

Study of Osmosis by Potato Osmoscope

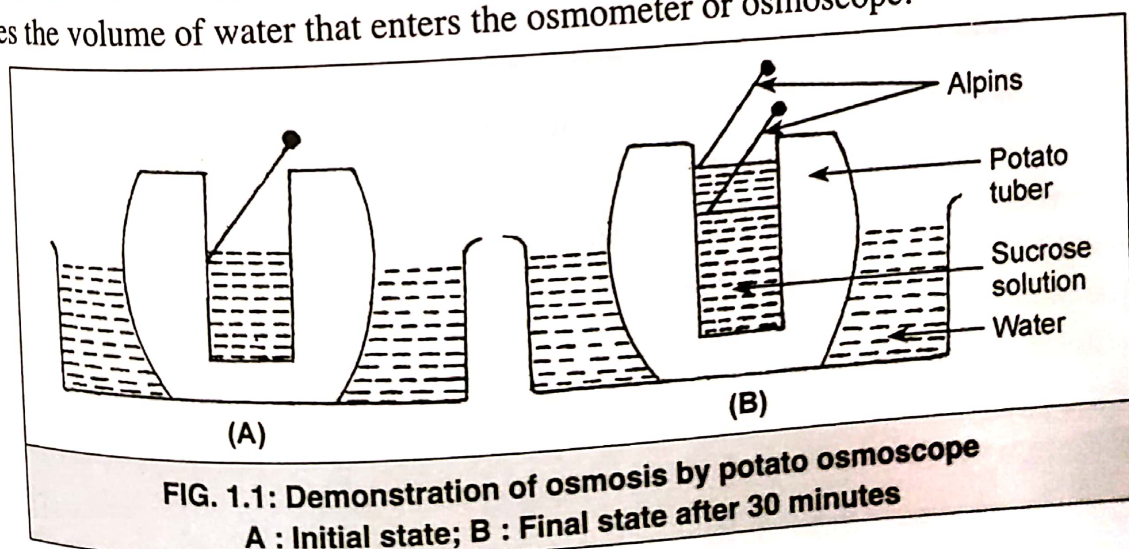
EXPERIMENT NO. 1

Aim of the experiment. To study the phenomenon of osmosis by potato osmoscope or osmometer.

Requirements. 1.0 M sucrose solution, wide beaker, distilled water, a pin, potato tubers, scalpel.

Procedure. A potato tuber of large size is taken and the outer skin is removed. Then at the middle of the tuber a hole of approximately 2 cm diameter and depth of 4 cm is prepared with the help of a scalpel carefully, as shown in the diagram (Fig. 1.1) Now 1.0 M sucrose solution (342 gms of sucrose in 1000 ml of water) is added in the hole of the tuber so as to fill up to approx 2 cm height and at that level an alpin is pieced obliquely. Then it is kept in a wide beaker with some distilled water in such a way that the water level outside is below the height of the potato osmoscope. Now it is kept for half an hour and then the rise in the level of sucrose solution is marked and another alpin is pierced at the upper level.

Observation. Due to osmosis, water from outside enters through the wall of the tuber towards the sucrose solution so that after half an hour the height of the level of sucrose solution is increased. The difference in the initial level and final level is measured which indicates the volume of water that enters the osmometer or osmoscope.



Precautions.

1. The hole in the potato tuber should be made carefully.
2. Sucrose solution should be added in the hole of the osmoscope carefully.
3. Water level in the outside should be kept at a lower level than that of the height of the osmometer.

Conclusion. From the above experiment it is concluded that in osmosis solvent molecules (here water) pass from its higher concentration to its lower concentration (*i.e.* to sucrose solution where water concentration is less), or in other words in osmosis, solvent molecules pass from lower concentration of solution to higher concentration of solution when separated by a semi-permeable membrane. Potato tuber acts as a semi-permeable membrane. Due to osmosis there is a rise in the level of sucrose solution in the hole of the potato tuber.

N.B. Sucrose solution of 1.0 M (M = molar) is prepared by adding 342 gms of sucrose (mol. wt. 342) in 800-900 ml. of distilled water and stirred and the final volume is made upto 1000 ml by adding distilled water.

Determination of Osmotic Potential of Plant Cell Sap by Plasmolytic Method of Leaves of *Rhoeo* or *Tradescantia*

► EXPERIMENT NO. 2

Aim of the experiment. To determine the osmotic potential of plant cell sap by plasmolytic method of leaves of *Rhoeo discolor* or *Tradescantia*.

Requirements. Watch glass, glass slide, cover slip, 1.0 M sucrose solution (342 gm of sucrose / 1000 ml of water), compound microscope, graph paper.

Pant material. Fresh leaf of *Rhoeo discolor*/*Tradescantia*.

Theory. Plasmolysis is the phenomenon of shrinkage of protoplasm when cells are immersed in hypertonic solution leading to exo-osmosis where solvent (water) molecules pass from the cell to the outside. Solution may be :

- (a) **Hypotonic** : When its concentration is lower than that of the cell sap. It leads to endo-osmosis where solvent (water) molecules enter the cell making it turgid.
- (b) **Isotonic** : When its concentration is equal to that of the cell sap so that there is no movement of the solvent molecules. (Here turgor pressure or wall pressure is zero).
- (c) **Hypertonic** : When the concentration of the external solution is higher than that of the cell sap. It leads to exo-osmosis making the cell flaccid.

Osmotic pressure (π) is the pressure applied by the solution to prevent the inward flow of water across a semi permeable membrane. But **osmotic potential** (ψ_π), also know as **solute potential** (ψ_s), is the potential of water molecules to move from a hypotonic solution to a hypertonic solution across a semipermeable membrane. Thus osmotic potential is equal in magnitude, but opposite in sign to osmotic pressure so that

$$\psi_\pi = -\pi$$

Osmotic pressure (π) = CRT, where C = concentration of the solute in solution

R = Universal gas constant (0.082)

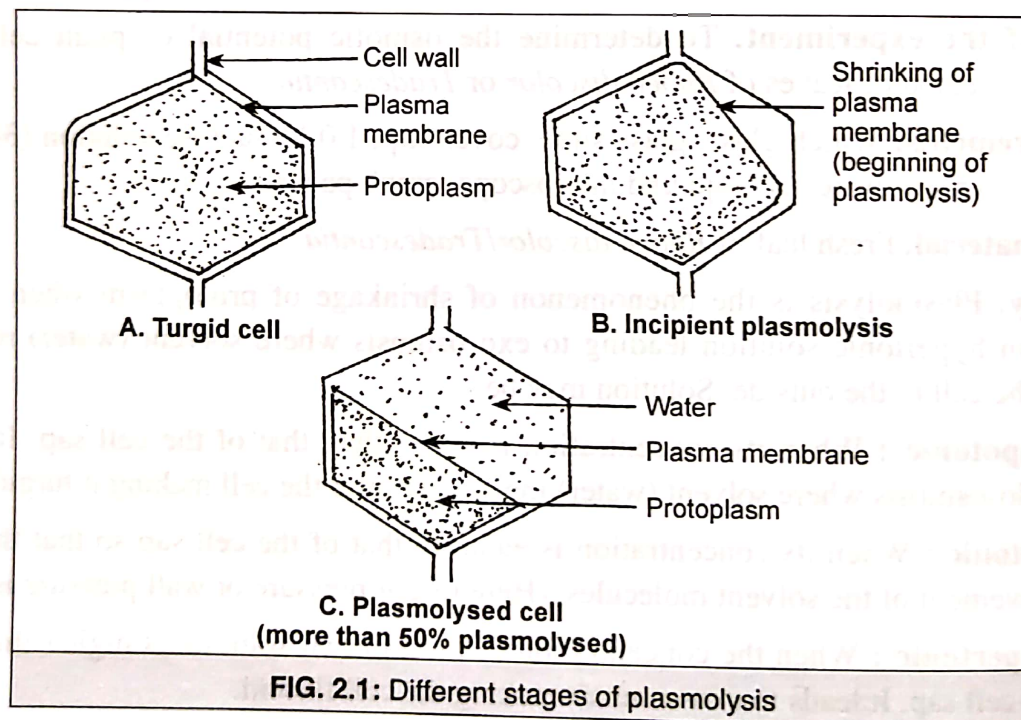
T = Absolute Temperature (273+ room temp. in °C)

Hence Osmotic potential (ψ_{π}) = $-\pi = -CRT$

$$\psi_{\pi} = -CRT$$

By using the above equation, osmotic potential of the cell sap of leaves or any other plant material can be calculated. The value should be expressed in bar as atmospheric pressure is approximately equal to bar (1 atm. = 1.01 bar)

Procedure. A fresh leaf of *Rhoeo discolor* is taken, broken at the mid-rib portion and the lower fine violet or pink coloured epidermal peels are taken and made into small pieces. These pieces are kept in watch glasses containing distilled water (control) or 0.1, 0.2, 0.3, 0.4 and 0.5 M (Molar) concentration of sucrose solution separately. These concentrations of sucrose solutions are prepared from stock 1.0 M sucrose solution by adding distilled water in specific proportions. In each concentration, the peels are kept for 15 minutes and then mounted on a glass slide with a cover slip and observed through a compound microscope by the high power resolution (Fig. 2.1) The data are presented in a table and plotted in a graph. Now the osmotic potential is calculated.



Observation

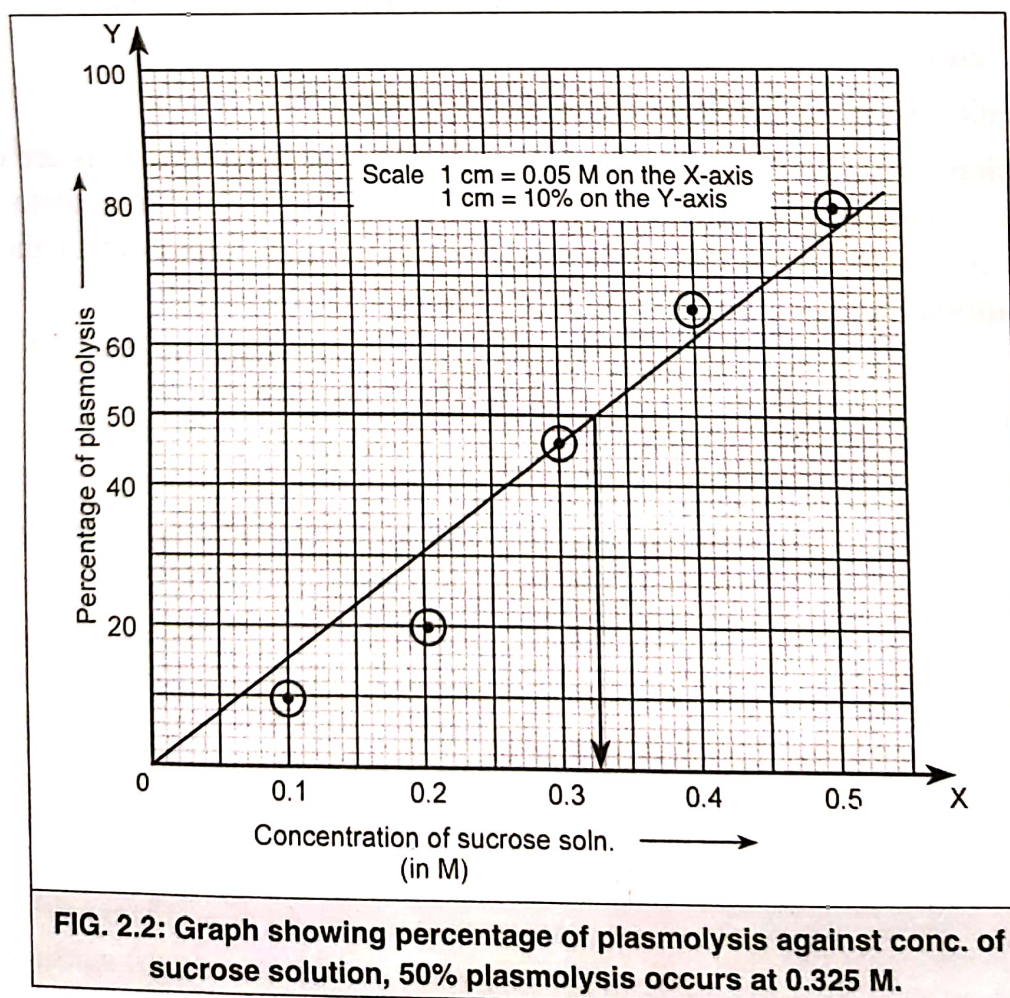
- (1) The peel in distilled water exhibited turgid cells with fully expanded pink or violet protoplasm due to endo-osmosis where water (solvent) enters the plant cell from outside.
- (2) When the peels are kept in increasing concentration of sucrose solution from 0.1 M to 0.5 M, gradually the percentage of plasmolysed cells increased. Plasmolysis occurs due to shrinkage of protoplasm when the cells are immersed in **hypertonic** solution

(higher concentration than that of the cell sap) and it is due to exo-osmosis where water (solvent) goes out of the cell. It is generally at more than 0.3 M sucrose solution.

- (3) The starting of shrinkage of protoplasm is known as **incipient plasmolysis**.
- (4) At higher concentration of sucrose, (at 0.5 M or more), the protoplasm strinks to a larger extent so that it forms a small spherical mass at one side or centre of the cell.
- (5) The concentration of sucrose solution where 50% plasmolysis has occurred is the **isotonic** condition when the concentration of the cell sap and that of the external medium is similar. It is approximately of 0.3 M for the epidermal peels of *Rhoeo discolor*.

TABULATION

No. of obs.	Conc. of sucrose soln. in M	No. of cells in the focus	No. of cells plasmolysed	% of plasmolysis
1	0 (Control)	14	0	0
2	0.1	15	1	6.66
3	0.2	14	3	21.42
4	0.3	13	6	46.15
5	0.4	14	9	64.28
6	0.5	14	12	85.71



Calculation: From the sample graph, concentration of the solution at isotonic condition = 0.325 M say at 27°C of room temperature.

$$\begin{aligned}\text{Osmotic potential, } (\psi_{\pi}) &= -CRT \\ &= -0.325 \times 0.082 \times (273 + 27) \\ &= -0.325 \times 0.082 \times 300 \\ &= -7.995 \text{ atmospheres} \\ &= -7.995 \times 1.01 = -8.07 \text{ bars}\end{aligned}$$

Precautions.

- (1) Fresh healthy leaf of *Rhoeo discolor* should be taken.
- (2) Thin peels (violet colour) should be taken carefully.
- (3) After taking the peels, it should immediately be kept in distilled water or in test solutions.
- (4) Counting of cells should be done through high power of compound microscope. The cells where 50% or more than 50% of protoplasts have shrunk, are counted as plasmolysed cells.
- (5) The test solutions should be covered with watch glasses.
- (6) Preparation of different concentrations of sucrose solution should be done accurately and carefully.
- (7) The plotting in the graph should be done carefully.

Conclusion. From the above experiment, it can be concluded that cells are turgid when kept in water or hypotonic solution, but plasmolysed when immersed in hypertonic solution. Plasmolysis is due to exo-osmosis where solvent molecules comes out of the plant cell. Isotonic condition (50% plasmolysis) is found to be at 0.325 M.

Osmotic potential of the cell sap of *Rhoeo discolor* was found to be -8.07 bars at 27°C from the sample calculation.

Study of Structure of Stomata of Dicot and Monocot Leaves

Aim of the experiment : To study the structure of stomata of dicot and monocot leaves.

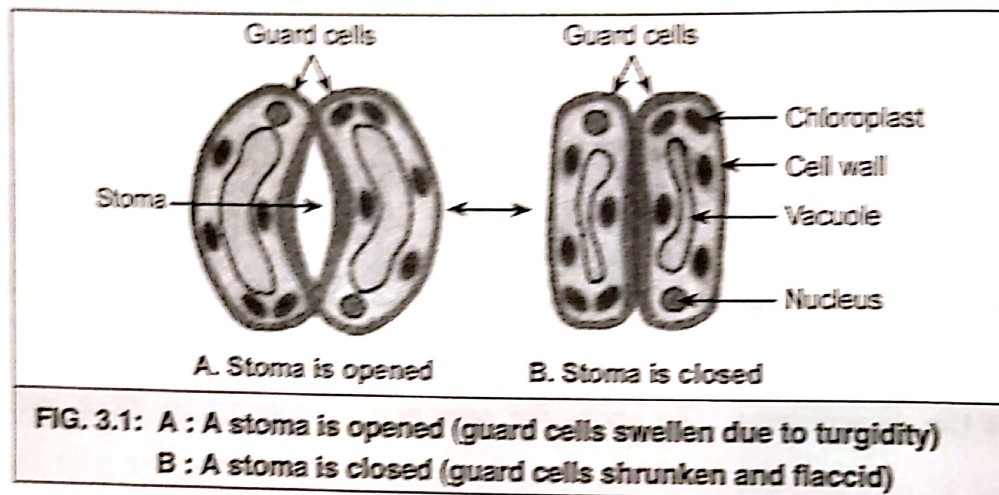
Requirements : Fresh leaves of *Nerium* or china rose (dicot) and *Rhoeo discolor* or *Tradescantia* or *Dracaena* (monocots), miscroslide, cover slip, compound microscope, distilled water.

Theory : Stomata are minute pores present in soft and aerial parts of the plant. The stomata are of elliptical shape consisting of two specialized epidermal cells called **guard cells**. The guard cells are kidney shaped in dicotyledons and dumbell-shaped in monocotyledons.

The wall of the guard cell surrounding the pore is thickened where as the rest of the wall is thin, elastic and semipermeable. Guard cells are surrounded by modified epidermal cells, called **subsidiary cells** or **accessory cells**, which support in the movement of guard cells. Surrounding the subsidiary cells, epidermal cells are present. The size and shape of stomata and guard cells vary from plant to plant and in monocot and dicot plants. When fully open, the stomatal pore measures about 3-12 μm in width and 10-40 μm in length. Stomatal area is generally 1-5% of the total area of the leaf.

The stomatal pore is widened or opened when the guard cells are turgid and closed when the guard cells are flaccid (Fig. 3.1) Moreover stomata are generally present in the lower surface of the leaf in dicots (hypostomatic type) and on both the sides of the leaf in case of monocots (amphistomatic type). However exceptions are there to this. In hydrophytes, where the leaves float, stomata are present on the upper surface of the leaves and this is known as **epistomatic type**. In submerged hydrophytes, (e.g. *Potamogeton*) stomata are absent and this condition is known as **astomatic type**. Stoma is the site for transpiration and gaseous exchange for photosynthesis and respiration.

Procedure : Fresh leaves of *Nerium* and *Rhoeo discolor* are taken. The lower epidermal peels of *Nerium* and both upper and lower epidermal peels of *Rhoeo discolor* leaves are taken carefully and cut into small pieces. These pieces are kept at the centre of three clean microslides and one or two drops of distilled water are put and then cover slips are put carefully. Now the prepared microslides are observed through the high power of a compound microscope.



Observation : Stomata are seen clearly through the high power objective of a compound microscope. In case of dicot leaf of *Nerium* many stomata are observed in the lower epidermal peel and the stomata here have kidney shaped guard cells. On the other hand stomata are present both in the lower and upper epidermal peels of the monocot leaf of *Rhoeo discolor* and here the guard cells are dumb-bell shaped. Subsidiary cells are present around the guard cells. Chloroplasts are also present in the guard cells.

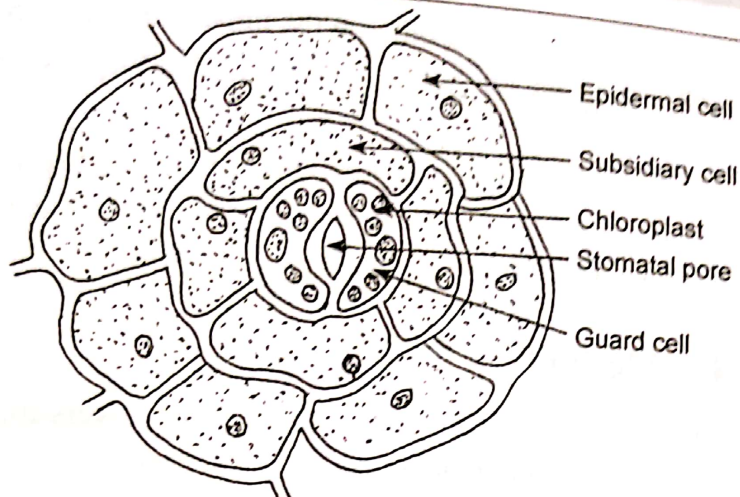
Precautions

- Fresh, healthy, clean and uninjured leaves of both dicot and monocot plants should be taken.
- Stomata are identified by the presence of two guard cells.
- Stomata should be observed through the high power of the compound microscope.

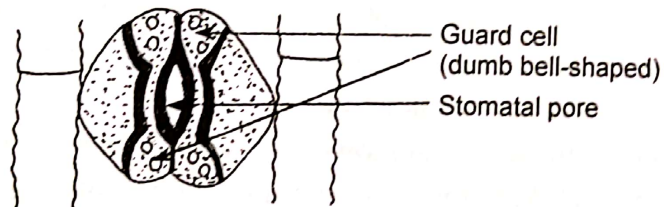
Conclusion : A stoma is an epidermal specialized tissue consisting of stomatal pore, guard cells, subsidiary cells, and epidermal cells. The guard cells are kidney-shaped in dicot leaves and dumb-bell shaped in monocot leaves. Stoma is the site for transpiration and gaseous exchange. When the guard cells are turgid, the stomatal pore opens and when flaccid, it is closed.

N.B. : Basing on the distribution, different types of stomata are grouped as follows :

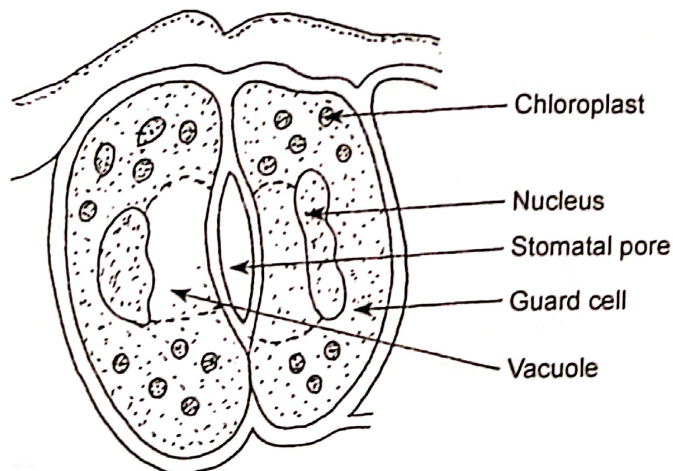
- Hypostomatic types.** When stomata are present only on the lower leaf surface and absent in upper leaf surface which is found in dorsiventral leaves like apple, mulberry etc. (also known as Apple or mulberry type)



A. Structure of a typical stoma of a dicot leaf



B. Stoma of a monocotyledonous leaf showing dumb bell shaped guard cells



C. A Stoma of *Tradescantia* highly magnified

FIG. 3.2: A. Structure of a typical stoma, B. A stoma of monocot leaf showing dumb bell-shaped guard cells, C. A stoma of *Tradescantia* highly magnified.

- (2) **Amphistomatic type.** When stomata are present on both the surfaces of the leaf *i.e.*, in case of isobilateral leaf. It may be of two types :
- Potato type.** Stomata occur more in the lower surface than the upper surface, *e.g.*, potato, tomato, brinjal and members of the family Solanaceae.
 - Oat type.** Stomata are equally distributed in both the surface of a leaf *e.g.*, oat, wheat, rice, grasses.

- (3) **Epistomatic.** Stomata are present only on the upper surface of the leaf *e.g.* water lily where the leaves float on the water surface (also known as **water lily type** of stomata).
- (4) **Astomatic.** When stomata are completely absent in the leaf surfaces, *e.g.* submerged leaves of *Potamogeton*. In these leaves if stomata are present, then they are non-functional (also known as **Potamogeton type** of stomata).

Fevikwik Adhesive Method

Requirements. Fevikwik adhesive tube, microslide, coverslip, forcep, compound microscope, Plant material - leaves of China rose and *Dracaena*.

Procedure. Fresh, healthy, clean and uninjured leaves of China rose (*Hibiscus rosa-sinensis*, dicot) and *Dracaena* (monocot) are taken. A drop of fevikwik adhesive is put over the upper surface of a China rose leaf and over the lower surface of another China rose leaf and the adhesive is spread into a thin film by the help of a glass rod. Like wise fevikwik adhesive is applied over the upper and lower surfaces of two separate *Dracaena* leaves. Now these four leaf samples are kept for 10-15 minutes for complete drying of the adhesive. Then with the help of a forcep, the thin adhesive films are removed from each leaf sample and kept over glass slides with few drops of water and mounting of coverslip and observed through compound microscope.

Observation. Impression of the stomata are seen on the adhesive film clearly when seen through the high power objective of a compound microscope. In case of China rose leaf, a large number of stomata are seen in the film of the lower surface, but no stoma is observed in the film of the upper surface. In case of *Dracaena* leaf, stomata are seen in both the films of the upper and lower surfaces.

Stomata are kidney-shaped in the guard cells of China-rose. But these are more elongated and slightly dumb-bell shaped in *Dracaena*.

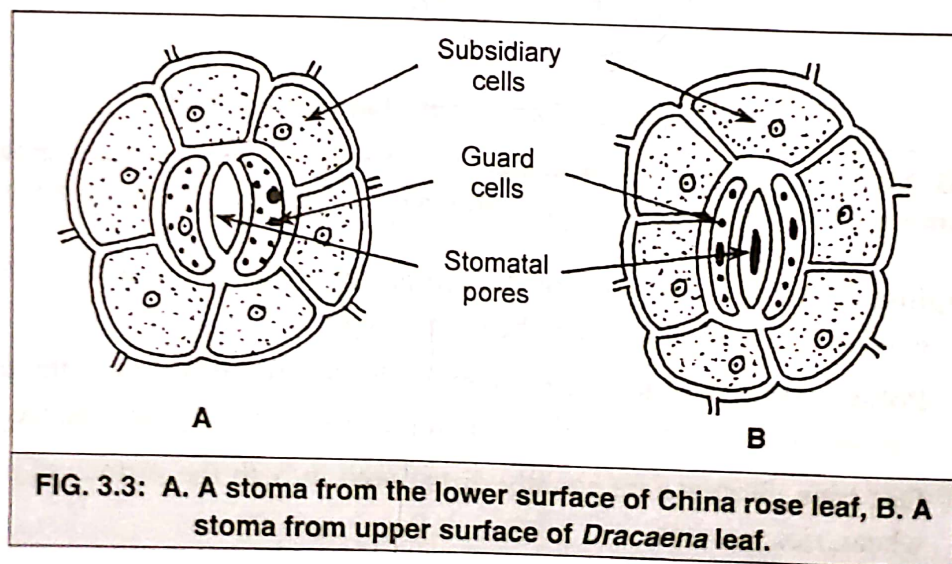


FIG. 3.3: A. A stoma from the lower surface of China rose leaf, B. A stoma from upper surface of *Dracaena* leaf.

Precautions

- (a) Fresh, clean, healthy and uninjured leaves should be taken.
- (b) Feviquik adhesive should be carefully spread over a portion of the leaf lamina with the help of a clean glass rod tip.

Conclusion. From the above experiment, it is concluded that in case of a dicot leaf (dorsiventral) stomata are present only in the lower epidermis and absent in the upper epidermis and the guard cells are kidney-shaped. But in case of a monocot leaf (isobilateral), stomata are present in both lower and upper epidermes and the guard cells are more elongated and slightly dumb-bell-shaped.

Determination of Rate of Transpiration by Cobalt Chloride Method

► EXPERIMENT NO. 4

Aim of the experiment. To determine the rate of transpiration by cobalt chloride method.

Requirements. Cobalt chloride solution (5%), microslides (glass slides), clips, filter paper, stop watch, punching machine, desiccator, beaker. **Plant material** - A well watered potted dicot plant, like China rose (*Hibiscus rosa-sinensis*).

Theory. The loss of water from the aerial parts of the plant in the form of water vapour is known as transpiration which mainly occurs through stomata. In dicot plants, the leaves are dorsiventral where stomata are only present in the lower (dorsal) surface and absent in the upper (ventral) surface. Hence, the rate of transpiration is higher in the lower surface than that of the upper surface.

In case of isobilateral leaves of monocots, stomata are present in both dorsal and ventral surfaces so that transpiration occurs in both the surfaces. Transpiration is more in mature than young and old leaves.

Procedure. A filter paper is dipped in 5% cobalt chloride solution for some time. Then it is taken out and dried at room temperature. Through the help of a punching machine, round discs are cut from the dried cobalt chloride paper and kept in a desiccator so that the discs are completely dried. The cobalt chloride paper is pink when wet and blue when dried. The dried

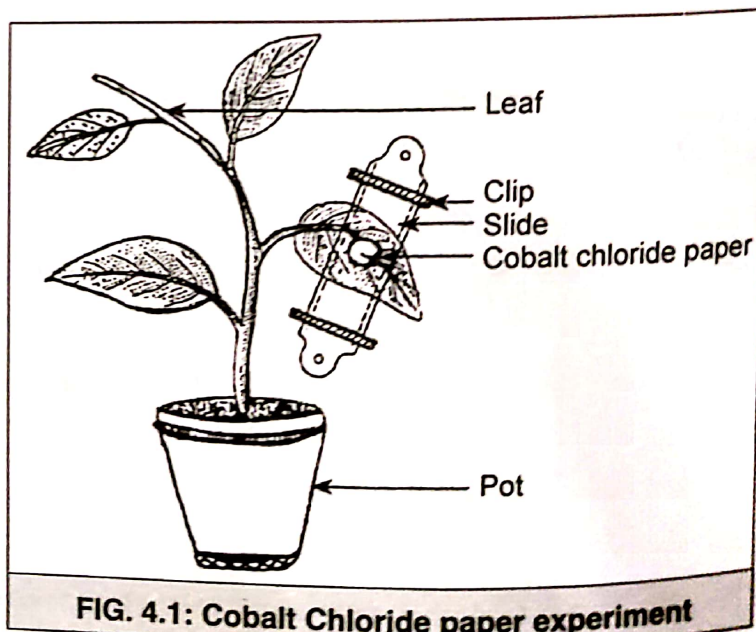


FIG. 4.1: Cobalt Chloride paper experiment

discs are placed one each, on the upper and lower surface of a leaf of the potted plant. Then one microslide on the upper and another microslide on the lower side of the leaf are kept closely together with the help of clips. Now the time taken by each disc of cobalt chloride paper to change its blue colour to pink is noted. Observations are taken for young, mature and old leaves.

Sl. No.	Nature of leaf	Time taken to change the colour in min.		
		Upper Epidermis	Lower Epidermis	Total (Upper + Lower Epidermis)
1	Young			
2	Mature			
3	Old			

Observation. The time taken for change of colour from blue (dried) to pink (wet) is more in case of the cobalt chloride paper kept on the upper surface than that of the lower surface of each type the leaf. It is due to higher rate of transpiration from the lower surface than that of the upper surface so that the lower disc gets wet earlier and the colour changed to pink. However time taken for change of colour is the least in the mature leaf, intermediate in young and higher in old leaves.

Precautions

- The potted plant should be well watered before the start of the experiment.
- 5% cobalt chloride solution (5 gms of cobalt chloride in 100 ml. of water) should be prepared carefully.
- The leaf surface should not be wet while keeping the disc of filter paper.
- The microslides should be cleaned and dried before putting on the leaf surface.

Conclusion : The rate of transpiration is more in the lower surface than in the upper surface of a dicot leaf. It is the highest in the mature, intermediate in the young and lowest in the old leaves as determined by cobalt chloride method.

N. B. Transpiration Index : The transpiration index of the plant can be calculated as follows :

The transpiration rate of an open water-evaporating surface (physical evaporation) can be known by taking a Petri dish containing water and the blue paper-strip is held by suitable arrangement as close to the water surface as possible, without actually touching it and the time taken for colour change (to pink) is noted. The time taken in seconds for a standard colour change of cobalt chloride paper over a water-evaporating surface (S) divided by the time taken for the same colour change on the leaf (E) and multiplied by 100 is the transpiration index which is a measure of the water loss from the leaf surface as a percentage of the evaporation from free water surface.

$$\text{Transpiration index (TI)} = \frac{S}{E} \times 100$$

Demonstration of Transpiration by Ganong's Potometer

► EXPERIMENT NO. 5

Aim of the experiment. To demonstrate transpiration by Ganong's potometer.

Requirements. Ganong's potometer, beaker, water, fresh healthy twig of China rose (*Hibiscus rosa-sinensis*)

Theory. Transpiration is a physiological process where water is lost from the aerial parts of the plant in the form of water vapour. It may occur through stomata, cuticle or lenticel out of which stomatal transpiration accounts to about 90-95% of the total transpiration. The rate of transpiration is the amount of water lost per unit time which can be measured by using Ganong's potometer. Potometer is an instrument used to measure the rate of transpiration.

Procedure. A Ganong's potometer is taken which consists of a glass tube bent twice at right angle in opposite direction (Fig. 5.1). The upper end is widely fitted with a rubber cork

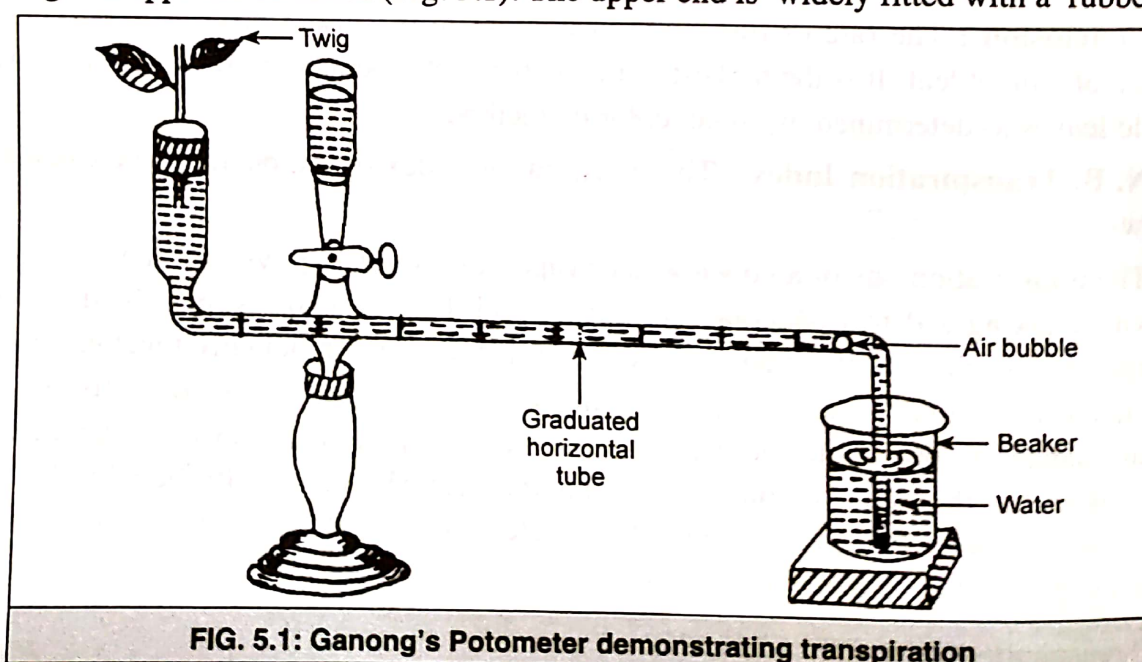


FIG. 5.1: Ganong's Potometer demonstrating transpiration

having a central hole through which an obliquely cut twig of China-rose is inserted with water. The lower end of the tube is dipped in a beaker full of water. The entire apparatus is made air tight and an air-bubble is conveniently introduced into the lower tube. The apparatus is kept for sometime under light and the movement of air bubble in the horizontal tube per unit time (say 2 minutes) is noted.

A table with sample data is given below.

TABULATION

Type of apparatus	No. of observations	Initial reading in cm	Final reading in cm	Diff. in reading in cm	Mean diff. in cm
Ganong's Potometer	1				
	2				
	3				

Observation. It is observed that the air bubble in the horizontal tube of Ganong's potometer gradually moves towards the twig. The movement of this air bubble per unit time (say 2 minutes) is found to be x (put here the exact value you found).

Precautions.

- (1) Fresh, healthy and uninjured twig of China rose should be taken.
- (2) The twig should be cut obliquely inside water.
- (3) The apparatus should be made air tight.
- (4) Minimum three readings should be taken in each experimental set up.

Conclusion. From the above experiment it is concluded that due to transpiration in the aerial parts of the twig, water is absorbed or pulled in through the cut end of the twig from the horizontal tube so that air bubble or water column is gradually moved. the rate of transpiration per unit time can be measured by using Ganong's potometer.

N.B. If the experiment is to be carried out under different environmental conditions, then the above experiment may be performed by keeping the experimental set-up under (i) shade (inside the laboratory without fan), (ii) wind (on the laboratory table with fan having full speed) and sunlight (in the garden or lawn having full sunlight). The data may be presented in a histogram.

Demonstration of Ascent of Sap/Transpiration Pull

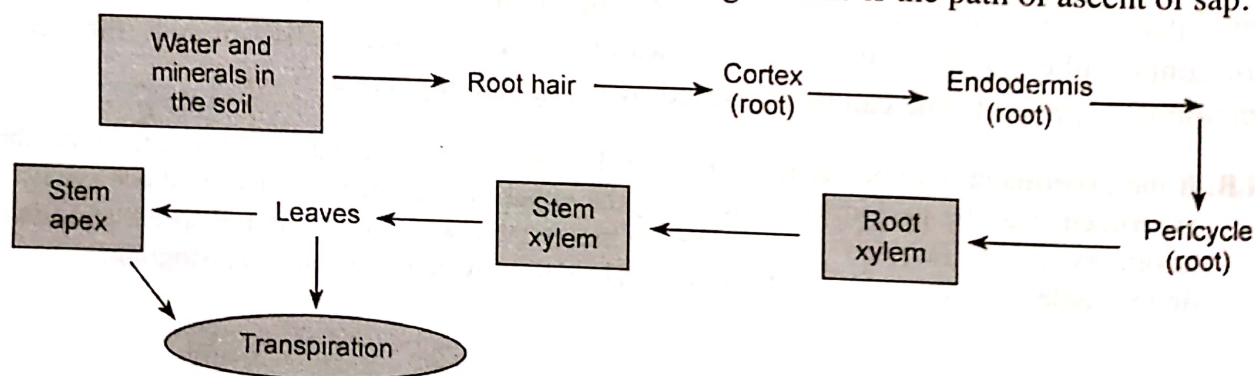
► EXPERIMENT NO. 6

Aim of the experiment : To demonstrate ascent of sap or transpiration pull.

(A) Ascent of Sap

Requirements : Beaker, water, wax or vaseline, suitable holder to hold the cut shoot, two small shoots of a woody plant like mango.

Theory : The process by which water and minerals are translocated from base to the apex or transpiring organs (leaves) of the plant is known as **ascent of sap**. Generally it takes place through the complex xylem tissue. The following scheme is the path of ascent of sap.



Procedure : Two fresh small shoots of mango (*Mangifera indica*) are taken. Their cut ends are so prepared that in one shoot, the phloem tissues are blocked with wax, while in the other shoot, the xylem tissues are blocked with wax. Now the cut ends of the two shoots are carefully submerged in water in a large beaker and each shoot is kept upright with the help of a suitable hold-stand. The two shoots are kept at room temperature in the laboratory for an hour and then the condition of the two shoots are noted.

Observation : After one hour of incubation it was observed that the shoot with its xylem blocked showed wilting while the shoot with its phloem blocked remains unaffected. This indicates that ascent of sap occurs through the xylem and not through phloem.

Precautions :

- (1) Fresh, healthy and uninjured shoots of mango plant should be taken
- (2) The bark (phloem zone) towards the cut ends should be removed carefully.
- (3) Wax or vaseline should be applied carefully to the cut end of the bark in one set and to the cut central xylem portion of the shoot in another set.
- (4) The shoots should be carefully kept upright with the help of a hold-stand.

Conclusion : From the above experiment it is concluded that ascent of sap occurs through the xylem tissues of the shoot or stem. Due to ascent of sap, the leaves remain intact or turgid, so wilting does not occur. If the xylem is blocked then ascent of sap does not take place and wilting of the leaves occurs.

(B) Transpiration Pull

Requirements : Farmer's potometer, beaker, water, twig of china rose (*Hitriscus rosa-sinensis*)

Theory : Ascent of sap occurs due to transpirations pull, the theory known as cohesion-tension theory put forth by Dixon and Joly (1895). Here the driving force is created in the transpiring surfaces of the leaves, so that water is pulled from the soil through the roots and stems just like a bucket of water is pulled from the well with the help of a rope.

Procedure : A Farmer's potometer is taken which consists of a wide-mouthed glass bottle fitted up with a rubber cork. Through three holes of the rubber cork, a bent glass tube with a scale, a reservoir glass tube with a pinch cock are fitted and in the third hole, a cut twig of China rose is fitted carefully. The apparatus is made air tight and kept in sunlight for sometime. Then the movement of water column in the horizontal tube per unit time is noted which indicates the rate of transpiration pull exerted in the leaves.

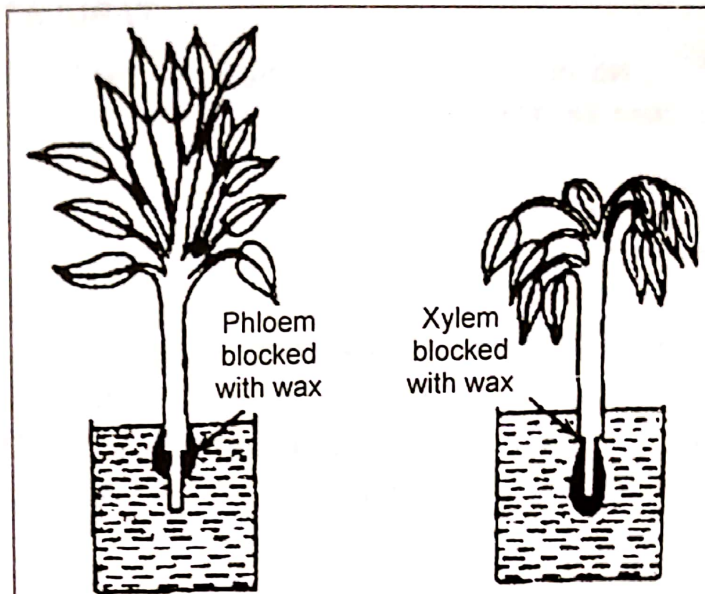
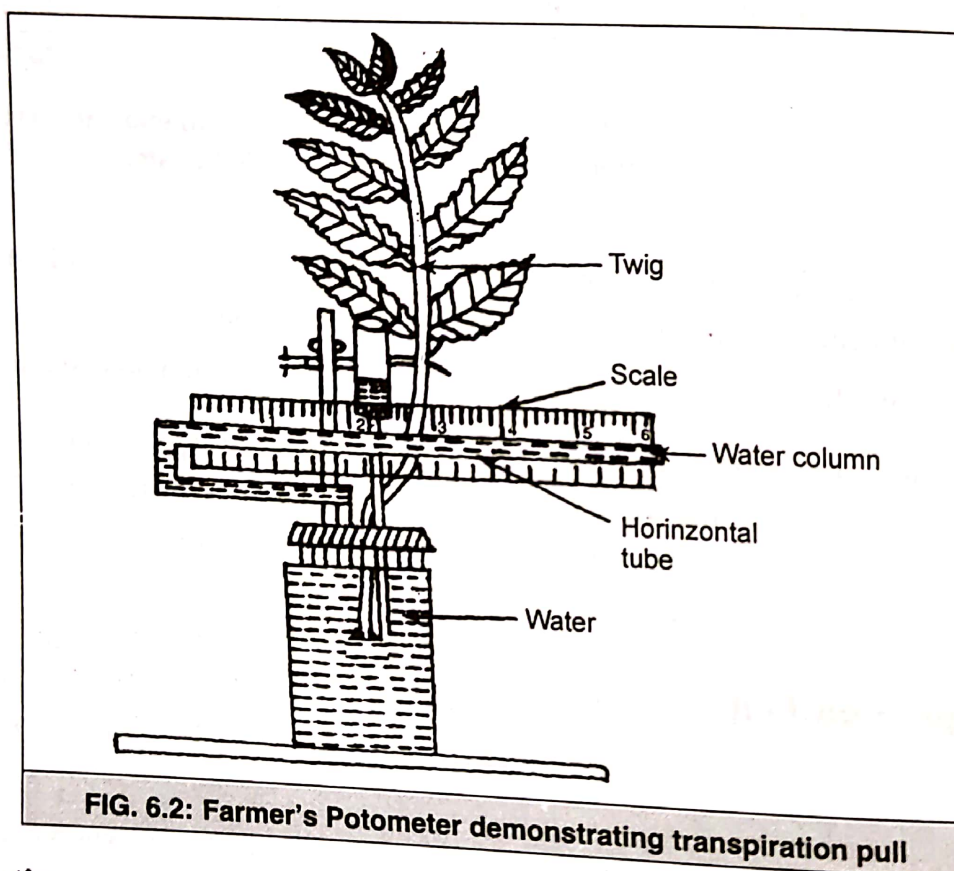


FIG. 6.1: Experiment to show the ascent to sap in the stem.

TABULATION

No. of observations	Initial reading in cm	Final Reading in cm	Diff. in reading in cm	Mean diff. in reading in cm
1				
2				
3				

Observation : After keeping the apparatus with the twig in the sunlight for sometime, it was observed that the water column in the horizontal tube moves and this movements of water per unit time (say 2 minutes) is noted. With some intervals three observations are noted. The mean of the readings is then calculated.

**Precautions :**

- (1) Fresh, healthy and uninjured twig of China rose should be taken.
- (2) The twig should be cut obliquely inside water.
- (3) The apparatus should be made airtight.

Conclusion : From the above experiment it is concluded that due to transpiration pull in the aerial parts of the shoot, water is absorbed or pulled in through the cut end of the twig and translocated through the stem to the leaves. Thus ascent of sap or conduction of water and minerals takes place due to transpiration pull which can be measured quantitatively indirectly by the amount of water absorbed per unit time.

Effect of Temperature on Membrane Permeability by Colorimetric Method

► EXPERIMENT NO. 7

Aim of the experiment : To study the effect of temperature on membrane permeability by colorimetric method.

Requirements : Size 4 cork borer, scalpel, forcep, water baths, beaker, thermometer, measuring cylinder, test tubes, tissue paper, marker pen, fresh beet root.

Theory : The plasmamembrane of the plant cell is generally semipermeable which allows passage of selective solutes or solvents through its minute pores. The permeability of this membrane is regulated by a number of factors out of which temperature is one. Generally with the increase in temperature, the rate of permeability will increase upto a certain optimum temperature like wise with decrease in temperature the permeability is also decreased up to a certain minimum temperature (0°C or $< 0^{\circ}\text{C}$) below which no permeability occurs.

Procedure : With the help of a size 4 cork borer, cylindrical pieces of a fresh beet root are cut and soaked over right in a beaker of distilled water. The pigment from any cells that have been cut will leak into the water. Rinse away any pigmented water in the morning and replace with fresh distilled water in a beaker.

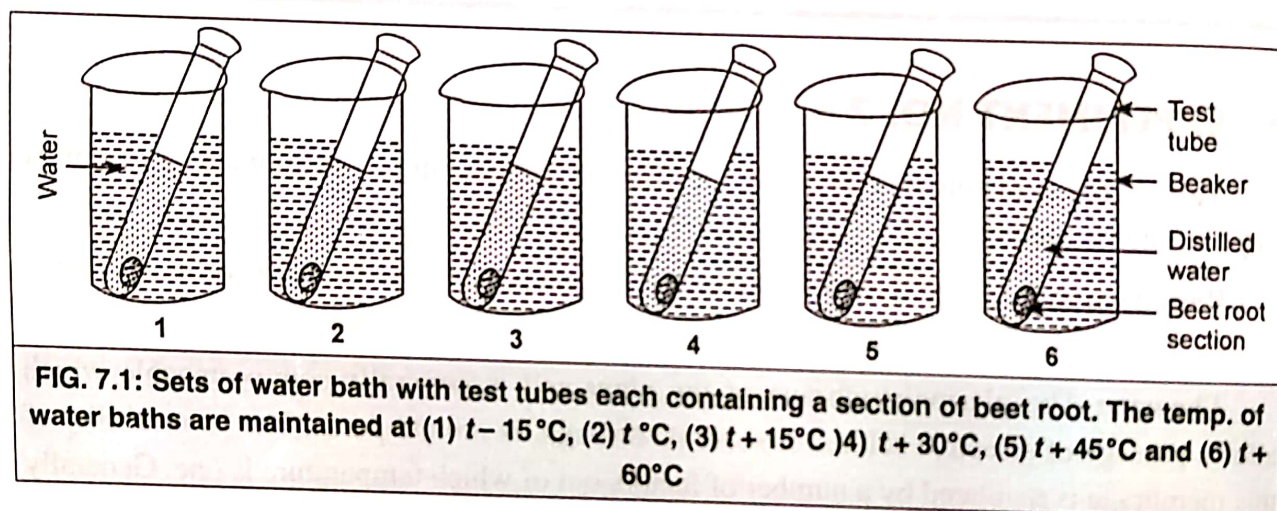
While starting the experiments, the beet root cores are taken from the beaker and each core is cut in section of 2 cm thickness. Now these 2 cm sections are put in a wide mouthed test tube with plenty of distilled water. Next five test tubes are labelled with a marker pen and 5 ml of distilled water is added to each test tube and these tubes are kept in five separate water baths with temperature maintained at (1) room temp. (t) (say 25°C), (2) $t + 15^{\circ}\text{C}$, (3) $t + 30^{\circ}\text{C}$, (4) $t + 45^{\circ}\text{C}$, (5) $t + 60^{\circ}\text{C}$ and (6) $t - 15^{\circ}\text{C}$. The tubes are kept in the specific water

baths for 5-10 minutes to equilibrate to the temperature of the water bath. The temp. of each bath is known with the help of a thermometer. Either hot or cold water is added to maintain the temp. The beet root sections are removed from the distilled water and blotted gently on a tissue paper. Now one piece of the 2 cm thick beet root section is put in each test tube with the help of a forcep carefully and kept for 30 minutes in the water bath.

After the incubation period, the test tubes are taken from the water bath, shaken gently to make sure that all the pigments are well-mixed into the water. Now the beet root sections are removed from each test tube with the help of a forcep carefully.

The optical density or absorbency (A) of each test sample is measured with the help of a digital colorimeter at 530 nm, against distilled water as the blank.

The data may be plotted in a graph against temperature in the x-axis. The pattern of graph (temp. curve) is to be recorded.

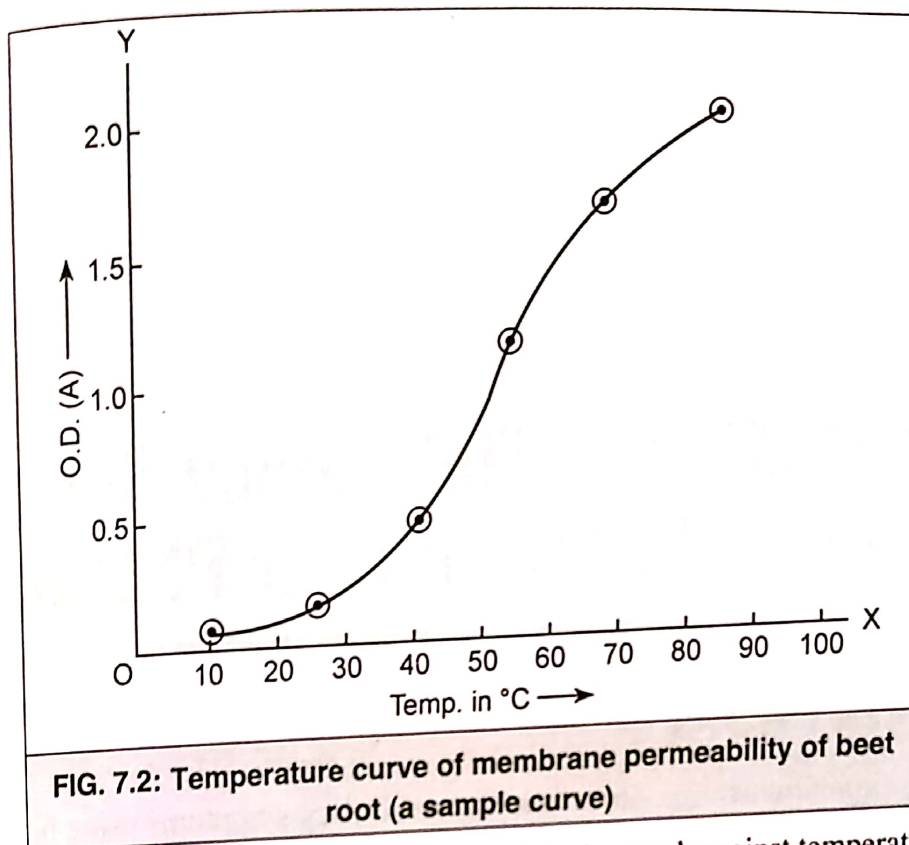


Tabulation

TABLE 7.1 Optical Density of Test Samples Against Temp.

Sl. No. of Test tubes	Temperature	Observation	Optical Density (A) at 530 nm
1	10 ($t(25) - 15$)	Very slightly pink	
2	25 (t)	Very pale pink	
3	40 ($t + 15$)	Pale pink	
4	55 ($t + 30$)	Pink	
5	70 ($t + 45$)	More Pink	
6	85 ($t + 60$)	Dark Pink	

N.B. The actual O.D. (A) should be recorded in column 4



N.B. The exact value of O. D. (A) should be plotted in the graph against temperature.

Observation : The observation of colour of the test sample of each test tube is noted in the table (Tab. 7.1) It is observed that the depth of the colouration (pink) of the test solution increased with increase in temperature which is very slightly pink at 10°C and dark pink at 85°C. As per the graph (7.2) the optical density (O.D.) is slowly increased with increase in temperature upto 40°C and then sharply increased upto 70°C and then the rate is slightly decreased upto 85°C, but it is the highest value among all the test samples. The O.D. is a measure of the rate of membrane permeability.

Precautions :

- (1) Fresh, healthy and uninjured beet root should be taken.
- (2) The thickness (or length) of each beet root sections should be uniformly of 2 cm.
- (3) Before incubating in the water bath, the beet root sections should be carefully soaked gently in tissue paper.
- (4) Before taking O.D., the test solutions should be carefully shaken.
- (5) Temperature of the water bath may be maintained by adding hot or cold water as per requirement.

Conclusion : From the above experiment, it is concluded that the membrane permeability of beet root is increased initially slowly with increase in temperature and with further increase in temperature it is enhanced sharply upto around 70-80°C, but also enhanced upto the end of the experiment. It indicates that membrane permeability increases up to close to boiling point. It is very negligible at and below 10°C. There may not be any permeability at freezing point.

Study of Mineral Deficiency Symptoms using Plant Materials or Photographs

► EXPERIMENT NO. 8

Aim of the experiment : To study mineral deficiency symptoms using plant materials or photographs.

Requirements : Different plant materials or photographs showing mineral deficiency symptoms.

Theory : Mineral ions are required for normal growth and development of plants. There are 16 essential elements out of which some are required in traces known as trace elements or **micronutrients** like molybdenum, copper, zinc, boron, manganese, chlorine and some are required in comparatively high amount known as **macro-nutrients** like nitrogen, sulphur, phosphorus, magnesium, calcium, potassium etc. The nutrients in soil occur as minerals and are called mineral nutrients.

The mineral deficiency symptoms can vary from a depression of growth rate to obvious stunting, deformity, discolouration, chlorosis, distress and even mortality. Visual symptoms distinctive enough to be useful in identifying a deficiency are rare. Most deficiencies are multiple and moderate. However, while a deficiency is seldom that of a single nutrient, nitrogen is commonly the nutrient in shortest supply. For details of mineral deficiency symptoms, refer chapter two (E) of theory section of this book.

Observation and Identification : The supplied plant materials or photographs are observed and identified as to which mineral deficiency is there as follows.

1. Nitrogen deficiency

(I) Plant material : Cucumber fruit (Fig. 8.1)

Symptoms :

- (i) The supplied cucumber fruit is misshaped and not having the normal shape.

(ii) It is chlorotic.

(iii) It has reduced growth

Identification : Due to the above characteristic symptoms the supplied cucumber fruit is deficient in nitrogen.

(II) Photograph of *Citrus* plant (Fig. 8.2)

Symptoms :

(i) The plant shows stunted growth.

(ii) Chlorosis is observed in the older leaves which started at the tips.

(iii) The growth of the leaves is reduced.

Identification : Due to the above symptoms, the *Citrus* plant is identified to have nitrogen deficiency.



FIG. 8.1: Cucumber fruit having deficiency of nitrogen.



FIG. 8.2: Severe nitrogen deficiency in *Citrus* plant.

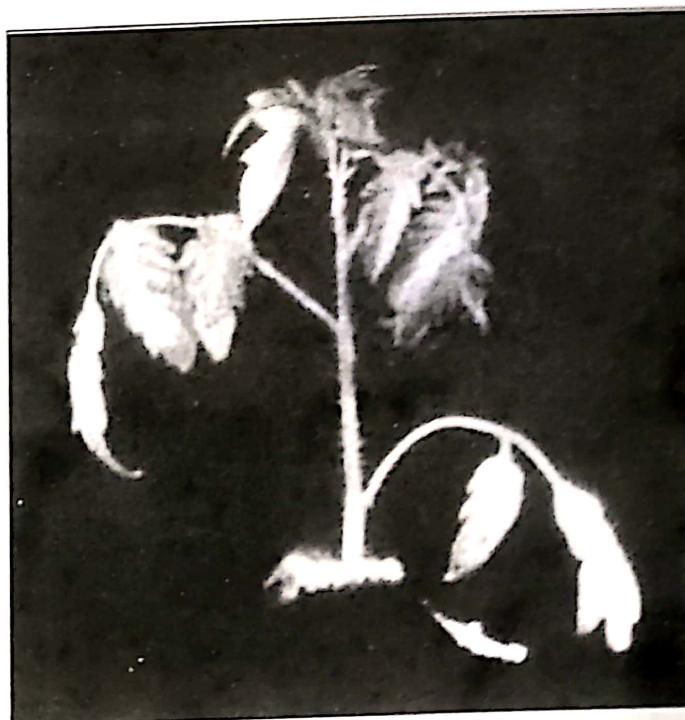


FIG. 8.3: Tomato shoot with leaves having purple interveinal tissue on their undersides.

(2) Phosphorus deficiency

Supplied material/photograph : Tomato shoot (Fig. 8.3)

Symptoms :

(i) Stunted growth of the shoot is marked.

(ii) The leaves have purple interveinal tissues on their undersides.

(iii) Purpling of the stem is marked

Identification : Due to the above symptoms, the tomato plant is identified to have phosphorus deficiency.

(3) Potassium deficiency

Supplied photograph : Banana plant (Fig. 8.4)



FIG. 8.4: Banana plants with leaves showing chlorosis. (Potassium deficient).

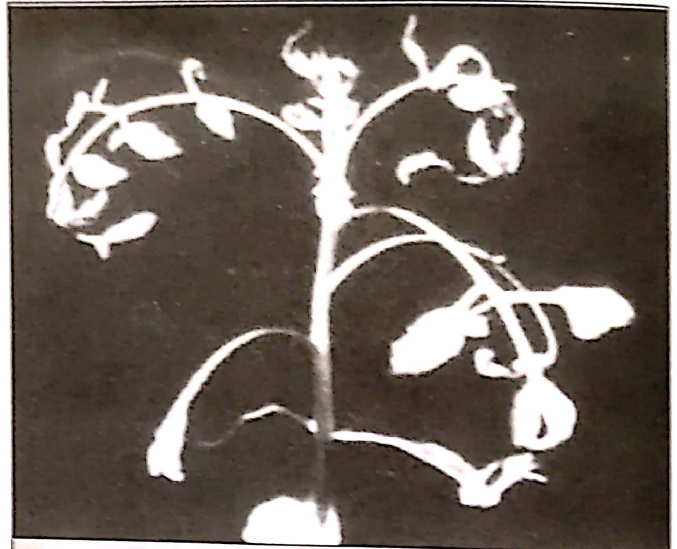


FIG. 8.5: Calcium deficient tomato plant

Symptoms :

- (i) The older leaves of the banana plant is first chlorotic and then necrotic.
- (ii) The most common symptom is chlorosis along the edges of the leaves.
- (iii) The tip of the midrib bends downward.
- (iv) Slow and stunted growth of the plant is marked.

Identification : Due to the above symptoms, the supplied photograph of banana plant is identified to have potassium deficiency.

(4) Calcium deficiency

Supplied material/photograph : Tomato plant (Fig. 8.5)

Symptoms :

- (i) The most important symptom is that the young leaves are twisted and cupped.
- (ii) The growing tips of the leaves are deep brown.
- (iii) The stem is weakened.
- (iv) The terminal bud is deteriorating.

Identification : Due to the above symptoms, the supplied material/photograph of tomato plant is identified to have calcium deficiency.

(5) Magnesium deficiency

Supplied material/photograph : Corn plant (Fig. 8.6)

Symptoms :

- (i) The plant shows interveinal chlorosis of older leaves, which generally appears first.

- (ii) Leaf tissues between veins (interveinal) become yellowish while leaf veins remain green.

Identification : Due to the above symptoms, the supplied plant material/photograph of corn (*Zea mays*) is identified to have **magnesium** deficiency. Moreover magnesium is a part of chlorophyll molecule and hence its deficiency causes chlorosis of leaves.



FIG. 8.6: Magnesium deficient corn plant.



FIG. 8.7: Sulphur deficient tomato leaves

(6) Sulphur deficiency

Supplied material /photograph : Tomato leaves (Fig. 8.7)

Symptoms :

- (i) The leaves are uniformly chlorotic.
- (ii) The younger leaves are also chlorotic with evenly, lightly coloured veins.
- (iii) Growth of the leaves is retarded.
- (iv) Symptoms may be similar to N deficiency.

Identification : Due to the above symptoms, the supplied material/photograph of tomato leaves is identified to have sulphur deficiency.

(7) Boron deficiency

Supplied plant material/photograph *Carica papaya* fruit (Fig. 8.8)

Symptoms :

- (i) The papaya fruits have bumps so that the normal shape of the fruit is distorted.
- (ii) It is a crop-specific symptom i.e. lumpy and distorted fruit in case of papaya. Other crops have other specific symptoms of boron deficiency.



FIG. 8.8: Boron deficient papaya fruits

Identification : Due to the above symptomatic characters, the supplied material/photograph is identified to have boron deficiency.

(8) Copper deficiency

Supplied plant material/photograph : Onion plant (Fig. 8.9)

Symptoms :

- (i) The onion leaves have white tips due to chlorosis.
- (ii) These leaves twist in spirals or right angles.
- (iii) Reduced growth of the plant is observed.

Identification : Due to the above symptomatic characters, the supplied material/photograph is identified to have copper deficiency (right side plant)



FIG. 8.9: Copper deficient onion leaves

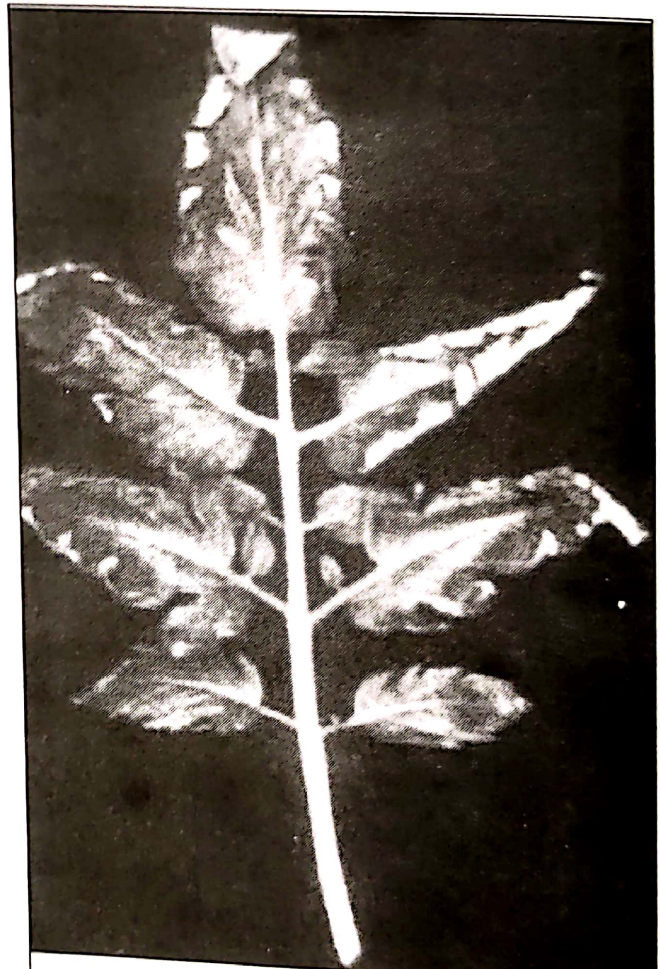


FIG. 8.10: Chlorine deficient tomato leaves.

(9) Chlorine deficiency

Supplied material/photograph : Tomato leaf (Fig. 8.10)

Symptoms :

- (i) The leaflets of the supplied tomato leaf is chlorotic.
- (ii) The leaf edges are rolled upward.
- (iii) Wilting symptom is marked.

Identification : Due to the above symptomatic characters, the supplied material/ photograph of tomato leaf is identified to have chlorine deficiency.

(10) Iron deficiency

Supplied material/photograph : Soybean plant (Fig. 8.11)

Symptoms :

- (i) The supplied material/photograph is of soybean plant where the young leaves have interveinal chlorosis.
- (ii) The youngest leaves may be white, because iron, like magnesium, is involved in chlorophyll production.

Identification : Due to the above symptomatic characters, the supplied material/ photograph of soybean is identified to have iron deficiency.



FIG. 8.11: Iron deficient soybean plants



FIG. 8.12: Manganese deficient orange leaves.

(11) Manganese deficiency

Supplied plant material/photograph : Orange branch (Fig. 8.12)

Symptoms :

- (i) The supplied material/photograph is of an orange branch having leaves developing a mottled pattern of light and dark green.
- (ii) Chlorosis is marked as tiny yellow spots.

Identification : Due to the above symptomatic characters, the supplied material/ photograph of an orange branch is identified to have manganese deficiency.

(12) Molybdenum deficiency

Supplied plant material/photograph : Tomato leaves (Fig. 8.13)

Symptoms :

- (i) The supplied plant material / photograph is of tomato leaves.
- (ii) The leaves show interveinal chlorosis.
- (iii) Leaf margins roll inwards.
- (iv) Necrotic spots appear at the leaf margins.
- (v) Growth of the leaves is retarded.

Identification : Due to the above symptomatic characters, the supplied materials/photograph of tomato leaves is identified to have molybdenum deficiency.

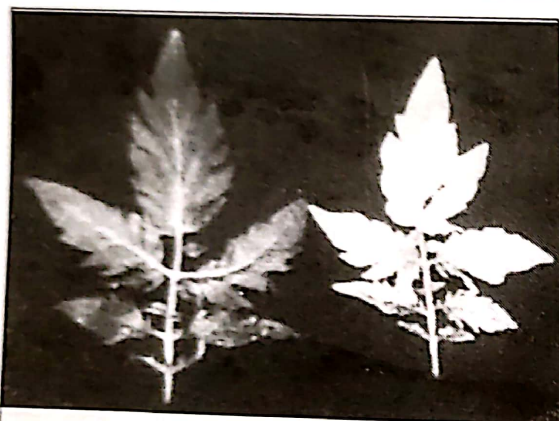


FIG. 8.13: Molybdenum deficient tomato leaves



FIG. 8.14: Zinc deficient onion leaves are twisted (right); plants at left are normal

(13) Zinc deficiency

Supplied plant materials/photograph : Onion leaves (Fig. 8.14).

Symptoms :

- (i) The photograph shows normal onion plants in left and mineral deficient plants in the right.
- (ii) The mineral deficient onion leaves are twisted and rolled downward.
- (iii) Growth is reduced so that the leaves are abnormally small, mottled and chlorotic.
- (iv) Chlorotic banding appears at the basal part of the leaves.

Identification : Due to the above symptomatic characters, the supplied material/photograph of onion plants in the right are identified to have **zinc** deficiency.

Separation of Pigments using Paper Chromatography Technique

► EXPERIMENT NO. 9

Aim of the experiment. To separate and study the plant pigments by paper chromatography technique.

Requirements. Acetone, petroleum ether, wide long test tubes, capillary tube, funnel, split cork, beaker, mortar and pestle, Whatman's No. 1 chromatography paper and filter paper.

Plant material. Fresh leaves of *Bougainvillea* or *Ocimum sanctum*.

Theory. Chromatography is the technique of separation of substances or mixtures according to their partition coefficients (*i.e.* relative solubility) in two immiscible phases *i.e.* one stationary phase (*e.g.* a solid) and one mobile phase (*e.g.* liquid). In paper chromatography, the components of a mixture are first absorbed on an insoluble material like paper sheet and then the components are separated in a suitable liquid solvent.

Paper chromatography is mainly useful for the detection and separation of amino acids or pigments. The components are identified from their R_f (Resolution front) value as follows.

$$R_f = \frac{\text{Distance travelled by the compound from the origin}}{\text{Distance travelled by the solvent}}$$

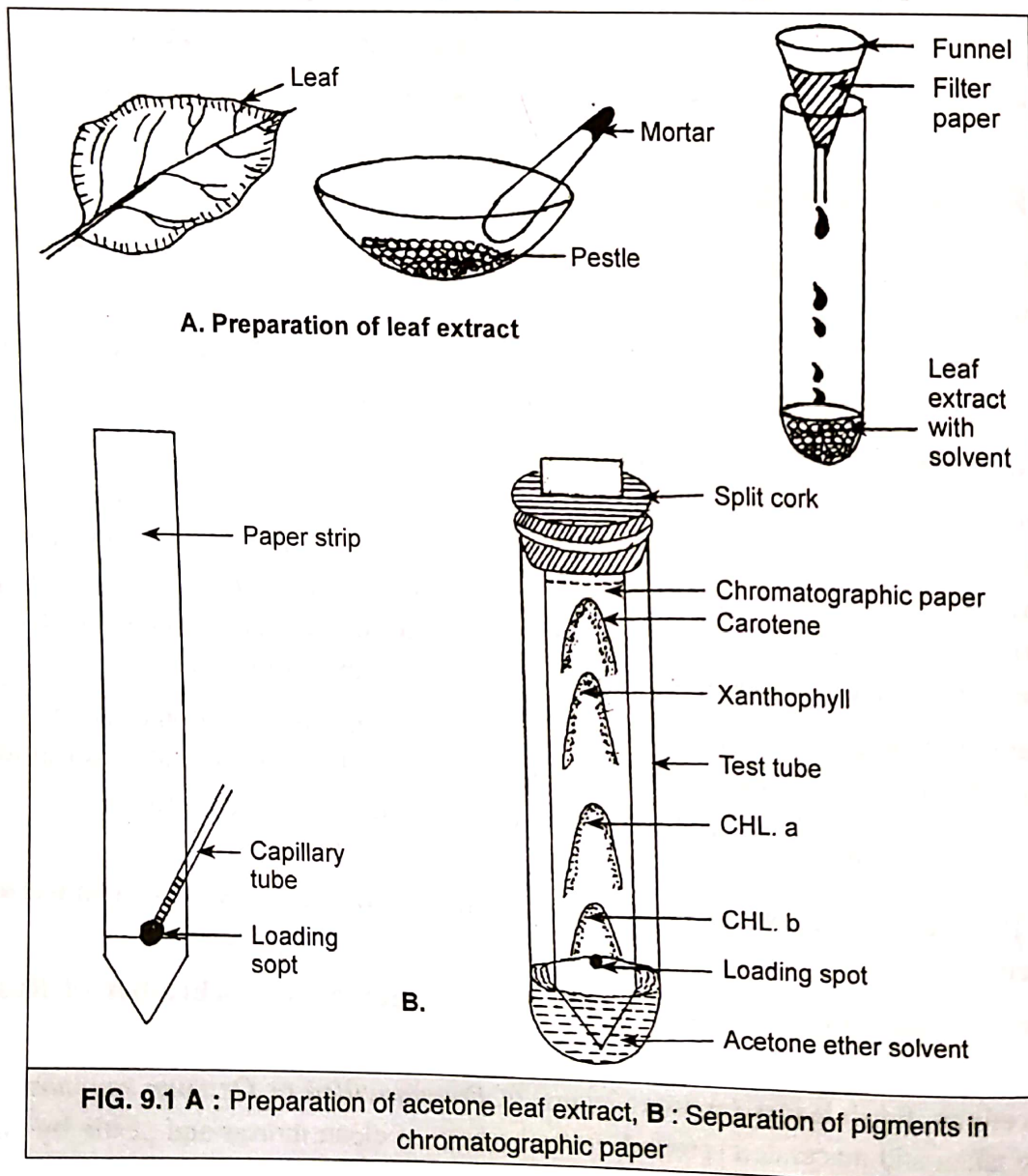
The R_f value is fixed for a compound for a particular solvent and varies when the solvent is changed.

Paper chromatography was first discovered by **Izmailov** and **Schraiber** of Russia in 1938.

Procedure. Fresh leaves (approx. 2 gms) of *Bougainvillea* or *Ocimum sanctum* (sacred basil) are taken and macerated (ground) with the help of clean mortar and pestle by taking 5 ml. of acetone. Now it is filtered with the help of a funnel and filter paper to get the acetone extract of the leaf pigments. A narrow strip of chromatography paper is taken and one end is cut into a narrow notch. Then a drop of the acetone extract of the leaf is put at the middle of

the strip near the notch by the help of the capillary tube. It is kept for 10-15 minutes to dry. This process is repeated till 4-5 drops at the same spot are dried. Now 5 ml of acetone-ether solvent (in the ratio 1 : 9) is poured in the wide-long test tube. Then the narrow chromatography paper loaded with leaf pigment extract is hanged carefully with the help of the split cork in such a way that the pigment spot lies just 1 cm above the acetone-ether solvent level. The split cork is tightly fitted to make it air tight. The apparatus now is kept for 20-30 minutes till the solvent rises up sufficiently in the chromatography paper. Lastly the chromatography strip is taken carefully and allowed to dry at room temperature (Fig. 9.1).

Observation. Distinct different coloured bands are seen in the narrow chromatography paper. The lower most band is yellowish-green, then towards upper in sequence are dark-



green band, yellowish band and orange-yellow band. The distance of each band from the loading spot and the distance travelled by the solvent are measured, from which the R_f (Resolution front) value is calculated, which is fixed for a particular pigment.

Calculation. The R_f value for different pigments is calculated as follows :

$$R_f = \frac{\text{Distance travelled by the pigment}}{\text{Distance travelled by the solvent}}$$

Sl. No.	Colour of the Spot	Pigment	R_f Value
1.	Yellowish-green	Chlorophyll-b	0.45
2.	Dark green	Chlorophyll-a	0.65
3.	Yellow	Xanthophyll	0.71
4.	Orange-yellow	Carotene	0.95

Precautions.

- (1) Fresh leaves of *Bougainvillea* or *Ocimum sanctum* should be taken and washed before grinding and soaked gently with blotting paper.
- (2) The loading spot should be concentrated and taken carefully.
- (3) Preparation of acetone-ether solvent in 1 : 9 ratio should be prepared carefully.
- (4) The chromatography paper strip should not touch the wall of the test tube.
- (5) The loading spot should be just 1 cm. above the level of the solvent when hanged in the test tube.

Conclusion. The yellowish-green, dark green, yellow and orange-yellow spots are due to presence of chlorophyll-b, chl-a, xanthophyll and carotene respectively. The R_f value for a particular pigment is a constant number. The difference in the distance travelled by different pigments is due to their difference in molecular weight, the lighter pigments travel a higher distance than the heavier pigments. The R_f values of Chl. a, Chl. b, xanthophyll and carotene are 0.65, 0.45, 0.71 and 0.95 respectively.

Determination of Rate of Photosynthesis under Varying CO_2 Concentration

► EXPERIMENT NO. 10

Aim of the experiment. To determine the rate of photosynthesis under varying CO_2 concentration

Requirements. Sodium bicarbonate (NaHCO_3) beaker, compound balance and weight box, glass rod, Wilmott's bubbler.

Plant material. Fresh, healthy, uninjured twig of *Hydrilla*.

Theory. Photosynthesis is an anabolic process, where carbohydrates are produced with liberation of molecular oxygen from water and carbon dioxide in the green parts of the plant in presence of sunlight. The rate of photosynthesis is affected by many factors like light intensity, light quality, light duration, dissolved carbon dioxide, water content, chlorophyll content and temperature, etc. CO_2 concentration is also an important factor in regulation of photosynthesis.

Procedure. To study the effect of dissolved carbon dioxide on photosynthesis, a *Hydrilla* twig is fitted in the Wilmott's bubbler with 100 mg of sodium bicarbonate added to the water and kept in sunlight and the no. of bubbles evolved per unit time is noted. Likewise observations are noted

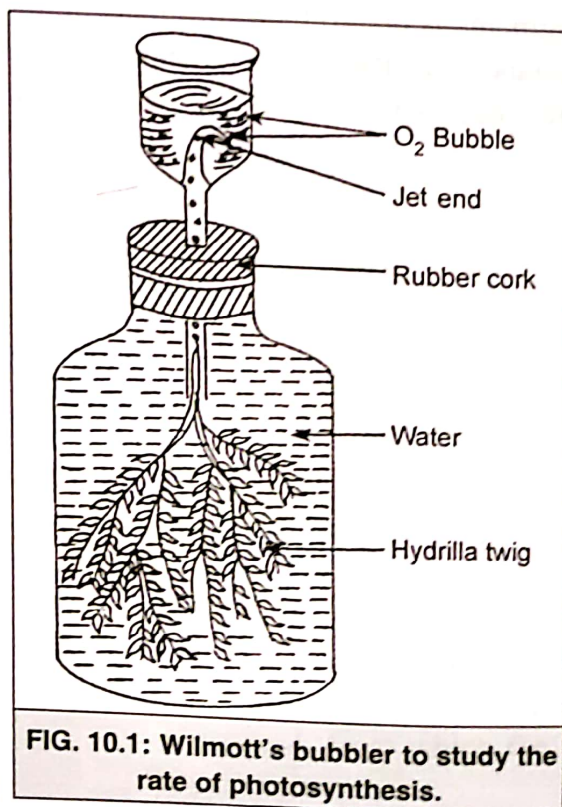


FIG. 10.1: Wilmott's bubbler to study the rate of photosynthesis.

with 200 mg, 300 mg, 400 mg and 500 mg of sodium bicarbonate added to Wilmott's bubbler respectively. The data are noted in a tabular form. The rate of photosynthesis (no. of bubbles evolved/2 min.) is plotted against the concentration of CO_2 (NaHCO_3) in a graph.

TABULATION

No. of observations	NaHCO_3 in mg	No. of bubbles evolved per unit time (say 2 minutes)	Mean no. of bubbles /unit time
1	100		
2			
3			
1	200		
2			
3			
1	300		
2			
3			
1	400		
2			
3			
1	500		
2			
3			

N.B. The students should record the expect value in column 3 and 4.

Observations : Regarding the effect of dissolved carbon dioxide (CO_2) on photosynthesis, it is observed that the mean no. of bubbles evolved per unit time (= rate of photosynthesis) is highest at 300 mg NaHCO_3 and lowest at 500 mg NaHCO_3 . The rate of photosynthesis increases with increase in the concentration of dissolved CO_2 upto 300 mg and with further increase in CO_2 conc., the rate of photosynthesis decreases.

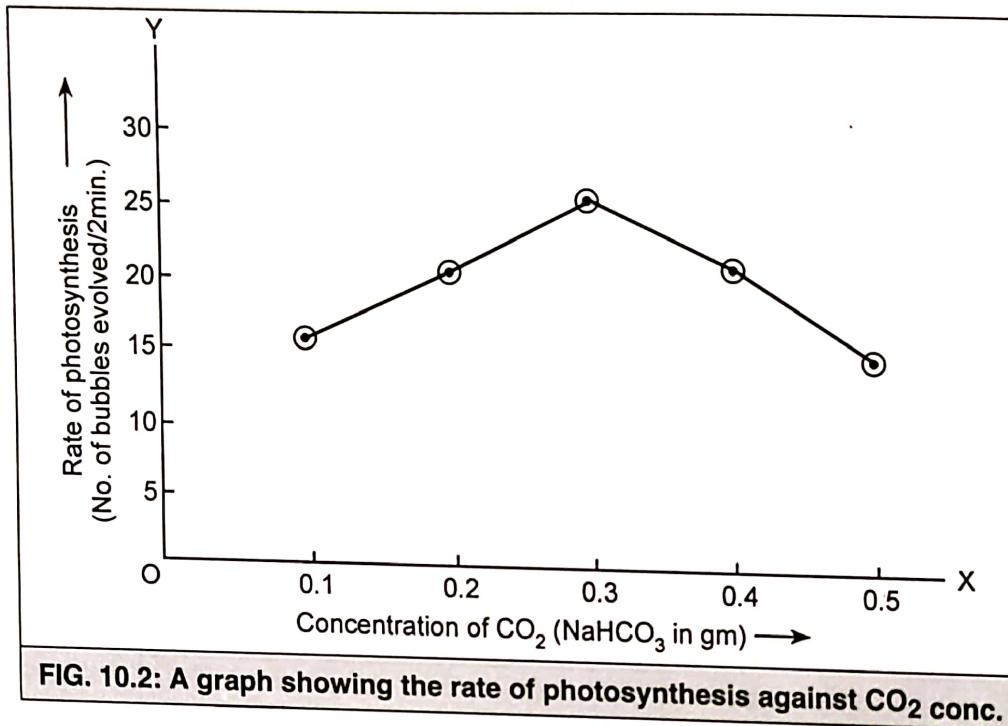
Precautions :

- (1) Fresh, healthy and uninjured twig of *Hydrilla* should be taken.
- (2) The apparatus should be made air tight.
- (3) For studying the effect of dissolved CO_2 , each time 100 mg of NaHCO_3 should be added so that it would be 100, 200, 300, 400 and 500 mg subsequently in the Wilmott's bubbler.
- (4) The same *Hydrilla* twig should be used for all the experimental set up with different concentration of NaHCO_3 .

(5) The graph should be plotted carefully.

Conclusion. From the above experiment, it is concluded that :

The rate of photosynthesis increases with increase in the concentration of dissolved CO_2 upto an optimum concentration (*i.e.* 300 mg NaHCO_3) and then it decreases with subsequent increase in the conc. of dissolved CO_2 as it has toxic effect.



N.B. The above graph is a sample one. The students should plot the exact value.

Study of Effect of Light Intensity on Oxygen Evolution in Photosynthesis by using Wilmott's Bubbler

► EXPERIMENT NO. 11

Aim of the experiment. To study the effect of light intensity on oxygen evolution in photosynthesis by using Wilmott's bubbler.

Requirements. Sodium bicarbonate (NaHCO_3), beaker, water, Wilmott's bubbler, glass rod.

Plant material. Fresh, healthy, uninjured twig of *Hydrilla*.

Procedure. To study the effect of light intensity, a fresh, healthy, uninjured, twig of *Hydrilla* is cut obliquely inside water and fitted in the Wilmott's bubbler with water as shown in Fig. 11.1. A pinch of sodium bicarbonate is added to the water in the Wilmott's bubbler and it is made air tight. The fitted apparatus is kept in shade (low intensity of light) for sometime and then the number of bubbles (oxygen) evolved per unit time (2 or 3 minutes) is counted and noted.

Now the apparatus is kept in sunlight at 10 am at 11 am and at 12.00 noon. The number of bubbles evolved per unit time in each observation is noted as per the table shown. The data are also plotted in the form of a histogram.

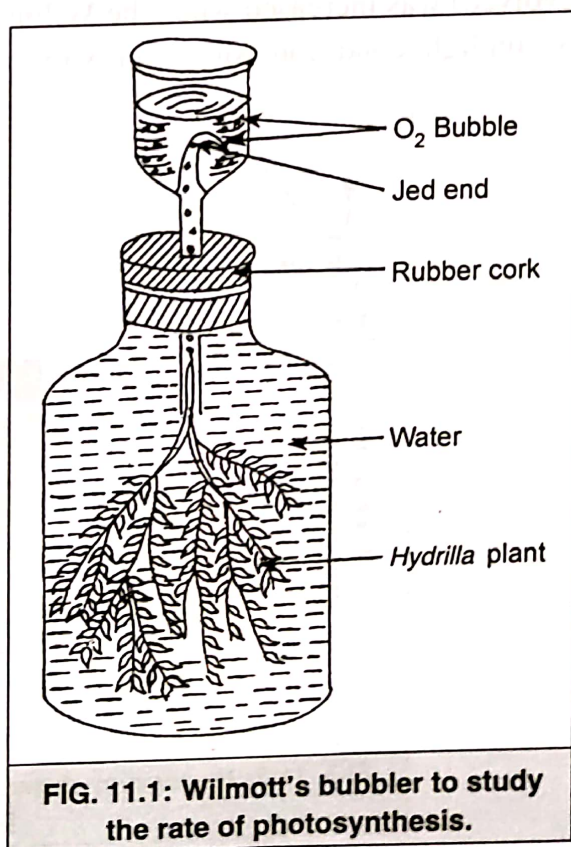


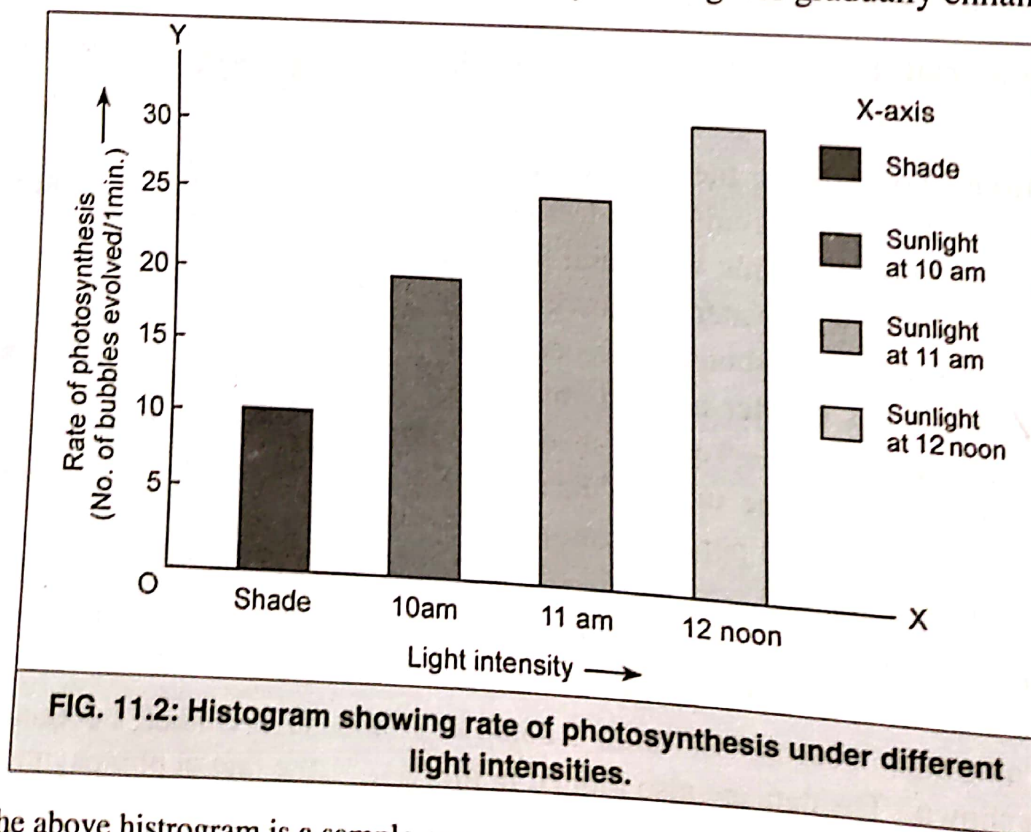
FIG. 11.1: Wilmott's bubbler to study the rate of photosynthesis.

TABULATION

No. of observations	Light intensity	No. of bubbles evolved per unit time (say 2 minutes)	Mean no. of bubbles/unit time
1	Shade		
2			
3			
1	Sunlight (at 10 am.)		
2			
3			
1	Sunlight (at 11.00 a.m.)		
2			
3			
1	Sunlight (at 12.00 noon)		
2			
3			

N.B. The students should note the exact data in column no. 3 and 4.

Observation : It was observed that the rate of photosynthesis (no. of oxygen bubbles evolved) was increased when the Wilmottis bubbler is shifted from shade (low light intensity) to sun light condition. Moreover, when the intensity of sunlight is gradually enhanced i.e.



N.B. The above histogram is a sample one.

from 10 am to 11 am and then to noon (12.00 noon), the rate of photosynthesis also increased and it is highest at noon time when the sunlight intensity is highest among the conditions studied here. The histogram also revealed the same trend.

The students have to plot the exact data they have obtained from the observations.

Precautions :

- (1) Fresh, healthy, uninjured *Hydrilla* twigs should be taken.
- (2) In the experiment a pinch of NaHCO_3 should be taken to enhance the concentration of CO_2 in water.
- (3) For each set of observation, the fitted apparatus should be kept for sometime.
- (4) For each condition, minimum 3 readings should be taken.
- (5) The same *Hydrilla* twig should be used for all sets of experiment.

Conclusion. From the above experiment it is concluded that:

The rate of photosynthesis *i.e.* evolution of oxygen bubble/min increases with increase in the light intensity. It is lowest under shade and highest under sunlight at noon.