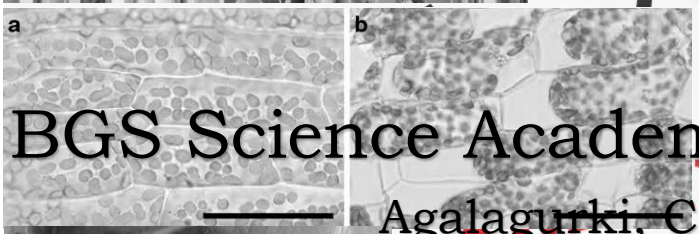
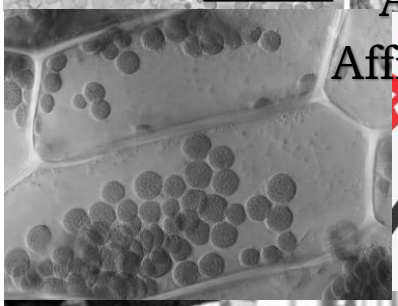




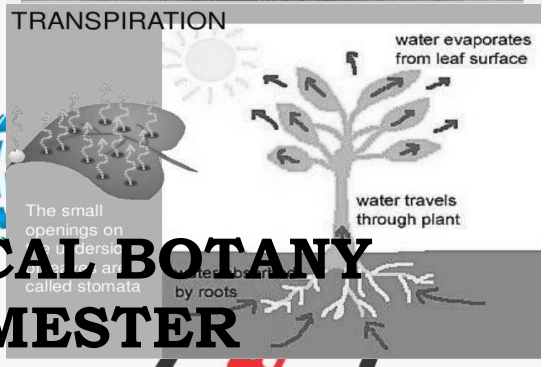
BGS Science Academy & Research Centre

Agalagurki, Chikkaballapura

Affiliated to Bangalore University

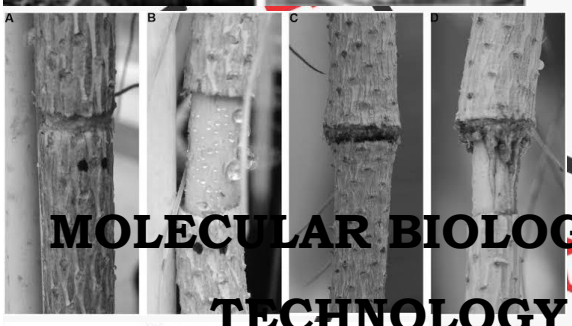


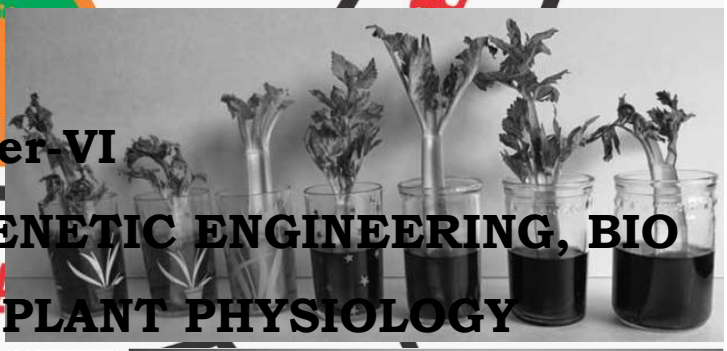




A MANUAL OF PRACTICAL BOTANY

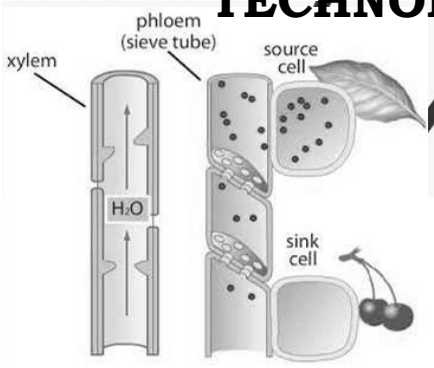
III B.Sc. FIFTH SEMESTER

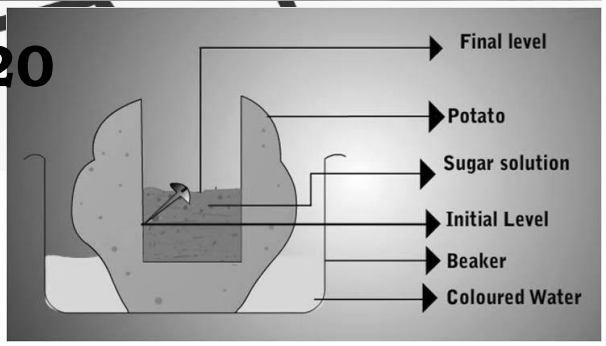




Paper-VI

MOLECULAR BIOLOGY, GENETIC ENGINEERING, BIO TECHNOLOGY AND PLANT PHYSIOLOGY





2019-2020

DEPARTMENT OF BOTANY
Laboratory Instructions

- Do not forget to carry laboratory apron, observation book and other required accessories.
- Handle equipments, microslides, glasswares, chemicals and specimen bottles with proper care.
- Report breakages / damages to the batch incharge or laboratory assistants.
- Keep the laboratory clean and tidy.

PRACTICAL PAPER VI

MOLECULAR BIOLOGY, GENETIC ENGINEERING, BIOTECHNOLOGY AND PLANT PHYSIOLOGY

Total Units: 13

1. Qualitative Test for Starch, Proteins, Reducing Sugars and Lipids. **2 units**
2. Determination of Osmotic Potential of the cell sap by Plasmolytic method. **1 unit**
3. Determination of Stomatal Index. **1 unit**
4. Structures of Stomata in Hydrophytes, Mesophytes and Xerophytes. **2 units**
5. Streaming of Protoplasm to show cyclosis. **1 unit**
6. Determination of pH of plant samples by using indicators. **1 unit**
7. Study of Osmosis (OSMOSCOPE) and Transpiration (POTOMETER) Experiments. **3 units**
8. Study of Phloem Transport by Ringing Experiment. **2 units**

PRACTICAL QUESTION PAPER - VI

MOLECULAR BIOLOGY, GENETIC ENGINEERING, BIOTECHNOLOGY AND PLANT PHYSIOLOGY

Time: 3 hours **Max marks: 35**

1. Conduct the biochemical test of sample **A** and **B**. **3 X 2 = 6 marks**
2. Determine the osmotic potential of the cell sap by Plasmolytic Method / Stomatal index of material **C.** **8 marks**
3. Determine the pH of the given sample **D.** **2 marks**
4. Set up and comment on the experiment **E.** **6 marks**
5. Comment on experiment **F** and **G.** **4+4= 8 marks**
6. Class records **5 marks**

SCHEME OF VALUATION

1. Samples – Starch, Protein, Reducing sugar and Lipids for **A** and **B** (Positive test- **1** mark, Negative test- **2** marks).
2. **C** – Conducting the experiment **-3** marks, Principle– **2** marks, Procedure – **1** mark, Results- **2** marks
3. **D**- Extract from root, stem, leaves of a plant to be given (Determination of pH- **1** mark, Comment- **1** mark).
4. Experiments of **E**-
   a. Potato Osmoscope.
   b. Thistle Funnel experiment.
   c. Farmer's Potometer
   d. Ganong's Potometer

   (Requirements- **1** mark, Principle - 1 mark, Procedure and Conducting - **3** marks, Results- **1** mark)
5. Experiments of **F** and **G** –
   a. Streaming of Protoplasm (Cyclosis).
   b. Balsam Plant experiment.
   c. Bell Jar Experiment.
   d. Transpiration Pull.
   e. Mass Flow Hypothesis
   f. Ringing Experiment.

   (Identification- **1** mark, Comment- **3** marks)
6. Class Records – **5** marks

CONTENTS

QUALITATIVE TEST FOR STARCH, PROTEIN, REDUCING SUGARS AND LIPIDS

TEST FOR STARCH:

PRINCIPLE:

Starch is an important polysaccharide, which is the storage form of carbohydrate in plants. It is abundantly found in roots, tubers, stem, fruits and cereals. Starch exists in two forms –α- amylose and amylopectin.

α- amylose is composed of linear chains of D- glucose in α (1 -4) glycoside linkages. The chain has a reducing end and a non –reducing end. Although poorly soluble in water, α- amylose forms micelles in which □*2*) can insert into the middle of the amylose helix to give a blue colour that is characteristic and diagnostic for starch.

In contrast to α- amylose, amylopectin also shows a highly branched chain of glucose units. The linear linages in amylopectin are α (1-4), whereas the branch linkages are α (1-6). As in the case for α- amylose, amylopectin forms micellar suspensions in water, Iodine reacts with such suspensions to produce a red- violet colour.

REQUIREMENTS:

Test solution, Iodine solution, Test tubes, test tube holder or stand, dropper etc.

PREPARATION OF SOLUTIONS:

TEST SOLUTION- Prepare 1% starch solution (dissolve 1gm of starch in 100ml in distilled water) and heat it till starch dissolves completely. Use distilled water as a Negative control.

I2 in 100ml of water).

EXPERIMENT	OBSERVATION	INFERENCE
Take 2ml of 1% starch solution + few drops of Iodine solution.	Blue colour of Starch Iodide appears.	Presence of Starch.

PROCEDURE:

Take 2 ml of 1% Starch solution in a test tube and add a few drops of Iodine Solution. Blue colour of Starch iodide appears confirming the presence of starch. On boiling, blue colour disappears while on cooling it reappears.

RESULT:

Blue colour indicates the presence of starch.

TEST FOR PROTEIN:

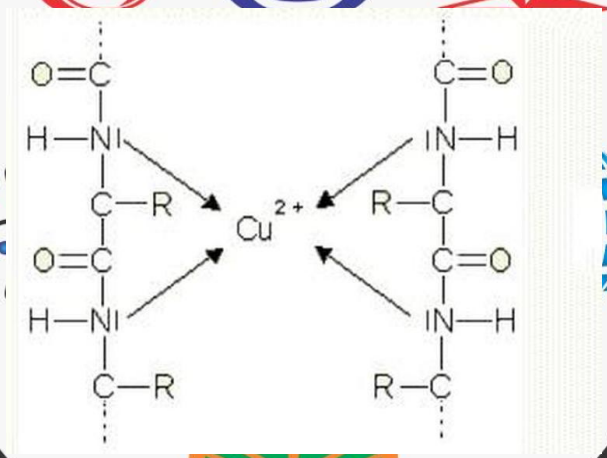
PRINCIPLE:

Proteins are the most abundant class of bio – molecules constituting of more than 50% of the dry weight of plant cells. Proteins are linear polymers of Amino acids. They are the agents of biological functions. The estimation of protein content is the fundamental need in any enzymological study.

BIURET REACTION:

Substances containing two or more peptide linkages produce a blue violet colour with dilute copper sulphate solution in a strong alkali. This is called Biuret Reaction, after the compound Biuret which gives the test. The blue violet colour is developed due to the formation of a complex between cupric ion and two adjacent peptide chains.

FORMULA:



REQUIREMENTS:

O4

Test solution:

Any protein solution (such as green gram or Bengal gram).

PROCEDURE:

O4solution. A blue violet colour appears due to the formation of a complex between cupric ion and adjacent peptide chains.

EXPERIMENT	OBSERV	INFER

	ATION	ENCE
Take 2 ml of test solution in a test tube and add few drops of Biuret reagent or add 2 – 3 drops of 20% □4	Solution turns into pale purple or violet colour	Indicate the presence of protein

RESULT:

Pale purple or Violet colour indicates the presence of proteins.

TEST FOR REDUCING SUGAR:

PRINCIPLE:

Carbohydrates are poly hydroxy aldehydes or ketones that occur in nature as monosaccharides, disaccharides, oligosaccharides and polysaccharides. The monosaccharide and disaccharide exhibit reducing property owing to the presence of free hydroxyl group of the anomeric carbon atom.

Monosaccharide undergoes dehydration under acidic condition to furfural or hydroxyl methyl furfural, which forms the bases for qualitative reactions of sugars.

O4 to cupric oxide which is responsible for the change of colour. The amount of cupric oxide formed depends upon the amount of reducing sugar available in the sample. Therefore the blue colour may be converted to pale green, yellow, orange red or brick red depending upon the concentration of reducing sugar in the sample.

REQUIREMENTS:

Test tubes, Test solutions, Benedict's reagent

Benedict's reagent:

Dissolve 100g Sodium carbonate and 173g of sodium citrate dihydrate in a final volume of 850ml water. Slowly with stirring, and a solution of 17.3g Copper sulphate pentahydrate in 100ml of water. Bring the final volume to 1 litre. Benedict's reagent is commercially available.

PROCEDURE:

Take 2 ml of test solution in a test tube. Add 5 drops of Benedict's reagent and incubate for 5 mins in boiling water bath. The colour changes from blue to green and then to reddish orange indicating the presence of reducing sugars.

EXPERIMENT	OBSERVATION	INFERENCE
2ml of test solution + Benedict's reagent and keep it in hot water bath for 5 min	Colour changes from blue to green and then to reddish orange	Presence of reducing sugars

RESULT: Change of colour from blue to green and then to reddish orange indicates the presence of reducing sugars**.**

TEST FOR LIPIDS:

PRINCIPLE:

Lipids are heterogenous collection of substances, which are characterized by their low solubility in water but high solubility in organic solvents such as Chloroform, Benzene, Carbon tetrachloride and Ether. On hydrolysis, most lipids yield fatty acids. The simple lipids may be classified into four groups namely- Neutral Lipids, Phosphatides, Glycolipids and Waxes.

Lipids provide the structural framework to the living tissues of plants and animals. Plasma membrane and cell organelles like chloroplasts, mitochondria, endoplasmic reticulum, lysosomes and Golgi complex contain the lipids in the forms of layers. In plant cell, lipids are found in the form of small droplets dispersed in the cytoplasm.

Lipids serve as prime fuel reserve for metabolism and provide more energy than carbohydrates and proteins.

Reichert- Meissl Value:

Is defined as the number of milliliter of 0.1N KOH solution required to neutralize the volatile water soluble acids obtained by the hydrolysis of 5.0g of fat.

REQUIREMENTS: KOH, Sudan black III alcohol.

PROCEDURE:

Add 2ml of test solution to 2ml of water in a test tube. Add few drops of Sudan III (dissolve 0.1% in alcohol) and shake. A red stained oil layer separates out on the surface of water, which remains uncolored.

EXPERIMENT	OBSERVATION	INFERENCE
Add 1ml of water and few	Red colour oil layer separates	Presence of lipids

drops of test solution and 2 drops of Sudan black III. Shake and allow to settle	out on the surface of water which remains colourless	

RESULT: A red stained oil layer separates out on the surface of water which remains colourless indicating the presence of lipids.

ALTERNATE TEST FOR LIPIDS:

PRINCIPLE:

Lipids do not mix with water easily, but they do mix with alcohol. Alcohol combines with water very well. Therefore when a mixture of lipids and alcohol is released into water a finely dispersed mass of liquid molecules is produced and this gives rise to a cloud like colloidal suspension.

MATERIALS REQUIRED: Absolute alcohol, given sample containing lipids, distilled water, test tube

PROCEDURE:

A small quantity of the given sample is taken in a test tube and 2 ml of absolute alcohol is added to it. The test tube is shaken thoroughly and the well shaken mixture is poured into distilled water in a test tube.

OBSERVATION: The released mixture is quickly converted into a cloudy precipitate in the aquatic medium.

DETERMINATION OF OSMOTIC POTENTIAL OF CELL SAP BY PLASMOLYTIC METHOD

AIM: To determine the osmotic potential of cell sap by plasmolytic method.

PRINCIPLE:

If a living cell is placed in a solution having a greater osmotic pressure than the cell sap, exosmosis will occur. The water will pass out of the cell and there will be a gradual loss of turgour. As a result of this, several changes in cell appear as stated-

1. Pulling away of the plasma membrane from the cell wall.
2. Shrinkage of the protoplasm and its manifestation in the corner of the cell as a spherical mass in the centre, this phenomenon is termed Plasmolysis.

Incipient Plasmolysis:

If withdrawal of the Protoplast has just started, the cell is in a state of incipient plasmolysis or threshold plasmolysis.

Isotonic: when the concentration of solution outside the cell is equal to vacuole, it is in tune or isotonic with the sap.

Hypertonic: if the concentration is higher, the solution is said to be hypertonic.

Hypotonic: if the concentration is lower, the solution is said to be hypotonic.

Plasmolysis can be demonstrated if epidermal tissue from the *Rhoeo* sp or onion scale leaves is immersed in a hypertonic solution of sucrose. Because of the pigmentation of the sap in leaf cells of these plants, the shrinking protoplasm can be easily seen under microscope.

REQUIREMENT:

Onion scale leaves or *Rhoeo discolor* leaf, Slides, Cover glass, Watch glass or cavity blocks, Microscope, Pipettes, Test tubes, Measuring jar, Balance, Test tube stand, 1M stock solution .

PROCEDURE:

Take the leaves of *Rhoeo discolor* and prepare solutions of different concentrations. Prepare the standard solution of sucrose by dissolving 85.5g of sucrose in 100ml water and add 150ml of water in it to make its final volume 250ml. From this standard solution, prepare 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 M solutions by further dilution as follows-

1. 1 ml of SS + 9ml of Water = 0.1 M solution (SS=Standard Solution)
2. 2 ml of SS + 8ml of Water = 0.2 M solution
3. 3 ml of SS + 7ml of Water = 0.3 M solution
4. 4 ml of SS + 6ml of Water = 0.4 M solution
5. 5 ml of SS + 5ml of Water = 0.5 M solution
6. 6 ml of SS + 4ml of Water = 0.6 M solution

Now, peel off the lower colored epidermis of the leaf of *Rhoeo discolor* and cut into six small pieces. Dip one piece in each solution for some time and observe separately under microscope after mounting on the slide for plasmolysis.

Suppose, the plasmolysis does not occur in 0.1, 0.2 and 0.3 M solution and occurs in 0.4, 0.5 and 0.6 M solutions. It indicates that the isotonic solution should be in between 0.3 and 0.4 M sucrose solution. Now, prepare further dilutions of the solutions between 0.3 to 0.4 that is 0.2, 0.34, 0.6 and 0.38 M. Repeat the experiment with these dilutions and find out the concentration where 50 percent of the cells show incipient plasmolysis. This concentration of sucrose solution will be isotonic with the cell sap.

□□)of the cell sap can be calculated by the formula-

$$(□□) = -CRT$$

1− 1

1 □−1 mol□*−1*

T = absolute temperature (273+ T) K

The above formula is suitable only for non–electrolytes. E.g., sucrose solution is an electrolyte. Eg. NaCl, its osmotic pressure is calculated first and then multiplied by its degree of dissociation. Ionization constant for NaCl is 1.8.

RESULT:

The Osmotic Potential of cell sap by plasmolysis method = _____ bars.

DISCUSSION:

When a plant cell is kept in a hypertonic solution, water from cell sap flows out due to a reverse concentration gradient. As the solution in the external medium is more concentrated, it has more osmotic potential and hence water moves out of the cell, resulting in shrinkage of the cell volume. This phenomenon of water loss from cells which is similar to exosmosis is called plasmolysis.

The plasmolytic method of determining the osmotic potential was first derived by Devries (1884). The method involves finding out the osmotic potential of the cell in different concentrations of solutions. At a particular concentration of cell sap, it is equal to the concentration of the external medium. Usually osmotic potential obtained by this method is

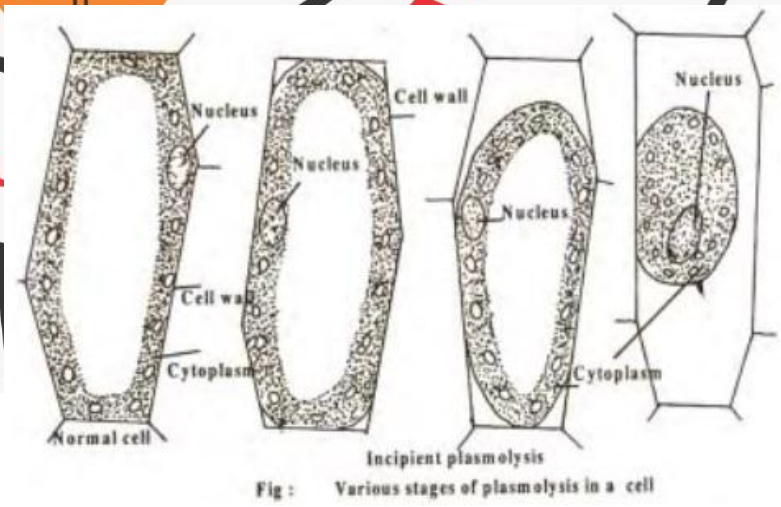


Fig : Various stages of plasmolysis in a cell

slightly higher than actual value. Hence osmotic potential is then calculated using the formula of Hofler (1917).

The process of plasmolysis and deplasmolysis are important because they help in measurement of permeability of cell membranes.

SL. NO.	MOLAR CONCENTRATION OF SUCROSE SOLUTION	VOLUME OF STOCK SOLUTION	VOLUME OF DISTILLED WATER
1	0.1M	1.0ml	9.0ml
2	0.2M	2.0ml	8.0ml
3	0.3M	3.0ml	7.0ml
4	0.4M	4.0ml	6.0ml
5	0.5M	5.0ml	5.0ml
6	0.6M	6.0ml	4.0ml
7	0.7M	7.0ml	3.0ml
8	0.8M	8.0ml	2.0ml

SL. NO.	MOLAR CONCENTRATION OF SUCROSE SOLUTION	NO OF CELLS IN UNIT AREA	NO OF CELLS PLASMOLYSED	PERCENTAGE OF PLASMOLYSIS	NATURE OF PLASMOLYSIS
1	0.1M				
2	0.2M				
3	0.3M				
4	0.4M				
5	0.5M				
6	0.6M				
7	0.7M				
8	0.8M				

DETERMINATION OF STOMATAL INDEX BY QUICK FIX METHOD

AIM:

To determine the stomatal index and study of stomatal structure by quick fix method or direct peeling (*Vinca, Beetle, Commelina* leaves).

REQUIREMENTS:

Rhoeo discolor leaves, Beetle leaf, Glass slide, Cover slip, Microscope, Petriplate, Quick Fix or cellophane tapes.

PRINCIPLE:

Stomata are the minute pores present on the epidermis of stem and leaf. Each stomata consists of an aperture surrounded by two kidney shaped cells called Guard cells. The wall of the guard cells lining the aperture is thicker than the surrounding cells. Guard cells are surrounded by two or more subsidiary cells.

Stomatal frequency differs with environmental conditions, especially due to the availability of water. Stomatal index is an expression of the percentage proportion of the ultimate division of the dermatogens of the leaf that have been converted into stomata. Thus under a given set of conditions, a species tends to form a definite proportion of stomatal index.

PROCEDURE:

Apply thin film of quick fix on the surface of leaf. Remove the dried quick fix by peeling it away from the leaf surface and mount it on a slide in a drop of water. Observe it under high power of magnification of a microscope. Draw a diagram of the stomata and identify.

OR

Lower epidermal peeling of the given leaf is directly taken. It is cut into small strips, stained in saffranin, mounted in glycerine and observe under high power objectives.

STOMATAL INDEX:

The quick fix peel mounted on the slide is observed under microscope. Count the number of stomata visible under the field of microscope and also the number of epidermal cells in the same area.

STOMATAL INDEX, I = S/E+S ×100

Where,

S= No. of Stomata in an unit area,

E= Number of Epidermal cells in the same unit area.

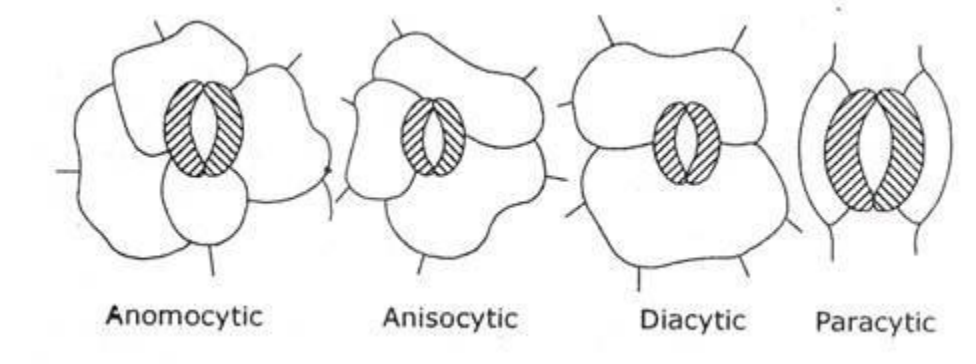
Based on the subsidiary cell arrangement with respect to guard cells, the stomata are classified into following types:

a) **ANOMOCYTIC STOMATA OR IRREGULAR CELL TYPE** where the subsidiary cells are indistinguishable from the epidermal cells. Eg. Ranunculus Type

b) **ANISOCYTIC STOMATA OR UNEQUAL CELL TYPE** where, of the subsidiary cells one is small and the other two are larger. Eg. Cruciferous Type

c) **DIACYTIC STOMATA OR CROSS CELL TYPE** where the stomata remains surrounded by a pair of subsidiary cells which lie at right angles to the longitudinal axis of pore and guard cells. Eg. Acanthaceae Type

d) **PARACYTIC STOMATA OR PARALLEL CELL TYPE** where stoma remains enclosed by a pair of subsidiary cells which are parallel to the longitudinal axis of pore and guard cells. Eg. Rubiaceae Type



SL. NO.	NAME OF THE PLANT	NUMBER OF STOMATA PER UNIT AREA	NUMBER OF EPIDERMAL CELLS
1	*Rhoeo discolor*		
2	Beetle Leaf		

STOMATAL INDEX, I= $\frac{S}{E+S}$ ×100

RESULT:

Stomatal Index of *Rhoeo* leaf is

Stomatal Index of Beetle leaf is

STREAMING OF PROTOPLASM TO SHOW CYCLOSIS:

(Demonstration experiment)

AIM:

To observe the protoplasmic streaming movements in *Elodea* leaf (Or staminal hairs of *Tradescantia*).

INTRODUCTION:

All living organisms are composed of the essential living substances protoplasm which is jelly like colloidal substance. The protoplasm undergoes series of chemical changes that regulates the entire cellular activity. The major characteristic feature of all living system is expressed by protoplasm and its components via movement, permeability and metabolic reactions. The streaming movement of protoplasm also indicates its living nature. The principle mechanism of streaming movement is the constant cycle of depolymerisation and repolymerisation of cytoskeletal elements.

REQUIREMENTS:

Young leaves of *Elodea* or *Vallisneria* (Leaf of any Hydrophytes), Slides, Cover Slips, Compound microscope

PROCEDURE:

Collect fresh leaves or dissect out the stamina hair, place them on the slide and cover with the help of cover slip adding a drop of water. Observe under high power of the microscope especially the chloroplast in the Elodea leaf.

OBSERVATION AND RESULTS:

The chloroplasts appear to move in a circulating fashion around the vacuole. In the case of *Elodea*, there is a large central vacuole and the chloroplasts move in a clockwise or anti clockwise direction. This is known as rotation.

The movement of the chloroplast seen under the microscope is actually due to the circulation or streaming of protoplasm. The movement probably occurs due to the Sol gel formation of protoplast. Microtubules of cytoplasm may also contribute to the movement.

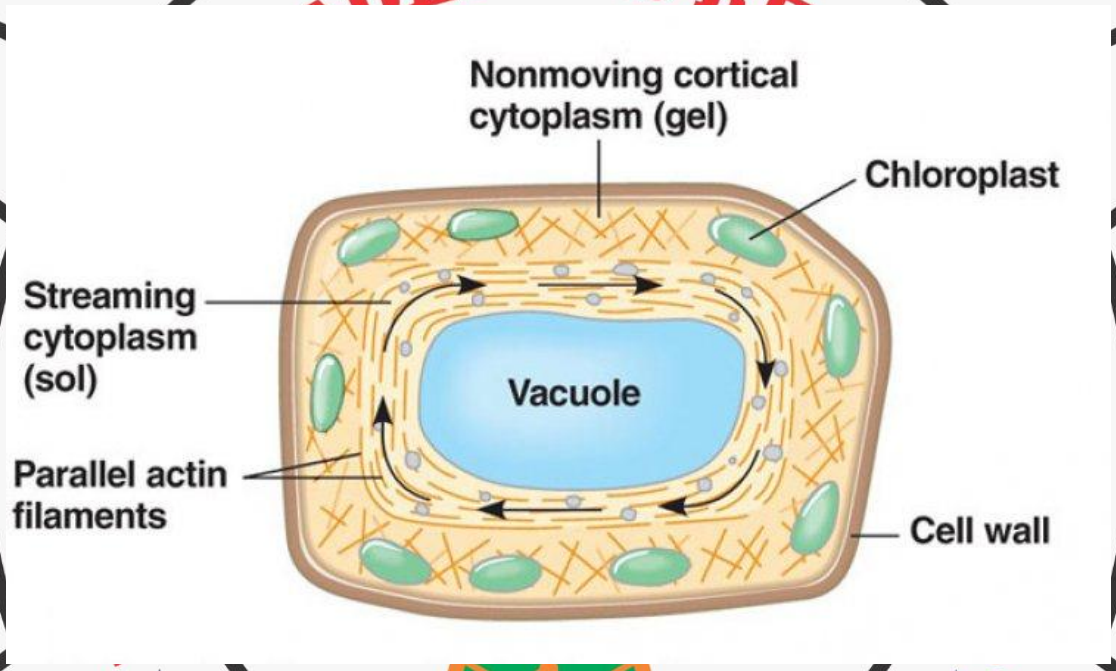
The chloroplasts were found moving around the vacuole in both clockwise and anti-clockwise direction.

DISCUSSION:

The streaming movement of protoplast is called Cyclosis. This is of 2 types, namely:

1) Rotation and 2) Circulation

- Rotation is the movement of the protoplasm that moves in clockwise or anticlockwise direction. The cells of *Hydrilla* etc show rotation.
- Circulation is the movement of protoplasm in different directions around a number of small vacuoles. It is seen in *Tradescantia* staminate hair. The rate of Cyclosis normally decreases with the maturity of the tissue.



Demonstration of Streaming movement in the protoplasm in a living plant cell

DETERMINATION OF APPROXIMATE pH OF PLANT SAMPLE USING INDICATOR

AIM:

To determine the approximate pH of plant sample using indicator.

PRINCIPLE:

In most living systems (Vascular sap and Protoplasm), the pH is maintained at remarkably constant value. This is made possible through the presence of buffer system in plants. The fact that certain acids, bases and salts dissociate to relatively light extent permits the formation of buffer system.

REQUIREMENTS:

Plant samples, roots, stem and leaves of any herbaceous plant (Dicot & Monocot), universal pH indicator / pH paper, pestle and mortar, distilled water.

PROCEDURE:

Wash the plant material thoroughly in water. Cut the root, stem and leaves into small pieces separately. Prepare three separate aqueous extracts in distilled water by homogenizing the tissue in a mortar. Test the pH of root, stem and leaf extract using pH indicator.

NOTE: For the purpose of comparison different plant samples may be used or alternatively same species growing in different areas may be used for comparison.

RESULT:

Name of plant	pH of Stem	pH of Root	pH of Leaf

DISCUSSION:

Plants are found growing in a pH range of 3 to 9. pH is the hydrogen ion concentration of any solution. All □+ ion. The pH scale ranges from 0 to 14 and can be used to decide the strength of solutions from normal acid to normal base. Most of the physiological reactions occur at a specific pH. Any alteration of the pH will disturb the rate of reaction. All the physiological reactions occur under the catalytic action of enzymes which are □*6* to p□*8*. In a single plant, the different parts show different pH and different species having different range of pH. The pH of the plants varies depending on the nature of soil and other external factors.

BELL JAR EXPERIMENT:

AIM: To demonstrate transpiration by vapour condensation inside Bell jar.

REQUIREMENTS: Bell jar and potted plant, polythene sheet, glass plate, grease.

PROCEDURE: Take a well-watered potted plant. Cover the pot with a polythene sheet to check the evaporation of water from the soil surface. Now place the potted plant over a glass plate and cover it with the bell jar. Make the apparatus air tight by smearing grease in the place between glass plate and bell jar. Leave the apparatus undisturbed.

OBSERVATION: After sometime, drops of water will appear on the inside of bell jar.

INFERENCE: Water drops are formed on the inside of bell jar. It is due to the condensation of water vapour which is produced during transpiration.

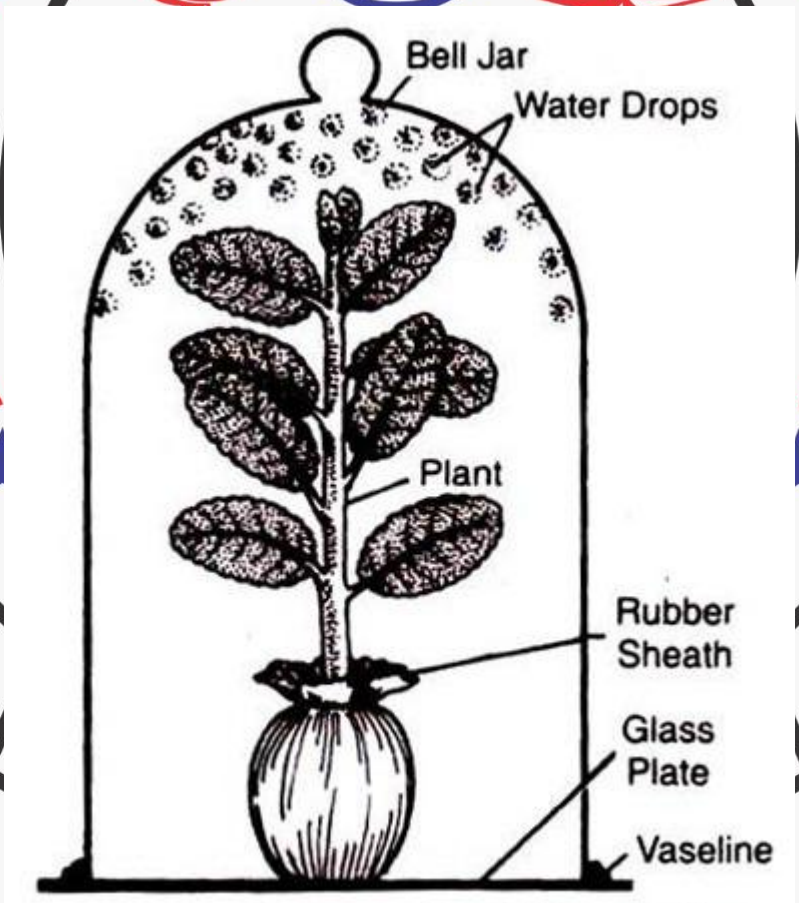


Fig. 21. Demonstration of transpiration.

DEMONSTRATION OF SUCTION PRESSURE DUE TO TRANSPIRATION:

AIM: To demonstrate suction pressure due to transpiration.

PRINCIPLE: Leaf cells lose water during transpiration. It increases the diffusion pressure deficit. These cells, therefore, withdraw water from the neighbouring cells. The later, in turn, get water from the xylem sap of the leaf. The sap in the leaves thus comes under a pull from the transpiring leaf cells. This pull or suction is due to the increased diffusion pressure deficit of the cells as a result of transpiration.

MATERIALS REQUIRED: A long narrow glass tube, rubber tubing, leafy shoot cut under water, water, mercury, beaker and stand.

PROCEDURE: A long narrow glass tube with rubber tubing at one end is completely filled with water. The cut end of a leafy twig is inserted through the rubber tubing without any gap. The lower end of the tube is dipped in beaker containing mercury. The whole setup is kept in sunlight.

RESULT: After some time, there will be a rise in level of mercury. It shows that water present in tube is being pulled or suckered by the transpiring twig.

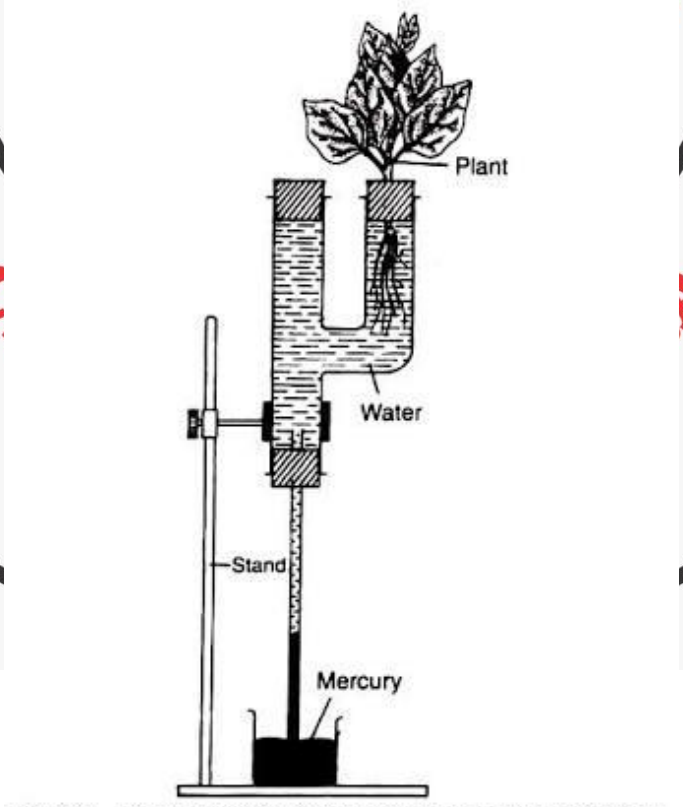


Fig. 22. Demonstration of water-lifting power of transpiration.

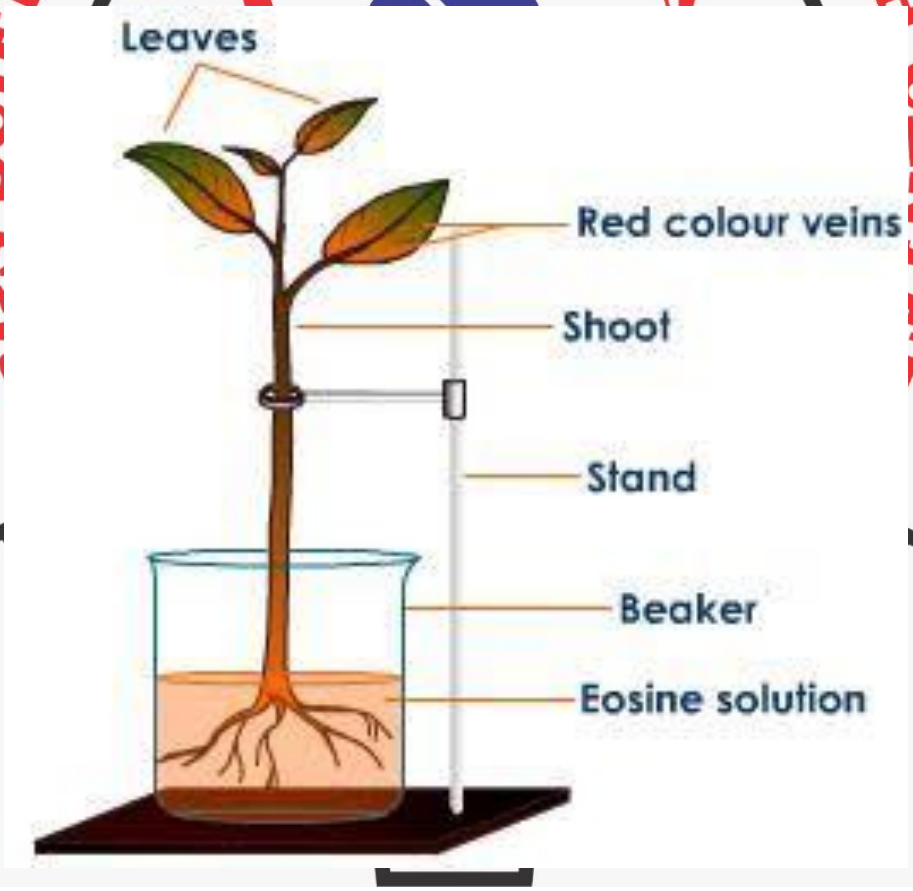
BALSAM PLANT EXPERIMENT

AIM: To determine the experiment that xylem is the pathway of ascent of sap.

MATERIALS: A beaker, water, eosin stain, stand, white balsam plant with roots.

PROCEDURE:

- Put few drops of eosin stain in a beaker containing water.
- Insert a balsam plant into it. Fix to stand, keep it for few hours.
- Red lines can be seen in the transparent stem and veins of the leaves.
- When a section of stem is observed under the microscope it shows that only the xylem vessels are coloured.
- This experiment confirms xylem is the pathway of ascent of sap.



BALSAM PLANT EXPERIMENT

STUDY OF PHLOEM TRANSPORT BY RINGING EXPERIMENT

AIM: To demonstrate the ringing experiment to study the phloem transport.

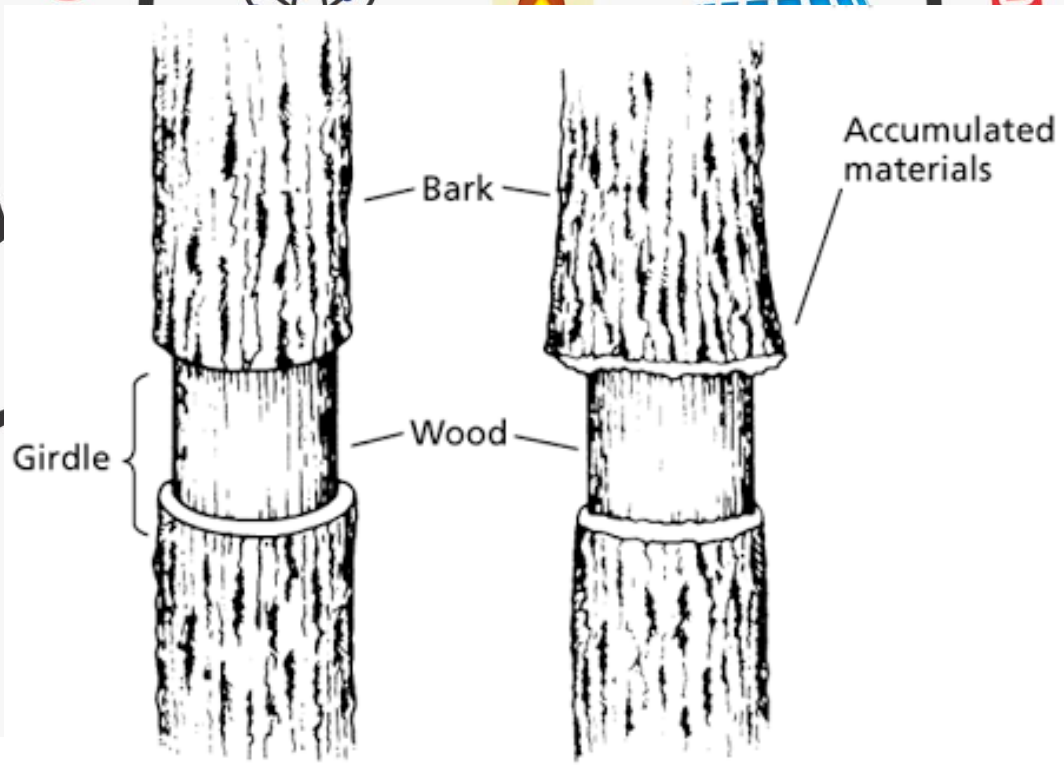
REQUIREMENTS: Healthy potted plant, knife.

PROCEDURE: Malphigi (1671) used "Ringing experiment" to show the role of xylem in ascent of sap.

A healthy potted plant is selected, a ring of bark (all the tissue outer the xylem) is removed from the stem (2-3cm in length) just outside the xylem should not be injured. The experiment is left as such for few days.

OBSERVATION: Whereas in case of twig, there is no downward movement of solutes takes place because phloem tissue is removed, therefore bulging of stem takes place in cut end portion and roots are developed.

RESULT: The bulging or swelling of stem takes place in the cut end and root is developed in part where phloem removed because in this region, no translocation of solutes takes place. So, it comes to end portion. It undergoes swelling and growth of roots takes place. Therefore, it clears that phloem is necessary for translocation of solute.



RINGING EXPERIMENT

MUNCH MASS FLOW HYPOTHESIS

AIM: To explain Munch mass flow hypothesis.

REQUIREMENTS: Osmometer X and Y, sucrose solution, semi-permeable membrane, horizontal tube.

PROCEDURE: The most widely accepted theory of translocation of solutes prepared by Ernst Munch (1923) it is variously called the mass flow, solutions flow, pressure flow theory be proposed a physical model that behaved very much like the phloem system in a living plant it consist of two bulbs 'X' and 'Y' made up of semi-permeable membrane and connected with each other by a tube 'T'. Bulb X is filled with concentrated sucrose solution and Y contains only pure water. Both bulbs are immersed in water. While doing so, water immediately begin to enter the X (which contain sucrose solution). Since it has lower water potential, as a result, pressure increases in this chamber and it forces some of the solutions to rise into the connecting tube 'T' and eventually flows across the other bulb 'Y' taking some of the sucrose solution along. When sucrose solution enters the bulb Y, it forces some of the water to flow out through the membrane into the water bath. The flow of solution from X to Y continues until the solution in both bulbs become equally concentrated at such a movement, both the bulbs would have equal water potential.

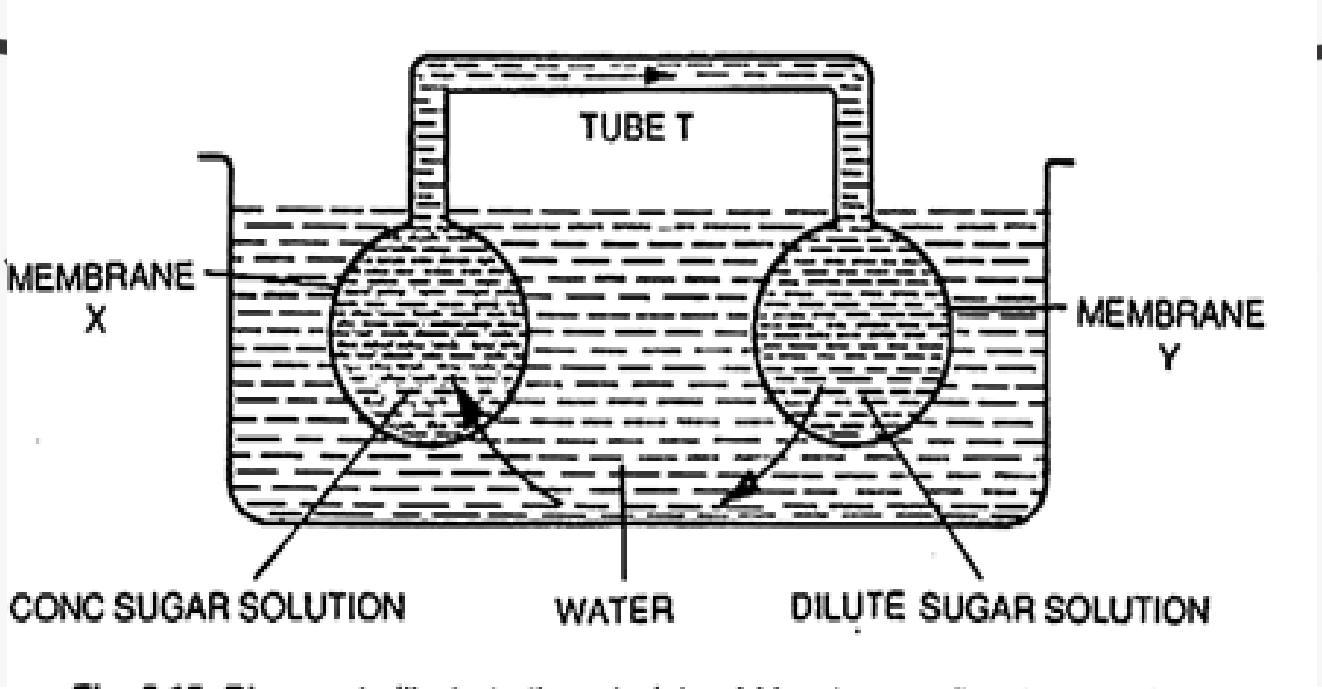


Fig. 5.15. Diagram to illustrate the principle of Munch mass flow hypothesis.

POTATO OSMOSCOPE

AIM: To demonstrate osmosis through plant membrane with the help of potato osmosis.

REQUIREMENTS: Potato tuber, sugar solution, water, pin, beaker and Petri plates.

PROCEDURE: A large sized potato tuber is taken, its skin is peeled off on the base is cut to make it flat. A hallow cavity is made in the center with the help of the scoop. The cavity is filled with strong sugar solution. The initial level of sugar solution is marked with a pin inserted in the wall of the tuber. The potato tuber is now placed in a beaker of water.

OBSERVATION: After few hours, there is a rise in the level of sugar solution in the cavity.

INFERENCE: the rise in the level of sugar solution is due to osmotic diffusion of water into the sugar solution. The experiment clearly demonstrates that living cells of potato act as differentiate permeable membrane.

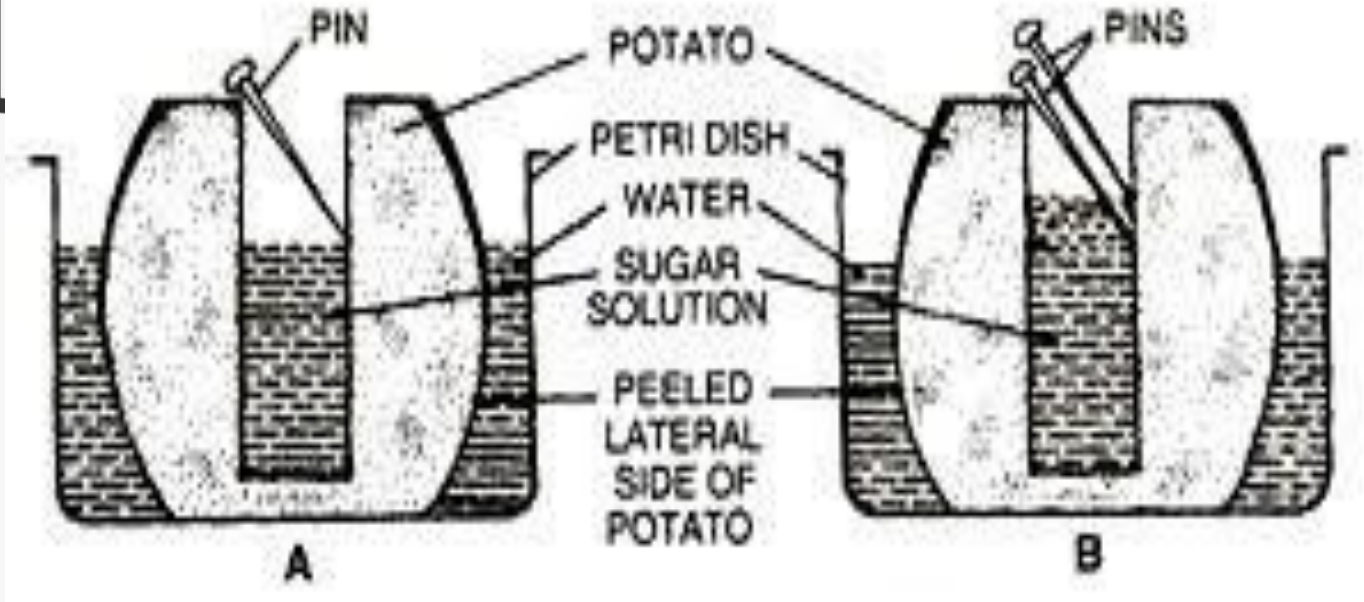


Fig. 11.8. Potato osmoscope experiment to demonstrate osmosis. A, Original level; B, Final level.

OSMOSIS: THISTLE FUNNEL OSMOSCOPE

AIM: To demonstrate the phenomenon of osmosis using an organic membrane.

REQUIREMENTS: Sugar solution, organic membrane, water, Thistle funnel, beakers, stand and string.

PROCEDURE: Tie the organic membrane to the mouth of the thistle funnel tightly. See that membrane is devoid of any pores. Invert the thistle funnel and pour sugar solution into it. Note the initial level of sugar solution in the stem of funnel by tying a string. Place it in as beaker containing water. See that the mouth of the funnel is immersed completely in water. Clamp it firmly to the stand. Leave the setup undisturbed for some time.

OBSERVATION: There is a raise in the level of sugar solution in the thistle funnel.

RESULT: One side diffusion of water from the region of its higher concentration to the region of its lower concentration in a system of two liquid separated by a semi-permeable membrane occurs by osmosis.

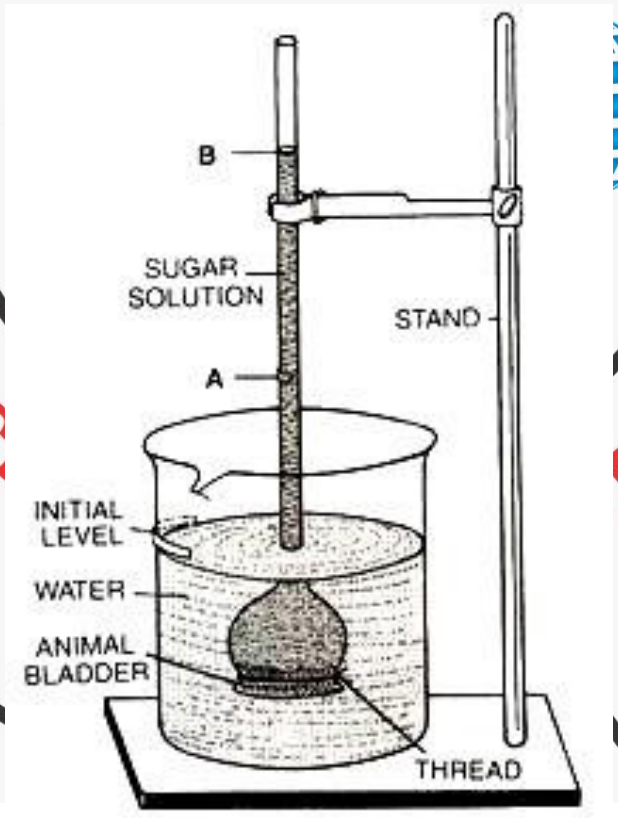


Fig. 11.7. Thistle funnel experiment to demonstrate osmosis.

TRANSPIRATION: GANONG'S POTOMETER EXPERIMENT

AIM: To measure the rate of transpiration.

PRINCIPLE: Transpiration in leaves creates a tension in the xylem to absorb water. So, transpiration of water is equal to the absorption of water by roots or stem.

REQUIREMENTS: Ganong's potometer, beaker, water and plant twig

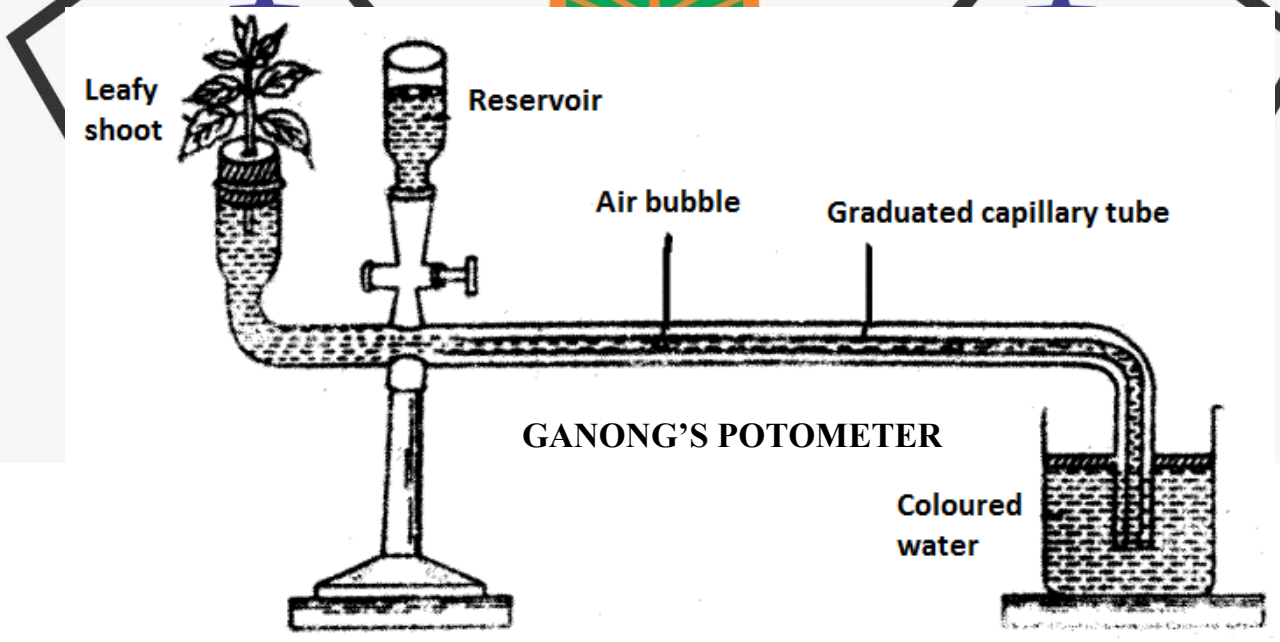
DESCRIPTION OF APPARATUS: Ganong's potometer consists of a horizontal graduated tube with 2 vertical reservoirs at one end. The tube is bent at the other end with a small terminal opening. The inner reservoir has a stopper at its base. The complete apparatus is fixed on a wooden stand.

PROCEDURE: Fill the apparatus with water. Select a healthy leaf twig and cut it under water. Fix the twig to the wide end of the horizontal tube through a hole in the cork. Dip the other end of horizontal tube in a small beaker containing water. Make the apparatus air tight by smearing grease around the cork. Take out the bent end from the beaker and insert an air bubble in the horizontal tube, again place the tube in the beaker. Now keep the apparatus in bright light.

OBSERVATION: After sometime it will be observed that the air bubble slowly moves towards the other end. The rate of its movement can be measured with the help of a graduated horizontal tube.

INFERENCE: As transpiration occurs in the leaves of the twig, water is drawn from the reservoir and ultimately from the beaker. As the water is drawn from the beaker the air bubble moves along the horizontal tube. The rate of movement of air bubble will be the rate of transpiration. The rate varies with the changes in the environmental conditions.

The rate of transpiration at different conditions can be measured from the movement of air bubble. By allowing the water to flow from the inner reservoir, the position of the air bubble can be regulated.



MEASUREMENT OF TRANSPIRATION USING FARMER'S POTOMETER

AIM: To measure the rate of transpiration by using farmer's potometer.

PRINCIPLE: The method is based on the assumption that the rate of absorption of water is approximately equal to the rate of transpiration.

REQUIREMENTS:

Farmer's photometer, water, Vaseline, shoot cut under water and beaker.

PROCEDURE: Farmer's potometer comprises a wide mouthed bottle having rubber cork with 3 holes. A thistle funnel with a stop cork is fitted through one hole. It acts as a water reservoir. A long narrow bent tube of known diameter with a scale is fitted to a bottle through another hole. In the third hole, a leafy twig (cut under water) is introduced in such a way that its lower cut end remains well under water.

The whole apparatus is filled with water and the reservoir is closed by stop cork and the apparatus is made air tight. An air bubble is introduced into the narrow tube. Afterwards the free end of the tube is dipped in water contained in a beaker. As transpiration proceeds, the air bubble travels forward. Note the time taken by air bubble to pass from one end of the scale to the other. The water reservoir is opened slowly to push the air bubble back to the beginning of the scale.

The rate of transpiration is calculated in terms of ml/hr. the volume of the water transpired can be calculated by using the formula

$$V = \prod r^2 l$$

Where, V= Volume,

$\prod$ = 22/7,

r= Radius of narrow tube,

l= length or distance travelled by the bubble.

OBSERVATION: Air bubble moves through the graduated horizontal tube.

INFERENCE: the distance travelled by the air bubble will give the volume of water that is lost due to transpiration. With the help of farmer's potometer the effect of different factors such as temperature, wind, velocity, humidity, light etc on rate of transpiration, can be measured.

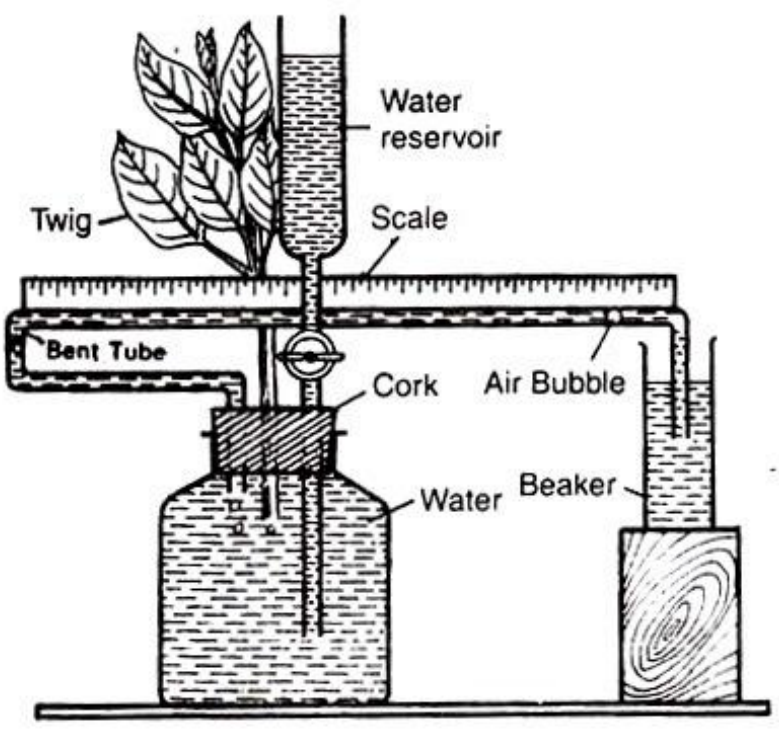
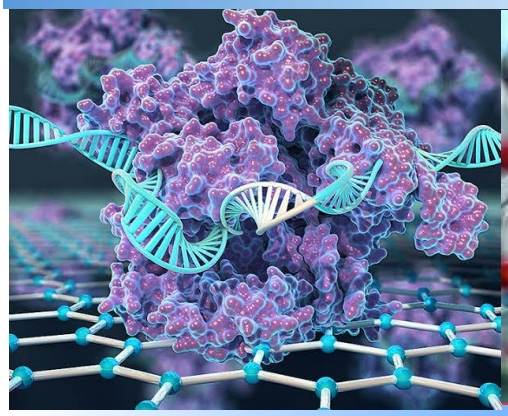


Fig. 30. Farmer's potometer.

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