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* (4^{th} 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.

Experiment to Study the External Features of Plants (With Diagrams)

Experiment:

Objective:

To study the external features of root, stem, leaf and flower of monocot and dicot plants.

Apparatus and materials required:

Glass slides, forceps, hand lens, scissors, dissecting microscope, a complete monocot plant such as onion or paddy or wheat or maize, and a complete dicot plant such as mustard or sunflower or pea.

Theory:

The flowering plants, or angiosperms, are differentiated into root, stem, leaves and flowers. They bear seeds enclosed in a fruit. They are divided into monocotyledons and dicotyledons on the basis of the kind of seeds they bear. Monocotyledons bear seeds which have a single cotyledon. The seeds of dicotyledons have two cotyledons.

Procedure:

1. Take a monocot plant. Separate root, stem, a leaf and a flower of this plant with the help of scissors and place these parts on different slides separately with forceps.

2. Then take a dicot plant and repeat the process.

3. Now observe and compare the external features of root, stem, a leaf and a flower of the monocot and dicot plants using hand lens and subsequently by dissecting microscope.

Observation:

Root:

Identifying features of root:

1. The part of a plant that generally develops from the radicle of embryo is called root.

2. It fixes the plant firmly into the ground and provides rigidity against wind and water.

3. It absorbs water and minerals from soil.

4. It grows towards the centre of gravity, i.e., it is positively geotropic.

5. It possesses unicellular root hairs.

6. It normally grows away from light, i.e., it is negatively phototropic.

7. It does not bear buds, leaves and flowers, and lacks nodes and internodes.

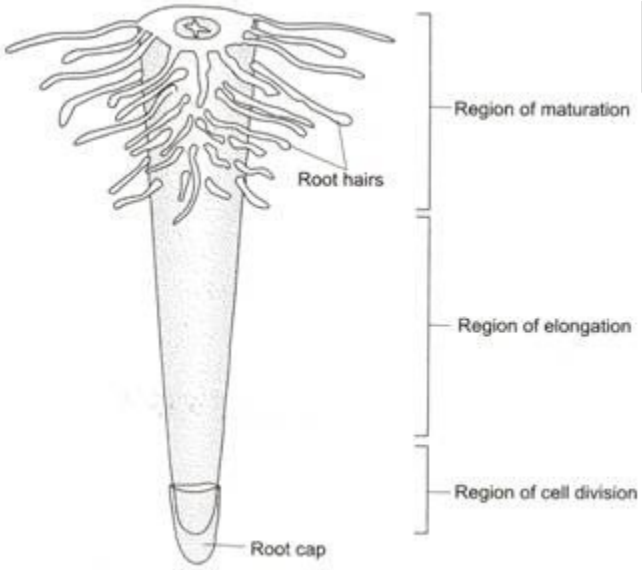
8. The root has four regions from the apex to the base:

(i) Root cap

(ii) Region of cell division (apical meristem)

(iii) Region of elongation

(iv) Region of maturation



Regions of the root

9. The root cap protects the growing root apex while the main growing region of the root lies just behind the root cap.

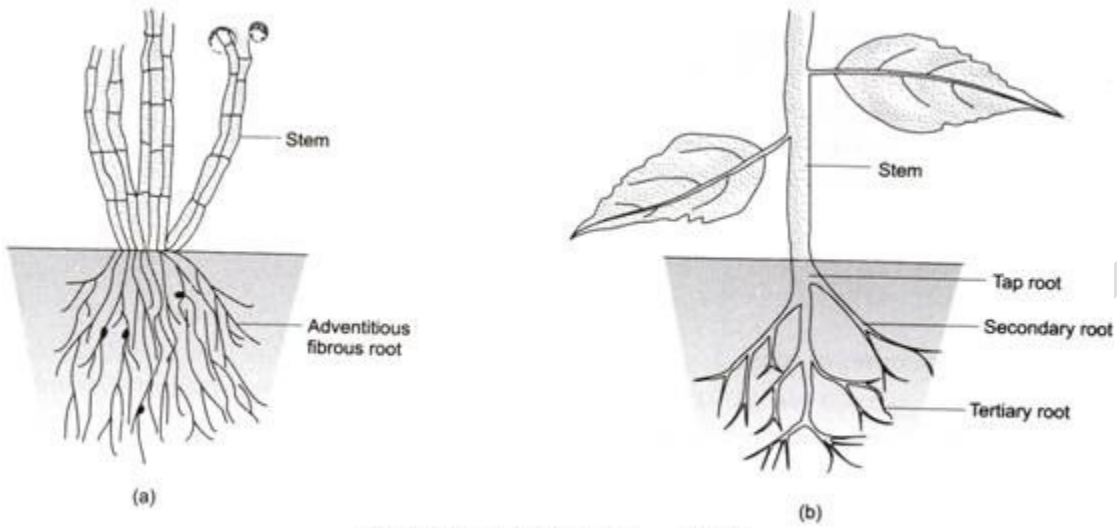
Monocot root:

1. In monocots, primary root does not persist for a longer period. It is soon replaced by a cluster of long, threadlike roots which originate from the base of the stem. These roots are called fibrous roots.

2. Roots developing from any other part of the plant than radicle are called adventitious roots.

3. Fibrous root is a type of adventitious root.

4. Due to absence of secondary growth in thickness these roots remain slender.



Types of root (a) Monocot root (b) Dicot root

Dicot root:

1. In most of the dicots, root develops directly from the radicle.

2. It grows longer, thickens and is known as primary root.

3. It persists and becomes stronger to form tap root.

4. It generally produces lateral branches called secondary roots.

5. Branches of the secondary roots are called tertiary roots.

6. Tap root along with its branch system is called tap root system.

Stem:

Identifying features of stem:

1. The part of the plant that develops from the plumule of embryo is called stem.

2. It forms the axis and is the ascending part of the plant.

3. It is differentiated into nodes and internodes.

4. It bears leaves and branches at the nodes. The part of stem that lies between two nodes is called internodes.

5. It is positively phototropic, i.e., grows towards light and negatively geotropic, i.e., grows away from the gravity.

6. The shoot (stem and its branches) is usually green and photos5mthetic.

7. The apex of stem is called shoot tip. It bears apical bud which is responsible for elongation of the plant. Shoot apex lacks cap.

8. Stem bears either unicellular or multicellular hair, or trichomes.

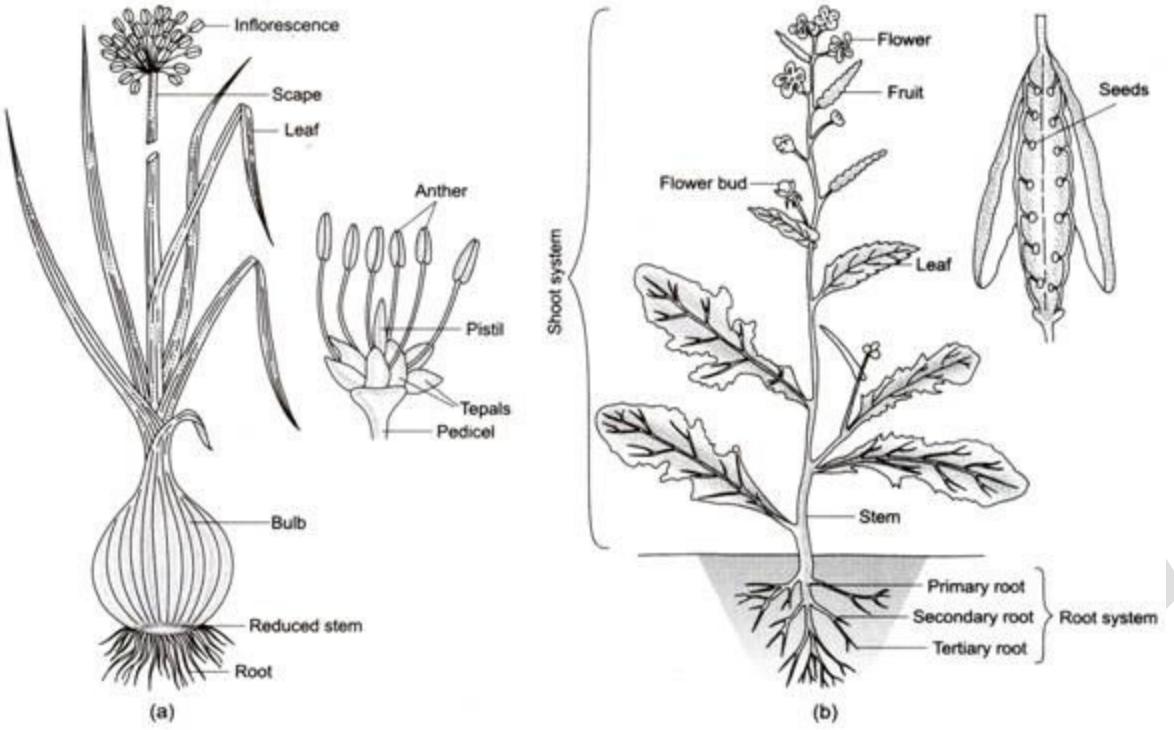
9. The main function of the stem is to support leaves and branches and hold them in a position to receive maximum light. Thus it forms the main skeleton of the plant.

Monocot stem:

1. It is aerial, erect, herbaceous or woody, usually unbranched.

2. It is usually differentiated into solid nodes and hollow internodes. In maize, internodes are also solid.

3. In some members stem is modified into underground organs like rhizome (e.g., ginger), corm (e.g., Colocasia) or bulb (e.g., onion).



External features of plants (a) A monocot plant (onion) (b) A dicot plant (mustard)

Dicot stem:

1. It is normally long, erect, herbaceous or woody, cylindrical and branched.

2. It has distinct nodes and internodes. Both the nodes and internodes are solid.

3. Sometimes it is creeping and modified into tendril.

4. It is often four-angled (quadrangular) or five-angled (pentangular).

5. In potato, the underground stem is modified into tubers.

Leaf:

Identifying features of leaf:

1. It is the lateral appendage of the stem that arises at the node.

2. It bears a bud in its axil.

3. It is attached to the stem with the help of a structure called the leaf base.

4. A stalk called petiole develops from the leaf base which bears a green flattened structure called lamina.

5. Lamina, or leaf blade, has midrib, veins, and leaf apex and leaf margin.

6. The leaves are grouped into two categories—simple and compound—on the basis of incision. Simple leaves have a single lamina. When the incision of the lamina goes down to the midrib, the leaf becomes compound having a number of leaf segments called leaflets.

7. The main functions of leaves are synthesis of food (photosynthesis), transpiration, and exchange of gases through its pores called stomata.

8. Sometimes leaves get modified for storage, defense, support, reproduction and trapping insects.

Monocot leaf:

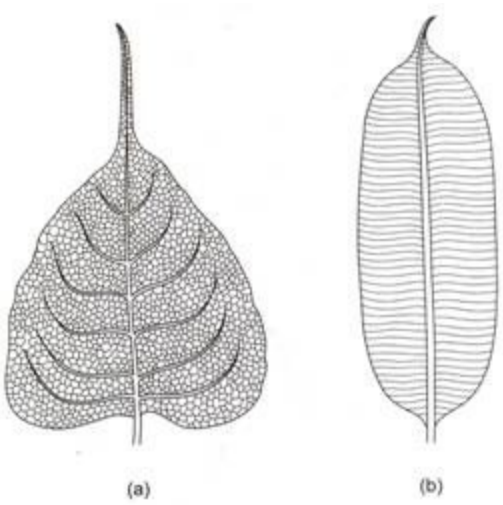
1. Leaves are arranged isobilaterally, i.e., both surfaces are similar.

2. The venation (arrangement of veins and veins on the lamina) is parallel. In monocots veins run parallel to each other from base to the tip of the lamina. Veins connecting the adjacent longitudinal veins are inconspicuous.

3. Leaves are usually long and narrow, running parallel to the stem.

4. Leaves are mostly simple.

5. Leaf sheath (expansion of leaf base into a broad sheath) is usually present.



Venation in leaves (a) Reticulate (b) Parallel

Dicot leaf:

1. Leaves are arranged dorsiventrally, i.e., upper and lower surfaces are distinctly different.

2. In dicot leaves, venation is reticulate, i.e., irregularly distributed to form a network.

3. In most dicots, the leaf base bears two lateral appendages called stipules.

4. Leaves are either simple or pinnately compound.

5. Leaf sheath is usually absent.

Flower:

Identifying features of flower:

1. The reproductive part of an angiosperm (higher) plant is flower, which develops from floral buds.

2. The flower is considered to be a modified shoot.

3. The stalk of the flower is called pedicel and the tip of the pedicel continues as an enlarged axis called thalamus or receptacle.

4. All the floral parts are arranged on the thalamus in a definite sequence.

5. A typical flower consists of four sets of floral parts, or whorls: calyx (sepals), corolla (petals), androecium (stamens) and gynoecium (carpels).

6. The first two whorls, i.e., calyx and corolla are not directly involved in reproduction and are called accessory whorls.

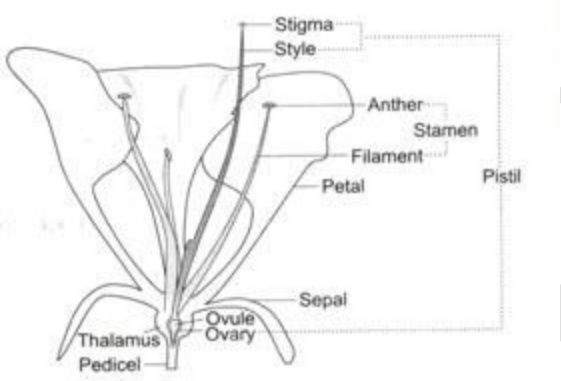


Diagram of different parts of a flower

7. The inner two whorls, i.e., androecium and gynoecium are directly concerned with sexual reproduction and are called essential whorls.

8. Sepals form the outermost whorl called calyx. They are usually green and leaf like, and arise at the base of the flower.

9. Petals form the corolla. They are generally brightly coloured and sometimes fragrant to attract insects.

10. The third whorl androeciunt is the male reproductive part of the flower and consists of stamens. Each stamen consists of a slender filament and an anther at the tip.

11. Gynoecium, or pistil, is the centrally placed fourth whorl which bears the female reproductive organ called carpel. Each pistil consists of a basal swollen ovary, a narrow stalk-like style, and stigma at the tip. The ovary contains one or many ovules.

Monocot flower:

1. Calyx and corolla are not distinct in monocot flowers. Instead, perianth is present which is composed of tepals.

2. In monocots, flowers appear in clusters.

3. The flower is typically trimerous (each whorl is in multiple of three).

4. Stamens are usually versatile, i.e., filament is attached to the back of anther at a point only.

Dicot flower:

1. Dicot flowers usually have distinct floral parts, i.e., calyx, corolla, androecium and gynoecium.

2. Calyx is composed of sepals, and corolla is composed of petals.

3. Flower is mostly pentamerous (each whorl in multiple of five), sometimes tetramerous (each whorl in multiple of four).

4. In dicots, flowers usually appear separately.

5. Stamens are usually basified, i.e., filament is attached to the base of the anther.

Objective

Our objective is to prepare temporary stained glycerine mounts of transverse sections of the stem and root of Dicot and Monocot plants.

Theory

Stems and roots of plants are made up of different types of tissues. These tissues form different layers in the composition of stems and roots. Plant anatomy is the general term for the study of the internal structure of plants.

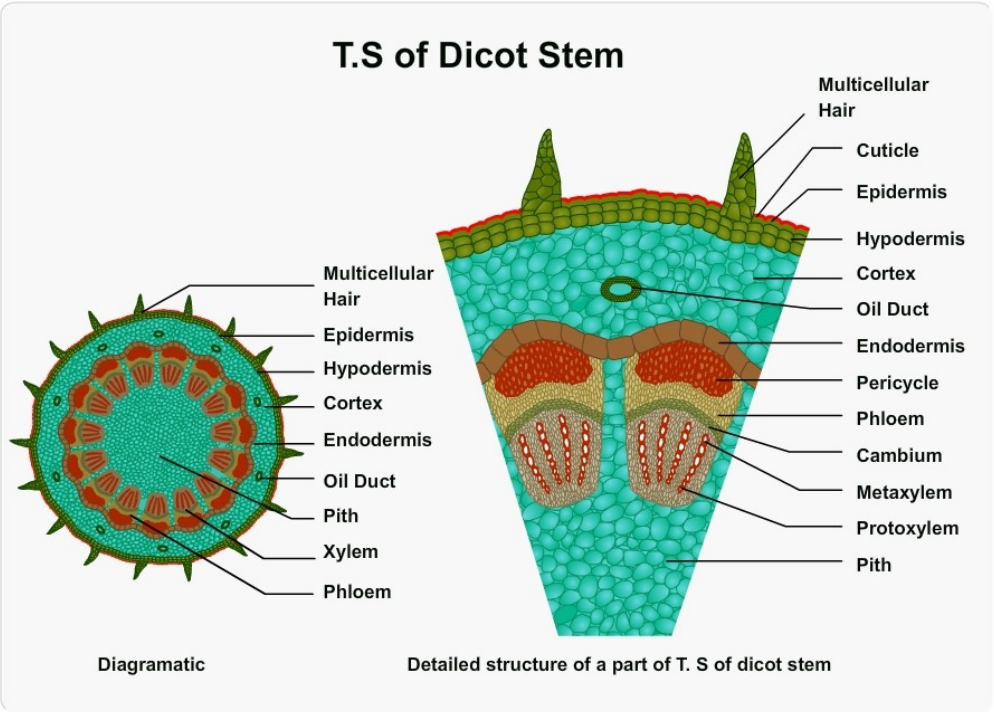
To study the structural details of the stem or root of a monocot or dicot plant, it is essential to be familiarized with the sectioning and staining techniques used with plant materials. It is also necessary to take the sections with uniform thickness so that the light passes through them equally and the different tissues found in the material are clearly visible under the microscope. To examine the tissues clearly, it is desirable to stain the section with suitable stains, as different stains colour the tissues differently.

Anatomy of Plant Stems and Roots

We will now look at the anatomy of dicot and monocot stems and roots.

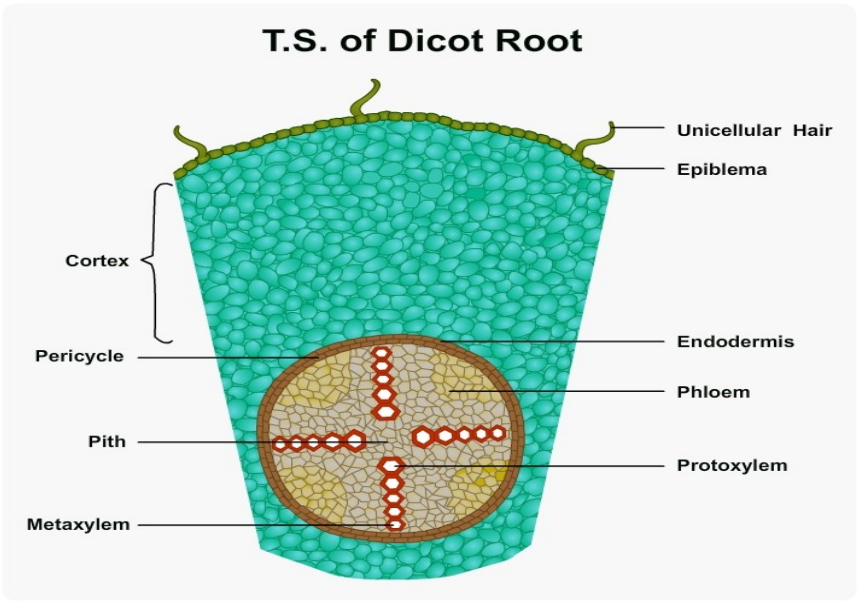
Comparison of the Anatomy of Dicot and Monocot Stems

Features	Dicot Stem	Monocot Stem



Comparison of the Anatomy of Dicot and Monocot Roots

Features	Dicot Root	Monocot Root
	Single layer of compactly	yer of compactly cells.
		in walled root hairs.
		iated into cortex, mis, pericycle and
		e alternate bundles of d phloem.
		undles are exarch.
		essels are oval.



Points to Note

Conjoint: Xylem and phloem occurs in same bundle.

Collateral: Phloem lies towards the outerside and xylem occurs towards the inner side.

Open: Cambium is present between phloem and xylem.

Closed: Cambium is absent between phloem and xylem.

Exarch: Protoxylem lies towards the outerside and metaxylem towards the centre.

Endarch: Metaxylem lies towards the outer side and protoxylem towards the inner side.

Materials Required


Sunflower Stem & Root

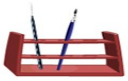

Maize Stem & Root


Compound microscope


Glycerine


Safranin solution

Distilled water


Needle & Brush


Cover slip


New Blade

Filter paper

Glass Slide


Dropper

Procedure

- Take a blade and a sunflower stem to be sectioned.
- Dip the top of the material and the blade in water.
- Hold the material to be sectioned horizontally between the thump and the first finger of your left hand.
- Hold the blade in the right hand with the edge of the blade facing you and handle at right angles to it.
- Cut the sections of the material quickly using the blade.
- Transfer the sections in Petri dish containing water.
- Using a brush, select a thin uniform and complete section and place it on the glass slide.
- Using a dropper, take some safranin solution.

- Pour one drop of safranin solution over the section and allow it to stand for two minutes.
- Remove excess stain using a filter paper.
- Pour a drop of glycerine over the section.
- Place a coverslip gently on the section with the aid of a needle.
- Remove the excess glycerine using a filter paper.
- Repeat the same procedure for the sunflower root, maize stem and maize root and prepare the slides.
- Observe each section under the microscope.

Study of Plasmolysis

Our Objective

To demonstrate plasmolysis in peels of Rhoeo plant in hypotonic and hypertonic solutions using salt solution.

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

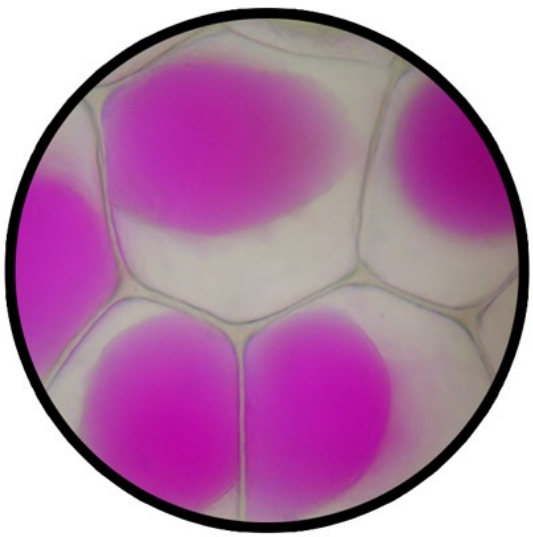
The cell membrane is a semipermeable membrane that separates the interior of all cells from the surrounding environment. The semipermeable membrane allows some particles, ions, or water molecules across the membrane, but blocks others. Water molecules constantly move inside and outside the cell across 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.

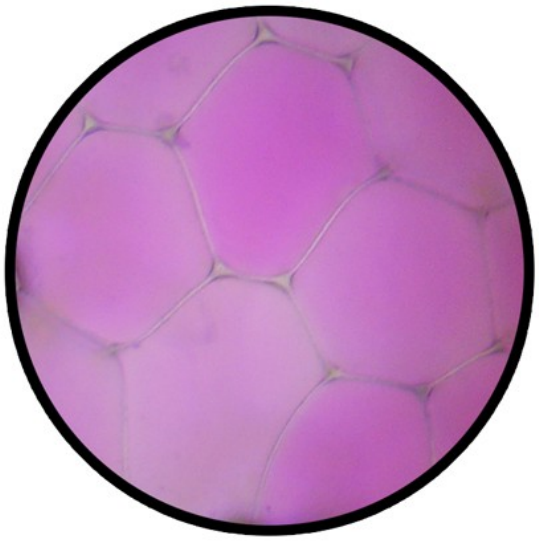
When a plant cell is placed in concentrated salt solution, water concentration inside the cell is greater than that which is outside the cell. Therefore, water moves through the cell membrane into the surrounding medium. Ultimately the protoplasm separate from the cell wall and assumes spherical shape.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



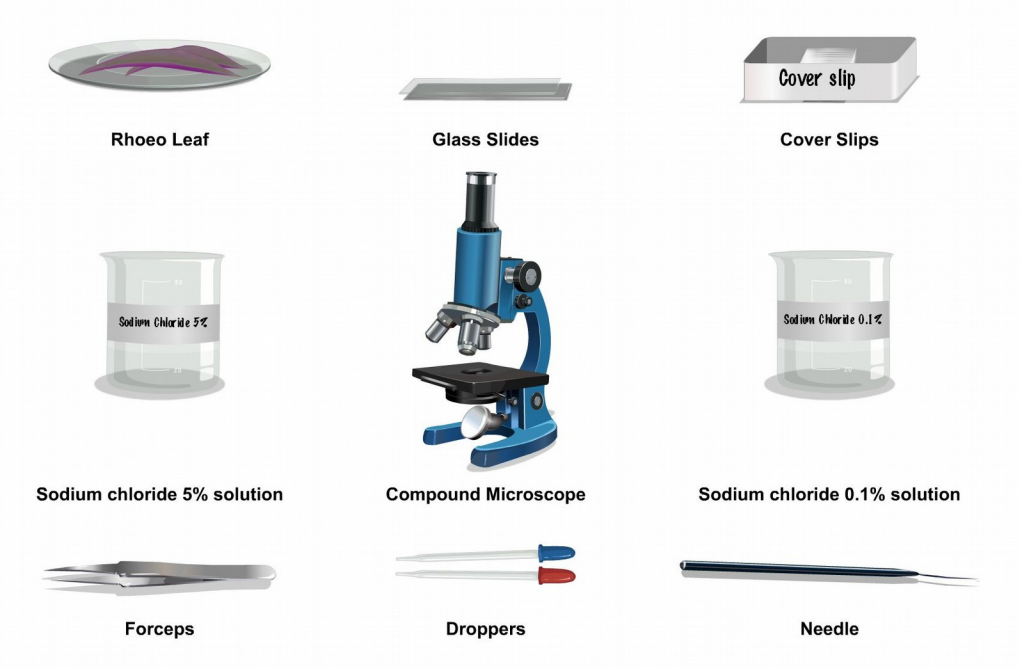
Plasmolysed cells in hypertonic solution



Deplasmolysed cells in hypotonic solution

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

Materials Required



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.

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.

Experiment on Plasmolysis (With Diagram)

Object:

To demonstrate the phenomenon of plasmolysis.

Requirements:

Tradescantialeaf, water, sugar solution, slides, cover glass, microscope, blade.

Method:

1. From the lower surface of the leaf of Tradescantia, peel off small segments of epidermis by a blade.

2. Put few peelings on a slide, mount in a drop of water, put a cover glass and study under microscope.

3. Mount some of the peelings in the drops of sugar solutions of different concentrations. Study 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).

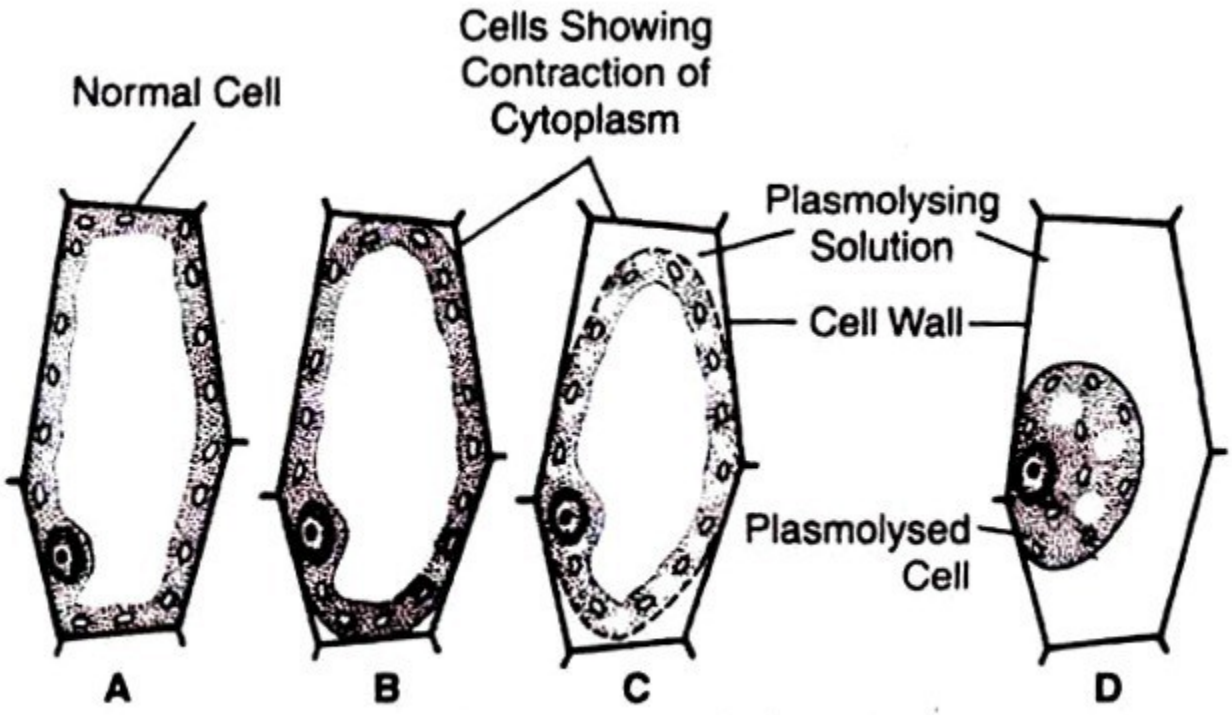


Fig. 7. Phenomenon of plasmolysis.

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.

Demonstration of Stomata on a Leaf Peel

Objective

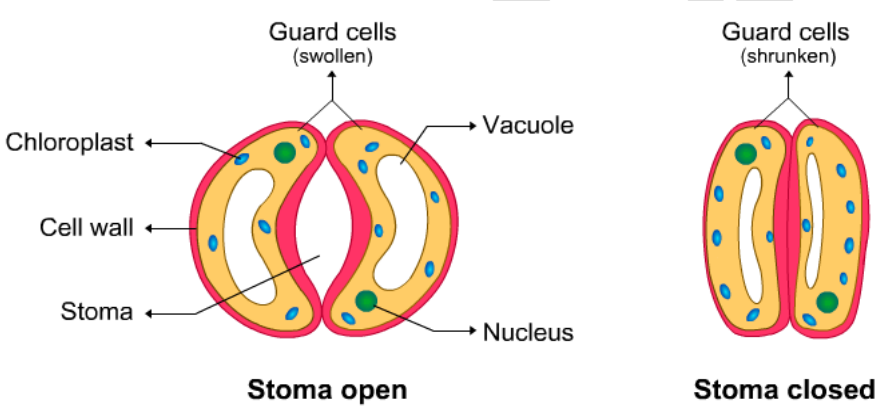
Our objective is to prepare a temporary mount of a leaf peel to show stomata.

Theory

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

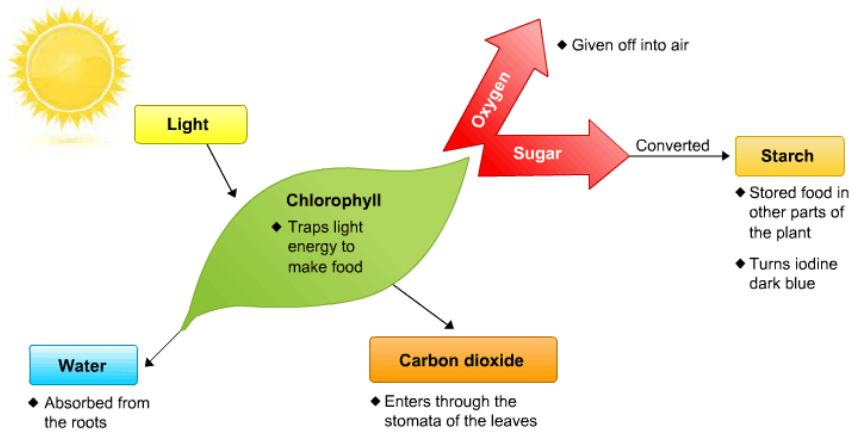
surface of the leaf. On the other hand, monocot plants have the same number of stomata on both surfaces of the leaf. In the case of floating plants, stomata is found only on the upper epidermis.

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

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.

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.

Experiment to Observe Temporary Mount of a Leaf Peel to Show Stomata

Experiment:

Objective:

To prepare a temporary mount of a leaf peel to show stomata.

Apparatus and 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.

Theory:

Stomata are small openings found widely scattered on the epidermis of leaves and young stems. They are mostly found on the lower surface of a dicot leaf and on both the surfaces of a monocot

leaf. Stomata regulate the exchange of gases and water vapour between the atmosphere and leaves.

Procedure:

1. Remove a healthy leaf from the potted plant.

2. Remove a part of the peel from the lower surface of the leaf. You can do this by folding the leaf over and gently pulling the peel apart using forceps. Keeps the peel in a watch glass containing water.

3. Put a few drops of safranin stain in a watch glass.

4. After 2-3 minutes take out the peel and place it on a clean glass slide.

5. Put a drop of glycerin over the peel and place a clean coverslip gently over it with the help of a needle.

6. Remove the excess stain and glycerin with the help of blotting paper.

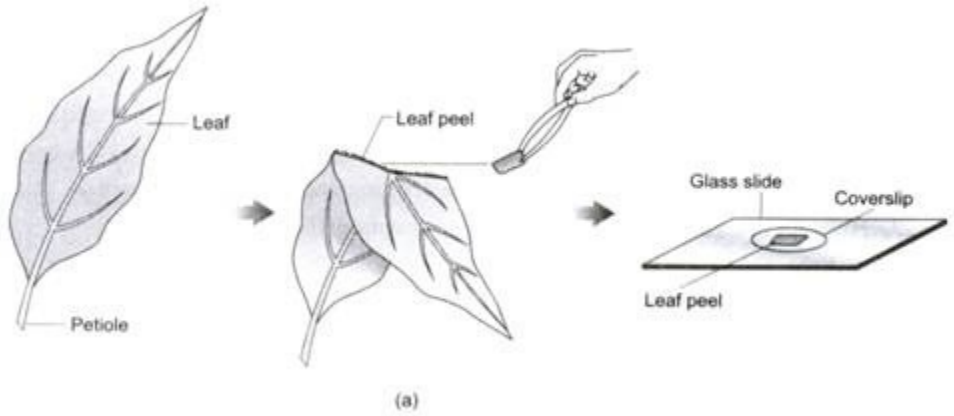
7. Observe the slide under the low-power and high-power magnifications of the compound microscope.

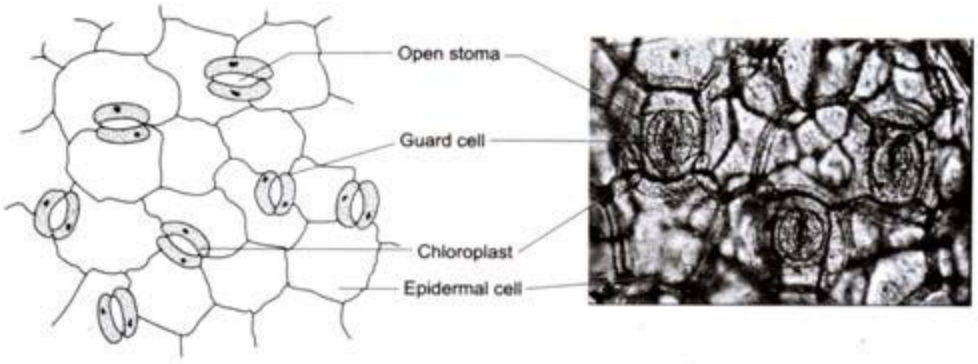
Observations:

1. The epidermal cells are visible. These are irregular in outline and have no intercellular spaces.

2. Many small pores (stomata) are seen scattered among the epidermal cells.

3. Each pore is guarded by two bean-shaped guard cells, each containing chloroplasts and a nucleus.





(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

4. The inner concave boundary of each guard cell is thick, whereas its outer boundary is thin.

5. The stomata may be open or closed. The guard cells regulate the opening and closing of the stomata.

Result:

Stomata are present in the epidermal cells of the lower surface of the leaf.

Precautions:

1. Cut the peel to a proper size and avoid folding it.

2. Always place the peel at the centre of the slide and hold the slide at the edges.

3. Do not overstrain or under strain the peel.

4. Always handle the peel with a brush as a needle may damage the cells.

5. Take care to prevent the peel from drying by using glycerin.

6. Place the coverslip gently, avoiding any air bubbles.

7. Remove excess stain and glycerin with a blotting paper.

3. Demonstration of opening & closing of stomata

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

Aim of the Experiment:

To demonstrate the process of guttation with entire potted plant.

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).

Method:

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. 17).

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.

Paper Chromatography

Objective

Our objective is to separate and study plant pigments by paper chromatography.

Theory

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

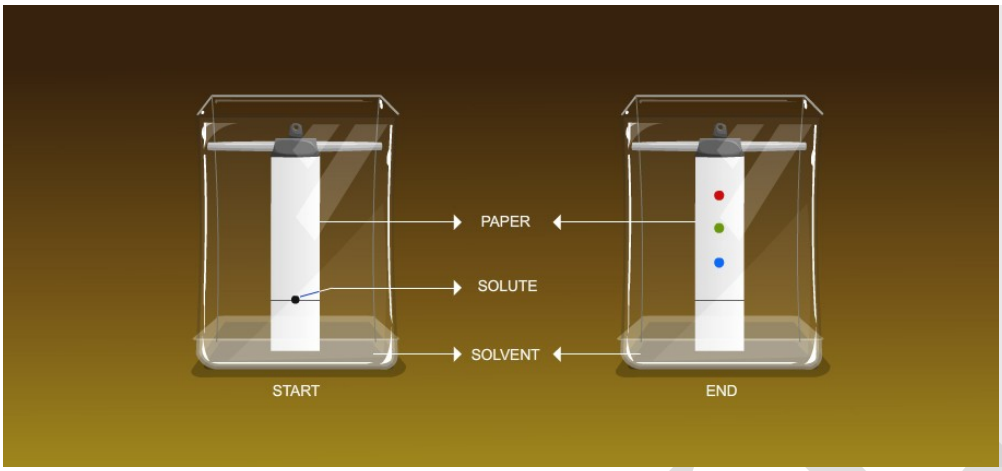
Photosynthetic plants convert light energy from the sun to chemical food energy. During photosynthesis, molecules referred to as pigments are used to capture light energy. Pigments are chemical compounds which reflect only certain wavelengths of visible light. Plant leaves contain four primary pigments: 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

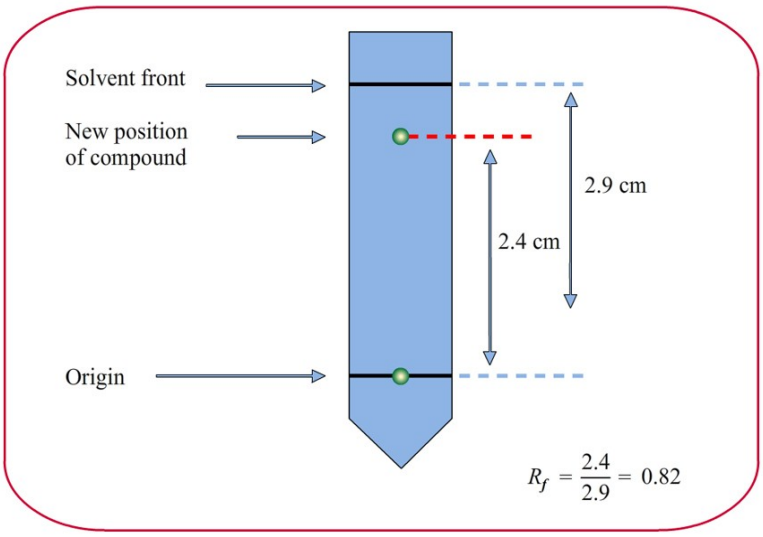
Chromatography is a technique used to separate molecules on the basis of differences in size, shape, mass, charge, solubility and adsorption properties. The term chromatography is derived from Greek words Chroma-colour and Graphe-write. There are many types of chromatography: paper chromatography, column 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.

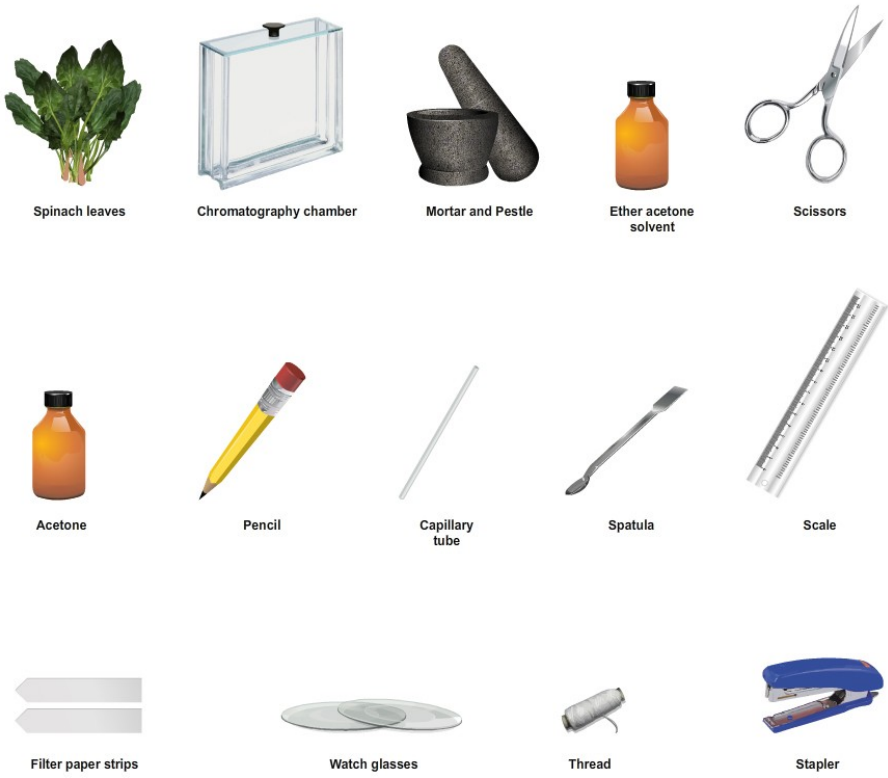


Retention Factor

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



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.

Demonstration of Aerobic Respiration in Plants:

Experiment:

Aerobic respiration in plants can be experimentally proved with the help of a simple apparatus like:

(i) Respiroscope which consists essentially of a stout vertical tube which is bent into a bulb at the one end (Figure 19a), or

(ii) With the help of a long-necked round-bottomed flask fitted with a centrally-bored cork at the mouth through which passes a glass tube (Figure 19b).

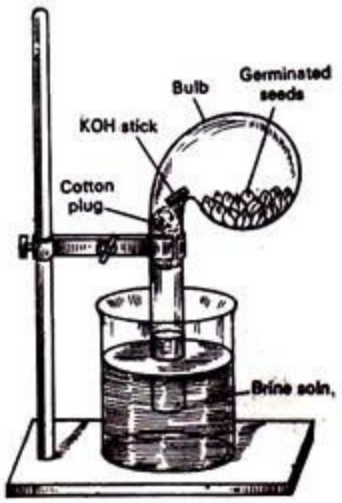


Fig. 19(a).

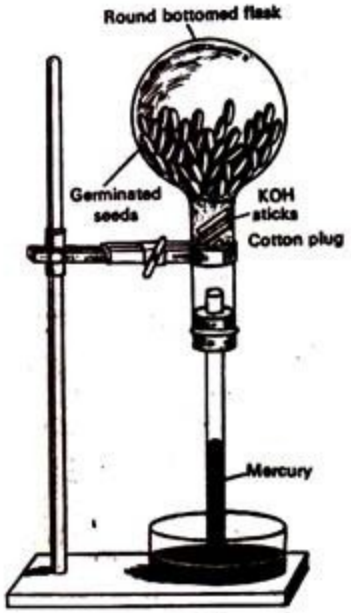


Fig. 19(b).

The respiroscope or the inverted flask is fixed vertically to a stand and a few germinating gram seeds or flower petals are placed in the bulb of the respective or in the inverted flask plugged with cotton at the base the vertical tube of the respiroscope or inverted flask is dipped just below the surface of water or mercury in a beaker.

A few caustic potash (KOH) pellets are introduced in the bent portion of the respiroscope or in the long neck of the round bottom flask and kept in position with loosely held cotton wool Care should be taken that respiratory materials and KOH pellets do not come in contact.

Precautions should be taken that the free end of the tube does not touch the bottom of the water or mercury trough. Fittings must be air-tight to avoid any leakage.

Observation:

The apparatus is allowed to stand for a few hours when it is seen that water or mercury has risen in the vertical tube of the apparatus proving the production of partial vacuum.

Inference:

Due to respiration of germinating seeds or flower petals CO_2 has been released which is at once absorbed by KOH pellets. Thus the partial vacuum produced by the absorption of O_2 by the respiring material could not be filled up by the released CO_2. Hence, water or mercury is drawn upward into the tube.

Demonstration of Anaerobic Respiration:

Experiment:

A few germinating gram seeds are taken in a test tube which is completely filled with mercury and is then inverted just below the surface of mercury in a trough. It is then vertically held with a clamp and stand (Figure 20).

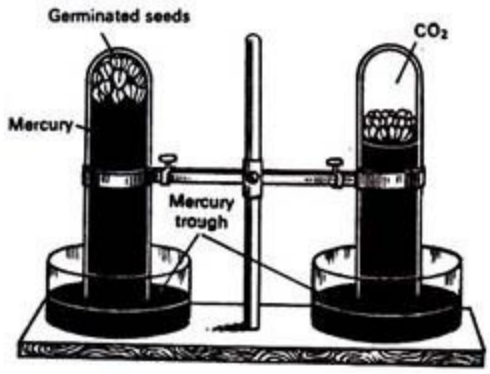


Fig. 20.

Observation:

Observation from time to time reveals that a gas is formed within the test tube by the displacement of mercury in the test tube. A few KOH pellets arc introduced through the open end of the test tube when mercury again rises filling the test tube.

Inference:

Here the respiration of germinating seeds takes place in complete absence of O_2 supply and the gas produced is CO_2 as evidenced by its absorption by KOH. This proves that anaerobic respiration has taken place.

PREPARATION OF ROOT SAMPLE FROM LEGUMINOUS PLANT

AIM:

To enrichment and isolate of rhizobium from root nodules.

PRINCIPLE:

Rhizobium can fix atmospheric N2 only in root nodules of legumes and that to when it is bacterial stage of its life cycle. It posses that entire complement of gene spot N2 fixation, which

are normally latent and become active only under special condition. Rhizobium makes N2 available to plant and inturn the bacteria derive nutrients from the tissue of the plant.

Yeast mannitol agar with congo red is used for cultivation of rhizobium species and for studying root nodules.

Yeast extract serve as a good source of readily available aminoacids, vitamin B complex and necessary growth factor for rhizobium. It also posses the oxidation-reduction potential of medium in range favourable for rhizobium and serve as hydrogen donor in respiratory process.

Mannitol is fermentable sugar alcohol sources. Mg provide the cation essential for congo red inhibits penicillin susceptible strain colonies of rhizobia.

MATERIALS REQUIRED:

Agar, K2HPO4, mannitol, yeast extract, MgSO4.7H2O, Nacl, beaker, measuring cylinder, inoculating loop, test tube, aluminium foil, pair of scissors, distilled water, beach alcohol, ziplac bags, slide, coverglass, saffranin, congo red, oxidized hydrogen peroxide and dextase tube.

MEDIA COMPOSITION:

INGREDIENTS	**gms/litre**
Yeast extract	1.000
Mannitol	10.000
Di pottasium phosphate	0.500
Magnesium sulphate	0.200
Sodium chloride	0.100
Congo red	0.025
Agar	20.000
Final pH (at 25˚C)	6.8±0.2

PROCEDURE:

COLLECTION OF NODULES:

The groundnut plants were uprooted and loosely adhering soil was removed by gentle shaking, the mature nodules along with roots were washed in running water until the removal of soil

particles adhering. The collected nodules were kept in polythene shuts and transported to laboratory for further analysis.

ISOLATION AND PURIFICATION OF RHIZOBIUM

The collected nodules were washed 5 to 6 times with distilled water they were surface sterilized using 0.1% mercuric chloride solution for 1 minute, 70% ethanol for 4.5 minutes and washed in distilled water it was transferred to 70% ethanol for 20 minutes finally washed in distilled water to remove all the traces of stains.

The sterilized nodules were crushed with pestle and mortar by adding small aliquots of sterile water, which was $10^{-}7$ diluted.

The diluted suspension $10^{-}5$ to $10^{-}7$ were selected and 0.1ml of suspension was inoculated in petriplate containing sterile YEMA medium with congo red. The inoculated plates were incubated at 29˚C for 3 days.

They were picked out using a sterile loop and uniformly streaked on YEMA solution. The isolates were purified and stored for further studies.

RESULT:

Pure culture of rhizobium was isolated from root nodules of leguminous plant.

Estimation of stress indicators proline and osmolyte

Aim

To estimate stress indicator proline and osmolyte.

Introduction

Proline is a basic aminoacid 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 has not yet been established for the increased level of proline ,one of the hypothesis refer to be protein to aminoacid and conveasion to proline for storage.

Many workers have reported a several fold increses 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 sulphursalicyclicacid,protein are precipitated as computer. Other inferonce materials are also presumably removed by absouption to the protein sulphosalicyclic 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% aquaeoussulphosalicyclic acid

Glacial acetic acid

Toluene

proline

Procedure

Extract 0.5g of plant material by homogenizing in 10ml of 3% of aqueroussilphosalicyclic acid.

Filter the homogenate through wattman no 2filter paper.

Take 20ml of filtrate in test and add 2ml of glacial acetic acid and add 2ml of ninhydrin.

Heat it in the boiling water bath for 1 hour.

Terminate the reaction by placing the tube in ice bath.

Add 4ml of toluene to the reaction.

Add stir well for 20-30 seconds.

Separate the toluene layer and warm to room temperature.

Measure the red colour intensity at 520nm.

Run a series of standard with pure toluene in a similar way to prepare a curve.

Find out the amount of proline in the test example for the curve.

Result

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

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