).

Unit 6 Algae: Organization, Reproduction and Classification, Section 6.2 Range of Organization, Sub-section 6.2.1 Structure of an Algal Cell: Prokaryotic and Eukaryotic forms, Section 6.4 Classification, Sub-section 6.4.9 Heterokontophyta, Class Phaeophyceae and Unit 7 Algae: Morphology and Life Cycles, Section 7.2 Morphology and Life Cycles, Sub-section 7.2.5 *Fucus.*

4.6.2 Method of Study

You shall conduct this exercise by the following methods:

- study of morphology of thallus by using herbarium / museum specimen of *Fucus*;
- study of the internal tissue organization of thallus by observing the permanent slides of mid-rib and wing of *Fucus*;
- study of the location and morphology of reproductive structures - receptacles in the thallus in the provided specimen; and
- study of the structures of male, female or hermaphrodite conceptacles through permanent slides, and making their labelled figures.

4.6.3 Materials Required

In addition to *Biology Laboratory Student's Kit* you would require:

Plant Materials

(A) Herbarium / museum specimens of vegetative thallus of *Fucus*.

(B) Herbarium / museum specimen of thallus of *Fucus* showing receptacles.

(C) Permanent slides: t.s. midrib, t.s. wing, t.s. receptacle - male, female or bisexual.

4.6.4 Procedure

(1) Observe, draw, label the structure of vegetative thallus of *Fucus* and study its holdfast, midrib, wing, cryptostomata, air bladders, and dichotomous branching.

(2) Observe and make a labelled drawing of the location, shape, number of receptacles in a reproductive thallus of *Fucus*.

(3) Observe permanent slides of wing and midrib of *Fucus* and make labelled drawings of its various tissues: cuticle, meristoderm, cortex, medulla, and hyphae.

(4) Observe, draw, label the shape of a receptacle. Locate and draw - its ostiole, periphyses, wall of conceptacle, oogonia, antheridial filaments with the help of permanent slides.

(5) Write explanatory notes for the various observations made by you.

(6) Write the classification of *Fucus* and mention the identification points.

4.6.5 Observations

Refer to Fig. 4.5.

(A) Study of Thallus

External Features

Study the herbarium / museum specimen provided to you and look for the following features:

(1) Thallus is flat and dichotomously branched.

(2) It is attached to the substratum by a disc-shaped holdfast.

(3) A stout midrib helps the thallus to stand erect from holdfast. The midrib is more pronounced in older regions than in younger regions of the thallus.

(4) Thallus bears a number of flat strap-like, dichotomously branched blades with smooth and entire margins. Blades are also called wings.

(5) Thallus may appear bloated / inflated at places due to the presence of air bladders.

(6) A number of small openings can be observed on the surface of the wing. These are openings of sterile conceptacles within the thallus. The openings are called cryptostomata or cryptoblasts.

(7) Fertile thalli become swollen at the terminal ends of the wings. These are called receptacles.

(8) Male, female or bisexual conceptacles are present within the receptacles.

Internal Structure

T.s. wing

(1) A mature wing is differentiated into meristoderm, cortex, and medulla.

(2) The peripheral layer of cells is covered with cuticle and is called meristoderm. It consists of rectangular and closely placed cells filled with dark brown plastids.

(3) Cortex is composed of relatively large cells, size of which gradually increases towards the centre (medulla). The outer cortical cells are more rich in plastids than the interior cells.

(4) The photosynthetic pigments are chlorophyll a, c and water-soluble fucoxanthin - a xanthophyll.

(5) The central portion of the wing is made up of narrow, filamentous, loosely arranged cells. The region is called medulla. The large intercellular spaces in medullar region are filled with mucilage.

(6) Running longitudinally along the medullary cells are elongated cells - hyphae. Hyphae provide mechanical support to the thallus.

T.s. midrib

(1) Internally midrib consists of three zones – meristoderm, cortex and medulla.

(2) Covered with cuticle small, rectangular, compactly arranged single peripheral cells constitute meristoderm. These cells are rich in plastids, the chromatophores.

(3) The cortex is a much wider zone. Compactly arranged at periphery the cells of cortex become loose and larger towards the centre.

(4) Medulla shows irregular and longitudinal arrangement of hyphae. They make a compact and solid centre.

(B) Study of Reproductive Structures

V.s. bisexual receptacle

(1) A receptacle appears biconvex in outline.

(2) In the body of thallus the receptacle has similar tissue differentiation as that of a wing.

(3) On either side of the receptacle and towards the periphery are present a number of pear-shaped cavities. These are broader at base and have small openings - the ostioles at the apex. Ostiole opens towards outside.

(4) The wall of receptacle is made up of a lightly woven network of hyphal and medulla cells.

(5) A large number of sterile, uni-seriate, multi-cellular, hair-like structures arise from the inner surface. These are called paraphyses. They are free except at the point of attachment. They also project outside through ostiole.

(6) From the base as well as from the broader inner surface of cavity arise male and female sex organs - antheridia and oogonia respectively.

(7) Antheridia are located on highly branched antheridial hairs or on short hairs arising from the wall of the conceptacle.

(8) An antheridium is stalked, unicellular, oval with double layer wall.

(9) An antheridium produces many pyriform, uni-nucleate and bi-flagellate antherozoids.

(10) The two flagella are unequal in length and are laterally inserted. The anterior of the two is longer in length and is of tinsel type. The shorter, whiplash flagellum is posterior in position.

(11) Oogonia arise singly directly from the wall of the conceptacle.

(12) Each oogonium is shortly stalked, globose, swollen and has three-layered thick wall.

(13) A mature oogonium produces eight oospheres or ova (eggs).

V.s. male receptacle

The male receptacle broadly possesses similar organization as observed in a bisexual conceptacle except that it produces only antheridia.

V.S. female receptacle

The female receptacle broadly possesses similar organization as observed in a bisexual conceptacle except that it produces only oogonia.

4.6.6 Classification and Identification

Kingdom: Protista	:	1) Eukaryotic. 2) Uni-/multi-cellular organisms.
Division: Heterokontophyta	:	1) Motile swarmers with 2 unequal laterally placed flagella. 2) Anterior flagellum is longer-tinsel type. 3) Posterior flagellum is shorter-whiplash type.
Class: Phaeophyceae	:	1) Multi-cellular, filamentous, pseudoparenchymatous or parenchymatous, small to very large thallus body. 2) Cell walls rich in cellulose, alginic acid and fucoidin. 3) Photosynthetic food storage material is principally laminarin. 4) Photosynthetic pigments in the chromatophores are chlorophyll a, c fucoxanthin, and carotenoids besides phaeophycean tannins. 5) Plasmodesmatal connections in between the cells of parenchyma (if present). 6) Zoospores and antherozoids are biflagellate. 7) Sexual reproduction iso- to oogamous.
Order: Fucales	:	1) Thallus parenchymatous with distinct apical cell. 2) Gametophytes reduced to egg cells and spermatozoids. 3) Sexual reproduction oogamous. 4) Antherozoids possess 2 equal laterally placed flagella. 5) Asexual reproduction is absent.
Family: Fucaceae	:	1) Apical cell of thallus is 4 sided in mature plant. 2) Thallus dichotomously branched and in one plane. 3) Thallus with midrib. 4) Thallus dilated at frequent intervals by conspicuous intercalary vesicles.

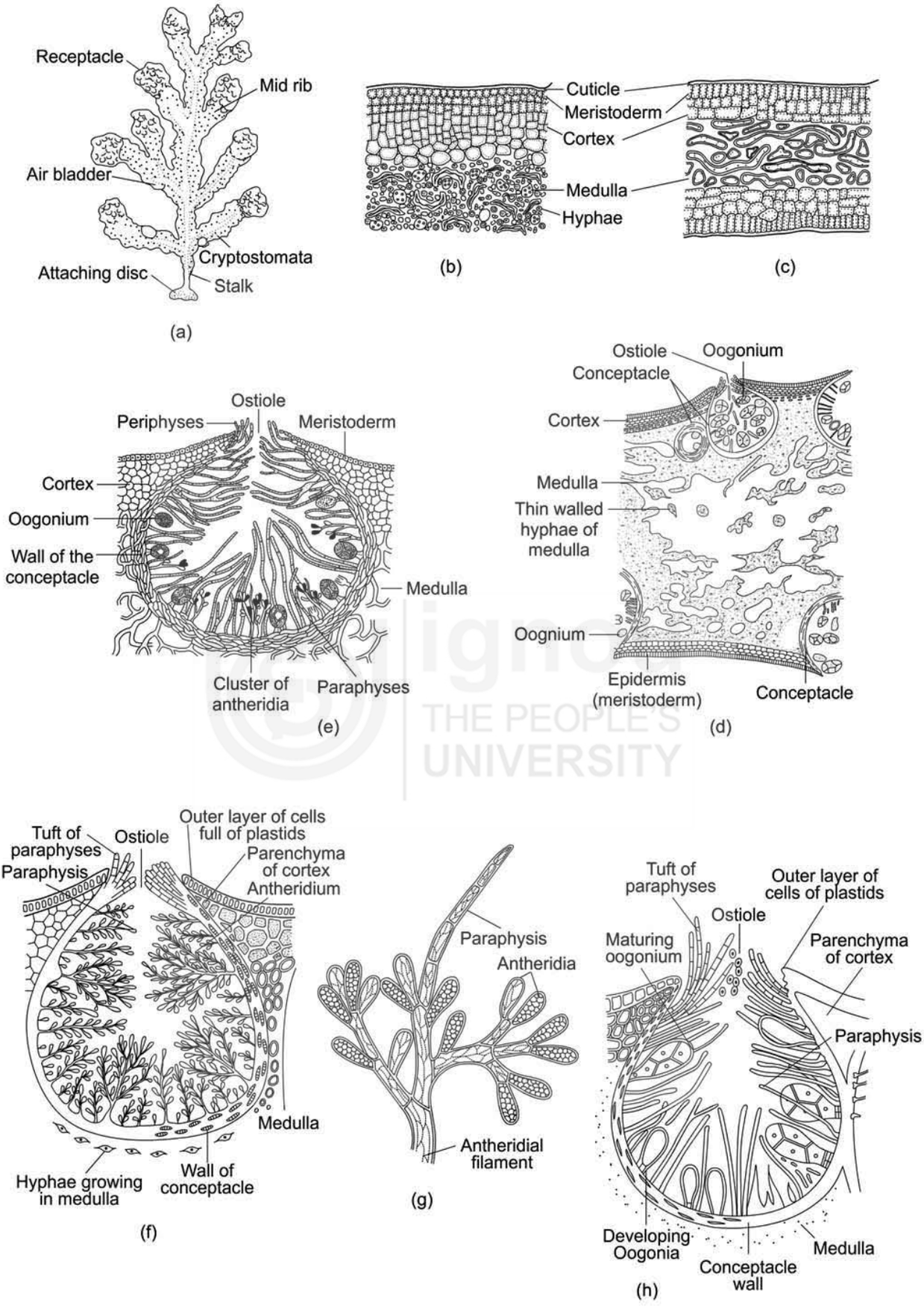


Fig. 4.5 (a-h): ***Fucus*****. (a) Habit sketch. (b) T.s. stipe, a part magnified. (c) T.s. blade, a part in enlarged view. (d) V.s. receptacle. (e) V.s. bisexual conceptacle. (f) V.s. male conceptacle. (g) An antheridial filament. (h) V.s. female conceptacle.**

5) Sex organs in conceptacles.

6) Oogonia with 8 oospheres.

Genus: *Fucus* : 1) Erect plant body.

2) Holdfast disciform or irregular.

3) Branching dichotomous or sub-pinnate.

4) Branches star-shaped with more or less distinct midrib.

4.7 *POLYSIPHONIA*

4.7.1 Introduction

The genus *Polysiphonia*, a red alga belongs to Division Rhodophyta. It is exclusively marine and lacks motility throughout its life cycle. It is commonly found along the Altantic and Pacific Coasts in littoral and sub-littoral regions. A few species are epiphytes on mangroves or brown algae. Some species of *Polysiphonia* are also found along the Indian coasts. The colour of its thalli ranges from red to dark purple due to the presence of the pigments r-phycoerythrin and r-phycocyanin.

Floridean starch is the principal assimilatory product. The multi-cellular branched thalli are polysiphonous and macroscopic. Chromatophores possess pyrenoids without surrounding sheath of starch grains. The male and female sex-organs are called spermatium and carpogonium respectively. Post-fertilization changes are complex and result in the formation of carposporophytes. The carposporophytes are parasitic on female thalli. The thalli are dioecious, and the tetrasporophyte represents diploid phase in the life cycle. Hence, the alternation of generations is triphasic and vegetative habit of all the three phases is isomorphic.

Structure

Objectives

After doing this exercise, you should be able to:

- identify this alga from its habit;
- differentiate the male haploid, female haploid and diploid tetrasporophytic thalli;
- observe the carposporophytic phase on a female thallus;
- prepare temporary preparations of *Polysiphonia* thallus representing all the 3 phases;
- observe, draw and label the branched and polysiphonous habit;
- observe and differentiate the apical cells, trichoblast, spermatium, carpogoniate filaments, and tetraspores through temporary-mounts or permanent slides in *Polysiphonia*;
- write your observations, the unique characteristics, and classification of *Polysiphonia* by indicating the identification points.

Study Guide/Prior Knowledge

For doing satisfactory work, you must read the following before coming to the laboratory:

B.Sc. UGC CBCS, Core Course Botany Paper–I Biodiversity (Microbes, Algae, Fungi and Archegoniates).

Unit 6 Algae: Organization, Reproduction and Classification, Section 6.2 Range of Organization, Sub-section 6.2.1 Structure of an Algal Cell: Prokaryotic and Eukaryotic forms, Section 6.4 Classification, Sub-section 6.4.3 Division Rhodophyta. Unit 7 Algae: Morphology and Life Cycles, Section 7.2 Morphology and Life Cycles, Sub-section 7.2.6 *Polysiphonia.*

4.7.2 Method of Study

You shall conduct this exercise by following methods:

- study the temporary-stained preparations of vegetative and reproductive thalli of *Polysiphonia*.
- study the permanent slides of vegetative and reproductive thalli of *Polysiphonia*.
- draw neat and labelled sketches of various features of vegetative and reproductive structures of *Polysiphonia* observed under a dissection microscope and compound microscope.
- write explanatory notes on the observations made.
- jot down the classification giving the points for its identification.

4.7.3 Materials Required

In addition to *Biology Laboratory Student's Kit* you would require:

1. Plant Material

(A) Fresh / fixed thalli of *Polysiphonia* - vegetative, male, female (carposporophytic), and tetrasporophytic.

(B) Permanent slides of thallus - vegetative, male - spermatium, female - carpogonium and carposporophyte, and tetrasporophyte.

2. Stains and Chemicals

Safranin (1.0%), Glycerine (1.0%), and distilled water.

3. Glassware and Apparatus

Dissection microscope, compound microscope, micro slides, microcoverslips, watch glasses, and petridishes.

Preparation of Temporary-stained Slides

(1) Pick-up the vegetative thallus of *Polysiphonia* with a needle or forceps. Spread it on a clean micro slide. You may use a dissection microscope. Soak out fixative or water with the help of a blotting paper. Put a drop of safranin on the thallus. Soak out the stain. Wash the thallus with distilled water to clean it. Place a drop of glycerine over the thallus. Put a micro

coverslip over it. Avoid any air bubble. Remove or soak the excess peripheral mounting medium with a blotting paper.

(2) Observe under the compound microscope / dissection microscope.

(3) Draw, label, and write explanatory notes on the observations made.

(4) Repeat (1), (2) and (3) for male, female and tetrasporphytic thalli.

(5) Observe the permanent slides of whole mount of vegetative thallus, male thallus, female thallus, thallus with carposporophyte and tetrasporophytic thalli.

(6) Write the classification of *Polysiphonia* and mention its identification points.

4.7.4 Observations

Refer to Fig. 4.6.

(A) Study of Vegetative Thallus

i) External Features

Observe the following characteristics in a whole mount or permanent slide:

(1) The plant body is filamentous, multi-cellular, branched and polysiphonous.

(2) The branching is dichotomous.

(3) Each branch terminates into single-celled apex. Proximal to this apical cell are present a number of flat cells.

(4) The main axis of the thallus is made up of a series of parallel filaments around a large central cell.

(5) This central cell is large, barrel-shaped. It is surrounded by 4 to 24 peripheral cells. These peripheral cells are called pericentral cells.

(6) Near the apical region of a filament below the apical cell are present trichoblasts that are uni-seriate, multi-cellular, dichotomously divided structures that are gradually tapering at tip.

(7) Thick-walled, richly-lobed, uni-cellular rhizoids are present at the proximal end of the thallus. Rhizoids arise from the peripheral cells of the creeping system.

(8) Thus, the thallus is called heterotrichous with a horizontal (creeping system) and an erect system. The erect system bears the characteristics mentioned above (1) to (6).

ii) Structure of a Cell

(1) It has thick cell wall.

(2) It is uni-nucleate.

(3) It has a large central vacuole.

(4) Around the central vacuole are present a large number of small, discoid chromatophores.

(5) Pyrenoid is characteristically absent in the chromatophore.

(6) Reserve food is stored in the form of starch grains - floridoside, also called floridean starch.

(7) The neighbouring cells (any two peripheral / pericentral cells and peripheral and central axial cell) are connected with one another by cytoplasmic lining known as pit-connections.

(B) Study of the Reproductive Structures

Observe the male, female and tetrasporophytic thalli through temporary and/or permanent slides. The basic characteristics of the vegetative regions of thallus are similar to the one discussed above. Observe the characteristic features of sex-organs, and spores along with their location on the thallus as given below:

i) Male sex-organ – Spermatangium

(1) The spermatium (uninucleate protoplast of spermatangium), also called antheridium, represents male sex organ.

(2) A large number of spermatia are produced in clusters the male thallus.

(3) A cluster of spermatangia are produced by a fertile trichoblast situated near the apex.

(4) Each spermatangium is oval in shape, naked (without an outer membrane) and it contains many spermatia (sing. spermatium).

(5) The spermatium is non-motile, small, uni-nucleate, oval to spherical in shape.

(6) A male thallus represents haploid phase in the life cycle of *Polysiphonia*.

ii) Female-sex-organ

Procarp bearing carpogonium

(1) The female, haploid thallus bears female sex-organ-carpogonium.

(2) A carpogonium has a swollen base and a long, tubular, receptive cell at its tip is called trichogyne.

(3) A carpogonium possesses a single haploid female nucleus.

(4) Each carpogonium is present on the female thallus inside a structure called procarp.

(5) A procarp is an urn-shaped body. It has multi-cellular wall called pericarp.

(6) Each procarp opens at its tip through a pore called ostiole.

(7) The trichogyne of the carpogonium protrudes out of the ostiole.

iii) Cystocarp

(1) A cystocarp is a post-fertilization structure.

(2) The thallus on which cystocarp is formed is called carposporophyte.

(3) The cystocarp is oval or urn-shaped structure attached to a lateral branch.

(4) It opens with an ostiole at its anterior, narrower end.

(5) A single-layered peripheral covering layer of cells is called pericarp.

(6) The cystocarps are sites of production of carpospores.

(7) Carpospores are produced from the base of the cystocarp.

(8) Each carpospore is oval, uni-nucleate and diploid.

(9) The unfertilized female, photosynthetic thallus bearing procarps represents the haploid phase in the life of *Polysiphonia*.

(10) The cystocarp, a post-fertilization structure, bears a small, diploid phase, which is completely parasitic on the haploid female thallus.

iv) Tetrasporophyte

(1) A tetrasporophytic thallus morphologically is completely identical to the male or female thalli representing gametophytic generation(s).

(2) All the cells of the thallus are diploid.

(3) A carpospore produces a tetrasporophytic thallus.

(4) The thallus bears tetrasporangia (2n) in a longitudinal series.

(5) The tetrasporangia are cut off by pericentral cells.

(6) Each tetrasporangium is a small, spherical body borne on short, one-celled stalk.

(7) Following meiosis, each tetrasporangium produces four haploid tetraspores arranged tetrahedrally.

(8) Each tetraspore is uni-nucleate.

(9) Upon germination a tetraspore develops into a haploid male or female thallus.

4.7.5 Classification and Identification

Kingdom : Protista (Protoctista)	:	1) Eukaryotic 2) Uni- / multi-cellular organisms.
Division: Rhodophyta	:	1) Motility absent throughout the life cycle. 2) Photosynthetic pigments are chlorophyll a and d, phycoerythrins and phycocyanins. 3) The cell wall consists of cellulose, pectin, polysulphates and polysaccharides.

Class: Rhodophyceae	:	1) Chromatophores are red to dark purple. 2) Photosynthetic reserve is floridean starch. 3) Female reproductive organ has a receptive structure - trichogyne. 4) Male gametes are non-motile. 5) Post-reproductive structure cystocarp is present.
Order: Ceramiales	:	1) Thalli are uni- or multi-axial or filamentous. 2) Auxillary cell is cut-off after fertilization and is borne on supporting cell of a 4-celled carpogonial filament.
Family : Rhodomelaceae	:	1) Thallus is polysiphonous. 2) Production of two kinds of laterals - ordinary branch and trichoblast. 3) Main axis surrounded by pericentrals. 4) Thallus bushy, branches delicate.
Genus : *Polysiphonia*	:	1) Uni-nucleate, dome-shaped apical cell. 2) Trichoblast, uni-cellular, colourless and bears sex-organs. 3) Trichoblasts drop off from the older parts of the thallus. 4) Tetrasporangia borne singly.

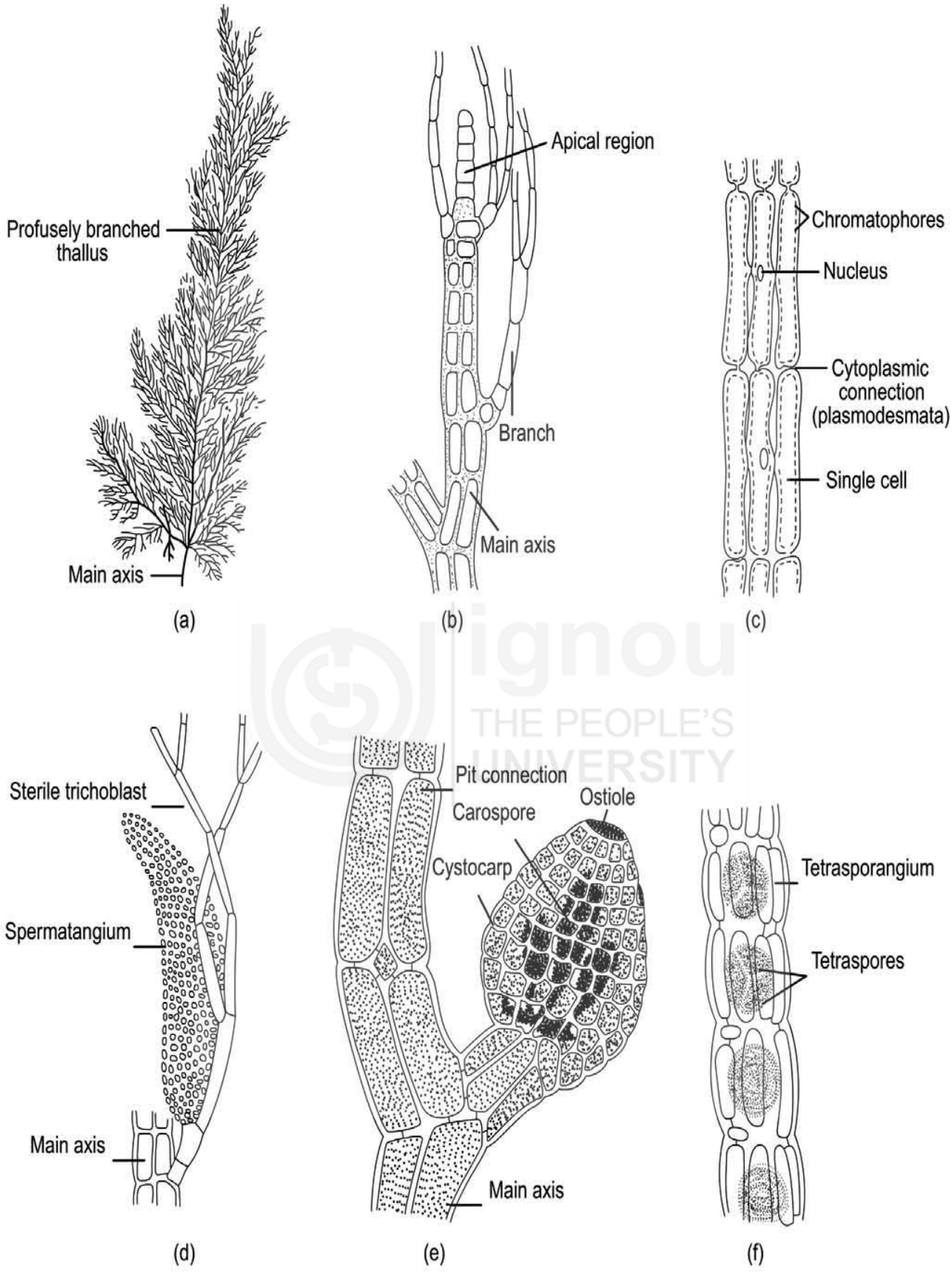


Fig. 4.6 (a-f): ***Polysiphonia*****. (a) A plant body. (b) Apical region of the plant enlarged. (c) A few pericentral cells. (d) An antheridial branch. (e) A cystocarp. (f) A portion of the axis of a tetrasporophyte showing tetrasporangia.**

EXERCISE 5

RHIZOPUS, PENICILLIUM

Structure

5.1 INTRODUCTION

In Unit 9 and Unit 10 of Block 3 you studied that fungi are heterotrophic, and live as saprophytes, parasites, or symbionts. They are found in almost every kind of habitat. The fungal cell may be acellular, cellular, tube-like, filamentous or branched. The fungal cell / mycelium could be aseptate or septate. It could also be uni-nucleate, bi-nucleae (dikaryotic), multi-nucleate or coenocytic.

You have also studied that the various organisms classified as fungi vary in their form, cell-wall composition, in the absence or presence of flagella, the number and kind of flagella, types of spores they produce during asexual and sexual reproduction. However, each group of fungi exhibits certain features which help us to distinguish one from the other group. You have also studied that based on the parameters chosen, a number of classifications of fungi have been proposed. You are advised to refer to Unit 9 for the classification followed in these exercises.

Objectives

After doing this exercise you should be able to:

- use an appropriate procedure for examination of morphological and cellular features of a fungal specimen with unaided eye as well as by using optical devices;

- ❖ examine the habit, general morphology, cellular organization and fructifications of a fungal colony / specimen;
- ❖ study asexual reproductive bodies in the genera *Rhizopus*, and *Penicillium* in the given fixed or fresh materials and/or from permanent slides;
- ❖ study sexual fruiting body structure in *Rhizopus;*
- ❖ record your observations in the form of sketches and write detailed explanatory notes on the specimens examined;
- ❖ identify and list the morphological, asexual, sexual reproductive features that help designate the given fungal genus to the various taxa of the classification;
- ❖ know the fixatives, staining material, mounting media used to study fungal material.

Study Guide/Prior Knowledge

For doing satisfactory work in laboratory you are advised to read the following before coming to the laboratory.

B.Sc. UGC CBCS, Core Course Botany Paper I – Biodiversity (Microbes, Algae, Fungi and Archegoniates).

Unit 9 Fungi: Introduction; Section 9.2 General Characteristics, Section 9.4 Range of Thallus Organization, Section 9.5 Cell Wall, Section 9.7 Reproduction, Section 9.8 Classification; Unit 10 Fungi: True Fungi, Section 10.2 General Characteristics, Sub-section 10.4.1 *Rhizopus*, and Sub-section 10.4.2 *Penicillium*.

5.2 *RHIZOPUS*

5.2.1 Introduction

Rhizopus, a terrestrial, saprophytic fungus belongs to the Division Zygomycota. The somatic body of the fungus consists of a branched, coenocytic mesh of mycelia whose cell walls are made up of chitin and glucans. Asexual reproduction takes place by means of non-motile single-celled multinucleate aplanospores. Sexual reproduction takes place by fusion of two-generally equal gametangia resulting in the formation of zygospore / zygosporangium. Motility is absent throughout the life cycle.

R. stolonifer is the most commonly occurring saprophytic species. It is responsible for the rot of sweet potato and fruits of apple, tomato and strawberries.

Objectives

After doing this exercise you should be able to:

- prepare temporary stained preparations of the somatic and asexual structures/phases of the fungus;
- identify the structure of mycelia of *Rhizopus*;
- observe and study asexual fruiting body, sporangium and aplanospores in *Rhizopus*;

Structure

- study different stages of sexual reproduction in *Rhizopus* through permanent slides;
- draw, label, describe the morphological characteristics of the vegetative, asexual and sexual structures in *Rhizopus*;
- write the classification of *Rhizopus* along with its identification points.

Study Guide/Prior Reading

For doing satisfactory work you must read the following before coming to the laboratory. Unit 9 Fungi: Introduction. Unit 10 Fungi: True-Fungi, Section 9.2 General characteristics, Section 9.5 Cell wall composition, Section 9.7 Reproduction, Section 9.8 Classification, Section 10.2 General characteristics; Sub-section 10.4.1 *Rhizopus*.

5.2.2 Method of Study

1. You shall prepare temporary stained preparations of somatic and asexual phases in the life cycle of *Rhizopus*.
2. You shall observe and study the sexual phase in the life cycle of *Rhizopus* through permanent slides.

5.2.3 Materials Required

In addition to *Biology Laboratory Student's Kit* you require the following:

1. **Plant Materials**
   - **(A) Fresh Material** - Cultures of *Rhizopus* can be made by keeping damp bread at suitable day temperatures, under a bell jar, for 2-3 days prior to the day of laboratory work.
   - **(B) Fixed Materials** - Somatic mycelia, asexual and sexual phases in the life cycle of *Rhizopus*.
2. **Permanent Slides** - Asexual and sexual phases in the life cycle of *Rhizopus*.
3. **Stains** - Cotton blue, aniline blue, lactophenol, glycerine 1.0%.
4. **Glassware and Apparatus** - Dissection microscope, compound microscope, microslides, microcover slips, watch glasses/small petri dishes.

5.2.4 Procedure

- You will prepare temporary-stained preparations as instructed by your teacher and observe under the compound light microscope.
- You shall draw and label the structures you observe in these preparations under the dissecting as well as a compound microscope.
- You shall draw, label and observe the vegetative, asexual and sexual structures of *Rhizopus* through permanent slides.

5.2.5 Observations

Pick-up a few thread-like white cottony material provided to you, stain it with cotton blue, mount in lactophenol and study the following aspects and compare your observations and sketches with the figures provided in Fig. 5.1.

(A) Somatic Morphology

Mycelium

1) These are thread-like branched, tube-like structures.
2) These are aseptate and multinucleate. Hence, are also termed as coenocytic.
3) The major portion of the hypha possesses conspicuous vacuoles.
4) The tip of the hypha has relatively more dense contents.

Stolon and *Rhizoids*

1) In the older mycelia, some parts of the hyphae produce branched *rhizoids*. The rhizoids penetrate the substratum for absorption of nutrients.
2) Some portion of the hyphae grow horizontally parallel to/above the substratum and is termed as *stolon* or *runner*.
3) The portion of the stolon that bends downwards produces another group of rhizoids when they touch the substratum.

(B) Asexual Reproductive Structures

The asexual reproductive structures look-like dark-black mass of mycelia. You shall observe the following aspects under the microscope.

Sporangiophore

1) Some hyphae grow upwards in tufts.
2) Such upward growth is very conspicuous at the point of stolon where the rhizoids are formed.
3) The number of sporangiophores per tuft could be variable.
4) This structure is termed sporangiophore because it bears a sporangium at its distal end.

Sporangium

1) A sporangiophore is swollen at the tip and forms a sporangium.
2) A sporangium is generally globose.
3) A sporangium has a columella in the centre and a space between the columella and the wall of sporangium is the site of formation of asexual spores.
4) A cell wall separates columella from the spore-producing zone.
5) A large number of such spores can be observed per sporangium.

Aplanospores

1) The asexual spores in *Rhizopus* are called aplanospores.
2) These are angular or rounded.
3) They are multinucleate.
4) They may be blue, brown or even colourless.

5) They possess distinct cell wall, which is either smooth or cuticularised.

6) The spores are non-motile and are liberated by breaking of sporangial wall.

7) Each spore germinates to produce an aseptate, multinucleate, tube-like, coenocytic mycelium.

(C) Sexual Reproductive Structures

Rhizopus reproduces sexually towards the end of growing season. Sexual reproduction is of the conjugation type. Some spp. of *Rhizopus* are homothallic while others are heterothallic. For initiation of sexual reproduction two hyphal strands are required. In heterothallic strains, these strands are termed (+)/(-). Morphologically, however, both the strains are identical.

You shall study the sexual reproductive structures in *Rhizopus* through permanent slides.

Zygophores

1) The reproductive hyphae produce a lateral outgrowth. The hypha producing such a lateral outgrowth is called zygophore.

2) Two zygophores, oppositely placed, are attracted towards each other.

3) In heterothallic species, each of the zygophore is derived from the opposite strain.

Progametangia

1) The horizontal outgrowth enlarges in size and becomes club-shaped.

2) These structures are called progametangia (sing.: progametangium)

3) The two oppositely placed progametangia make physical contact at their tips.

4) Each progametangium gets a cross-wall resulting in the formation of a larger basal cell and a dense, smaller apical cell.

5) The basal larger cell is called suspensor.

6) The denser apical cell is called gametangium (pl. gametangia).

7) The 2 zygophores, 2 suspensor cells and 2 gametangia form a H-shaped structure.

Aplanogametes

1) The protoplasmic content of each gametangium transforms into a gamete termed as aplanogamete.

2) The cell-walls of two oppositely placed gametangia dissolve at the point of contact.

3) Two aplanogametes meet and fuse. The resultant cell is called fusion cell. This contains the protoplasm and nuclei of both the aplanogametes.

Zygospore

1) The fusion cell becomes large and is called *Zygospore*.

2) It produces a thick two-layered wall around it.

3) The outer wall is darker and warty. It is called **exine** or **exospore**.

4) The inner wall is thick and is called **intine** or **endospore**.

5) Prior to the germination of zygospore, the diploid nucleus divides meiotically producing a large number of haploid nuclei.

6) Zygospore absorbs water, swells and bursts open.

7) The outer exine breaks, inner intine wall grows out as a tube with protoplasm and a large number of haploid nuclei. This structure is called **promycelium.**

Zygosporangium

1) A promycelium grows vertically.

2) It swells at the tip to produce a *zygosporangium* (germ sporangium).

3) It produces a large number of unicellular, non-motile, germ-spores.

4) These are meiospores.

5) Each of these meiospores germinate to form a somatic mycelium.

5.2.6 Classification and Identification

Kingdom: Fungi	:	1) Achlorophyllous 2) Cell wall consists of chitin and chitosome (glucans) 3) Reserve food materials are glycogen and oil
Division: Zygomycota	:	1) Thick-walled Zygospore 2) Coenocytic mycelium 3) Asexual aplanospores 4) Absence of motility 5) Absence of centrioles (you may have not observed)
Class: Zygomycetes	:	1) Extensive mycelia 2) Asexual reproduction predominant 3) Sexual reproduction – both homo- and heterothallic
Order: Mucorales	:	1) Aseptate mycelium 2) Active protoplasmic streaming 3) Septa, when present, without pore 4) Septum formation only to delimit sporangia/gamentangia 5) Saprobes (predominantly) 6) Karyogamy in zygospore

Family: Absidiaceae	:	1) Stolon and rhizoids often found
		2) Zygospore and suspensors are often opposed
		3) Sporangia with persistent cell wall
Genus: *Rhizopus*	:	1) Sporangium with a definite apophysis
		2) Sporangiophores arising from stolons opposite the rhizoids
		3) Aplanospores smooth or marked by striations

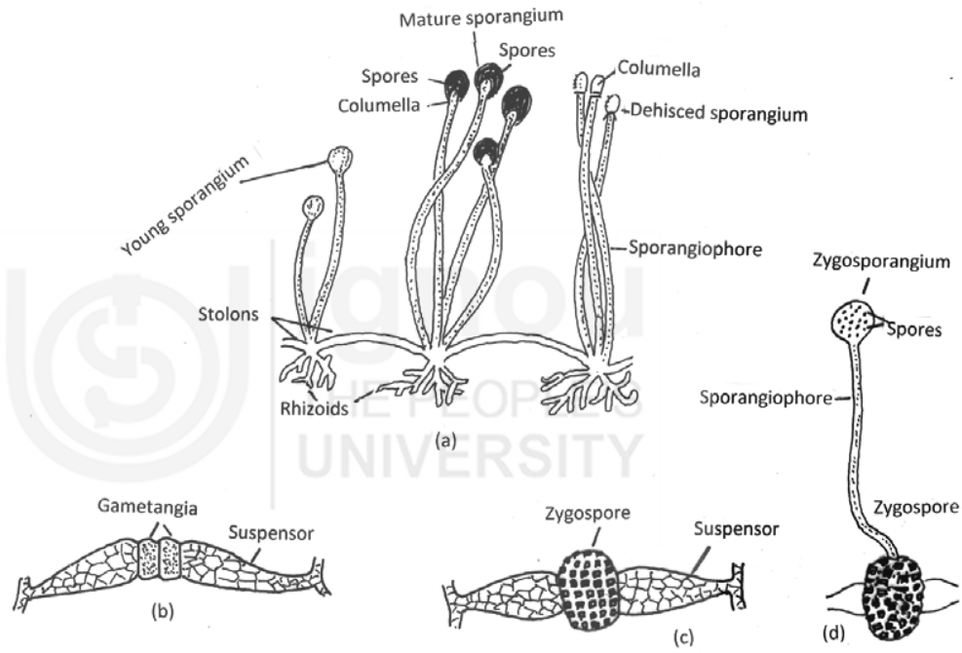


Fig. 5.1: *Rhizopus*. a) Vegetative mycelium with sporangiophores and sporangia; b-d) Sexual phases of life cycle.

5.3 *PENICILLIUM*

5.3.1 Introduction

Penicillium, commonly known as green- and blue-mould, is a fungus which belongs to the Division Ascomycota. The somatic body of the fungus consists of branched, septate mesh of hyaline mycelia. The individual hyphal cells are multinucleate. *Pencillium* occurs is soil and air as saprophyte. However, it is a potentially pathogenic fungus. Asexual reproduction takes place by the formation of greenish, bluish or yellow conidia. These conidia are produced by brush-like broom-shaped conidiophores termed as penicillus. The sexual phase of *Penicillium* has not been clearly worked out. However, the genera, *Eupenicillium* (*Carpenteles*) and *Talaromyces* produce asexual fruiting bodies

similar to *Penicillium*. The pear-shaped or globose asci are formed within a fruiting body called cleistothecium. The sex organs are highly reduced. The motility is completely absent in *Penicillium*.

Objectives

After doing this exercise you should be able to:

- prepare temporary stained preparations of somatic and asexual structures/phases of *Penicillium*;
- identify the structure of mycelia of *Penicillium*;
- observe and study the asexual fruiting body, the penicillus in *Penicillium;*
- study the structure of cleistothecium, asci and ascospores in *Penicillium* through permanent slides;
- to draw, label, describe the morphological characteristics of the somatic, asexual and sexual structures in *Penicillium*; and
- provide the classification of *Pencillium* along with the identification points.

Study Guide/Prior Reading

For doing this exercise you must read the following before coming to the laboratory. Unit 9 Fungi: Introduction. Unit 10 Fungi: True-Fungi, Section 9.2 General Characteristics, Section 9.5 Cell wall composition, Section 9.7 Reproduction, Section 9.8 Classification, Section 10.2 General characteristics, Sub-section 10.4.2 *Pencillium*.

5.3.2 Method of Study

You shall prepare temporary-stained preparations of somatic and asexual phases in the life cycle of *Penicillium* and observe and study them.

Thereafter, you shall observe and study the sexual phase in the life cycle of the fungus through permanent slides.

5.3.3 Materials Required

In addition to *Biology Laboratory Student's Kit* you require the following:

1. **Plant Materials**

   (A) Fresh Material - Cultures of *Penicillium* or old wet leather belts or wet rotting orange rinds with *Penicillum*, other fruits, jellies and food stuffs.

   (B) Fixed Material - Somatic and asexual phases of *Penicillium*.

2. **Permanent Slides** - Somatic mycelia, asexual and sexual fruiting bodies of *Penicillium*.
3. **Stains** - Cotton blue, aniline blue, lactophenol, glycerine 1.0 %.
4. **Glassware and Apparatus** - Wash bottle, strips of blotting paper, dissecting microscope, compound microscope, micro slides, micro coverslips, watch glasses / small petridishes.

5.3.4 Procedure

- You will prepare the temporary-stained preparations as instructed by your teacher and observe the structures under the compound microscope;
- You shall draw, label the structures you observe in the preparation(s); and
- You shall observe, draw, label the vegetative, asexual and sexual structures of the *Penicillium* through permanent slides.

5.3.5 Observations

(A) Somatic Structures

Pick-up a few thread like hyaline-cottony material provided to you. Stain them with cotton blue, mount in lactophenol and study the following aspects and compare your observations, sketches with the figures provided at the end of this exercise (Fig. 5.2).

Mycelium

1) Mycelium is freely branched and septate.
2) Each hyphal cell is uni- to multi-nucleate.
3) The vertical septum separates neighbouring hyphal cells. The septum is incomplete and allows the free movement of protoplasmic contents including nuclei across it.
4) Hyphal cells could be coloured due to pigments on the surface of their cell walls.

(B) Asexual Reproductive Structures

Prepare a slide from the material provided. Generally the mycelial mass looks green, yellow or blue at this stage.

Sporangiophores

Long, erect and branched conidiophores arise from the mycelium.

Penicillus

1) Conidiophore grows vertically and branches at its upper end. The ultimate branches are known as metulae (Sing. metula).
2) Branch of conidiophores ends in bottle-shaped sterigmata.
3) Each sterigmata bears a group of conidia.
4) Development of conidia is basipetal.
5) Branched conidiophores with its conidia look like a small 'Penicillus' (brush-like).

Conidiospores

1) Conidia are generally blue, sometimes green or yellow providing a characteristic colour to the colony.
2) Conidia are globose to ovoid in shape and appear as glass beads under the microscope.

(C) Sexual Reproductive Structures

You shall study the sexual structures with the help of permanent slides.

Cleistothecium

1) The fruiting body - ascocarp is called cleistothecium.
2) The wall of cleistothecium is called peridium.
3) The peridium is made of sterile septate hyphae.

Ascus

Globose or pear-shaped asci lie scattered in a cleistothecium.

Ascospore

1) Each ascus has eight uni-nucleate, wheel-shaped ascospores.
2) Ascospores are released by rupture of the cleistothecium.

5.3.6 Classification and Identification

Kingdom: Fungi	:	1) Achlorophyllous 2) Cell wall consists of chitin and chitosomes 3) Reserve food material is glycogen and oil.
Division: Ascomycota	:	1) Septate hyphae 2) Hyphal cells multinucleate 3) Cell wall growth centripetal, wall incomplete 4) Cell wall pore associated with Woronin bodies. 5) Produce asci and ascospores
Class: Ascomycetes	:	1) Fruiting body is ascus 2) Ascospore formed after karyogamy and meiosis 3) Septate hyphae, multi-nucleate hyphal cells 4) Septum incomplete, with Woronin bodies 5) Reduced male gametangium 6) Formation of ascogonium, ascogenous hyphae 7) Formation of croizer prior to ascocarp development
Order: Eurotiales	:	1) Asci in free mycelium or within sessile stipitate ascocarp

2) Mycelium forms mesh/solid wall of ascocarp

3) Sexual reproduction by trichogyne and undifferentiated hyphae

4) Spherical to ovoid asci

5) Single-celled ascospore of varied shapes

Family: Trichocomaceae : 1) Pseudoparenchymatous cleisotothecia

2) Stromata in cleistothecium

3) Teleomorphic genera have phialidic anamorphs

Genus: *Penicillium* : 1) Phialidic brush-like conidiophores

2) Conidiophore branched

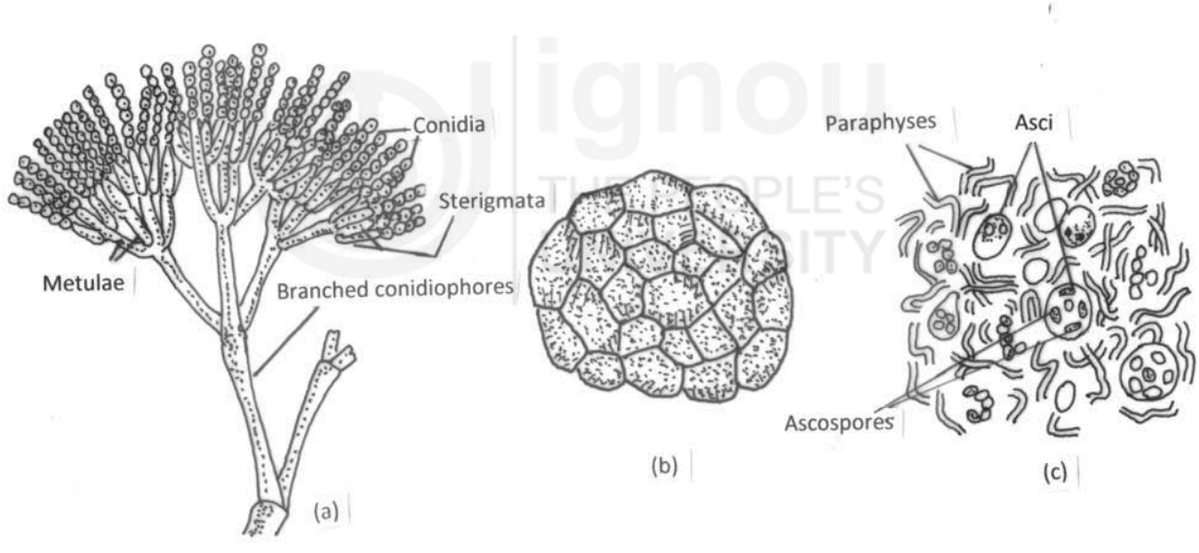


Fig. 5.2: *Penicillium*. a) Mycelium with asexual fruiting bodies; b) Whole mount of cliestothecium; c) Smear mount of cliestothecium showing asci and ascospores.

EXERCISE 6

ALTERNARIA

Structure

6.1 INTRODUCTION

Alternaria is a very common outdoor saprophytic fungus. It belongs to Fungi-Imperfecti, in which no sexual (perfect) phase in the life cycle is reported. It is represented by its anamorph phase only. Hence, it is placed in class Loculoascomycetes of the Division Ascomycota. It is also an example of mitosporic fungi. The somatic phase is represented by dark, septate and branched mycelia. The individual hyphal cells are multi-nucleate. The septum is perforated. Asexual reproduction takes place by non-motile, multi-cellular conidia, produced acropetally. The conidia occur in chains at the tip of conidiophores. A long beak is characteristic of the terminal cell of a conidium. The beak helps in dispersal of conidia.

A. solani is the causal organism of the disease – Early blight of potato. *Alternaria* also occurs as weak parasite of many plants, e.g., *A. brassicae* and *A. brassicicola* cause grey and dark leaf spots of *Brassica* spp., *A. burnsi* parasitizes *Cuminum cyminum* (cumin), *A. triticina* parasitizes *Triticum* spp. (wheat), *A. alternata* infects *Helianthus* annuus (sunflower), *A. lini* causes blight of linseed (*Linum usitatissimum*), *and A. palandui* causes blight of *Allium sativum* (garlic).

You may use any of the above for laboratory work.

Objectives

After doing this exercise you should be able to:

- prepare a temporary-stained tease mount of the somatic and asexual structures of *Alternaria*;
- identify the structure of mycelium, and conidium of *Alternaria*, especially the beak, longitudinal and transverse septa in multicellular conidia;
- differentiate between healthy and diseased leaves of potato with the help of 'target-board' symptoms; and
- provide the classification of *Alternaria* along with identification points.

Study Guide/Prior Reading

For doing satisfactory work you must read the following before coming to the laboratory:

Unit 9 Fungi: Introduction, Section 9.2 : General Characteristics, Section 9.4 Range of Thallus Organisation, Section 9.7 Reproduction, Section 9.8 Classification. Unit 10 Fungi: True Fungi, Sub-section 10.4.3 *Alternaria*.

6.1.2 Method of Study

You shall:

1) prepare temporary-stained tease preparation of somatic and asexual phases in the life cycle of *Alternaria*.
2) observe the healthy and diseased specimen/photographs of potato leaves infected with *Alternaria*.
3) observe the 'target-board' symptoms of early-blight of potato.

6.1.3 Materials Required

In addition to *Biology Laboratory Student's Kit* you require the following:

1. **Plant Materials -**
   (A) Fresh or fixed culture of *Alternaria* and diseased leaves.
   (B) Photograph or herbarium sheet of potato leaves showing early blight symptoms.
2. **Permanent slides -** *Alternaria* conidiophores and conidia.
3. **Stains -** Cotton blue, aniline blue, lactophenol, glycerine (1.0%).
4. **Glassware and Apparatus -** Wash bottle, strips of blotting papers, a compound microscope, a magnifying glass, micro slides and microcoverslips, watch glasses, small petri dishes.

6.1.4 Procedure

Prepare the temporary-stained tease preparation as instructed by your counsellor and observe under the compound microscope.

- Draw and label the structures you observe in these preparations under a compound microscope.
- Compare the healthy and diseased leaves of potato in herbarium sheets with the help of a magnifying glass observe, draw, lesions on the diseased leaves.
- Use photographs of diseased-leaves, if the herbarium specimen is not available.

6.1.5 Observations

(A) Somatic Structures

Prepare a temporary stained preparation from the fixed material provided and / or tease out the infected lesion region from the infected leaf provided. Observe the following aspects, note the points and draw figures in your record book. You may refer to Fig. 6.1 at the end of this exercise.

Mycelium and hyphal cells

1) Mycelium is branched and septate.
2) Each hyphal cell is uni-nucleate.
3) Within the host tissue, the mycelia are intercellular.

(B) Study of Asexual Reproductive Structures

Conidiophores

1) Conidiophores are not much differentiated from the somatic hyphae.

Conidia

1) Conidia are usually yellowish brown.
2) Condium occurs either singly or in chain and is borne on conidophore.
3) Conidium is long, dark-coloured, muriform (beaked), multi-cellular and dictyosporous (with both longitudinal and transverse septae)
4) Condium is ovoid or spindle-shaped.

(C) Study of Host-Parasite Relationship

Early blight of Potato is caused by the infection of *Solanum tuberosum* by *Alternaria solani*.

Observe the herbarium specimen of infected potato leaf and look for the following:

1) Disease appears first on lower leaves as small, isolated, scattered, pale brown spots on leaflets.
2) Later, the spots become covered with greenish blue growth of fungus.
3) Further growth of fungus results in development of necrotic spots.
4) Concentric rings appear in necrotic spots in the older leaflets and darkened areas on the stem. These concentric rings look-like 'target-boards'.

5) A narrow chlorotic zone surrounds such spots.

6) Severe infection leads to shrivelling of leaves, followed by their abscission.

7) The infected host looks blighted.

8) Tubers may also get infected, become brown to black and develop necrotic spots.

Scrape with needles the infected spots and prepare a temporary-stained mount. Observe the mycelium, conidiophores and conidia and draw labelled sketches.

6.1.6 Classification and Identification

Kingdom: Fungi	:	1) Achlorophyllous 2) Cell wall consists of chitin and chitosomes 3) Reserve food material is glycogen and oil
Division: Ascomycota	:	1) Septate hyphae 2) Hyphal cells multi-nucleate 3) Cell wall growth centripetal, wall incomplete 4) Cell wall pore associated with Woronin bodies 5) Sexual phase, if any, possesses ascus and ascospore
Class: Loculoascomycetes (Deuteromycetes)	:	1) No perfect stage in life cycle, represented by anamorph only 2) Sexual reproduction absent 3) Well developed, septate branched hyphae 4) Septal pore incomplete 5) Asexual reproduction by conidia 6) Conidia and conidiophores form specialized structures
Order: Moniliales	:	1) Conidia producing Ascomycetes 2) Saprophytic as well as parasitic 3) Conida produced directly from hyphae 4) Conidiophore free or in aggregate 5) No pycnidium or acervulus formation

Family: Dematiaceae	:	1) Both hyphae and conidia are dark 2) Conidia not associated with any fruiting body 3) Saprophytic and / or parasitic 4) Hyphae light coloured but conidia are dark
Genus: *Alternaria*	:	1) Conidiophores distinct, erect 2) Conidia dictyospores, multi-celled, with both transverse and longitudinal partitions

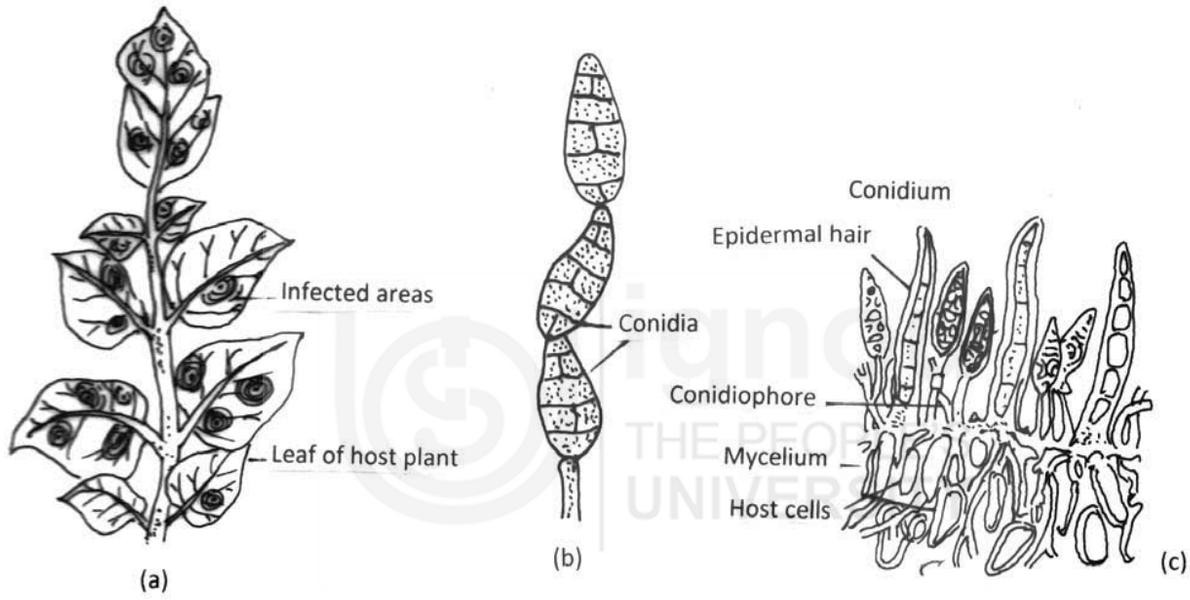


Fig. 6.1: ***Alternaria*. (a) Target-board disease symptom on the leaves of the host. (b) W.m. of conidia. (c) A portion of infected leaf (tease mount / v.s.), conidiophores and mycelia.**

EXERCISE 7

PUCCINIA

Structure

7.1 INTRODUCTION

Puccinia belongs to Division Basidiomycota. Of the more than 700 species of *Puccinia* about 150 are reported from India. *Puccinia* is an obligate parasite on cereals, millets and many other crops of economic importance. The commonest species, *Puccinia graminis* parasitizes wheat and rarely on oats and rye.

Puccinia requires two hosts to complete its life cycle. The primary host is wheat while barberry plant acts as secondary host. Such pathogens, which require two hosts to complete their life cycle, are termed heteroecious. There is a total of five stages in the life cycle of *Puccinia*. Of these the stages, uredospores and teleutospores occur on wheat plants whereas pycniospores and aecidiospores are produced on barberry leaves. The intercellular mycelium, with prominent intracellular haustoria, are dikaryotic in wheat while in barberry leaves, the mycelium is monokaryotic. The asexual reproduction can occur through uredospores. Karyogamy takes place in promycelium in teleutospores. The haploid basidiospores infect barberry leaf. Spermiation, and dikaryotization occur in barberry leaf. Dikaryotic aecidiospores infect wheat leaves to restart the life cycle.

The infective phase of primary host is highly harmful to the wheat crop.

Objectives

After doing this exercise you should be able to:

- identify the infected leaves of the primary and the secondary hosts and demarcate the infective pustules (regions);
- identify and distinguish between uredosori and teleutosori;
- identify and distinguish between the uredospores and teleutospores;
- observe, locate and study the pycnidia and aecidial cups in permanent slides;
- prepare temporary-stained mounts of v.s. of infected leaves of wheat;
- draw, label, and describe the morphology of somatic, asexual and sexual phases of *Puccinia* in its two hosts;
- prepare tease mounts of spores from the infected regions of wheat leaves; and
- provide the classification of *Puccinia* along with the identification points.

Study Guide/Prior Reading

For doing satisfactory work you must read the following before coming to the laboratory:

Unit 9 Fungi: Introduction, Section 9.2 General Characteristics, Section 9.4 Range of Thallus Organisation, Section 9.7 Reproduction, Section 9.8 Classification. Unit 10 Fungi: True Fungi, Section 10.2 General Characteristics, Sub-section 10.4.4 *Puccinia*.

7.1.2 Method of Study

1) You shall study the morphology of infective spots on leaves of both the primary host (wheat) and the secondary host (barberry) from fresh material or herbarium specimens.
2) You shall prepare temporary-stained preparations of v.s. of wheat leaf passing through the uredosorus and teleutosorus.
3) You shall prepare the tease mounts of uredospores and teleutospores.

7.1.3 Materials Required

In addition to *Biology Laboratory Student's Kit* you require the following:

1. Plant Materials

Fresh or fixed infected leaves of wheat and barberry.

2. Permanent slides

V.s. of wheat and barberry leaves passing through the infective regions – uredosorus / teleutosorus in wheat, and pycnidial and aecidial cups in barberry leaves.

3. Stains

Cotton blue, aniline blue, lactophenol, glycerine 1.0%.

4. Glassware and Apparatus

Wash bottle, strips of blotting papers, dissecting microscope, compound microscope, a magnifying glass, micro slides, microcoverslips, watch glasses / petridishes.

7.1.4 Procedure

- Observe, demarcate the healthy and infective pustules on wheat and barberry leaves and make their neat and labelled sketches.
- Cut v.s. of wheat leaves passing through the infective pustules, and prepare their temporary-stained mounts.
- You shall observe, draw, label the fungal components and the host's tissues.
- You shall observe, draw, label and distinguish various kinds of spores in the life cycle of *Puccinia*, and record their characteristics.
- Provide the classification of *Puccinia* along with identification points.

7.1.5 Observations

Observe and draw the given fresh or dried herbarium specimen of infected wheat leaf. You may refer to Fig. 7.1 for this exercise.

(A) Herbarium specimen of Black Stem Rust of Wheat

1) Red, oval or lemon-shaped infective pustules can be observed on leaves, especially in inter-veinal region of lamina, leaf sheaths and sometimes also on the stem.
2) The infected regions appear dark brown or black, oblong to linear lesions. These lesions may merge to form large patches.

Uredosorus and Uredospores

Observe the uredosorus and uredospores in your temporary-stained preparations or in a permanent slide. You shall observe:

1) The uredosorus in v.s. of leaf reveals the ruptured host epidermis due to the pressure exerted by the underlying uredospores.
2) The dikaryotic intercellular and branched mycelium is aggregated below the epidermis.
3) The uredospores are produced in upright manner in massive groups from the mycelium.
4) Each uredospore is stalked, rounded or oblong in shape and is binucleate.
5) Each uredospore has 2 cell walls. The outer exine, is finely verrucose or echinulate. The inner intine, is smooth.

6) Each uredospore has four equatorial germ-pores.

7) The uredospores get dispersed by wind and can reinfect the wheat plants to produce another crop of uredosori. It reflects asexual mode of reproduction.

Teleutosorus and Teleutospores

Observe the black, oval pustules of teleutosorus and teleutospores in your temporary-stained preparations or in the permanent slide. You shall observe:

1) In a v.s. of leaf, the teleutosorus reveals the dikaryotic, intercellular, branched mycelium, a bunch of teleutospores and the ruptured epidermis of leaf.

2) Teleutospores are formed by the same mycelium that earlier produced uredospores.

3) A teleutospore is borne on the terminal end of an upright mycelium.

4) Each teleutospore is stalked, elongated and 2-celled. The apex of the teleutospore could be rounded, pointed or nearly flat.

5) The teleutospore has very thick but smooth exine and delicate, thin intine. The exine is black at maturity.

6) Each of the two cells of a teleutospore is binucleate. At later stage, you may observe only one nucleus per cell due to the fusion of 2 nuclei. Such a nucleus is diploid.

7) Each cell has only one germ pore.

8) The (2n) nucleus in one of the cells divides meiotically to produce 4 haploid basidiospores. You may/may not observe this stage.

(B) Study of infection on Secondary Host — Barberry leaf

- Observe the infected barberry leaf. Draw the adaxial surface and look for the pycnidial cups. The abaxial surface of leaf possesses aecidial cups.
- Observe, the permanent preparations for the study of *Puccinia* on alternate host. Make suitable labelled sketches.

Herbarium specimen of infected Barberry leaf

1) Upon landing on a barberry leaf, the haploid basidiospore germinates to produce branched, septate, monokaryotic mycelium. Observe such intercellular mycelium.

2) Such mycelia produce pycnidium on the upper (adaxial) surface of the leaf. Each pycnidium is flask-shaped with an opening - ostiole, at its apex.

3) The hyphae near the ostiole are unbranched, pointed and orange. These are called periphyses. They also project beyond ostiole.

4) Some of the periphyses are thin walled. These are called receptive hyphae. They also project out of ostiole but far beyond periphyses.

5) The cavity of the pycnidium is lined by many elongated, uni-nucleate structures called pycnidiophores or spermatophores.

6) These pycnidiospores can be observed arranged in a palisade-like layer. Each of them cut off a chain of uni-cellular, very small spores, called pycnidiospores or spermatia (singl. spermatium).

7) Pycnidiospores are discharged from ostiole. They are released in air, enter another pycnidium, somatic transfer into another monokaryotic mycelium results again in the formation of dikaryotic mycelium.

Aecidial cup and Aecidiospores

In a vertical section of an infected barberry leaf, aecidial cups are produced by newly formed dikaryotic mycelium on the abaxial (lower) side of leaf. You should observe, draw, label and write comments about them. You will observe that:

1) Aecidial cup is present generally on the abaxial side. You may observe both pycnidia and aecidial cup in the same leaf and that too in the same plane of section of host leaf;

2) Each aecidium is cup-shaped;

3) It possesses an outer protective layer called peridium;

4) The developed aecidium pushes through abaxial epidermis;

5) At the base of aecidium there are many elongated cells, called sporophores. These are arranged in palisade-like manner.

6) Each sporophore is dikaryotic. It cuts off alternately a larger and a smaller cell. The smaller cell is called disjunctor cell. The larger cell is aecidiospore.

7) As a lot of aecidiospores in young, early stage are tightly, closely placed, they appear hexagonal.

8) Soon after separation, following dissolution of disjunctor cell, the aecidiospores become large, and are round.

9) An aecidiospore is dikaryotic, with thick but smooth wall.

10) Soon after liberation, they land on wheat leaf to produce uredospores to complete autoecious or heteroecious life cycle.

11) May have 5 stages in life cycle labelled as stages 0, I, II, III, IV.

12) Life Cycle may be termed as :

Macrocyclic =	all 5 stages present;
Demicyclic =	life cycle without uredospores; and
Microcyclic =	life cycle without uredospores and aecidiospores.

7.1.6 Classification and Identification

Kingdom: Fungi	:	1) Achlorophyllous
		2) Cell wall consists of chitin and chitosomes
		3) Reserve food material is glycogen and oil
Division : Basidiomycota	:	1) Septate hyphae, with dolipore septum

2) Mycelial cells exhibit haploid monokaryotc, dikaryotic (2n nuclei) and monokaryotic diploid stages

3) Produce basidiospores

Class : Basidiomycetes :

1) Basidiospores are haploid

2) Basidium produces basidiospores, after plasmogamy, karyogamy and meiosis in that order

3) Mycelium may have clamp connections

5) Asexual reproduction, if occurs, is by conidia, oidia, and arthospores

Order : Uredinales :

1) Obligate, biotrophs (obligate parasites)

2) Rust fungi

Family : Pucciniaceae :

1) Rust fungi

2) Produce many kinds of spores, are heteroecious

3) Require two hosts for completion of sexual life cycle

4) Highly reduced, specialized kind of sex-organs

5) Spermiation is the mode of dikaryotization

6) Dikaryotic phase capable of asexual propagation

7) Mycelia with intracellular haustoria

8) No clamp connection in diakaryotic mycelia

9) Mycelial septa without dolipore

Genus : *Puccinia* :

1) Teleutospore - stalked

2) Teleutospores occur in groups but are free from each other

3) Heteroecious, macrocyclic

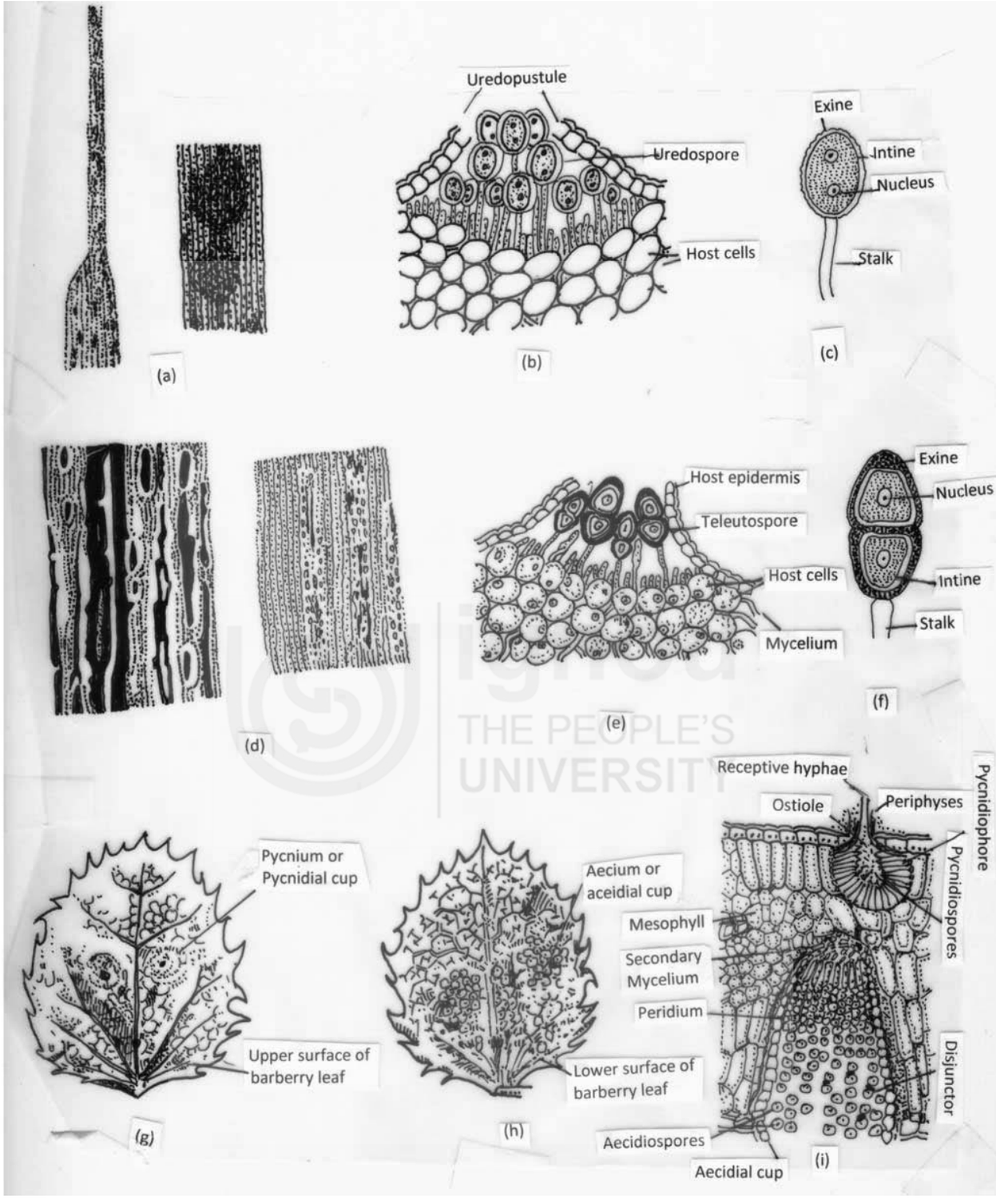


Fig. 7.1: *Puccinia*. a) An infected leaf and culm of wheat (left hand side) and its portion enlarged (right hand side). This is uredospore stage; b) V.s. wheat leaf through an uredosorus; c) An uredospore enlarged; d) A portion of wheat leaf showing infective patches, teleutospore stage (left hand side), and a portion of the same enlarged (right hand side); e) V.s. wheat leaf through a teleutosorus; f) A teleutospore; g) Adaxial surface of infective barberry leaf with pycnidia; and h) Abaxial surface of an infective barberry leaf having aecidia; i) V.s. a part of barberry leaf showing a pycnidium (adaxial surface) and an aecial cup (abaxial surface).

EXERCISE 8

AGARICUS

Structure

8.1 Introduction

The genus *Agaricus*, often synonymous with the term 'mushroom' has more than 200 species. It belongs to the Division Basidiomycota. *Agaricus*, like all other mushrooms is saprophytic. It spreads across the substratum with the help of perennial mycelia. However, to the naked eye, *Agaricus* is visible by its moderate to large, white fruiting body, the basidiocarp.

Apart from the commonest species *A. campestris* (field-mushroom), *A. arvensis* (horse-mushroom) and *A. tabularis* (ring-mushroom) are also quite common.

Agaricus thallus is represented by three kinds of mycelia: i) primary mycelia, are monokaryotic, septate, multicellular, with one haploid nucleus per cell; ii) secondary mycelia are dikaryotic, multicellular, septate with each cell possessing two nuclei (each representing opposite strain); and iii) tertiary mycelia — all the cells/ tissues/ mycelia that constitute the fruiting body — basidiocarp.

Extensively branched, septate primary mycelium, is capable of indefinite growth, represents haploid, monokaryotic somatic phase. It is perennial and proliferates in the substratum. It possesses characteristic dolipore septae between its neighbouring hyphal cells. The primary mycelium develops from a haploid basidiospore.

The secondary mycelium is produced by somatic hybridization of two compatible primary mycelia representing two strains. Hence, *Agaricus* is regarded as heterothallic. In secondary mycelia, each of the hyphal cell

possesses two nuclei, each belonging to opposite strain. It is called a dikaryon. A typical mode of cell and nuclear division, involving formation of a clamp-connection, help maintain the dikaryotic feature of secondary mycelium. This process is known as dikaryotization. The dikaryotic mycelia are represented by a well organized specialized tissue that leads to the development of a fruiting body, the basidiocarp – a characteristic of *Agaricus*. In the basidium formed within a basidiocarp, two nuclei fuse to produce a diploid nucleus (diploidization). Following meiosis, four haploid basidiospores are produced. Each of these germinates to produce a primary mycelium.

Objectives

After doing this exercise you should be able to:

- ❖ observe and differentiate morphologically the button stage and full grown basidiocarps of *Agaricus*;
- ❖ study, identify different parts of mature basidiocarp in *Agaricus*;
- ❖ prepare temporary-stained mounts of vertical sections through the gills of *Agaricus* basidiocarp;
- ❖ identify, draw, label the tissue differentiation within a gill of *Agarcius*; and
- ❖ provide the classification of *Agaricus* along with identification points.

Study Guide/Prior Reading

For doing this exercise you are advised to read the following before coming to the laboratory.

B.Sc. UGC CBCS, Core Course Botany Paper I – Biodiversity (Microbes, Algae, Fungi and Archegoniates).

Unit 9 Fungi: Introduction; Section 9.2 General Characteristics, Section 9.4 Range of Thallus Organization, Section 9.5 Cell Wall, Section 9.7 Reproduction, Section 9.8 Classification; Unit 10 Fungi: True Fungi, Section 10.2 General Characteristics, Sub-section 10.4.5 *Agaricus.*

8.1.2 Method of Study

- You will observe, draw, label and write explanatory notes on the morphology of button-stage and mature basidiocarps of *Agaricus*.
- Observe, study, draw, label various parts of v.s. of a mature gill of *Agaricus* tissue components of the gill, and write explanatory notes for each of these components.
- You will observe and draw, a basidium and basidiospore.

8.1.3 Materials Required

In addition to *Biology Laboratory Student's Kit* you require the following:

1. **Plant Materials**

   (A) Fixed/fresh/herbarium specimen of button-stage fruiting body of *Agaricus*.

   (B) Fresh and fixed mature basidiocarp of *Agaricus*.

2. **Permanent slide -** v.s. gills of mature basidiocarp of *Agaricus*.

3. **Stains -** Cotton blue, aniline blue, lactophenol, safranin (1.0%), glycerine 1.0%.

4. **Glassware and Apparatus -** wash bottle, compound microscope, micro slides, microcover slips, watch glasses/petri dishes.

8.1.4 Procedure

- With the help of a magnifying glass observe, draw, label the various parts of a button-stage basidiocarp of *Agaricus*. Write explanatory notes of your observations.
- Observe, draw, label the various parts of mature basidiocarp of *Agaricus*. Write the salient points.
- Cut v.s. of a gill of a mature basidiocarp of *Agaricus*, stain with cotton blue, mount in lactophenol. Observe under compound microscope the various types of tissues. Draw, label its parts and write explanatory notes about them.
- Observe and study the permanent slide of v.s. of gill of *Agaricus.*

8.1.5 Observations

Refer to Fig. 8.1.

(A) Morphology of Basidiocarp

Button-shape stage

Cut l.s. basidiocarp, stain with safranin and mount in glycerine, observe under a magnifying glass.

1) It is a developmental stage of the basidiocarp.
2) It is made up of ***dikaryotic***, secondary *mycelia*.
3) It is visible above the ground as small, globose body.
4) In longitudinal section, it shows a small **stipe** and **pileus** on top of it.
5) There is a constriction at site where stipe meets pileus.
6) At the level of constriction, two **lamellar cavities** or **chambers**, one on either side can be observed.
7) Each of these chamber or cavities have small lamellae. The lamellae are also called **gills**.

8) The edge of the pileus is connected to stipe by a thin sheet of tissue, called **veil** or **velum**.

Mature basidiocarp

1) The mature basidiocarp consists of a **stalk** or **stipe**, and an expanded **pileus** on top of it.
2) In a mature basidiocarp, the **veil** or **velum** ruptures and can be observed in the form of a ring, the **annulus** on the stipe, just below the pileus.
3) The upper surface of pileus is flesh coloured and is tough in texture.
4) Pileus on underside bears many lamellae or gills which hang down vertically.
5) These gills extend almost radially from stipe to the margins of the pileus.

(B) Anatomy of Gill

In t.s. of a gill, stained with safranin and mounted in glycerine, the following structures can be observed.

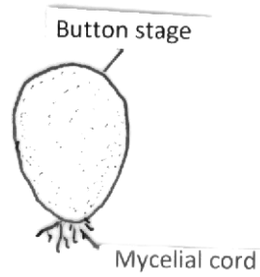
1) Three zones: trama, sub-hymenium and hymenium.
2) Central core of loosely arranged elongated sterile hyphae constitutes the **trama**.
3) Hyphal cells of trama curve outwards on either side of the gill and forms a more or less compact tissue of cells. This zone is called **sub-hymenium**.
4) Individual hyphal cells of a sub-hymenium terminate into elongated, club-shaped cells. This superficially placed cell layer of gills is known as **hymenium**.
5) At maturity, these cells of hymenium mature as fertile **basidia** (sing. basidium) or immature, sterile paraphyses (sing. paraphysis) cells. The paraphyses are regarded as undeveloped basidia.
6) Each basidium is club-shaped.
7) All the cells/tissue/mycelia that constitute the basidiocarp constitute **tertiary mycelia** in the life cycle of ***Agaricus***.
8) The basidium cell has 2 haploid nuclei or one diploid nucleus. This diploid nucleus is the product of syngamy (fusion of two nuclei) and the resultant cell a **dikaryon**.
9) This dikaryon stage is very short-lived. The nucleus undergoes meiosis to form 4 haploid nuclei.
10) Each basidium has 4 terminally placed small, tender stalks, the **sterigmata**. Each of the nucleus gets metamorphosed into a haploid cell, the **basidiospore**.
11) These 4 basidiospores are placed external to basidium, on top of sterigmata, one per sterigmatum.

12) Each basidiospore is haploid, uninucleate, oval in shape.

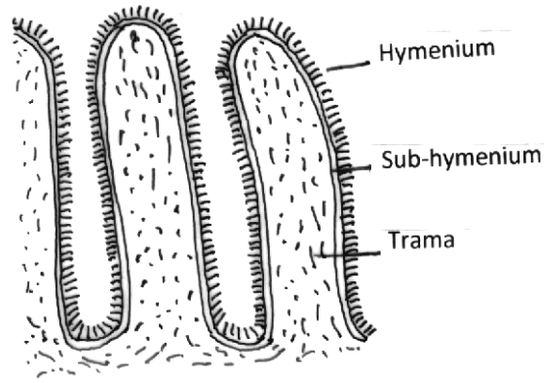
13) Upon release, each basidiospore germinates into a monokaryotic, branched, multicellular, primary mycelium.

8.1.6 Classification and Identification

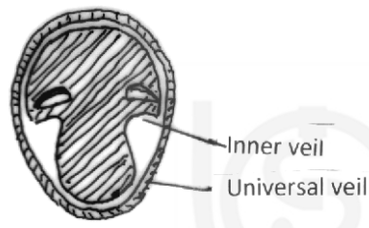
Kingdom: Fungi	:	1) Achlorophyllous
		2) Cell wall consists of chitin and chitosome
		3) Reserve food material is glycogen and oil
Division: Basidiomycota	:	1) Septate, hyphae with dolipore septum (usually present)
		2) Plasmogamy, karyogamy and meiosis precede basidiospore production
		3) Basidia generally club-shaped and non-septate
		4) Mycelium may have clamp-connection
		5) Asexual reproduction, if occurs, is by conidia, oidia, arthospores
Order: Agaricales	:	1) Basidia borne on lamellae (gills)
		2) Basidiocarp soft and putrescent
Family: Agaricaceae	:	1) Basidiocarp fleshy
		2) Gills narrow in section
Genus: *Agaricus*	:	1) Pileus centrally stipitate
		2) Annulus typically present in basidiocarp
		3) Gills free
		4) Stipe readily separating from pileus



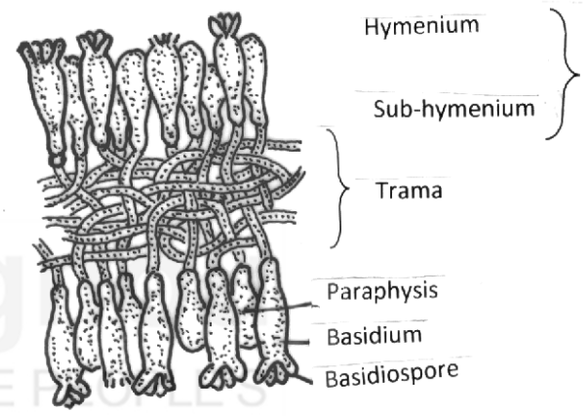
(a)



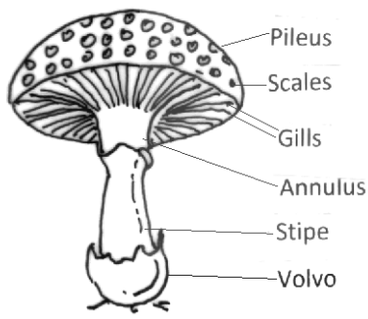
(d)



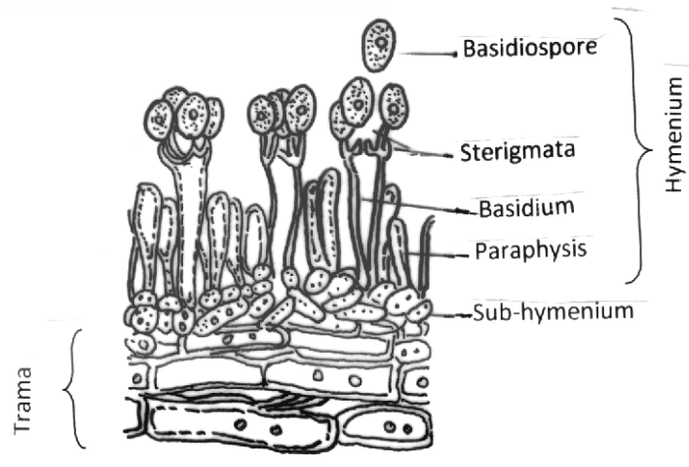
(b)



(e)



(c)



(f)

Fig. 8.1: *Agaricus*. (a-c) Diagrammatic representation of various stages of basidiocarp development. (a) Button stage. (b) Intermediate stage. (c) Mature basidiocarp. (d) V.s. basidiocarp showing gills. (e) A part of the gill magnified. (f) Magnified view of a gill showing various components of hymenium, sub-hymenium and trama. Note basidium and basidiospores.

EXERCISE 9

LICHENS

Structure

9.1 INTRODUCTION

Lichens exhibit association between two kinds of organisms. Their association is so intimate that it provides an appearance of a single organism. A lichen body possesses an algal and a fungal component. The algal component - phycobiont, makes the lichen autotrophic. It belongs to either blue green algae (cyanobacteria) or green algae. The fungal component - mycobiont, provides lichen the ability to reproduce. The nature of relationship between these algal and fungal components in a lichen body is a topic of debate and conjecture among the scientists. It is regarded as mutualistic (symbiotic), where both the components are equal partners and derive benefit from each other. It is also viewed that fungal component parasitises the phycobiont (parasitism). Some regard the relationship as helotism, where one component, the mycobiont, derives more benefit than the other, the phycobiont.

The lichens are classified on the basis of their attachment to the substratum as crustose, foliose and fruticose. They are also classified as ascolichens and basidiolichens depending on the kind of sexual fruiting bodies they produce. Consequently, the fungal component belongs to Divisions Ascomycota and Basidiomycota respectively. When the fungal partner belongs to class Deuteromycetes, the lichens are called Deuterolichens. Lichens can grow on a variety of habitats such as bare-rocks, bark of trees, extreme environmental stress conditions of humidity, drought, and snow. They are also regarded as the pioneer organisms of xeric succession. Apart from ecological significance, lichens are also used as fodder. They are important in brewery, cosmetics, perfume, tanning and dyeing industries.

Objectives

After doing the exercise you should be able to:

- identify the three kinds of lichen thali on the basis of their form;
- examine the anatomical features of foliose lichen thallus;
- observe the modifications in the anatomical features of fungus and alga in such symbiotic relationship;
- recognize and describe the vegetative, asexual and sexual reproductive structures in lichens; and
- record your observations about the specimens and permanent slides in the form of labelled diagrams and write explanatory notes.

Study Guide/Prior Reading

For doing satisfactory work you must read the following before coming to the laboratory.

Unit 11 Fungi: Lichens and Mycorrhiza. Sub-sections 11.2.1 General Account, and 11.2.2 Reproduction.

9.1.2 Method of Study

You shall conduct this exercise through herbarium/museum specimens, photographs and permanent slides.

9.1.3 Materials Required

In addition to *Biology Laboratory Student's Kit* you require the following:

1. **Plant Materials**

   Herbarium/museum specimens

   i) **Crustose lichen**

   Graphis/Lecanora/Haematomma/ any other

   ii) **Foliose lichen**

   *Physcia/Parmelia/Peltigera/Collema/Parmotrema /Gyrophora/*any other

   iii) **Fruticose lichen**

   *Usnea/Ramalina/*any other

2. **Permanent slides**

   V.s. vegetative thallus of foliose lichens: homoisomerous and heteromerous,

   W.m. soredium,

   V.s. pycnidium, and

   V.s. apothecium.

3. **Glassware and Apparatus**

   Hand lens, magnifying glass, dissection microscope, and compound microscope.

9.1.4 Procedure

(i) Observe, draw, label the morphological, anatomical and reproductive details of the various specimens/permanent slides provided.

(ii) Write explanatory notes and the identification points for all the observations made.

9.1.5 Observations

Refer to Fig. 9.1

(A) Morphological Features

Observations and study the specimen photographs provided and look for the following aspects.

i) Crustose lichen

(1) They appear as hard, granular crusts on the substratum such as rocks and bark of trees.

(2) They adhere very firmly and closely to the substratum.

(3) Thalli are partially or completely embedded in the substratum.

(4) Thalli are variously coloured, ash-colour is very common.

(5) A lot of polygonal areas called aerolae are visible on the surface of thallus.

(6) Fruiting bodies, if present, are formed on the upper surface.

ii) Foliose lichen

(1) Thallus is deeply incised into a lot of leaf-like, lobed margins.

(2) Thallus is flat.

(3) From the under surface of the thallus at certain regions arise rhizines. These rhizines help the thallus to attach to the substratum.

(4) Thallus could be brownish or greyish.

(5) At certain regions of the thallus, small, hard, dark and gall-like outgrowths can be observed. These are called cephalodia (sing. cephalodium).

(6) Cephalodia help retain moisture.

(7) Pycnidia and apothecia may be observed on the upper surface of thallus.

iii) Fruticose lichen

(1) Fruticose lichens are shrubby and possess cylindrical, flat or ribbon-like body.

(2) They are upright, pendulous and often branched.

(3) Rhizines form disc-like structures and help thallus attach to the substratum.

(4) Distal ends of the thalli may produce sexual fruiting bodies such as apothecia.

(B) Internal Structure

Observe the permanent slides under a dissection microscope and a compound microscope. Observe the following structures and draw labelled diagrams Refer BBYCT-131, Block-3, Fig. 11.3.

Foliose lichen

A foliose lichen could be of homoisomerous (*Collema, Leptogium*) or heteromerous (*Parmelia, Physcia*) internal organization.

i) Homoisomerous thallus

(1) The algal cells, (phycobiont) are irregularly scattered through the fungal (mycobiont) mycelia.

(2) Both the algal cells and fungal hyphae are embedded in a gelatinous matrix or the ground substance.

ii) Heteromerous thallus

(1) The thallus possesses four distinct zones or regions.

(2) These regions are: upper cortex, gonidial layer (algal layer), medulla, and lower cortex.

(3) The upper cortex may or may not be delimited by an epidermis like layer or hyphae.

(4) Upper cortex may possess breathing pores that help in gaseous exchange.

(5) Within upper cortex the hyphae are placed vertically without any intercellular spaces. If such intercellular spaces are present, then they are filled with gelatinous materials.

(6) An algal or gonidial layer occupies the zone beneath upper cortex. A large number of algal cells are held together in a network of fungal hyphae within this region.

(7) A loosely interwoven network of fungal hyphae constitutes medulla, a region present below the gonidial or algal layers.

(8) The lower side of the thallus (near the substratum) is made-up of lower cortex. The lower cortex consists of compact fungal cells, lying parallel or perpendicular to the lower surface.

(9) Rhizines are formed in the lower cortex zone.

(C) Reproductive Structures

Refer : BBYCT-131, Block-3, Figs 11.4 and 11.5.

i) Soredium (w.m.)

Observe the permanent slide of w.m. of soredium and study the following aspects.

(1) Each soredium is a structure for vegetative propagation.

(2) It is a bud-like outgrowth developing either from the entire surface or in localized patches called soredia.

(3) It is non-corticated, non-pigmented and whitish, arising out of medulla of thallus.

(4) It consists of a few medullary hyphal cells enmeshed around algal cells.

ii) Pycnidium (v.s.)

Observe and study the permanent slide showing v.s. pycnidial cup or pycnidum.

(1) Pycnidia or pycnidial cups are produced on the upper surface of the thallus.

(2) A pycnidum is a flask-shaped cavity with a small opening at its apex, called ostiole.

(3) It is lined by hyphae thoughout its entire inner cavity.

(4) The tip of these hyphae produce pycnidiospores.

(5) The pycnidiospores are released through the ostiole.

(6) When in contact with an algal cell, the pycnidiospore forms a new lichen thallus.

iii) Apothecium (v.s.)

Observe and study the v.s. of apothecium, asci and ascospores.

(1) An apothecium is a saucer-shaped fruiting body.

(2) An apothecium is lined with palisade-like layer of cells called hymenium.

(3) Within the hymenium are observed a series of elongated cells, the asci, intermixed with sterile hyphal cells, the paraphyses.

(4) Each ascus usually contains one to eight ascospores. Eight is the usual number.

(5) An ascospore when released, comes into contact with an algal cell and they form a new thallus.

(6) The thalloid vegetative region of the apothecium exhibits heteromerous body organization.

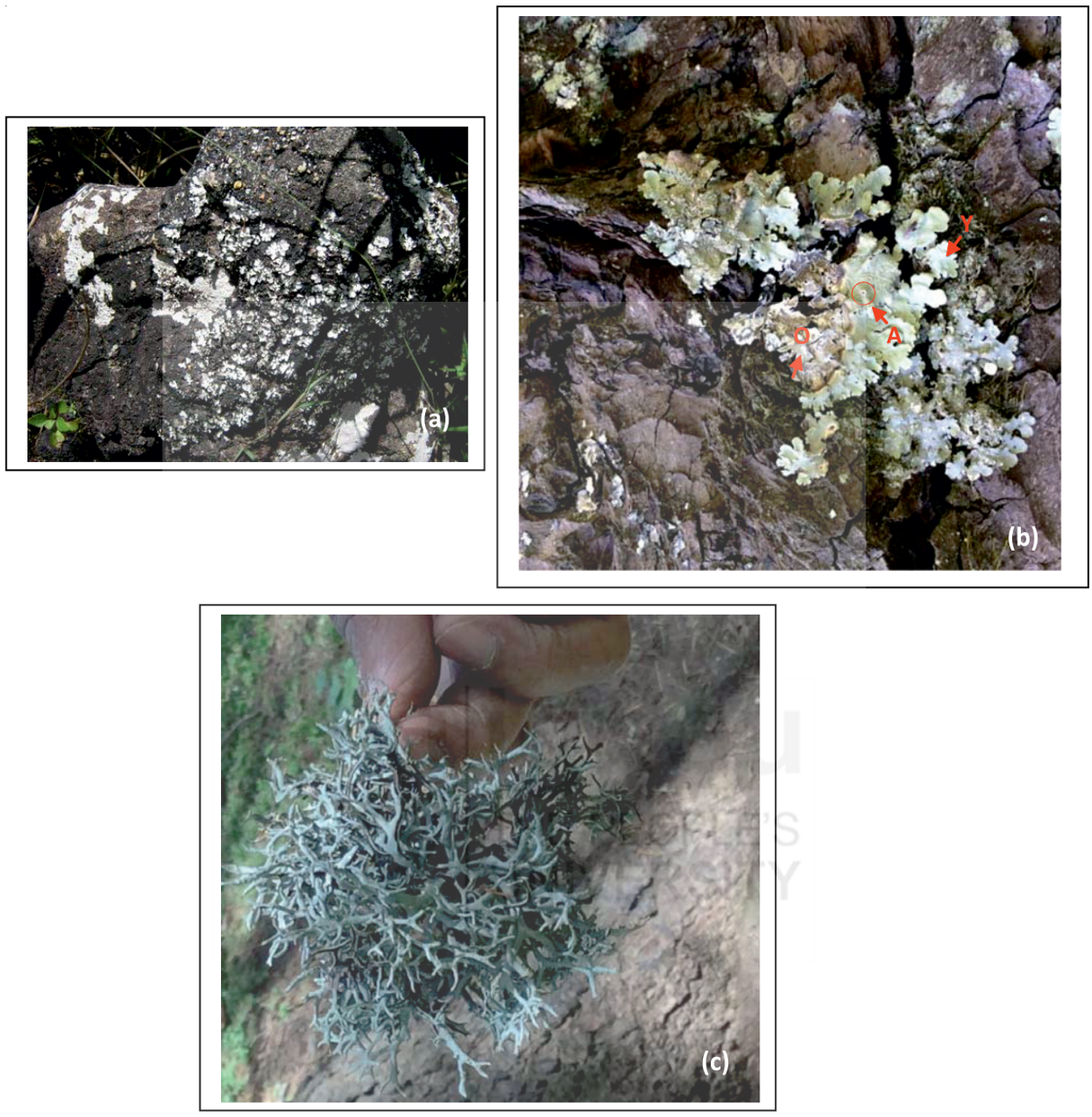


Fig. 9.1: Lichens. a) Crustose lichen; b) Foliose lichen; c) Fruticose lichen. In Fig. (b) note apothecium (A), old (O) and young (Y) portions of the thallus. (Courtsey: A.K. Kavathekar, 2017).

EXERCISE 10

MYCORRHIZA

Structure

10.1 INTRODUCTION

Mycorrhiza constitutes a mutualistic symbiotic association between a fungus and the roots of a plant. They are also called 'Root Fungi'. There are more than 6000 species of fungi that are known to be mycorrhizal. Almost 95 per cent of vascular plants have mycorrhizal association. During these symbiotic relationships, the fungus provides principally, the elements phosphorus and nitrogen to the host root and gets photosynthetic carbon and vitamins in return.

There are two principal kinds of mycorrhiza: ecotomycorrhiza and endomycorrhiza. More than 140 genera belonging to around 40 families of terrestrial plants are known to possess ectomycorrhiza. They form a thick peripheral network of fungal colony around the roots of the host. In endomycorrhiza the fungal structures are almost entirely present within the host-root. Outwardly, the roots look normal. Arbuscular Mycorrhiza (AM fungi) are the commonest of all mycorrhiza reported in more than 80 percent of plant species of which 90 per cent are vascular plants. These AM fungi were earlier known as Vescicular Arbuscular Mycorrhiza (VAM). Endomycorrhiza can be classified as: Arbuscular, Ericoid, and Orchidaceous.

Mycorrhiza also plays an important role in the ecology of soil where they occur. Apart from host-specific benefits, mycorrhiza helps natural soil ecosystems via seedling establishment and supply of recycled nutrients.

Objectives

After doing this exercise you should be able to:

- observe and recognize the host-fungal relationship in a mycorrhiza;
- differentiate between ectomycorrhiza and endomycorrhiza; and
- compare and contrast the characteristics of Harting net, arbuscule and pelton.

Study Guide/Prior Reading

For doing satisfactory work you must read the following before coming to the laboratory.

Unit 11 Fungi: Lichens and Mycorrhiza. Sub-sections 11.3.1 Ectomycorrhiza, and 11.3.2 Endomycorrhiza.

10.1.2 Method of Study

You shall study and compare the structures of ectomycorrhiza and endomycorrhiza through photographs and illustrations.

10.1.3 Materials Required

1. Photographs of rhizomorph of ectomycorrhiza on a root.
2. Microphotograph/illustration of Harting-net.
3. Microphotographs/electron micrographs/illustrations of arbuscule, pelton.
4. Photographs of mycorrhizal fungi: *Glomus* (AM-fungi); and *Rhizoctonia* (orchidaceous endomycorrhiza).

10.1.4 Procedure

- You shall conduct the exercise by observing, comparing the photographs/ illustrations/microphotographs of rhizomorph of ectomycorrhiza.
- Microphotograph/illustration of a Hartig net.
- Microphotograph/electron micrograph of arbuscule, and pelton.
- Photographs/illustrations of AM-fungi - *Glomus*, and orchidaceous endomycorrhiza - *Rhizoctonia*.
- Draw labelled diagrams of the observations made and write explanatory notes about them.

10.1.5 Observations

Refer to Fig. 10.1.

(a) Ectomycorrhiza

(i) Morphology

1. The root of the host species is completely surrounded by a sheath of fungal tissue.

2. This fungal sheath could be several mm thick.
3. The network of fungal mycelium could form a rhizomorph.

(ii) Hartig net

1. The fungal hyphae penetrate the root.
2. Within the root, the fungal hyphae penetrate between intercellular spaces of outermost layers of root and form a network of mycelium.
3. These intercellular hyphae form a net-like structure called Hartig net.
4. This net-like structure increases the surface area for fungus-host interface.

Endomycorrhiza

Morphology

1. The fungal structures are almost entirely present within the host root.
2. Outwardly, such 'infected' roots took normal.
3. Host-fungal association is endotrophic.
4. Endomycorhiza could be: arbuscular, ericoid, or orchidaceous.

(b) Arbusular Endomycorrhiza (AM)

- They are obligate biotrophs.
- They occur as coarse, intercellular, aseptate, coenocytic mycelia.
- Repeated, dichotomous branching of hyphae is called arbuscule.
- Within host cell/tissue they form large balloon-shaped intercalary or terminal vesicles.
- Such vesicles are thick-walled.
- These arbuscules invaginate plasmalemma of host cells. These can also be termed as haustoria.
- There exist an apoplastic compartment in between plant and fungal cell membrane. This space is termed as periarbuscular space.
- Through this periarbuscular space the exchange of nutrients between the host and the fungus takes place.
- These arbuscules are ephemeral. Dead arbuscules are digested by host cells. They could also be observed as irregular clumps of fungal remnants within the host cells.
- These vesicles are swollen, spherical or oval and multinucleate.
- These vesicles are rich in lipid-content.
- They reproduce by budding at hyphal tips.
- When in contact with soil, such mycelia can produce multinucleate chlamydospores - the resting spores.

- A very common, most prevalent arbuscular endomycorrhizal fungus is *Glmous* (Zygomycota).

(c) Ericoid Endomycorrhiza

1. The fungus *Hymenoscyphus* (Ascomycota) 'infects' the plants of *Erica* and a few other flowering plants to enter into endomycorrhizal relationship.
2. During harsh winter season, when host plants cannot produce their food through photosynthesis, the fungus provides C-nutrients to the host.
3. A non-chlorophyllous genus, *Monotropa* also enters into similar relationship with such mycorrhiza.

(d) Orchidaceous Endomycorrhiza

1. They are associated with orchids all through the life cycle of their host.
2. During this relationship there is net flow of C-nutrients from fungal partner to the host, especially during very early phase of life of the host plant.
3. Fungus penetrates host through the epidermal hair cells of non-chlorophyllous protocorm phase of orchids.
4. Inside cortex of protocorm, fungus develops a strong network of hyphae.
5. Within cortex of protocorm, they produce a mass of coiled hyphae.
6. Such a structure is called pelton.
7. Each pelton is ensheathed by host plasmalemma and produces a perifungal membrane - a modified form of plasmalemma.
8. Nutrients enter the host through such modified plasmalemma.
9. The fugus *Rhizoctonia* is the most prevalent orchidaceous endomycorrhizal fungus.
10. In such a relationship the orchid host is a parasite on the fungus, *Rhizoctonia*.

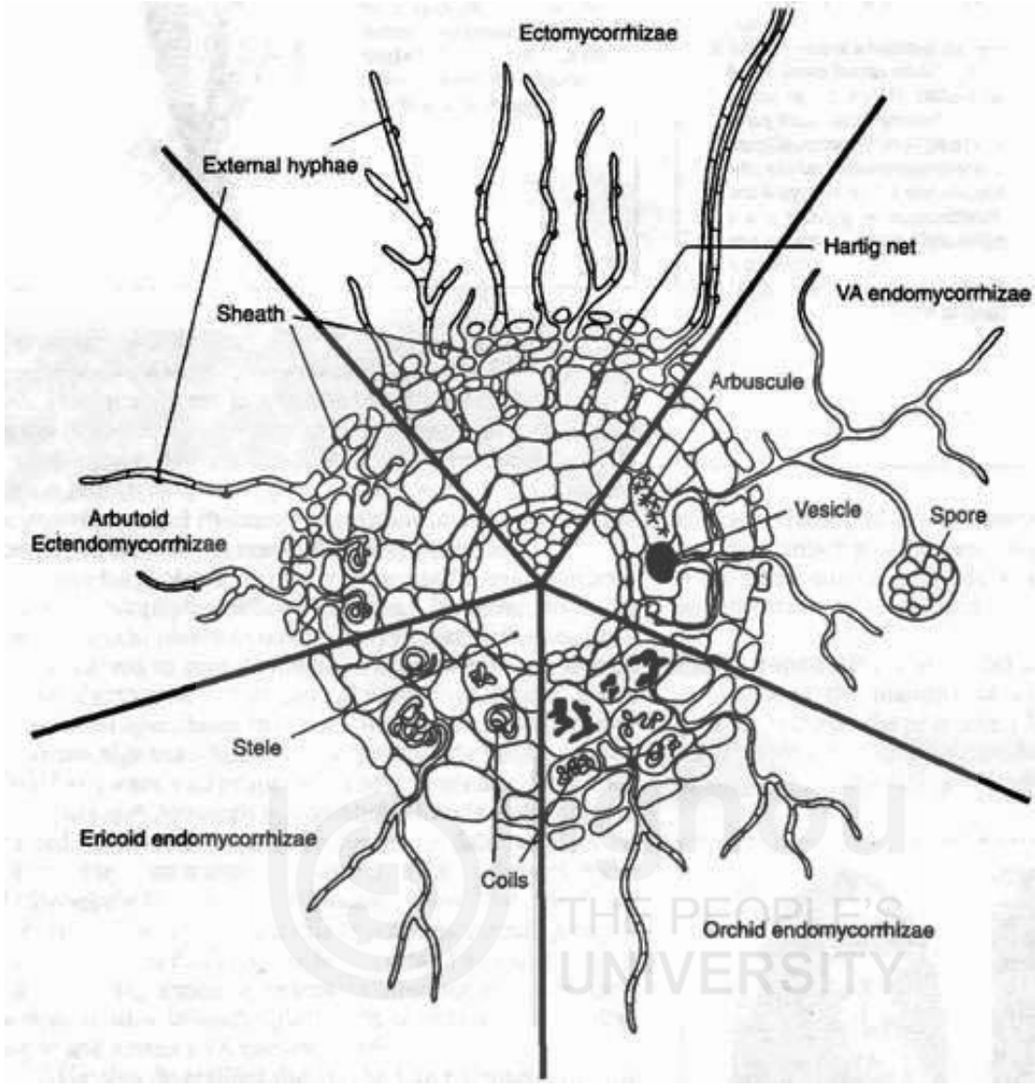


Fig. 10.1: Mycorrhiza. a) Ectomycorrhiza, note Hartig net; b) Vesicular arbuscular endomycorrhiza (VAM); c) Orchid endomycorrhiza; and d) Ericoid endomycorrhiza; e) Arbutoid endomycorrhiza. *Source*: www.davidmoore.org.uk

EXERCISE 11

MARCHANTIA

Structure

11.1 INTRODUCTION

Bryophytes are the simplest, most primitive non-vascular plants. They thrive in habitats that are more or less perpetually wet. It is believed that they were the first land plants and might have originated from fresh water photosynthetic ancestor, probably algae. During their evolution the bryophytes acquired certain adaptations to survive in the terrestrial habitats. These adaptations are: possession of protective cover, cuticle, minute reproductive structures, release of spores in air, all alike spores (homosporous), protective chemical present in spores, sporopollenin, presence of stomata, and structure associated with gaseous exchange in some bryophytes.

Although, bryophytes are land plants, they need water to transfer male gametes to carry out fertilization. More than 23,000 known bryophytes are divided into three sub-groups: liverworts, hornworts, and mosses. All of them are small, green, measuring in few centimeters and are devoid of roots.

Like all sexually reproducing plants the bryophytes have distinct alternation of generations between gametophytic and sporophytic phase. They represent the only group of land plants where the gametophyte is conspicuous, independent with no or poorly developed transporting tissue and are autotrophic. Their sex organs, antheridium (male) and archegonium (female) are multi-cellular and possess sterile cells as cover. The sporophyte on the other hand is partially or wholly dependent on the gametophyte for anchor and nutrition.

Together with vascular plants, the bryophytes are embryophytes, the plants that produce multi-cellular embryos.

In this and next exercise, you will study the morphological, anatomical and reproductive features in two genera – *Marchantia*, a liverwort and *Funaria*, a moss.

Objectives

After doing this exercise, you will be able to:

- follow right procedure for examination of specimens of *Marchantia* and *Funaria* for investigating their morphological, anatomical and reproductive characteristics;
- make suitable sketches of such characteristics observed and label their features;
- write reports on the observations made;
- compare and contrast the morphological, anatomical and reproductive features of two bryophytes investigated; and
- assign the given genera of bryophytes to various taxa of classification by providing suitable identification points.

Study Guide/Prior Reading

For doing satisfactory work you may read the following before coming to the laboratory.

B.Sc. UGC CBCS, Core Course Botany Paper – I Biodiversity (Microbes, Algae, Fungi and Archegoniates)

Unit 12 Introduction to Archegoniates, Unit 13 Bryophytes: Introduction, Unit 14 Bryophytes: Type Studies.

11.2 *MARCHANTIA*

11.2.1 Introduction

The genus *Marchantia*, a liverwort, though cosmopolitan in distribution thrives in moist places mainly in the temperate region. Of the more than 65 species of *Marchantia* about 11 including the most common *M. polymorpha* and *M. palmata* are reported from India.

Some salient characteristics of *Marchantia* are: dichotomously branched, dorsiventral thallus, distinct apical notch, reproducing asexually through gemmae produced in distinct gemma cups, the sex-organs - antheridia and archegonia produced on separate specialized structures antheridiophores and archegoniophores respectively, thalli are dioecious, sporophytic phase is completely dependent on the gametophyte for food, protection and anchor. The independent, autotrophic, dominant gametophytic phase characterizes the heteromorphic alternation of generations.

Structure

Objectives

This exercise would enable you to:

- ❖ follow the right procedure for examination of *Marchantia* thallus for investigating its morphological characteristics;
- ❖ make a sketch of the morphology of a specimen of *Marchantia*, observe and label its features;
- ❖ prepare materials for examination of internal structure of thallus of *Marchantia*;
- ❖ examine the anatomical structures of thallus of *Marchantia*, draw a diagram and label the parts observed;
- ❖ study the structures of sex-organs, and sporophyte in *Marchantia*;
- ❖ write explanatory notes on the observations made; and
- ❖ jot the classification of *Marchantia* mentioning its identification points.

Study Guide/Prior Reading

For doing satisfactory work you may read the following before coming to the laboratory.

B.Sc. UGC CBCS, Core Course Botany Paper – I Biodiversity (Microbes, Algae, Fungi and Archegoniates).

Unit 14 Bryophytes: Type Studies, Section 14.3 *Marchantia*.

11.2.2 Method of Study

In this exercise you would:

- observe vegetative and reproductive thalli of *Marchanita* with unaided eye and by using a dissection microscope/magnifying glass;
- make safranin-stained temporary whole mount preparations of rhizoids, scales, and gemmae;
- study v.s. thallus, rhizoids, scales, v.s. gemma cup, gemma, v.s. antheridiophore, antheridium, v.s. archegoniophore, archegonium, v.s. mature sporophyte through permanent slides;
- draw labelled sketches of your morphological and anatomical observations made and write explanatory notes.
- highlight the identification points for *Marchantia*.

11.2.3 Materials Required

In addition to *Biology Laboratory Student's Kit* you require:

1. **Plant Materials**

   (A) Museum/Herbarium specimen of vegetative thallus, thallus with gemma cups, thalli with antheridiophore and archegoniophore.

   (B) Fixed Materials of thallus of *Marchantia*.

2. **Permanent Slides** of v.s. thallus, w.m. rhizoids, w.m. scales, v.s. gemma cup, w.m. gemmae, v.s. antheridiophore, v.s. archegoniophore.
3. **Chemicals** - Safranin (0.5%), glycerine 1.0%, distilled water.
4. **Glassware and Apparatus** - magnifying glass/dissection microscope, compound microscope, microslides, microcover slips, watch glasses, petri dishes.

11.2.4 Procedure

You will study the salient morphological, anatomical and reproductive features of *Marchantia* by observing the following characters, drawing labelled sketches and writing explanatory notes.

11.2.5 Observations

Refer to Fig. 11.1.

(A) Morphological features

Observe the vegetative thallus of *Marchantia* and look for:

i) Thallus

(1) The thallus is prostrate, flat, dorsiventral, deep green (if the plant material is fixed the colour may be faded), and dichotomously branched.

(2) A shallow groove is prominent on dorsal surface.

(3) A distinct mid-rib as low ridge is observed on ventral surface.

(4) Along the mid-rib on ventral surface are numerous hairy structures called rhizoids.

(5) On either side of mid-rib on ventral surface are present numerous scales.

(6) A large number of hexagonal areas, visible to one's eye or through a magnifying glass are present on the entire dorsal surface. Within each of the hexagonal area a prominent pore is observed.

ii) Rhizoids

The rhizoids are unicellular hair-like extensions of lower epidermal cells of the thallus. These could be simple or tuberculate.

- **Simple rhizoids:** They possess smooth walls.
- **Tuberculate rhizoids:** The inner cell walls show knob-like projections. In surface view, the projections appear as circular dots.

iii) Scales

The scales are multi-cellular, dark structures are arranged in 2-4 rows along the either side of the mid-rib on the ventral surface of the thallus. They could be:

- **Simple**, or
- **Appendiculate**, the ones which possess a sub-rotund appendage at its tips.

(B) Anatomical features

V.s. thallus

Observe the following characteristic features of the internal tissue organization of vegetative thallus in vertical section.

- Two distinct zones, upper and lower (dorsal and ventral respectively) are observed. Upper zone is photosynthetic while the lower zone is primarily for storage and anchor.

Upper Zone Surface

(1) Upper zone is covered with a thin-walled single layer of cells, the epidermis.

(2) The cells of the epidermis are chlorophyllous.

(3) The epidermis is interrupted at regular intervals by barrel-shaped pores.

(4) A distinct air-chamber lies beneath every pore.

(5) Two adjacent air-chambers are separated by uni-seriate vertical partition, made up of chlorophyllous cells.

(6) Within each air-chamber lie, simple or branched, multi-cellular, filaments of cells rich in chloroplasts. These are also called photosynthetic filaments.

(7) These photosynthetic filaments arise from the base of the air-chamber.

Lower Zone Surface

(1) Lower zone is made of multi-layered, compactly arranged parenchymatous cells which are distinctly achlorophyllous.

(2) A few cells, scattered randomly, possess oil, and are called oil cells.

(3) A few cells, scattered randomly possess mucilage, and are called mucilaginous cells.

(4) A distinct, single layered epidermis delimits the lower surface. These cells are also achlorophyllous.

(5) A number of scales can be observed arising from the lower epidermal cells.

(6) A large number of rhizoids also arise from the ventral surface of epidermis, between the scales.

Reproductive features

(a) Asexual reproductive structures

Morphology of gemma cup

Observe a thallus with gemma cups:

(1) Gemma cups are found on the dorsal surface of the thallus, arising as a part of the thallus.

(2) It remains attached to thallus by its base.

(3) A large number of gemmae are present in a cup. The development of gemmae within a cup is asynchronous.

V.s. thallus with gemma cup

Observe the permanent slide and look for the following features.

(1) Outline is goblet-shaped. It possesses an outer wall and a central cavity.

(2) The outer wall shows outer photosynthetic zone and inner storage zone (similar to that of a vegetative thallus).

(3) From the floor of the central cavity arise numerous discoid gemmae.

(4) Intermingled with gemmae are many mucilage hairs or cells.

Structure of a gemma

Study permanent slide of w.m. of gemma(e) under a compound microscope and look for the following aspects.

Each gemma has one-celled stalk. It helps gemma attached to the base of gemma cup.

(1) A gemma is multi-cellular.

(2) A gemma is disc-shaped.

(3) It has 2 shallow notches on both the lateral sides.

(4) Each notch possesses a row of apical cells.

(5) Towards the periphery of the gemma, colourless oil cells are present.

(6) Rhizoidal cells are present among the central core of cells.

(7) All the cells of gemma, except oil and rhizoidal cells contain chloroplasts.

(b) Sexual reproductive features

Observe male and female reproductive thalli bearing antheridiophore and archegoniophore respectively. These are erect sexual branches, are continuation of the thallus and grow vertically upwards through the apical notches at the end of tip of the thallus/branch.

Antheridiophore

Morphology

(1) An antheridiophore is formed on the adaxial surface of thallus.

(2) It consists of a stalk and a disc at the apex.

(3) The disc consists of 8 lobes.

V.s. of antheridial disc

Study the v.s. antheridiophore/antheridial disc in a permanent slide and observe that:

(1) The upper epidermis is interrupted by barrel-shaped pores that open into air chambers.

(2) Within air chambers are present multi-cellular branched/unbranched, chlorophyllous filaments.

(3) In addition to the air chambers there are many flask-shaped cavities.

(4) Each cavity has an opening at its upper end.

(5) At the base of each cavity lies an antheridium, the male sex-organ.

Antheridium

(1) Each antheridium possesses a multi-cellular stalk and globular body.

(2) The body of antheridium is delimited by a multi-cellular, single-layered jacket.

(3) A very large number of androcytes makes up the bulk of antheridium.

(4) Each androcyte produces one biflagellate antherozoid.

(5) Both the flagella of an antherozoid are anteriorly placed and are whiplash type.

Archegoniophore

Morphology

(1) An archegoniophore is formed on the adaxial surface of the thallus.

(2) It consists of stalk, stellate and peltate disc at the top.

(3) A total of 9-rays are present in the disc.

(4) Groups of archegonia, the female sex organs, are found in between the rays.

V.s. of archegoniophore

Study the v.s. archegoniophore/ archegonial disc(s) in a permanent slide and observe the features mentioned below.

(1) The internal structure of a disc is similar to that of a thallus.

(2) Outermost layer is epidermis, interrupted by air-pores.

(3) Each air pore opens into an air-chamber.

(4) Within an air-chamber are present multi-cellular, branched/ unbranched chlorophyllous filaments.

(5) In the young stage, 8 groups of archegonia are formed on upper surface. Each group represents a growing point of a disc. The archegonial necks face upwards at this stage.

(6) As the disc matures, the growing apices get curved and lie close to the stalk. The archegonial neck faces downwards at this stage.

(7) The youngest archegonium in each group lies near the stalk and the oldest archegonium is now placed towards the periphery of the disc.

Observe and record only the structure in the permanent slide provided to you.

(8) In each group 12-15 archegonia are present.

(9) Each of this group of archegonia is enclosed by a 2-lipped, pendent sheath of involucres.

(10) This involucre sheath is called perichaetium.

(11) Perichaetium hangs down vertically from the lower surface of a disc.

(12) A green, cylindrical process arising from the periphery of disc, between the groups of archegonia is formed. It is called ray.

(13) In a mature archegoniophore there are 9 rays per stalk. However, only 2 rays are visible in v.s. of an archegoniophore.

Archegonium

(1) It has flask-shaped structure.

(2) It possesses a multi-celled, but small stalk, swollen venter and a long neck.

(3) A large egg cell is present within a single-layered venter.

(4) The long neck has 6 vertical rows of jacket-cells that surround 4 or more neck canal cells.

Sporophyte

(1) It is a post-fertilization product.

(2) As the post-fertilization events occur the walls of venter divide periclinally to produce 2 to 3 layered calyptra.

(3) The calyptra surrounds the developing sporophyte/sporogonium.

(4) A additional layer of cells arise from base of venter. It develops into perigynium/pseudo-perianth.

V.s. sporophyte

Study the v.s. archegoniophore showing v.s. sporophyte in a permanent slide and observe that:

(1) All the cells are diploid to begin with.

(2) Multi-cellular sporophyte at maturity is differentiated into foot, seta and capsule.

(3) Foot is bulbous or spreading structure directed towards the base of the archegonium.

(4) It anchors the sporophyte to gametophytic tissue.

(5) Seta is a short, thick, multi-celled, multi-layered middle segment of a sporophyte. It connects basal foot to the terminal part of sporophyte, the capsule. Compactly placed cells are elongated along the axial plane.

(6) The cells of seta elongate and push the capsule away from the point of attachment to gametophytic tissue.

(7) Capsule is the terminal free-end of the sporophyte.

(8) Capsule is spherical in shape. It possesses a single-layered wall, the jacket.

(9) Cell walls of the jacket cells possess thick-ring like bands.

(10) The multi-cellular, densely protoplasmic, mass of cells within jacket of capsule constitute sporogenous tissue.

(11) Each of the spore-mother cell (2n) following meiosis produces 4 haploid spores.

(12) A few cells of diploid sporogenous tissue develop into elaters without meiosis. Hence, each elater is a diploid cell.

(13) Elaters at maturity possess thick spiral thickenings.

(14) At the time of dehiscence, the capsule part of the sporophyte can be observed free of calyptra, pseudoperianth and perichaetium.

(15) Each spore has 2-walls. Outer exine and inner intine.

Observe and draw the sporophyte as observed in the permanent slide provided to you. In a given, permanent slide, there can be more than one developing sporophyte, each in a different stage of development. The one youngest sporophyte shall be near the stalk and the oldest one towards the periphery.

11.2.6 Classification and Identification

Kingdom: Plantae	:	1) Eukaryotic
		2) Cell wall cellulosic
		3) Autotrophic
Division: Bryophyta	:	1) True root absent
		2) Presence of archegonia and antheridia
		3) True vascular tissue absent
Class: Hepaticopsida	:	1) Rhizoids without septa
		2) Chloroplast without pyrenoid
		3) Capsule lacks columella
Order: Marchantiales	:	1) Presence of scales
		2) Two types of rhizoids
		3) Oil bodies in storage cells
		4) Sporogonium jacket - unistratose
Family: Marchantiaceae	:	1) Sex organs borne on stalked receptacles.
		2) Air pore barrel-shaped
		3) Capsule possesses elaters
Genus: *Marchantia*	:	1) Photosynthetic filaments within air chambers branched
		2) Scales ligulate as well as appendiculate
		3) Gemma cup goblet shaped.

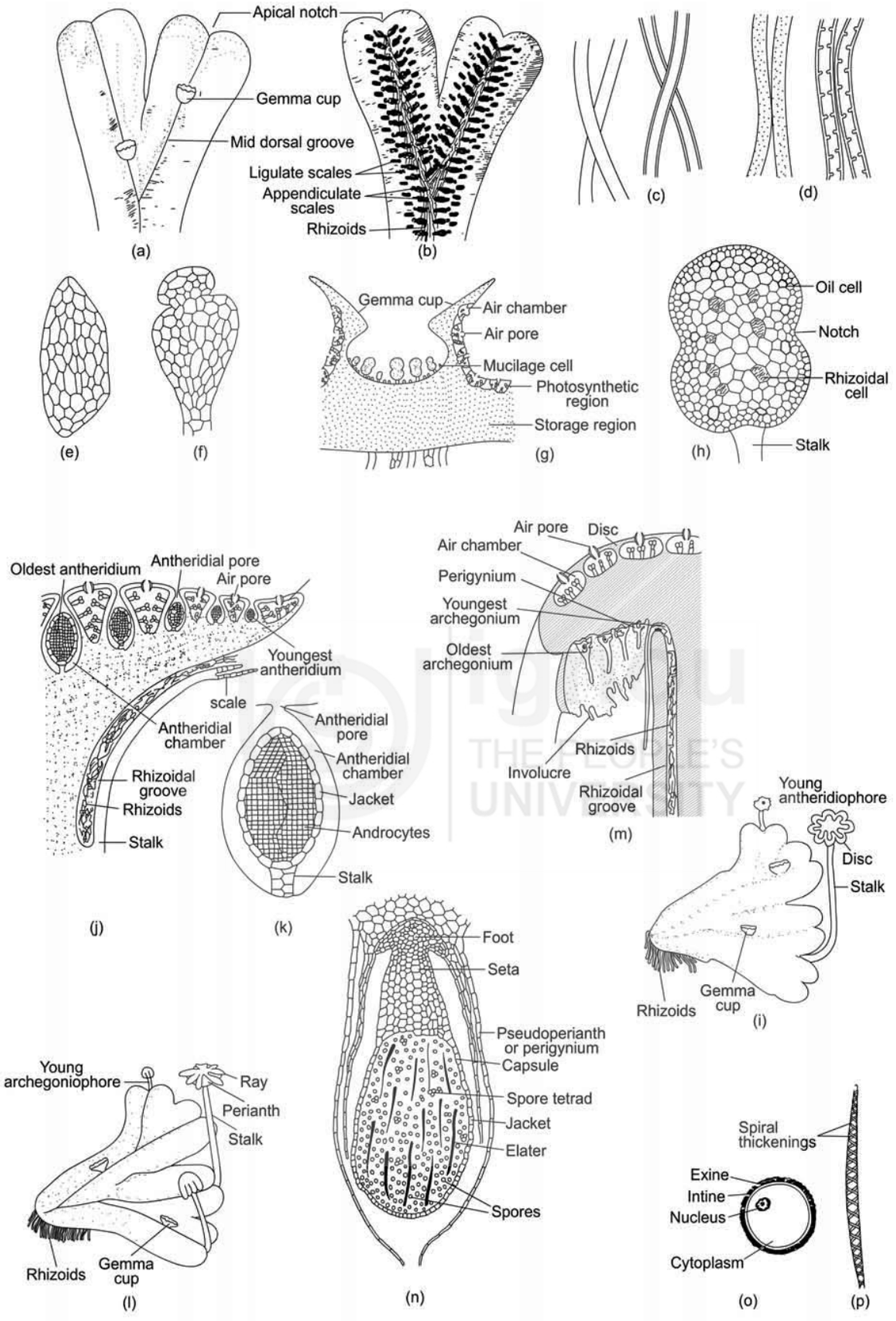


Fig. 11.1: ***Marchantia*****. a) Dorsal view of thallus; b) Ventral view of thallus; c) Parts of simple and tuberculate rhizoids. d) Enlarged view of simple and tuberculate rhizoids; e) Simple scale; f) Appendiculate scale; g) v.s. gemma cup; h) A gemma; i) Male thallus with an antheridiophore; j) l.s. antheridiophore; k) Antheridial chamber with antheridium; l) Female thallus with archegoniophore; m) l.s. archegoniophore; n) l.s. mature sporophyte; o) Spore; and p) Elater.**

EXERCISE 12

FUNARIA

Structure

12.1 INTRODUCTION

The genus *Funaria* is a very common moss. It is widely distributed throughout the world. One of the species *F. hygrometrica* is cosmopolitan and is best known of all the mosses. The adult plant body is a gametophyte, that is, the dominant phase in the life cycle. It has an upright axis and spirally arranged leaves on it. It is monoecious and autoeicous. The sporophyte is anchored to the gametophyte and is only partially independent for its nutrition. An independent branched, autotrophic, prostrate protonemal phase intervenes the upright, mature gametophytic and sporophytic phases. The alternation of generations is heteromorphic.

Objectives

After the conduct of this exercise pertaining to *Funaria* you are expected to:

- recognize and differentiate between gametophyte and sporophyte;
- describe the morphology of stem, leaves, rhizoids and protonema in the gametophyte;
- differentiate between antheridial and archegonial heads and study the organization of sex organs - antheridium and archegonium;
- identify various parts of the sporophyte;
- explain the internal organization of apophyses, theca and the upper region of capsule in a sporophyte;
- demonstrate procedures to prepare temporary mounts of leaf, rhizoids, peristome and spores; and
- write its classification mentioning the identification points.

Study Guide/Prior Reading

You may read the following before coming to the laboratory.

B.Sc. UGC CBCS, Core Course Botany Paper – I Biodiversity (Microbes, Algae, Fungi and Archegoniates)

Unit 14 Bryophytes: Type Studies, Sub-Section 14.4 *Funaria*.

12.1.2 Method of Study

You will conduct this exercise through:

- observation of a gametophyte and a sporophyte of *Funaria* with unaided eye and by a dissection microscope/magnifying glass.
- preparation of temporary stained mounts of leaves, rhizoids, peristome and spores.
- study of w.m. peristome, v.s. capsule in a sporophyte through permanent slides.
- permanent slides of w.m. of an antheridial and an archegonial head to differentiate between the sex organs - antheridium and archegonium.
- drawing labelled sketches of all the observations made and write explanatory notes on them.
- highlighting the identification points for classification of *Funaria*.

12.1.3 Materials Required

1. **Plant Materials**

   (A) Fixed/Fresh specimen of vegetative gametophyte, antheridial and archegonial heads and sporophyte of *Funaria*.

   (B) Herbarium specimen of *Funaria* with sporophyte.

2. **Permanent Slides** of w.m. leaf, rhizoid, protonema, antheridial head, archegonial head, v.s. capsule, operculum, and peristome.

3. **Chemicals -** safranin (0.5%), glycerine (1.0%), distilled water.

4. **Glassware and Apparatus** - Dissection microscope/magnifying glass, compound microscope, micro slides, microcover slips, petri dishes, watch glasses.

12.1.4 Procedure

You will study the salient morphological features of gametophytic plant body and reproductive structures and sporophyte of *Funaria* by observing the fixed, fresh, museum specimens, permanent slides and through temporary stained preparations.

12.1.5 Observations

You may refer to Fig. 12.1.

(A) Gametophyte

Pick up a leafy specimen of *Funaria* and observe the following features:

(a) Gametophore (vegetative)

(1) *Funaria* gametophyte is an erect, leafy plant about 1-3 cm high.

(2) The leaves are arranged spirally and crowded at apex of an axis.

(3) It may be branched. The branch, if any, arises from below a leaf. Such branching is termed extra-axillary.

(4) The axis is attached to soil with rhizoids.

i) Leaves

Observe the w.m. of a leaf under dissection microscope/ magnifying glass and look for the following features :

(1) They are sessile (without stalk).

(2) They are ovate in shape.

(3) A distinct thick mid-rib is present.

(4) Wings are less thick than the mid-rib.

(5) The cells of the wings are chlorophyllous with a number of chloroplasts within cells.

ii) Rhizoids

Observe the w.m. of rhizoids under a compound microscope and look for the following features:

(1) They are multi-cellular and copiously branched.

(2) The partition of cells is oblique.

(3) The cells are without chloroplasts and colourless. They may be brownish.

(b) Gametophore (reproductive)

Observe permanent slides of whole mounts of reproductive gametophores, w.m. of antheridial head, and w.m. of archegonial head and look for the following features:

i) Antheridial head

(1) The antheridia, the male sex organs, are formed at the tip of a main branch of a gametophores.

(2) Such tips of the axis are swollen and bear a crown of leaves called perichaetial leaves.

(3) At the distal end are present a number of antheridia.

(4) A large number of uni-seriate filaments with swollen terminal cells are present amongst the antheridia. These filaments are called paraphyses.

(5) Antheridia exhibit asynchronous development.

Antheridium

(1) Each antheridium is stalked and is club-shaped.

(2) The stalk is massive and multicellular.

(3) One-layered sterile cover, called jacket, surrounds the body of antheridium.

(4) The cells of jacket are chlorophyllous.

(5) The tip/terminal end of the jacket is with swollen cell(s) called operculum.

(6) A dense mass of large number of androcytes forms a bulk of antheridium.

(7) Androcytes produce biflagellate antherozoids.

(8) Both the flagella are terminal, equal in length and are of whiplash type.

ii) Archegonial head

(1) The female sex-organs, the archegonia are formed at the tip of the branch. During post-fertilization phase of life, such lateral branch overtakes the main axis in growth. It may provide an apparent view of a main axis. Such tips are distinctly swollen.

(2) Archegonia are surrounded by a cluster of leaves intermingled with them. These leaves are called paraphyses.

(3) The paraphyses are uniseriate, multi-cellular and with swollen tips. The cells could be chlorophyllous.

(4) Archegonia and paraphyses are surrounded by closely folding, unmodified chlorophyllous leaves. These are known as perichaetial leaves.

(5) Archegonia exhibit synchronous development and are present in a cluster.

Archegonium

(1) Each archegonium has a massive, multi-cellular stalk.

(2) It has a broad 2-layered venter.

(3) Within a venter is present a ventral canal cell.

(4) The neck of an archegonium has six long rows of cells with a common neck canal.

(5) The neck canal possesses six or more neck-canal cells.

(6) One large egg cell is present in the cavity formed by a wide venter.

Funaria, is monoecious but autoecious, i.e., both kinds of sex organs are present on the same plant but on separate branches. The main branch bears antheridia and lateral branch produces archegonia.

(B) Sporophyte

(i) Pick-up a specimen where a sporophyte is attached to a gametophyte and look for the following feature(s):

(1) A sporophyte develops on the lateral branch of *Funaria* plant.

(2) Following fertilization and during sporophyte development this lateral branch may overtake the main branch in growth and may provide a 'false-look' of a main axis.

(3) The length of the sporophyte may be more than the vegetative axis.

(4) A sporophyte develops from a diploid zygote formed within an archegonium on an archegonial head of a branch, and is thus physically attached to the gametophyte at its base.

(5) A sporophyte has three parts - foot, seta and capsule.

(6) Foot is the basal portion of a sporophyte, is poorly developed and is embedded within the archegonial head. You may or may not observe this part of the sporophyte.

(7) A seta, representing the middle part of a sporophyte, is a long, slender and often twisted structure. It's length pushes the capsule, the terminal part of a sporophyte, aerially.

(8) On top of seta is present capsule. It is slightly lobed and has a pear-shaped calyptra covers the capsule.

ii) L.s. capsule

Observe a permanent slide of l.s. capsule under a compound microscope and look for the following features:

A capsule has 3 distinct parts - apophysis, theca (capsule proper), and upper region.

Apophysis

(1) It is basal part of the capsule.

(2) It has conspicuous conducting strands in the centre that are continuous with those present in seta.

(3) The cental part is surrounded by chlorophyllous, parenchymatous intercellular spaces.

(4) The outer most single-layered epidermis possesses stomata.

Theca

(1) Theca is the fertile region of the capsule.

(2) It possesses a massive, multi-cellular, central group of cells called as columella.

(3) Columella projects into the cavity of operculum of upper region of capsule.

(4) Basal part of theca is connected to the central part of the apophysis.

(5) Around the columella is located 'U'-shaped spore sac.

(6) The spore sac is broken at the base, thus separating two arms of the alphabet 'U'.

(7) The spore sac consists of an outer wall of three or four layers and a middle wall of one layer. In the middle space sporogenous tissue are located.

(8) Sporogenous tissue differentiates into spore mother cells.

(9) Meiosis in spore mother cells produces a large number of haploid spores.

Draw only those stages out of (7), (8) and (9) that you observe in the permanent slides provided to you.

(10) Outside the spore sac lies an air space. This air space is divided into a number of chambers by transverse uniseriate and multicellular filaments. These filaments are called trabaculae.

(11) Two-three layers of capsule wall near theca are chlorophyllous. Other cells are colourless.

Upper Region

(1) The upper region of the capsule consists of operculum, peristome, annulus and rim.

(2) At the constriction immediately at the points where theca joins upper region lie a few layers of thin-walled cells. This zone is called rim.

(3) Just above the rim lies very delicate five or six layers of superimposed epidermal cells, called the annulus. The break within annulus helps dispersal of spores from the spore sac.

(4) Above the rim lie two layers of peristome.

(5) The mouth of capsule is covered by operculum.

iii) Peristome

Dissect out the two rows of peristome teeth by keeping a mature capsule on a microslide. Remove operculum with the help of needles while viewing through a dissection microscope/magnifying glass. Mount them in glycerine and observe the following:

(1) The peristome teeth are yellow-black or yellow-brown.

(2) The peristome consists of two rows of curved triangular plate-like teeth. Each row has sixteen teeth.

(3) Inner peristomal teeth are colourless, shorters, and delicate.

(4) The bases of inner peristome teeth are directly covered by the teeth of the outer peristome, but as they move away from the base, they curve, thus narrowing the slits between outer peristome teeth.

(5) Hygroscopic movements in the outer peristome teeth assist in the liberation of spores from the capsule.

iv) Spore

Observe the spores within a spore sac in l.s. capsule and by squeezing the mature capsule on a microslide. Put a drop of safranin over it. Drain-out excess stain and put a drop of glycerine. Cover with the a microcover

slip and observe this temporary whole mount under a compound microscope and look for the following features:

(1) The spores are uni-cellular, uni-nucleate.

(2) It possesses two-layered cell wall. The outer is coloured, smooth, and is called exine or exospore. The inner, is hyaline and is called intine or endospore.

(3) A nucleus, oil globules, chloroplasts and cytoplasm are visible inside the intine.

(C) Protonema

Observe the permanent slides of a protonema and look for the following features in it.

(1) Protonema consists of multi-cellular extensively branched filaments.

(2) The protonemal filaments form horizontal prostrate system and consist of green, chlorophyllous, photosynthetic cells.

(3) The cells are separated by transverse septa.

(4) At certain regions postively geotropic, multi-cellular, branched, and achlorophyllous rhizoids are formed.

(5) The cells of rhizoids are thick-walled and are brown in colour.

(6) Upright gametophores arise from the green, cells of protonema as buds.

(7) Each bud develops into a mature vegetative axis with spirally arranged leaves as laterals.

(8) Every spore (n) released by capsule can produce a protonema.

(9) A number of gametophores may arise from a protonema.

(10) Protonema is the gametophytic phase in the life cycle of *Funaria*.

12.1.6 Classification and Identification

Kingdom: Plantae	:	1)	Eukaryotic
		2)	Cell wall cellulosic
		3)	Autotrophic
Division: Bryophyta	:	1)	True root absent
		2)	Presence of archegonia and antheridia
		3)	True vascular tissue absent
Class: Bryopsida	:	1)	Gametophore erect and leafy
		2)	Rhizoids multicellular, with oblique septa
Order: Funariales	:	1)	Leaves ovate or spathulate
		2)	Peristome usually double
		3)	Capsule somewhat drooping
Family: Funariaceae	:	1)	Calyptra possesses a long beak

2) Capsule pyriform

3) Capsule somewhat drooping

Genus: *Funaria*

1) Leaves spirally arranged

2) Leaf phyllotaxy – 3/8

3) Stem with distinct epidermis, cortex and conducting strand

4) Leaves crowded at the apex

5) Apex of gametophores forms a bud-like apex.

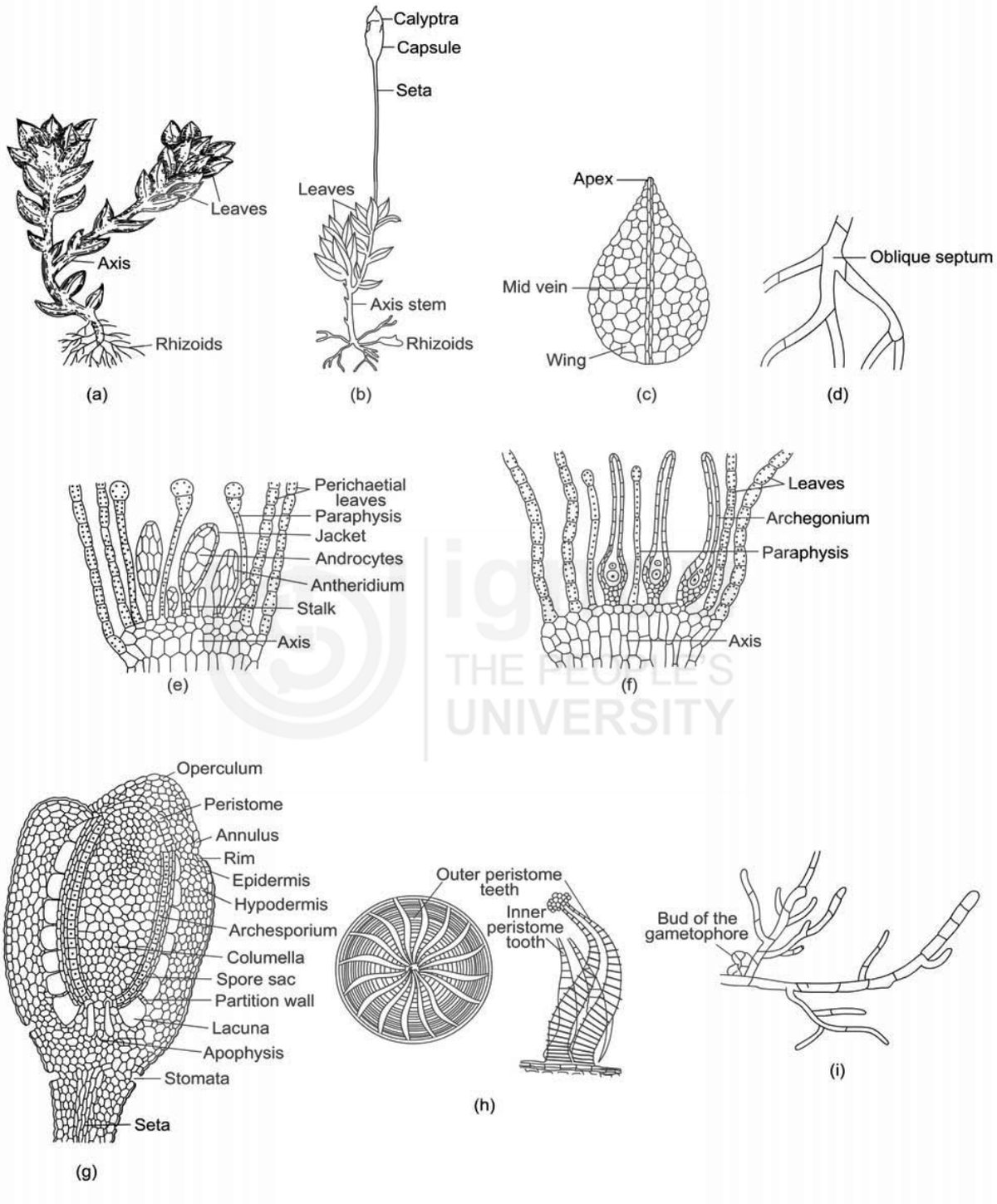


Fig. 12.1: ***Funaria*****. a) Gametophyte. Note it is branched; b) Gametophyte with a sporophyte; c) Leaf; d) Rhizoid. It is branched; e) Antheridial head in v.s.; f) Archegonial head in v.s; g) Sporophyte in l.s.; h) Peristome teeth, in top view (left hand side figure) and a portion enlarged (right hand figure); and i) Protonema, a portion enlarged. There is a bud on the gametophore.**

STUDY OF THE MORPHOLOGICAL, ANATOMICAL AND REPRODUCTIVE FEATURES OF SOME SELECTED GENERA OF PTERIDOPHYTES

INTRODUCTION

Pteridophytes include an ancient group of vascular plants of which some have survived till the present times. They include lycopods, horsetails and ferns. The lycopods are probably the oldest known vascular plants.

In contrast to bryophytes, the pteridophytes exhibit evolution of specialised vascular tissue. The evolution of microphylls and megaphylls as the main organs of photosynthesis had probably enabled pteridophytes to achieve larger plant size than the bryophytes.

The life cycle of pteridophytes involves an alternation of generations. The dominant, sporophytic generation shows a horizontal underground stem, called rhizome and above ground, an erect stem. The plant has roots, branches and leaves/ fronds. The plants produce spores in specialised structures ,the sporangia which may be borne solitary, terminal, in cone-like strobili, at the tip of stem or in the sporophylls. The sporangia cluster together in the form of sori which are characteristic of ferns.

The gametophytes are tiny-green, small, short-lived structures that bear sex-organs: antheridia and archegonia.

In these exercises you will examine the morphological, anatomical and reproductive features of a few selected genera, namely *Selaginella* (lycopod), *Equisetum* (horsetail) and *Pteris* (fern).

The rich fossil record of these plants provides scientists the initial history of successful migration, occupation and domination of land habitats by plants.

Objectives

In these exercises you should be able to:

- follow an appropriate procedure for examination of morphological, anatomical and reproductive features of the genera under study;
- identify the correct fixative, staining material and mounting media used to study the given genera;
- examine the habit, general morphological and anatomical organization of the vegetative organs of *Selaginella*, *Equisetum* and *Pteris*;
- study the reproductive structures in *Selaginella*, *Equisetum* and *Pteris*;
- observe the gametophytes with special attention to antheridia and archegonia in the permanent slides/photographs provided;
- record your observations in the form of sketches and give detailed explanatory notes on the specimen examined; and
- identify and list the morphological and reproductive features that will help to designate the given pteridophytic genus to the various ranks of taxa of the classification.

Study Guide/Prior Knowledge

For doing satisfactory work you must read the following before coming to the laboratory.

The UGC (CBCS) Course “Core Botany, Paper I : Biodiversity (Microbes, Algae, Fungi and Archegoniates. You are advised to read the Block-5 : Units 16, 17 and 18. The Unit 17: Pteridophytes: Type Studies, section 17.2 – *Selaginella*; section 17.3 – *Equisetum*, and section 17.4 – *Pteris*.

EXERCISE 13

STUDY OF MORPHOLOGICAL, ANATOMICAL AND REPRODUCTIVE FEATURES OF *SELAGINELLA*

Structure

13.1 INTRODUCTION

Plants belonging to genus *Selaginella* are mostly found in damp areas of the tropical and subtropical regions. However, a few species are markedly xerophytic and inhabit desert regions. *Selaginella* is also referred to as "resurrection plants" because of their ability to recover after prolonged drought. *Selaginella* could be epiphytic, vine-like or form creeping axes.

The most characteristic reproductive feature of *Selaginella* is heterospory and the development of microgametophytes and megagametophytes within the micro and megaspore respectively. The reproductive structure of *Selaginella* is a strobilus.

Objectives

After doing this exercise you should be able to:

- describe the habit of vegetative plant body of *Selaginella*, the dominant sporophytic generation;
- differentiate between the morphology of stem, root and describe unique structure, the rhizophore and observe the ligule;
- prepare a stained temporary mount of T.S. of stem and draw, label, describe anatomical characteristics of stem;
- locate, identify the reproductive structure, the strobilus;
- dissect out and prepare temporary whole mounts of micro and mega sporophylls and observe, draw micro and megasporangia;
- study, draw, describe and label a strobilus in a longitudinal section with the help of permanent slide; and
- assign the genus to various ranks of taxa of classification with distinct identification characteristics.

Study Guide/Prior Knowledge

For doing satisfactory work you must read the following units before coming to the laboratory.

The UGC (CBCS) Course - core Botany-, Paper I – Biodiversity (Microbes, Algae, Fungi and Archegoniates) Unit 16: Pteridophytes: an Introduction: Unit 17 – Pteridophytes: Type Studies; section 17.2 *Selaginella*.

13.2 METHOD OF STUDY

You shall conduct this exercise:

a) With the help of herbarium specimen of *Selaginella* plant. (If herbarium specimen is not available, you can use fixed plant material).

b) With the help of temporary – stained/ unstained preparations of leaf, sporophylls, stem, and strobilus.

c) Through permanent slides of t.s. stem and l.s. of strobilus.

d) Observing, drawing, labelling, and writing explanatory notes on the observations made.

13.3 MATERIAL REQUIRED

In addition to general Biology Kit and Laboratory Kit provided, you will require :

1. Plant Material :

A) Herbarium/Museum specimen with strobili, rhizophore and roots.

B) **Fixed Material :** stem; leaves; strobili.

(C) **Permanent slides** : w.m. ligulate leaf; t.s. stem; w.m. strobilus; microsporophyll; w.m. megasporophyll; l.s. strobilus

2. **Stains and Chemicals:** Safranin (0.5%); Glycerine (1.0%); Distilled water.

3. **Glassware and Apparatus :** Dissection microscope; magnifying glass; compound microscope; macroslides; micro cover slips; watch glasses; Petri dishes.

13.4 METHOD

(A) External Morphology :

(i) Observe the herbarium (fixed specimen) of *Selaginella* showing stem, roots, rhizophores and strobili.

(ii) Draw a sketch of branching pattern of the plant.

(iii) With the help of a magnifying glass, observe and draw a portion of stem and the arrangement of leaves.

(iv) Locate and draw the rhizophore, its point of origin and distal end.

(B) Dissect out and prepare a temporary-unstained mount of a leaf. Observe the dorsal and ventral view of the leaf. Locate the ligule on the dorsal side of the leaf with a magnifying glass or under a dissection microscope, draw the structure of the ligule on your note book.

(C) Cut a t.s. of the stem and prepare a safranin-stained temporary slide. With the help of a compound microscope observe, draw, label and describe the outline, vascular and non-vascular regions of the stem. Especially look for the meristeles and the trabaculae.

(D) For the study of reproductive features locate and dissect out a strobilus :

(i) Prepare an unstained-temporary mount of a strobilus. Look at the axis and the sporophylls.

(ii) Dissect out and prepare a temporary mount of a microsporophyll. Observe the position, outline, stalk of a microsporangium and number of microsporangia per microsporophyll on the dorsal side of sporophyll.

(iii) Dissect out and prepare a temporary mount of a megasporophyll. Observe the position, outline, stalk and number on the dorsal side of a megasporangium.

(iv) Observe the permanent slides showing l.s. of a strobilus. Draw the axis; micro and megasporophyll; micro and megasporangia; sporangial stalk; wall of sporangia, and the micro and megaspores; draw the position of ligule with reference to sporangia.

13.5 OBSERVATIONS

(A) Study of External Morphology

The dominant vegetative plant body of *Selaginella* represents diploid sporophytic generation. Observe, study and sketch the following:

Habit:

Observe the specimen, identify the plant body parts: root, stem, leaves and rhizophore (Fig.13.1).

(i) The specimen you observe could be prostrate, creeping, sub-erect or a climber. Branching is characteristic, terminal and unequal, forming weaker and stronger branches.

(ii) The plant body is divided into the organs: root, stem and leaves.

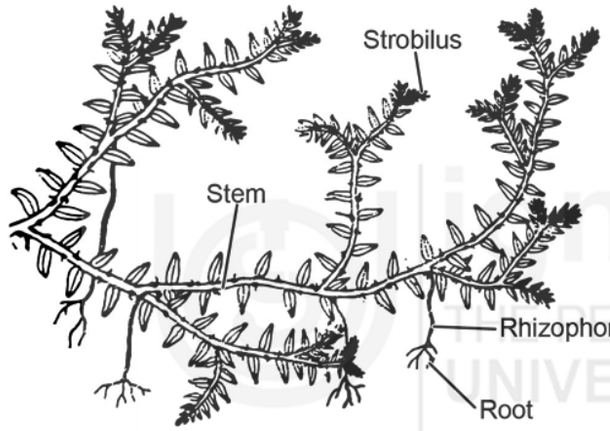


Fig. 13.1: A vegetative plant showing horizontal rhizome and upright shoot.

Root:

The primary root is short-lived (you may not observe it in the specimen provided); adventitious roots are present.

Stem:

The stem is covered with conspicuous leaves. Depending on the kind of leaves on the stem the genus is divided into two sub-genera:

(i) Sub-genus: *Homeophyllum* : It has upright stem and all the leaves are alike, spirally arranged, small and simple.

(ii) Sub-genus: *Heterophyllum* : It has prostrate, dorsiventral stem and the leaves are dimorphic (small and large). The leaves are borne in pairs on stem.

Rhizophore:

(i) At the point(s) where the stem branches, a cylindrical leafless organ can be observed.

(ii) This organ is called rhizophore.

(iii) Rhizophore is positively geotropic

(iv) On reaching the ground, it terminates into roots.

(B) Study of a Ligulate Leaf

Leaf:

(i) When dimorphic, you can see that the smaller leaf of each pair is inserted on the dorsal side of the stem while the larger leaf is inserted on the ventral side.

(ii) The large leaf always alternates with the large leaf, and small leaf with the small leaf (Fig.13.2).

(iii) Each leaf is sessile, generally obovate, with acute apex. The leaf possesses a distinct mid-rib.

(iv) At the base of each young leaf, on the adaxial face, a tongue-like outgrowth, the ligule is present.

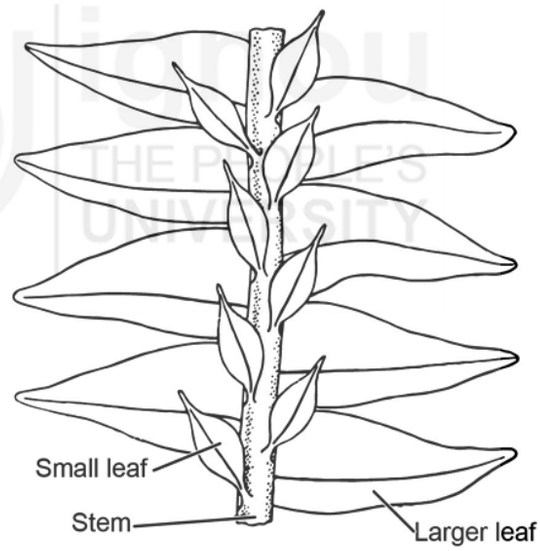


Fig. 13.2: A branch showing leaf arrangement.

Ligule:

(i) The ligule is differentiated into basal sheath, glossopodium and the body. A mature ligule is tongue-to-fan shaped.

(ii) The cells of the sheath are tubular and dead.

(iii) The cells in the glossopodium are vertically elongated, thin and greatly vacuolated.

(iv) The body of the ligule has isodiametric parenchymatous cells which possess dense protoplasm (Fig.13.3).

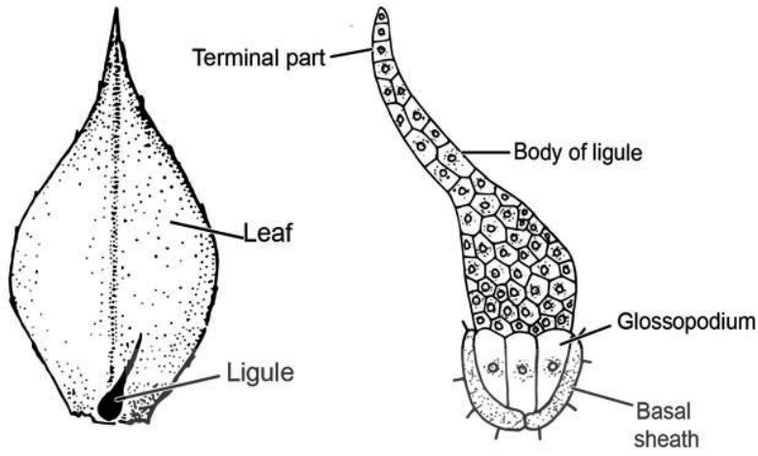


Fig. 13.3: A leaf with ligule and V.S. of ligule.

(C) Study of Stem (T.S.)

Cut a t.s. of stem and prepare a temporary-stained preparation. Stain the section in safranin, destain in water and mount in glycerine. Study under the compound microscope: observe, draw, label and describe the various tissue zones and cell types. You will see the following characteristics (Fig.13.4):

i) You will see that outline of t.s. of stem appears slightly wavy.

ii) Three distinct zones: outer **epidermis**, central **cortex** and inner **stele** are visible.

iii) The epidermis is single-layered, without any stomata and is covered with cuticle.

iv) The multilayered cortex can be segregated into three zones. The outermost 1-2 layers beneath the epidermis, consists of thick-walled cells and is known as **hypodermis**.

v) The middle zone of cortex is made up of multilayered parenchymatous cells without any intercellular spaces. All the cells of the cortex are thin-walled.

vi) The **endodermis** is the inner most layer of cortex which separates vascular tissue from the cortex. Some of its cells get radially elongated, called trabaculae (sing: trabacula). You can see conspicuous intercellular spaces between two adjacent trabaculae. The cells of the endodermis are characterized by the presence of transverse thickenings on their radial cell walls.

vii) The **stele** is generally a protostele. The number of steles per stem axis could be 1-16. The stem could be termed **polystelic**. A stele possesses both xylem and phloem and has exarch protoxylem. Larger vascular bundles may have mesarch protoxylem.

viii) The phloem consists of smaller cells with dense protoplasm. In every individual stele xylem is surrounded by phloem.

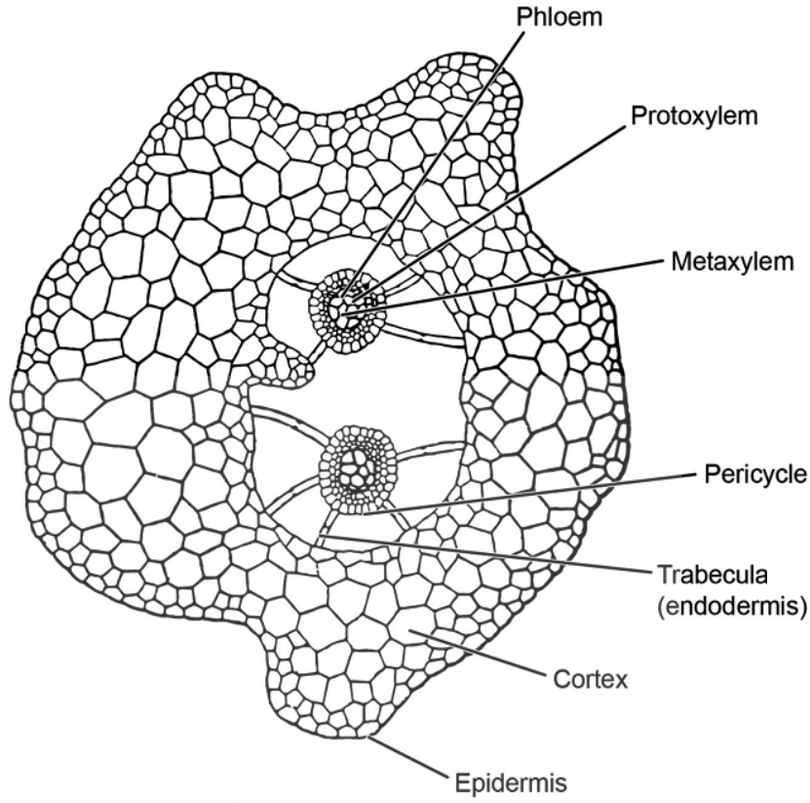


Fig. 13.4: T.S. of stem.

ix) A single-layer of parenchymatous cell which surrounds the xylem and phloem tissues is called **pericycle**. The cells of pericycle are connected to trabaculae.

(D) Study of Spore-bearing Organ (Strobilus)

Whole mount of strobilus:

Observe the distal (apical) regions of a reproductive branch of the plant. The spore producing organs, the sporangia, are aggregated as a strobilus. Cut a strobilus longitudinally; mount it on a slide in water or glycerine. Observe under a dissection microscope or with a magnifying glass. Draw the diagram and write comments:

(1) A strobilus is a condensed axis along the with the reproductive leaves.

(2) These spirally arranged isophyllous leaves bear sporangia in its axil on the dorsal surface. These are called sporophylls.

(3) The sporangium may produce many small, or large microspores, megaspores may count up to 4 in number. Consequently, they are referred to as microsporangium or megasporangium respectively. Similarly, the corresponding sporophylls are termed as microsporophylls and megasporophylls respectively.

(4) There are a number of sporophylls per strobilus. Generally a strobilus bears both the types of sporangia. Only *S. gracilis*, possesses one kind of sporangia in a strobilus, either mega-or microsporangia.

(5) When both kinds of sporangia occur in a strobilus, their arrangement differs from species to species :

(i) Only megasporangia one side and microsporangia on the other side of the axis e.g. *S.oregana*.

(ii) Microsporangia are present in large numbers and only one or two megasporangia are present at the proximal (basal) regions of the strobilus, e.g., *S. kraussiana*. Thus, *Selaginella* is heterosporous.

(6) Each sporophyll possesses a ligule. The sporangium is formed in between the axis and the ligule.

Whole mount of a microsporophyll :

Dissect out a microsporophyll from a strobilus. Mount in glycerine on a slide with the dorsal surface facing upwards. Study the following under a dissection microscope or with a magnifying glass:

(1) Each microsporophyll is almost sessile and has a broad base and a pointed tip (Fig.13.5).

(2) It is expanded nearer to the base (proximal end).

(3) It bears a microsporangium at the base on the dorsal surface of the sporophyll between a ligule and the axis of the strobilus.

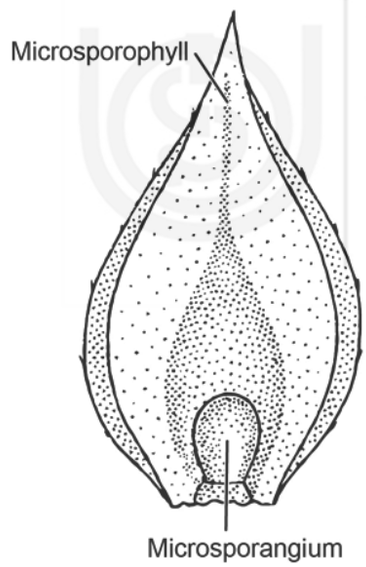


Fig. 13.5: A microsporophyll.

Whole mount of megasporophyll:

Dissect out a megasporophyll from a strobilus. Mount in glycerine on a slide with the dorsal surface facing upwards. Study the following under a dissection microscope or with a magnifying glass.

(1) Each megasporophyll is almost sessile and has broad base and a pointed tip (Fig.13.6).

(2) It is expanded nearer to the base (proximal end).

(3) It bears a megasporangium at the base on the dorsal surface of the sporophyll between a ligule and the axis of the strobilus.

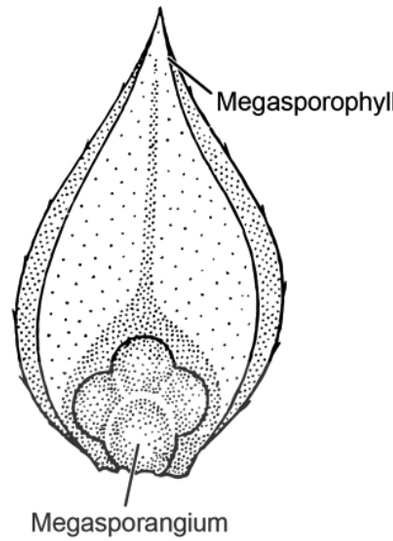


Fig. 13.6: A megasporophyll.

L.S. of strobilus (heterosporous):

You will be given a permanent slide of l.s. of a heterosporous strobilus, observe it under a compound microscope. Draw, label and study the following (Fig.13.7):

(1) A broad axis tapering at both ends.

(2) On either side of the axis sporophylls are present.

(3) Arrangement of micro and mega sporophyll could vary with species (draw the arrangement as you observe you in the permanent slide provided to you).

(4) Every sporophyll is achlorophyllous, swollen at base and possesses a tapering end.

(5) A ligule in l.s.can be observed which is placed external to the point of attachment of the sporangium on the sporophyll.

(6) Both types of sporangia have a small stalk and have two-layered jackets. The outer layer of jacket is chlorophyllous and has columnar cells. Cells of the outer jacket are thickened except at the apex. The inner layer of jacket has tangentially elongated cells. The tapetum layer, which nourishes the developing spores, may be observed.

(7) Two types of sporangia when ripe differ in their size, form, structure and colour.

(8) The microsporangium is smaller, uniform in outline. It is dark brown or red. It produces a large number of haploid spores following meiotic divisions in diploid microspore mother cells.

(9) The megasporangium is much larger, four lobed, pale green or orange. It produces 4 large haploid megaspores following meiosis in a diploid megaspore mother cell.

(10) The microspores are pyramidical in shape and possess thick, ornamented exine and a thin uniform intine.

(11) The megaspores are large in size, possess a characteristics triradiate ridge at its apex. It has thick sculptured exine and thin uniform intine.

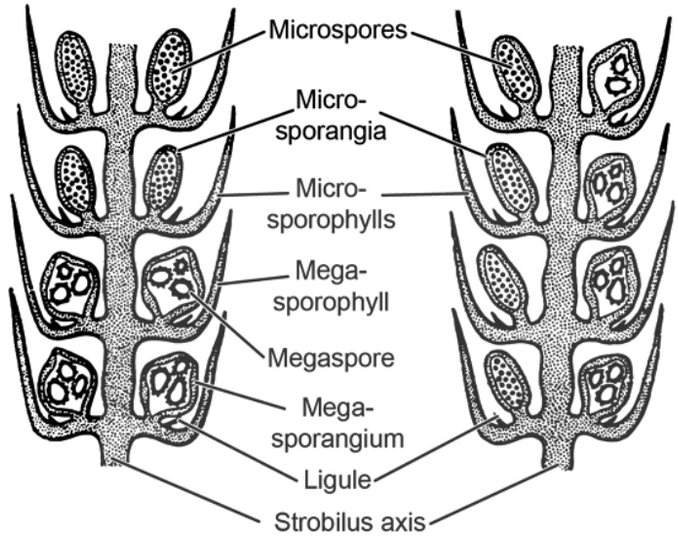


Fig. 13.7: L.S. of strobili showing distribution of micro and megasporophylls.

13.6 CLASSIFICATION AND IDENTIFICATION

KINGDOM: PLANTAE : 1) Autotrophic; Chlorophyllous
2) Cell wall cellulosic
3) Food storage product is starch

DIVISION: LYCOPHYTA : 1) Sporophytic body possesses roots, stem and leaves
2) Leaves small and simple; Leaves with one unbranched vascular bundle
3) Vascular bundle proto-or siphonostele
4) Sporangia borne adaxially on sporophylls
5) Cones/strobilii bears sporangia
6) Homo- or heterosporous

CLASS: LIGULOPSIDA : 1) Heterosporous
2) Leaves-ligulate

ORDER: SELAGINELLALES : 1) Each foliage leaf with a ligule on adaxial surface
2) Heterosporous

FAMILY: SELAGINELLACEAE : 1) Stem herbaceous, dorsiventral or erect
2) Gametophyes often reduced.

GENUS: *SELAGINELLA* : 1) Roots arise from rhizophores
2) Trabaculae present in stem
3) Stele generally protostelic

EXERCISE 14

STUDY OF MORPHOLOGICAL, ANATOMICAL AND REPRODUCTIVE FEATURES OF *EQUISETUM*

Structure

14.1 INTRODUCTION

Equisetum is popularly known as “horsetails” or “scouring rushes”. *Equisetum* is the only representative genus of the class Sphenopsida that is alive today. The plant, representing the dominant Sporophytic generation, is herbaceous and possesses perennial rhizome. The aerial shoots may be annual or perennial. The leaves are very small, simple, scale-like and usually achlorophyllous. However, the stems are photosynthetic. The stem exhibits intercalary growth. The rough texture of plant is due to deposition of silica on the outer surface. The compact cones/ strobilii are terminal. The spores are morphologically homosporous and could exhibit physiological incipient

heterospory. The spores characteristically possess elaters. Short-lived, irregularly shaped, chlorophyllous, lobed prothallus could be monoecious or-dioecious. The archegonial base is sunken in prothalli and is fertilized by multiflagellated antherozoids requiring water for the transfer of the male gamete. Young sporophyte remains attached to the gametophyte in its initial stages before becoming fully independent. The gametophytes live independent of the sporophyte.

Objectives

After doing this exercise you should be able to:

- describe the habit of vegetative plant body of *Equisetum* and differentiate between the morphology of rhizome, stem, leaves, roots and tubers if present;
- dissect out the whorl of small, scaly, achlorophyllous leaves;
- prepare a stained temporary mount of t.s. of the internode of stem and observe, draw, label and describe the anatomical characteristics of the stem;
- locate, identify the spore-bearing reproductive structure, the strobilus/cone at the terminal ends of fertile axis;
- distinguish between the axis and the sporangiophores in a strobilus and dissect out and prepare a whole mount of a sporongiophore and observe peltate sporangia;
- prepare temporary-stained mounts of t.s. and l.s. of a strobilus and draw labelled diagrams;
- prepare whole mounts of dry and wet spores and observe elaters; and
- assign the genus to various ranks of taxa of classification with distinct identification characteristics.

Study Guide/Prior Knowledge

For doing satisfactory work you must read the following before coming to the laboratory:

The UGC (CBCS) Course core- Botany, Paper I: Biodiversity (Microbes, Algae, Fungi, and Archegoniates) Unit 16: Pteridophytes : An Introduction and Unit 17 : section 17.3 : *Equisetum*.

14.2 METHOD OF STUDY

You shall conduct the exercise:

(a) With the help of herbarium specimen of the *Equisetum* plant. (If the herbarium specimen is not available you can use the fixed plant parts provided to you.)

(b) With the help of temporary stained/unstained preparations of stem (t.s.), strobilus (t.s.; l.s.).

(c) With the help of temporary stained/unstained whole mount preparation of spores; leaf whorls;

(d) Observing, drawing, labelling, and writing explanatory notes on the observations made.

14.3 MATERIAL REQUIRED

In addition to general Biology Kit and Laboratory Kit provided to you will require :

1. **Plant Material :**

   A) Herbarium/Museum specimen showing rhizome, aerial branches: vegetative and fertile; tubers;

   B) **Fixed Materials :** stem; strobilus;

   C) **Permanent slides:** t.s. stem; w.m. leaves; t.s. rhizome; l.s. strobilus; t.s. strobilus; w.m. spores; w.m. prothallus with sex organs.

2. **Stains and Chemicals :** Safranine (0.5%); Acetocarmine (1.0%); Glycerine (1.0%); distilled water; rectified spirit;

3. **Glassware and Apparatus :** Dissection microscope; magnifying glass; compound microscope; spirit lamp; match box; Bunsen burner; macroslides; microcoverslips.

14.4 METHOD

External Morphology:

(1) Observe the herbarium/fixed specimen of *Equisetum* showing rhizome, roots, tubers, stem and strobilii.

(2) Draw a sketch of vegetative and reproductive parts of the specimen.

(3) With the help of a magnifying glass, observe the ridges and furrows of stem, whorled, scaly leaves, their arrangement at nodes of the stem; the branching pattern.

(4) Cut a t.s. of internode of stem and prepare a safranin-stained temporary slide under a compound microscope observe the t.s.; study, draw, label and describe the outline, vascular and non-vascular regions of the stem, three kinds of air spaces (canals) and sunken-stomata etc.

(5) Observe t.s. of rhizome under a compound microscope. Study, draw, and label various tissues and their distribution.

(6) Prepare t.s. and l.s. of safranin-stained temporary slides of a strobilus and observe, draw, label, and describe the cone-axis, sporangiophore and peltate sporangia.

(7) With the help of a needle, scoop out a few spores from a sporangium. Prepare temporary slide stained with aceto-carmine (with or without heating). Observe the exine, intine, nucleus and elaters in the spores.

14.5 OBSERVATIONS

(A) STUDY THE EXTERNAL MORPHOLOGY

Observe the herbarium specimen of sporophyte of *Equisetum* (Fig.14.1).

Habit:

The plant is differentiated into roots, rhizome, aerial branches of stem and leaves.

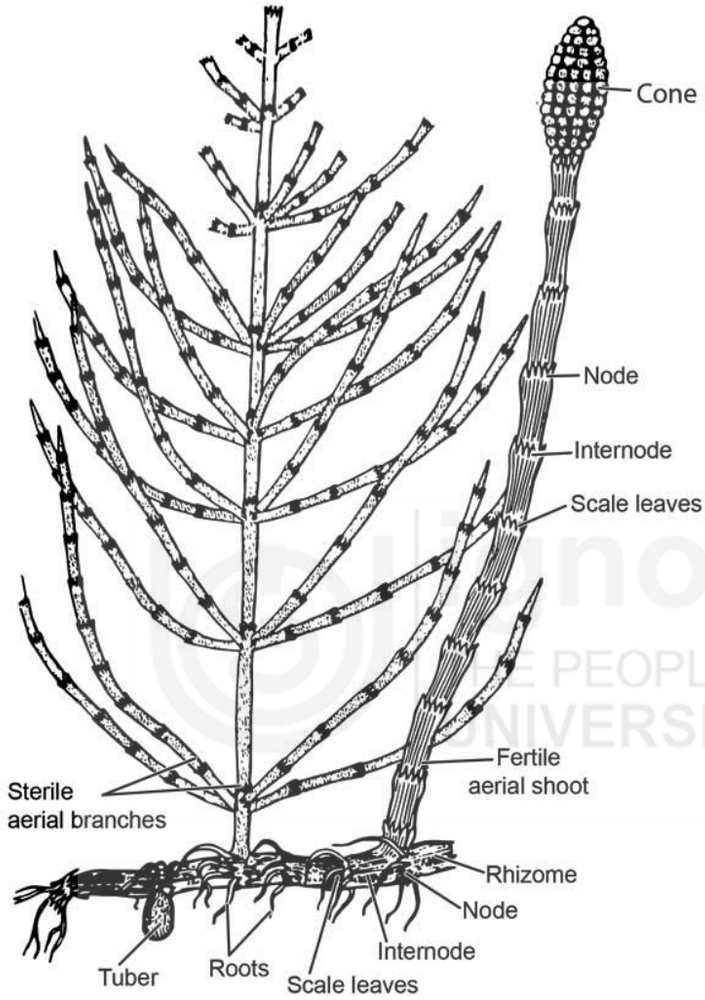


Fig. 14.1: A vegetative plant showing horizontal rhizome and upright shoot.

Rhizome:

The horizontal rhizome has distinct nodes and internodes; from the nodes arises upright aerial branches and positively geotropic roots.

Root:

The roots are slender and fibrous.

Stem:

(1) The aerial stem axes are of variable lengths and possess characteristic joints. The texture of stem is rough.

(2) The aerial stem (branch) could be: sterile or fertile .The sterile branches are green and branched; (ii) the fertile branches are non-green, unbranched and terminate in a cone. Fertile branches die after release of spores.

(3) Some species have green, branched fertile shoots, with a cone at the apex of each of lateral branch. Such branches do not die after the

spores are shed. (You will study and draw only the specimen you observe).

(4) Each internode of an aerial branch is longitudinally ribbed. The number of leaves and the ridges are same.

Leaf

(1) Every leaf is present directly above a ridge present on the internode below.

(2) The ridges on the stem of successive internodes alternate, as also the leaves of successive nodes.

(3) Leaves are simple, small, scaly, and whorled and fused laterally and possess longer or shorter free apices. Leaves are achlorophyllous.

(4) The branches develop at the node in between two leaves. The number of branches at a node is equal to the number of leaves.

(B) Study of Anatomy of Vegetative organs

Anatomy of stem (internode):

Observe the temporary-stained preparation of an internode of stem and look at the following characters (Fig.14.2):

(1) The outline is wavy with ridges and grooves.

(2) The epidermis, cortex and stele along with pith cavity constitute the major tissue zones.

(3) The epidermis is cuticularised, silicified and is made up of tangentially elongated cells. Sunken stomata are conspicuous.

(4) Cortex, beneath the epidermis is highly differentiated into outer and inner cortex. The outer cortex, below the ridge is sclerenchymatous. Small patches of sclerenchyma are also present below the groove.

(5) Radially elongated palisade cells rich in chloroplasts are present beneath the ridge. There are a fewer chlorophyllous cells beneath a grove.

(6) Large and thin walled parenchymatous cells constitute inner cortex. In this zone below the grooves, vallecular canals are present.

(7) The stele is ectophloic siphonostele that consists of a ring of vascular bundle.

(8) Position of endodermis is variable. It could be (i) as a simple ring outside the vascular bundles; (ii) in addition there is also an internal, and an external endodermis strip in between the bundles; (iii) each vascular bundle has its own endodermis (You should draw only what you observe in your slide).

(9) Layers of cells beneath endodermis constitute pericycle.

(10) Vascular bundles are collateral, conjoint, with endarch protoxylem, arranged in a ring. A vascular bundle is present below each ridge. Two metaxylem and one protoxylem strands are visible in each vascular bundle. Some protoxylem elements disintegrate to form a carinal canal in each bundle. Pith cavity is known as central canal.

(11) Presence of ridges and grooves; sunken stomata, thick cuticularised epidermis; large sclerenchyma zones beneath ridges, and the presence of chlorophyllous palisade parenchyma are the **xerophytic** adaptations of the *Equisetum* stem.

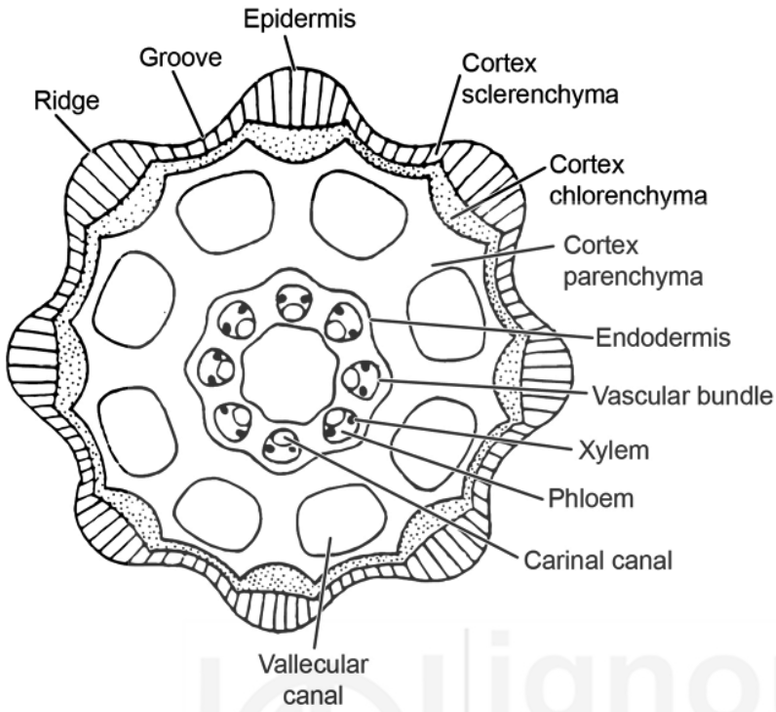


Fig. 14.2: T.S. of aerial shoot (diagrammatic).

(12) In contrast, presence of three kinds of air spaces: vallecular canal, carinal canal and pith cavity are the **hydrophytic** adaptations of the *Equisetum* stem.

(C) Anatomy of Rhizome

Study the anatomy of a rhizome with the help of a permanent slide and you will observe the following:

(1) The ridges and furrows make the outline wavy.

(2) Epidermis is highly cuticularised and stomata are present.

(3) Cortex possesses a few layers of sclerenchyma below the epidermis and a large zone of parenchyma to the inner side.

(4) Large vallecular canals are present in inner parenchymatous cortex, below the grooves.

(5) Endodermis is single-layered and encloses a ring of vascular bundles. Below every ridge there is one vascular bundle. Each vascular bundle is conjoint, collateral and endarch. A protoxylem cavity, carinal canal is present in every bundle.

(6) The centre of the rhizome has a large pith cavity.

(D) Study of spore-bearing structure- Cone (strobilus)

Observe the strobilus (cone) at the terminal end of a fertile axis (Fig.14.3).

(1) At the basal end of a cone observe the ring of scaly leaves called annulus.

(2) The cone appears as a biconvex structure with tapering ends and wide in the middle.

(3) Observe a large number of hexagonal (polygonal) discs on which numbers of sporangiophores are very compactly arranged.

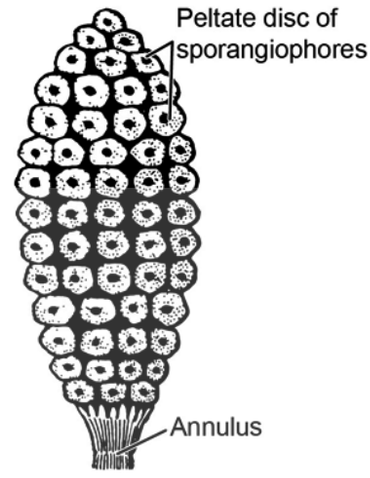


Fig. 14.3: Fertile cone.

L.S. of a strobilus:

With the help of a razor blade cut a median longitudinal section of the strobilus and prepare a temporary stained-preparation and observe (Fig.14.4):

(1) An axis of cone has a large number of attached sporangiophores.

(2) Cone-axis is centrally located.

(3) The stalk holds a polygonal peltate disc at right angles to it. These discs fit closely to form a protective cover for the sporangia below.

(4) Sporangium appears to be attached to the lower side of the discs.

(5) Each sporangium is elongated and possesses a spore-sac. This sac holds numerous haploid spores and is protected by a single jacket.

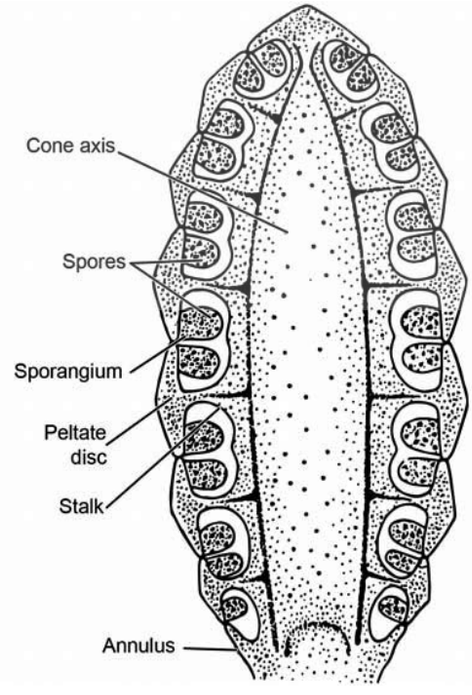


Fig. 14.4: L.S. of strobilus.

T.S. of a strobilus

Make temporary-stained preparation of the t.s. of strobilus (Fig.14.5) and you will observe:

1) A cone axis and a number of sporangiophores attached to it. The centrally located region is called cone axis.
2) Sporangiophores are arranged in a whorl. Each sporangiophore has one stalk and a disc. Stalks keep discs attached to the cone axis.
3) Peltate disc bears sporangia on its underside. Each sporangium appears elongated and cylindrical.
4) Each sporangium is covered by single layered jacket which surrounds a spore-sac. There are a number of haploid spores within a spore-sac.

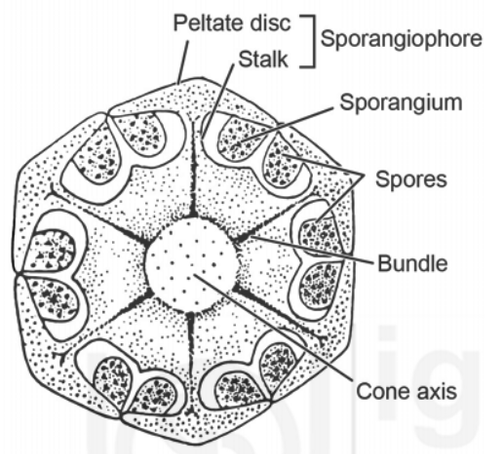


Fig. 14.5: T.S. of strobilus.

(E) Study of whole mount of sporangiophores:

Dissect out a sporangiophore along with its disc and sporangia (Fig.14.6); and observe the following characteristics:

(1) A long stout stalk has an umbrella-like thick disc at the top.
(2) There are a number of sporangia hanging from the disc.
(3) The sporangia exhibit longitudinal line of dehiscence
(4) Each sporangium has a number of spores.
(5) The spores are morphologically similar, hence are homosporous. However, in some species out of the four spores produced by a spore mother cell via meiosis; two produce male prothalli and other two develop into female prothalli. Hence, they exhibit physiological (incipient) heterospory.

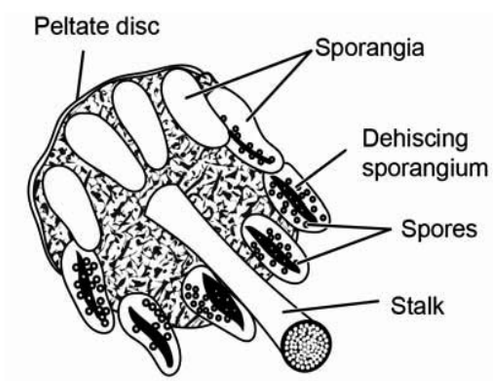


Fig. 14.6: Sporangiophore with sporangia.

(F) Study of whole mount of spores (Wet and Dry) :

Tease the sporangium with a needle. Mount spores in water. You can stain them with safranin or acetocarmine. Observe the preparation under a compound microscope.

(1) A spore consists of a four layered wall.

(2) Apart from intine and exine, the usual two wall layers, a middle cuticular layer and outermost layer called perispore are present.

(3) The perispore of each spore is differentiated into four narrow spirally wide bands, with flat-spoon tips. All the four bands are attached at a common point. These projecting bands are called elaters. Each spore has one haploid nucleus and is rich in cytoplasm (Fig.14.7).

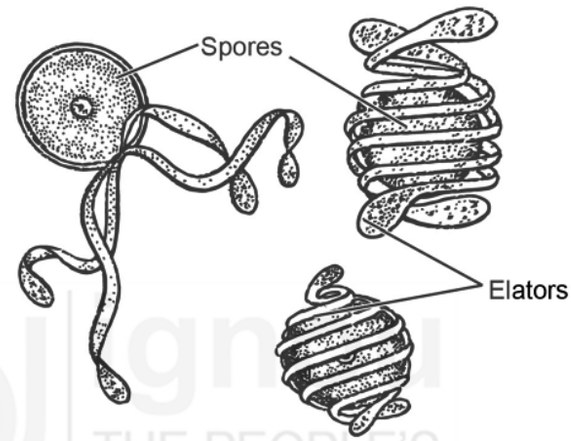


Fig. 14.7: Spore with uncoiled elaters and spores with coiled elaters.

Dry Spores:

(1) To observe them uncoiled, first let the wet spore dry on a slide (or warm gently over a flame). You may also use acetocarmine prior to warming. These elaters are hygroscopic and they coil and uncoil with the changes in the atmospheric humidity.

14.6 CLASSIFICATION AND IDENTIFICATION

KINGDOM: PLANTAE :	1) Autotrophic; Chlorophyllous
	2) Cell wall cellulosic
	3) Food storage product is starch
DIVISION: SPHENOPHYTA :	1) Sporophytic body with roots, stems and leaves.
	2) Stem is jointed with distinct nodes and internodes.
	3) Stem with proto- or siphonosteles without leaf gaps.
	4) Leaves scale-like, in whorls at nodes.
	5) Branches arise in whorls at nodes.

6) Sporangia borne on sporangiophores in a cone.
7) Mostly homosporous.
8) Gametophyte exosporic, green.
9) Anthrozoid multiflagellate.

CLASS: SPHENOPSIDA :
1) Stem branched; articulated; ridged.
2) Stem with distinct ridges and furrows.
3) Distinct nodes and internodes.
4) Leaves microphyllous.
5) Leaves scaly, in whorls at nodes.

ORDER: EQUISETALES :
1) Stem branched; branches borne in tranverse whorls.
2) Internodes alternate with each other.
3) Vascular bundle endarch, siphonostelic.

FAMILY: EQUISETACEAE:
1) Homosporous.
2) Sporangia borne on sporangiophores.
3) Presence of cones.
4) Secondary growth absent.

GENUS: *EQUISETUM*:
1) Leaves scaly and colourless.
2) Sunken stomata in grooves.
3) Palisade parenchyma in stem.
4) Presence of vallecular, carinal and central canals in stem.

EXERCISE 15

STUDY OF MORPHOLOGICAL, ANATOMICAL AND REPRODUCTIVE FEATURES OF *PTERIS*

Structure

15.1 INTRODUCTION

Pteris is a widely distributed genus with about 250 species of which 19 are found in India commonly known as fern. It is generally found in cool, damp, shady places in tropical and subtropical regions of the world. *Pteris vitatta* is a very common low altitude (up to 1200 m above sea level) fern that produces new leaves almost throughout the year. *P. quadriauratia* is the commonest road-side species throughout North-Western Himalayas while *P. cretica* occurs at 1200-2400 m altitude.

Pteris is a terrestrial, perennial herb possessing creeping or semi-erect rhizome covered by scales. Roots arise from rhizome. Leaves (fronds) could be simple or compound and exhibit circinate vernation. *Pteris* is a

homosporous genus with spore-producing structures which are formed under the sides of leaves in form of coenosori. During the life cycle, the dominant, perennial, vascular sporophytic phase alternates with an independent, short-lived, fragile, autotrophic gametophytic generation. The antherozoids are multiflagellated and need water to ensure fertilization.

Objectives

After conducting the exercise you should be able to:

- identify rhizome, roots, and fronds that represent the sporophyte of *Pteris*; study the frond-morphology and differentiate a sporophyll from a vegetative frond of *Pteris*;
- prepare temporary-stained preparations of transverse sections of rachis and study its anatomical features;
- observe and identify the anatomical characteristics of a rhizome of *Pteris* with the help of a permanent slide;
- prepare temporary-stained whole mount preparations of a portion of sporophyll; a few sporangia, and spores of *Pteris* for study;
- cut a vertical section and prepare a temporary-stained preparation of a sporophyll passing through coenosori and study;
- identify and prothallus (the gametophyte); sex-organs : antheridia and archegonia in permanent slides;
- observe, study and comprehend the relationships between a young, sporophyte and gametophyte with the help of a permanent slide;
- assign the genus to the various ranks of taxa of classification with distinct identification characterics to the *Pteris*.

Study Guide/Prior Knowledge

For doing satisfactory work you must read the following before coming to the laboratory :

The UGC- (CBCS) Course-Core-Botany – Paper I, Biodiversity (Microbes, Algae, Fungi and Archegoniates) Unit 16: Pteridophytes: Type studies; section: 17.4 *Pteris*.

15.2 METHOD OF STUDY

You shall conduct the exercise:

(a) by studying herbarium of the living plant of *Pteris*, its aerial parts comprising of rhizome, root and frond;

(b) with help of magnifying glass for studying branching pattern of a frond; rachis and pinnae; circinate vernation; and location of coenosori on the underside of leaflet margins;

(c) by studying the internal anatomical structure of rachis with the help of permanent and self prepared temporary-stained slide/s;

(d) with the study of internal anatomical structure of rhizome and rachis with the help of a permanent slide;

(e) with the study through whole-mount temporary-stained or unstained preparation of a part of sporophyll; scooped sporangia; and spores;

(f) with the study of prothallus; its shape; organization structure and distribution of antheridia; archegonia on a prothallus (permanent slides);

(g) by the study of prothallus-young sporophyte relationship with the help of a permanent slide;

(h) by labelled sketches, and explanatory notes on the observations made by you;

(i) enumeration of distinct identification points and assigning the genus *Pteris* to the various ranks of taxa of classification; and

(j) observing, drawing, labelling, and writing explanatory notes on the observations made.

15.3 MATERIAL REQUIRED

Along with the general Biology kit and Laboratory kit you will require :

1. **Plant Materials :**

   (A) Herbarium/Museum specimen showing rhizome, roots, fronds, sporophylls;

   (B) **Fixed Materials :** rachis; sporophyll; and

   (C) **Permanent Slides :** rachis t.s.; rhizome v.s; sporophyll w.m.; prothallus with sex organs (antheridium and archegonium); w.m. prothallus with young sporophyte.

2. **Stains and Chemicals :** Safranin (0.5%); Glycerine (1.0%); Distilled water.

3. **Glassware and Apparatus :** Dissection microscope; magnifying glass; compound microscope; macroslides; microcoverslip; Petri dishes and watch glasses.

15.4 METHOD

External Morphology

(1) Observe the herbarium specimen showing rhizome, root; frond (vegetative and reproductive).

(2) Draw the sketch of branching pattern of a frond; circinate vernation; venation in a leaflet; coenosori on the underside of margins of sporophylls;

(3) Prothallus; sex-organs; young sporophyte, if necessary use magnifying glass.

(4) Preparation of temporary-stained slides of rachis, v.s. of sporophyll.

(5) Use safranin for staining, water for destaining and glycerine as mounting medium.

15.5 OBSERVATIONS

(A) Study of External Morphology

Observe the herbarium specimen and look for the following characteristics (Fig.15.1):

(1) Small to very large frond.

(2) Stem short-creeping to erect; slender to massive. Stem with many scales: scales are basifixed; elongate to narrow.

(3) Leaves (fronds) petiolate; pale to dark coloured; pinnate. Lamina occasionally palmate.

(4) Petiole (rachis) adaxially grooved. Short spines sometime present on the axis. Veins simple or forked, free at margins or form coastal arches.

(5) Sporophylls similar to vegetative leaves.

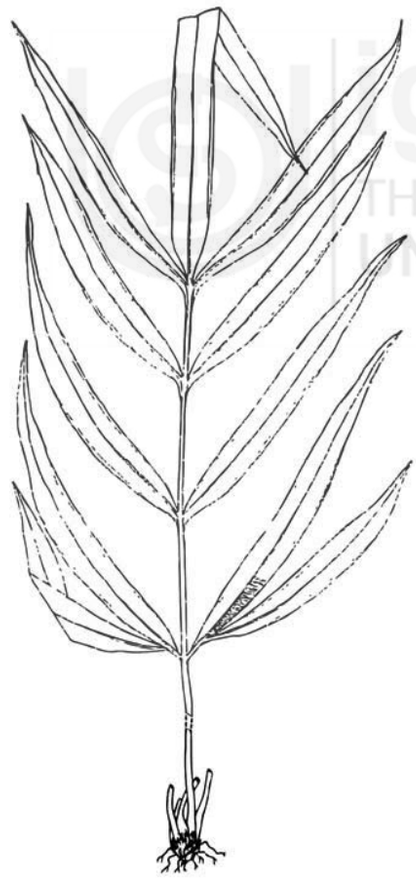


Fig. 15.1: Plant with rhizome, roots and vegetative frond.

(B) Study of anatomy of a rachis

Prepare a temporary-stained slide of rachis and observe under the compound microscope. (You may also observe a permanent slide of t.s. of rachis). Look for the following characteristics (Fig.15.2):

(1) The outline is horse-shoe shaped or hemispherical.

(2) The tissues are differentiated into 3 zones: epidermis, ground tissue and stele.

(3) Epidermis is the outer-most thick cuticularised layer of cells.

(4) Outer 2-3 layers of ground tissue are thick-walled, and often sclerenchymatous. These layers constitute hypodermis.

(5) Beneath hypodermis is present a large, parenchymatous ground tissue.

(6) Within the ground tissue lies a U-shaped or horse-shoe shaped stele.

(7) The stele is demarcated from ground tissue by a layer of endodermis.

(8) Beneath endodermis is present a few layers of parenchymatous pericycle.

(9) A large massive xylem occupies the core of the stele.

(10) The xylem is surrounded by phloem from all sides. Thus, the phloem is present in between xylem and pericycle.

(11) With the maturity of rachis (in older regions), the stele may consists of :

(i) two large meristeles; and

(ii) a large number of smaller meristeles.

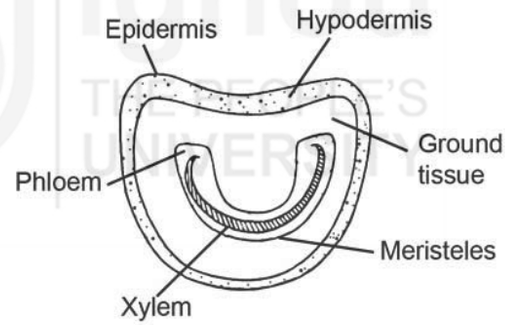


Fig. 15.2: T.S. of young rachis (diagrammatic).

(C) Study of anatomy of rhizome:

Observe the permanent slide of rhizome under a compound microscope, and look for the following characteristics:

(1) An outline of the section appears to be biconvex (Fig.15.3).

(2) The tissues are differentiated into epidermis, ground tissue and the stele.

(3) The epidermis is the outermost single layer of thickly cuticularized cells.

(4) The cortex is divided into 2 distinct zones :

(i) outer one or a few layers of sclerenchymatous cells lying beneath the epidermis, called hypodermis; and

(ii) the large, multilayered parenchymatous zone spread up to the centre of the organ. This is termed as ground tissue. These cells are rich in starch grains.

(5) The structure of the stele varies with the age of the rhizome (draw what you observe in the section provided to you). In the centre of the organ, and occupying a layer area a distinct stele can be observed.

(i) In very young rhizome, the stele is a protostele;

(ii) In plants with 2-3 leaves, the rhizome has ectophloic siphonostele; and

(iii) The rhizome of a mature plant exhibits a dictyostele.

(6) A dictyostele consists of a large number of distinct vascular bundles, each is called a meristele.

(7) These meristeles are arranged in 2 rings separated by 2 large bands of multicelled sclerenchymatous cells.

(8) Each meristele possesses its own endodermis and 1-2 layers of pericycle layers beneath endodermis. Each of these meristeles has central core of xylem surrounded by phloem all around.

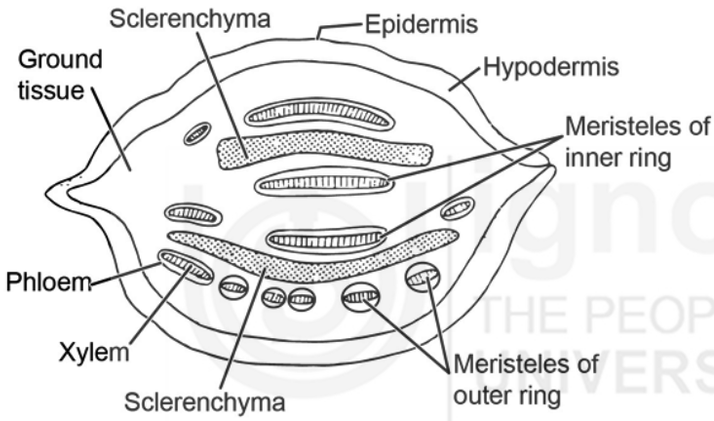


Fig. 15.3: T. S. rhizome (diagrammatic).

(D) Study of reproductive structures

(1) Observe the whole mount of abaxial surface of pinnules of a fertile frond.

(2) Sporangia occur in groups called sori. These sori are present very closely forming a confluent sorus. Such a sorus is termed coenosorus. In a coenosorus the identity of an individual sorus is lost.

(3) A sorus is protected by an indusium in ferns. In *Pteris,* the margins of the fertile pinnule cover the sori in the form of a flap. Thus it is not a true indusium. It is called false-indusium. The lower indusial flap is poorly developed.

Study of V.S. sporophyll

- Observe the permanent slide of v.s. of fertile pinnae (leaflet) of *Pteris*.
- Also, prepare a temporary-stained preparation of v.s. of sporophyll.

Observe the following characteristics (Fig.15.4):

(1) Upper (adaxial) region of the sporophyll has similar structural organisation as observed in the vegetative leaflet.

(2) The lower (abaxial) surface is under developed, and functions as protective cover for sporangia/sori. This is called false-indusium.

(3) On the innerside of the margins a placental tissue (receptacle) can be observed.

(4) From the receptacle arise a large number of sporangia. The development of each individual sporangium is from an individual initial cell. Thus, it is called leptosporangiate.

(5) The development of numerous sporangia from the receptacle is asynchronous so various sporangia are in different stages of the development. Such a sorus is called mixed sorus.

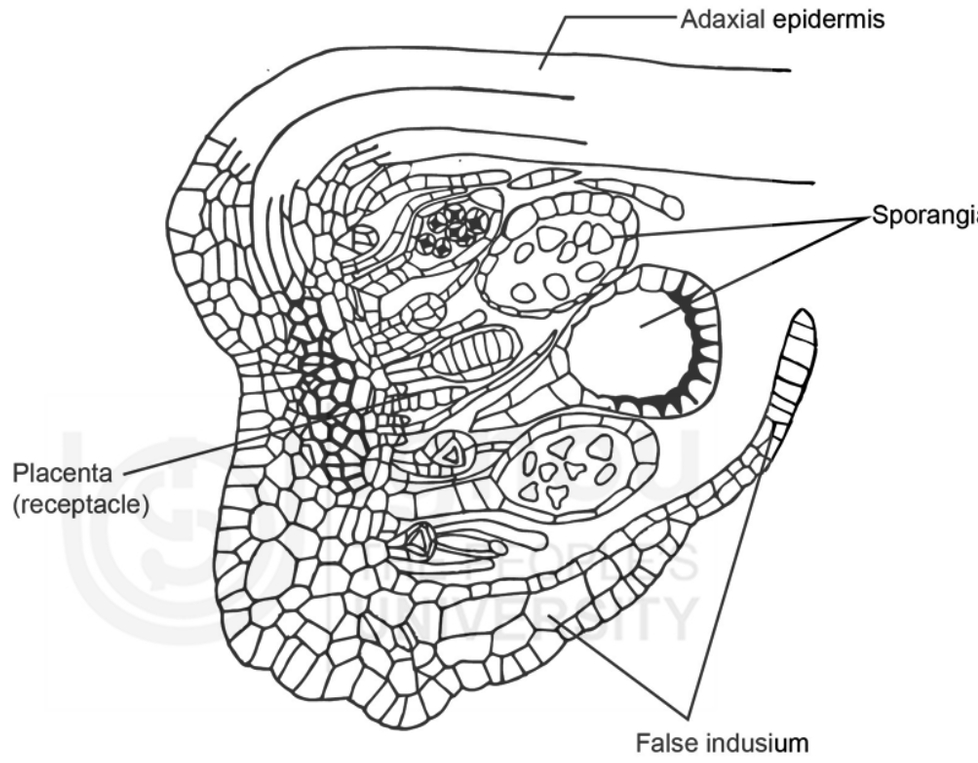


Fig.15.4: A part of coenosorus.

Study of sporangium-whole mount:

(a) Scratch a few sporangia from the sorus with a needle/forceps and prepare a stained-temporary preparation; or

(b) Observe a sporangium within a sori in v.s. of the preparation prepared by you or from a permanent slide.

You will find following features in V.S.

(1) Each sporangium is differentiated into a stalk and a capsule (Fig.15.5).

(2) A stalk is made up 3 rows of cells and is long and slender.

(3) The capsule is oval to biconvex in shape. It is covered by a single layer of cell called jacket.

(4) A ring of thick-walled cells forms an annulus.

(5) A few thin-walled cells of the ring is called the stomium.

(6) The capsule produces within it 32-64 haploid cells called spores. Meiosis in a diploid spore-mother cell results in the formation of 4 haploid spores.

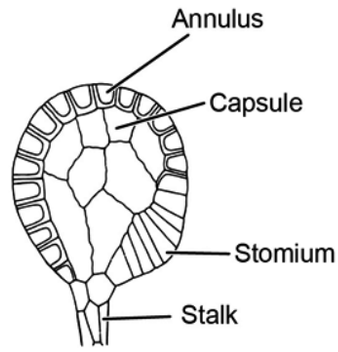


Fig. 15.5: Single microsporangium.

Study of Spore:

Take a sporangium, scoop out a few spores, stain in safranin, mount in glycerine and observe under compound microscope. Look for the following characteristics:

(1) All the spores are similar in size and shape-hence the *Pteris* is homosporous.

(2) Each spore is haploid with 2-layers of walls.

(3) The outer layer is made up of thick wall and is called exine.

(4) The inner thin layer of the wall is called intine.

(E) Study of the Gametophyte (Prothallus):

Observe the permanent slide of prothallus of *Pteris* under dissecting microscope and the sex organs under a compound microscope. Look for the characteristics given below:

(1) A haploid spore germinates into a gametophyte. Since the gametophyte of *Pteris* is a thalloid structure, it is also termed prothallus.

(2) It is dark-green, heart-shaped almost single-layered sheet of cells with distinct apical-notch.

(3) At mid-rib region, a several cell thick cushion can be observed. Prothallus is attached to substratum with the help of rhizoids. The rhizoids are produced on the lower side of the prothallus, from the cells of central region.

(4) Prothallus is very delicate, small, autotrophic structure and does not possess any vasculature.

Sex-organs:

Study the sex-organs of *Pteris* with the help of permanent slides of whole mounts of gametophyte (prothallus) under a compound microscope. Look for the following characteristics (Fig.15.6):

A Prothallus is generally monoecious.

Antheridium:

(1) Antheridium, the male sex-organ, arises on the ventral side of adult prothallus. Antheridia occur among the rhizoids.

(2) A mature antheridium wall consists of 3 cells

(i) the basal cell. It could be funnel-shaped;

(ii) the annular cell; and

(iii) The apical cap cell (cover cell).

(3) Repeated mitotic division of androgonial cell produces 32, multiflagellated spermatozoids (antherozoids).

(4) An antheridium dehisces through opening of cap cell.

Archegonium:

(1) Archegonium is the female sex-organ in *Pteris* and are restricted to the cushion region behind apical notch.

(2) Each archegonium is made up of a neck and a venter. The neck is 6-8 celled high with a single binucleate neck-canal cell. A venter consists of a single, small venter canal and a large egg cell.

(3) The venter is embedded within prothallus and the curved neck is slightly raised above the prothallus.

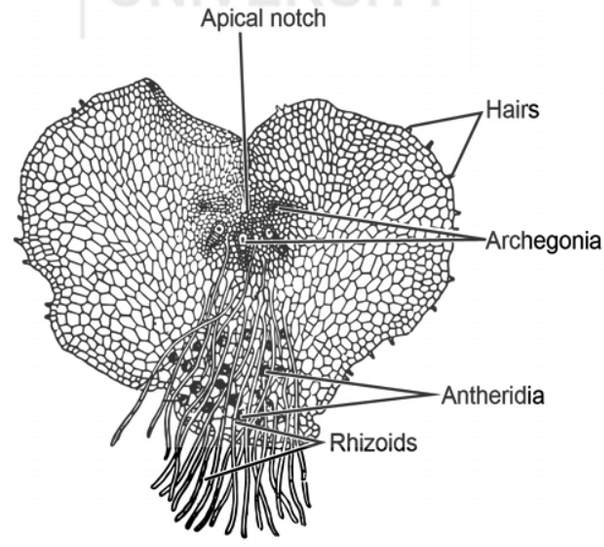


Fig. 15.6: A heart-shaped bisexual gametophyte with sex-organs.

(F) Young sporophyte

Observe the permanent slide of whole mount of gametophyte of *Pteris* with young sporophyte. Look for the following characteristics (Fig.15.7):

(1) Fertilization of egg by an antherozoid results in formation of a diploid zygote within venter of archegonium.

(2) The development of an embryo from zygote (2n) takes place while the archegonium is still attached to the prothallus.

(3) Young sporophyte is differentiated into young leaves, primary and secondary roots. Primary root grows on lower side.

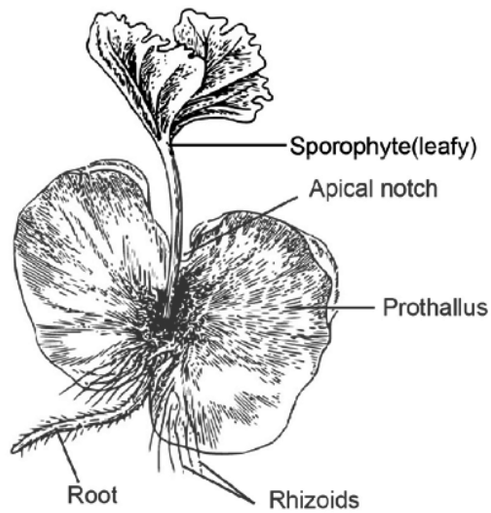


Fig. 15.7: A prothallus supporting a young developing sporophyte.

(4) Leaves are petiolate and erect. These emerge through apical notch of gametophyte. Young leaves are simple.

(5) Young sporophyte is dependent on prothallus till it forms its own photosynthetic leaf. It absorbs food through foot produced by young embryo.

(6) Autotrophic prothallus nourishes the young sporophyte in intial stages.

15.6 CLASSIFICATION AND IDENTIFICATION

KINGDOM: PLANTAE	:	1) Autotrophic; Chlorophyllous
		2) Cell wall cellulosic;
		3) Food storage product-starch
DIVISION: FILICOPHYTA	:	1) Large leaves; megaphylls/fronds.
		2) Stem with proto-, siphono-, dictyostelic, or polycylic
		3) Stem with leaf gaps
		4) Sporangia develop on margin, upon abaxial surface of leaf.
CLASS: LEPTOSPORANGIOPSIDA		1) Sporangium develops from a single initial.
		2) Limited number of spores per sporangium.

3) Homosporous-except water ferns.

4) Antheridia raised above prothallus

5) Limited number of antherozoids per antheridium

6) Foot, root, cotyledon, stem of young embryo have common origin.

ORDER: FILICALES : 1) Mixed sori

FAMILY: POLYPODIACEAE : 1) Annulus of sporangium is vertical

2) Each sporangium with 32-64 spores

GENUS: *PTERIS* : 1) Presence of coenosorus

2) False indusium develops from margins of sporophylls.

3) Sorus enclosed between indusial flaps.

STUDY OF THE MORPHOLOGICAL, ANATOMICAL AND REPRODUCTIVE FEATURES OF SOME SELECTED GENERA OF GYMNOSPERMS

INTRODUCTION

Non-flowering seed plants, the gymnosperms - comprise only a small component of the plant kingdom, but are distributed throughout the world. On many mountainous habitats they form dominant vegetation. A large number of gymnosperms are of immense economic importance in forestry, horticulture and as source of a number of forest produce. The presence of highly reduced gametophytic generation, siphonogamy, and development of seed are some of the characters they share with flowering plants. However, similar to bryophytes and pteridophytes, most gynmnosperms produce archegonia, the female sex-organs. The perennial, vascular plant body the well developed vegetative organs such as roots, stem and leaves, comprise the dominant sporophytic generation in the life of a gymnosperm. The young developing embryo with a maturing seed is nourished by relatively massive, haploid, pre-fertilization tissue, the female gametophyte. It is also referred to as 'haploid' endosperm.

In these laboratory exercises you shall study morphological, anatomical and reproductive features of two gymnosperms, *Cycas* and *Pinus*.

Objective

In these exercises you should be able to :

- follow an appropriate procedure for examination of morphological, anatomical and reproductive features of the genera under study;
- know the fixative, staining material, mounting media used to study the prescribed genera;
- examine the general morphological, anatomical organization of the vegetative organs of *Cycas* and *Pinus*;
- study the reproductive structures in *Cycas* and *Pinus*;
- record your observations in the form of sketches and give detailed explantory notes on the specimen examined; and
- identify and list the morphological, anatomical and reproductive features that help to designate the given gymnosperm genus to the various ranks of taxa of the classification.

Study Guide/Prior Knowledge

For doing satisfactory work you must read the following before coming to the laboratory.

The UGC (CBCS) Course Core Botany, Paper I: Biodiversity (Microbes, Algae, Fungi and Archegoniatles). Block-6: The Units 19: Gymnosperms: General Characteristics, Unit 20: Gymnosperms: *Cycas,* and Unit 21 Gymnosperms: *Pinus.*

EXERCISE 16

STUDY OF MORPHOLOGICAL, ANATOMICAL AND REPRODUCTIVE FEATURES IN *CYCAS*

Structure

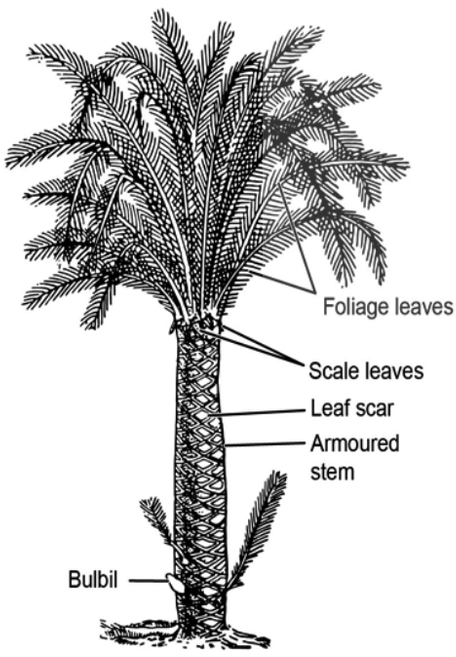


Fig. 16.1: Morphology of *Cycas* plant

16.1 INTRODUCTION

Cycas is cultivated and also found in wild and represents the sporophytic generation. Armour of alternating bands of dimorphic large and small leaf bases cover the aerial stem in a spiral manner. The short-lived primary root is replaced by large, fleshy adventitious root system. The plant also produces apogeotropic coralloid roots. The large leaves alternate with brownish scaly leaves, the cataphylls. *Cycas* is dioecious (Fig.16.1) and produces very large male cones in male plants. Large, hairy megasporophylls, with conspicuous naked ovules, are produced by female plants. Large archegonia, egg cells and massive female gametophyte are characteristics of *Cycas*. The sperm cells are flagellate, though the flagella are shed prior to syngamy. The stem also possesses manoxylic wood.

Objectives

After conducting this exercise you should be able to:

- make out the general morphological and anatomical organization of a coralloid root and distinguish it from adventitious roots;
- distinguish between a large foliar leaf and a small scaly leaf and describe the anatomy of a rachis and a leaflet;
- recognize a bulbil;
- identify, describe and comment on the various parts of a microsporophyll;
- prepare a whole mount of a microspore/pollen grain, draw and describe the structure of a male gametophyte;
- draw, label and describe the various parts of an ovule, including the regions of female gametophyte; and
- identify and list the morphological, anatomical and reproductive features that help designate the genus, *Cycas* to the various ranks of taxa of classification.

Study Guide/Prior Knowledge

For doing satisfactory work you must read the following before coming to the laboratory.

The UGC (CBCS) Course of Core Botany (CBCS)- Botany Paper I : Biodiversity (Microbes, Algae, Fungi and Archegoniates), Block-6 Unit 20: Gymnosperms *Cycas*; section 20.3 : Morphology; 20.4 : Anatomy, and 20.5 : Reproduction.

16.2 METHOD OF STUDY

You shall conduct the exercise by the following methods

(A) Study morphological characters through herbarium/fixed material of coralloid root; bulbil, and leaves;

(B) Preparation of temporary-stained preparations of t.s. coralloid root; v.s. leaflet, and t.s. root; v.s. microsporophyll; w.m. microspores/pollen grains.

(C) Study of coralloid root; leaflet, root; microsporophyll; microspores/pollen grains and ovule (t.s.) through permanent slides.

(D) Observing, drawing, labelling and writing explanatory notes on the observations made;

(E) Identification of the object(s) under study by providing suitable reasons.

(F) Assigning the genus *Cycas* to the various ranks of taxa of classification with suitable identifications points.

16.3 MATERIAL REQUIRED

In addition to general Biology kit and Laboratory kit you require :

1. **Plant Material :**

   (A) **Herbarium specimen** of frond (leaf); bulbil; male cone; megasporophyll.

   (B) **Fixed Material:** leaflets; bulbil; coralloid root; rachis; root; microsporophylls.

   (C) **Permanent Slides:** t.s. coralloid root; v.s. leaflet; t.s. root (normal); w.m. microspore/pollen grains; l.s. ovule.

2. **Stains and Chemicals:** Safranin (0.5%); Glycerine (1.0%); Distilled water.

3. **Glassware and Apparatus :** Dissection microscope; magnifying glass; compound microscope; macroslides; microcoverslips; Petri dishes; watch glasses.

16.4 METHOD

(A) You shall observe, draw, label and write the morphological comments for the plant materials:

Coralloid roots, fronds; bulbils;

(B) You shall prepare temporary- stained preparations of t.s. coralloid roots; v.s. leaflet; t.s. root (normal); v.s. microsporophyll; w.m. microspore/ pollen grain.

(C) You shall observe the permanent slides t.s. coralloid roots; v.s. leaflet; t.s. root (normal); v.s. microsporophyll; w.m. microspore/pollen grain and l.s. ovule.

(D) Observe the materials (B) and (C) carefully and draw labelled sketches on your note book what you observe and write explanatory identification notes.

16.5 OBSERVATIONS

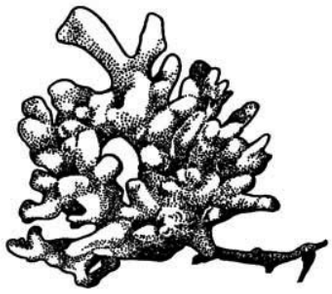


Fig. 16.2: A part of coralloid root.

(A) Study of Morphology of Vegetative Structures:

(i) Coralloid root (Fig.16.2)

- *Cycas* produces two kinds of roots: normal and coralloid.
- Secondary roots are negatively geotropic.
- These are repeatedly dichotomously branched.
- As a cluster they appear as coralloid.
- They project above the soil surface.
- They also exhibit elongation.

(ii) Bulbil

- Older parts of the stem exhibit branching.
- This branch develops as a small bulbil.
- A bulbil is an adventitious bud.
- It arises from the lower fleshy region of the leaf bases.
- When mature, it produces a crown of leaves, so typical of main stem.
- Later, it can grow as a branch
- A bulbil also serves as a means of vegetative propagation, both in nature and artificially (in horticulture).

(iii) Frond Leaf

- A stem bears a terminal group of leaves.
- The leaves are dimorphic: (a) green, photosynthetic fronds; (b) brown, hairy scale. Foliage and scale leaves alternate with each other.
- Young foliage leaves are circinately coiled and are covered with ramenta. Each foliage leaf is pinnately compound, with about 80-100 pairs of pinnae, that are closely arranged.
- These pinnae are placed opposite to each other on a rachis. The rachis has decurrent base.
- Each pinna is tough, leathery and entire. It possesses a mid-rib but has no lateral veins.
- The scale leaves are small, simple, and brown with aborted lamina. The scales are hairy. The scaly leaves are persistent (as are the leaf bases of foliage leaves).

(B) Study of Anatomy of Vegetative Structures:

Observe your temporary stained preparations or the permanent slides under the microscope. The salient features are mentioned below:

Root (T.S) Normal

Young Root (t.s.)

- The section is circular in outline (Fig.16.3).
- The tissues are differentiated as: epidermis, cortex and centrally located stele.
- The epidermis is one-layered of thin-walled cells. It also possesses unicellular root hairs.

- The cortex is multilayered. The parenchymatous cells of cortex are filled with starch grains. A few of them contain tannin. The tannin cells are scattered.
- Innermost layer of cortex, forms an endodermis and delimits the stele.
- Multi-layered pericycle separates vascular tissue from endodermis.
- A central stele is made up of radial and exarch vascular bundles. It has two protoxylem archs (diarch).

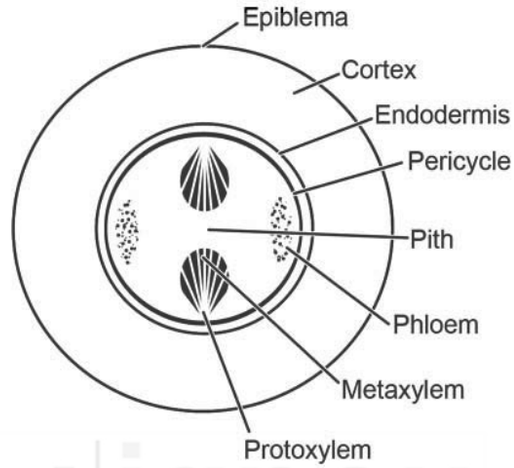


Fig. 16.3: T.S. of normal root (diagrammatic).

Older Root

- The root exhibits secondary growth and a few-layered cork-cells lie beneath epidermal layer. They appear as layers of bricks.
- A large cortical zone has starch-filled cells while a few scattered tannin-cells can also be observed.
- Endodermis and pericycle layers retain their identity.
- Secondary phloem becomes prominent, may crush primary phloem. Cambial arcs are observed along the inner edge of phloem in the stele.
- Secondary xylem is situated in between pith and cambium. Primary xylem (two archs) is retained in a mature secondary root.

Coralloid Root (T.S.)

The structure is almost similar to a normal young root while the cortex, has prominent outer, middle and inner zones (Fig.16.4).

- The outer and inner cortical zones are similar, multilayered and possess parenchymatous cells.
- The middle-zone is called 'algal-zone'. Its cells are radially elongated, and contain bacteria, fungi but mainly cyanobacteria filaments belonging to genus *Anabaena*.
- Vascular bundles are radial, exarch and triarch. The secondary growth is meagre or absent.

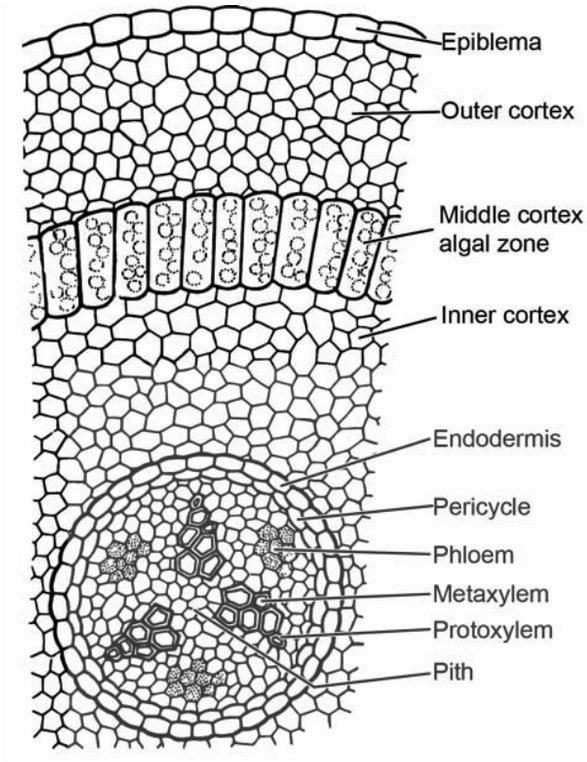


Fig. 16.4: T.S. of a coralloid root (diagrammatic).

T.S. Rachis

T.S. of temporary-stained preparation of a permanent slide of t.s. rachis. You will observe (Fig.16.5).

- The outline is cylindrical; shows insertion of pinnae on the adaxial (upper) side.
- The tissue differentiation shows: epidermis, hypodermis, ground tissue and a ring of vascular bundles.
- The epidermis is single-layer of thickly cuticularised cells, interrupted by stomata throughout the surface. The distribution of stomata is termed amphistomatic.
- The layers below epidermis constitute hypodermis. Most of the cells in this zone are thick-walled and sclerenchymatous. Few chlorenchymatous cells are intermixed with these cells.
- The 2 or 3 layers of hypodermis are on adaxial surface while several layers on the abaxial surface.
- The large, bulk of tissue beneath hypodermis is parenchymatous ground tissue.
- Many mucilagenous ducts are scattered throughout ground tissue. Each mucilaginous duct is 2-layered. The inner layer of cells is composed of epithelial cells while the outer layers of cells are tangentially elongated sclerenchymatous cells.
- The large numbers of vascular bundles are arranged in an inverted omega (Ù) shaped arc. Each vascular bundle is surrounded by a layer

of thick-walled bundle-sheath cells. The vascular bundles are conjoint, collateral and open.

- The positions of xylem, and phloem within a vascular bundle shows variation along the length of rachis :
- In upper, adaxial and most of the rachis the vascular bundles are diploxylic, i.e. with both centripetal and centrifugal xylem. The centrifugal xylem occurs in two small groups on either side of triangular, centrally placed centripetal xylem. The phloem is placed towards the abaxial side of rachis. In the basal region of the rachis vascular bundles show only endarch, centrifugal xylem and abaxial phloem.
- A little higher-up on the base of rachis, the vascular bundles show centrifugal xylem on abaxial side and centripetal xylem on adaxial side. In centre of these two xylem regions lies the protoxylem – a condition called mesarch.

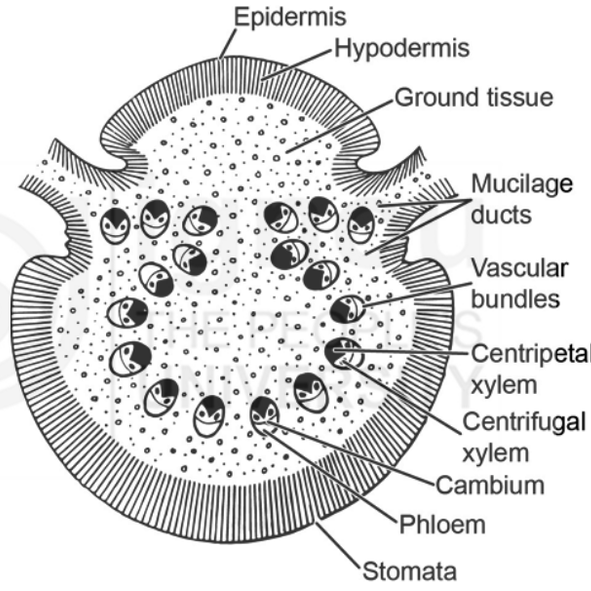


Fig. 16.5: Outline diagram of rachis in T.S.

V.S. Leaflet (Pinna)

Observe the V.S. of temporary-stained preparation of leaflet or a permanent slide of the same and observe the following (Fig.16.6):

- It shows a distinct mid-rib and the lamina (wings). The mid-rib is swollen, while the wings are lateral, narrower and flattened. Mid-rib could be little or prominently projected on the adaxial side. The margins of the wings could be revolute or straight.
- On adaxial surface a single-layer of thickly cuticularised epidermis is present.
- Hypodermis, the layers below epidermis are sclerenchymatous.
- Mesophyll is well developed and differentiated as palisade and spongy layers. The former is more adaxial while the latter is conspicuously

abaxial. Palisade mesophyll parenchyma could be present or absent in mid-rib. The cells of spongy-mesophyll parenchyma possess prominent intercellular spaces.

– On either side of the centripetal metaxylem of mid-rib vascular bundle and somewhat connected to it are two tracheid like cells-called transfusion tissue.

– Between the palisade and spongy parenchyma cells 3 or 4 layers of tracheid like cells are present. These cells are long, colourless, and they run transversly from the mid-rib to the margins of the wings. These layers of cells are called accessory transfusion tissue. These cells connect the xylem of the vascular bundles of midrib through transfusion tissue.

– A single-large central vascular bundle is present in the centre of a mid-rib. This vascular bundle is surrounded by parenchyma cells rich in calcium oxalate crystals. A thick-walled layer of cells forms a bundle-sheath that surrounds the mid-rib vascular bundle.

– This vascular bundle of mid-rib is conjoint, collateral, open and diploxylic. Phloem occurs on the lower side (abaxial). The vascular cambium occupies the zone in between xylem and phloem. The xylem is large, triangular patch of centripetal xylem and two small groups of centrifugal xylem.

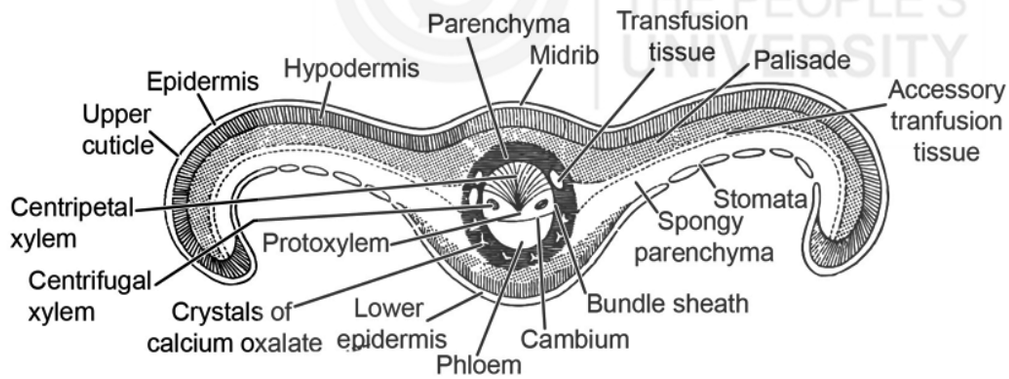


Fig. 16.6: V.S. of a leaflet showing thick-walled epidermis with cuticle, and stomata, on the lower region.

(C) Study of Reproductive Structures

Microsporophyll (w.m.)

Male *Cycas* plants produce male cones. These are short stalked, compact, large, ovate to conical in shape and terminal in position. Each male cone consists of an axis around which numerous microsporophylls are spirally arranged (Fig.16.7).

– Dissect out a microsporophyll from a male cone or pick up a fixed microsporophyll. See the sporangia on the lower surface with a magnifying glass and observe :

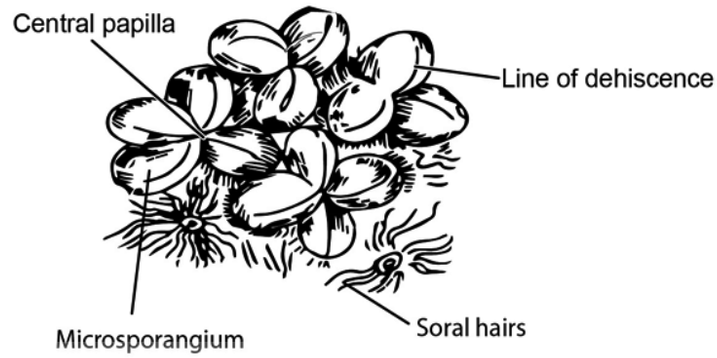


Fig. 16.7: Undehisced microsporangia arranged in groups forming sori.

- The single microsporophyll is woody, horizontally flat, triangular structure.
- It is differentiated into sterile and fertile regions.
- Fertile part is wedge-shaped and expanded distally from a narrow point of attachment.
- Sterile part is the distal region of a microsporophyll.
- Abaxial (lower) surface of the fertile part bears numerous microsporangia in groups of 4 or 5. Together, they are called sori (consisting of more than one sporangium (Fig.16.8)).
- With a sorus, each microsporangium is located around a central papilla.
- Many hairs are distributed on this surface mixed with sporangia.
- Each sporangium has a radial line of dehiscence.

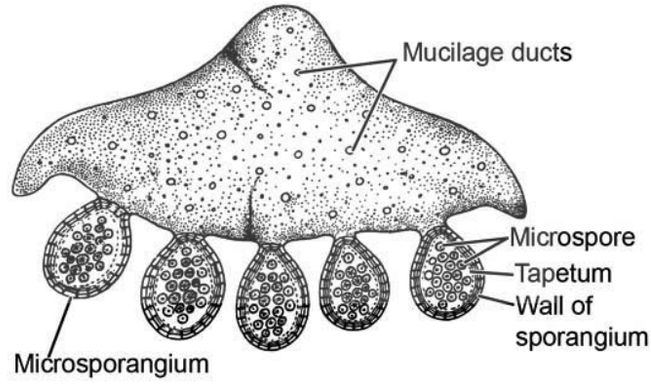


Fig. 16.8: V. S. Microsporophyll showing abaxial microsporangia and enlarged part.

Microspores/pollen grain (w.m.)

- Pick-up/pluck out a sorus and draw the central papilla and hairs.
- With the help of a needle pierce a sporangium, scoop out the microspores and prepare a temporary-stained preparation.

– A sporangium produces a large number of microspores. A microspore could be with one haploid nucleus and large amount of cytoplasm. Every microspore develops into a mature male gametophyte (a pollen grain).

– It may possess a large antheridial cell and a smally lens-shaped prothallial cell, or

– It may possess a small prothallial cell, an antheridial cell and a tube cell,

– Thus, at the time of release a mature pollen grain could be 3-celled.

– Each pollen grain is boat-shaped because of the presence of a depression on the distal side.

Note : In the prepared slide you may observe spores in any of the above stage of development. Observe, draw and comment on the morphology of a microspore/pollen grain that you observe.

L.S. Ovule

Observe the l.s. ovule (permanent slide) under a compound microscope and draw, label and you will see following features.

– Ovule is orthotropous (funiculus and micropyle are along the same plane/axis)

– It is unitegmic- The integument is very thick and is fused with nucellus except the upper region where a nucellar peak is formed; leaving a small opening called micropyle(Fig.16.9).

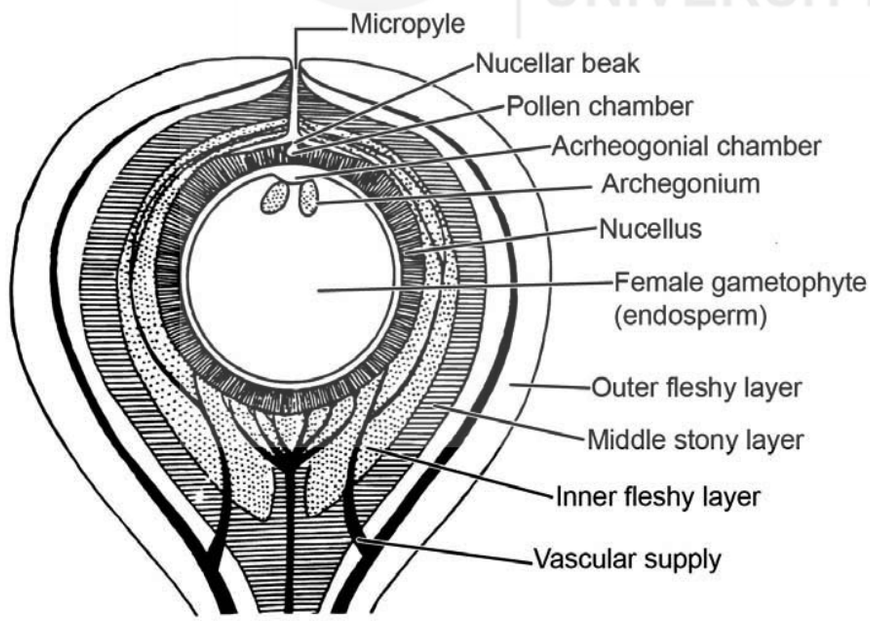


Fig. 16.9: L.S. ovule showing archegonial initial which divides to form a neck initial and a central cell.

– An integument possess 3 distinct regions :

- outer freshy layer, with a vascular supply

- inner fleshy layer with a vascular supply
- Middle Stony Layer Without a Vascular Supply.

– A nucellus lies just below the integument and forms a nucellar beak

– A few cells of this nucellar beak get dissolved to produce a chamber. This is termed pollen-chamber. This chamber lies in the centre of the beak.

– In the inner region of the ovule, lies a large, massive female gametophyte, whose cells are haploid. Within female gametophyte lie two archegonia, opposite to the pollen chamber. The archegonial chamber is just above the archegonia.

– The orange and coloured, fleshy ovules are oval in shape and each shows a small point at the distal end which represents the remnant of the micropyle.

Note: Depending on the stage of development of female gametophyte, in the permanent slide that you observe, the ovule may have nuclear, partly cellular, completely cellular, with or without archegonia. You study what you observe and record your findings.

16.6 CLASSIFICATION AND IDENTIFICATION

KINGDOM : PLANTAE	:	1) Chlorophyllous; autotrophic
		2) Storage food material is starch
		3) Cell walls are cellulosic
DIVISION: GYMNOSPERMAE	:	1) Absence of vessels in xylem
		2) Ovules-naked, attached to a scale
		3) Haploid “endosperm”
CLASS: CYCADOPSIDA	:	1) Wood manoxylic
		2) Leaves-large, frond-like
		3) Seeds with radial symmetry
ORDER: CYCADALES	:	1) Plants woody; stem unbranched
		2) Presence of mucilage canals
		3) Leaf trace diploxylic
		4) Dioecious
		5) Ovules orthotropous
		6) Flagellated spermatozoids

FAMILY: CYCADACEAE	:	1) Leaves with circinate vernation
		2) Presence of coralloid roots
		3) Coralloid roots with endophytic cyanobacteria
		4) Megasporophylls foliar
GENUS: *CYCAS*	:	1) Large as well as scaly leaves
		2) Foliage leaves pinnately compound
		3) Presence of transfusion tissue in leaf
		4) Diploxylic vascular bundles in leaf
		5) Vascular bundles arranged in an inverted omega-shaped in rachis
		6) Single, large male cone.

EXERCISE 17

STUDY OF MORPHOLOGICAL, ANATOMICAL AND REPRODUCTIVE FEATURES IN *PINUS*

Structure

17.1 INTRODUCTION

Pinus is the most well-known representative of the family Pinaceae and order Coniferales. The large tree represents the sporophytic generation. The plant is monoecious with male and female cones borne on separate branches. The male cones occur in a cluster while the female cone replaces a long shoot.

Pinus exhibits two kinds of roots : the normal tap root and the mycorrhizal root. The stem is erect and woody. The wood is pycnoxylic and devoid of vessel elements. Two types of branches long and dwarf are characteristic of *Pinus*. The foliage leaves are reduced as needles and exhibit xerophytic characteristics. The male gametophytes, the pollen grains, are winged. The female cones possess a number of hard, woody, ovuliferous bract scale complexes which are arranged spirally on an axis. Pollinated by wind, male gametes are transferred by pollen tubes. *Pinus* produces winged seeds. Developing seeds exhibit polyembryony and mature embryo could be polycotyledonary.

Objectives

After conducting this exercise you should be able to:

- examine the general morphological organization of a long and a dwarf shoot;
- prepare a temporary-stained transverse section of a needle and list the xerophytic characteristics of a needle of *Pinus* along with the anatomical details;
- prepare a temporary-stained transverse section of a stem and study the anatomical details;
- compare the anatomical details of secondary xylem (wood) of *Pinus* in t.s., t.l.s, and r.l.s with the help of permanent slides;
- examine the morphology of male and female cones;
- study the structure of a microsporophyll, microsporangia within a male cone through whole mounts and in t.s./l.s. and prepare a temporary stained preparation of a mature pollen grain and comment on its characteristics;
- observe, draw, label and describe the structure of a female cone in l.s. Also, study the morphology of a female cone; and
- identify and list the morphological, anatomical and reproductive features that help designate *Pinus* to various ranks of taxa of classification.

Study Guide/Prior Knowledge

For doing satisfactory work you must read the following before coming to the laboratory.

The UGC (CBCS) Course-Core Botany Paper I: Biodiversity (Microbes; Algae; Fungi, and archegoniates) Block-6 Unit 21: GYMNOSPERMS: *Pinus*; section 21.3 : Morphology; section 21.4 : Anatomy, and 21.5 : Reproduction.

17.2 METHOD OF STUDY

You shall conduct the exercise by the following methods :

(a) Study herbarium/fresh/fixed materials of : long shoot; dwarf shoots; needle; male cone; females cones (I yr, II yr and III yr).

(b) Preparation of temporary-stained slides of : t.s. needle; t.s. stem; w.m. microsporophyll; w.m. microspores/pollen grains.

(c) Study of above (b) and t.s. needle; t.s. stem; l.s. male cone; w.m. microspore/pollengrains and l.s. female cone through permanent slides, and morphology of female cones (Iyr, II yr, III yr).

(d) Observing, drawing, labelling, and writing explanatory notes on the observations made.

(e) Identification of the object(s) under study by providing suitable reasons.

(f) Assigning the genus *Pinus* to the various ranks of taxa of classification with suitable identification points.

17.3 MATERIAL REQUIRED

In addition to general Biology Kit and Laboratory kit, you require:

1. **Plant Materials :**

   (A) Herbarium/Museum specimens of twigs showing long-and dwarf shoots; needles; female cones (Iyr, IIyr and IIIyr); and male cones.

   (B) Fixed Material: needles; stem; male cones.

   (C) Permanent Slides: t.s. needle; t.s. stem; t.l.s stem; r.l.s stem; l.s. male cone; w.m. microspore/pollen grain; l.s. female cone.

2. **Stain and Chemicals:** Safranin (0.5%); Glycerine (1.0%); Distilled water;

3. **Glassware and Apparatus:** Dissection microscope; magnifying glass; compound microscope; microslides; microcoverslips; Petri dishes; watch glasses.

17.4 METHOD

(A) You shall observe, draw, label and write the morphological comments for the materials :

long shoot; dwarf shoot; needle; male cones; female cones.

(B) You shall prepare temporary-stained preparations of :

t.s. needle; t.s. stem; w.m. microspore/pollen grain

(C) You shall observe the permanent slides for the material (B) and t.l.s. stem; r.l.s stem; l.s. male cone; t.s. male cone; l.s. female cone.

(D) Observe the material (A), (B) and (C), draw labelled sketches. Write explanatory identification notes on your observations.

17.5 OBSERVATIONS

(A) Study of Morphology of Vegetative Structures:

Observe a twig of specimen provided and look for the following:

Long Shoot:

(1) Aerial part of the plant consists of a branched stem.

(2) The branching is monopodial and branches are arranged in whorls.

(3) The branches are dimorphic (two types). The larger branches are long shoots, also called branches of unlimited growth. It bears an apical bud enclosed in bud scales.

(4) Each long shoot arises as a lateral bud in the axil of a scale leaf. These lateral buds grow horizontally on the main stem to a certain length; it is referred to as nodal growth. When a long shoot falls off, it leaves a scar on the stem.

Dwarf Shoots

They are also called branches of limited growth, brachyblasts or foliar spurs. They are borne on long shoots. They arise in the axial of a scaly leaf (Fig.17.1).

(2) Each dwarf shoot bears two opposite scaly leaves, called prophylls followed by 5-13 spirally arranged scaly cataphylls in 2/5 phyllotaxy.

(3) Each dwarf shoot bears two kinds of leaves: 2-6 long, needle like foliage leaves and scale leaves which are protective in nature.

(4) The numbers of needles per dwarf shoot is constant for a species and is used as a taxonomic character. *P. monophylla* has 1; *P. sylvestris*, *P. merkusii* bears 2; *P. roxburghii* bears 3; *P. wallichiana* and *P. durangensis* have 5 needles per dwarf shoot.

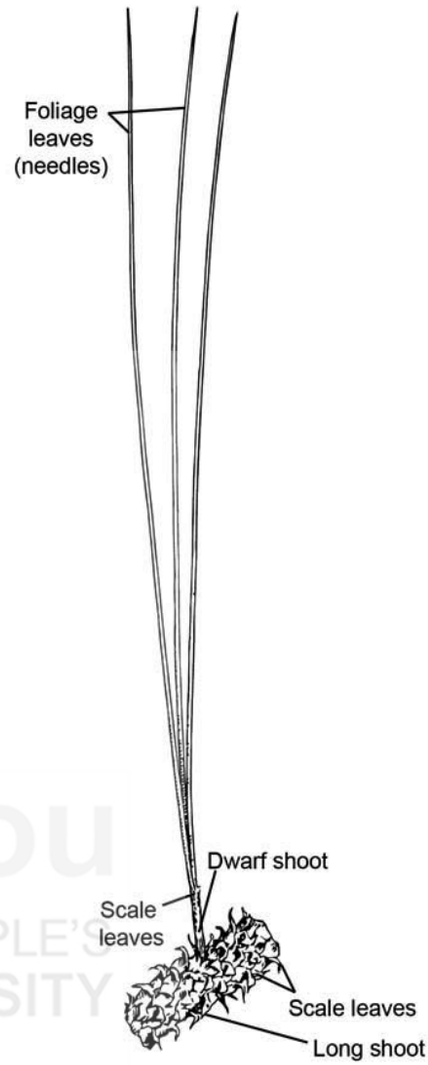


Fig. 17.1: Dwarf shoot bearing three needles.

(B) Study of Anatomy of Vegetative Structures :

T.S. of Needle :

Study the temporary- stained preparation of t.s. of a needle or a permanent slide of t.s. of a needle(Fig.17.2), and look for the following:

(1) A needle could be round (*P.monophylla*); semi-circular (*p. merkusii)*; triangular (*P. roxburghii*); or a very narrow segments of a circle (*P. durangensis*) in outline.

(2) The needle is differentiated into epidermis, mesophyll and stele.

(3) Epidermis is single layer of tangentially elongated thickly cuticularised cells. Epidermis possesses sunken stomata all over its surface.

(4) One to few, thick walled layers of cells, the hypodermis, is present beneath the epidermis. Below the corners, if any, hypodermis could be multilayered; Hypodermal cells are sclerenchymatous. Sub-stomatal chambers are conspicuous within hypodermis layers.

(5) Mesophyll, which lies beneath hypodermis is made up of polygonal parenchymatous cells, densely filled with the chloroplasts.

(6) The chloroplasts bear many plate-like/peg-like infoldings projected into cell-lumen of chloroplast cell.

(7) Resin canals are present both in hypodermis and in the mesophyll tissue.

(8) Endodermis is very conspicuous. It's cells are barrel-shaped and tangentially thickened.

(9) A multi-layered parenchymatous pericycle is prominently observed on inner side of endodermis.

(10) Generally 2 distinct vascular bundles lie in the centre of the needle. *P. strobes* has only one vascular bundle.

(11) The two vascular bundles are separated from one-another by a T-shaped thick-walled transfusion tissue.

(12) Each vascular bundle is conjoint, collateral, open with endarch protoxylem. Phloem is located abaxial to the xylem. A very little secondary growth may be visible.

(13) The needle of *Pinus* exhibits the following xerophytic features:

i) Narrow acicular form of the leaf

ii) Presence of thick cuticle

iii) Amphistomatic nature

iv) Sunken stomata

v) Thick, sclerenchymatous hypodermis

vi) Infolded peg-like structures in mesophyll

vii) Presence of transfusion tissue.

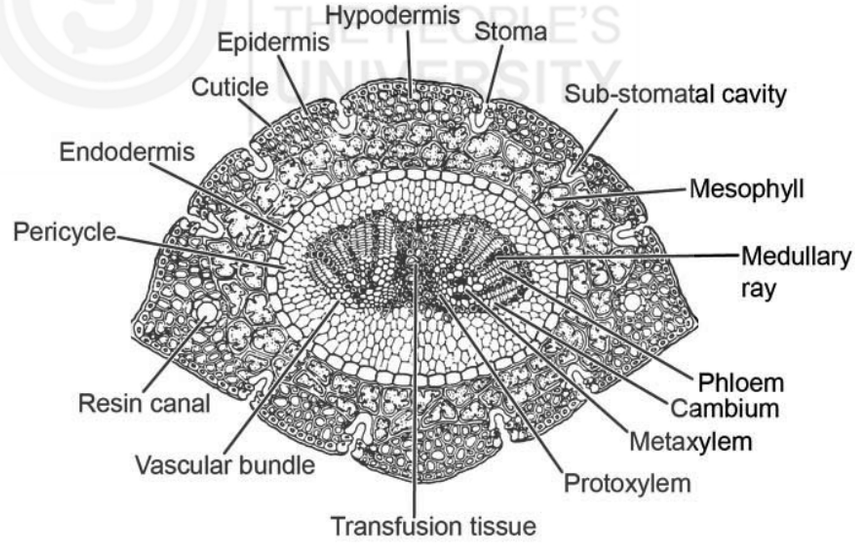


Fig. 17.2: T.S. needle.

Stem (T.S.) - Old:

Prepare a temporary-stained slide of stem or observe a permanent slide of t.s. of stem and observe under a compound microscope, look for the following characteristic features (Fig.17.3).

(1) The major tissue differentiation in the t.s. of old stem could be demarcated as: cork, cortex; primary and secondary vascular tissue, and pith (from periphery to the centre.)

(2) The outermost region is formed by successive layers of thick suberised cork cells.

(3) Below the cork, are present a few layers of regularly arranged cork-cambium cells.

(4) Secondary cortex is present below the cork-cambium. These cells are parenchymatous.

(5) Parenchymatous primary cortex is many layered and is present below secondary cortex. There are number resin canals in the zone.

(6) Two zones of phloem: primary and secondary can be observed beneath cortex. The primary phloem occurs as small patches of crushed tissues. Secondary phloem is found as a well distinguished ring. Sieve cells and parenchyma constitute the phloem.

(7) A few layers of vascular cambium lie in between secondary phloem and secondary xylem. Within vascular cambium tangentially elongated, fusiform initials and radially placed ray initials can be observed.

(8) A large volume of secondary xylem tissue can be observed (older the stem, more of this tissue).

(9) The secondary xylem shows distinct and sharp annual thin-walled and large xylem elements form a ring of spring wood. A thick-walled and small xylem element forms a ring of autumn wood. Secondary xylem elements are very compactly placed, hence, the wood is termed pycnoxylic.

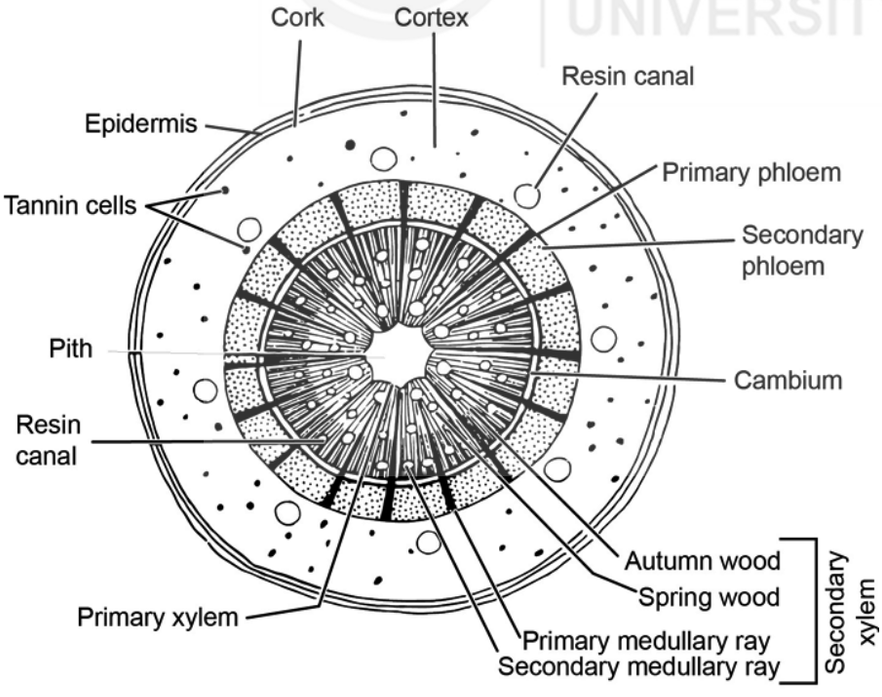


Fig. 17.3: T.S. Stem showing secondary growth.

(10) Spring and autumn wood tissue formed in one calendar year constitutes annual rings. In older stems, a number of such annual rings could be observed.

(11) Secondary xylem (wood) is composed of tracheids and xylem parenchyma. Vessels and xylem fibres are absent. The wood of *Pinus* is called non-porous.

(12) Xylem rays traverse from secondary phloem to secondary xylem.

(13) Primary xylem, lies within inner boundaries of secondary xylem, and is endarch.

(14) A small patch of parenchymatous tissue, lies in the centre of the stem. A few cells of pith contain tannin.

Observe the permanent slides of t.l.s. and r.l.s. of wood (secondary xylem of wood).

TLS of wood

(1) Tracheids and xylem rays are cut transversely in this plane.

(2) The bordered pits can be distinctly observed. They show overarching borders, forming a dome-like structure. It encloses in the centre a small disc-like structure called torus (Fig.17.4).

(3) Xylem rays are uniseriate and are made up of short, more or less rounded cells, three-or-four cell high.

(4) Ray may contain dead cells at tips, called ray tracheid.

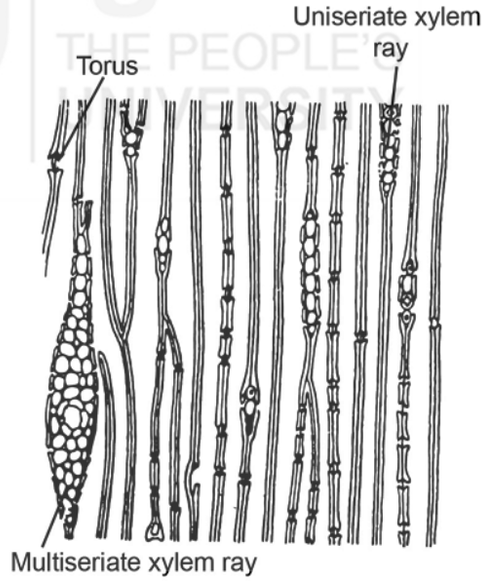


Fig. 17.4: TLS of secondary xylem.

R.L.S. of Wood

(1) The section shows secondary xylem, ray tracheids and ray parenchyma.

(2) Secondary xylem is composed of tracheids with bordered pits on their radial cell walls. You can observe bordered pits in this section in surface view (Fig.17.5).

(3) Bordered pits are circular areas surrounded by special cellulose

thickenings called crassulae or Bars of Sanio. It the pits are close to one other, the bars fuse to form Rims of Sanio.

(4) Xylem rays run horizontally. In this plane, they are cut length-wise and exhibit their length and specifically the height. They are uniseriate.

(5) Xylem rays are made-up of ray parenchyma cells and ray tracheids. The starch-filled central rows of cells are living with simple pits on their walls. The peripheral cells are dead and are called marginal ray cells and possess bordered pits on their walls. They are also called ray-tracheid cells.

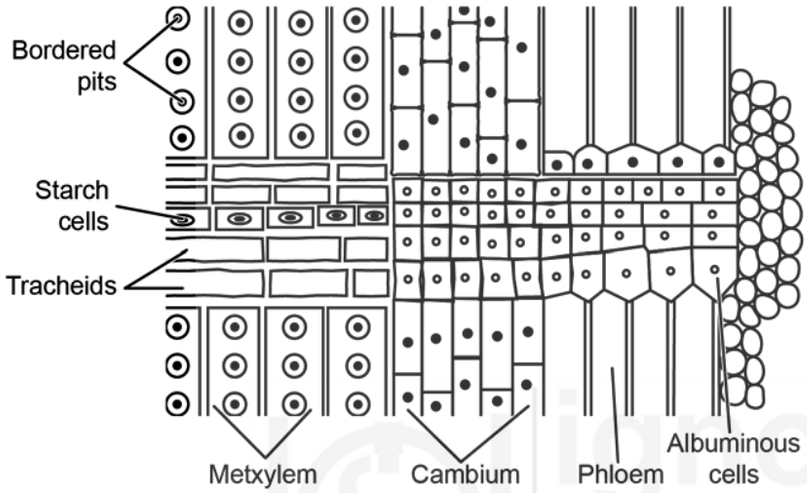


Fig 17.5: RLS of secondary xylem.

(C) Study of Reproductive Structures:

Male Cone:

Male cones are modified dwarf shoots. Each male cone arises in the axis of a scaly leaf. The main shoot, on which these are produced, continues to grow further.

– Observe a cluster of male cones on a long shoot in the fresh/preserved material provided to you.

Microsporophyll (W.M.):

Pick-up one male cone and observe under a dissection microscope or a magnifying glass. Pluck out a microsporophyll with the help of forceps. Place the microsporophyll on a macroslide, mount in glycerine and observe (Fig.17.6).

(1) The microsporophyll has an expanded triangular central part and stalk-like base. The terminal part projects into a tip.

(2) The microsporophyll on its abaxial side bears two ovoid microsporangia or pollen sacs on its lateral sides.

(3) These microsporangia bear a number of haploid microspores in various stages of development.

(4) Each of the microspore matures into a male gametophyte - the pollen grain.

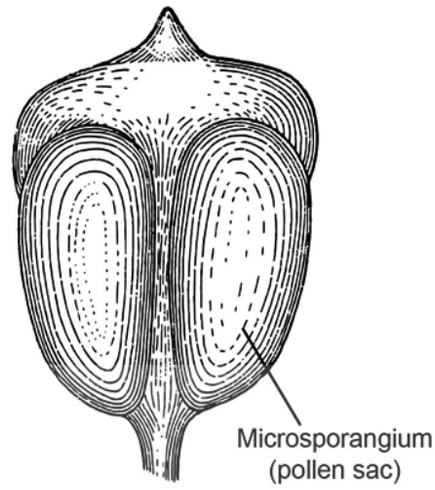


Fig. 17.6: A microsporophyll.

L.S. of Male cone:

Observe a permanent slide of a male cone (l.s.) under a compound microscope and look for the following features (Fig.17.7):

(1) Every male cone is a cluster of microsporophylls. Male cone has single, centrally located cone axis. Around this axis are placed a number of spirally arranged microsporophylls.

(2) The pointed tips of the microsporophylls are projected to the distal end of the cone.

(3) Lower-most microsporophylls of a cone are sterile, i.e., they do not produce/bear any microsporangia.

(4) Each microsporophyll bears on its lateral side only one microsporangium with a cavity.

(5) Each microsporangium has its own wall which encloses many microspores in the cavity.

(6) The wall of the microsporangium consists of epidermis, wall layers and tapetum.

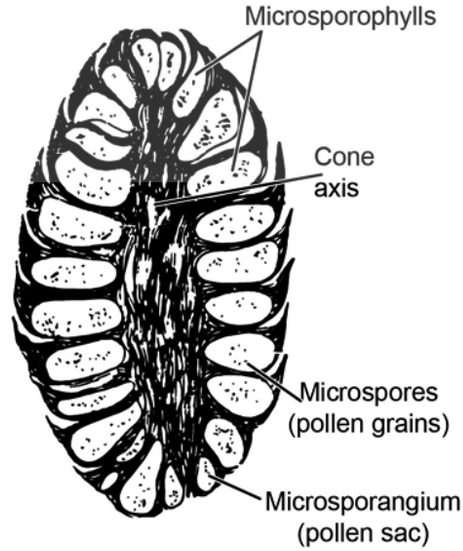


Fig. 17.7: LS Male cone.

T.S. of Male Cone:

Observe the permanent slide of a male cone (t.s.) under a compound microscope and observe the following features:

(1) An expanded central cone-axis.

(2) On the periphery of the cone-axis are present a number of microsporophylls in a circle.

(3) Each microsporophyll bears two microsporangia on its lower surface.

(4) Every microsporophyll has an expanded triangular central part and stalk-like base. Terminal part projects as a tip.

(5) Every microsporangium has its epidermis, tapetum and spore-sac.

(6) Within a spore-sac are present a large number of microspores at various stages of development.

(7) Each microspore matures into a male gametophyte, the pollen-grain.

Microspore / pollen-grain (w.m.)

Scoop out a few microspore/pollen grains from a microsporangium with the help of a needle, stain and mount with acetocarmine and observe under the microscope.

(1) A young microspore is globular or spherical in shape

(2) Microspore is uninucleate, when produced. It is a haploid cell.

(3) As it develops into a pollen grain, the thick outer cell wall of microspore, the exine, expands in the form of wings on the slides.

(4) Within exine is present a smooth inner cell wall, the intine.

(5) The uninucleate cell ultimately produces two prothallial cells and an antheridial cell. The antheridial cell is largest of the all the cells (Fig.17.8).

(You may observe one or more stages of the microspore to pollen grain developmental stage in your w.m. temporary preparation. Draw only what you observe. You may observe the preparations of your batchmates for some other stages).

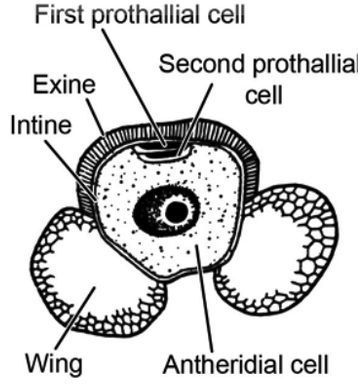


Fig. 17.8: Pollen grain at shedding stage.

Female Cone:

Observe the 1st, 2nd, and 3rd year female cones. Draw the position, arrangement and structure of sporophylls.

(1) The female cones are larger than male cones.

(2) These cones are borne at the apices of the young elongated shoots. They represent modified shoots of unlimited growth.

(3) A shoot may bear one to four female cones in three years. The female cones are reddish-brown.

(4) First-year female cones are compact and the sporophylls are compactly arranged.

(5) Second year female cones are larger in size and woody in nature. The sporophylls are still compactly arranged.

(6) By the third year, the sporophylls become loose and get separated from one another as the cone-axis elongates.

(7) In a female cone, there are sporophylls that are arranged spirally around the cone-axis.

L.S. of Female cone:

Observe the permanent slide of l.s. female cone under the compound microscope as well under the dissection microscope. Draw and label the parts and look for the following features (Fig.17.9):

(1) A central cone axis and a large number of sporophylls on both the sides.

(2) Every sporophyll has two kinds of paired scales :

(i) a bract scale or the cone scale, and

(ii) an ovuliferous scale or seminiferous scale.

(3) Many thin, small bract scales are arranged spirally around the cone-axis. They are directly borne on the cone-axis.

(4) In axils of each of these bracts scale is present; a megasporophyll or ovuliferous scale.

(5) The bract scale and the ovuliferous scale together form an ovuliferous-bract scale complex.

(6) These two kinds of scales in a complex which are fused at base near the cone-axis, but are free at a distance away from it.

(7) The ovuliferous scales in the middle part of the cone are the largest while they are smallest at both the apical and basal ends of a cone.

(8) Within an ovuliferous scale are present two ovules.

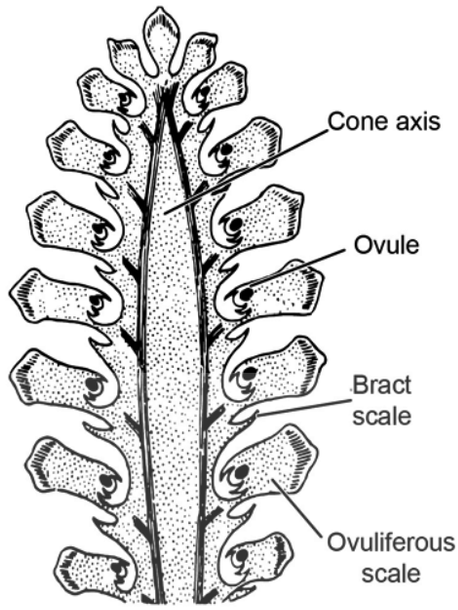


Fig. 17.9: L.S. of female cone.

L.S. of Ovule:

Observe an ovuliferous scale complex carefully for the structure of an ovule. Draw and label the following features:

(1) An ovule is an elongated structure.

(2) The ovule is unitegmic.

(3) The integument has 3 layers: outer thin; middle stony and inner fleshy one. As the ovule matures, the outer layer disappears.

(4) The nucellus is fused with the inner fleshy layer except at its tip. This region forms an elongated, slender micropyle. The opening of micropyle faces the cone-axis.

(5) In the nucellus, lies a small cavity just opposite the micropyle. This cavity serves as pollen-chamber.

(6) The haploid, massive, ultimately cellular female gametophyte develops within the nucellus.

(7) An archegional chamber is formed in the apical region of the female gametophyte.

(8) Upto six archegonia are formed within the female gametophyte near the base of an archegonial chamber.

(9) The massive haploid female gametophyte serves as 'endosperm' for the developing embryo(s) during seed development.

17.6 CLASSIFICATION AND IDENTIFICATION

KINGDOM: PLANTAE : 1) Chlorophyllous; autotrophic.

2) Storage food material is starch.

		3) Cell walls are cellulosic.
DIVISION: GYMNOSPERMAE	:	1) Absence of vessels in xylem.
		2) Ovules-naked attached to a scale.
		3) Haploid 'endosperm'.
CLASS: CONIFEROPSIDA	:	1) Leaves needle shaped.
		2) Wood pycnoxylic.
		3) Resin canals present.
		4) Compact male and female cones.
		5) Non-flagellated male gametes.
		6) Seeds bilaterally symmetrical.
ORDER: CONIFERALES	:	1) Two types of branches : dwarf and long shorts.
		2) Leaves dimorphic: foliar and scaly.
		3) Roots are mycorrhizal.
		4) Pollen grains winged.
		5) Embryo with two-many cotyledons.
FAMILY: PINACEAE	:	1) Resinous wood.
		2) Plants monoecious.
		3) Sporophylls spirally arranged.
		4) Two microsporangia per microsporophyll.
		5) Female cone woody.
		6) Exhibit polyembryony.
		7) Seeds dry and winged.
GENUS: *PINUS*	:	1) Secondary xylem with annual rings.
		2) Dwarf shoots with little secondary growth.
		3) Mesophylls with peg-like ingrowths.
		4) Needles acicular, xerophytic.
		5) Male cones borne laterally in clusters.
		6) Female cones borne singly, and terminally.
		7) Bract and ovuliferous scales spirally arranged.
		8) Two ovules on abaxial surface of an ovuliferous scale.

FEEDBACK FORM

Dear Student,

We look forward to your feedback regarding the **design, contents, time for study language, presentation style,** and **delivery** of the course. Your feedback on any other aspect is also welcome. The mailing address is given at the back of this page.

Thank you,

Yours sincerely
Course Coordinator
Biodiversity (Microbes, Algae, Fungi and Archegoniates)
Course Code: BBYCL-132

Please write your feedback here.

Please write your feedback here.

Please fold it here.

Please affix postal stamp here

To,

The Course Coordinator
Biodiversity (Microbes, Algae, Fungi and Archegoniates)
Course Code: BBYCL-132
School of Sciences
Indira Gandhi National Open Univerrsity
Maidan Garhi,
New Delhi-110068

Please fold it here.

Please write your Enrolment No. and Address here.