SEMESTER III

17BTU311 PLANT PHYSIOLOGY PRACTICAL 4H - 2C

Total hours/week: L:0 T:0 P:4 Marks: Internal: 40 External: 60 Total: 100

Practical

1. Preparation of stained mounts of anatomy of monocot and dicot's root, stem & leaf.
2. Demonstration of plasmolysis by *Tradescantia* leaf peel.
3. Demonstration of opening & closing of stomata
4. Demonstration of guttation on leaf tips of grass and garden nasturtium.
5. Separation of photosynthetic pigments by paper chromatography.
6. Demonstration of aerobic respiration.
7. Preparation of root nodules from a leguminous plant.
8. Estimation of stress indicators – Proline and osmolyte estimation

References

1. Dickinson, W.C. (2000). *Integrative Plant Anatomy.* USA: Harcourt Academic Press.
2. Nelson, D.L., & Cox, M.M. (2004). *Lehninger: Principles of Biochemistry* (4th ed.). New York: USA, W.H. Freeman and Company.
3. Salisbury, F.B., & Ross, C.W. (1991). *Plant Physiology*. Wadsworth Publishing Co. Ltd.
4. Taiz, L., & Zeiger, E. (2006). *Plant Physiology* (4th ed.). MA: USA, Sinauer Associates Inc.

Lecture Plan

S. No	Lecture Duration Period	Name of the practical	Support Material/Page Nos
1	4	Preparation of stained mounts of anatomy of monocot and dicot's root, stem & leaf	Taiz, L., & Zeiger, E. (2006). *Plant Physiology* (4th ed.). MA: USA, Sinauer Associates Inc
2	4	Demonstration of plasmolysis by *Tradescantia* leaf peel	
3	4	Demonstration of opening & closing of stomata	
4	4	Demonstration of guttation on leaf tips of grass and garden nasturtium	
5	4	Separation of photosynthetic pigments by paper chromatography	
6	4	Demonstration of aerobic respiration	
7	4	Preparation of root nodules from a leguminous plant	
8	4	Estimation of stress indicators – Proline and osmolyte estimation	

1. Preparation of stained mounts of anatomy of monocot and dicot's root, stem & leaf

Aim: To prepare temporary stained glycerine mounts of transverse sections of stem and root of Dicot and Monocot plants.

Material required: Sharp razor, brush, dropper, needles, watch glass, microscopic slides, cover-slips, safrannin, glycerine and compound microscope.

Procedure:

1. Take 2-3cm long pieces of the material.
2. Hold the material between thumb and first finger of your left hand.
3. Hold the razor in the right hand with edge of the blade facing you and handle at right angle to it.
4. Dip the top of the material in water.
5. Then start cutting transverse sections as fast as possible in a watch glass containing water.
6. Select the thinnest section of the material with the help of a delicate brush.
7. Take a clean watch glass with water, transfer thin sections of the material.
8. Put a few drops of saffranin stain in the watch glass with water.
9. Leave it for 3-5 minutes.
10. Drain off stain and wash with water if necessary.
11. Put the thinnest section in the centre of the slide.
12. Put a drop of glycerine over the material.
13. Cover it with a coverslip with the help of needle.
14. Observe it under a compound microscope after staining and mounting.
15. Study under the microscope: Focus the slide under lower of microscope and then change to high power if needed

Precautions:

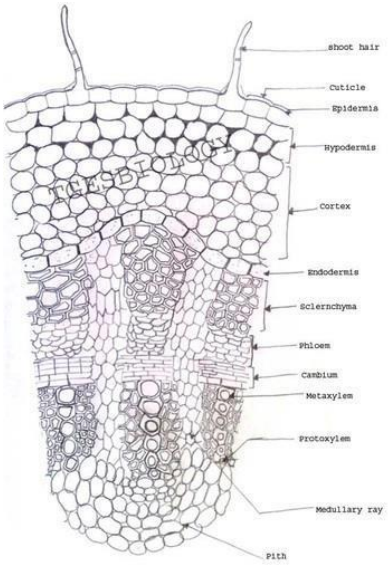
- Safranin is to be used to stain only the lignified tissues, over staining can be removed by washing in water.
- Air bubbles must be avoided in the sections.
- Use only brush to transfer or to handle the sections. Do not use needles for this purpose.
- Discard the incomplete and oblique sections.

A. Temporary slide preparation of Dicot stem

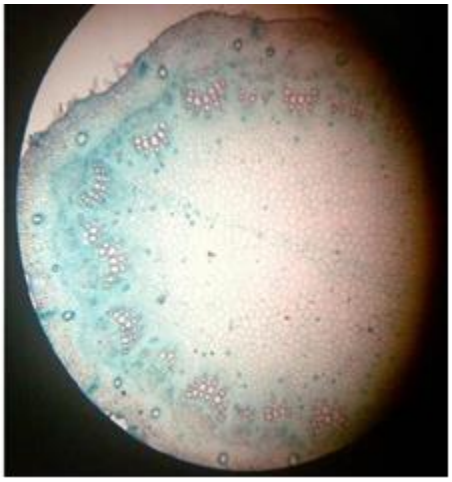
Identifying characteristics-

- Multicellular hair present on the epidermis.
- Hypodermis collenchmatous.
- Xylem endarch (metaxylem towards periphery and protoxylem towards centre)
- Vascular bundles are arranged in a ring.
- Vascular bundles conjoint, collateral and open.(cambium present)

Inference: The given specimen is the section of dicot stem.



T.S of dicot stem (for drawing)



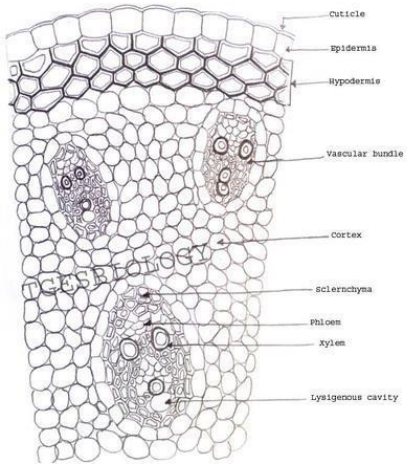
T.S of dicot stem as seen under microscope.

B. Temporary slide preparation of monocot stem

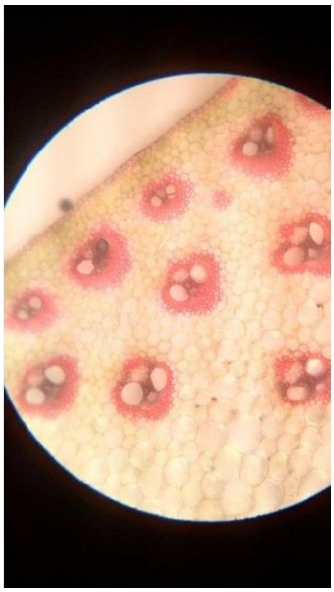
Identifying characteristics-

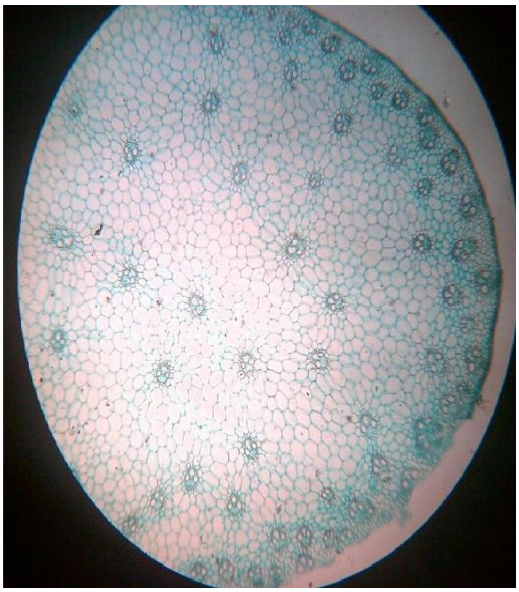
- Hypodermis is sclerenchymatous.
- Cortex is not differentiated into endodermis and pericycle.
- Vascular bundles are scattered in the ground tissue.
- Vascular bundles are conjoint,collateral and closed, i.e cambium is absent.
- Each vascular bundle is surrounded by a bundle sheath
- Xylem is y-shaped and metaxylem lies towards periphery.

Inference: The given specimen is the section of monocot stem.



T.S of monocot stem (for drawing)





Section of monocot stem as seen under microscope

Section of monocot stem as seen under microscope with details of vascular bundles.

C. Temporary slide preparation of dicot root

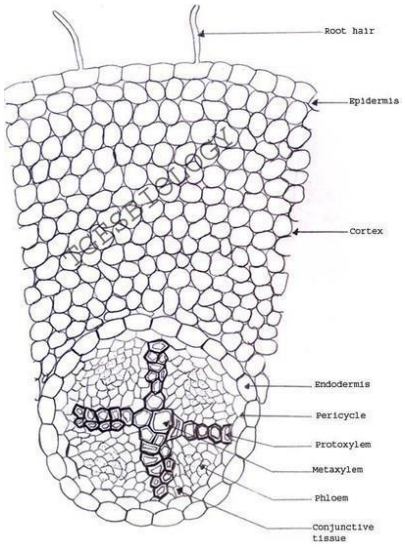
Identifying characteristics:

- Unicellular hair are present on the epidermis.
- Hypodermis is absent
- Vascular bundles are radial. Xylem and phloem are present on separate radii.

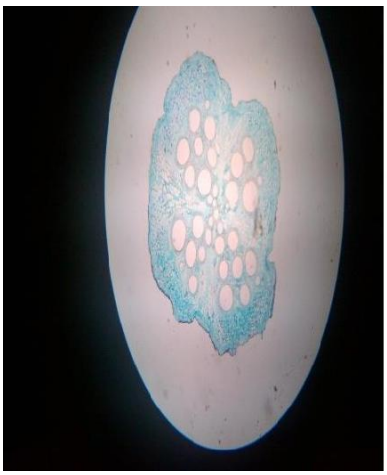
B Xylem and phloem bundles are less than 6.

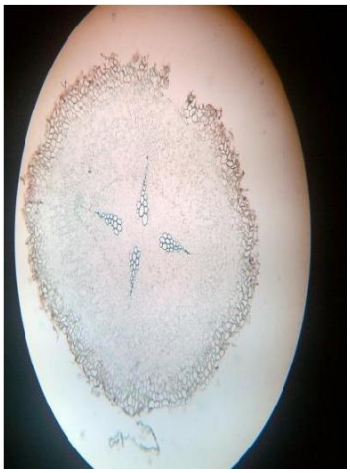
C Protoxylem lies towards periphery and metaxylem lies towards centre.

Inference: the given specimen is the section of dicot root



T.S of dicot root (for drawing)





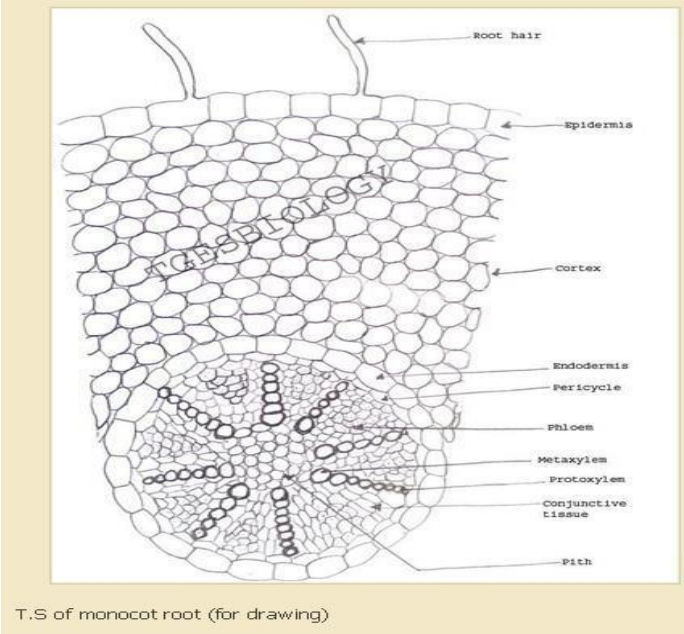
Section of dicot root as seen under the microscope

D. Temporary slide preparation of monocot root

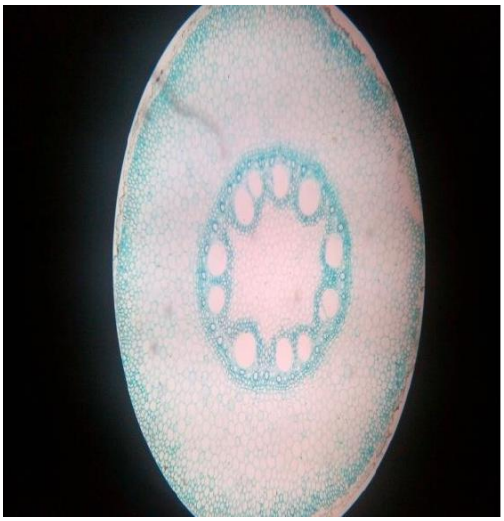
Identifying characteristics:

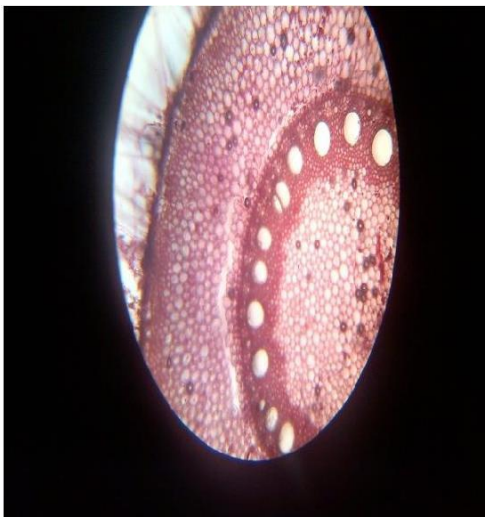
- Unicellular hairs are present on the epidermis.
- Hypodermis is absent
- Vascular bundles are radial. xylem and phloem are present on separate radii.
- Xylem or phloem bundles are more than 5.
- Metaxylem lies towards centre.

Inference: the given specimen is the section of monocot root.



T.S of monocot root (for drawing





Section of monocot root as seen under the microscope.

2. Demonstration of plasmolysis by *Tradescantia* leaf peel

Aim: To demonstrate the phenomenon of plasmolysis by *Tradescantia* leaf peel.

Principle:

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. Normally people use Rheo or Tradescantia plant epidermal cell for experiment because they have coloured cell sap which can be clearly visible.

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.

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 Requirements:

Tradescantia leaf, water, sugar solution, slides, cover glass, microscope, blade.

Procedure:

- Take two glass slides and place them on the table.
- Take a rhoeo leaf from the Petri dish.
- Fold the leaf and tear it along the lower side of the leaf.
- Using a forceps, pull out two small segments of thin transparent layer from the lower epidermis of the rhoeo leaf.
- Place the epidermal peels on both glass slides.
- Using a dropper, take some sodium chloride 0.1% solution from the beaker.
- Put 1 to 2 drops of solution on one slide.
- Using another dropper, take sodium chloride 5% solution from the beaker.
- Put 1 to 2 drops of solution on the next slide.
- Place a cover slip over the peel of both slides using a needle.
- Place the slides one by one under the compound microscope.
- Observe them under the microscope.

Observations:

Peelings mounted in the water show clear cell structure. But the peelings placed in the sugar solution show the Concentra in their cell contents. More the concentration of sugar solution more is the contraction and shrinkage of cell contents. Peelings mounted in very high concentrated sugar solution, when observed under microscope, show complete shrinkage of their cell contents

which become round or ball-like.

Results:

Shrinkage of the cell contents in the peelings mounted in conc. sugar solution is due to the fact that the osmotic pressure of the outer sugar solution is higher than that of the osmotic pressure of the cell sap. So the water from the cell sap diffuses into the external sugar solution through the semipermeable plasma membrane of the cell. Thus there is a shrinkage of the cell contents and this phenomenon is known as plasmolysis (Fig. 7). If slightly plasmolysed (incipient plasmolysis.) cells are now kept in pure water, these will show the phenomenon of endosmosis and the cells will recover soon. This indicates the phenomenon of deplasmolysis.

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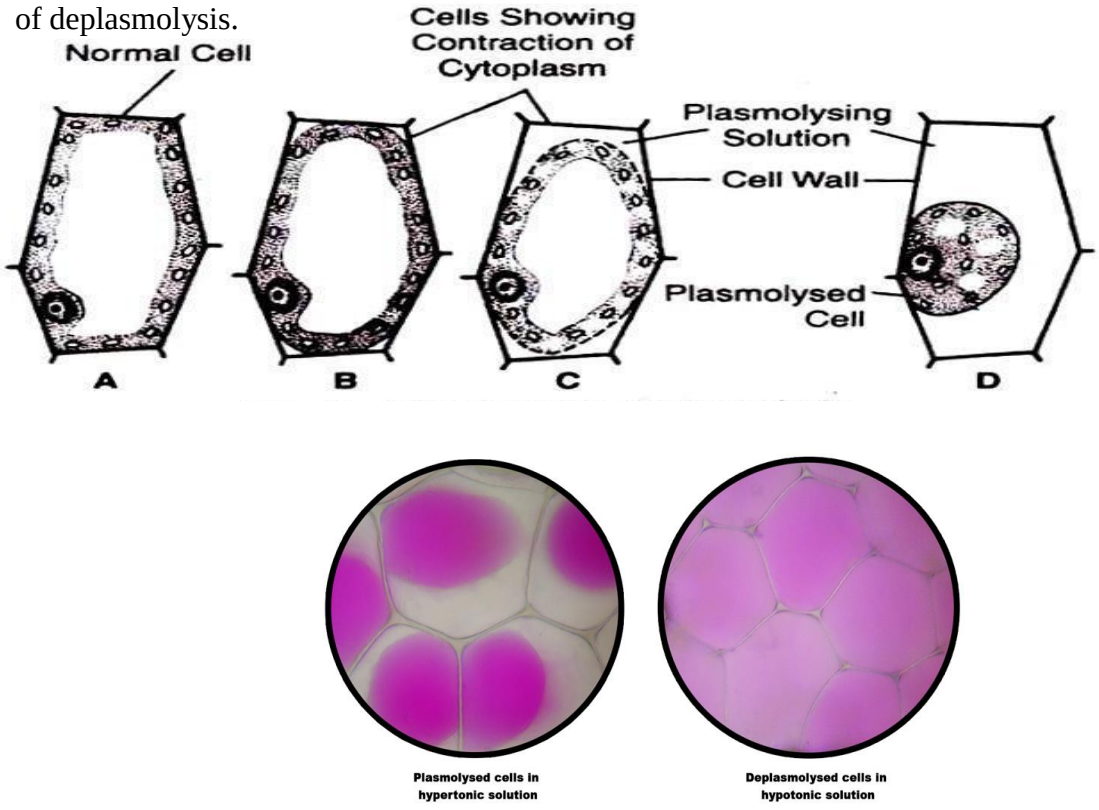


Fig. Demonstration of plasmolysis and deplasmolysis in peels of the Rheo plant.

Conclusion

When plant cells are immersed in sodium chloride 5 % solution or concentrated salt solution, water moves through the cell membrane into the surrounding medium because the water concentration inside the cell is greater than that which is outside the cell. Ultimately the protoplasm causes shrinkage and assumes spherical shape. This is called plasmolysis. When a plant cell is immersed in sodium chloride 0.1% solution or dilute salt solution, the water moves into the cell because of the higher concentration of water outside the cell than inside the cell. The cell then swells and becomes turgid.

Precautions

- Take the epidermal peel from the lower surface of rhoeo leaf.
- Do not let the peel dry out.

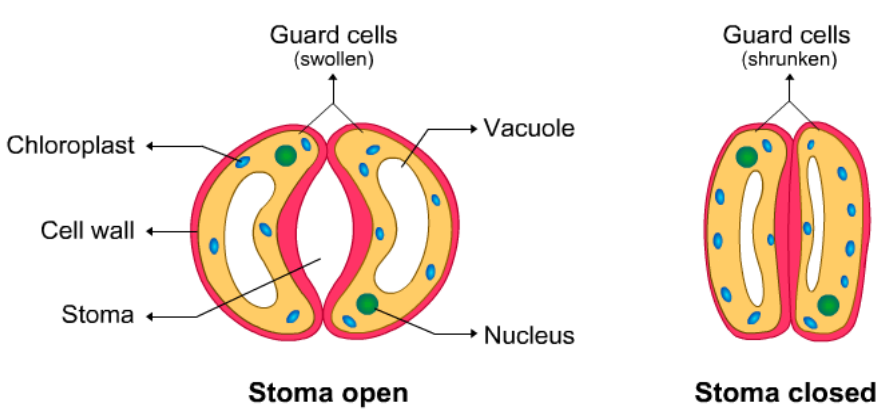
3. Demonstration of opening & closing of stomata

Principle:

Stoma (plural stomata), a word derived from Greek which means 'mouth', is a pore found in the epidermis of leaves, stems and all other plant parts found above the ground. Stomata are thus named because they permit the exchange of gases between the atmosphere and the inside of the leaf.

Structure of Stomata

The size and shape of stomata vary in different plants. The structure of the stomata consists of a kidney shaped epidermal cell with an opening in the centre known as the pore. The stomata are bordered by a pair of specialised parenchyma cells known as the guard cells that are responsible for regulating the size of its opening, thus saving the plant from water loss. When fully stretched, the guard cells expand and thereby open the stomata. When the guard cells lose water, they shrink and the stomata will close. Guard cells also have large vacuoles. The cells that surround the guard cells are known as subsidiary or accessory cells. The guard cells in dicot plants are kidney shaped and dumbbell shaped in monocots.



Position of Stomata

Although stomata are found on the epidermis of leaves, the exact positioning is different from plant to plant. Stomata are found on either both sides or on just one side of the leaf. When the stomata are present on both sides of the leaf, they are called amphistomatic; epistomatic, if present on the upper side; and hypostomatic, if present on the lower side.

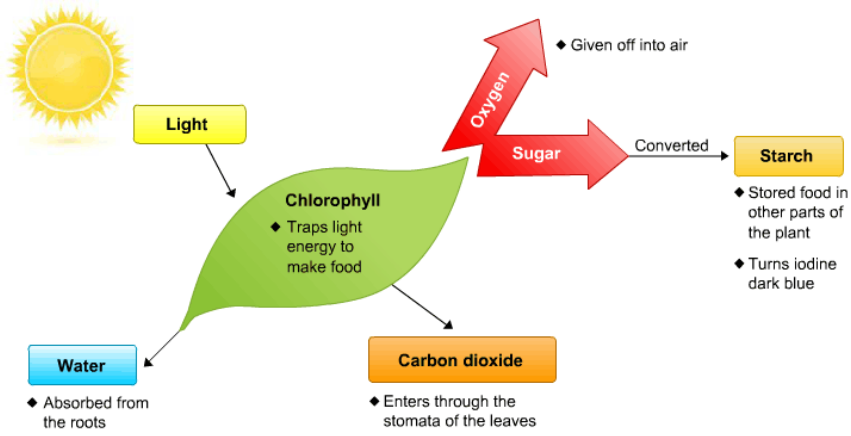
The distribution of stomata varies in different plants. There are more stomata on plant surfaces in conditions like lower atmospheric carbon dioxide concentrations, moist environments and higher light. In dicot plants the number of stomata is greater on the lower surface than on the upper surface of the leaf. On the other hand, monocot plants have the same number of stomata on both surfaces of the leaf. In the case of floating plants, stomata is found only on the upper epidermis.

Functions of Stomata

The major function of stomata is the exchange of gases by taking carbon dioxide from the atmosphere and giving out oxygen that is used by human beings and animals. They help in photosynthesis and transpiration.

Photosynthesis

Photosynthesis is a process used by plants to manufacture food with the help of sunlight, carbon dioxide and water. It is through the stomata that the plant takes carbon dioxide from the atmosphere. The water molecule is broken down into hydrogen and oxygen, and it is through the stomata that the oxygen is then released as a by-product in the atmosphere. Stomata are the medium of gaseous exchange and cellular respiration in plants.



Transpiration

Transpiration is a process of evaporation of water from the surface of the plant. This keeps the plant cool and transfers minerals and other materials to different parts of the plant. As the plant takes water from the soil, the openings absorb other minerals. For a plant to take water from the soil, water needs to evaporate from the surface of the plant. Once this happens, pressure is developed that forces the roots to absorb water from the soil and transfer it to the tips of the plant. It is through the stomata that the major work of evaporation of water is done.

Materials Required:

A potted Tradescantia or Bryophyllum plant, forceps, needles, watch glasses, glass slides, a dropper, coverslips, a brush, blotting paper, safranin, glycerine and a compound microscope.

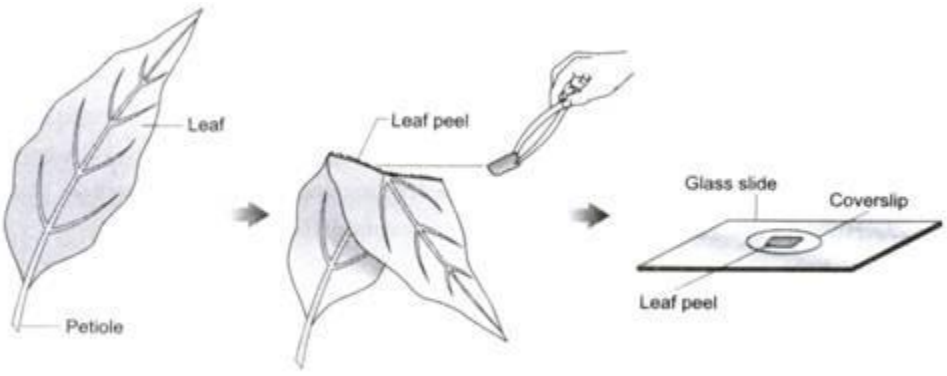
Procedure

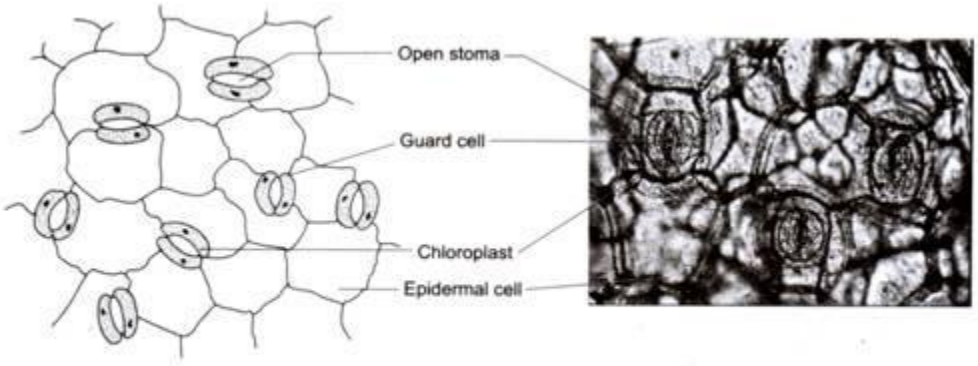
1. Pluck a fresh leaf from a balsam plant.
2. Fold the leaf and carefully tear along the bruised area of the lower side of the leaf.
3. We can see a colourless narrow border along the torn edge.
4. Carefully pull out the thin membranous transparent layer from the lower epidermis using a forceps.

5. Put the epidermis into a watch glass containing distilled water.
6. Take few drops of Safranin solution using a dropper and transfer this into another watch glass.
7. Using a brush transfer the epidermis into the watch glass containing the Safranin solution.
8. Keep the epidermis for 30 sec in the Safranin solution to stain the peel.
9. To remove excess stain sticking to the peel, place it again in the watch glass containing water.
10. Place the peel onto a clean glass slide using the brush.
11. Take a few drops of glycerine using a dropper and pour this on the peel.
12. Using a needle, place a cover slip over the epidermis gently.
13. Drain out the excess glycerine using a blotting paper.
14. Take the glass slide and place it on the stage of the compound microscope.
15. Examine the slide through the lens of the compound microscope.

Observations

- The epidermis is made of uniseriate layers of cells that have distinct cell walls, a nucleus and cytoplasm, and are closely packed.
- The epidermal layers are broken at places. These openings are the stomata.
- Each stoma is guarded by a pair of bean shaped cells that are guard cells.





(a) Mounting a leaf peel (b) Epidermal layer in the peel taken from a dicot leaf showing open stomata (c) High-power magnification of stomata

Precautions

- The epidermal peel should be taken from a freshly-plucked leaf.
- Take the epidermal layer from the lower surface of a leaf, as it has more stomata.
- Always use a clean glass slide.

4. Demonstration of guttation on leaf tips of grass and garden nasturtium.

Aim: To demonstrate the process of guttation with entire potted plant.

Principle:

Most water is lost from plants in the form of vapors. However, some water may be exuded in liquid form, a process called guttation.

Requirements:

A potted plant of garden nasturtium, water, bell jar (Instead of garden nasturtium other plants like oat seedlings, wheat seedlings, tomato, Colocasia, etc. may also be taken).

Procedure:

1. Take a potted plant of garden nasturtium and water it copiously.
2. Cover the pot along the plant with a bell jar and place it in a cool and dark place.
3. Connect the apparatus to an aspirator and make it air-tight (Fig).

Fig. 17. Demonstration of guttation.

4. Keep the experiment a for a few hours and observe the changes.

Observations:

Slow exudation of water begins at the tip of each leaf. These water drops gradually enlarge and may fall off or run down the side of the leaf.

Results:

This exudation of water is due to the phenomenon of guttation. When the plant is copiously watered then water is forced from the xylem vessels through intercellular spaces and out of plant from pore-like structures (called hydathodes, water pores or water stomata's, Fig. 19) present at the margins of the leaves.

Water exudes through hydathodes with the help of a pressure developed in the sap of the xylem elements. It is believed to be a pressure identical with the root pressure. The exuded water also contains amino acids, mineral salts, sugars and traces of other solutes.

Guttation occurs abundantly when the conditions are such that absorption of water by the roots is very high and the rate of transpiration is very slow. Guttation can also be demonstrated with a single freshly cut leaf of garden nasturtium when it is fixed on one end of a U-tube fitted with a cork and filled with water. From the other end of the U-tube add a little amount of mercury which helps in forcing the water in the petiole.

5. Separation of photosynthetic pigments by paper chromatography

Principle:

Before going into detail, let's understand the role of pigments in plants.

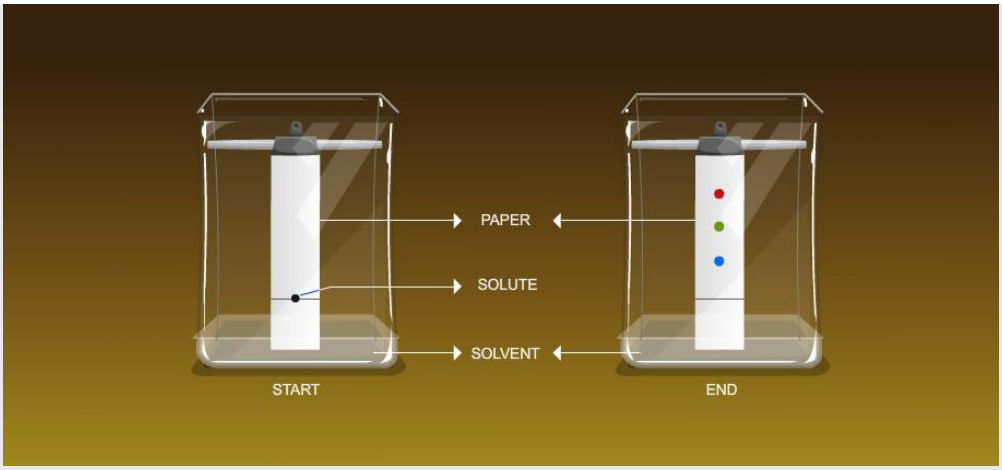
Photosynthetic plants convert light energy from the sun to chemical food energy. During photosynthesis, molecules referred to as pigments are used to capture light energy. Pigments are chemical compounds which reflect only certain wavelengths of visible light. Plant leaves contain four primary pigments: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

To separate and visualize the four primary pigments of green plants, we can use a simple technique called chromatography.

Chromatography

Chromatography is a technique used to separate molecules on the basis of differences in size, shape, mass, charge, solubility and adsorption properties. The term chromatography is derived from Greek words Chroma-colour and Graphe-write. There are many types of chromatography: paper chromatography, column chromatography, thin layer chromatography and partition chromatography. These techniques involve the interaction between three components: the mixture to be separated, a solid phase and a solvent.

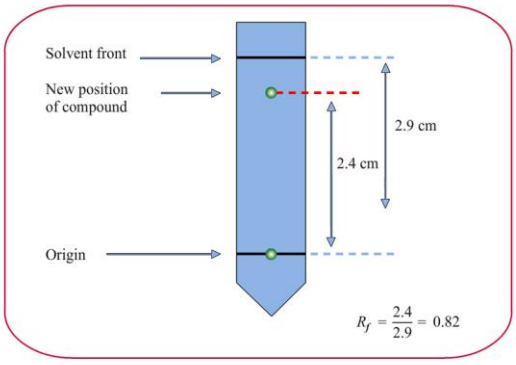
In paper chromatography, the mixture is spotted onto the paper, dried and the solvent is allowed to flow along the sheet by capillary attraction. As the solvent slowly moves through the paper, the different compounds of the mixture separate into different coloured spots. The paper is dried and the position 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. Different plant pigments can be separated by using the technique of paper chromatography.



Retention Factor

Retention factor or Rf value is applied in chromatography to make the technique more scientific than a mere analysis. The retention factor or Rf is defined as the distance travelled by the compound divided by the distance traveled by the solvent.

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



Materials Required:

Green spinach leaves, scissors, mortar and pestle, spatula, chromatographic chamber, filter paper strip.

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

R_f Value of the each pigment spot can be calculated by the equation;

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

Measure the distance of each pigment band from the loading spot and also the distance travelled by the solvent. Calculate the Rf value using the equation and record the values in the table.

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.

6. Demonstration of aerobic respiration

Aim: To show experimentally that carbon dioxide is released during respiration.

Principle:

Respiration is a catabolic process which involves the breakdown of food or complex organic molecules into simpler products, with the release of energy. This process can take place either in the presence of oxygen (aerobic respiration) or in its absence (anaerobic respiration).

The overall reaction mechanism of aerobic respiration involves the oxidation of carbohydrate and the subsequent production of CO_2, H_2O and energy.

$C_6H_{12}O_6+6O_2 \rightarrow 6CO_2 + 6H_2O$ + Energy

Materials Required:

A conical flask, a beaker, a cork with a hole, a glass tube bent at right angles at two places, a small test tube, KOH solution, thread, coloured water. Vaseline and germinating seeds of gram or pea.

Procedure:

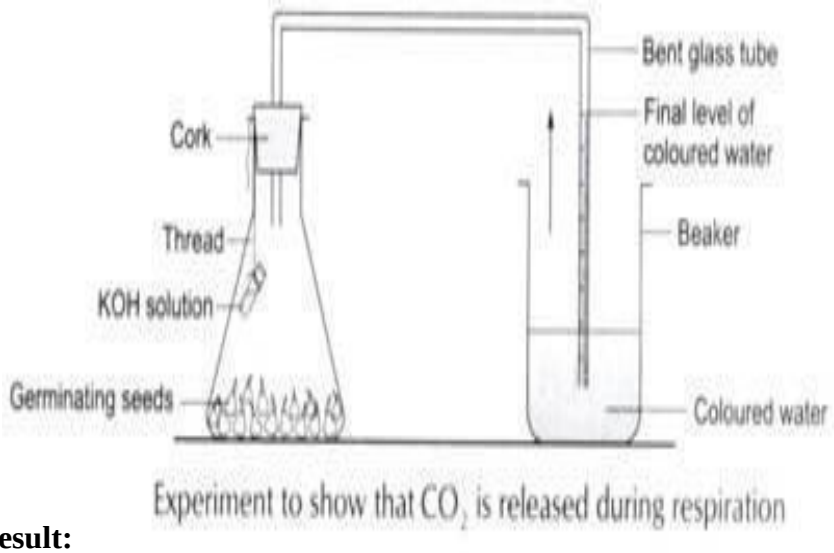
1. Take the conical flask and place some germinating gram or pea seeds in it.
2. Insert the shorter end of the glass tube through the hole in the cork and fix it on the conical flask.
3. Before fixing the cork, hang a test tube containing KOH solution inside the conical flask with the help of a thread.
4. Take coloured water in the beaker and keep the longer end of the glass tube dipped inside it.

5. Make the conical flask airtight by applying Vaseline on its rim.

6. Note the initial level of water in the tube.

7. Observe and note the rise in the water level after an hour, without disturbing the apparatus. Observations:

Water level rises up in the bent tube.



Experiment to show that CO_2 is released during respiration

Result:

The rise in the level of water indicates that CO2 is produced by germinating seeds during respiration. Actually, the germinating seeds respire and produce CO2, which is absorbed by KOH solution. This creates a vacuum in the conical flask. The air present in the bent glass tube moves into the conical flask. This pulls the water in the bent tube further up.

Precautions:

1. Keep the conical flask airtight.
2. Fix the shorter end of the glass tube in such a way that it does not touch the seeds.
3. Use freshly prepared KOH solution.

7. Preparation of root nodules from a leguminous plant.

Principle:

Bacteria of genus Rhizobium are fast growing Gram-negative rods that are normally involved in nitrogen fixation for the atmosphere. Rhizobia are commonly found in the root nodules of legumes; we obtained our samples from the root nodules of a greenhouse soybean plant inoculated with Bradyrhizobium japonicum (formerly called Rhizobium japonicum) and of naturally growing clovers on campus. Our goal was to derive an enrichment culture protocol that allowed for optimal growth of Rhizobia from both samples. The soybean nodule was manually inoculated with Rhizobium, so results from enrichment and isolation techniques for the soybean Rhizobium could serve as a basis for determining legitimate growth of Rhizobium on the clover sample, of which we weren't sure of the presence of the bacteria. Yeast mannitol agar (YMA) has been recommended (Microbial Media, www.nexusresearchgroup.com) for the isolation of Rhizobium, so we used this media for our protocol. YMA contains yeast extract, which provides the bacteria with a nitrogen source, a necessary element for natural Rhizobium function. YMA also contains mannitol, a major one of the many carbohydrates that Rhizobium can utilize for metabolism. Our enrichment culture protocol also involves incubating the bacteria at room temperature (20-25 C) at standard room pH (6-8) and regular lighting for at least two days. As this method follows Bergey's description of optimum Rhizobium growth conditions (Bergey's

5th ed.), we expected our enrichment culture to be successful in growing the desired organism and having the organism achieve desired results via isolation techniques.

Materials Required: Agar, K2HPO4, Mannitol, Yeast extract, MgSO4.7H2O, NaCl, beakers, flasks, graduated cylinders, innoculating loops, test tubes, aluminium foil, pair of scissors, distilled water, bleach, alcohol, ziploc bags, tweezers, scaples, glass rods, petri dishes, slides, cover slips, Iodine, Safranin, Crystal violet, Maneval's solution, Congo red, Oxidase,

Hydrogen Peroxide and dextrose tubes.

Procedure: A. Making the medium

A total of 3 liters of Yeast Mannitol Agar (YMA) [Microbial Media, p. 1007] was made:

Agar	15.0g
Mannitol	10.0g
K2HPO4	0.5g
Yeast Extract	0.4g
Mg2SO4.7H2O	0.2g
NaCl	0.1g

Components were poured into a large flask and the volume was brought to 1 L by adding distilled water. The flask opening was covered with foil, and medium was gently heated to boiling with the stir bar mixing the solution. The flask was autoclaved for 15 minutes at 15 psi at 121 C. Plates were poured and allowed to cool overnight.

B. Preparation of bacteria samples from root nodules

Leguminous plant inoculated with Rhizobium was obtained from the garden. Brown nodules ~7mm wide and a little bit of root were cut from the root system of the soybean plant.

The roots and nodules were rinsed with water

About 5-6 1 cm long sections, each with one or two nodules, were cut from the leguminous roots, and each with a nodule, were immersed in 1% chlorine bleach solution in sterile petri dishes for 15 minutes.

(All tweezers, scalpels, and glass rods used to manipulate the nodules were contained in 70% alcohol for sterlilization.)

Bleach solution was poured off, and nodules were immersed in 70% alcohol. The lid was closed and the dish was swirled for about a minute.

The solution was again poured off, and nodules were immersed in distilled water. The dish was swirled with the lid closed for a minute. This step was repeated two more times for rinsing.

The tweezer sitting in alcohol was passed over flame for sterilization. The largest root nodules from the clover were transferred to separate drops of water on a petri dish and crushed. The two soybean nodules were also crushed in water.

A large number of Yeast Mannitol Agar plates were streaked with either soybean or clover samples, using sterile technique. The plates were inverted and left to grow at room temperature (20-25 C) for two or three days.

C. Growth, Isolation and Confirmation of the samples

Impure colonies on a single plate were restreaked on separate plates. Several innocula of soybean colonies were restreaked, and several innocula of clover samples looking like soybean colonies were restreaked. Soybean and clover samples were restreaked every two or three days.

Unused media plates and observed plates were stored in the cold room for further analysis.

8. Estimation of stress indicators – Proline and osmolyte estimation

Aim

To estimate stress indicator proline and osmolyte from plant sample.

Introduction

Proline is a basic amino acid found in high percentage in basic protein. Free protein proline is said to play a role in plants under a stress condition. Though the mechanisms have not yet been established for the increased level of proline, one of the hypotheses refer to be protein to amino acid and conversion to proline for storage.

Many workers have reported a several fold increases in the proline contain under physiological and pathological stress condition. Hence one analysis of proline in plants has become routine in pathology and physiology division of agricultural science.

Principle

During extraction, it acquire sulphur salicyclic acid, protein are precipitated as computer. Other inference materials are also presumably removed by absorption to the protein sulpho salicyclic acid complex. The extracted proline is made to react with ninhydrin in acidic condition (pH 1.0)to form chlorophore (red colour) and read at 520nm.

Reagents required

Acidic ninhydrin warm 1.25g ninhydrin 30ml glacial acetic acid and 20ml ortho phosphoric acid ,with agitation until dissolve store at 40’c and use within 24hours, 3% aquaeous sulpho salicyclic acid, Glacial acetic acid, Toluene, proline

Procedure

1. Extract 0.5g of plant material by homogenizing in 10ml of 3% of aqueous silpho salicyclic acid.
2. Filter the homogenate through Wattman no. 2 filter paper.
3. Take 20ml of filtrate in test and add 2ml of glacial acetic acid and add 2ml of ninhydrin.
4. Heat it in the boiling water bath for 1 hour.
5. Terminate the reaction by placing the tube in ice bath.
6. Add 4ml of toluene to the reaction.
7. Add stir well for 20-30 seconds.
8. Separate the toluene layer and warm to room temperature.
9. Measure the red colour intensity at 520nm.
10. Run a series of standard with pure toluene in a similar way to prepare a curve.
11. Find out the amount of proline in the test example for the curve.

Calculation

Express the protein contain on fresh weight basis by follows

M moles per of tissues = ug proline *ml toluene/115.5*5/9sample

where 115.5 is the molecular weight of proline,

=0.15*5/115.5*5/0.5

=0.15 OD

Result

The concentration of proline estimation from the given sample was 0.15 OD at 520nm.