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A practical manual on

Fundamentals of Crop Physiology

Credits: 3(2+1)

Semester: II

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Experiment - 1

Aim: - Structure & Function Of Plant Cell

The term cell is derived from the Latin ‘cella’ means storeroom or chamber.

The term cell was first used by the English botanist Robert Hooke in 1665, to describe the

individual units of the honeycomb-like structure in cork under compound microscope.

Plants are multicellular organisms composed of millions of cells with specialized function. All plant cells have the same basic eukaryotic organization.

Cell Wall

A fundamental difference between plant and animal cells is that the plant cell is surrounded by a rigid cell wall, mostly made of polysaccharides (cellulose, hemicellulose, pectin) and lignin.

Plants have two types of cell walls, primary and secondary.

Primary cell walls are thin and characteristic of young, growing cells.

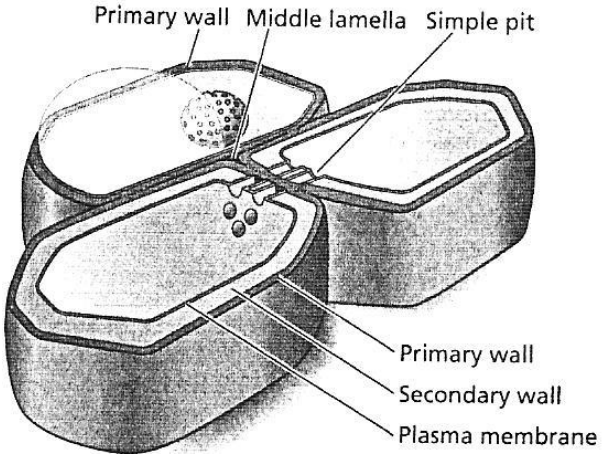
Secondary cell walls are thicker and stronger, and they are deposited when most cell enlargement has ended.

Secondary cell walls have their strength and toughness due to lignin; a glue like material.

The lignified secondary walls provide the plants the structural reinforcement necessary to grow vertically above the soil.

Bryophytes which lack the lignified cell walls are unable to grow more than a few centimeters above the ground.

In plants, the neighboring cells are cemented together by a middle lamella (intercellular layer).



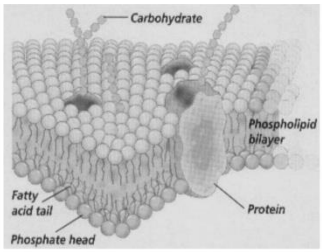
Plasma Membrane (Plasmalemma)

All cells are enclosed in a membrane that serves as their outer boundary, separating the cytoplasm from the external environment.

This plasma membrane allows the cells to take up and retain certain substances while excluding others. Thus, plasmalemma accounts for selective traffic of solutes across membrane.

All biological membranes consist of a double layer (bilayer) of phospholipids in which proteins are embedded.

The membrane is not a static structure, but it is a dynamic structure. Both lipid and protein molecules are free to move and are usually in a constant motion. However, these molecules readily move in the plane of membrane, a process known as lateral diffusion.



Phospholipids

Phospholipids are a class of lipids in which 2 fatty acids are linked to glycerol, which is linked to a phosphate group.

A head group such as choline is also attached to phosphate group. Phosphatidylcholine is a phospholipid common to most membranes.

The head groups are highly polar (hydrophilic) whereas the hydrocarbon chains of fatty acids are highly nonpolar (hydrophobic).

Thus, phospholipids display both hydrophilic and hydrophobic properties, hence they are amphipathic.

In the bilayer, the amphipathic lipids are arranged is such a way that their hydrophobic tails point toward each other and the hydrophilic heads make the surfaces.

The bilayer is stable in aqueous environment because its surfaces readily associate with water.

Proteins

The proteins which are embedded in lipid bilayer are globular.

These proteins can be divided into two types, integral and peripheral. Integral proteins are deeply embedded in the lipid bilayer. Most integral proteins span the entire width of the lipid bilayer so one part of the protein interacts with the outside of cell, another part interacts with hydrophobic core and the third part interacts with interior of cell (cytosol).

Ion channels are always integral proteins. Certain receptors that participate in signal transduction are integral proteins.

Nucleus

The nucleus is surrounded by a double membrane called the nuclear envelope. The space between these two membranes is called the perinuclear space. The joining sites of the two nuclear membranes are called the nuclear pores. The material filled in the nucleus is called nucleoplasm (or nuclear sap). About 8% of the surface area of the nuclear membrane is occupied by pores. These pores allow the transport of substances between cytosol and nucleus.

Nucleus is the site of storage and replication of chromosomes, composed of DNA and its associated proteins (histones). The DNA-protein complex is known as chromatin.

Nucleus contains a densely granular region called the nucleolus, which is the site of ribosome (ribosomal RNA) synthesis.

Ribosomal proteins are synthesized in cytosol and transported into nucleus via nuclear pores, where they bind with rRNA to form 40S and 60S subunits. These subunits pass into cytosol and aggregate to form 80S ribosomes.

The genes are transcribed in nucleus to form mRNA, tRNA and rRNA. mRNA and tRNA pass from nucleus to cytosol where they are used for protein synthesis.

The nucleotide sequence of mRNA is translated into amino acid sequence of proteins by ribosomes. tRNA assists by transferring amino acids to mRNA codons.

Endoplasmic Reticulum

Cells have an elaborate network of internal membranes called endoplasmic reticulum (ER).
ER is continuous with the outer membrane of nuclear envelope (but not plasmalemma).

The ER lumen of one cell is connected to adjacent cell via plasmodesmata.
There are 2 types of ER, smooth and rough, which are interconnected.

Rough ER is covered with ribosomes which synthesize proteins to be delivered to lumen of ER.
Smooth ER lacks ribosomes.

Golgi Apparatus

Golgi apparatus (or Golgi complex) is made of one or more dictysomes (or Golgi bodies) which are stacks of 3-10 flattened sacs (cisternae) and vesicles. Plant cells contain up to several hundred Golgi bodies dispersed in cytoplasm. The cisternae close to plasmalemma are called *trans* face, and the cisternae close to center of cell are called *cis* face. The medial cisternae are between *trans* and *cis* cisternae. Golgi body is a dynamic structure; new cisternae are continuously produced from endoplasmic reticulum at *cis* face while old cisternae are lost in the form of vesicle at *trans* face. Golgi apparatus has intermediary position between ER and extracellular space.

Ribosomes

Ribosomes are composed of rRNA and protein.

Ribosomes play an important role in protein synthesis.

Plant cells contain 3 distinct types of ribosomes, which occur in cytoplasm, mitochondria and chloroplast.

The mitochondrial and chloroplastic ribosomes are smaller (70 S) than cytoplasmic ribosomes (80 S).

The cytoplasmic ribosonmes may be found in cytosol or attached to endoplasmic reticulum.

The rRNA molecules of cytoplasmic ribosomes are formed by transcription of nuclear genes in nucleolus. Whereas rRNA of mitochondrial and chloroplastic ribosomes are formed by transcription of mitochondrial and chloroplastic genes respectively.

The proteins of cytoplasmic ribosomes are coded by nuclear genes and synthesized in cytosol. Most of the proteins of mitochondrial and chloroplastic ribosomes are also synthesized in cytosol by nuclear genes.

Mitochondria

Mitochondria are cytoplasmic organelles.

Mitochondria are the sites of oxidative phosphorylation (ATP synthesis).

Mitochondria are surrounded by two membranes. The outer membrane is smooth and the inner membrane is highly convoluted. The folds of inner membrane are called ‘cristae’.

The components of respiratory electron transport chain are found in inner membrane.

The inner membrane is also characterized by the presence of stalked particles with spherical heads containing ATPase. ATPase catalyses the synthesis of ATP.

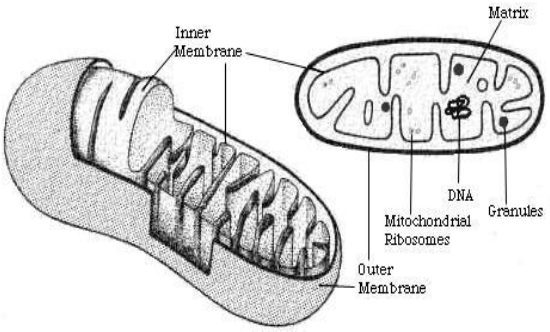
The inner membrane is highly impermeable to the passage of protons (H^+), which allows the formation of electrochemical gradient necessary for ATP synthesis.

The compartment enclosed by inner membrane is called ‘matrix’. Matrix contains the enzymes of Krebs cycle (TCA cycle or citric acid cycle).

Mitochondria contain their own protein synthesizing machinery (ribosomes, tRNA etc.). Mitochondrial ribosomes are smaller (70 S) than those found in cytosol (80 S).

Mitochondria contain circular, histone-free DNA molecule, similar to those of bacteria.

Mitochondrial genome of plants consists of about 200 kb (200,000 base pairs), which is much larger than animal mitochondria.



Plastids

Plastids are the organelles which are peculiar to plant cells.

Plastids that contain high concentration of carotenoid pigments are called ‘chromoplasts’. They give yellow, orange and red colors to many fruits (tomato), roots (carrot) and flower petals.

Nonpigmented plastids are called ‘leucoplasts’. An important type of leucoplast is ‘amyoplast’ which is a starch-storing plastid.

Chloroplasts are the plastids that contain green pigment, chlorophyll. They are found in green tissues of plant, especially leaf. They are absent in roots.

The chloroplast is surrounded by the inner and outer membranes.

Chloroplasts also contain third system of membrane called thylakoid. All the chlorophyll is contained within this membrane, which is the site of light reactions of photosynthesis.

Thylakoid membranes are highly folded and appear like stacked coins. These stacked membranes are known as grana lamellae (or grana thylakoid). The membranes without stacking are known as stroma lamellae (or stroma thylakoid). Each stack is called granum.

The inner space within a thylakoid is known as lumen.

The region of the chloroplast that is inside the inner membrane and surrounds thylakoids is known as stroma. The carbon reactions take place in stroma.

Chloroplasts contain their own DNA and protein-synthesizing machinery.

Chloroplast genome is smaller (145 kb) than mitochondrial genome (200 kb). Chloroplast DNA is circular and histone-free.

Ribosomes occur free in stroma or bound to the outer surface of thylakoid membrane. As is mitochondria, most of the chloroplast's proteins are encoded by nuclear genes, synthesized in cytosol and transported to organelle.

Central Vacuole

Mature plant cells contain large, water-filled central vacuole (usually one or two). Central vacuole can occupy 80-90 % of the total volume of cell.

Each vacuole is surrounded by a vacuolar membrane or tonoplast.

The vacuole contains water, inorganic ions, organic acids, sugars and enzymes.

Like animal lysosomes, plant vacuoles contain hydrolytic enzymes including proteases, ribonuleases and glycosidases.

Vacuole has storage function as well as to provide rigidity to plant cell.

Microbodies

Plant cells also contain microbodies, which are spherical organelles surrounded by a single membrane.

The two main micobodies are peroxisomes and glyoxysomes.

Peroxisomes are present in photosynthetic cells of plant leaf. Their function is the removal of

potentially toxic hydrogen peroxide (H_2O_2) using the enzyme catalase.

$$H_2O_2 \xrightarrow{\text{Catalase}} H_2O + \tfrac{1}{2}\ O_2$$

Glyoxysomes are present in oil-storing seeds. They can convert stored fatty acids into sugars that can be translocated in the plant to provide energy for growth.

Cytoskeleton

The cytosol in organized into a 3-dimensional network of filamentous proteins called 'cytoskeleton'.

Cytoskeleton serves as scaffolding for the movement of organelles and other components. Cytoskeleton plays an important role in maintenance of cell shape as well as in cell division. Basically 2 types of cytoskeletal elements are found in plant cells; microtubules and microfilaments.

Microtubules are hollow cylinders with an outer diameter of 25 nm.

Microtubules are composed of polymers of the globular protein 'tubulin'.

A single microtubule consists of thousands of tubulin monomers arranged in 13 columns called protofilaments.

Microfilaments are solid with 7 nm diameter.

Microfilaments are composed of protein globular actin (or G-actin).

Experiment - 2

Theory

Stomata are minute pores found on the epidermis of leaves and young shoots of plants that are used to control exchange of gases. The pore is surrounded by a pair of specialised cells called the guard cells that are responsible in regulating the size of the opening.

Water is released through the stomata into the atmosphere in the form of water vapour through the process called transpiration. Besides this, the exchange of oxygen and carbon dioxide in the leaf also occurs through the stomata.

Distribution of Stomata

Distribution of stomata varies between monocots and dicots, between plant species, and between the underside and top side of the leaves on a plant.

Stomata are found more on plant surfaces thriving under higher light, lower atmospheric carbon dioxide concentrations and in moist environments.

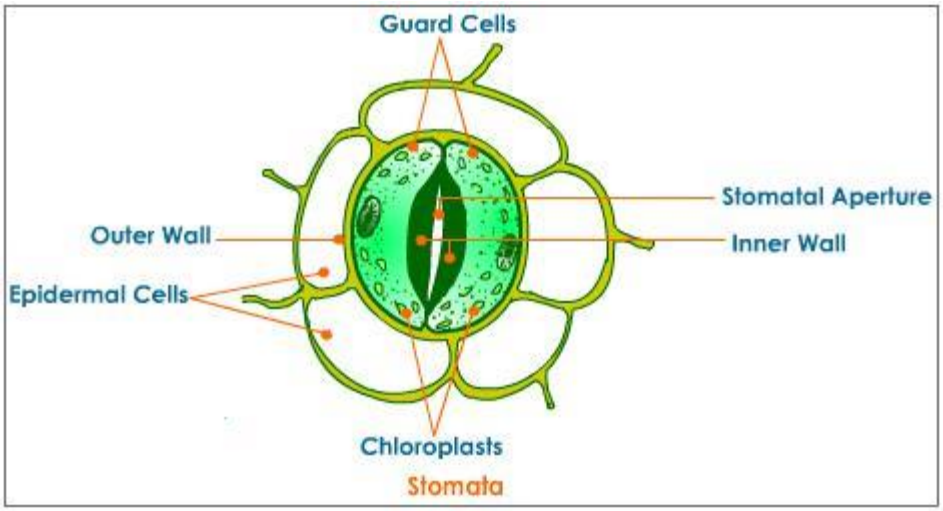
Usually the lower surface of a dicot leaf has a greater number of stomata while in a monocot leaf they are more or less equal on both surfaces. In most of the floating plants, stomata are found only on the upper epidermis.

Materials Required

Fresh leaf, Glycerine, Safranin solution, Forceps, Needle and brush, cover slip, compound microscope, Distilled water, dropper, blade, watch glass, glass slide

Procedure

- Pluck one fresh leaf of a four-o'clock plant.
- Take two watch glasses and pour some distilled water into the both watch glasses.
- Split the leaf from the four-o'clock plant obliquely.
- Take the peel from the upper surface of the leaf using the forceps.
- Place the peel into a watch glass containing water.
- Take another peel from the lower surface of the leaf using the forceps.
- Place the peel into the other watch glass containing water.
- Using a dropper, take few drops of Safranin solution and put it into the two watch glasses.
- Take two clean glass slides and place the leaf peel on the slides one by one, using a brush.
- Take a blade and cut a small rectangle or square piece from each peel.
- Take some glycerine using a dropper and put one drop of glycerine on both slides.
- Take a cover slip and place it gently on the peel with the help of a needle.
- Take the glass slide and place it under compound microscope.
- Observe under the microscope.
- Count the number of stomata in the peels of both upper and lower epidermis of the leaf appearing in the microscopic field.



Experiment - 3

Objective

To determine the percentage of water imbibed by gram seeds.

Theory

Gram seeds when soaked in water swell up due to imbibition. As a result of absorption or imbibition of water, the size of the raisins increases. The difference in mass between the swollen and dry raisins gives the amount of water imbibed by the raisins.

Imbibition is the process of adsorption of water by substances without forming a solution. Swelling of seeds when immersed in water is an example of imbibition. Imbibition is the temporary increase in the volume of the cell. Imbibition is a passive transport of materials that does not require energy during the process.

The substance that imbibes water is called imbibant and the liquid which is imbibed is called adsorbent. The process of imbibition occurs mainly due to the presence of hydrophilic or lyophilic colloids. Water is imbibed through the sub microscopic capillaries present on the surface of the body.

The movement of water into the plant parts continues until a dynamic equilibrium is attained. Imbibition of water increases the volume of the imbibant, which results in imbibitional pressure.

Materials Required

Blotting paper, petridish, gram seeds, spatula, electronic balance, distilled water, small beaker

Procedure

- Take about 20 dry raisins that have intact stalks.
- Weigh the raisins on an electronic balance and note the weight.
- Pour some distilled water into a beaker.
- Transfer the raisins from the balance into the beaker.
- Allow the raisins to soak for 2-3 hours.
- Remove the swollen raisins from the water using a spatula and put them in a Petri dish containing a blotting paper.
- Gently dry the raisins using another blotting paper.
- Weigh the swollen raisins on the electronic balance and note the weight.

Observations

The weight of dry raisins is,x… gm

The weight of swollen raisins is, y…gm

Calculations

Weight of water absorbed by the raisins = $(y-x)gm$

Percentage of water absorbed by the raisins = $\frac{(y-x)}{x}$

Precautions

Raisins should be clean and dry and should have intact stalks.

Experiment - 4

AIM: To demonstrate the process of osmosis with varying solution concentration

Material Required: Potato tubes, potato peelers, knives, ruler, petri-dshes, and sucrose solutic 0.8M.

Procedure:

- Prepare 1 molar solution of sucrose.
- Now prepare the rest of the solutions as following.

1. Take 4 petri-dishes and label them 1,2,3,4 and 5.
2. With the help of knife take 4 potato strips of the size of 3 cm x 0.5 cm x 0.5 cm .
3. Record the initial weight and length.
4. Place each piece in petri-dishes labeled 1, 2, 3,4 and 5 containing 0.2M, 0.4M, 0.6M , 0. in each.
5. Cover the petri-dishes and keep them aside.
6. After 30 minutes, take out the piece from the petri-dish 1, dry it on a filter paper and mea
7. Repeat the procedure with the pieces kept in petri-dishes2, 3,4 and 5.
8. Record the length and weight of the pieces in a tabular form.

Serial dilution table for preparation of final sucrose concentration.

	Amount of distilled water to be added	**Amount of sucrose solution to be added**	**Final sucrose concentration**
1	8ml	2ml	0.2M
2	6ml	4ml	0.4M
3	4ml	6ml	0.6M
4	2ml	8ml	0.8M
5	0ml	10ml	1 M

OBSERVATION:

Table showing change in the size and mass of the potato tissue

Sucrose solution	At the start		After 30 minutes		Change in	
	Length	Weight	Length	Weight	Length	Weight
0.2M						
0.4M						
0.6M						
0.8M						
1M						

Experiment - 5

Aim:- To demonstrate the process of plasmolysis using onion cells.

Theory

Plasmolysis is the process of shrinkage or contraction of the protoplasm of a plant cell as a result of loss of water from the cell. Plasmolysis is one of the results of osmosis and occurs very rarely in nature, but it happens in some extreme conditions. We can induce plasmolysis in the laboratory by immersing living cell in a strong salt solution or sugar solution to lose water from the cell.

The cell membrane is a semipermeable membrane that separates the interior of all cells from the surrounding environment. The semipermeable membrane allows some particles, ions, or water molecules across the membrane, but blocks others. Water molecules constantly move inside and outside the cell across cell membranes. This free flow of water has the very important consequence of enabling cells to absorb water.

Plasmolysis and deplasmolysis

When a plant cell is immersed in concentrated salt solution (hypertonic solution), water from the cell sap moves out due to exosmosis. Exosmosis is the passage of water from higher water concentration to lower water concentration through a semipermeable membrane.

When a plant cell is placed in concentrated salt solution, water concentration inside the cell is greater than that which is outside the cell. Therefore, water moves through the cell membrane into the surrounding medium. Ultimately the protoplasm separate from the cell wall and assumes spherical shape. It is called plasmolysis.

When a plasmolysed cell is placed in a hypotonic solution, (i.e., the solution having solute concentration lower than the cell sap), the water moves into the cell because of the higher concentration of water outside the cell than in the cell. The cell then swells to become turgid. It is called deplasmolysis.

If we place living cells in isotonic solution (i.e., both solutions have the same amount of solute concentration), there is no net flow of water towards the inside or outside. Here, the water moves in and out of the cell and is in equilibrium, so the cells are said to be flaccid.

Materials-required:
Onion bulb, watch glass, petri-dish, slides, cover-slips, forceps, brush, needles, microscope and 20% concentrated sucrose solution.

Procedure :

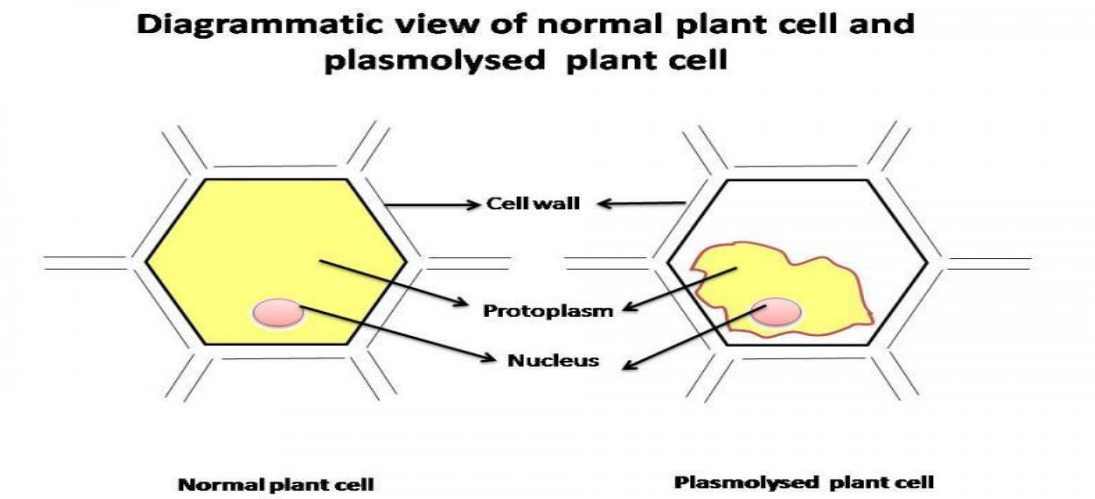
- Take an onion bulb, with the help of forceps pull a thin transparent peel gently.
- Keep this peel in water filled watch glass.
- Transfer the peel gently on a clean slide in a drop of water with the help of a brush and needle.
- Examine it under high power of a microscope.(40X)
- Observe the individual cells and make a sketch of the cells showing the cell wall and cell membrane. **(observation 1)**
- With the help of dropper put the sucrose solution on the slide by the sides of cover-slip so that it reaches the peel under the cover-slip.
- Examine the peel again after 10 mins. **(observation 2)**
- Drain out the concentrated sugar solution from the peel and add few drops of water into the peel.
- Observe the cells again after 10 mins**.(observation 3)**

Observation table:

	Condition of the cell	Explanation
Observation 1		
Observation 2		
Observation 3		

Inference:

- When the peel of onion is kept in concentrated solution (hypertonic), the protoplasm shrinks as the water starts moving out due to exosmosis.
- This phenomenon of shrinkage of protoplasm when the cells are kept in a concentrated solution is known as plasmolysis.
- Further, when the cells are kept in water (hypotonic solution) the protoplasm again regains its original shape due to movement of water into the cells by the process of endosmosis. This phenomenon is called deplasmolysis.



Experiment -6

Objective

Our objective is to compare the rate of transpiration between the upper and lower surfaces of a leaf.

Theory

Transpiration is the process of water movement through a plant and its evaporation into the atmosphere from its aerial parts. In leaves and in young shoots the epidermal layer contains minute microscopic pore like structures called stomata. Transpiration occurs chiefly through the stomata of the leaves. The stomata are mainly concerned with exchange of gases during the process of photosynthesis and respiration. Each stomata has a slit like opening called the stomatal pore, which is surrounded by two special cells called the guard cells. These special cells help to regulate the rate of transpiration by opening and closing the stomata.

Materials Required:

A healthy potted plant, forceps, filter paper strips, wire gauze, 3% cobalt chloride solution, clips, petridish, glass slide.

Procedure:

- Take 3 % cobalt chloride solution from beaker and pour into the Petri dish.
- Take some filter paper strips and dip them in the cobalt chloride solution.
- Keep the strips in the solution for 3-5 minutes. They become pink in colour when wet.
- Remove the strips from the solution using forceps.

- Place the strips on the wire gauze to allow them to dry.
- The filter paper becomes blue in colour on drying.
- Select one healthy leaf and clean the leaf to remove the water droplets using a filter paper.
- Take the dry pieces of cobalt chloride paper from the wire gauze.
- Place the dried strips of cobalt chloride paper: one on the upper and the other on the lower surface of a leaf of the potted plant.
- Take two glass slides and place one over the upper and the other over the lower side of the leaf.
- Clip the slides together using binder clips.
- Note the time taken by the cobalt chloride paper to change its blue colour to pink.

Note: Dry cobalt chloride paper that is blue in colour turns pink when it comes in contact with water. Using this property of cobalt chloride paper we can study the rate of transpiration from the two surfaces of a leaf by comparing the loss of water vapour from the two surfaces of the leaf.

Observation

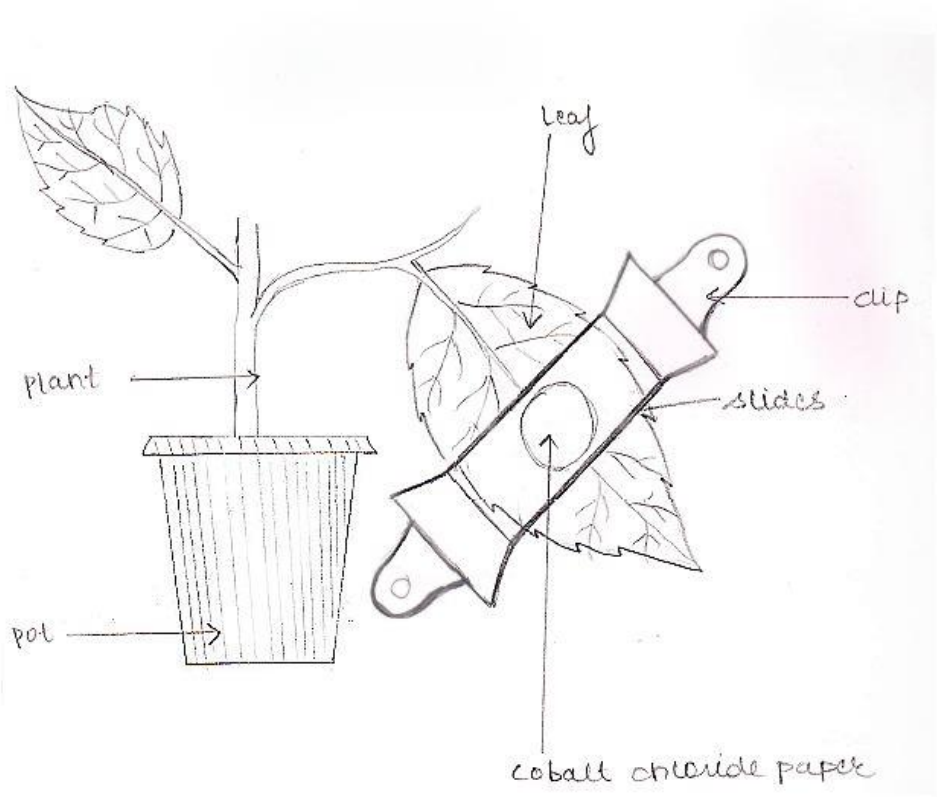
The time taken to change colour of the cobalt chloride paper from blue to pink on the lower leaf surface is less than the upper surface.

Conclusion

The quick change in the colour of cobalt chloride paper on the lower surfaces indicates higher rate of loss of water vapour from this surface than the upper one.

Precautions

- Always use a well watered potted plant for the experiment.
- Always handle the dried cobalt paper with dry hands or forceps.
- The leaf surface should not be wet while applying the cobalt chloride strips.



Experiment -7

Aim:- To separate and study plant photosynthetic pigments by paper chromatography.

Theory:-

Photosynthetic plants convert light energy from the sun to chemical food energy. During photosynthesis, molecules referred to as pigments are used to capture light energy. Pigments are chemical compounds which reflect only certain wavelengths of visible light. Plant leaves contain four primary pigments: chlorophyll a (dark green), chlorophyll b (yellowish-green), xanthophylls (yellow) and carotenoids (orange).

In paper chromatography, the mixture is spotted onto the paper, dried and the solvent is allowed to flow along the sheet by capillary attraction. As the solvent slowly moves through the paper, the different compounds of the mixture separate into different coloured spots. The paper is dried and the position of different compounds is visualized. The principle behind the paper chromatography is that the most soluble substances move further on the filter paper than the least soluble substances.

Materials Required:

Spinach leaves, chromatography chamber, motar and pestle, ether acetone solvent, scissors, acetone, pencil, capillary tube, spatula, scale, filter paper strips, watch glass, thread, stapler

Procedure:

- Take a few freshly plucked green spinach leaves.
- Using scissors, cut the spinach leaves into small pieces and let them fall into the mortar.
- Take a measuring cylinder that contains 5ml of acetone and pour it into the mortar.
- Grind the spinach leaves using the mortar and pestle.
- Place the extract into a watch glass using a spatula.
- Take a strip of filter paper having a narrow notch at one end of the strip.
- Take a pencil and a scale and draw a horizontal line with a pencil about 2-3 cm away from the tip of the notch.
- Put a drop of the pigment extract in the middle of the line with the help of a capillary tube.
- Allow the drop to dry and repeat till four or five drops are placed on the paper.
- Take the chromatographic chamber and pour ether acetone solvent in it.
- Fold one end of the filter paper strip and staple it.
- Using a thread, hang the filter paper strip in the chromatographic chamber.
- The loading spot should remain about 1 cm above the solvent level.
- Leave the chromatographic chamber undisturbed for some time.
- We can observe, as the solvent moves through the paper, it spreads the different pigments of the mixture to various distances.
- When the solvent rises about 3/4th up the strip, remove the strip carefully and let it dry.

Observation

The dried chromatographic paper strip shows four distinct paper bands. Different pigments can be identified by their colours.

Calculations

Rf Value of the each pigment spot can be calculated as;

Rf = (Distance travelled by the compound)/(Distance travelled by the solvent)

Result

The topmost orange yellow band of pigments in the separation corresponds to carotene. The yellowish band appearing below it indicates the xanthophylls. The third from above dark green band represents chlorophyll a. The lowermost yellowish green band is that of chlorophyll b.

Precaution

- Spinach leaves should be fresh and green.
- The loading spot should be 2-3 cm away from the tip of the notch.

- While hanging the strips in the chromatography chamber, the loading spot should remain about 1 cm above the solvent level.

Pigment	Distance travelled by different pigments	Distance travelled by the solvent	R_f value
Carotene	6.65cm	7 cm	0.95
Xanthophyll	5.25cm	7 cm	0.71
Chlorophyll a	4.55cm	7 cm	0.65
Chlorophyll b	3.15cm	7 cm	0.45

Experiment -8

Aim

To study the rate of respiration in germinating seeds having different substances such as carbohydrates, fats and proteins.

Theory

Respiration is the process during which simple carbohydrates, like glucose, break down into simpler substances and liberate carbon dioxide and energy. The compound used, or oxidized, during respiration is called a respiratory substrate. Carbohydrates, fats, and proteins are examples of respiratory substrates, and carbohydrates are the preferred respiratory substrate among them. The rate of respiration can be measured in terms of gas exchange, that is, consumption of the respiratory substrate oxygen, or evolution of carbon dioxide.

Materials Required

Test tube, Distilled water, germinating seeds of bean, a cork with a hole, measuring cylinder, a conical flask, thread, freshly prepared potassium hydroxide solution,

Procedure

- Using a spatula, place about 30 germinating bean seeds in a conical flask.
- Pour 4ml of potassium hydroxide solution into a measuring cylinder.
- Transfer the potassium hydroxide solution from the measuring cylinder into a small test tube.
- Tie a cotton thread around the neck of the test tube.
- Suspend the test tube in the conical flask above the germinating seeds.
- Close the mouth of the conical flask with a cork.

- Insert one end of a delivery tube into the conical flask through the cork and dip the other in a beaker containing water.
- Observe the position of the water level in the delivery tube.
- Keep the apparatus undisturbed for two hours.

Observation

After two hours, you will see that the level of water has risen in the delivery tube at the end dipped in the beaker of water.

Conclusion

The rise in water level at the end of the bent glass tube proves that the germinating seeds release CO_2 during respiration and requires large amount of O_2 which creates a pressure inside the conical flask due to which the water is sucked in upward direction.