B.L.D.E.A's S.B. Arts & K.C.P. Science College, Bijapur.

Department of Botany

B.Sc 1st Semester

Laboratory Manual

Name:_____

Roll No:_____

Year:_____

CONTENTS

B.SC. I - MICROBIAL DIVERSITY AND TECHNOLOGY

EXPERIMENTS

Experiment 1

Safety measures in microbiology laboratory and study of equipment/appliances used for microbiological studies.

Safety measures in microbiology laboratory

- Microbiology laboratories handle microorganisms such as bacteria, fungi, viruses and parasites, which might be potentially hazardous. In order to protect researchers and laboratory workers from the potential risks associated hazardous microbes, it is essential to follow safety measures to protect researchers and laboratory workers. This further need to prevent contamination, and minimize the risk of exposure to pathogens. Knowing the importance of the safety and research, all major laboratories strictly adhere to the following safety measures and the same to be followed in all microbiology laboratory.
- **Standard Operating Procedures (SOPs):** Every microbiology lab must have their own SOPs based on their nature of work and type of microbe handling in the laboratory. The same to be established and followed for all the operations, including specific protocols for handling, storing, and sterilization.
- **Restricted access**: Access to the microbiology laboratory must be restricted to authorized personnel only. Any visitors need to be accompanied with laboratory worker and before that laboratory's safety protocols should be informed.
- **Personal Protective Equipment**: Generally regarded as PPE kit. All personnel must wear appropriate PPE, including aprons/ lab coats, gloves, eye protection viz., safety goggles or face shields. All these gears must be worn in all the time until the person take an exit from the laboratory. It is also advisable to wear respiratory protection (if necessary).
- **Training and Education**: All laboratory personnel should receive appropriate training on microbiological safety practices, emergency procedures, and the proper use of equipment and PPE. Regular refresher training should also be provided. Care and attention must be given to the new or unique isolates (like COVID-19).
- **Biosafety Cabinets (BSCs)**: Biosafety cabinets, such as Class II biological safety cabinets, provide a containment enclosure to handle microorganisms safely. They protect both the worker and the surrounding environment by minimizing the release of aerosols and maintaining a sterile work area.
- **Containment facility and measures**: Microbiology laboratory should be designed with appropriate facilities i.e., as per the standard norms of Bio Safety levels (BSL), Microorganisms should be contained in appropriate containers viz., biohazard containers or autoclave bags according to the type of organism is using to avoid exposure and cross

contamination. Containment measures should also include the use of biosafety cabinets and fume hoods.

- **Decontamination:** Laboratory regularly decontaminated, and all equipment must be sterilized prior and post use to prevent cross-contamination. Laboratory personals should also wash their hands regularly and decontaminate any spills immediately.
- **Risk Assessment:** A thorough risk assessment must be performed to identify potential hazards and develop appropriate measures to alleviate the risks such as assessing the pathogenicity of microorganisms, probability of exposure, and further implementing control measures to minimize or eliminate risks.
- **Waste management:** All waste generated in the microbiology laboratory viz., contaminated materials, biohazard waste etc., should be disposed of properly. The care should to taken to dispose the waste by proper segregation and labelling according to the appropriate biohazard level and finally disposing off with established protocols.
- **Emergency protocols:** Laboratories must be provided with emergency procedures in place during unforeseen incidents or accidents. The procedures include evacuation plans, first aid and communication protocols.

Microbiology laboratory equipment's

Common and specific equipment's and instruments used in microbiology laboratory are Microscopes, Hot air oven, Autoclave/Pressure Cooker, Inoculation needles/loop, Petri plates, Incubator, Laminar flow hood, Colony counter, Haemocytomer, Micrometer etc.

1. Microscope

Microscopes are essential and basic tools in microbiology laboratories, allowing researchers to observe and study microscopic organisms and structures in detail.

Every microscope contains specific parts for bringing increased magnification and resolution of the object/ microbe to be observed. Here are the main parts of a microscope with a brief explanation of their functions:

- **Eyepiece:** Also called as ocular. The eyepiece is the lens situated at the top of the microscope to look through. It typically provides magnification of 10x or 15x.
- **Objective lenses:** Every microscope has multiple objective lenses attached to a rotating nosepiece. The objective lenses provide varied magnification i.e., low (4x), medium (10x), high (40x) and oil immersion (100x) power. In general, each objective lens has its own magnification power.
- **Stage:** The stage is a flat platform where the slide or specimen is placed for examination. It usually has a mechanical stage that allows precise movement of the slide horizontally (x-axis) and vertically (y-axis) for accurate positioning and scanning of the specimen.

- **Coarse and fine adjusting knobs:** These knobs are used to bring the specimen into focus. The coarse knob is used for initial and large adjustments to focus, while the fine focus knob is used for fine-tuning to obtain a clear and sharp image.
- **Condenser:** The condenser is located beneath the stage and helps concentrate and focus the light onto the specimen. Condenser consists of lenses and adjustable diaphragm mainly to optimise the amount of light passing through.
- **Iris diaphragm:** The iris diaphragm is situated within the condenser and it controls the amount of light passing through the specimen. Adjusting its aperture size can reduce or enhance the contrast and resolution of the image.
- **Light source:** The light source, usually a built-in lamp, provides illumination for the specimen. In most microscopes, the light is transmitted through the specimen from below, but in some specialized microscopes, such as fluorescence microscopes, the light is directed from above. The light source can either external source or it can be fitted in the microscope itself.

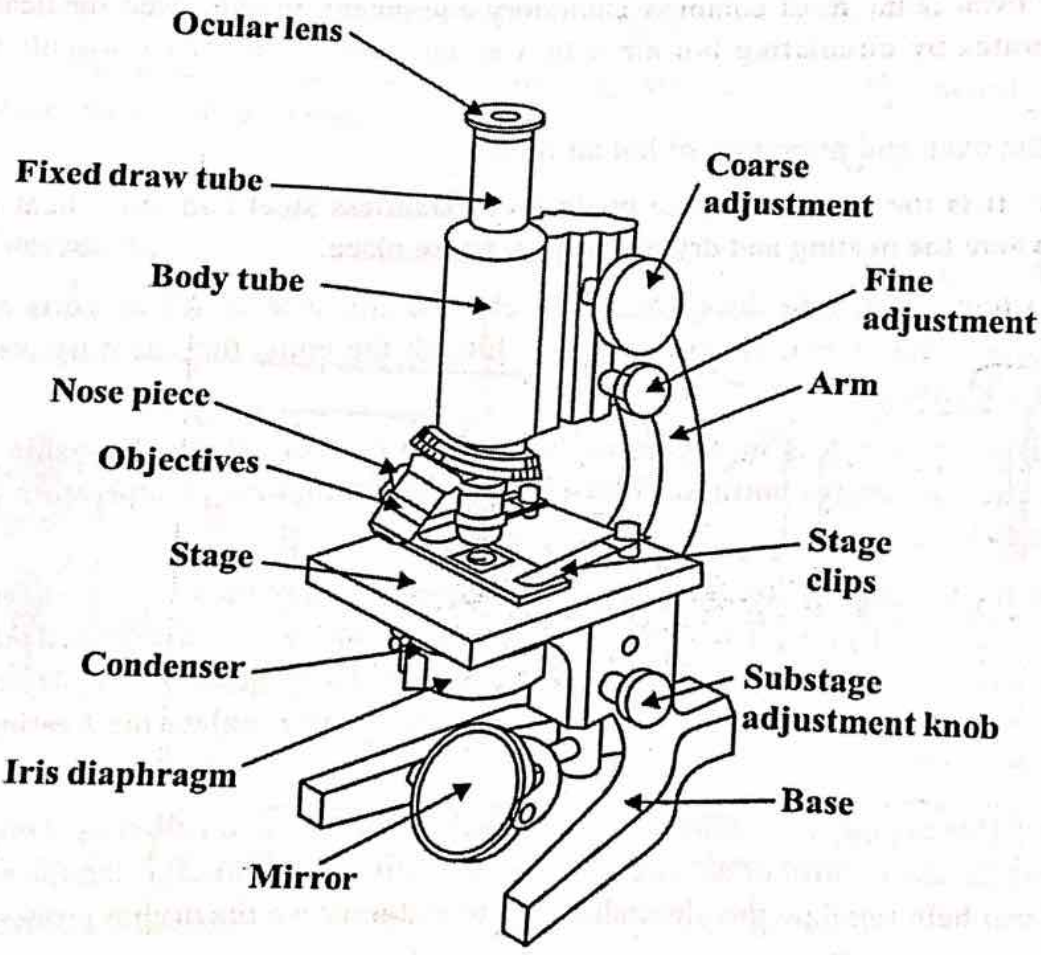


Figure 1: Compound Microscope

Principle of Microscopy:

The principle of microscopy is based on the interaction of light waves with the specimen. Light passes through or reflects off the specimen, and the microscope's lenses magnify and focus the resulting image for observation. The objective lenses capture the light rays and create an enlarged real or virtual image that is then further magnified by the eyepiece. The combination of the objective and eyepiece lenses determines the overall magnification.

The microscopic can utilize different techniques and additional components, such as polarizers, filters, phase contrast, dark field, or fluorescence systems, to enhance the visualization of specific features or structures in the specimen. And some high and cut end research requires sophisticated and high-resolution yielding microscope i.e., Electron microscopy (SEM and TEM) to observer the morphology, shape and structure of the object. These techniques allow scientists to observe microscopic details and analyse various properties of the specimens under investigation.

2. Hot air oven

A hot air oven is the most common laboratory equipment, mainly used for heating and drying. It operates by circulating hot air within an enclosed chamber to provide uniform heating.

Parts of the oven and principles of hot air oven:

- **Chamber**: It is the enclosed space made up of stainless steel and other heat resistant material where the heating and drying processes take place.
- **Heating Element**: Inside the chamber an electric coil or a series of coils are fitted for generating heat. When electricity flows through the coils, they heat up, raising the temperature inside the oven.
- **Blower**: Blower or fan is incorporated to circulate the hot air evenly inside the oven chamber. The fan ensures uniform distribution of heat, minimizing temperature variations within the oven.
- **Temperature Control**: A temperature control system is provided with the hot air oven either as knob or TFT press. The control is essential for maintaining the desired temperature inside the oven. It typically consists of a thermostat and a temperature display panel. The user can set the desired temperature, and the system will regulate the heating element accordingly.
- **Air Vents**: Hot air ovens usually have adjustable air vents also called as damper. These vents allow for the control of air circulation and ventilation within the chamber. Adjusting the vents can help regulate the air exchange rate and improve the drying process.

Working principles of Hot Air Oven Operation: it involves the series of events

- **Convection**: The primary principle behind a hot air oven is convection heating. Convection

involves the transfer of heat through the movement of hot air. The hot air rises, creating a convection current, and is then circulated by the blower/fan. This circulation ensures that the heat is evenly distributed throughout the oven chamber, leading to uniform heating of the samples.

- **Uniform Heating**: the system contains heating element and the blower, the combined work of these two ensures uniform heating inside the oven. The fan circulates the heated air, preventing the formation of hotspots and temperature variations. Uniformity is crucial for consistent and accurate temperature control.
- **Drying and Sterilization**: Hot air ovens are commonly used for drying and sterilizing various materials and equipment. Earlier removes moisture from the samples and promotes drying. And later is mainly works at high temperatures to kill microorganisms, making it suitable for sterilization purposes.
- **Precise temperature control**: Hot air ovens are designed to provide precise temperature control. The temperature control system, including the thermostat and temperature display panel, allows users to set and maintain required temperature. This control is crucial for experiments requiring precise and stable temperature conditions.

Note: The specific design and features may vary based on the manufacturer and the research application of the oven.

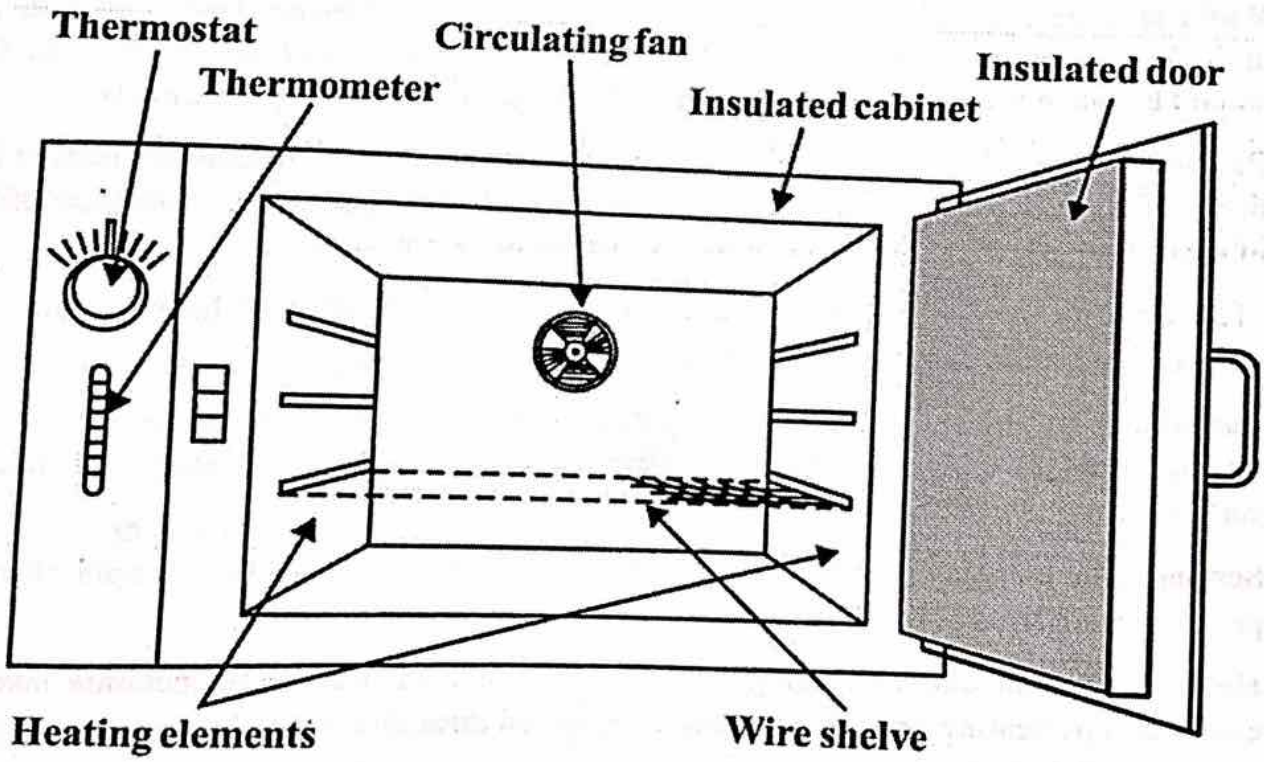


Figure 2: Hot air oven

3. Autoclave/ Pressure Cooker

An autoclave conventionally also called as pressure cooker, the device is used to sterilize equipment and supplies by subjecting them to high-pressure steam. It is commonly used in medical and laboratory settings to ensure the elimination of pathogen and contaminants.

Parts of an Autoclave:

- **Chamber**: The main part of the autoclave is chamber, where the items to be sterilized are placed. It is usually made of a strong metal such as stainless steel and is designed to withstand high-pressure and high temperature.
- **Door/ cap/ lid**: The autoclave chamber needs to be sealed with a cap that locks tightly during the sterilization process. It is equipped with a gasket or sealing ring to prevent steam from escaping out of the chamber.
- **Control Panel**: The control panel (typically located on the front of the autoclave) contains various controls and indicators for setting and monitoring the sterilization process. It may include settings for temperature, pressure, time and cycle duration.
- **Heating System**: Autoclaves use a heating system, often electric or steam-based, to generate the heat required to produce steam. Heating system raises the temperature inside the chamber, that enables the heating of water beyond its boiling point and the steam circulates inside the chamber to the desired level for sterilization.
- **Water storage**: Autoclaves require a water source to generate steam. That is often located at the bottom of the autoclave, holds the water needed for the sterilization process. Care should be taken toward using water, which should not choke or clog the outlets.
- **Pressure Release Valve**: This safety feature allows the controlled release of pressure from the autoclave after the sterilization process is complete. It helps to prevent sudden releases of steam and allows for a gradual return to atmospheric pressure.

The working principle of an autoclave involves a combination of heat, pressure, and steam to achieve sterilization. The following are the steps of each.

- **Loading of samples**: The items to be sterilized are placed inside the autoclave chamber. Arranging of the material is important ensure the free circulation of steam and reach all surfaces.
- **Sealing**: The autoclave lid to be closed and locked securely, ensuring a tight closure to prevent steam from escaping.
- **Heating**: The autoclave's heating system is activated, raising the temperature inside the chamber. The heating process can be accomplished through electric heaters or by injecting steam into the chamber.
- **Pressure build-up**: As the temperature increases, the water in the reservoir generates steam under high pressure. The pressure inside the chamber gradually rises.

- **Sterilization:** The high-pressure steam maintains a consistent temperature and penetrates the items inside the chamber, effectively killing microorganisms. The time duration of the sterilization process depends on the items to be sterilized.
- **Pressure Release:** After the completion of sterilization process, the pressure inside the chamber is gradually released using the pressure release valve. This allows the steam to escape slowly, preventing sudden pressure drops.
- **Cooling:** Final step is cooling. Once the pressure has been released, the autoclave starts to cool down. The items inside the chamber are waited to cool so that, the materials can be safely removed.

Note: working and parts of autoclave varies. Some models may have additional features and variations in their working principles, but the basic concept of using heat, pressure, and

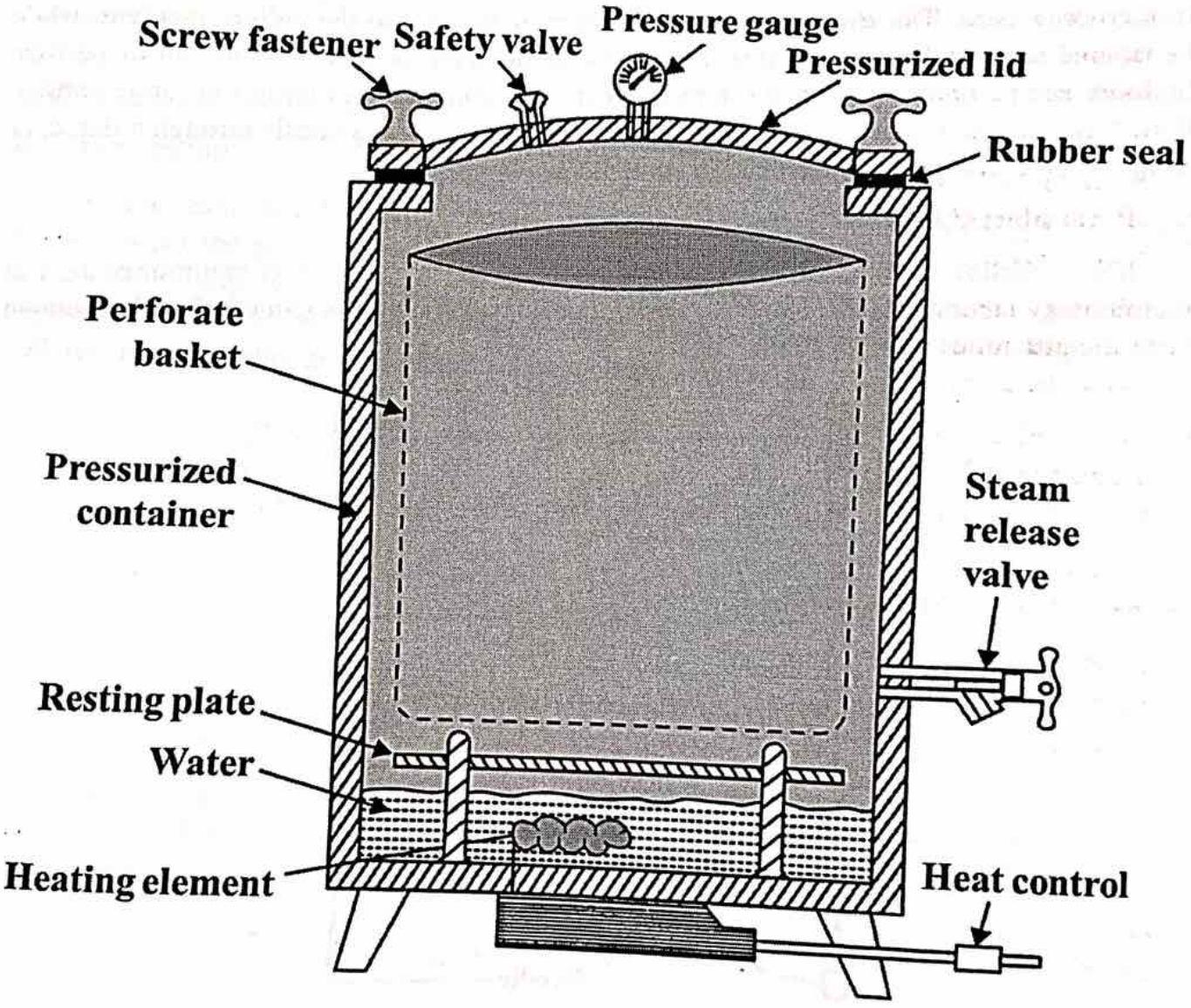


Figure 3: Autoclave

steam for sterilization remains consistent. Autoclave is the upgraded version of traditional cooker, which everyone using in home.

4. Inoculation needles/loop

An inoculation needle or inoculation loop is a small and equally important tool used to transfer microorganism culture, such as bacteria or fungi, from one medium to another. It is made of thin, elongated metal or plastic rod with a sharp or tapered end.

Technical details: Inoculation needles/ loop made up of various materials (stainless steel, platinum, or disposable plastic). The choice of material depends on the specific application and whether sterilization or single-use is preferred. The metallic needle is attached to a handle or shaft that provides stability and control during the inoculation process. The handle can be made of plastic, fibre or metal, offering a comfortable grip for the user. Further the tip of the inoculation needle is designed to be sharp or tapered to facilitate the aseptic transfer of microorganisms. The sharpness allows for easy insertion into the culture medium, while the tapered shape helps to minimize damage to the medium. It is very important to sterilize the loop/ needle before and after use to prevent contamination and ensure accurate results. Sterilization methods can include flame sterilization by passing the needle through a flame, or using an autoclave or other sterilization equipment.

5. Petri plates/ Petri dishes

It is a shallow, cylindrical, transparent plastic or glass dish that is commonly used in microbiology laboratories for culturing microorganisms. Petri dish is named after the German bacteriologist Julius Richard Petri.

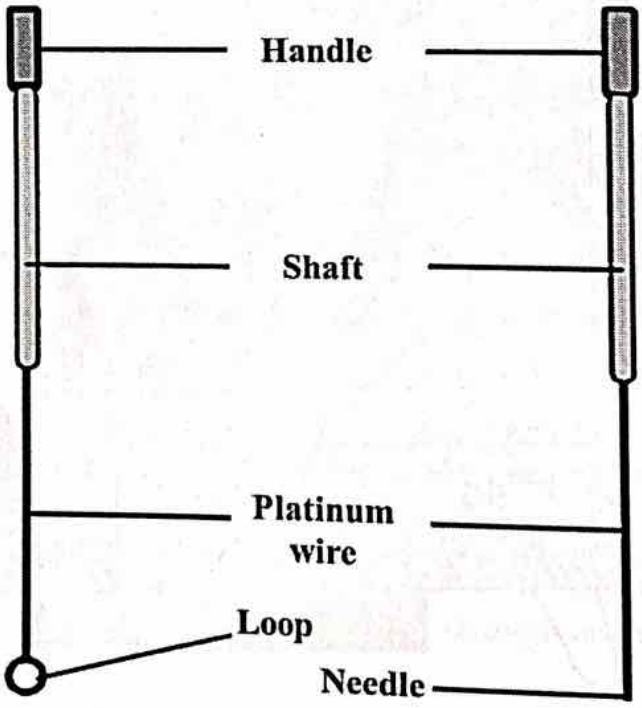


Figure 4: Inoculation needles and loop

Petri plates are typically made of sterile material and have a flat, round shape with a lid. The dish is filled with a gellified media (nutrient agar or agarose gel) which provides a solid surface for microbial growth.

Microorganisms viz., bacteria, fungi, or yeasts, can be inoculated on the agar media using a sterile loop or swab. The lid is then placed on the plate to prevent contamination from the environment. The petri plate is then incubated at a suitable temperature for the growth of the specific microorganism being cultured.

After incubation period microorganism colonies will be observed on the surface of the nutrient agar media, and each colony refer to a single organism or a group of identical organisms that formed by single cell. These colonies can be studied for their size, shape, colour and other characteristic feature, this allows microbiologists to identify and study different types of microorganisms.

Petri plates are basic and essential tools in microbiology research, clinical diagnostics and educational laboratories. Since they provide controlled conditions for studying and isolating microorganisms. They are widely used to grow and study bacteria, fungi, and other microorganisms, aiding in various scientific and medical investigations.

6. Incubator

An incubator is a laboratory device provides controlled conditions or adjusting ideal conditions for the growth of organisms based on the type of work. Incubator is mainly used in the fields of biology, medicine and agriculture. Incubators are commonly used for hatching eggs by providing natural hatching temperature in artificial mode, growing bacteria or cell cultures, and nurturing premature babies in neonatal care units.

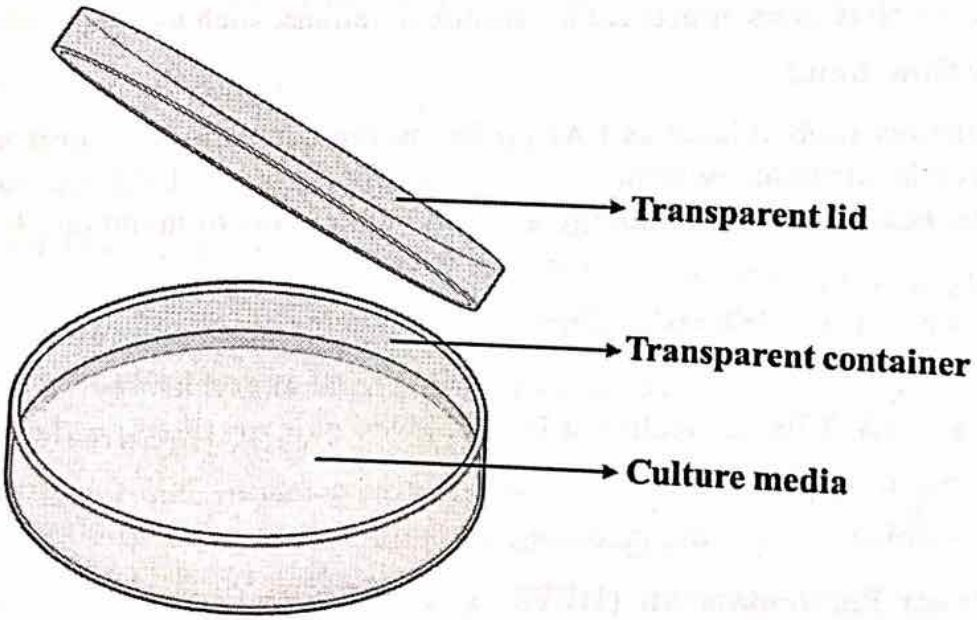


Figure 5: Petri plates

Note: The specific parts and principles of an incubator can vary depending on its intend purpose; however following are the general overview on the parts of an Incubator:

- **Chamber**: It is the place where the organisms or materials to be placed. It is usual enclosed to maintain a controlled environment.
- **Temperature Control**: Incubators have a heating element, such as a heating coil infrared lamp, required to regulate the temperature inside the chamber. Some incubator may also have a cooling system for specific applications.
- **Temperature Sensor**: A sensor, i.e., thermostat is used to measure the temperature inside the incubator and provide opportunity for temperature regulation
- **Humidity Control**: Based on the requirements of the type of organisms or material to be incubated. Some incubators have a humidifier system to maintain a specific humidity level. This may involve a water reservoir or evaporation pads.
- **Air Circulation**: To ensure constant and uniform temperature and humidity inside the chamber, incubators have fans or air vents that control airflow within the chamber.
- **Control Panel**: A user interface for adjusting or setting and monitoring the temperature, humidity, and other parameters will be seen on the top side of the incubator. It may include digital displays, buttons, and control knobs.
- **Doors and Seals**: Incubators have doors with airtight seals to prevent temperature and humidity fluctuations and to protect the organisms inside. External observation can be done, for that a window may also be present.
- **Safety Features**: Incubators may have safety mechanisms like alarms or automatic shut-off systems, to alert users or prevent hazardous situations, such as overheating.

7. Laminar flow hood

Laminar airflow (abbreviated as LAF) refers to the controlled and uniform movement of air in a specific direction within an enclosed environment. LAF commonly used in pharmaceuticals, healthcare, microbiology and cell culture labs to maintain clean and sterile environment.

The key parts and principles of laminar airflow systems are as follows:

- **Air Supply**: A system that generates clean and filtered air, which serves as the source of the laminar airflow. This can include a fan or blower unit, air filters, and ductwork.
- **Pre-filters**: Filters are installed within air supply system to stop larger particles inside flow and contaminants from the incoming air.
- **High-Efficiency Particulate Air (HEPA) Filters**: HEPA filters are the key component of a laminar airflow system. They are designed to remove >99 to 99.97 of particles larger than 0.3μ in size. HEPA filters ensure that the air flowing through them is exceptionally clean and free from any contaminants larger than 0.3μ.

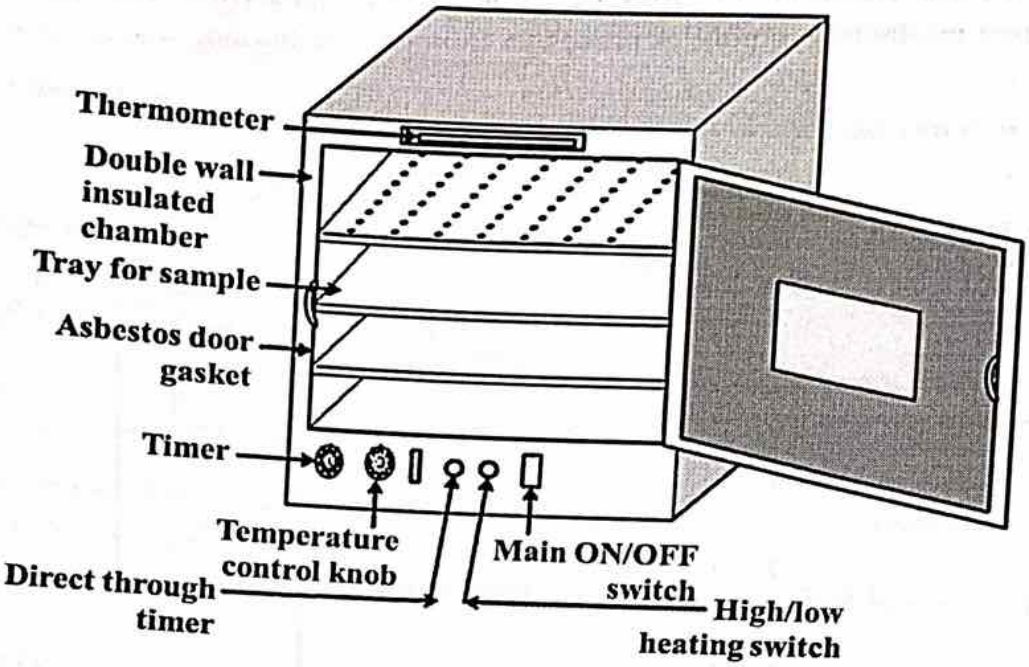


Figure 6: Incubator

- **Air Plenum**: A chamber located above the HEPA filters to collect the filtered dust before release into the controlled environment. Further, it helps to distribute the air evenly across the filter surface, maintaining laminar flow.
- **Work area**: The specific area where the laminar airflow is required. This can be a cleanroom, laboratory bench, or any other controlled environment where the air needs to be kept clean and sterile.

Principle of Laminar Airflow:

Laminar airflow is to provide a constant flow of highly filtered air in a controlled environment, maintaining a clean and sterile workspace. Following are the key principles.

- **Unidirectional Flow**: LAF systems create a unidirectional airflow, that is air moves in a straight line with constant velocity, usually from the top to the bottom (Vertical laminar airflow) or from one end to the other of the work area (Horizontal laminar airflow). This helps to prevent the introduction of contaminants within the workspace.
- **High Filtration Efficiency**: Key component of LAF is HEPA filters, which are used to remove airborne particles viz., dust, microbes, and other contaminants by trapping and filtering the air having size larger than 0.3 microns. Then filtered air is released into the work area, ensuring a clean environment for sensitive processes, equipment, or products.
- **Sterility and Contamination Control**: Motto behind using LAF to maintain a sterile environment by reducing the concentration of airborne particles and microorganisms.

However, the unidirectional flow prevents contaminants from entering the workspace, protecting products, samples, or processes from contamination.

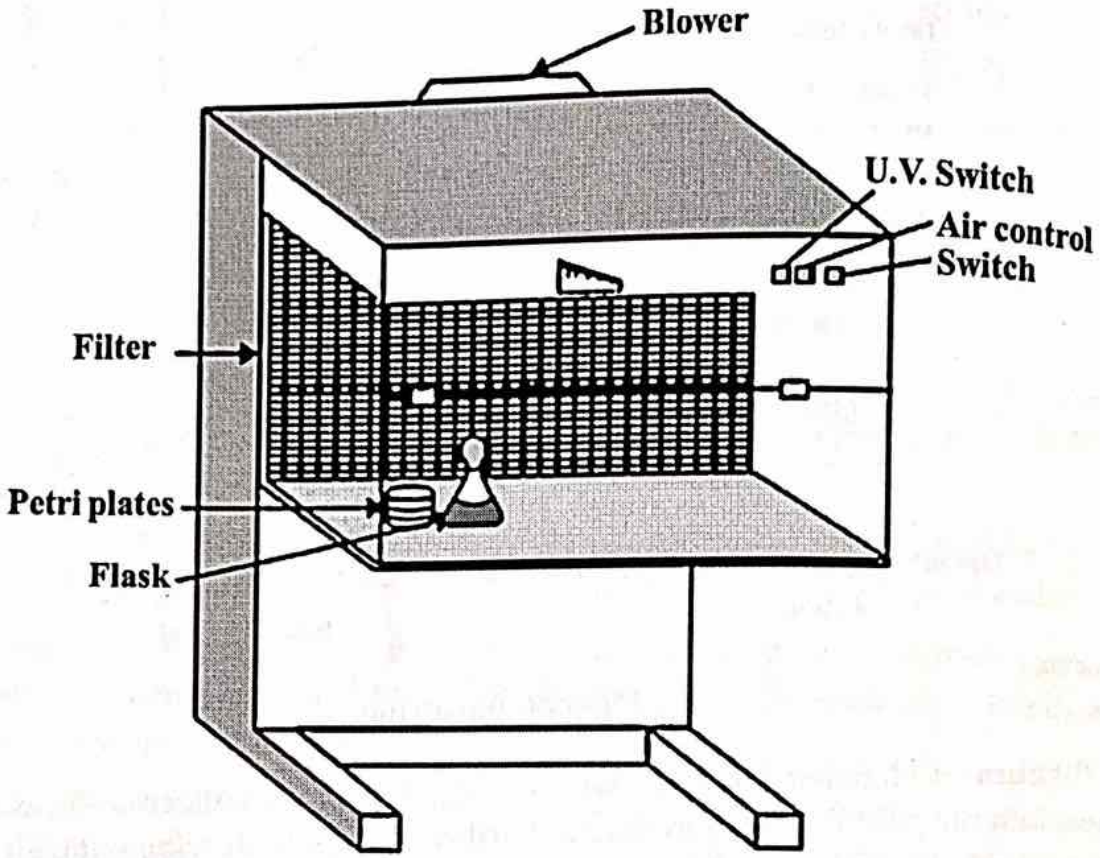


Figure 7: Laminar Air Flow

8. Colony counter

A colony counter is a traditional laboratory instrument used to count the number of colonies that grow on nutrient plates. It is commonly used in microbiology and biotechnology for assessing bacterial growth, determining antibiotic efficacy, and microbial contamination.

The main parts of a colony counter are:

- **Illumination Source:** A light source (either LED or fluorescent lamp) is used to illuminate the agar plate. The light enhances the visibility of the colonies for accurate counting.
- **Magnifying Lens:** A magnifying lens is positioned above the agar plate to enlarge the colonies size for improving visibility.
- **Counting Grid:** A matrix of lines (Grid) is overlaid on the lens external or integrated into the device. The grid helps in organizing and counting the colonies for accurate enumeration.
- **Display:** A digital or analog display scale is incorporated into the colony counter to show the count value. The display allows the user to easily track and record the colony count during the counting process.

- **Counting Buttons:** Buttons or switches are provided with the machine to increment or decrement the count displayed on the screen. These buttons enable the user to keep an accurate data of the colonies while counting.
- **Reset Button:** A reset button is used to clear the count and start the counting process for fresh.
- **Some optional features:** Adjustable lighting intensity, filters for improved contrast, or connectivity options to transfer data to a computer or printer may be the additional part of the colony counter.

Principle of Colony Counting:

The working principle of a colony counter is to provide an efficient method for counting and recording the number of colonies on a nutrient agar plate.

- **Illumination and Magnification:** The nutrient agar plate consisting of microbial colony is illuminated using a light source, which makes the clear visible of colonies. Magnifying lens may further enlarge the colonies for better visibility and making easier to distinguish individual colonies.
- **Counting Grid:** purpose of having the gird is to align the colonies within squares and incrementally counts them to determine the total colony count.

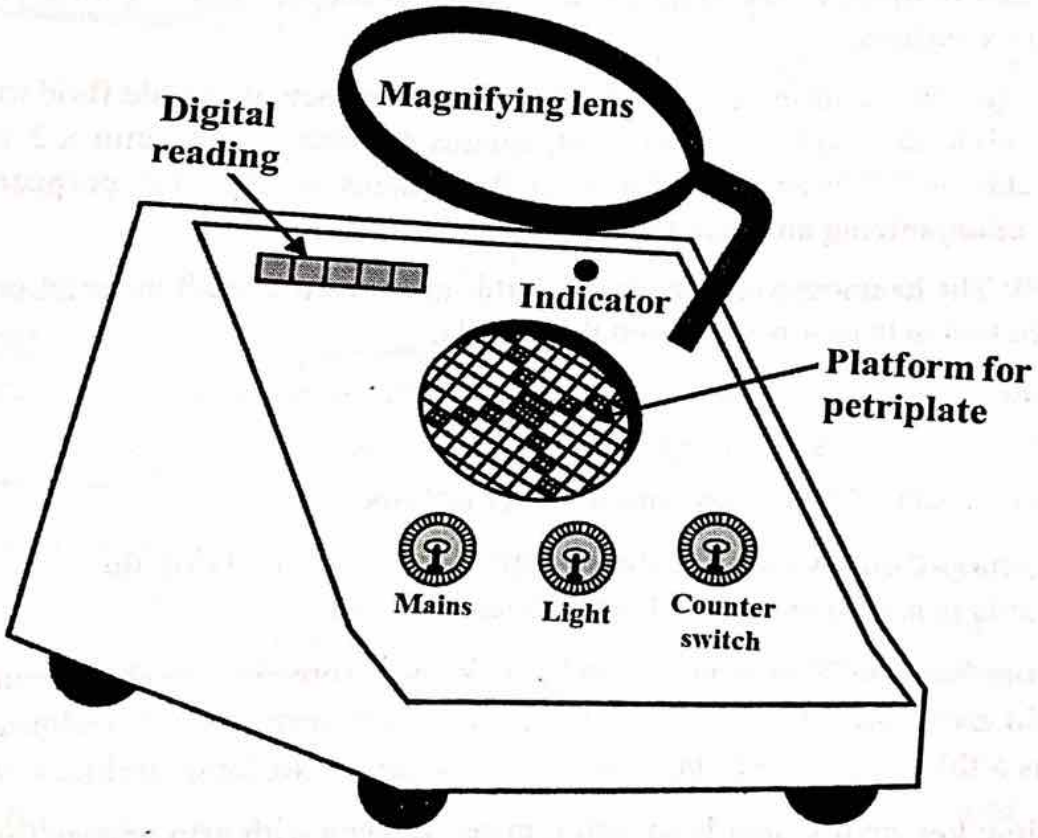


Figure 8: Colony counter

- **Manual Counting:** Colony counting is typically done manually; user will press the corresponding counting buttons to increment the count displayed on the screen.
- **Accuracy and Precision:** The accuracy and precision of colony counting depend on the skill and ability of user to differentiate individual colonies and accurately press the counting buttons.
- **Record-Keeping:** The user's recorded count can be manually transcribed or digitally stored for further analysis or documentation.

Colony counters simplify and streamline the colony counting process, providing a faster and more standardized approach compared to manual counting with a pen and paper.

9. Haemocytomer

A haemocytometer, also known as a counting chamber or a cell counting chamber, originally invented by Louis-Charles Malassez.

It is a specialized laboratory device exclusively used for counting cells, protoplast or particles in a known volume of fluid.

Parts of a Haemocytometer:

- **Glass Slide:** The haemocytometer primarily consists of a glass slide having specific dimensions and markings. The slide has a rectangular area with a counting grid pattern engraved on its surface.
- **Counting Grid:** The counting grid is a central area where actual sample fluid to be loaded on the glass slide that contains a series of squares or rectangles (3 mm x 3 mm). Each square or rectangle is further divided into smaller squares or grids. The purpose of having grid pattern in organizing and counting the cells.
- **Sample Well:** The haemocytometer central part is made with a small indentation or groove on its surface that acts as a well to hold the sample.
- **Side Grooves:** The haemocytometer often have side grooves to allow the sample to spread evenly across the counting grid area by capillary action.

Working principle of Haemocytometer is as follows

- **Sample Preparation:** A small volume (approximately 1ml) of the cell suspension containing cells is mixed and loaded into the sample well.
- **Spreading the Sample:** Capillary action helps in even spreading of the sample across the counting grid area. Soon the sample fills the space between the cover slip and the glass slide enables a thin and uniform layer over the counting grid for counting.
- **Cell Counting:** key component is counting in accordance with grid pattern that consists of squares and smaller grids within them. By placing the haemocytometer under a microscope and visually examines the grid squares.

- **Cell Enumeration:** Enumeration of cells that lie within the squares (even the cells touch the top or left boundary lines of each square) are counted, wherein cells touching the bottom or right boundary lines are not neglected from counting. The smaller grids within each square can be used for counting cells that overlap the lines. Counting can be done in multiple squares to ensure representative sampling.
- **Calculation of Cell Concentration:** The number of cells recorded from each grid squares. Also, the known volume of fluid on the haemocytometer chamber and the dilution factor are used to know the cell concentration in the original sample.

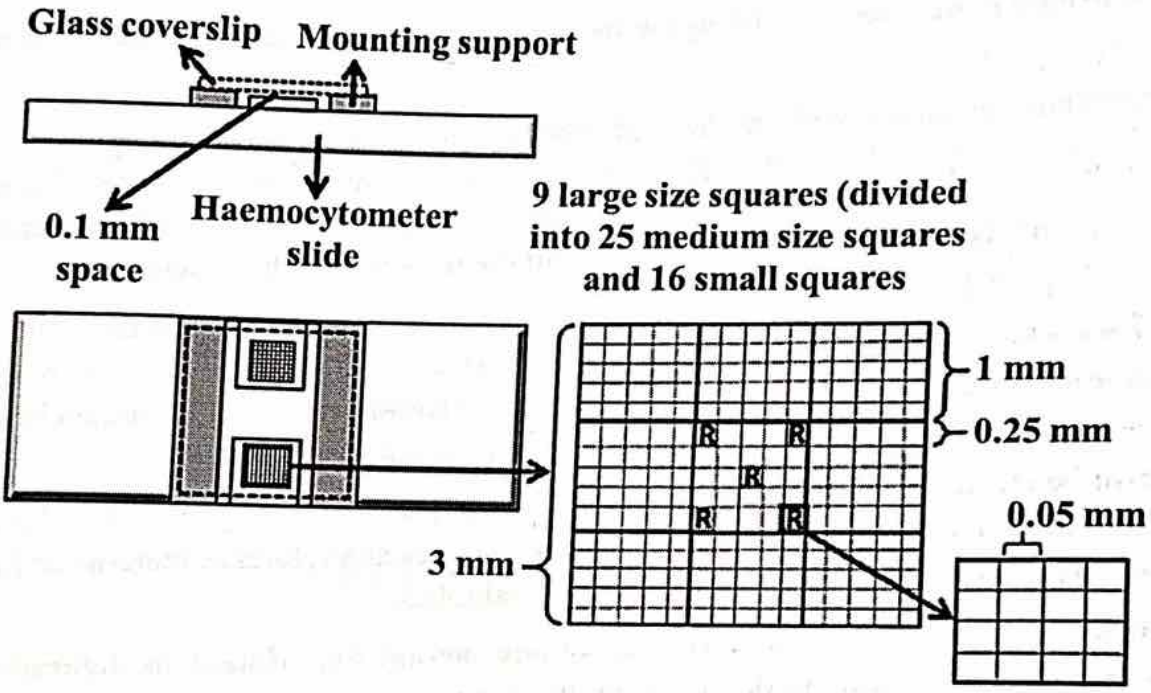


Figure 9: Haemocytometer

Experiment 2
Enumeration of soil/food /seed microorganisms by serial dilution technique.

Principle: It a common laboratory technique used to make a series of solutions with different concentrations from a stock solution. It is the significant experiment in microbiology, biochemistry, and analytical chemistry mainly used when working with substances that are highly concentrated and to get precise dilutions from the stock. The technique further allows researchers to explore a wide range of concentrations while conserving the original stock solution. It is essential to maintain aseptic and accurate techniques throughout the process to obtain reliable results.

Protocol for serial dilution experiment

- **Preparation of stock solution:** This involves preparation of concentrated solution of the substance that needs to be dilute. The concentration of the stock solution will be depending on the desired range of concentrations.
- **Labelling of tube/ vials:** Take a set of clean, sterile test tube/ vials, and label them with the appropriate dilution factors. For example, to maintain 1:10 serial dilution, labelling of the tubes as 10^{-1}, 10^{-2}, 10^{-3}, and so on i.e., up to the required dilution factor. Usually for bacterial and fungal cultures dilution factor might be 10^{-7} and 10^{-5} respectively.
- **Stock preparation:** sample (100mg) to be dissolved in 10 ml distilled water. This serves as stock solution.
- **Transferring aliquots:** with the help of pipette/ micropipette, transfer 1ml of the stock solution into the first (labelled as 10^{-1}) tube containing 9 ml of distilled water. Repeat the same to increase the dilution factor i.e., 1 ml from 10^{-1} labelled tube to 10^{-2} labelled tube and from 10^{-2} labelled to 10^{-3} labelled tube, till the required dilution factor.
- **Dilution of solution:** Each labelled tubes contains 9 ml of water and each time dilution to be increased by transferring 1 ml into next tube, so that total volume will be 10 ml in each test tube and dilution factor will be 1:10. Finally, the solution in the tubes should be mixed thoroughly to ensure even distribution of the solute in the solution.
- **Control:** It always a good practice to have a control tube containing only the diluent i.e., only the water/ buffer devoid of sample. This tube serves as a reference material to find out the concentration of the solute in the experimental tubes.
- **Analysis:** Once the dilution series are ready, actual experiment to determine the microorganism population in the different dilution tube can be analysed i.e., determining the bacterial, fungal or microbial counts and also to perform enzyme kinetics.

Experiment 3

Preparation of culture media (NA/PDA) sterilization, inoculation, incubation of *E coli* / *B. subtilis*/ Fungi and study of cultural characteristics.

Aim: Preparation of Nutrient Agar (NA) and Potato Dextrose Agar (PDA) media for culturing of microbes.

Principle: Any of the nutrient media compose of basic nutrients which can nourish and allow the growth of organisms in the cultures. Whereas nutrient agar (NA) and potato dextrose agar (PDA) are the best basic media used for culturing of bacteria and fungi.

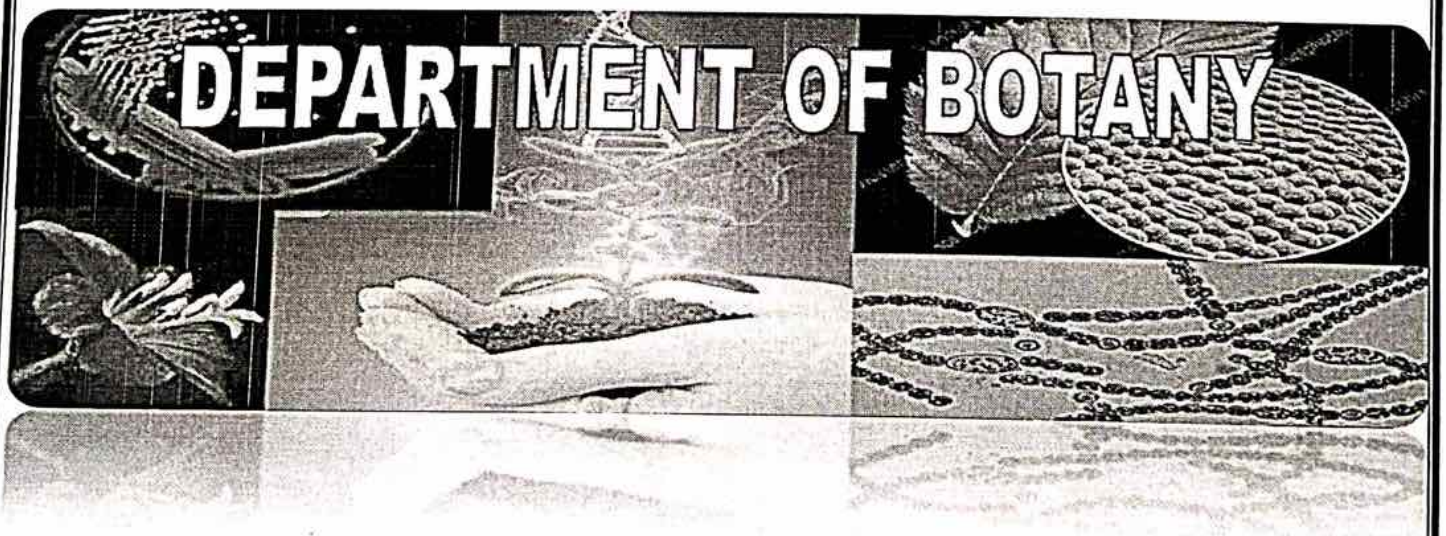
Requirements:

For NA media: Sodium chloride, Agar, Yeast/ Beef extract, Peptone dissolved in Distilled Water.

B.L.D.E.ASSOCIATION's
S.B. ARTS AND K.C.P. SCIENCE COLLEGE, VIJAYAPUR

DIVERSITY OF NON-FLOWERING PLANTS

B. SC – II SEMESTER LABORATORY MANUAL



Sl. No	Title of the experiment
1	Study of morphology, classification, reproduction and lifecycle of Nostoc, Oscillatoria.
2	Study of morphology, classification, reproduction and lifecycle of Oedogonium, Spirogyra, Ectocarpus and Batrachospermum.
3	Study of morphology, classification, reproduction and lifecycle of Riccia & Anthoceros/ Funaria.
4	Study of morphology, classification, anatomy, reproduction and lifecycle of Selaginella and Equisetum.
5	Study of morphology, classification, anatomy, reproduction and lifecycle of Pteris, Azolla/ Psilotum
6	Study of morphology, classification, anatomy, reproduction and lifecycle of Pteris, Azolla/ Psilotum
7	Study of morphology, classification, anatomy, reproduction and lifecycle of Pteris, Azolla/ Psilotum
8	Study of morphology, classification, anatomy & reproduction in Gnetum.
9	Study of important Blue green algae causing water blooms in the lake.
10	Study of different algal products and fossils impressions and slides.

PracticalNo:01

Study of morphology, classification, reproduction and lifecycle of Nostoc & Oscillatoria.

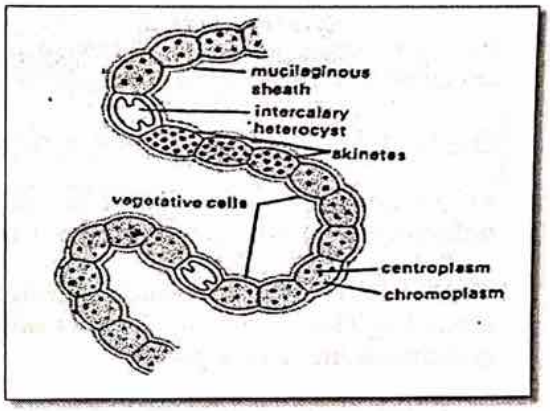
1. Nostoc:

Nostoc is a genus of blue-green algae or cyanobacteria. They are prokaryotic and perform photosynthesis. They are found mainly in freshwater as free-living colonies or attached to rocks or at the bottom of lakes. They are also found on tree trunks.

Classification:

Domain: Bacteria
Phylum: Cyanobacteria
Class: Cyanophyceae
Order: Nostocales
Family: Nostocaceae
Genus: Nostoc

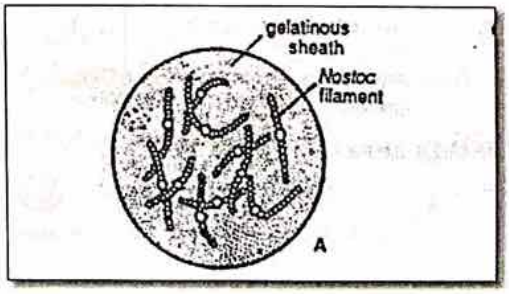
Morphology:



- The body of Nostoc is called as thallus.it is a simple body and consist of many threads like structure called filament.
- Each filament is composed of many rounded cells which are arranged in a chain like manner or in beaded form. All the cells are similar.
- The whole filament is covered by a gelatinous sheath.
- In the filament, some cells are large in size and thick-walled, called heterocysts. These cells perform two functions, reproduction and Nitrogen fixation.
- Each cell of Nostoc is rounded in shape. It is covered by an outer cell wall. The protoplasm of the cell consists of two parts:

a)Chromoplasm: It is the outer colored part along the cell wall, which contains blue green pigments (phycocyanin). It also prepared its own food due the presence of Chlorophyll pigment.

b) Centroplasm: It is the inner colorless part of the cell, which contains stored food granules. It also acts as nucleus. The cell does not contain true nucleus.



Reproduction& Life cycle of Nostoc:

InNostoc reproduction takes place by vegetative and asexual methods. The sexual reproduction is absent.

1) Vegetative Reproduction: Vegetative reproduction is mainly two types

a) Fragmentation: During dry period, the colony becomes dry and breaks into small pieces called fragments. This process is called fragmentation. These fragments are carried to different areas by wind or water. Then they grow into new plants. fragmentation is common in terrestrial species.

b) Hormogones: The vegetative part of the trichome lying between two adeacent heterocusts is called hormogonium.

During favorable conditions, the filaments of nostoc break into small pieces at the region of heterocysts. Each piece is called hormogone.

Each hormogone consists of two or more cells. The hormogone grows into new filament within the envelope of the colony or it comes out of the parent colony and grows into new colony.

2) Asexual Reproduction: Nostoc reproduces asexually by three methods they are

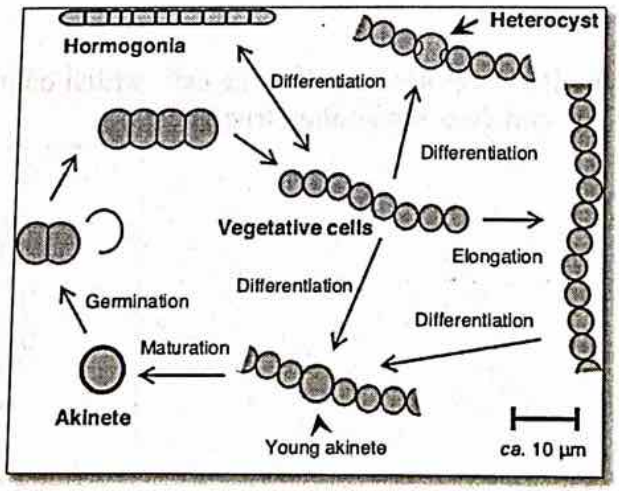
a) Akinetes: Akinetes are also called as resting or arthrospores. They are produced during unfavorable conditions. The akinetes may develop in between the heterocysts.

The vegetative cell first enlarges in size and stores plenty of reserve food. Then it secretes a thick wall around it. This thick walled cell is called akinete. When the conditions become favorable, the akinete germinates into a new plant.

b) Heterocysts: Heterocysts are thick walled cells found in nostoc filament. They are asexual reproductive bodies. They may be terminal to intercalary in position.

The protoplast of heterocyst divides into two then into four cells. This four celled germling comes out by the rupture by the heterocyst wall and grows into a new plant. The reproduction by heterocyst is very rare.

c) Endospores: The endospores ate produced inside the heterocysts. The protoplast of the heterocyst divides into small rounded structures called endospores. The endospores germinate into new filaments.



2.Oscillatoria:

Oscillatoria is a genus that includes simple filamentous cyanobacteria or blue-green algae. Under flowing freshwater, it develops a thin, bluish-green mucilaginous coating on the edges or surface of objects.

Classification:

Domain: Bacteria

Phylum: Cyanobacteria

Class : Cyanophyceae

Order: Oscillatoriales

Family: Oscillatoriaceae

Genus :*Oscillatoria*

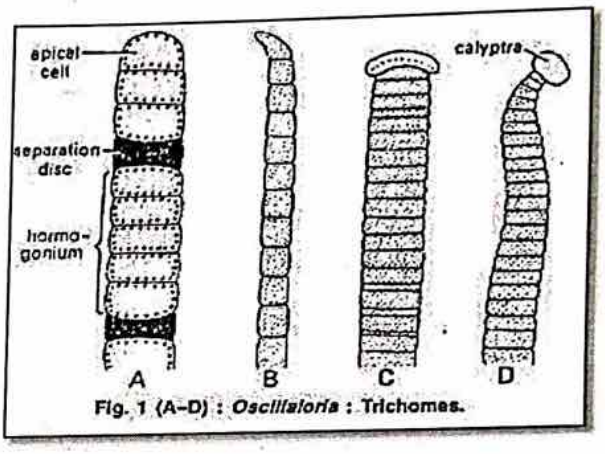


Fig. 1 (A–D) : *Oscillatoria* : Trichomes.

Morphology:

The features given below are useful in identifying Oscillatoria:

- It is a blue-green algae of fresh water bodies.
- Thallus is filamentous, unbranched, multicellular.
- The cells are arranged one above the other like a pack of cards.
- Each cell has a definite cell wall.
- Some cells of the filament may be dead and appear as blank spaces in the filament.
- Fresh specimen of the filaments shows oscillatory movements and hence the name Oscillatoria.

Cell Structure:

- Each cell is made up of an inner protoplasm, a middle plasma membrane, and an outside cell wall. Hemicellulose and pectin make up the rigid cell wall. The cell wall is a bi-layered structure and has many pores. A mucilage sheath also encircles the cell wall.
- The protoplasm is divided into an inner colourless portion termed centroplasm and an exterior pigmented portion termed chromoplasm. Many thylakoids are widely scattered throughout the chromoplasm.
- The chromoplasm also has small gas vacuoles and many photosynthetic pigments.
- Membrane-bound organelles are absent in these cells. However, small ribosomes and reserve food materials are present in the protoplasm.
- The nuclear material is composed of irregularly arranged DNA fibrils. Their nuclear material is called nucleoid as there is no outer nuclear membrane.

❖ Also, the cell structure somewhat resembles bacteria. Thus, *Oscillatoria* is also called cyanobacterium.

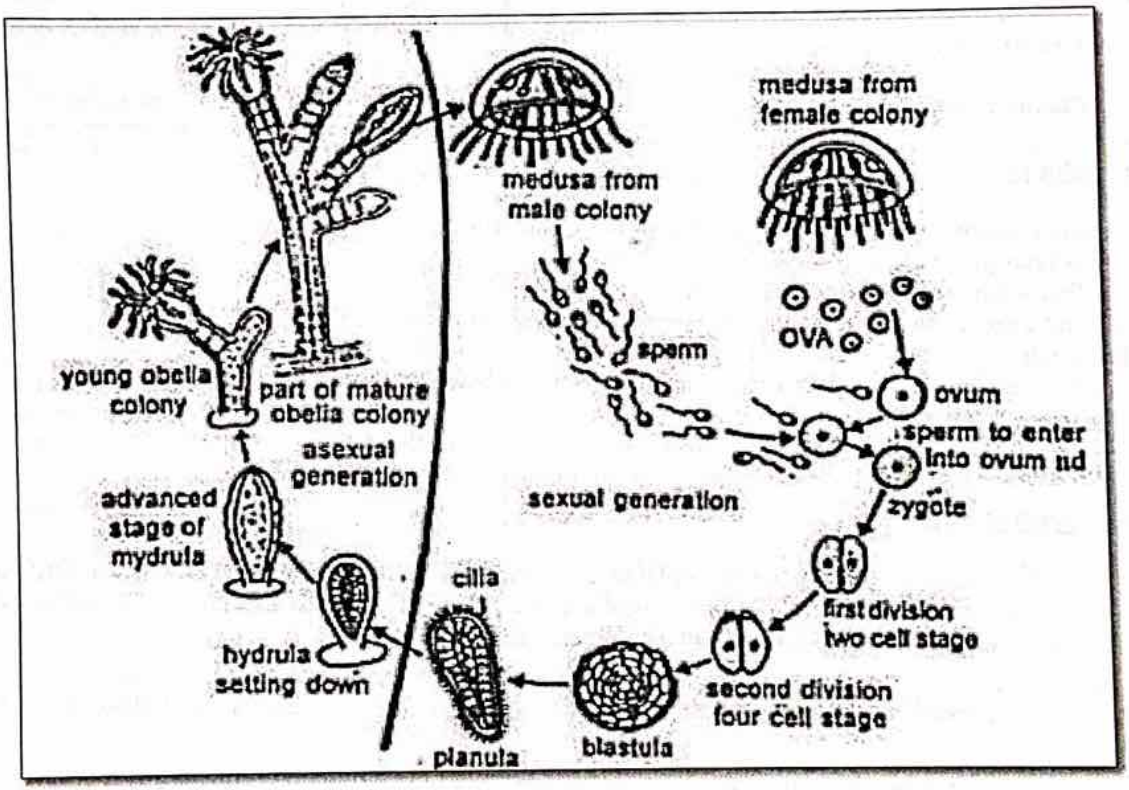
Oscillatoria Reproduction:

Reproduction in *O*scillatoria occurs only through vegetative methods like fragmentation and hormogone formation.

Fragmentation is made possible by dead cells that divide a filament into distinct sections or hormogonium, which then proliferates.

Hormogonia or hormogones are produced in mature filaments under favorable conditions. Hormogonia are small fragments of filaments present between adjacent necridia (mucilage-filled dead cells). The hormogones are freely released into the water when the filament ruptures at necridia. They later multiply and form new filaments.

Life cycle of Oscillatoria:



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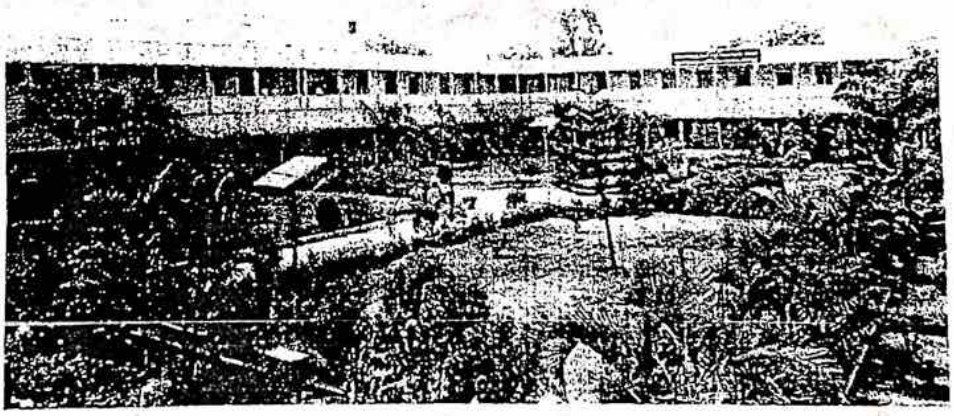
Affiliated to Rani Channamma University, Belagavi.

ರಾಣಿ ಚನ್ನಮ್ಮ ವಿಶ್ವವಿದ್ಯಾಲಯ, ಬೆಳಗಾವಿ
RANI CHANNAMMA UNIVERSITY, BELAGAVI
विद्वान् सर्वत्र पूज्यते

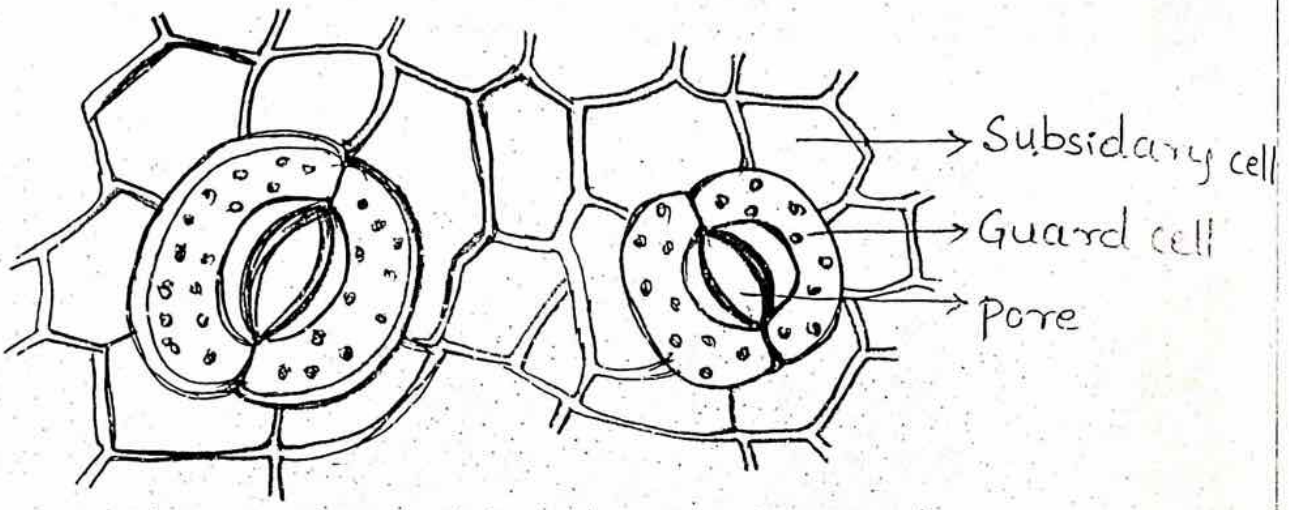
Bsc. III SEMESTER

LAB MANUAL

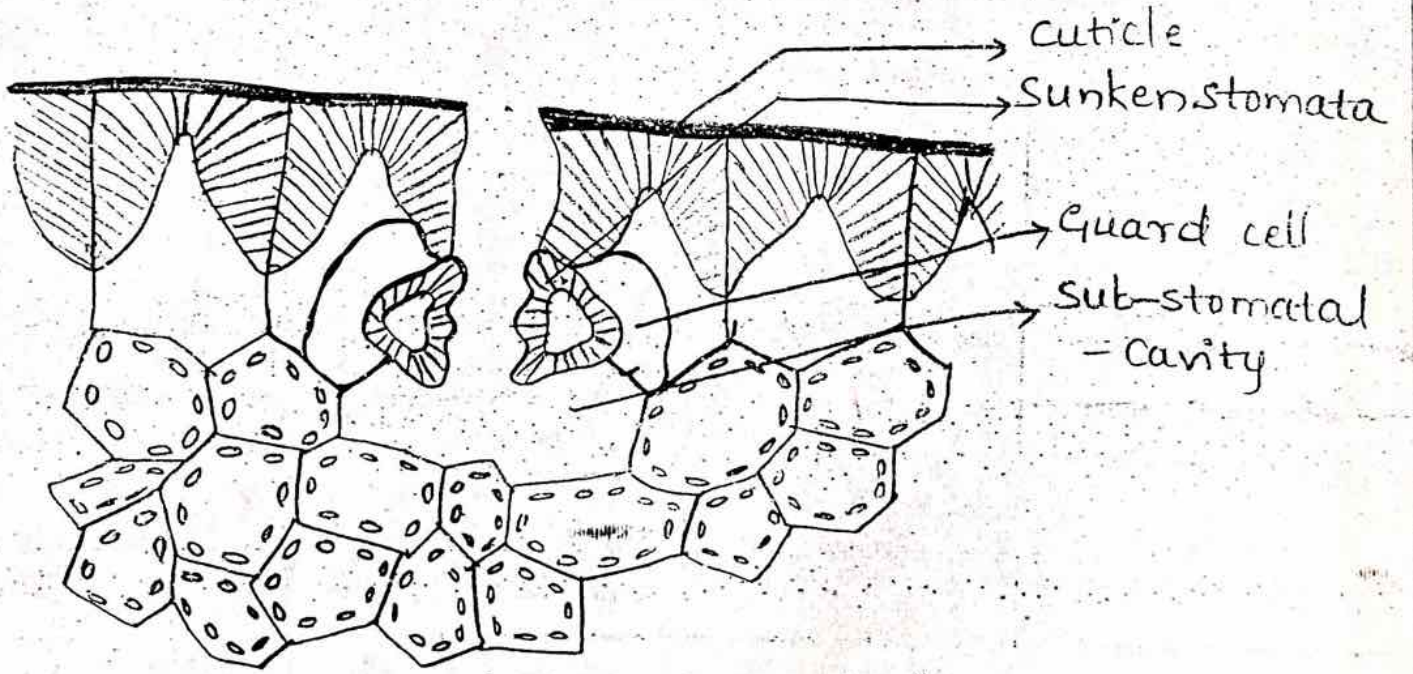
Dept. of Botany



STOMATA



SUNKEN - STOMATA



Expat.no.

Date:

ANATOMICAL STRUCTURES

1. Tyloses:

- Materials:-Stem of cucurbits, safety blade, slides, cover slips, water, microscope, safranin.
- Method: - cut a thin section of stem, stain in safranin for about 5 minutes. Wash in water till proper destaining is obtaining. Mount in glycerine and study under microscope.
- Observation: - In the plants the walls of the xylem vessels produces balloon like structure into the lumen of the vessels are called tyloses. They are found in many angiospermic families and in the vessels of coleus, cucurbita.
  They fully deposits starch, resin gums and other substances. They help in preventing rapid entrance of water, air and fungus from the diseases.

2. Stomata:

- Materials:- Leaves of banyan tree,slides,coverslip,microscope, water, safranine,glycerine,forceps,etc.
- Observation:- The stomata are generally present on the lower surface. A typical dicotyledonous stomatal apparatus consists of guard cells and the surrounding accessory/ subsidiary cells.
  The guard cells are bean shaped and the subsidiary cells are epidermal cells associated with guard cells.
  Functions:- They helps in the transpiration of plants and means of exchange of gases.

3. Sunken stomata:

- Materials:- Leaves of nerium, slides, coverslips,microscopes, water, safranine, glycerine, forceps,etc.
- Observation:-They are present in the xerophytic plants and some multiple epidermis develops in the nerium leaf. These are types of stomata which remain sunken in pots are formed,such stomata called as sunken stomata.

Function:- They helps in exchange of gases and transpiration. When stomata are open water escapes even when water loss is harmful to the plant. So they either by reduction of leaf surface per unit area.

4. **Lenticels:**
   - Materials: - Stem, slides, coverslip, blade, microscope, safranin, glycerine, etc.
   - Observations: - These are usually found in the epidermis of the plants. Lenticels are as a small portion of the pelloderm where the activity of the phallogen is more than elsewhere and the cork cells produced by it are loosely arranged and possess numerous intercellular spaces.

     Functions: - The exchange of gases during night / when stomata are closed.

5. **Sclereids:**
   - Materials: - Guava fruit, slides, needle, coverslip, blade, microscope, safranin, etc.
   - Observations: - sclereids/sclerotic cells are non-paranchymatous cells, usually isodiametric /irregular in shape.

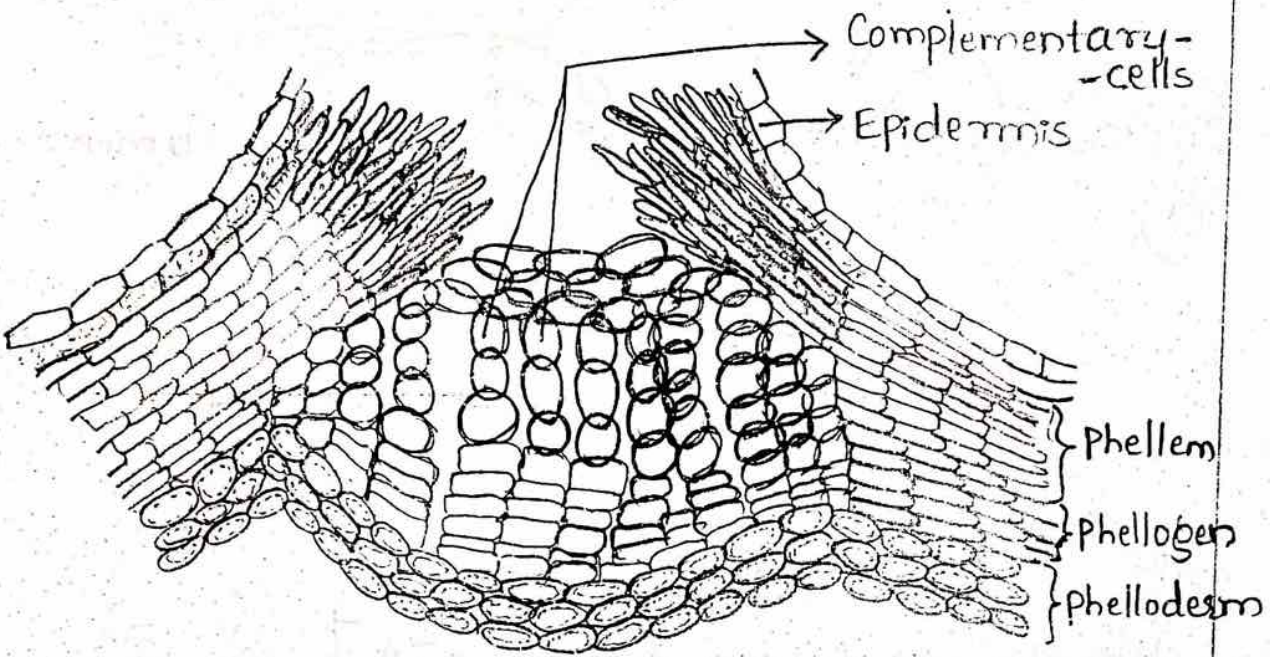
     Normally sclereids become dead with maturity. They may occur singly/in groups/ patches in different plants of parts.

     Brachysclereids also called stone cells. They are usually isodiametric ones like parenchyma cells. Macrosclereids are rather rod shaped elongated cells which form a palisade like epidermal layer on the seed coats.

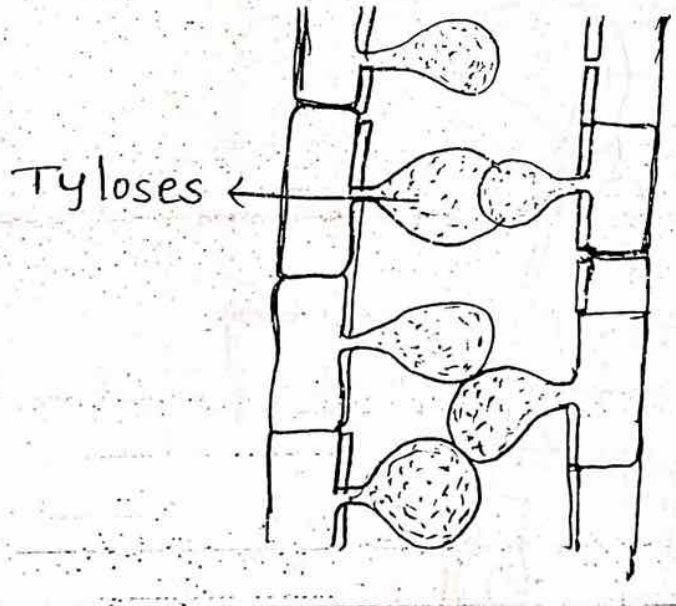
     Osteosclereids are bone like sclereids consisting of columnar cells, more/less dilated at the ends. Asterosclereids have irregularly branched arms.

6. **Vascular bundles:-**
   - The complex tissues, xylem and phloem are usually associated in the formation of the vascular bundles.
   - **Collateral:** - This is the most common type of vascular bundle in the stems and leaves of angiosperms. Here xylem and phloem remain side by side arranged on the same radius, phloem on the outer side.
   - **Bicollateral:** - In addition to the external phloem, another patch of phloem occurs on the inner side, may be called internal phloem.
   - **Radial:** - The components are arranged separately. Here xylem and phloem are arranged separately in different radii

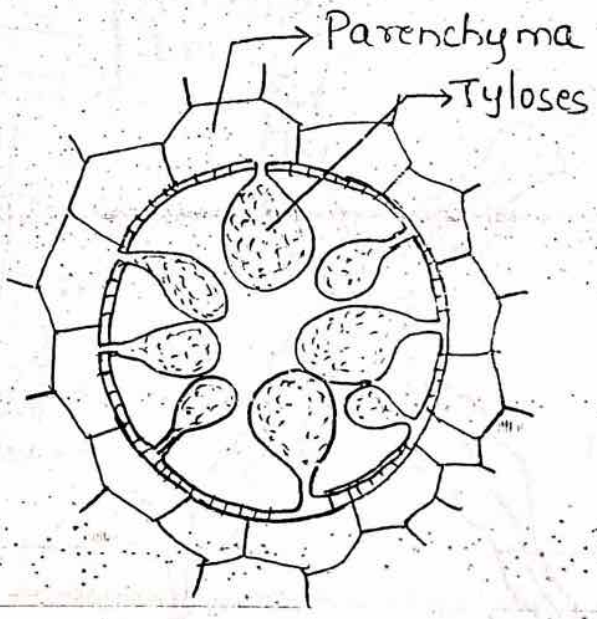
LENTICEL



TYLOSES



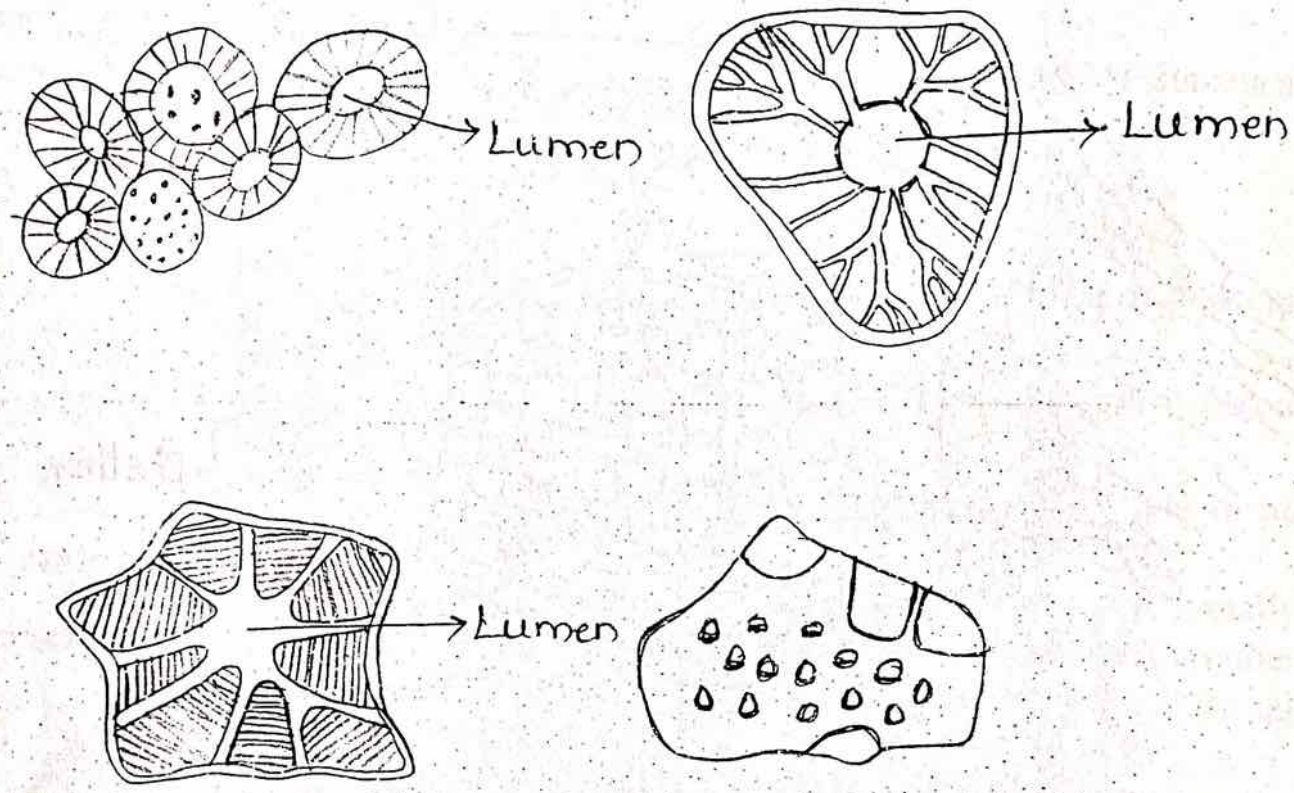
L.S. OF VESSEL
WITH TYLOSES

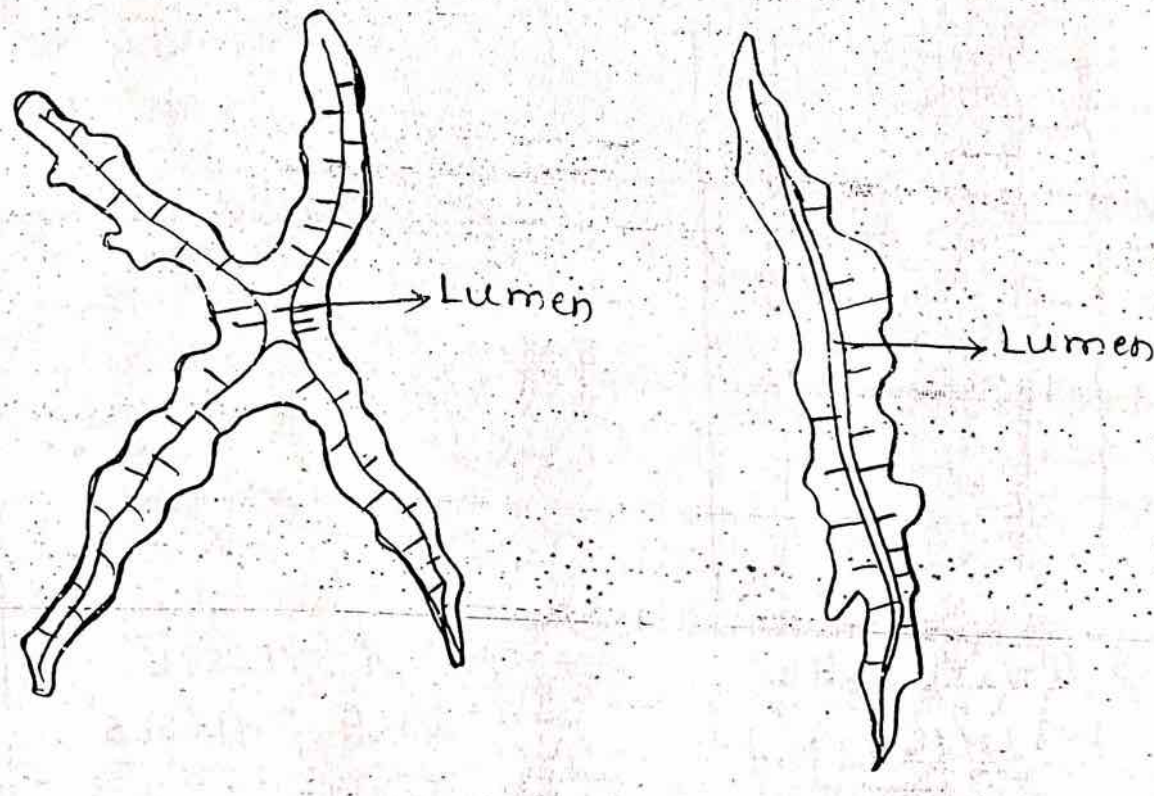


T.S. OF VESSEL
WITH TYLOSES

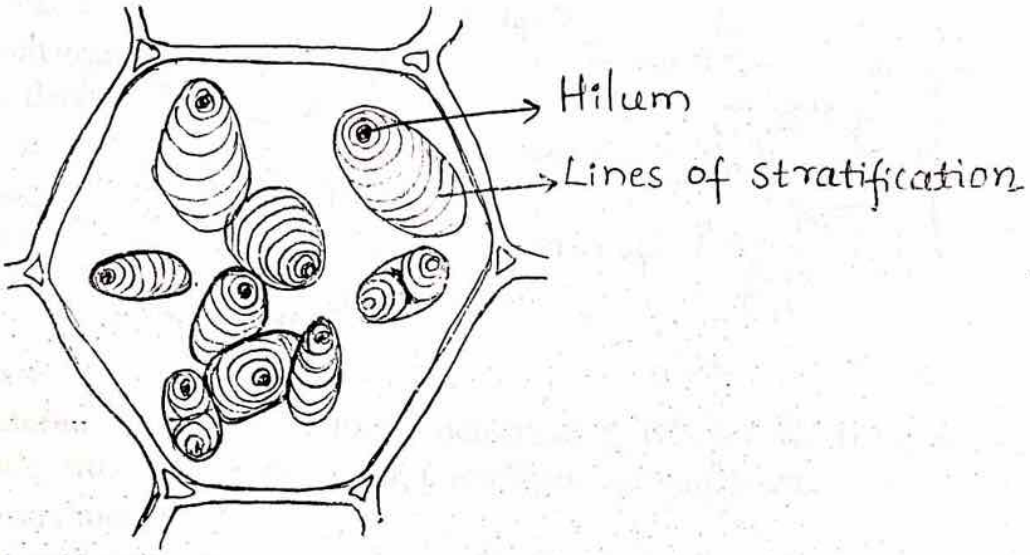
MOUNTINGS

SCLEREIDS :-

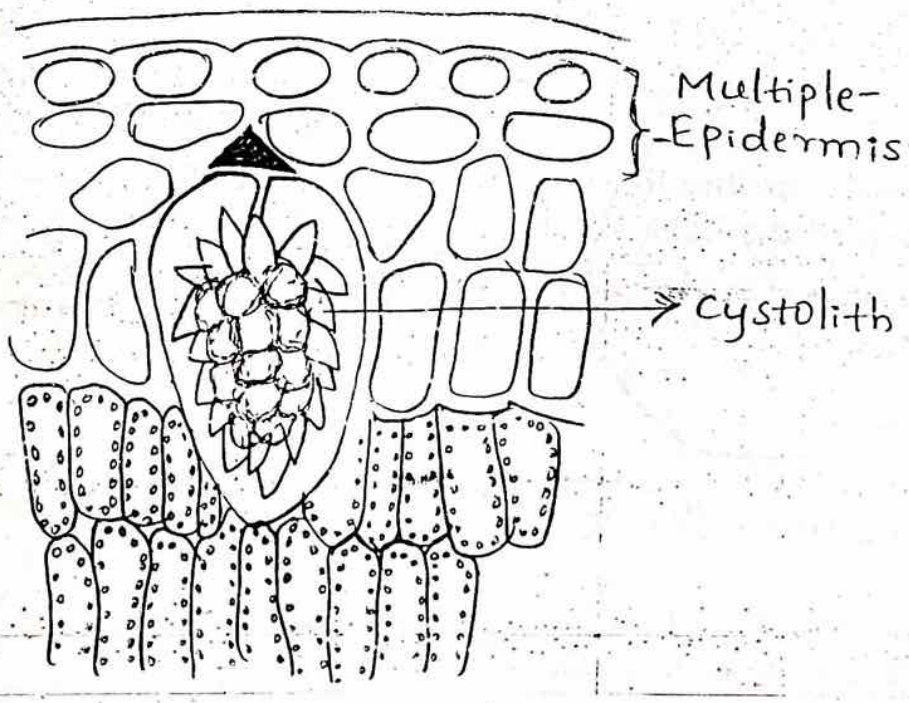




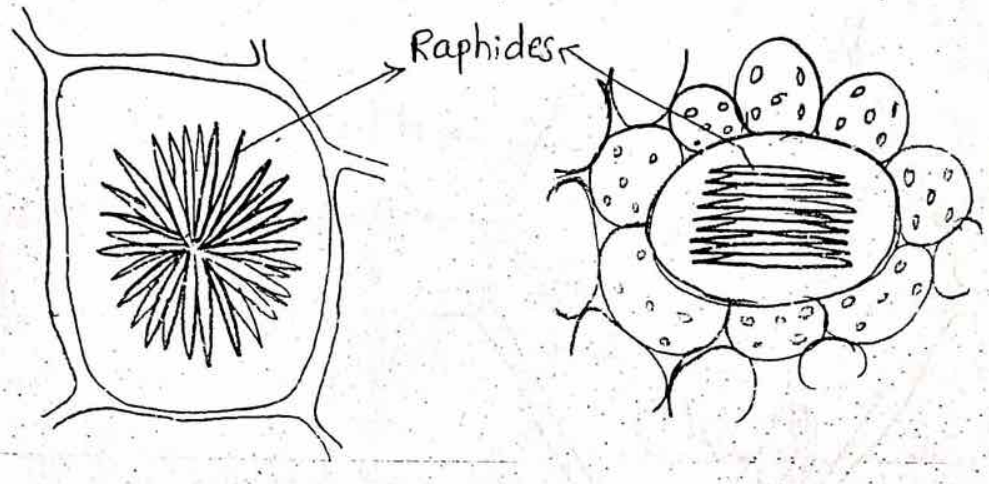
STARCH GRAINS



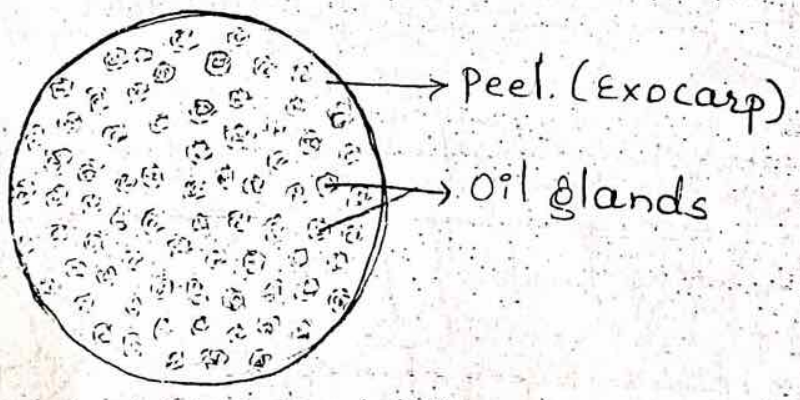
CYSTOLITHS



RAPHIDES



Oil glands

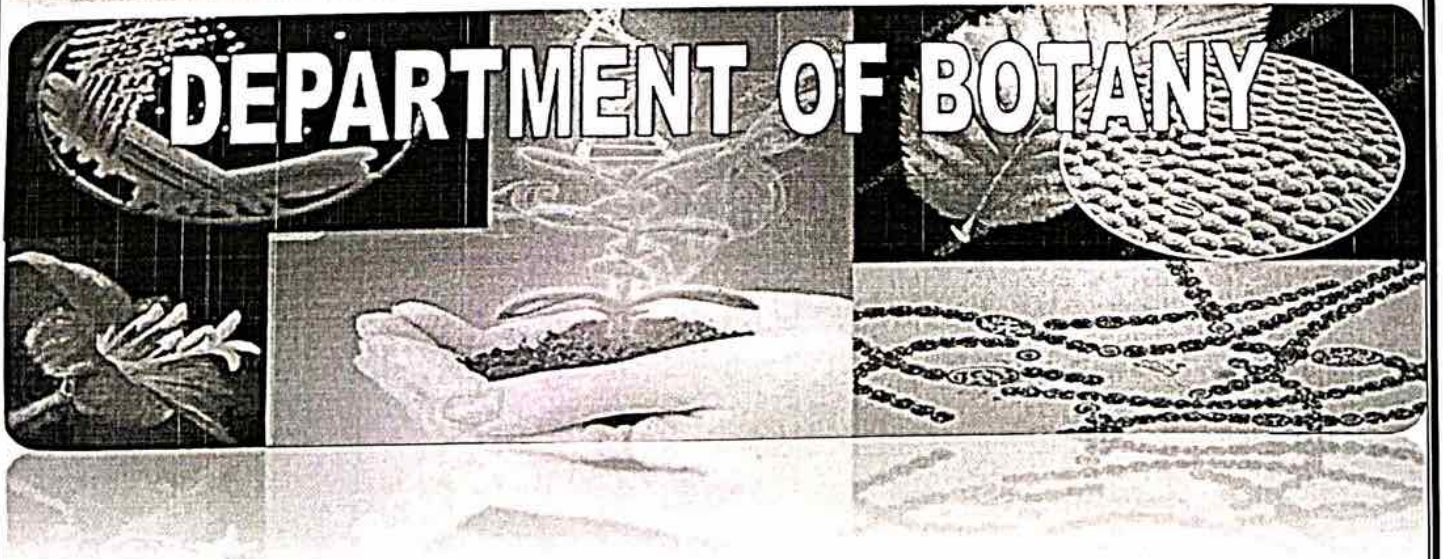


B.L.D.E.ASSOCIATION's

S.B. ARTS AND K.C.P. SCIENCE COLLEGE, VIJAYAPUR

ECOLOGY AND CONSERVATION BIOLOGY

B. SC – IV SEMESTER LABORATORY MANUAL



Practical No-01
Study of Ecological instruments – Wet and Dry thermometer, Altimeter, Hygrometer, Soil thermometer, Rain Gauge, Barometer.

Aim: To study the ecological instruments working principle and applications.

1. Wet and dry bulb thermometer:

Wet and dry bulb thermometer is an instrument for measuring air temperature and humidity. From the appearance, it consists of two identical ordinary thermometers, one of them is used for measuring air temperature, which we call dry bulb thermometer; and the other is called wet bulb thermometer.

The spherical part of wet bulb thermometer is wrapped in gauze soaked in distilled water, and the lower end of gauze is immersed in distilled water to keep it soaked.

Principle of wet and dry bulb thermometer:

The specific heat capacity of wet bulb wrapped in wet cloth is larger than that of dry bulb, and the temperature change is small. The dry bulb measures the accurate temperature, and its temperature difference is related to the relative humidity in the environment

Uses:

1. WBTs from wet-bulb thermometers are used primarily in determining the relative humidity of a volume of air or to calculate dew-point temperatures.
2. WBTs are also useful indicators in determining whether it is safe to engage in outdoor activities.
3. The evaporative cooling that occurs when air passes over the wet-bulb thermometer's cloth mimics the cooling of the human body from perspiration, so a WBT can be read as the minimum temperature at which sweating can cool the body in a particular environment, given that environment's current air temperature and humidity.

2. Altimeter

- An altimeter also called an altitude meter, it is an instrument used in the measurements of the altitude of objects above a fixed level.
- An altimeter is a device that measures altitude the distance of a point above sea level.
- Sky divers and mountaineers also use altimeter to pinpoint their location in the sky or on the ground.
- The most common types of altimeters are barometers.
- An altitude increase, air pressure decreases. (This is because the density of air is lower at higher altitude and it exerts less pressure on earth below.)
- An altimeter reading changes as elevation changes.

3. Hygrometer:

A hygrometer is a specially designed apparatus that calculates relative humidity in an open or enclosed area.

The term hygrometer is formulated from the Greek word 'Hygros' which means moist or wet.

Uses:

- A hygrometer helps to calculate moisture content in the air or enclosed areas. This is essential in manufacturing industries, hospitals, museums, agricultural fields, food preservation, meteorology, etc.
- Hygrometer are frequently used in pharmaceutical storage and manufacturing system. Most pharmaceutical products are hygroscopic; therefore variation in humidity could alter their chemical compositions.

4. Soil Thermometer:

A soil temperature thermometer specially designed to measure soil temperature.
Gardeners find these tools useful for planning plantings and also used by climate scientists, farmers, and soil scientists.
Soil temperature can provide a great deal of useful information when charted overtime.
How to use:
A soil temperature thermometer can perform a measurement in six steps.
1. Determined the proper depth to perform the measurement. If measuring for a mixed garden the depth should be at least 5-6 inches.
2. Use a screw driver to make a pilot hole. This ensures that the thermometers probe will not be damaged if forced into hard soil.
3. Insert the thermometer into the pilot hole and follow the directions supplied with thermometer.
4. Provide shade if the sun is bright, this can be achieved by simply putting your hand between the sun and the thermometer. This ensures that the reading is accurate.
5. Take a reading in the morning and take late morning, afternoon and then average out the results, if you are seeding a lawn take measurements on all four sides of your house because some areas warm quicker than others.

5. Rain guaze:

Rain gauze is an instrument used by meteorologists and Hydrologist to measure amount of rain fall at a given time.
Description of Apparatus:-The standard Rain Gauze consists of metallic cylinder fitted with wide funnel leading to graduated cylinder inside.

Method of Working:-

1. Place the rain Gauze in an open place during Rainfall.
2. When water fall into the funnel of apparatus, it get collected in the inner graduated cylinder.
3. Cylinder is marked in mm and will measure upto 250 mm.(9.8 inches) of rainfall.
4. Each horizontal line on the cylinder is 0.5 mililitre (0.02 Inches).
5. Length of 1 inch of rain measures as one inch when it collects inside.
6. Rainfall as low s 0.1 inch can be measured with this instrument. 0.01 is considered as trace.
7. The excess over flow in the large cylinder is carefully poured into another graduated cylinder and measured to give total rainfall.

Applications of Rain gauze

1. Rain gauze helps to measure the fluid amount in a defined precipitated space over time Reference.
2. Helps to know the amount of precipitation over an area.

3. Pluviometer reads the amount of rainfall of an area.
4. It also helps to critically mark drought areas.
5. Helps to study climatic adversities in time and help to prepare for an upcoming disaster.

6. Barometer:

Barometer is a device for monitoring atmospheric pressure, primarily used for weather forecasting and altitude calculations. It is used to gauge air pressure as it changes with altitude, either above or below sea level.

The working principle of a barometer depends on balancing mercury weight in the glass tube with respect to the atmospheric pressure. If the weight of the mercury is less than atmospheric pressure, the mercury level will increase. And if the weight of the mercury is more than the atmospheric pressure, the mercury level will decrease. The rising and falling of the mercury are measured using the inch scale marked on the glass tube.

Uses:

- It is used in science and industry to measure weather.
- It is used in watches and mobile devices.
- This tool can be used to measure altitude.
- There are portable instruments that use aneroid barometers.
- Altimeters for aircraft employ aneroid barometers.
- Since atmospheric pressure and elevation are intimately correlated, GPS software uses the barometer in smartphones to determine the height the device is at.

Practical No: 02

Determination of p^H of soil

Aim: To determine the p^H of different soil samples.

Principle:
p^H is a Property to known the chemical nature of soil; if the reaction is predominated by hydrogen ions it is acidic. If the reaction is not predominated by hydrogen ions, it is said to be basic. p^H of soil can be estimated by p^H meter or p^H paper or by universal indicator.

Requirements:
Soil sample, Distilled water, p^H paper, Beaker, Funnel, Glass rod, Weighing balance, Filter paper.

Procedure:
1. Take equal weight of sandy soil, Black soil, & Red soil in three separate beakers
2. Add 100 ml of distilled water and stir it for about 5-10 minutes.
3. Fix three Funnels with filter paper in three separate stands & kept empty beaker below the each funnel.
4. Pour the soil-water suspension into the funnel and filtrate is collected in the beaker.
5. Then dip the p^H paper in filtrate and air dry it. After that compare the p^H paper colour with standard index.

Observation:

p^H of the soil filtrate as follows:

Soil sample	pH value	Nature of Filtrate

Result:
1. The p^H of the given soil sample is ----------

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Affiliated to Rani Channamma University, Belagavi.

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RANI CHANNAMMA UNIVERSITY, BELAGAVI

विद्वान् सर्वत्र पूज्यते

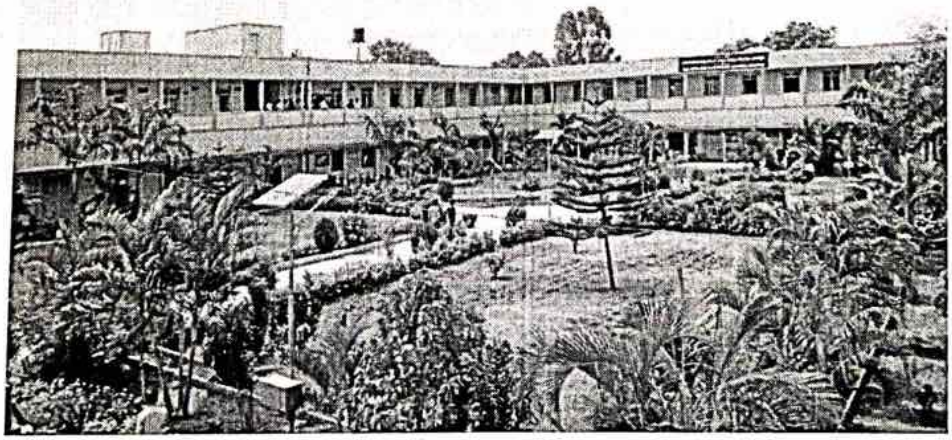
Bsc. Vth SEMESTER

LAB MANUAL

Paper-1

(Diversity of Angiosperms and their Systematics)

Dept. of Botany

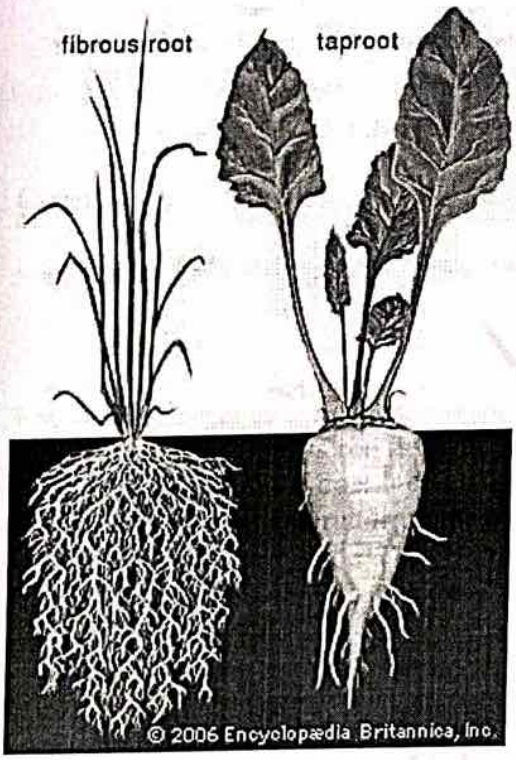


Morphologyof Root and their modification

Root, in botany, that part of a vascular plant normally underground. Its primary functions are anchorage of the plant, absorption of water and dissolved minerals and conduction of these to the stem, and storage of reserve foods. The root differs from the stem mainly by lacking leaf scars and buds, having a root cap, and having branches that originate from internal tissue rather than from buds.

Types of roots and root systems

The primary root, or radicle, is the first organ to appear when a seed germinates. It grows downward into the soil, anchoring the seedling. In gymnosperms and dicotyledons (angiosperms with two seed leaves), the radicle becomes a taproot. It grows downward, and secondary roots grow laterally from it to form a taproot system. In some plants, such as carrots and turnips, the taproot also serves as food storage.Grasses and other monocotyledons (angiosperms with a single seed leaf) have a fibrous root system, characterized by a mass of roots of about equal diameter. This network of roots does not arise as branches of the primary root but consists of many branching roots that emerge from the base of the stem.



Different kinds of roots

- Fibrous roots
- Creeping roots

- Tap-roots
- Tuberous roots
- Adventitious roots

Different kinds of roots

• The roots of a maize plant, a millet plant and a rice plant are alike.

• The roots of a mango tree, an orange tree and a lemon tree are alike.

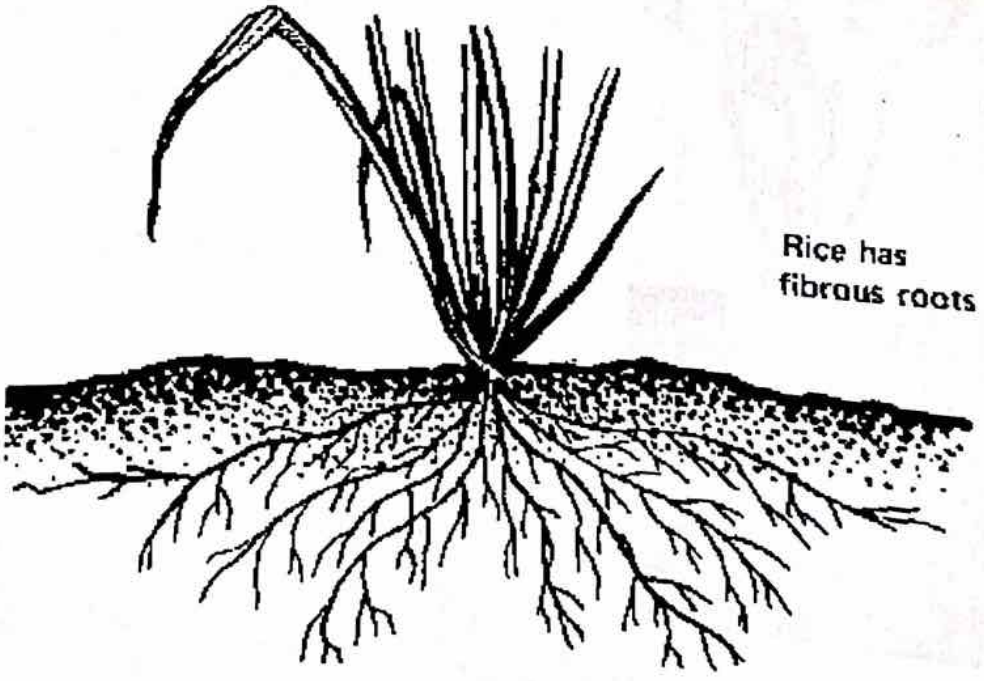
• The roots of maize, millet and rice are not like those of the mango tree, the orange tree and the lemon tree.

• Different plants have different roots.

Fibrous roots

Some plants have small, thin roots, all of the same length.

• These roots form a tuft, as for instance the roots of onion, rice, millet, maize.



Rice has fibrous roots

• A plant that has many small roots of the same length, the same thickness, the same shape, has fibrous roots.

Creeping roots

Some plants have roots that are shallow and long.

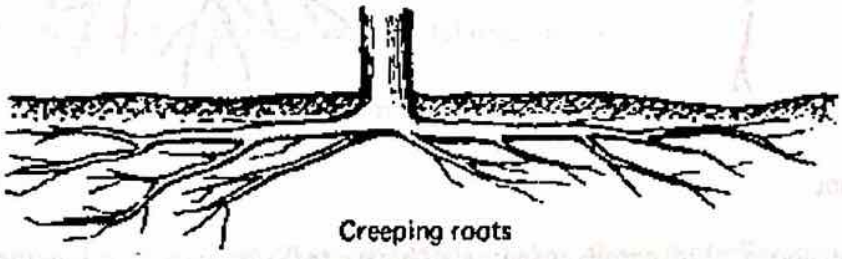
• Creeping roots do not go deep into the soil.

• These roots go a long way from the base of the plant.

They cover a large area.

They have to find in a small depth of earth the food necessary for the life of the plant.

Many trees have creeping roots.



Creeping roots

• A plant that has shallow, very long roots has creeping roots.

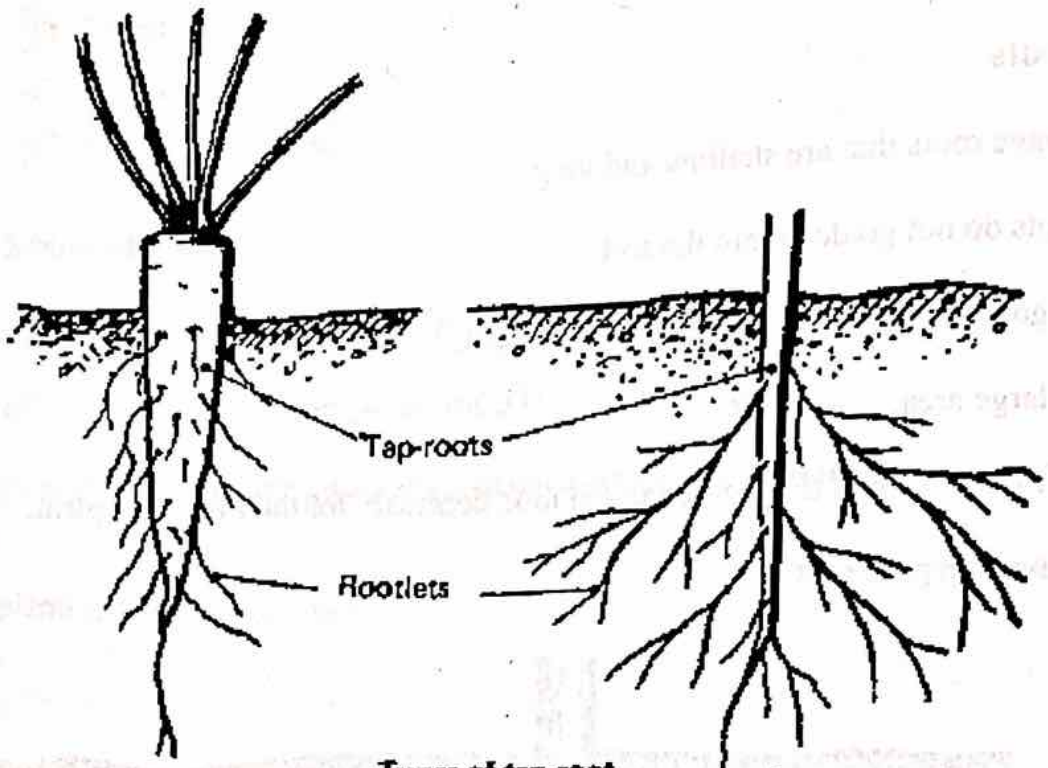
Tap-roots

Some plants have only one root, very thick, deep, straight, called a tap- root.

• Smaller roots grow on this thick root; they are called rootless.

• Tap-roots go deep into the soil.

They cannot penetrate soil that is too hard.



Types of tap-root

Types of tap root

• Cotton, coffee, cocoa, okra, carrots, papayas all have a root that goes deep into the soil, is very thick and straight.

They have a tap- root.

Tuberous roots

Some plants have very thick roots.

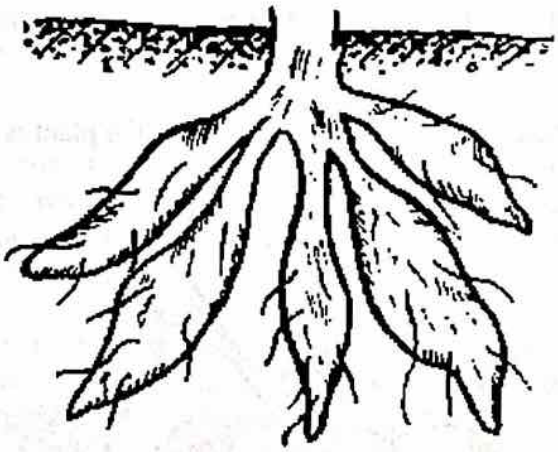
• These roots store up food.

• These roots are thick because they have taken up a lot of food from the soil.

The food is stored up in order to feed the whole plant.

The plant is said to have built up reserves.

For example, cassava.



Cassava roots

Cassava roots

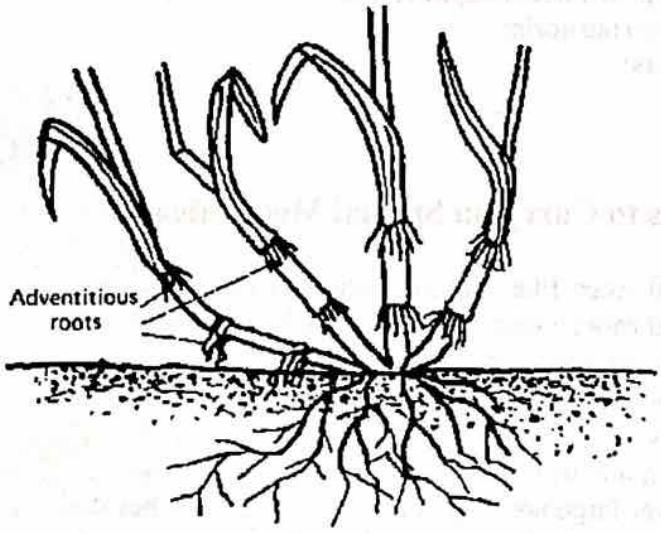
• A plant that stores up reserves in thick roots has tuberous roots.

Adventitious roots

In some plants roots start from the stem above the soil, that is, above the collar, and afterwards go down into the earth.

• Adventitious roots grow above the collar.

For example, mangrove, bamboo, maize and rice all have adventitious roots.

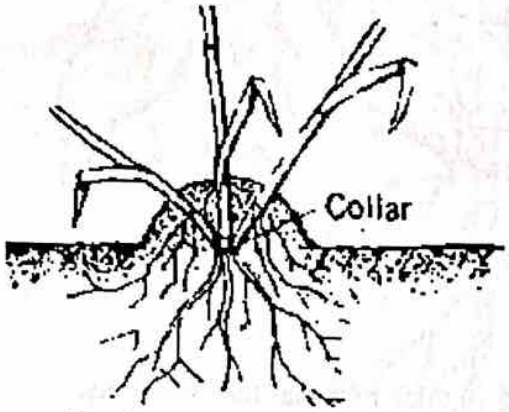


A rice plant

A rice plant

• Soil put around the collar helps adventitious roots to grow; the plant is earthed up.

• A plant with roots on the stems has adventitious roots.



Earthing up encourages adventitious roots to develop

Earthing up encourages adventitious roots to develop

Modifications of Roots for Physiological and Mechanical Functions

- Storage **Roots**: Storage **roots** become fleshy by the accumulation of stored up food. ...
- Epiphytic or Aerial Absorbing **Roots**: ...
- Assimilatory **Roots**: ...
- Reproductive **Roots**: ...
- Respiratory **Roots** or Pneumatophores: ...
- Sucking **Roots** or Haustoria: ...
- MycorrhizalRoots:

Modifications to Carry on Special Mechanical Functions:

1. Prop Roots:

- In some tropical trees like Ficusbengahtensis (banyan), the horizontal aerial branches give rise to aerial roots which are provided with root-caps and hang vertically down from the boughs like so many strings. These grow down and, on reaching the soil and becoming anchored, they begin to stouten and ultimately become almost as strong as the main trunk. They support or prop up the horizontal branches like so many pillars .
- Ultimately, the main trunk may die when the prop roots fully replace it. Long living banyan trees cover large areas by' their spreading branches supported on prop roots.
- The banyan tree at the Indian Botanical Garden, Shibpur, Calcutta is about 200 years old, covers a large area and has produced more than 900 prop roots. A similar tree in the

Theosophical Society compound Adyar, Madras and another in the Buitenzorg Botanical Garden, Java, are equally remarkable.

2. Stilt Roots:

- Certain shrubs and small trees like screw-pine (Pandanusfoetidus) grow on the edges of tanks, marshes, etc., where the anchorage is not very strong. In these cases, short roots grow obliquely downwards from near the base of the stem and act like stilts providing additional support as well as anchorage to the stem.

- The adventitious roots growing from the lower nodes of maize plants act in a similar way. Such stilt roots are also seen in mangrove plants like Rhizophora..

FIG. 233 FIG. 234 FIG. 235

FIG. 233. Prop root of banyan. FIG. 234. Stilt root of *Pandanus*. FIG. 235. Stilt root of maize.

3. Root Buttresses:

In some large trees, instead of stilt roots, there are great plank-like roots radiating from the base of the tree like wings . These are called buttresses and are actually partly root and partly stem. They may be seen in old trees of Bombaxceiba, Terminaliacatappa, Ficus sp., etc.

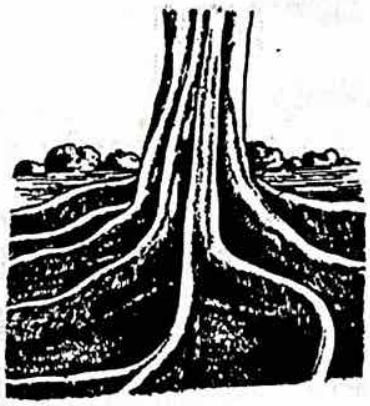


FIG. 236. Root buttress.

4. Climbing Roots:

Annonaceae: Characters, Distribution and Types

Characters of Annonaceae:

Wood aromatic, leaves exstipulate, floral parts usually numerous, free spirally arranged; stamens with distinctive enlarged and flat connective; gynoecium multipistilate, apocarpous.

A. Vegetative characters:

Habit and habitat:

Trees, shrubs or lianas.Artabotrys climbs by means of hooks. Oil ducts present in the bark, leaves and perianth leaves. Terrestrial and perennial. Evergreen, deciduous, cultivated as well as wild.

Root:

Tap, deep and extensively branched.

Stem:

Erect, branched, solid, woody, sometimes woody climbers. Leaves – Simple, entire, alternate, exstipulate, distichous, gland dotted.

B. Floral characters:

Inflorescence:

Often solitary, axillary, sometimes cauliflourous in groups.

Flower:

Actinomorphic but zygomorphic in Monodora due to difference in size of petals, hermaphrodite, unisexual in Stelechocarpus, complete, trimerous, hypogynous, perigynous (Eupomatia) spirocyclic, often aromatic.

Calyx:

Sepals 3, sepaloid, polysepalous, connate at the base, valvate.

Corolla:

Petals 6 in two whorls of 3 each, valvate or slightly imbricate.Sometimes no distinction into sepals and petals so perianth in 3 or more whorls of 3 each.

Androecium:

Stamens numerous spirally arranged on the axis which forms a large convex receptacle, filament short and thick, anthers long, extrorse, truncate connective, bithecous.

Gynoecium:

Carpels numerous or a few, usually free, spirally arranged on the raised receptacle, apocarpous, superior, unicarpellary, unilocular; ovules one to many, anatropous; style short or none, stigma small, Monodora (Africa) with syncarpous ovary and parietal placentation.

Fruit:

An aggregate of berries, united to form a single compound fruit (Annonasquamosa).

Seed:

Large, numerous, often embedded in a copious, white fleshy pulp, endospermic.

Pollination:

Entomophilous, due to gaudy and scented flowers.

Floral formula:

$\oplus \, ♀ \, K_3 \, C_{3+3} \, A_{\infty} \, \underline{G_{\infty}}$ or (∞)

Distribution of Annonaceae:

The family Annonaceae is commonly called Custard-apple family. Rendle included 62 genera and 820 species in this family. Lawrence recognised 80 genera and 850 species. Takhtajan (1966) included 120 genera and 2,100 species in this family. The family is widely distributed in the tropical regions of the world. Some genera are also found in the temperate climates. In India it is represented by 129 species.

Economic Importance of Annonaceae:

1. Food:

The fleshy fruits of various Annona specifics are juicy and edible, and also used in preparation of soft drinks and jellies. Recent analysis shows that they contain about 18 per cent sugar.

Edible fruits are also obtained from various species of Annona and Asimina.

2. Timber:

Bocageavirgata, B. laurifolia, Cyathocalyxzeylanicus, Duguetiaquitarensis, Oxandralanceolata and Eupomatialaurina yield useful timber.

3. Oil:

The flowers of Desmoschinensis furnish 'Macassar oil' a perfume. The perfume is also obtained from Mkiluafragrans and specially liked by Arab women.

4. Fibre:

The bark of Goniothalamuswightii produces strong fibres.

5. Ornamental:

Artabotrysodoratissimus and Annona discolor are grown in garden for their scented flowers. Desmeschinensis is an ornamental tree.

Common plants of the family:

1. Annonasquamosa L. – (H. Sarifa or sitafal) custard apple or sugar apple – well known fruit tree.

2. Annonareticulata L. – Bastard apple or Bullock's heart – a fruit tree.

3. Artabotrysuncinatas – A small climbing shrub with hooked peduncles – and glabarous leaves.

4. Cyathocalyxzeylanicus (H. HariChampa) – A tall tree with deflexed or horizontal branches.

5. Polyalthialongifolia – The. "Ashok" An ornamental tree.

6. Uvariacordata (Dunal) – Alston is a climber noted for its cordate leaves and red flowers.

Important Types of Annonaceae:

1. Annonasquamosa (H. sarifa or Sitafal, Fig. 26.1):

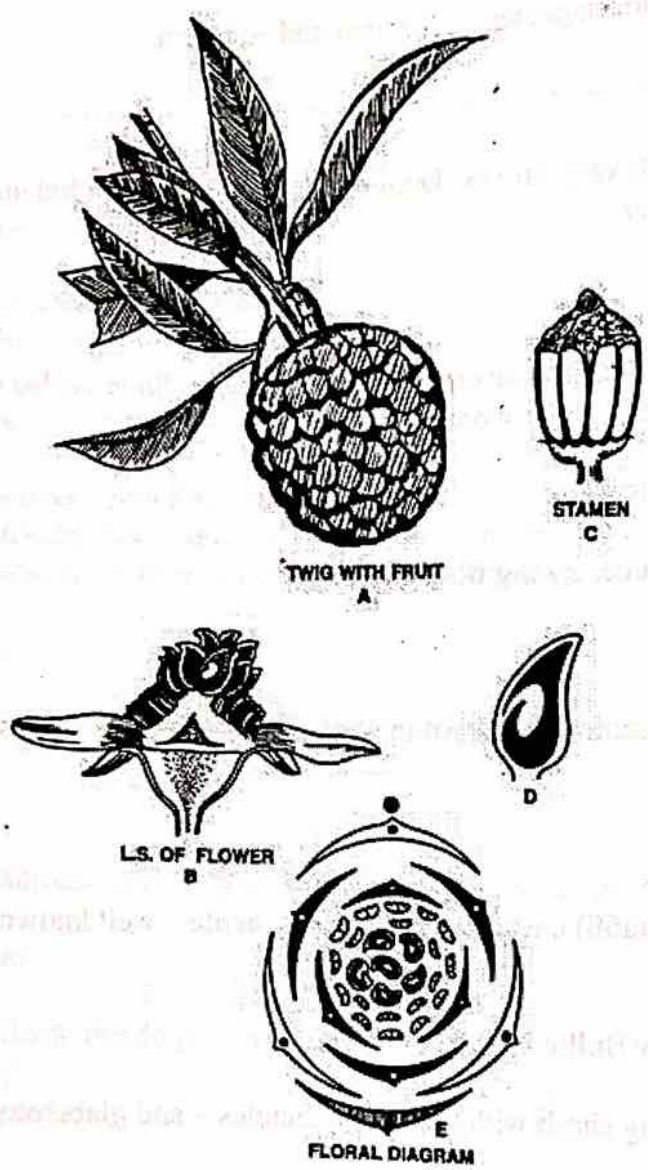


Fig. 26.1. *Annona squamosa*. A–Twig with fruit; B–L.S. of flower; C–Stamen; D–Carpel; E–Floral diagram.

Habit:
A small tree.
Root:
Tap, branched.
Stem:
Aerial, woody, branched.

Leaf:

Simple, alternate, exstipulate, entire, oblanceolate, obtuse.

Inflorescence:

Axillary, two or more flowers arise in the axil of each leaf.

Flower:

Bracteate, hermaphrodite, complete, actinomorphic, spirocyclic, hypogynous.

Calyx:

Sepals three, polysepalous, united at the base, valvate.

Corolla:

Petals three, polypetalous, fleshy, pale-yellow, valvate.

Androecium:

Stamens numerous, spirally arranged on a conical receptacle, filaments short, anthets oblong, adnated, and appendaged.

Gynoecium:

Multicarpellary, apocarpous, spirally arranged, superior, each unilocular, ovule one, basal placentation, style short, stigma long, tapering, papillose.

Fruit:

An etario of berries.

Floral formula:

$Br \oplus \varnothing K_3 C_{3+3} A_{\infty} G_{\infty}$

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LAB MANUAL

B.SC V SEMESTER (NEP)

PAPER-2: GENTICS & PLANT BREEDING

EXERCISE 1

Aim: Preparation and study of mitosis in onion root tips through Squash preparation.

Principle: Somatic growth in plants and animals takes place by the increase in the number of cells. A cell divides mitotically to form two daughter cells wherein the number of chromosomes remains the same (i.e., unchanged) as in the mother cell. In plants, such divisions rapidly take place in meristematic tissues of root and shoot apices, where the stages of mitosis can be easily observed.

Requirement: Onion bulbs, wide mouth glass/jar/bottle, glacial acetic acid, ethanol 2-4% acetocarmine/acetoorcein stain, N/10 HCl, spirit lamp, slide, cover slips, blotting paper, DPX / molten wax /nail polish and compound microscope.

Procedure

Growing of root tips

- Select a few medium-sized onion bulbs. Carefully remove the dry roots present.
- Grow root tips by placing the bulbs on glass tubes (of about 3–4 cm. diameter) filled with water.
- Care should be taken so that the stem portion of the bulb (basal part) just touches the water.
- A few drops of water may be added periodically to compensate evaporation losses.
- New roots may take 3–6 days to grow. Cut 2–3 cm long freshly grown roots and transfer them to freshly prepared fixative, i.e., aceto-alcohol (1:3:: glacial acetic acid : ethanol).
- Keep the root tips in the fixative for 24 hours and then transfer them to 70% ethanol (for preservation and use in future).
- Onion root-tip cells have a cell cycle of approximately 24-hour duration, i.e., they divide once in 24 hours, and this division usually takes place about two hours after sunrise.
- Therefore, roots grown on water should be cut only at that time to score maximum number of dividing cells.

Preparation of slide

- Take one or two preserved roots, wash them in water on a clean and greasefree slide.
- Take a watch glass place the roots in it, Add 1 drop of N/10 HCl followed by 2–3 drops of aceto-carmine or aceto-orcein stain
- Warm the watch glass on spirit lamp until getting slight fumes. Care should be taken that the stain is not dried up nor boil it.
- Transfer the root on to the new clean glass slide. Now cut the comparatively more stained (2–3 mm) tip portion of the root and retain it on the slide and discard the remaining portion. Finely chop the tip of root with sharp blade (care should be taken not to dry out the tip)
- Put one or two drops of aceto-carmine, mount a cover slip on it avoiding air bubbles.
- Place the slide on strip of blotting paper to wrap it, hold folds of blotting paper using the fingers in such a way that the cover slip mounted on the slide is properly held.
- Now slowly tap the cover slip using the blunt end of a pencil so that the meristematic tissue of the root tip below the cover slip is properly squashed and spread as a thin layer of cells.
- Take off the strip of blotting paper without disturbing the blotting paper.
- Carefully seal the margins of the cover slip using DPX or molten paraffin wax or nail polish.
- This preparation of onion root tips cells is now ready for the study of mitosis.

Study of slide

Place the slide on the stage of a good quality compound microscope. First observe it under the lower magnification (10 X objective) to search for the area having a few dividing cells. Examine the dividing cells under 40X or higher magnification of the microscope to observe the detailed features of mitosis.

Observation

The stages of mitosis can be broadly categorised into two parts: karyokinesis (division of nucleus) followed by cytokinesis (division of cytoplasm, and ultimately of the cell).

Those cells, which are not in the phases of cell division are considered to be in interphase. You may observe that most of the cells in a microscope field are in interphase

Interphase

The cells are mostly rectangular, oval or even circular in shape, with almost centrally situated densely stained nucleus. The chromatic (coloured) material of the nucleus is homogeneous and looks granular. The boundary of the nucleus is distinct. One or few nucleoli (sing: nucleolus) can also be observed inside the nucleus

Stages of Mitosis

(a) Prophase

- Intact nuclear outline is seen. The chromatin (seen as a homogeneous material in the nucleus at interphase) appears as a network of fine threads (chromosomes). Nucleoli may or may not be visible.
- If the cell under observation is in the early stage of prophase then the chromatin fibres (chromosomes) are very thin. However, in the cells at late prophase, comparatively thicker chromatin fibres would be visible. Besides this, in the late prophase the nuclear membrane may not be noticed.
- The process of mitosis is initiated at this stage wherein coiling and thickening of the chromosomes occurs
- Shrinking and hence the disappearance of the nucleolus and nuclear membrane takes place
- The stage reaches its final state when a cluster of fibres organizes to form the spindle fibres

(b) Metaphase

- The nuclear membrane disappears. Chromosomes are thick and are seen arranged at the equatorial plane of the cell which forms metaphase plate.
- Each chromosome at this stage has two chromatids joined together at the centromere, which can be seen by changing the resolution of the microscope. Nucleolus is not observed during metaphase.
- Chromosomes turn thick in this phase. The two chromatids from each of the chromosomes appear distinct
- Each of the chromosomes is attached to the spindle fibres located on its centromere.

(c) Anaphase

- This stage shows the separation of the chromatids of each chromosome. The chromatids separate due to the splitting of the centromere.
- Each chromatid now represents a separate chromosome as it has its own centromere.
- Each of the chromatid pair detaches from the centromere and approaches the other end of the cell through the spindle fibre
- The chromosomes are found as if they have moved towards the two poles of the cell.
- The chromosomes at this stage may look like the shape of alphabets 'V', 'J' or 'I' depending upon the position of centromere in them.
- Different anaphase cells show different stages of movement of chromosomes to opposite poles, and they are designated to represent early, mid and late anaphase.

(d) Telophase

- Chromatids have reached the other end of the cell, The spindle fibres disappears.
- Chromatids reach the opposite poles, lose their individuality, and look like a mass of chromatin.
- Chromatin fibres are formed as a result of uncoiling of daughter chromosomes.
- The appearance of two daughter nuclei at the opposing ends due to the reformation of the nucleolus and nuclear membrane.
- At this phase, splitting of the cell or cytokinesis may also occur.
- Cytokinesis In plants, a cell plate is formed in the middle after telophase.
- The plate can be seen to extend outwards to ultimately reach the margin of the cell and divide the cell into two. Such cell plates are characteristic of plant cells.

EXERCISE 2

Aim: Preparation and study of meiosis in *Tradescantia*

Principle: Meiosis is a type of cell division in which the number of chromosomes is halved (from diploid to haploid) in the daughter cells, i.e., the gametes. The division is completed in two phases, meiosis I and meiosis II. Meiosis I is a reductional division in which the chromosomes of homologous pairs separate from each other. Meiosis II is equational division resulting in the formation of four daughter cells. Stages of meiosis can be observed in a cytological preparation of the cells of testis tubules or in the pollen mother cells of the anthers of flower buds.

Tradescantia is an ideal plant for studying meiosis, because a single flowering stem contains a lineup of buds, from the smallest at the bottom to the largest at the top. These largest have already gone through meiosis and produced pollen. Young anthers in buds about six down from the top usually contain cells undergoing meiosis to yield microspores.

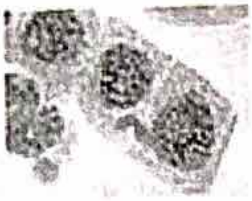
Requirement: *Tradescantia* flowers, 2-4% acetocarmine/acetoorcein stain, N/10 HCl, spirit lamp, slide, cover slips, blotting paper, DPX / molten wax / nail polish and compound microscope.

Fixation

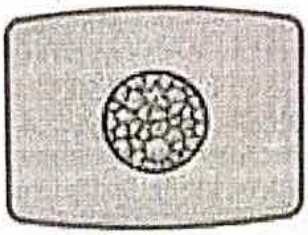
Flowers buds are fixed in canyons fluid (1 part acetic acid: 6 parts ethyl alcohol). The amount of fixatives must always be at least 10 times more than the volume of the plant tissue. After 48 hours, the material is transferred and preserved in 70% ethyl alcohol.

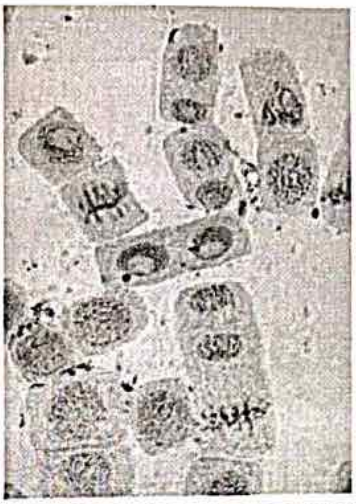
Procedure

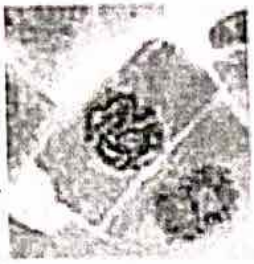
- Isolating Anther Cells that are Undergoing Meiosis
- Each flowering stem of Tradescantia is terminated by a group of flowers, below which are two leaf-like bracts. Cut a group of flowers, put it into a beaker of water.
- Remove the bract from one side.
- Count back six buds from the open flower; choose buds three through six. Push aside the petals and sepals to reveal the stamens.
- Carefully remove the six stamens and put them in a small drop of Farmer's fixative on your microscope slide. (The fixative kills everything in the stamens all at once, so that the chromosomes will be in excellent form for observation.)
- Add a small drop of aceto-carmine stain to the anther contents. Tap and poke at the stamens with blade just as you did the root tips, with the idea of opening up the stamens so that the cells undergoing meiosis will spill out onto your slide.
- Remove all the big chunks once you're sure that all the anthers are punctured.
- Gently warm the slide over spirit lamp.
- Carefully cover your preparation with a coverslip. You can reduce the number of bubbles by placing one end of the coverslip in the stain, and letting it fall over the preparation.
- Look at your preparation under the medium power of your compound microscope.
- Decide which stages of pollen development you have in you flower from the drawing of pollen development.
- You may well have more than one stage within a given flower, so be sure to scan the slide carefully.
- If you see good mitosis or meiosis, you may want to do a squash to spread the chromosomes out.
- To do a squash, fold your slide between layers of blotting paper, place it on a lab bench, and press directly down on the middle of your slide with the bottom of your thumb or tap with blunt end of pencil.
- Dont move your thumb from side to side or you will smudge your preparation.
- Place the glass slide under a microscope and observe the various stages of the meiotic cell cycle. If the cells are of desired stage, the cover glass is sealed with DPX/paraffin wax.



a. Interphase

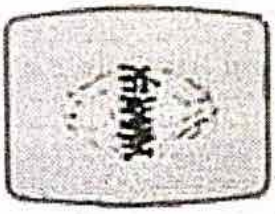




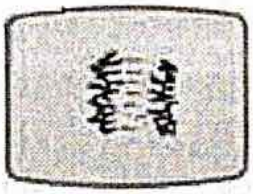


b. Prophase

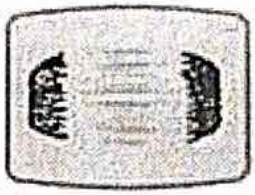
c. Metaphase

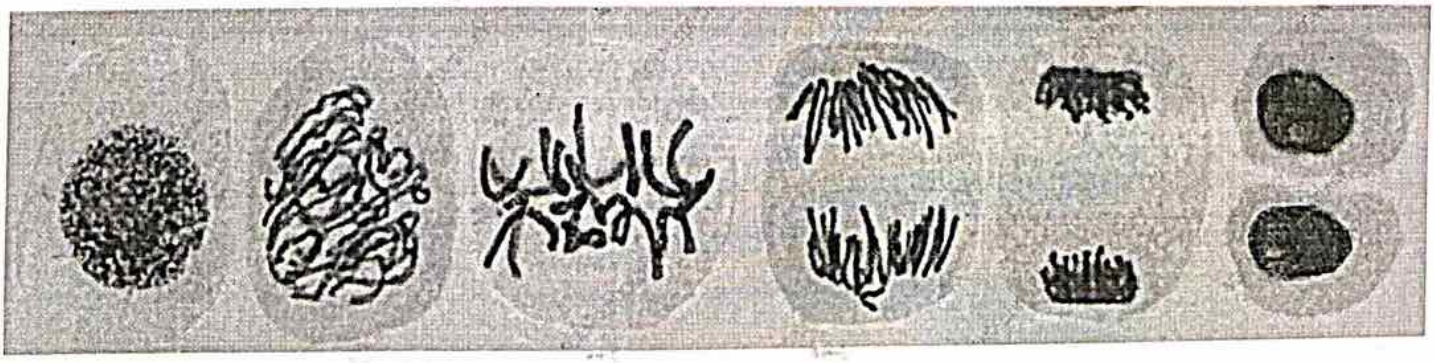


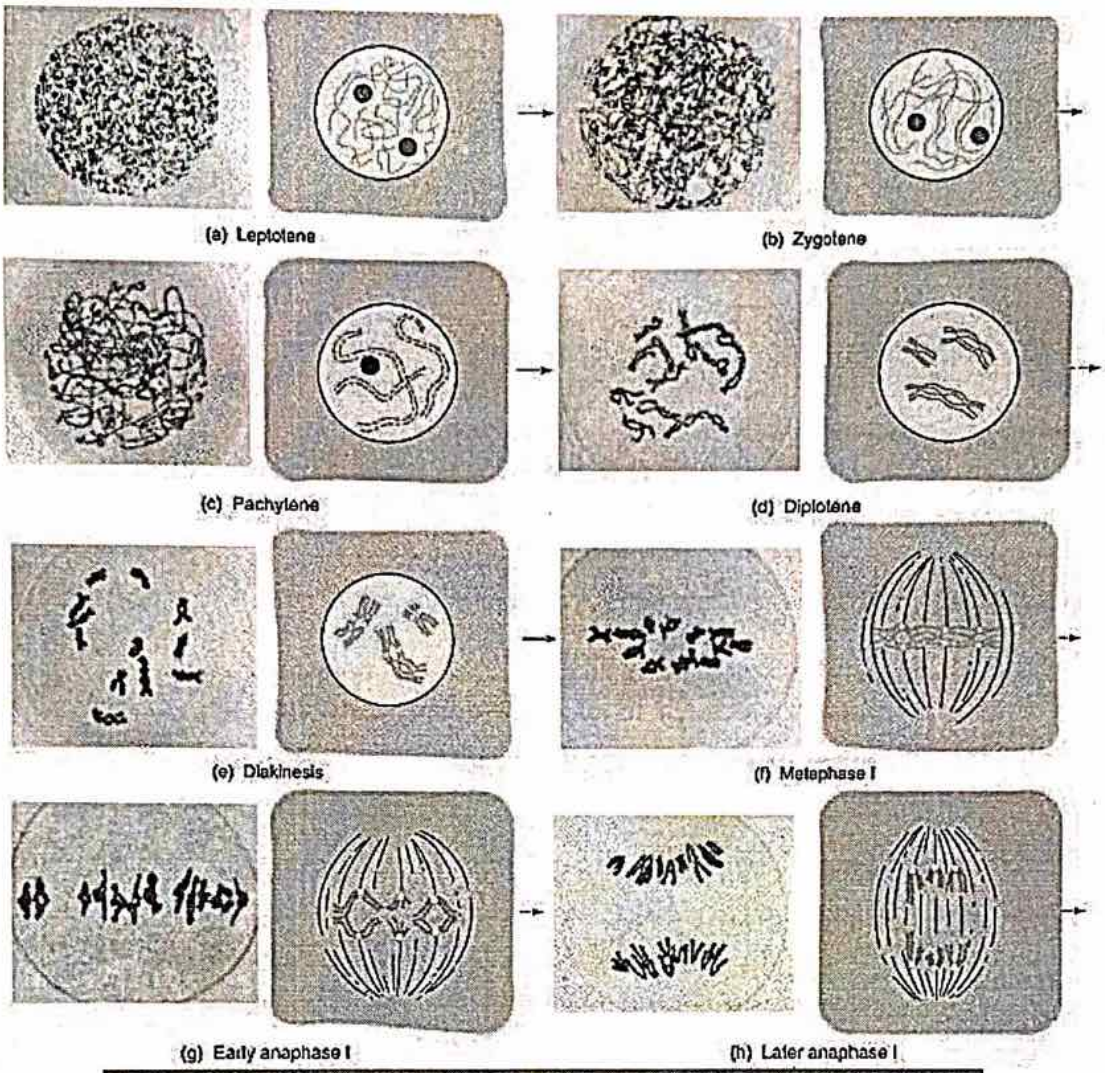
d. Anaphase

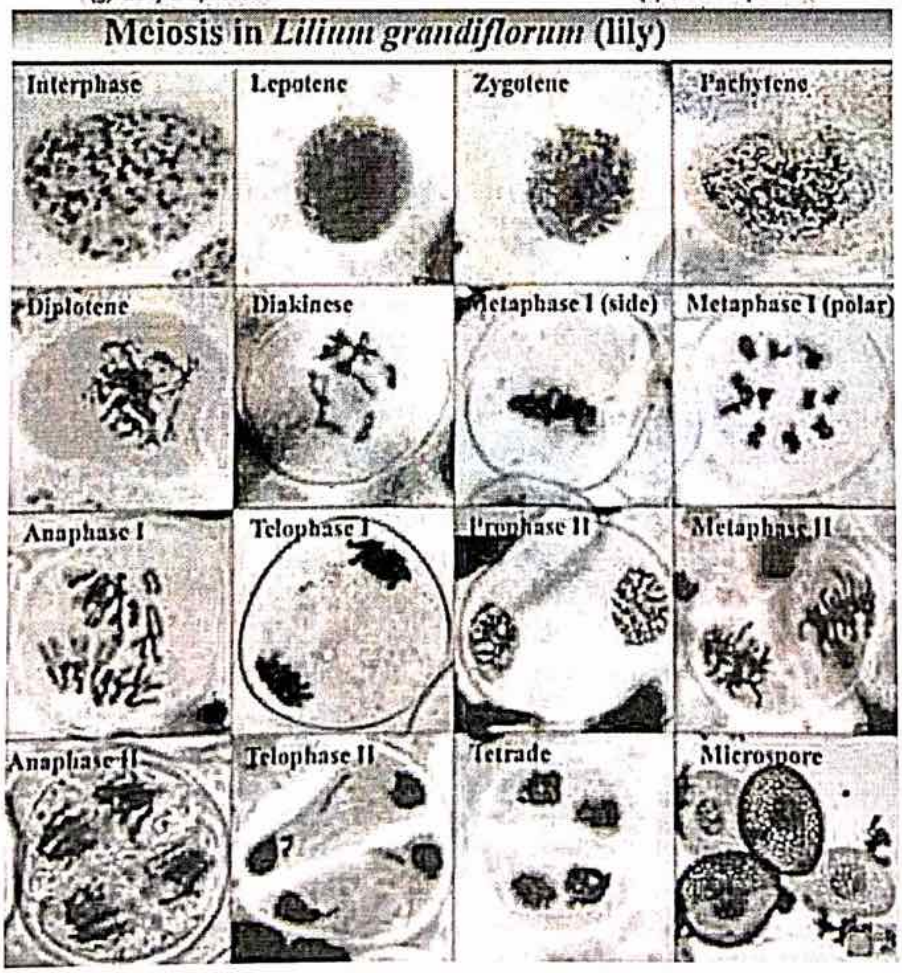


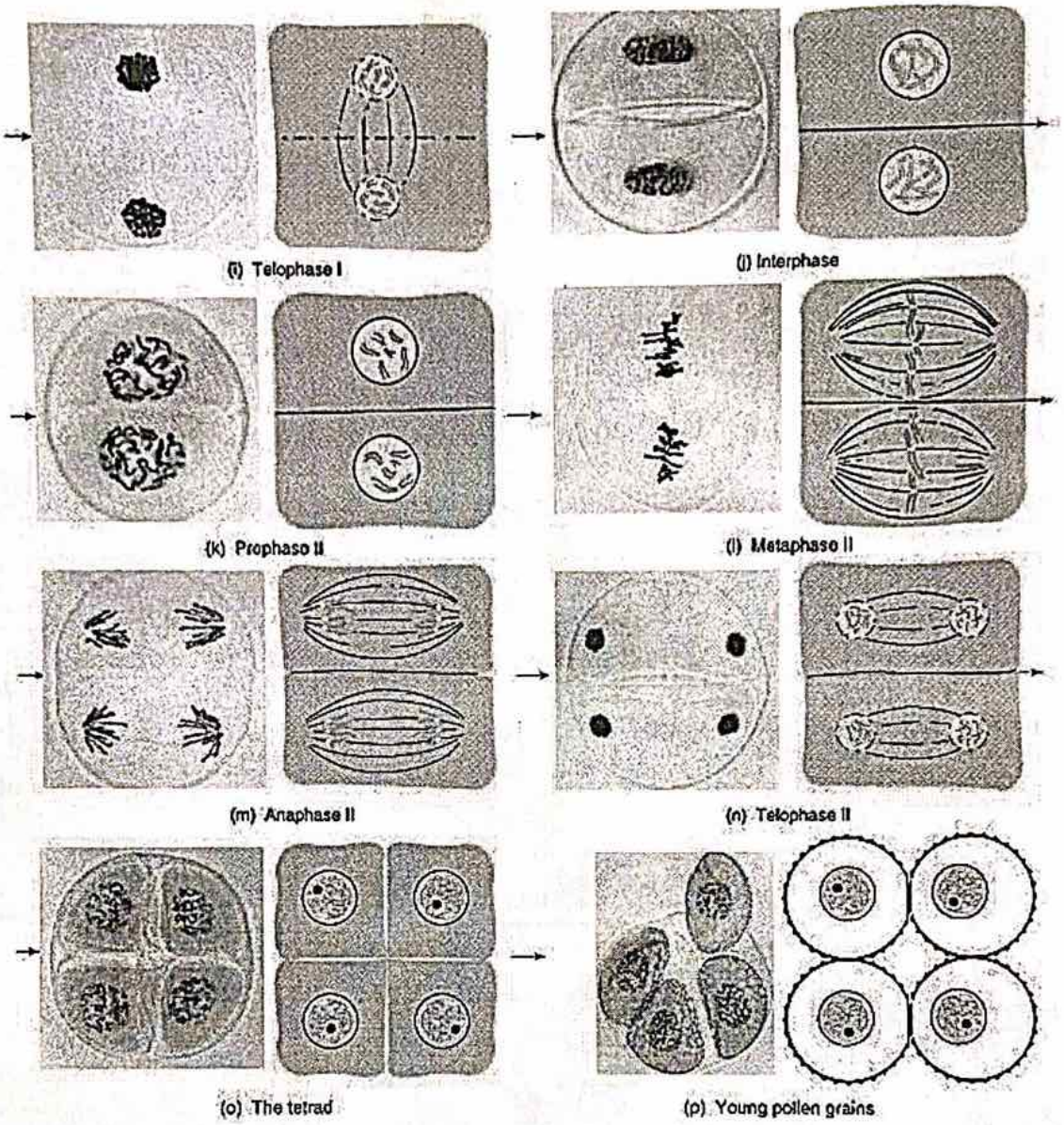
e. Telophase






(a) Leptotene
(b) Zygotene
(c) Pachytene
(d) Diplotene
(e) Diakinesis
(f) Metaphase I
(g) Early anaphase I
(h) Later anaphase I


Meiosis in *Lilium grandiflorum* (lily)
Interphase
Lepotene
Zygotene
Pachytene
Diplotene
Diakinese
Metaphase I (side)
Metaphase I (polar)
Anaphase I
Telophase I
Prophase II
Metaphase II
Anaphase II
Telophase II
Tetrade
Microspore


(i) Telophase I
(j) Interphase
(k) Prophase II
(l) Metaphase II
(m) Anaphase II
(n) Telophase II
(o) The tetrad
(p) Young pollen grains

Prophase-I

- During prophase-I, DNA is exchanged between homologous chromosomes in a process called homologous recombination. T his often results in chromosomal crossover.
- The paired and replicated chromosomes are called bivalents or tetrads.
- The process of pairing the homologous chromosomes are called Synapsis.
- At this stage, non-sister chromatids may cross-over at point called Chiasmata.

Leptotene

- The first stage of prophase-I is the Leptotene stage.
- Leptotene (Greek; Leptonema- thin threads)
- During this stage, individual chromosomes begin to condense into long strands within the nucleus.
- However, the two sister chrochromatids are still so tightly bound that they are indistinguishable from one another.

Zygotene

- Zygotene (Greek; zygonema- paired threads)
- Zygotene, occurs as the chromosomes approximately line up with each other into homologous chromosomes.
- The combined homologous chromosomes are said to be bivalent.

Pachytene

- In pachynema, the homologous chromosomes become much more closely associated. This process is known as Synapsis.
- The synapsed homologous pair of chromosomes is called a tetrad, because it consists of four chromatids.
- It can't be observed until the next stage, but the synapsed chromosomes may undergo crossing over in pachynema where exchange of geetic material between chromatid arms of homologous chromosomes.
- The chromosomes continue to condense.

Diplotene

- Diplotene (Greek; diplonema- two threads)
- In this stage, crossing over completes.
- The homologous chromosomes separete from one another little.
- Nuclear membrane and nucleolus begins to disappear.
- The homologous chromosomes show distinct separation from each other except at few regions where attachments are seen.
- These are chiasmata (sing. chiasma) representing the site of exchange of the parts between two homologous chromosomes (i.e. crossing over).

Diakinesis

- Chromosomes condense further during the diakinesis stage.
- In this stage, the homologous chromosomes separate further, and the chiasma terminalize due to contraction of the tetrad. Chiasmata can be still observed.
- The homologous pair of chromosomes appear more shortened, thick and prominent.
- All the homologous pairs appear scattered in the cell.
- Spindle fibre begin to form

Metaphase-I

- Metaphase-I is the second phase of meiosis.
- The spindle fibres organized between two poles and get attached to the centromere of chromosomes.
- Homologous chromosomes are still in pairs, and are arranged along the equatorial plane of the cell.
- At this stage, the number of bivalents can be counted. Chiasmata may still be seen in a few bivalents.

Anaphase-I

- The chromosome pairs appear to have moved towards the two opposite poles of the cell.
- This stage can be identified by the presence of two chromatids in each chromosome.
- In this stage, the sister chromatids remain attached at their centromeres and move together toward the poles.
- At the later stage, the anaphase - I may show the assembly of chromosomes at two poles. This results into the reduction of number of chromosomes to half.

Telophase-I

- The homologous chromosome pairs reach the poles of the cell .
- The homologous chromosome pairs complete their migration to the two poles.
- The chromosomes present at the two poles appear decondensed and form two distinct nuclei
- A nuclear envelope reforms around each chromosome set, the spindle disappears, and cytokinesis follows.

Prophase-II

- Meiosis-II begins without any further replication of the chromosomes. The nuclear envelope breaks down and the spindle apparatus forms.
- The dyads chromosome becomes thicker and shorter.
- Nuclear membrane and nuceolus disappear.
- Spindle fibre starts to form.

Metaphase-II

- The chromosomes having two chromatids attached at the centromere are observed arranged at the equatorial plane of the cell.
- Centromeres are arranged in aline called equatorial plate of invisible spindle apparatus.
- Spindle fibres organize between poles and attaches to centromere of chromosome.
- Each chromosome of metaphase II has two chromatid whereas in metaphase I these are paired homologous chromosomes each having two chromatids thus forming tetrad.
- In the metaphase I of meiosis, a few chiasmata are observed, where as no chiasmata are observed during metaphase II.

Anaphase-II

- Centromere of each chromosome divides and sister chromatids separates to form two daughter chromosome.
- Spindle fibre contracts and pull the daughter chromosome apart towards opposite pole.
- The two chromatids of each chromosome after separation appear to lie at the two poles of the cell.
- Anaphase II can also be distinguished from the anaphase I of meiotic division on the basis of chromatids: In anaphase I, each chromosome has two distinct chromatids, but in anaphase II, each chromosome is represented by one chromatid only.

Telophase-II

- Nuclear envelope forms around each set of chromosomes.
- Nucleolus appears in each nucleus.
- Chromosome elongates to form thin networks of chromatin.
- Nuclear membrane and nucleolus reappears.

EXERCISE - 1: HYBRIDIZATION TECHNIQUES - EMASCULATION, BAGGING

Aim: : To perform emasculation, bagging and tagging for controlled pollination.

Principle: Controlled pollination is a process of plant hybridization generally used in horticulture and **genetic** experiments. Traditional **plant breeding** programmes require the involvement of the human hand to promote the **reproductive process** in **plants**. This can be achieved by controlled **pollination**. In this process, the flowers after emasculation are wrapped in a transparent bag to safeguard them from undesired **pollen grains**. When these flowers are ready, they are brushed or dusted with the selected pollen to achieve the desired characteristics. Emasculation is a major step in hybridisation of bisexual plants as it prevents pollination by self undesired pollen grains and provides complete control over selecting desirable plant characters required for crop improvement.

Requirements: : Ornamental plants/ wild plants bearing large bisexual flower, magnifying lens, tweezers, small sharp scissors, brush, alcohol, rubber bands, paper bags, paper clips and tags.

Procedure:

Hybridization technique involves many steps such as (i) Selection of parents. (ii) Selfing of parents or artificial self-pollination. (iii) Emasculation. (iv) Bagging (v) Tagging (vi) Crossing (vii) Harvesting and storing the F, seeds (viii) Raising the F_1 generation.

1. Selection: The selection of parents depends upon the aims and objectives of breeding. Parental plants must be selected from the local areas and are supposed to be the best suited to the existing conditions.

2. Selfing: Selfing of parents or artificial self-pollination is essential for inducing homozygosity for eliminating the undesirable characters and obtaining inbreeds.

3. Emasculation: Removal of stamens or anthers or killing pollen of a flower without disturbing the female reproductive organ is known as emasculation. In bisexual flowers, emasculation is essential to prevent of self-pollination. In monoecious plants, male flowers are removed. (castor,coconut) or male inflorescence is removed (maize). In species with large flowers e.g. (bhindi, cotton, pulses) hand emasculation is accurate and it is adequate.

There are different ways to achieve emasculation

a. **Hand Emasculation-** In species with large flowers, removal of anthers is possible with the help of forceps. It is done before anther dehiscence. The corolla of the selected flower is opened with the help of forceps and the anthers are carefully removed using forceps. Sometimes corolla maybe totally removed along with epipetalous stamens e.g. sesame. In cereals, one third of the empty glumes will be clipped off with scissors to expose anthers. Gynoecium should not be injured. An efficient emasculation technique should prevent self pollination and produce high percentage of seed set on cross pollination. Hand emasculation is often done between 4 and 6 o'clock.

b. **Suction Method-** It is useful in species with small flowers. Emasculation is done in the morning immediately after the flowers open. A thin rubber or a glass tube attached to a suction hose is used to suck only the anthers from the flowers. Considerable self-pollination (upto10%) is likely to occur here. Washing the stigma with a jet of water may help in reducing self-pollination, However self pollination cannot be eliminated in this method.

c. **Hot Water Treatment-** Pollen grains are more sensitive than female reproductive organs to both genetic and environmental factors. In case of hot water emasculation, the temperature of water and duration of treatment vary from crop to crop. It is determined for every species. For rice, 10minutes treatments with 40-44^{o}C is adequate. for instance, sorghum needs a temperature of 42–48°C for ten minutes while rice needs a temperature of 40–44°C for ten minutes. Treatment is given before the anthers dehiscence and prior to the opening of the flower. Hot water is generally carried in thermos flask and whole inflorescence is immersed in hot water.

d. **Cold treatment** - Compared to hot water treatment, cold treatment is less efficient. For instance, when rice is treated with cold water at 0.6 °C, the pollen grains are destroyed without harming the gynoecium. For wheat, a temperature of 0.2 °C for 15–24 hours is necessary.

d. **Alcohol Treatment-** It is not commonly used. The method consists of immersing the inflorescence in alcohol of suitable concentration for a brief period followed by rinsing with water. For instance, treating sweet clover and Lucerne for 10 seconds with 57 percent alcohol was quite successful.

e. **Genetic Emasculation-** Genetic/ cytoplasmic male sterility may be used to eliminate the process of emasculation. This is useful in the commercial production of hybrids in maize, sorghum pearl millet, onion, cotton, and rice, etc. In many species of self-incompatible cases, also emasculation is not necessary, because self-fertilization will not take place. Protogyny will also facilitate crossing without emasculation.

f. **Use of Gametocide(Chemical hybridizing agents ,CHA)-** These are chemicals which selectively kills the male gamete without affecting the female gamete. eg. Ethrel, Sodium methyl arsenate, Zinc methyl arsenate in rice, Maleic hydrazide for cotton and wheat.

4. Bagging: Immediately after emasculation the flower or inflorescence are enclosed with suitable bags of appropriate size to prevent random cross-pollination.

5. Tagging: The emasculated flowers are tagged just after bagging. Generally circular tags of about 3 cm or rectangular tags of about 3 x 2 cm are used. The tags are attached to the base of flower or inflorescence with the help of thread. The following is recorded on the tag with pencil.

(i) Name of Female plant:
(ii) Name of Emasculator:
(iii) Date and time of emasculation:
(iv) Date of controlled pollination (v) Name of male parent, e.g., A x B {iv & v} filled in later stag

6. Crossing: The pollen grains collected from a desired male parent is transferred to the emasculated flower. This is normally done in the morning hours during anthesis. The flowers are rebagged immediately after artificial crossing.

7. Harvesting: Crossed heads or pods of desirable plants are harvested and after complete drying they are threshed. Seeds are stored properly with original tags.

8. Raising: In the coming season, the stored seeds are sown separately to raise the F_1 generation. The plants of F_1 generation are progenies of cross seeds and therefore are hybrids.

Fig. 12.1 Showing process of Emasculation

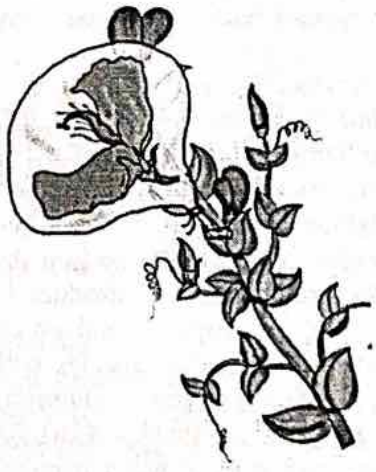


Fig. 12.2 Bagging of an emasculated flower

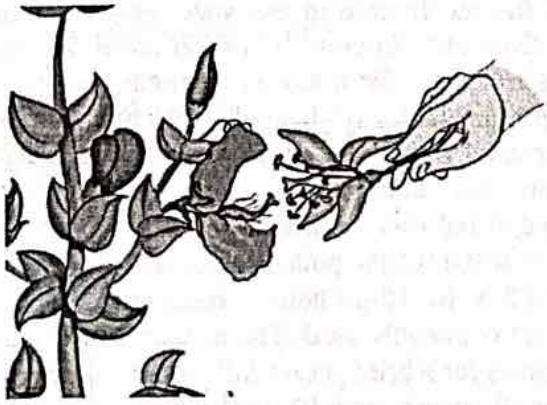


Fig. 12.3 Showing cross pollination on an emasculated flower

EXERCISE - 2: VERIFICATION OF MENDEL'S LAW THROUGH SEED RATIO

Aim: To verify Mendel's Law through seed ratios.

MENDEL'S LAW OF SEGREGATION (PURITY OF GAMETES)

Principle: When two pure lines with contrasting features of a character are crossed, the F1 hybrids show only of one of the parental traits. The phenotype that appears is called dominant and the one that does not appear is called recessive. When the F1 plants were self-pollinated, in the F2 generation both the parental traits appeared in the ratio 3 dominant: 1 recessive. The reappearance of the recessive character in F2 generation verifies the law of segregation.

Requirements: 16 Round and 16 Wrinkled seeds of the same size, cups, beakers or petri dishes.

Procedure:

1. Put 4 or 8 or 16 Round seeds in one beaker and 4 or 8 or 16 Wrinkled seeds in the other to represent male and female gametes respectively. Let the Round seed be indicated as 'R' and Wrinkled seed as 'r'.
2. Take one seed from each beaker and place them in pairs on the table (it represent fertilization). This makes 16 pairs and each pair with one Round –R and one Wrinkled seed-r representing heterozygous F1 individuals (Rr).
3. Take two beakers labelled A and B respectively to represent male and female individuals. Put 8 F1 progeny i.e., 8 pairs of Wrinkled and Round seeds in each beaker (representing males and females).
4. Stir the seeds using a pencil or pen to mix the seeds.
5. Take one seed from each beaker randomly and place them in pairs on the table. This makes 16 pairs representing 16 F2 individuals.
6. Note the genotype (RR or Rr or rr) of each pair, and their possible phenotype (Keep in mind that Round is dominant over Wrinkled).
7. Determine the genotypic and phenotypic ratios of the pooled data.

Observation: Record the results in the following table.

Generation	Trial	Total no. of plants	Genotypes (s)			Phenotype(s)		Phenotypic ratio	Genotypic ratio
			RR	Rr	rr	Round	Wrinkled		

Results: The seed propability results are as per the Mendelian Phenotypic ratio 3:1 and Genotypic ratio 1:2:1 or close to the ratio. Hence Mendel's Law of segregation is verified.

MENDEL'S LAW OF INDEPENDENT ASSORTMENT

Principle: In a dihybrid cross, the segregation of one gene pair is independent of the segregation of the other pair. For example in Mendel's dihybrid crosses, when two pure lines differing in two characters were crossed and their F1 individuals were self-pollinated, two new combinations appear Yellow in addition to parental combinations in the F2 individuals. The typical dihybrid cross phenotypic ratio is 9:3:3:1 which is a product of a typical monohybrid cross phenotypic ratio for two characters i.e., 3:1 X 3:1 = 9: 3:3:1

Requirements: 16 Round, 16 Wrinkled, 16 Yellow, 16 Green seeds of same shape and size, beakers.

Procedure:

1. Put 16 seeds of each colour in four separate beakers.
2. Keep the beakers containing Round and Yellow seeds on one side, and those containing Wrinkled and Green seeds on another side. They represent pure line parents bearing Round shape seed with Yellow colour seed (dominant traits — RRYY) and Wrinkled shape seed with Green colour seed (recessive traits — rryy). The Round and Wrinkled seed represents two alleles of a gene for seed shape while the Yellow and Green seed represents two alleles of a gene for seed colour.

DOMINANT PARENT		RECESSIVE PARENT	
BEAKER -01 ROUND SEEDS	BEAKER -02 YELLOW SEEDS	BEAKER -03 WRINKLED SEEDS	BEAKER -04 GREEN SEEDS

3. Take one Round, one Wrinkled, one Yellow and one Green seed and place them together on the table. Repeat the same till all the seeds are utilized. Note: Each cluster containing one Round, one Wrinkled, one Yellow and one Green seed represent F1 individuals (RrYy).
4. Take four beakers and label them as seed shape in male, seed colour in male, seed shape in female and seed colour in female respectively.

F_1 - MALE		F_1- FEMALE	
BEAKER -01	BEAKER -02	BEAKER -03	BEAKER -04
ROUND & WRINKLED SEEDS	YELLOW & GREEN SEEDS	ROUND & WRINKLED SEEDS	YELLOW & GREEN SEEDS

5. In the beakers labelled as seed shape in male put Round and Wrinkled seeds from the first 8 clusters and similarly in the beakers labelled as seed shape in female put Round and Wrinkled seeds from the other 8 clusters.
6. In the same way in the beaker labelled as seed colour in male put Yellow and Green seeds from the first 8 clusters and similarly in the beaker labelled as seed colour in female put Yellow and Green seeds from the other 8 clusters. The beakers represent F1 male and female individuals.
7. Stir the seeds of each beaker using a pencil or pen to mix the seeds.
8. Take one seed from each beaker randomly and place them together on the table. Repeat this same till the last seed is utilized. This makes 16 clusters each having 4 seeds representing 16 F2 individuals.
9. Record the genotypic and phenotype of each of the 16 F2 individuals.
10. Repeat the experiment a few more time and tabulate the result. Determine the phenotypic ratio.

Observation: Record the results in the following table.

Generation	Trials	Total number of plants	Genotypes									Phenotypes			
			1. YYRR	2. YYRr	3. YyRR	4. YyRr	5. YYrr	6. Yyrr	7. yyRR	8. yyRr	9. yyrr	1. Yellow, Red	2. Yellow, White	3. Green, Red	4. Green, White
F1	1														
	2														
	3														
Total															
F2	1														
	2														
	3														
Total															

Result: Large sampling gives the ratio nearer to Mendelian dihybrid phenotypic 9:3:3:1. Hence Mendel's Law of Independent Assortment is verified.

EXERCISE - 3: POLLEN VIABILITY / FERTILITY

Aim: To study Pollen Viability / Fertility.

Pollen Viability is one measure of male fertility. Viable pollen is responsible for high crop yield. In hybridization programme pollen fertility and viability have a paramount importance. In other contexts, pollen may be stored for germplasm conservation, to make hybrids between plants that flower at different times or places, or for later use in hybridization programmes, and the quality must be monitored. There are direct and indirect measures of pollen viability.

Direct tests consist of depositing the pollen on receptive stigmas and determining whether seeds are produced. Such testing has the advantage of providing an unequivocal measure for the population of pollen grains deposited on the stigma, but it has several disadvantages, such as time-consuming, labor intensive and lager number of fresh samples. Pollen germination can also be scored in vitro.

Indirect methods rely on the correlation between ability to fertilize an ovule and some physiological or physical characteristic that can be determined more rapidly. Indirect- methods that correlate with pollen germination include (1) the fluorochromatic procedure (FCR), (2) testing pollen for enzyme activity, and (3) testing stain ability IKI (iodine potassium iodide) and 1% TTC (2,3,5-triphenyl tetrazolium chloride). The correlation is greatest for FCR and lowest for stain ability

Germination Test

In vitro germination tests have been used to indicate viability of pollen. In general, there is a linear relationship between pollen viability and germination capability.

Pollen of most species will germinate and grow a tube when placed in a solution of calcium, boron, and an osmoticant. Although it provides a control- led experimental system, germination in-vitro does not completely mimic growth in-vivo such as temperature and germination medium. In-vitro germination can be affected by time of pollen collection and storage conditions as well as pollen density on the culture medium. A large numbers of pollens have been successfully germinated under laboratory conditions on relatively simple media. The composition of a germination medium to obtain optimal responses has to empirically formulate for each species. Brewbaker and Kwack (1963) medium found to be suitable for most species.

Ingredients	Brewbaker-Kwack Medium	Roberts Medium	Hodgkin and Lyon's Medium
Sucrose (5 - 20%)	Adjust according to species		
Boric Acid	100 mg/l	10 mg/l	100 mg/l
Calcium Nitrate	300 mg/l	362 mg/l	400 mg/l
Potassium nitrate	100 mg/l	100 mg/l	100 mg/l
Tris	-	60-130 mg/l	-
Manganese sulfate	-	-	4.86 g/l
TAPS	-	-	200 mg/l

One must keep in mind that time and method of collection and storage age of pollen affect viability. Pre germination relative humidity probably affects the internal solute potential and wall properties of the pollen grain and has a subsequent effect on pollen germination. Any work requiring maximally viable pollen should be conducted shortly after anther dehiscence. Germination is most successful immediately after anthesis and viability deteriorates rapidly in most species. Several methods of in vitro raising pollen cultures are available. The selection of a method depends on requirement of the study and facility. The methods are as follows.

- **Hanging Drop Culture:** The hanging drop culture method has been most commonly used in the past. Essentially, it involves suspending the pollen grains in a drop of nutrient medium (on a cover glass) hanging over a shallow depression of cavity slide. To prevent evaporation of the culture medium, the hanging drop culture is sealed with wax. The set up is incubated in humid chamber.
- **Sitting Drop Culture:** The sitting drop culture method is simpler than the hanging drop method. It involves culturing of pollen grains in a drop of culture medium placed on a cavity microslide. The culture is then maintained in a humid chamber to prevent evaporation.
- **Suspension Culture:** In large samples of pollen grains in 2 to 10-ml culture medium in suitable vials incubated on a shaker and observed for germination by placing drop in slide.

Aim: *To calculate the percentage of pollen germination to test Pollen Viability/ Fertility*

Principle: In nature, pollen grains germinate on the compatible stigmas of the carpel. They can also be induced to germinate in a synthetic medium. During germination, the intine grows out as pollen tube through one of the germ pores in the exine. *In vitro* germination tests have been used to indicate viability of pollen. In general, percentage of pollen germination depicts the pollen viability/Fertility.

Requirements: Fresh or preserved mature pollen grains of locally available suitable flower *(Hibiscus, Vinca, Petunia, Crotalaria, Tradescantia, Lily, Balsam* or *Jasmine* 10% sucrose solution (10g sucrose dissolved in 100ml water), distilled water, petridish, blotting paper, cavity slides, coverslips, brush, needle and microscope.

Procedure:

1. **Hanging Drop Culture method,**
   - Take a drop of 10% sucrose (nutrient medium) solution on a clean cover slip and dust mature pollen grains on it.
   - The margins of the coverslip is applied with wax or Vaseline.
   - Place the cover slip inverted on to a clean slide or a cavity slide to hanging the media drop over shallow depression of slide.
   - The set up is incubated in humid chamber for 15 minutes and then the observation is recorded.
2. **Sitting Drop Culture method**
   - Take a drop of 10% sucrose (nutrient medium) solution in the cavity depression of the cavity slide, dust mature pollen grains on it.
   - Place the coverslip over the cavity region of the slide without over spilling the media.
   - Place the slide on a wet blotting paper placed in the petridish which acts as a humid chamber.
   - Leave the pollen grains undisturbed for 15 minutes and then make the observation.

Observation: Pollen grains germinate and produce pollen tubes. Count the total number of pollen grains and number of pollen grains germinated in 3 — 5 microscope fields. Tabulate the observation and calculate the percentage of pollen grains germinated.

1. Name of the plant used as pollen source :
2. Number of pollen grains in a field of microscope = N
3. Number of germinated pollen grains in the same field of microscope = n
4. Percentage of pollen germination = n X 100/ N

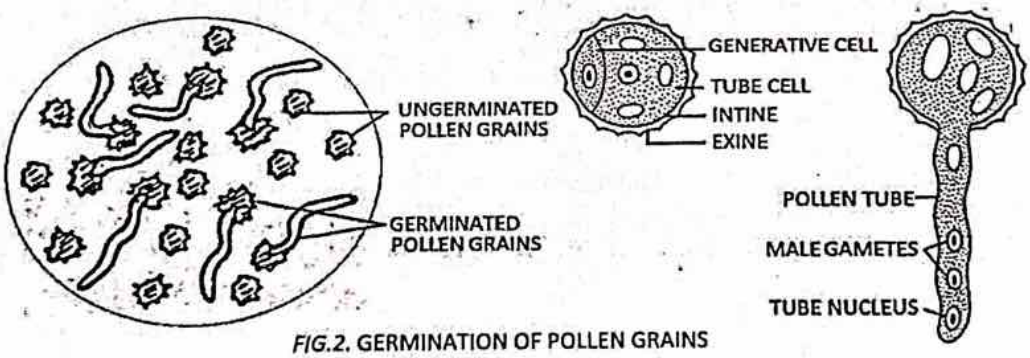


FIG.2. GERMINATION OF POLLEN GRAINS

Number of observation	Total number of pollen - N	Total number of pollen germinated - n	% of pollen germination nx100/N
1			
2			
3			
Average			

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ECO CLUB

LAB MANUAL

B.SC VI SEMESTER (NEP)

PAPER-1: CELL BIOLOGY

EXERCISE 1

Aim: Preparation and study of mitosis in onion root tips through Squash preparation.

Principle: Somatic growth in plants and animals takes place by the increase in the number of cells. A cell divides mitotically to form two daughter cells wherein the number of chromosomes remains the same (i.e., unchanged) as in the mother cell. In plants, such divisions rapidly take place in meristematic tissues of root and shoot apices, where the stages of mitosis can be easily observed.

Requirement: Onion bulbs, wide mouth glass/jar/bottle, glacial acetic acid, ethanol 2-4% acetocarmine/acetoorcein stain, N/10 HCl, spirit lamp, slide, cover slips, blotting paper, DPX / molten wax /nail polish and compound microscope.

Procedure

Growing of root tips

- Select a few medium-sized onion bulbs. Carefully remove the dry roots present.
- Grow root tips by placing the bulbs on glass tubes (of about 3–4 cm. diameter) filled with water.
- Care should be taken so that the stem portion of the bulb (basal part) just touches the water.
- A few drops of water may be added periodically to compensate evaporation losses.
- New roots may take 3–6 days to grow. Cut 2–3 cm long freshly grown roots and transfer them to freshly prepared fixative, i.e., aceto-alcohol (1:3:: glacial acetic acid : ethanol).
- Keep the root tips in the fixative for 24 hours and then transfer them to 70% ethanol (for preservation and use in future).
- Onion root-tip cells have a cell cycle of approximately 24-hour duration, i.e., they divide once in 24 hours, and this division usually takes place about two hours after sunrise.
- Therefore, roots grown on water should be cut only at that time to score maximum number of dividing cells.

Preparation of slide

- Take one or two preserved roots, wash them in water on a clean and greasefree slide.
- Take a watch glass place the roots in it, Add 1 drop of N/10 HCl followed by 2–3 drops of aceto-carmine or aceto-orcein stain
- Warm the watch glass on spirit lamp until getting slight fumes. Care should be taken that the stain is not dried up nor boil it.
- Transfer the root on to the new clean glass slide. Now cut the comparatively more stained (2–3 mm) tip portion of the root and retain it on the slide and discard the remaining portion. Finely chop the tip of root with sharp blade (care should be taken not to dry out the tip)
- Put one or two drops of aceto-carmine, mount a cover slip on it avoiding air bubbles.
- Place the slide on strip of blotting paper to wrap it, hold folds of blotting paper using the fingers in such a way that the cover slip mounted on the slide is properly held.
- Now slowly tap the cover slip using the blunt end of a pencil so that the meristematic tissue of the root tip below the cover slip is properly squashed and spread as a thin layer of cells.
- Take off the strip of blotting paper without disturbing the blotting paper.
- Carefully seal the margins of the cover slip using DPX or molten paraffin wax or nail polish.
- This preparation of onion root tips cells is now ready for the study of mitosis.

Study of slide

Place the slide on the stage of a good quality compound microscope. First observe it under the lower magnification (10 X objective) to search for the area having a few dividing cells. Examine the dividing cells under 40X or higher magnification of the microscope to observe the detailed features of mitosis.

Observation

The stages of mitosis can be broadly categorised into two parts: karyokinesis (division of nucleus) followed by cytokinesis (division of cytoplasm, and ultimately of the cell).

Those cells, which are not in the phases of cell division are considered to be in interphase. You may observe that most of the cells in a microscope field are in interphase

Interphase

The cells are mostly rectangular, oval or even circular in shape, with almost centrally situated densely stained nucleus. The chromatic (coloured) material of the nucleus is homogeneous and looks granular. The boundary of the nucleus is distinct. One or few nucleoli (sing: nucleolus) can also be observed inside the nucleus

Stages of Mitosis

(a) Prophase

- Intact nuclear outline is seen. The chromatin (seen as a homogeneous material in the nucleus at interphase) appears as a network of fine threads (chromosomes). Nucleoli may or may not be visible.
- If the cell under observation is in the early stage of prophase then the chromatin fibres (chromosomes) are very thin. However, in the cells at late prophase, comparatively thicker chromatin fibres would be visible. Besides this, in the late prophase the nuclear membrane may not be noticed.
- The process of mitosis is initiated at this stage wherein coiling and thickening of the chromosomes occurs
- Shrinking and hence the disappearance of the nucleolus and nuclear membrane takes place
- The stage reaches its final state when a cluster of fibres organizes to form the spindle fibres

(b) Metaphase

- The nuclear membrane disappears. Chromosomes are thick and are seen arranged at the equatorial plane of the cell which forms metaphase plate.
- Each chromosome at this stage has two chromatids joined together at the centromere, which can be seen by changing the resolution of the microscope. Nucleolus is not observed during metaphase.
- Chromosomes turn thick in this phase. The two chromatids from each of the chromosomes appear distinct
- Each of the chromosomes is attached to the spindle fibres located on its centromere.

(c) Anaphase

- This stage shows the separation of the chromatids of each chromosome. The chromatids separate due to the splitting of the centromere.
- Each chromatid now represents a separate chromosome as it has its own centromere.
- Each of the chromatid pair detaches from the centromere and approaches the other end of the cell through the spindle fibre
- The chromosomes are found as if they have moved towards the two poles of the cell.
- The chromosomes at this stage may look like the shape of alphabets 'V', 'J' or 'I' depending upon the position of centromere in them.
- Different anaphase cells show different stages of movement of chromosomes to opposite poles, and they are designated to represent early, mid and late anaphase.

(d) Telophase

- Chromatids have reached the other end of the cell, The spindle fibres disappears.
- Chromatids reach the opposite poles, lose their individuality, and look like a mass of chromatin.
- Chromatin fibres are formed as a result of uncoiling of daughter chromosomes.
- The appearance of two daughter nuclei at the opposing ends due to the reformation of the nucleolus and nuclear membrane.
- At this phase, splitting of the cell or cytokinesis may also occur.
- Cytokinesis In plants, a cell plate is formed in the middle after telophase.
- The plate can be seen to extend outwards to ultimately reach the margin of the cell and divide the cell into two. Such cell plates are characteristic of plant cells.

EXERCISE 2

Aim: Preparation and study of meiosis in *Tradescantia*

Principle: Meiosis is a type of cell division in which the number of chromosomes is halved (from diploid to haploid) in the daughter cells, i.e., the gametes. The division is completed in two phases, meiosis I and meiosis II. Meiosis I is a reductional division in which the chromosomes of homologous pairs separate from each other. Meiosis II is equational division resulting in the formation of four daughter cells. Stages of meiosis can be observed in a cytological preparation of the cells of testis tubules or in the pollen mother cells of the anthers of flower buds.

Tradescantia is an ideal plant for studying meiosis, because a single flowering stem contains a lineup of buds, from the smallest at the bottom to the largest at the top. These largest have already gone through meiosis and produced pollen. Young anthers in buds about six down from the top usually contain cells undergoing meiosis to yield microspores.

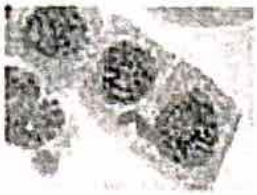
Requirement: *Tradescantia* flowers, 2-4% acetocarmine/acetoorcein stain, N/10 HCl, spirit lamp, slide, cover slips, blotting paper, DPX / molten wax / nail polish and compound microscope.

Fixation

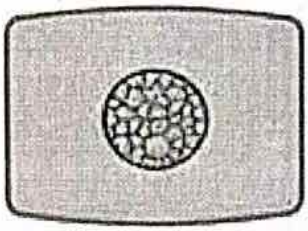
Flowers buds are fixed in canyons fluid (1 part acetic acid: 6 parts ethyl alcohol). The amount of fixatives must always be at least 10 times more than the volume of the plant tissue. After 48 hours, the material is transferred and preserved in 70% ethyl alcohol.

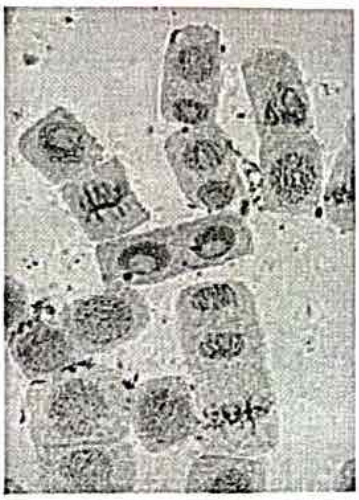
Procedure

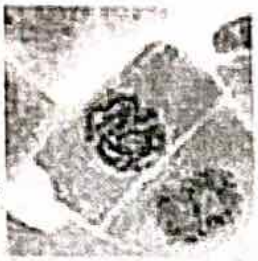
- Isolating Anther Cells that are Undergoing Meiosis
- Each flowering stem of Tradescantia is terminated by a group of flowers, below which are two leaf-like bracts. Cut a group of flowers, put it into a beaker of water.
- Remove the bract from one side.
- Count back six buds from the open flower; choose buds three through six. Push aside the petals and sepals to reveal the stamens.
- Carefully remove the six stamens and put them in a small drop of Farmer's fixative on your microscope slide. (The fixative kills everything in the stamens all at once, so that the chromosomes will be in excellent form for observation.)
- Add a small drop of aceto-carmine stain to the anther contents. Tap and poke at the stamens with blade just as you did the root tips, with the idea of opening up the stamens so that the cells undergoing meiosis will spill out onto your slide.
- Remove all the big chunks once you're sure that all the anthers are punctured.
- Gently warm the slide over spirit lamp.
- Carefully cover your preparation with a coverslip. You can reduce the number of bubbles by placing one end of the coverslip in the stain, and letting it fall over the preparation.
- Look at your preparation under the medium power of your compound microscope.
- Decide which stages of pollen development you have in you flower from the drawing of pollen development.
- You may well have more than one stage within a given flower, so be sure to scan the slide carefully.
- If you see good mitosis or meiosis, you may want to do a squash to spread the chromosomes out.
- To do a squash, fold your slide between layers of blotting paper, place it on a lab bench, and press directly down on the middle of your slide with the bottom of your thumb or tap with blunt end of pencil.
- Dont move your thumb from side to side or you will smudge your preparation.
- Place the glass slide under a microscope and observe the various stages of the meiotic cell cycle. If the cells are of desired stage, the cover glass is sealed with DPX/paraffin wax.



a. Interphase

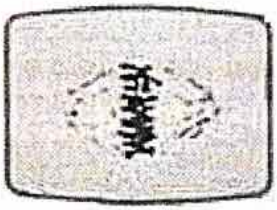


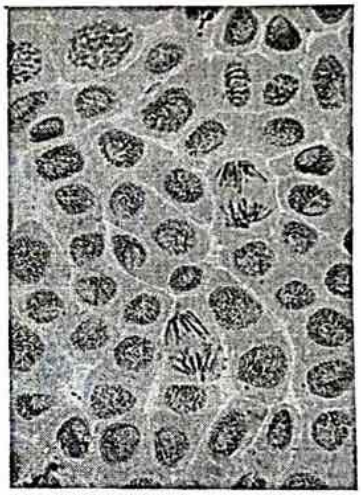




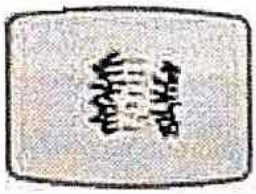
b. Prophase

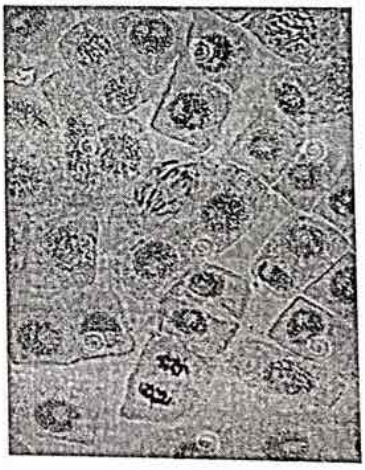
c. Metaphase



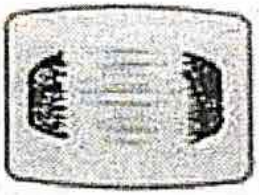


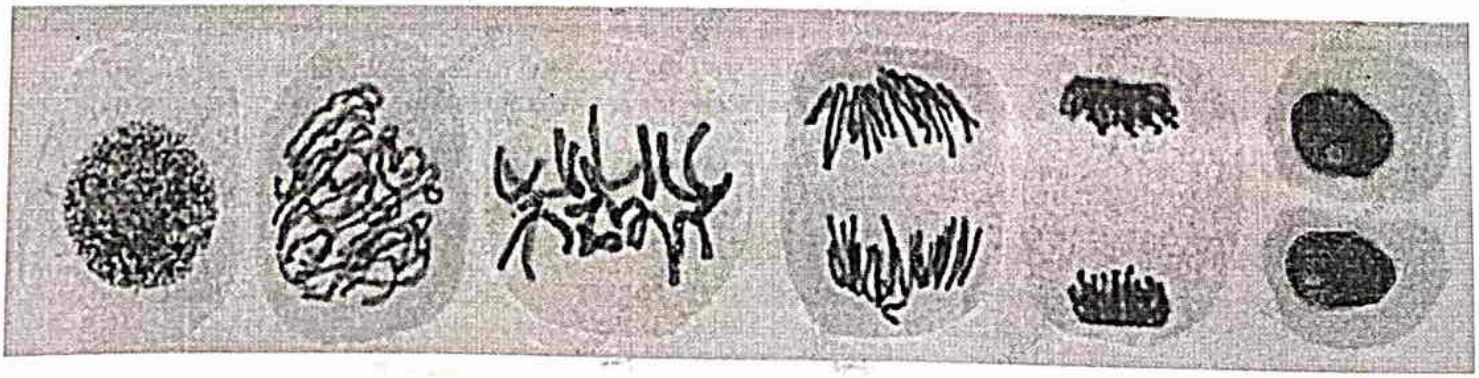
d. Anaphase

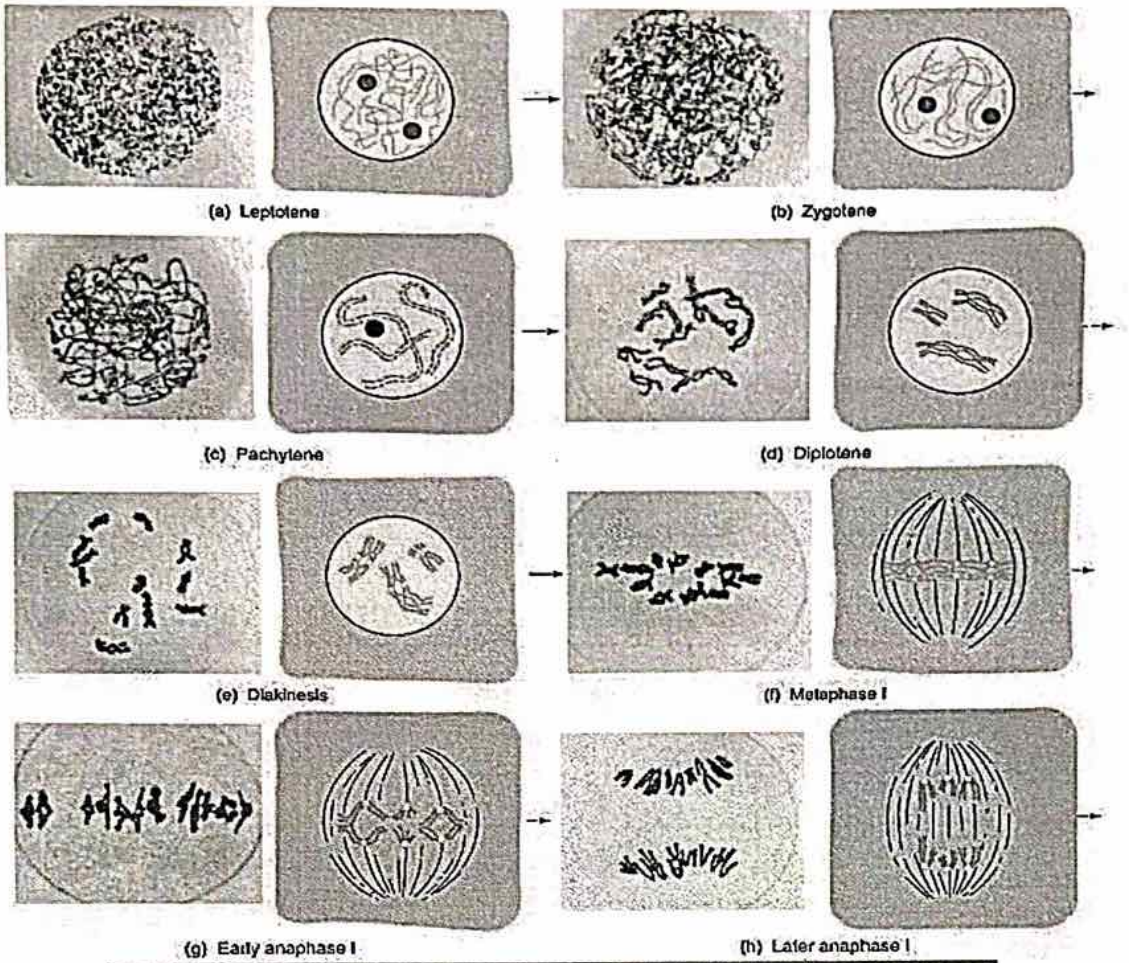


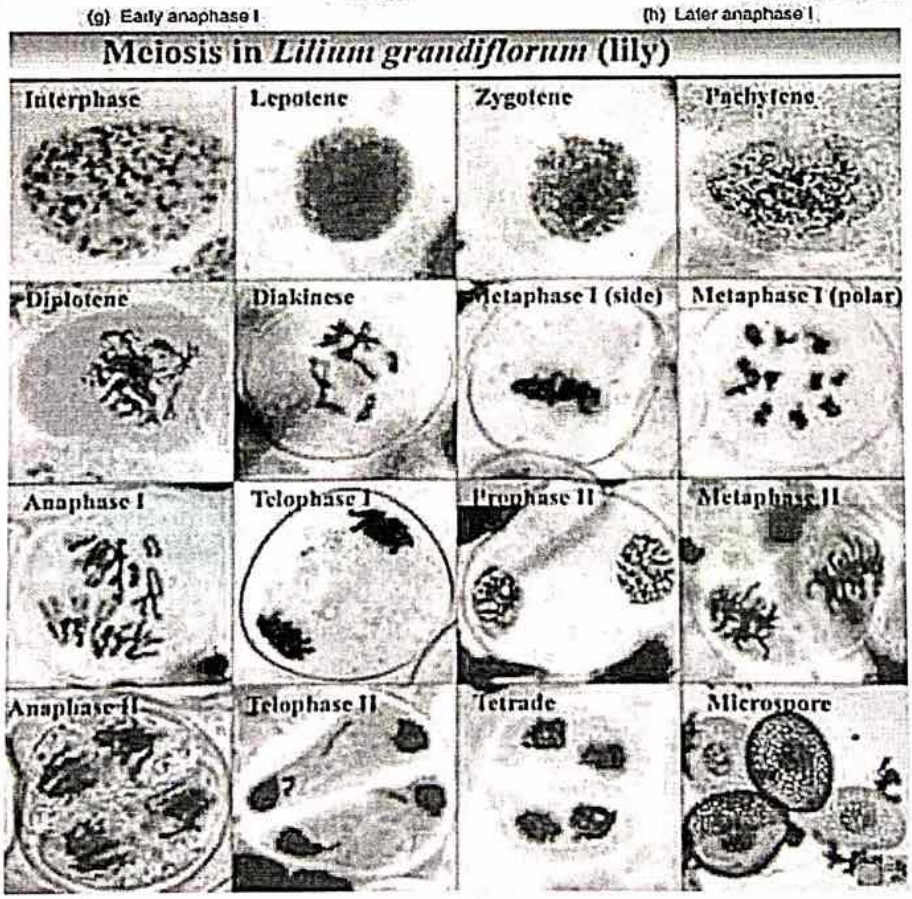


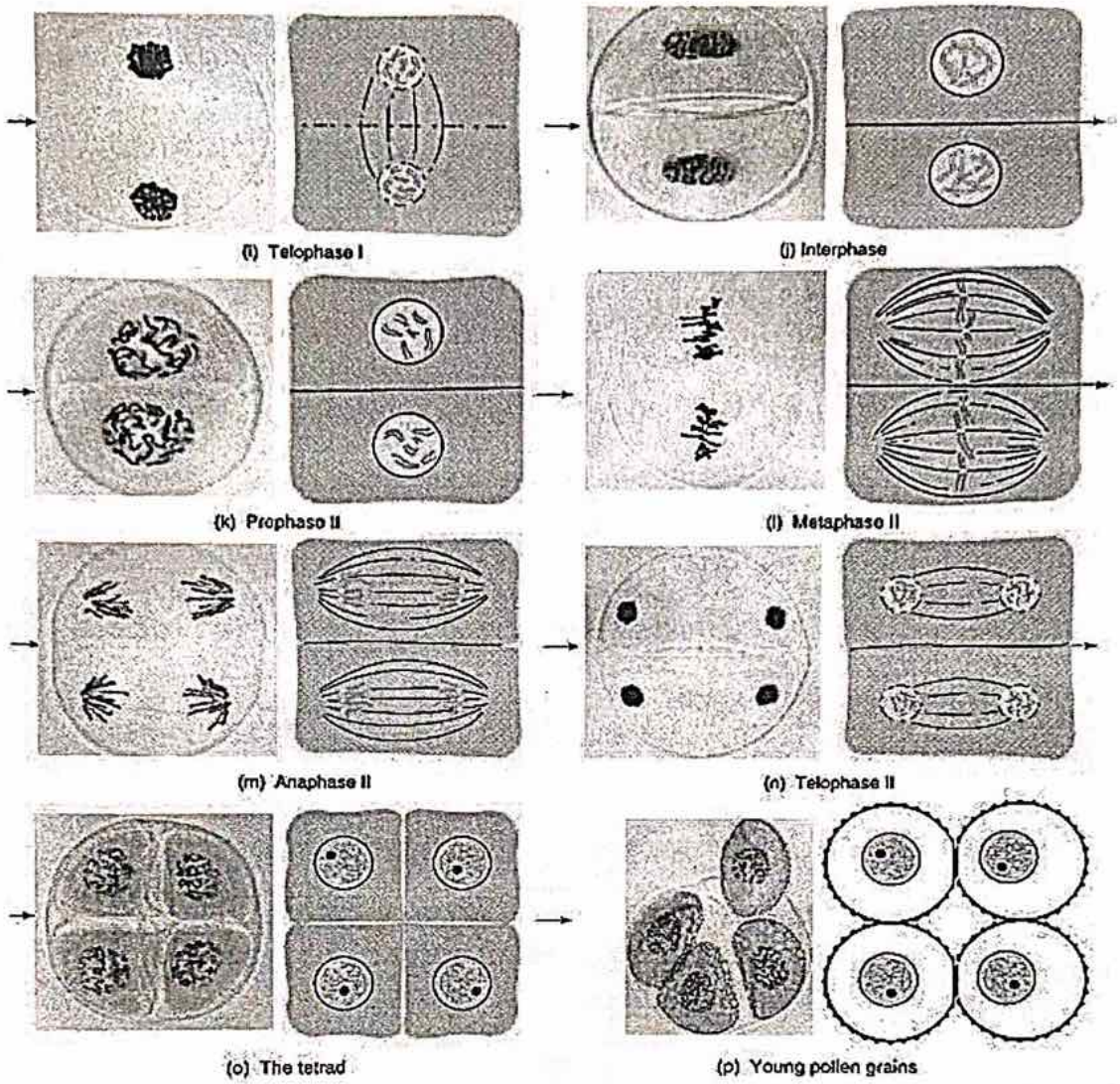
e. Telophase






(a) Leptotene
(b) Zygotene
(c) Pachytene
(d) Diplotene
(e) Diakinesis
(f) Metaphase I
(g) Early anaphase I
(h) Later anaphase I


Meiosis in Lilium grandiflorum (lily)
Interphase
Lepotene
Zygotene
Pachytene
Diplotene
Diakinese
Metaphase I (side)
Metaphase I (polar)
Anaphase I
Telophase I
Prophase II
Metaphase II
Anaphase II
Telophase II
Tetrade
Microspore


(i) Telophase I
(j) Interphase
(k) Prophase II
(l) Metaphase II
(m) Anaphase II
(n) Telophase II
(o) The tetrad
(p) Young pollen grains

Prophase-I

- During prophase-I, DNA is exchanged between homologous chromosomes in a process called homologous recombination. T his often results in chromosomal crossover.
- The paired and replicated chromosomes are called bivalents or tetrads.
- The process of pairing the homologous chromosomes are called Synapsis.
- At this stage, non-sister chromatids may cross-over at point called Chiasmata.

Leptotene

- The first stage of prophase-I is the Leptotene stage.
- Leptotene (Greek; Leptonema- thin threads)
- During this stage, individual chromosomes begin to condense into long strands within the nucleus.
- However, the two sister chrochromatids are still so tightly bound that they are indistinguishable from one another.

Zygotene

- Zygotene (Greek; zygonema- paired threads)
- Zygotene, occurs as the chromosomes approximately line up with each other into homologous chromosomes.
- The combined homologous chromosomes are said to be bivalent.

Pachytene

- In pachynema, the homologous chromosomes become much more closely associated. This process is known as Synapsis.
- The synapsed homologous pair of chromosomes is called a tetrad, because it consists of four chromatids.
- It can't be observed until the next stage, but the synapsed chromosomes may undergo crossing over in pachynema where exchange of geetic material between chromatid arms of homologous chromosomes.
- The chromosomes continue to condense.

Diplotene

- Diplotene (Greek; diplonema- two threads)
- In this stage, crossing over completes.
- The homologous chromosomes separete from one another little.
- Nuclear membrane and nucleolus begins to disappear.
- The homologous chromosomes show distinct separation from each other except at few regions where attachments are seen.
- These are chiasmata (sing. chiasma) representing the site of exchange of the parts between two homologous chromosomes (i.e. crossing over).

Diakinesis

- Chromosomes condense further during the diakinesis stage.
- In this stage, the homologous chromosomes separate further, and the chiasma terminalize due to contraction of the tetrad. Chiasmata can be still observed.
- The homologous pair of chromosomes appear more shortened, thick and prominent.
- All the homologous pairs appear scattered in the cell.
- Spindle fibre begin to form

Metaphase-I

- Metaphase-I is the second phase of meiosis.
- The spindle fibres organized between two poles and get attached to the centromere of chromosomes.
- Homologous chromosomes are still in pairs, and are arranged along the equatorial plane of the cell.
- At this stage, the number of bivalents can be counted. Chiasmata may still be seen in a few bivalents.

Anaphase-I

- The chromosome pairs appear to have moved towards the two opposite poles of the cell.
- This stage can be identified by the presence of two chromatids in each chromosome.
- In this stage, the sister chromatids remain attached at their centromeres and move together toward the poles.
- At the later stage, the anaphase - I may show the assembly of chromosomes at two poles. This results into the reduction of number of chromosomes to half.

Telophase-I

- The homologous chromosome pairs reach the poles of the cell .
- The homologous chromosome pairs complete their migration to the two poles.
- The chromosomes present at the two poles appear decondensed and form two distinct nuclei
- A nuclear envelope reforms around each chromosome set, the spindle disappears, and cytokinesis follows.

Prophase-II

- Meiosis-II begins without any further replication of the chromosomes. The nuclear envelope breaks down and the spindle apparatus forms.
- The dyads chromosome becomes thicker and shorter.
- Nuclear membrane and nuceolus disappear.
- Spindle fibre starts to form.

Metaphase-II

- The chromosomes having two chromatids attached at the centromere are observed arranged at the equatorial plane of the cell.
- Centromeres are arranged in aline called equatorial plate of invisible spindle apparatus.
- Spindle fibres organize between poles and attaches to centromere of chromosome.
- Each chromosome of metaphase II has two chromatid whereas in metaphase I these are paired homologous chromosomes each having two chromatids thus forming tetrad.
- In the metaphase I of meiosis, a few chiasmata are observed, where as no chiasmata are observed during metaphase II.

Anaphase-II

- Centromere of each chromosome divides and sister chromatids separates to form two daughter chromosome.
- Spindle fibre contracts and pull the daughter chromosome apart towards opposite pole.
- The two chromatids of each chromosome after separation appear to lie at the two poles of the cell.
- Anaphase II can also be distinguished from the anaphase I of meiotic division on the basis of chromatids: In anaphase I, each chromosome has two distinct chromatids, but in anaphase II, each chromosome is represented by one chromatid only.

Telophase-II

- Nuclear envelope forms around each set of chromosomes.
- Nucleolus appears in each nucleus.
- Chromosome elongates to form thin networks of chromatin.
- Nuclear membrane and nucleolus reappears.

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RANI CHANNAMMA UNIVERSITY, BELAGAVI

विद्वान् सर्वत्र पूज्यते

Bsc.VI SEMESTER

LAB MANUAL

(Plant Physiology & Biochemistry)

Dept. of Botany

TABLE OF CONTENTS

Experiment No.1:

To study permeability of membrane using different organic solvents.

Materials required:

Test tube, beaker colorimeter, cork borer, beetroot, organic solvents (Benzene, Diethyl ether, Ethanol, Acetic acid, Acetone etc.) cork borer

Procedure:

1. Take healthy beet roots and peel off the outer skin using the scalpel.
2. Use 1 cm. cork borer to take 6 cylinders from beet root.
3. Cut them into uniform sized cylinders.
4. Wash them repeatedly in running water for 10 minutes to remove pigments from the cut/damaged tissue.
5. Maintain the first beaker as control by taking 50 ml of distilled water.
6. Remaining five beakers are taken with 50 ml of different organic solvents (Benzene, Diethyl ether, Ethanol, Acetic acid, Acetone etc.) respectively.
7. Transfer the beet root cylinders one in each to the six beakers.
8. Incubate all the cylinders in the beakers for 1 hour, at room temperature
9. Since all the organic solvents are highly volatile, during incubation time all the beakers containing various organic solvents should be covered with Petri plates.
10. After 1 hour, shake the beakers, remove the cylinders and transfer the solution to the cuvette.
11. Measure the Optical Density (OD) at 530 nm. and plot the graph with Optical Density versus organic solvents.

Sl.No.	Size of Beet Root cylinder	Incubating medium	Concentration of Organic Solvent	Volume of Organic Solvent	Incubation period (at Room Temp.)	Optical Density (at 530 nm.)
1	1 cm.	Distilled water	1 N.	50 ml.	1 Hour	
2.	1 cm.	Ethanol	1 N.	50 ml.	1 Hour	
3.	1 cm.	Diethyl ether	1 N.	50 ml.	1 Hour	
4.	1 cm.	Methanol	1 N.	50 ml.	1 Hour	
5.	1 cm.	Acetone	1 N.	50 ml.	1 Hour	
6.	1 cm.	Benzene	1 N.	50 ml.	1 Hour	

Conclusion:

Since the permeability of the cell membrane is affected by various organic solvents, anthocyanin pigments get released into the respective organic solution. This is

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evidenced by the intense red Colour of the solution at various concentrations in different organic solvents. The release of the anthocyanin pigments take place only when the cell has been killed partially or completely in various organic solvents. The killing of the cells causes the loss of selective permeability of the cytoplasmic membrane, which becomes partly or completely permeable to solute particles.

Result: Maximum permeability is seen in _____.

Table for the preparation of 1 N. solution:

Sl.No.	Incubating medium	Concentration required	Volume of pure Organic Solvent	Volume of distilled water	Total volume
1	Ethanol	1 N.	2.3 ml.	47.7 ml.	50 ml.
2.	Diethyl ether	1 N.	3.7 ml.	46.3 ml.	50 ml.
3.	Methanol	1 N.	3.2 ml.	46.8 ml.	50 ml.
4.	Acetone	1 N.	2.9 ml.	47.1 ml.	50 ml.
5.	Benzene	1 N.	3.9 ml.	46.1 ml.	50 ml.

-0-0-0-0-0-

Experiment No.2:

a. Measurement of pH of plant samples by using p^H paper

Principle:-

The most biological reaction center around the ability of molecules to exist in electrically charged forms (+ and -). In its most predominant form the charge of any molecule is determined by its ability to release or attract H^+ ion concentration in terms of grams ion per litter in the range of 10^{-1} to 10^{-14}, which makes of its some what troublesome in calculation. To over come this, Saresem in 1909 introduced the term pH defined this as pH = -log $[H^{+}]$ (pH is defined as the negative logarithm of hydrogen ion activity, where activity understood to mean effective concentration). It is measured on a scale of 0 -14. PH -7 represents the neutral condition. Below pH -7, it is acidic and beyond pH -7, it is alkaline. According to this the $[H^{+}]$ concentration of 10^{-8} of ions per litter will be pH = 8.0, it is alkaline.

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The pH may be measured electrometrically with pH meter or calorimetrically with p^H indicators. Electrometric measurements of pH are more accurate, while colorimetric measurement recommended for on the spot analysis in the field

Requirements:

Plant source or extract, p^H papers, standard colour chart, forcep, beakers, pestle- mortar.

Procedure:

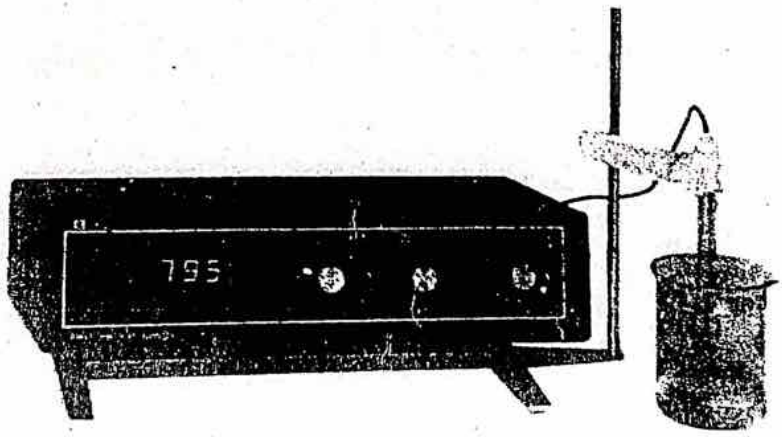
1. The plant source (Leaf, stem, root, fruit, seed, etc.) are ground with distilled water thoroughly and filter the solution with what man No.1, use the filtrate for reading p^H.
2. Torn off a strip of indicator paper with the forcep and insert it for a few seconds into the filtrate sample to be tested.
3. Compare the colour developed on treatment with indicator paper to the standard colour chart provided on the cover of p^H paper booklet to get the p^H value directly.

Result: The p^H of the given sample is _____

b. Measurement of pH of plant samples by using p^H meter.

Requirements:

Plant source or extract, p^H meter, beakers, pestle- mortar.



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Procedure:

Calibration of p^H meter for using:

1. The instrument has to be switched on atleast about 24 hrs before use (20 min) for about 15 minutes before use.
2. The electrode is washed with distilled water and then wiped with tissue paper
3. The temperature should be adjusted to room temperature.
4. Rinse the electrode with a small amount of buffer solution (at p^H 4).
5. Take a buffer solution is a beaker and immerse the electrodes gently.
6. Find the p^H of the buffer by using the selector knob. If it does not show p^H 4, which is the concentration of the buffers adjust the knob in such a way that it shows p^H 4.
7. Allow the instrument to be in this position until the reading remains constant.
8. The p^H of the selector knob should be turned back to the standby position.
9. Remove the electrode from the buffer and rinse in water.
10. Take the buffer with p^H 9.2 and repeat the procedure used for p^H 4 buffer.
11. Adjust the selector knob so that it shows required p^H.
12. The p^H meter now is properly standardized to take the readings from the sample.

Procedure for determining p^H of an unknown sample:

1. Wash and clean the electrodes properly and wipe them dry.
2. Adjust the temperature to the prevailing room temperature.
3. Immerse the electrode in the sample solution.
4. Turn on the selector knob and read the p^H value.
5. Remove the electrode from the sample, rinse with distilled water, wipe it again keep it immersed in beaker containing distilled water.

Result: The p^H of the given sample is _____.

-0-0-0-0-