Indira Gandhi
National Open University
School of Sciences

BBYCL-138
PLANT PHYSIOLOGY AND METABOLISM: LABORATORY

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PLANT PHYSIOLOGY AND METABOLISM: LABORATORY

This practical course is designed to give you hands on experience on various aspects of Plant Physiology which you have studied in theory course. These experiments will be related to the concept of water relations, photosynthesis, respiration and enzymes. The course is of 2 credits. The course consists of 4 demonstration experiments and 8 practical experiments. Demonstrations are for water relations and plant hormones while experiments pertain to photosynthesis, water relations, respiration and enzymes.

You would be able to successfully conduct the practical sessions within the prescribed time frame if you are well prepared with theoretical background, a detailed list of materials used, procedure to be followed and the observations that you need to make. You should study the lab manual carefully before joining the lab work.

After performing these practical exercises, your theoretical concepts will be clearer and you will have a better understanding of the processes such as transpiration, plasmolysis, photosynthesis, respiration, working of enzymes and plant hormones. You will also be able to tell about the importance of all the physiological events in relation to growth of plants. The questions asked at the end of each demonstration will be useful to you as they will provide you an insight of the processes operative in plants. The course is aimed to develop your practical skills as you will note the observations after performing the practical exercises.

You need to be both physically and mentally alert while performing the laboratory exercises. By the end of the course it is expected that you will get trained and develop the ability to work independently. You will also gain confidence after noting down the observations by yourself. It is necessary that you observe demonstration experiments carefully and note down the observations during the practical exercises, analyze them and reach a conclusion. It is advised that you discuss your results with the counsellor as this will help in clearing your doubts and help you to reach a logical conclusion.

You should realise that conducting practical session is an expensive exercise. It requires participation of a counsellor and technical staff. Therefore, the whole practical session should be taken seriously and utilised maximally to develop scientific temperament. Laboratory exercises are interesting and enjoyable. We hope that you will enjoy the practical session of Plant physiology. It is once more suggested that you go through the theoretical background required for conducting the practical exercise.

Objectives

After completing the exercises, you should be able to :

- describe the importance of water and hormones in the growth of plants through demonstration experiments;
- discuss the concept of plasmolysis, osmotic potential, and transpiration;
- provide information about the variations in distribution of stomata in plants;
- appreciate the process of photosynthesis and the environmental factors affecting it;
- be aware of the process of respiration;
- recognise the working of enzymes and factors affecting their activity; and
- discuss the basic principle involved in the technique of paper chromatography.

Instruments and other requirements

Before you start the laboratory course, you should carry some of these items along with you:

- sharpened pencils
- observation notebook
- an eraser
- a lab coat
- sanitizer

Instruments such as Compound microscope, Lux meter, Potometer, and Centrifuge will be provided to you from the laboratory. Glassware, chemicals and other miscellaneous items will also be provided to you from the laboratory.

Laboratory Etiquettes

One should be keen, hardworking, sincere, and with an analytical frame of mind to attain maximum knowledge from the laboratory courses. The present laboratory course is designed in such a way so that your knowledge about the subject will be enhanced and you will be able to develop analytical and observational skills. One needs to follow the following points before coming to the laboratory :

- read the laboratory manual exercises along with theory given in the course book.
- plan your experiment in such a manner that you complete the assigned work in the suggested time frame .
- use the facilities judiciously.
- strictly follow the instructions given by the counsellor.
- get the observations checked regularly by the counsellor after completing the experiment.
- handle the laboratory instruments and materials carefully.
- always discuss the doubts with the counsellor.

By following these points, the success and satisfaction will be yours. We wish you best of luck.

1

DEMONSTRATION OF BOLTING

Structure

1.1 INTRODUCTION

Gibberellins are a group of naturally occurring hormones having many physiological effects in plants. The effects are generally growth promotive.

One of the most remarkable effects of gibberellins is in converting a genetically dwarf plant into a plant of normal height. Addition of gibberellins to a cabbage plant converts the 'head' or dwarf stem into a stem which is 6-8 feet tall. Rosette plants of sugarbeet is an extensive case of dwarfing. **Lang**(1956) showed that such a stem can undergo rapid growth or 'bolting' if it is treated with gibberellins. Bolting is the elongation of the floral axis stalk in some dwarf biennial plants to produce flowers. The plant for one season grows vegetatively and in other season produces floral axis and fruits subsequently. The application of gibberellins to the plant at vegetative phase causes the plant to produce floral axis prematurely. This property has been exploited by agriculturalists in getting the crop within short span of time.

Many worker shave demonstrated the stimulation of cell division in the sweet peas. It is believed that the dwarfism in the mutant variation of a plant is due to blocking of the gene responsible for controlling the capacity for normal gibberellin production.

1.2 REQUIREMENTS

Plant Material: Two groups of potted plants of *Launaea* (Rosette habit) of same age (Four in each group).

Chemicals: GA_3 solutions {0.1 mg/L (0.1 ppm); 1 mg/L (1 ppm); 5mg/L (5ppm) and 10 mg/L(10ppm)}.

Miscellaneous: Sprayer/ cotton swab.

1.3 PROCEDURE

Expose the shoot apex of the rosette plants and

1. Select 4 weeks old potted plants of *Launaea* or *Lactuca sativa* (lettuce) of roughly equal size.
2. A total of 18 plants are divided into six groups of 3 plants each to demonstrate the effect of different concentrations of GA_3.
3. The six groups are categorized as i) Control (no GA_3) but only distilled water; ii) 0.01mg/L GA_3); iii) 0.1 mg/L GA_3; iv) 1 mg/L GA_3; v) 5 mg /L GA_3 and vi) 10 mg/ L GA_3.
4. Prepare 100ml solution of each group.Carefully shift the leaves to expose the shoot apex and apply the specific GA_3 concentration with the help of a cotton swab or spray the GA solution to runoff level. Repeat the application of hormone every third day for two weeks.

You will observe that the control plants retained their dwarf habit where as the plants sprayed with GA_3 showed elongation of internodes and bolting.

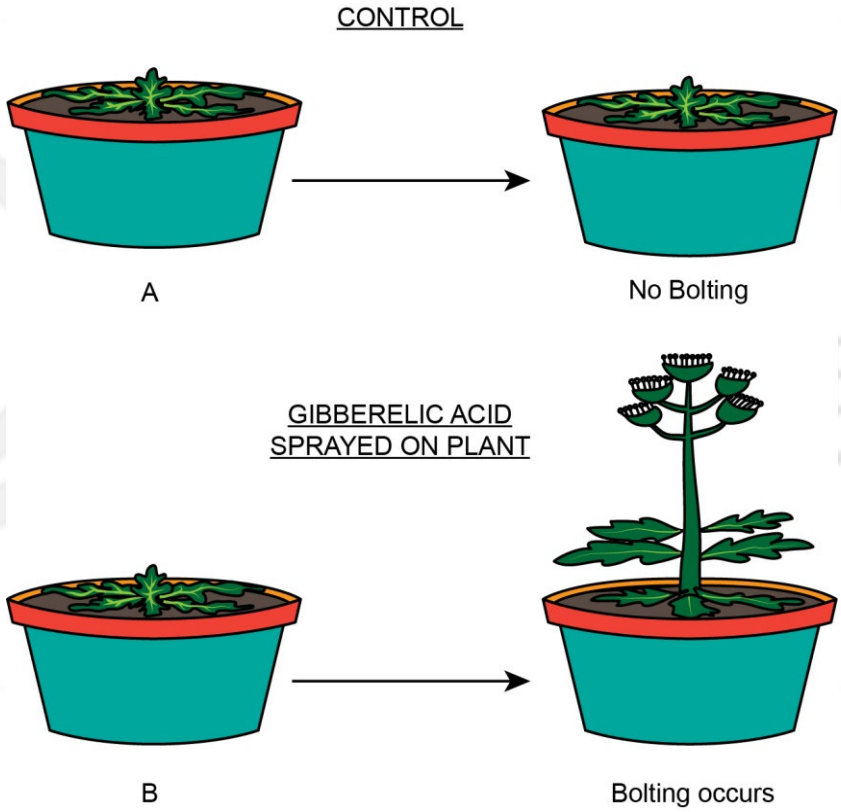


Fig. 1.1: Demonstration of bolting.

Now answer the following questions

1. What does the above experimental set-up demonstrate?
2. What causes the floral axis elongation?
3. What is the commercial advantage of extending the internode length?
4. Which chemicals promote dwarfing? Is dwarfing of any commercial importance?

Hints

1. Demonstration of the effect of GA_3 on rosette plants to induce bolting.
2. Bolting occurs due to increase in cell number as well as cell elongation.
3. For example, grape plants can be treated with gibberellins to extend the internode length. In this way the flowers are spaced further enabling the fruits to grow freely/with more room.
4. Dwarfing is promoted by antigibberellins e.g., AMO-1618, Cycocel (CCC), Phosphon D and Ancymidol. These chemicals make the plant short thereby preventing lodging. The antigibberellins block the gibberellin biosynthesis.

2

DEMONSTRATION OF THE EFFECT OF AUXINS ON ROOTING

Structure

2.1 INTRODUCTION

Auxins are one of the most important groups of plant hormones because of their many-sided roles in plants. These substances were also the first growth factors to be identified as plant hormones. **F.W. Went** succeeded in isolating these growth substances and named them as auxins. Auxins are synthesized from the amino acid tryptophan.

In nature root formation by a plant has been shown to be possible only if there are developing buds or leaves on them. Dormant buds fail to induce rooting. Ringing the cuttings immediately below developing buds from a normal plant also prevents rooting. Evidently the rooting in all such cases depends on the presence of a hormone. **Thimann** and his collaborators have shown that root priming substance and auxins are identical. The auxins have been found to increase the rate of formation and final number of root initials. This property of auxins has been taken advantage of in propagation of plants by stem cuttings in plants.

Thimann and Went (1930) found that indole acetic acid (IAA) and other growth substances are essential for initiating adventitious root formation in cuttings. They are applied at concentrations ranging from 100-1000 ppm (parts per million).

2.2 REQUIREMENTS

Glassware : 4 conical flasks (250 ml).

Plant Material : Stem cuttings of *Morus alba*.

Chemicals : i) IAA solutions (10^{-3}M, 10^{-4}M, 10^{-5}M). ii) Distilled water.

Miscellaneous : Cork stoppers.

2.3 PROCEDURE

Firstly four conical flasks were taken and one of them was filled with distilled water (control).In other three conical flasks, IAA solutions of different concentrations (10^{-3}M, 10^{-4}M, 10^{-5}M) were filled. All the flasks were sealed with cork stopper. Freshly cut pieces of the branches of *Morus alba* were inserted in the holes of cork stoppers so that lower ends of cuttings were touching the solution. These conical flasks were left for one week.

You will observe that in control flask having only distilled water there was no root initiation, but the flask having 10^{-4} M IAA solution had maximum root initiation. On the other hand, the cutting in flask having 10^{-5} M IAA solution showed poor root initiation. You will also observe that the stem cutting of flask having 10^{-3} M IAA solution showed no root initiation.

In the control flask without IAA, no root initiation was observed because endogenous auxins were present in extremely low concentrations thus, having no significant effect. In flask having 10^{-5} M 1AA solution, little root initiation occurred as the amount of auxin was less than the optimum concentration. In flask having 10^{-4} M IAA solution maximum root initiation was observed because it was having optimum concentration of IAA. In flask with 10^{-3} M 1AA solution the stem cutting got deformed due to supraoptimal concentration of IAA causing damage.

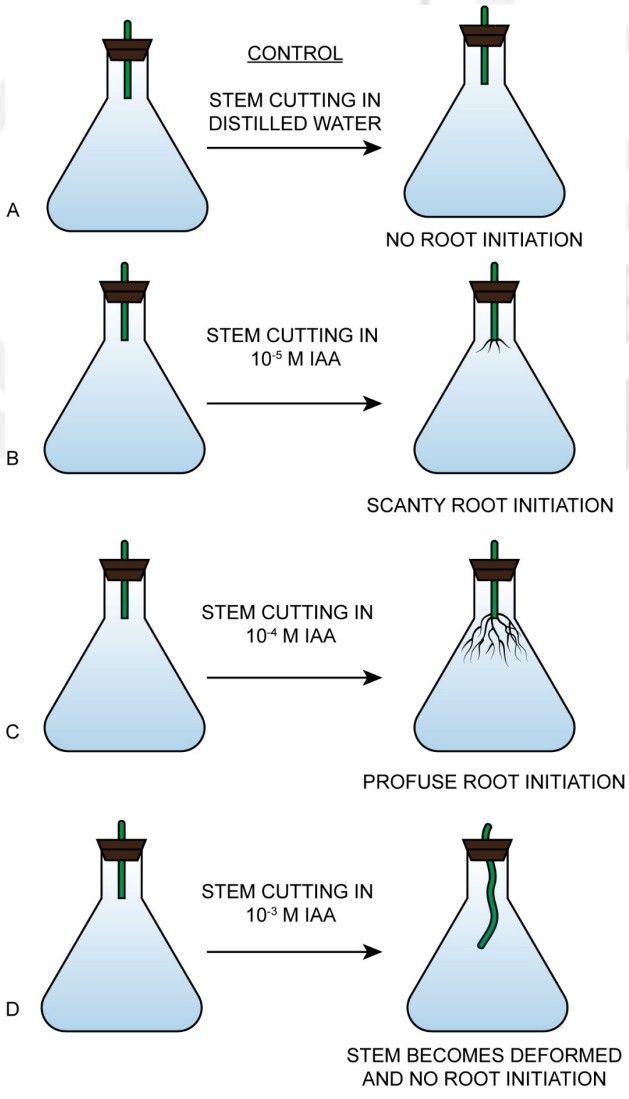


Fig. 2.1: Demonstration of effect of auxin on rooting in stem cuttings

Now answer the following questions

1. What does this experimental set-up demonstrate?
2. What will happen if the concentrationof IAA in the flask is increased ten-fold?
3. Name six synthetic auxins.

Answers

1. Demonstration of root induction by IAA in stem cuttings.
2. Supraoptimal concentrations of auxins are lethal to a plant.
3. NAA, IBA , 2,4-D, 2,4,5-T, MCPA, PCPA .

3

DEMONSTRATION OF ROOT RESPIRATION

Structure

3.1 INTRODUCTION

Cellular respiration is vital for organisms and consists of a series of pathways. The stored/reserve materials act as respiratory substrates and get oxidized to release ATP. Since all cells respire, roots are no exception. Usually the aerial parts of a plant are used to demonstrate rate of respiration or respiratory quotient (RQ). Roots also respire and thus, aerated soils are essential for normal plant growth. Waterlogging for long time chokes the roots and results in death of the plant.

3.2 REQUIREMENTS

Plant Material : Two small rooted plants of *Tagetes* with adventitious roots

Chemicals : Dilute NaOH solution, phenolphthalein

Miscellaneous : cork stopper with a hole.

3.3 PROCEDURE

In this demonstration experiment, a small, rooted plant *(e.g., Tagetes*, or wheat) with intact adventitious roots was taken and placed in a flask which had slightly alkaline water (with dilute NaOH solution) and coloured red with phenolphthalein. A second flask was taken which served as control and was without any plant but had only red coloured alkaline water.Stopper was fixed tightly. Both flasks were allowed to stand in diffuse light and the solutions in them were examined after some time.

You will observe that the control flask does not show change in the colour while the one with the roots becomes colorless as the colour of the solution fades. The respiring roots release CO_2, which reacts with water to form carbonic acid (H_2CO_3)

$$CO_2 + H_2O \longrightarrow H_2CO_3$$

Carbonic acid neutralizes NaOH present in the flask and the alkalinity of the solution starts decreasing, thus fading the red colour (phenolphthalein is colourless in the neutral medium).

$$H_2CO_3 + 2NaOH \longrightarrow Na_2CO_3 + H_2O$$

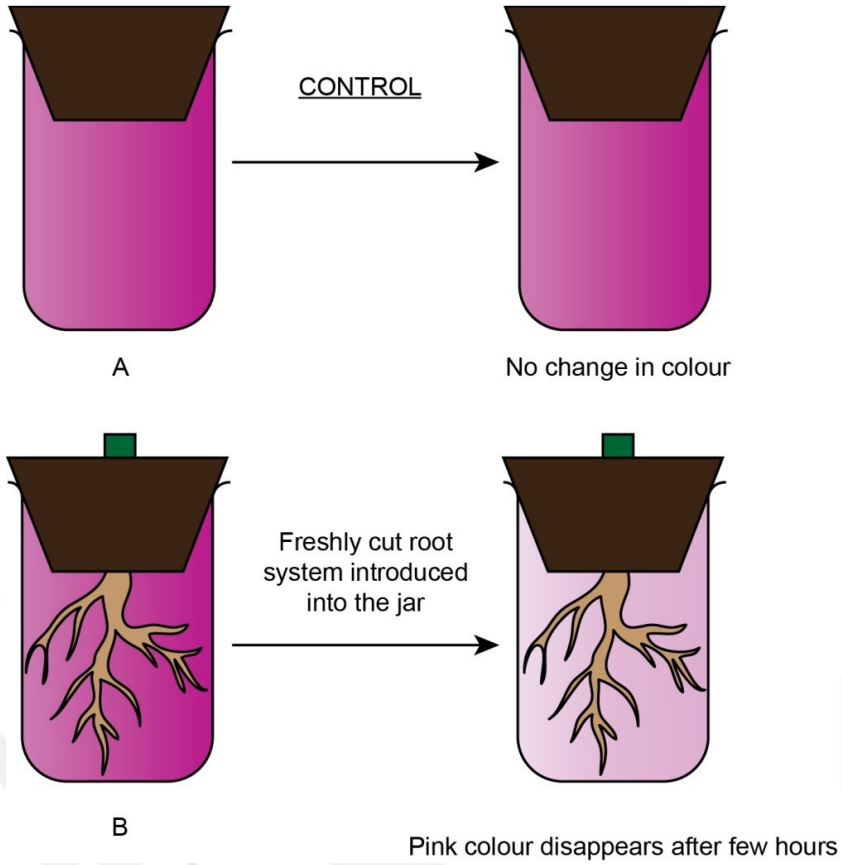


Fig. 3.1: Demonstration of root respiration

Questions

1. Explain the physiological mechanism involved in this set up, giving an equation.
2. What would happen if the solution in flask contained a buffer solution of pH 7.8?
3. What will happen if a drop of HCL is added to the experimental flask?
4. What results do you expect, if the roots in the experimental flask are pre-boiled?
5. In which way is root respiration helpful in the nutrient uptake from the soil? Explain.

Hints

1. See procedure
2. Solution will not turn neutral or acidic. Pink colour does not fade away.
3. Pink color will fade.
4. No change in colour as the roots are killed.
5. For active uptake of nutrients energy in the form of ATPis required,which is provided by the respiring roots.

4

DEMONSTRATION OF SUCTION DUE TO TRANSPIRATION

Structure

4.1 INTRODUCTION

You know that the plant transpires actively in nature and water is lifted upwards as a continuous column. You can see that water column does not collapse because of strong cohesive force among the water molecules as well as great adhesive force between water molecules and the hydrophilic walls of the tracheary elements. The continuous water column exists between the roots and the transpiring parts of the plant which are leaves. Thus, due to transpiration, a suction force or transpiration pull develops in the leaves of the plant, which is transmitted below to the roots via stem resulting in water uptake from the soil. The water lost by the plant during transpiration is compensated by the water absorbed by it from the capillary tube of the potometer. This results in rising of the mercury column.

4.2 REQUIREMENTS

Plant Material: Two small rooted plants of *Tagetes* (Marigold)

Apparatus: A simple or H-shaped potometer

Chemicals: Mercury

Miscellaneous: Cork stopper with a hole

4.3 PROCEDURE

This set upcomprises a simple potometer which includes a hollow glass tube with one end submerged in a trough containing mercury, and the other end fitted with the shoot of an actively transpiring plant (such as guava, marigold, sunflower or *Geranium*) under airtight conditions. Put the setup under the fan

to increase transpiration .You will observe a rise in the level of mercury column after some time (30 minutes or so), which indicates suction due to transpiration.

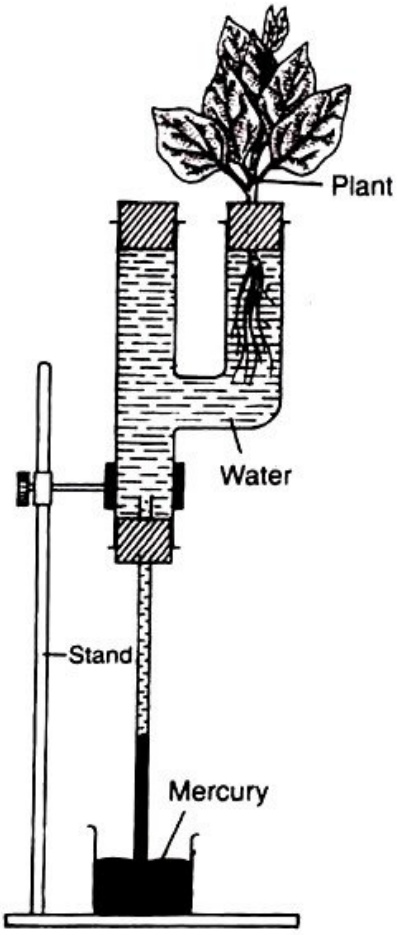


Fig4.1: Simple potometer to show suction due to transpiration.

Now try to answer these Questions

1. List the factors affecting the process being demonstrated.
2. What will happen if the leaves are smeared with grease or wax?
3. Why is mercury used in the set up?
4. What will happen if the set up is shifted to a humid environment?
5. Which plant hormone regulates stomatal closure during water deficit conditions?

Answers

1. Transpiration is affected by many factors like: sunlight, temperature, atmospheric humidity,air movement, CO_2,and water availability.
2. Stomates will not transpire and so mercury column will not rise.
3. It does not react with water.
4. Rate of transpiration will be low due to less vapour pressure deficit.
5. ABA (Abscisic acid).

EXPERIMENT 1

TO DETERMINE THE OSMOTIC POTENTIAL OF PLANT CELL SAP BY PLASMOLYTIC METHOD

Structure

1.1 INTRODUCTION

One of the important conditions for maintenance of the physiological active state of a plant is the optimum water balance. Water is an important constituent of the plant cell. It is the solvent for entry and transport of substances and for metabolic reactions. In Unit 1 you have learnt about the physical principles that govern the net water fluxes from one cell to the next cell and the bulk movement of water in soil-plant atmospheric system. Water potential(Ψw)is the driving force which causes water to move in plant system. Osmotic potential ($\Psi s/\Psi_\pi$) is an important component of water potential and is related to it by the equation.

$$\Psi w = \Psi s + \Psi p$$

1.2 MATERIALS REQUIRED

- Leaves of *Rhoeo discolor*
- Sucrose solution (0.25 M), distilled water
- Petri dishes, glass slides, cover slips, beakers andpipettes(1ml,10ml)
- Microscope, forceps, needle, razor blade, stopwatch, graph paper

1.3 PRINCIPLE

You know that biological membranes are differentially permeable to different solvents. Osmosis is the movement of the solvent molecules from region of higher concentration to lower concentration through semipermeable membrane.

Osmotic potential is the amount by which water potential is reduced as a result of the presence of the solute .It is also called **solute potential**. The osmotic potential of cell-sap is considered to be equal to that of solution at which 50% of the cells show plasmolysis and osmotic potential can be calculated by the formula given below

Osmotic potential = - ***C*RT**

Where ***C*** = molarconcentration at incipient plasmolysis

R = Universal gas constant(0.082 atm/mol)

T = absolute temperaturein ^{0}K (t + 273)

1.4 PROCEDURE

1. Prepare a series of sucrose solutions ranging from 0.15 molar to 0.35 molar by using 1 molar sucrose as a stock solution. The solutions can be prepared with the help of dilution table given below.

2. Take leaves of *Rhoeo discolor* and peel out the epidermis. This plant is used because they have anthocyanin pigments in their vacuolar sap. Anthocyanin pigments are water –soluble and respond to change in pH values of external medium.

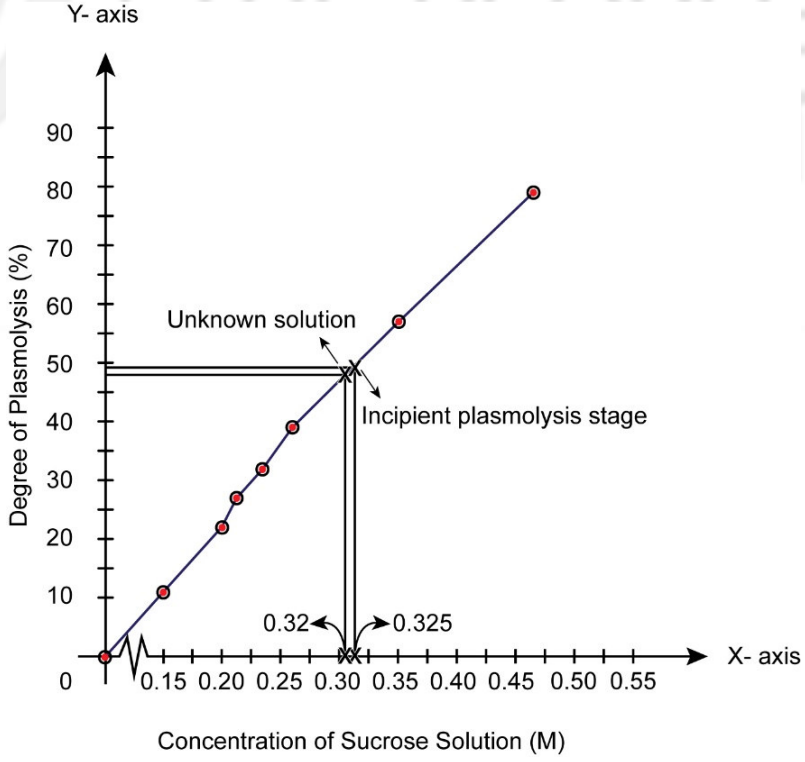


Fig1.1: Plotting of graph between concentration of sucrose solution and degree of plasmolysis.

3. In each petri plate, place three peels after a time gap of five minutes. Wait for ten minutes.

4. Take out the peel and mount it on the slides in their respective solutions and observe under microscope (6x, 40x).

5. Count total number of pigmented cells visible and number of cells that had undergone partial or complete plasmolysis. Plot a graph between the percentage plamolysed cells (Y-axis) against the sucrose concentrations (X-axis). From this graph we can find out the concentration at which 50% of the cells are plasmolysed – the stage of **incipient plasmolysis**.

6. Calculate osmotic potential of the cells by the formula given above.

Dilution Table

Stock solution = 1.0**M** (Molar) sucrose (342g sucrose in some distilled water. Raise the final volume to 1000 ml).

Concentration of Sucrose Solution (M)	Amount of 1 M Sucrose	Amount of Solvent (distilled water)	Total Volume
Control	–	10.0 ml	10 ml
0.15 M	1.5 ml	8.5 ml	10 ml
0.20 M	2.0 ml	8.0 ml	10 ml
0.22 M	2.2 ml	7.8 ml	10 ml
0.24 M	2.4 ml	7.6 ml	10 ml
0.26 M	2.6 ml	7.4 ml	10 ml
0.30 M	3.0 ml	7.0 ml	10 ml
0.35 M	3.5 ml	6.5 ml	10 ml

1.5 OBSERVATIONS

Observation Table

Sucrose concentration (M)	No. of pigmented cells examined (mean)	No. of cells plasmolysed (mean)	Degree of plasmolysis
Control	a)	a)	
	b)	b)	
	c)	c)	
0.15 M	a)	a)	
	b)	b)	
	c)	c)	
0.20 M	a)	a)	
	b)	b)	
	c)	c)	

0.22 M	a)	a)
	b)	b)
	c)	c)
0.24 M	a)	a)
	b)	b)
	c)	c)
0.26 M	a)	a)
	b)	b)
	c)	c)
0.30 M	a)	a)
	b)	b)
	c)	c)
0.35 M	a)	a)
	b)	b)
	c)	c)

Calculations

I. For given plant material

C = isotonic concentration = 0.32 M (from the graph)

R = gas constant = 0.082

T = absolute temperature = 306.5 °K (say at 33°C)

$\Psi s/\Psi_\pi$ = - CRT

= - 0.32 × 0.082 × 306.5

$\Psi s/\Psi_\pi$ = - 8.0425 bars

1.6 RESULTS

No plasmolysis is observed in the control (distilled water). With an increase in sucrose concentration, the number of plasmolysed cells increases because the external medium becomes hypertonic with respect to cell sap. As the plasmolysiscontinues, the cytoplasm along with cell membrane starts shrinking. A space is created between the cell wall and the cell membrane, which gets filled up with the plasmolysing solution (external sucrose solution and water from the cell sap). The sucrose concentration where 50% of the cells are plasmolysed, i.e. **isotonic concentration**, is determined from the graph. The concentration of an unknown solution can also be determined from the graph and used for calculating osmotic potential of that solution.

1.7 PRECAUTIONS

1. The epidermal peels should be one-celled thick and free from green tissue, and air bubbles.

2. All cells of the peel should invariably contain anthocyanin pigment.
3. Before use, keep the peels fully immersed in water to avoid the entry of air bubbles.
4. The peels should be examined after a fixed duration of time, the time to be adjusted before starting the experiment (trial run for fixing time period may be done).
5. Mounting of the peels should be done in their respective concentrations, and not in water to prevent deplasmolysis.
6. The petri dishes containing different sucrose solutions should be covered to prevent evaporation.
7. Even if the cells are slightly plasmolysed, they should be categorized as having undergone plasmolysis.

EXPERIMENT 2

TO STUDY THE EFFECT OF TWO ENVIRONMENTAL FACTORS (LIGHT AND WIND VELOCITY) ON TRANSPIRATION BY AN EXCISED

Structure

2.1 INTRODUCTION

Plant absorbs water from its surroundings (especially roots) but only a small fraction of the absorbed water is actually utilized by them. The bulk of the water absorbed is not retained and is evaporated into the air from the leaves and other aerial parts of the plant. The loss of water in the form of vapour from the aerial parts of the plant is known as **transpiration**.

Loss of water can take place from any part of the plant which is exposed to air. Leaves are, however, the principal organs of transpiration. The transpiration which occurs from the leaves is known as **foliartranspiration.** Most of the foliar transpiration takes place through the stomatal openings and is therefore, known as **stomatal transpiration.** A small fraction of the foliar transpiration also takes place from the general surface of the leaf through the cuticle and is known as **cuticular transpiration.** Loss of water vapours also takes place through the lenticels of woody stems and fruits, and is called **lenticular transpiration.**

2.2 MATERIALS REQUIRED

- Plant material – Twigs of *Tecoma*plant
- Apparatus - Potometer
- Glassware – i) A graduated pipette (1 mL)

  ii) 1 Beaker (1000 mL)
- Miscellaneous – Rubber tube, Stopwatch, Iron stand (with two holders), Sharp blades, Lux meter, Anemometer (optional) and modelling clay

2.3 PRINCIPLE

The rate of transpiration is influenced directly or indirectly by a number of factors, of which light intensity and wind velocity are among the chief ones. **Light** affects the rate of transpiration directly by opening the stomata. In the absence of light the stomata are closed and the stomatal transpiration is completely checked. High light intensity causes more stomata to open hence high rate of transpiration. It also affects the transpiration by heating the surface of leaf, hence more vaporisation of water present in cells of leaf.

Wind velocity also has remarkable effect on rate of transpiration. If wind is not blowing the water vapours are assumed to accumulate above the transpiring leaves. It lowers the steepness of the vapour- pressure gradient,which decreases the rate of transpiration. When wind is blowing, the water vapours quickly move away from the leaf surface resulting in increase in the steepness of vapour pressure gradient, hence rapid rate of transpiration. A gentle breeze is more effective in increasing the rate of transpiration than wind of greater velocity. It is believed that the wind has a cooling effect on the evaporating surface of leaf. This lowers the vapour pressure gradient, which reduces the rate of transpiration. High wind velocity also reduces the rate of transpiration by increasing the rate of loss of water from the mesophyll cells which eventually results in water stress- the flaccidity and closure of stomata.

Thus, wind velocity, like light, also affects the rate of transpiration as a combination of positive and negative influences.

2.4 PROCEDURE

1. First take a simple potometer with one end of the rubber tube attached to a graduated pipette.
2. Then fill it with water till the water level rises to the top most mark on the scale of the pipette.
3. Now cut a twig of *Tecoma* plant obliquely under water with a sharp blade and insert it at the free end of the rubber tube under water to avoid entry of air bubbles in the twig.
4. Then place the apparatus in sunlight for about 10 minutes (Lag period) and record the fall in water level in the pipette.

5. You have to repeat this procedure two more times.
6. Then place the apparatus in the shade but diffused sun light should be present there.
7. Keep the apparatus for a lag period of 10 minutes and again record the rate of fall in the water level. This process should be done three times.
8. Now repeat the same process in the room and take three readings.
9. Record the light intensity by a luxmeter or photometer. Now put the apparatus under very low wind velocity produced by a table fan.
10. Give a lag period of 10 minutes and record the fall in water level per minute.
11. Repeat the procedure for medium wind velocity and high wind velocity by giving the lag period in each case.
12. Compare the readings and plot the graphs.
13. You can measure the wind velocity with the help of an anemometer.

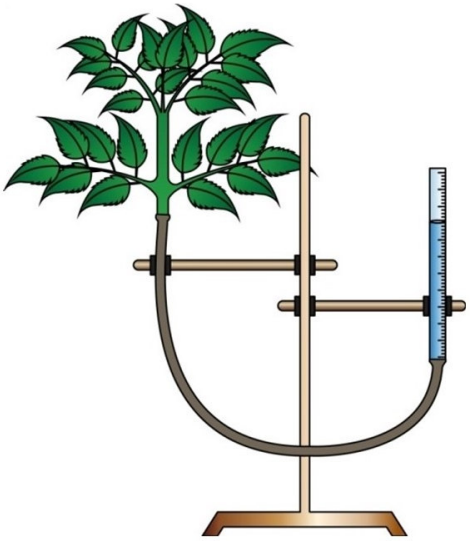


Fig 2.1: Measurement of the rate of transpiration with the help of a potometer.

2.5 OBSERVATIONS

Observation Table

a) Effect of Light Intensity on Rate of Transpiration

Sl. No.	Light Intensity	Initial Level of Pipette (mL)	Final Level of Pipette (mL)	Volume of H_2O Absorbed (mL/min)	Rate of Transpiration
1.	Lux	1) 2) 3)			

2.	Lux	1) 2) 3)			
3.	Lux	1) 2) 3)			

b) Effect of wind Velocity on Rate of Transpiration

Sl. No.	Wind Velocity	Initial Level of Pipette (mL)	Final Level of Pipette (mL)	Volume of H_2O Absorbed (mL/min)	Rate of Transpiration
1.	Low	1) 2) 3)			
2.	Medium	1) 2) 3)			
3.	High	1) 2) 3)			

2.6 RESULTS

The rate of transpiration is maximum when the potometer is kept under highlight intensity but decreases as it is shifted to a region of low light intensity.

The rate of transpiration is low at low wind velocity but increases at the medium wind velocity. The rate of transpiration again comes down at high wind velocity.

2.7 PRECAUTIONS

1. A healthy twig must be used for the experiment.
2. An oblique cut must be made in the stem while it is under water.
3. The apparatus must be air tight.
4. Constant log period must be given before changing the environmental conditions.

EXPERIMENT 3

TO DETERMINE THE STOMATAL INDEX AND STOMATAL FREQUENCY IN A MESOPHYTE AND XEROPHYTE

Structure

3.1 INTRODUCTION

The number, distribution and behaviour of stomata vary in plant leaves belonging to different habitats. These parameters directly affect the rate of transpiration. Stomata play an important role in the exchange of carbon dioxide and oxygen gases and loss of water in a plant.Stomata are present in the epidermal layer of the leaf. The stomatal pore is surrounded by a pair of guard cells which control its opening and closing. When the turgor pressure in guard cells increases, stomata opens and when it decreases the guard cells collapse and close the stomata. In this exercise you will determine stomatal index and stomatal frequencyin a mesophyte and xerophyte leaf.

Objectives

After performing this exercise, you should be able to:

- ❖ compare stomatal index and stomatal frequency in mesophytes and xerophytes and appreciate its ecological importance.

3.2 MATERIALS REQUIRED

- Leaves of a mesophyte : *Withania somnifera* or any other mesophyte
- Leaves of xerophytic plant: *Bryophyllum or* anyother xerophytes
- Distilled water

- Stage micrometer
- Ocular micrometer
- Petri dishes, slides, and cover slips
- Compound microscope, razor blade, blotting paper, brush and needle

3.3 PRINCIPLE

The epidermal surface of a leaf has large number of stomata. You can see stomata onlyunder a microscope. The number of stomata varies from species to species and it ranges from 1000 to 60,0000 per square centimeter. You can find stomata on both the sides of leaf, but in many species, they are present on either side. Stomata playan important role in regulating gaseous exchange. Generally, they are open in the light and closed in the dark. You will calculate stomatal frequency by the help of micrometry.

Stomatal Index (%) SI $= \frac{S}{E+S} \times 100$

S = Number of stomata in field of vision

E = Number of epidermal cells in the same area

Stomatal Frequency SF = Number of stomata per unit area (per cm^2)

$$= \frac{\text{Number of stomata in a given microscopic field}}{\text{Area of microscopic field}}$$

Area of microscopic field = πr^2 [r = radius of the microscopic field]

Salisbury concluded that stomatal frequency differs with environmental conditions, especially on availability of water.

Micrometry technique is used to determine dimensions of any microscopic material. Micrometers are of two types – stage and ocular micrometer.

i) Stage Micrometer : It is in the form of a glass slide with markings etched which can measure upto an accuracy of 100^{th} part of a mm. i.e., 100 divisions = 1 mm. Stage micrometer is placed on the stage of the microscope and is used to calibrate the ocular micrometer and to calculate area of microscopic field.

ii) Ocular micrometer : It is in the form of a circular glass disc with 100 equidistant divisions but of unknown dimensions. It is placed between the ocular and the objective lenses. Since the ocular micrometer is not calibrated, it is done using the stage micrometer for calculating the least count.

3.4 PROCEDURE

1. Take out epidermal peels from the lower surface of the leaves of *Withania* and *Bryophyllum*.
2. Stain them with dilute safranin, mount in glycerine and observe under high power (6 X × 30 X) of the compound microscope.

3. Calculate the **least count**, i.e., dimension of each small division on the ocular micrometer by coinciding it to the divisions of the stage micrometer.

4. Count the number of stomata and number of epidermal cells in the microscopic field. Record three concordant readings.

5. Calculate the 'stomatal index' and 'stomatal frequency' using the appropriate formulae.

3.5 OBSERVATIONS

Record your observations in the following table:

Observation table

Least count = μm

Plant Material	Number of stomata (S)		Number of Epidermal Cells (E)	Stomatal (SI) Index $\frac{S}{S+E}\times 100$	Area of the field of Vision (A)	Stomatal Frequency S/A
	Number	Mean				
Withania somnifera	A	a				
Bryophyllum	B	b				
	C	c				

3.6 RESULTS

Withania leaves show higher stomatal index and stomatal frequency as compared to *Bryophyllum* leaves. The latter being a succulent,hasa smaller number of stomata to reduce the rate of transpiration.

3.7 PRECAUTIONS

1. Peels should be taken from the same leaf by applying unequal pressure.

2. Avoid counting of stomata near edges of the field of vision (border effect).

3. Peels should be one-celled thick and free from green mesophyll tissue.

4. Magnification of the microscope should remain the same throughout the experiment.

5. Peels should be immediately kept in water to prevent the entrance of air bubbles.

EXPERIMENT 4

TO DEMONSTRATE HILL REACTION

Structure

4.1 INTRODUCTION

The process of photosynthesis is one of the most remarkable activities of a living green plant. Carbohydrates produced through photosynthesis contributeto the basic raw materials which directly or indirectly give rise to all organic compounds of virtually all plants and animals. The total global CO_2 available to plants for photosynthesis is about 11.2×10^{14}tons.

Photosynthesis consists of two phases, - light phase and dark phase. Light phase is photochemical phase called **"Hill reaction"**. It occurs inside thylakoids especially in grana region.

Dark phase is biochemical phase and occurs in the stroma. It is called Blackman's reaction. During light reaction water is photolyzed (split in the presence of light) and releases oxygen and electrons. These electrons travel through a series of carriers: cytochromes, plastoquinone and ferredoxin, to finally reduce $NADP^+$ TO NADPH.

Robin Hilland his colleagues (1937) first demonstrated the evolution of O_2 by illuminated suspension of isolated chloroplasts of *Stellaria media* when provided with artificial electron acceptors like ferricyanide in the absence of CO_2. Ferricyanide is reduced to ferrocyanide by photolysis of water.

The photoreduction of the dye by isolated chloroplasts is commonly referred to as **Hill Reaction**.

$$2\,A + 2H_2O \xrightarrow[\text{Chloroplasts}]{\text{Light}} 2AH_2 + O_2$$

A is the hydrogen acceptor and also called as **Hill's oxidant**.

4.2 MATERIALS REQUIRED

Glassware	:	Test tube, Funnels, Beakers (25 mL), Measuring cylinder 25 mL), Pipette (5 mL), Pipette (1 mL), Centrifugation tubes
Plant Material	:	Fresh spinach leaves
Chemicals	:	0.5 M sucrose solution
		0.1 M Na_2HPO_4
		0.01M DCPIP (2, 6-dichlorophenol indophenol)
		Distilled water
Miscellaneous	:	Blender
		Ice-cubes and ice bucket
		Muslin cloth
		Test tube stand
		Black paper
		Spectrophotometer
		Centrifuge and table lamp

4.3 PRINCIPLE

Hill reaction demonstrates that the artificial electron acceptors can substitute naturally occurring acceptors. In this exercise you will observe photoreduction of a dye 2,6-Dichlorophenol indophenol (DCPIP) which is blue in an oxidized state (quinone form) but becomes a colourless compound when reduced (phenol form)

$$\text{DCPIP (blue)} + H_2 \xrightarrow[\text{Chloroplasts}]{\text{Light}} \text{DCPIP-}H_2 \text{ (Colourless)} + \tfrac{1}{2}\, O_2.$$

After performing this experiment you should be able to:

- Isolate chloroplasts from leaves, and
- show photoreduction of dye (Hill oxidant) by illuminated suspension of chloroplasts.

4.4 PROCEDURE

i) **Preparation of dye:** Dissolve 10 mg of dye in 100 mL of distilled water to make 0.01 M DCPIP.

ii) **Preparation of buffer:** Mix 68.5 mL of 0.2 M NaH_2PO_4 [Sodium dihydrogen orthophosphate] and 31.5 mL of 0.2 M Na_2HPO_4 [disodium hydrogen phosphate] to make 100mL of 0.2 M buffer (Phosphate), pH 6.5

iii) **Isolation of Chloroplasts**

A) Homogenization

1. Pre-chill the glassware and reagents.
2. Take 50 g of spinach leaves (refrigerated in dark), remove petioles and midrib.
3. Add 100 mL of cold 0.5 m sucrose solution, and blend contents at top speed for about 15 sin a glass blender and stop for few seconds.
4. Then, again blend at low speed for 10 seconds.

B) Filtration

Filter the homogenate through muslin cloth in a beaker with the help of pre-chilled funnel.

C) Centrifugation

1. Now pour the filtrate into two pre-chilled centrifuge tubes in equal amounts and centrifuge at low speed (500 × g) for 3 min.
2. Take out the supernatant solution in another centrifuge tube and again centrifuge at high speed (2000 × g) for about 7 minutes.
3. Take out the pellet in a test tube and discard the supernatant solution.
4. Resuspend the pellet in 5 mL of cold 0.5 M sucrose solution. Keep it in dark in an ice bucket.

Demonstration of Hill reaction

Take 3 test tubes and pour into them the following materials:

Test tube I : 1 mL of chloroplast suspension + 2 mL of phosphate buffer + 2 mL distilled water + 1 mL DCPIP

Test tube II : 1 mL of chloroplast + 1 mL of phosphate buffer + 2 mL distilled water + 1 mL DCPIP

Test tube III : 1 mL phosphate buffer + 3 mL distilled water + 0.5 mL DCPIP (control).

1. Keep test tube I under light conditions.
2. Keep test tube II under light deficient conditions by wrapping black paper or aluminum foil around it.
3. Keep test tube III as control test tube.
4. Leave test tubes undisturbed for some time.
5. Take readings for optical density and % transmission in a spectrophotometer

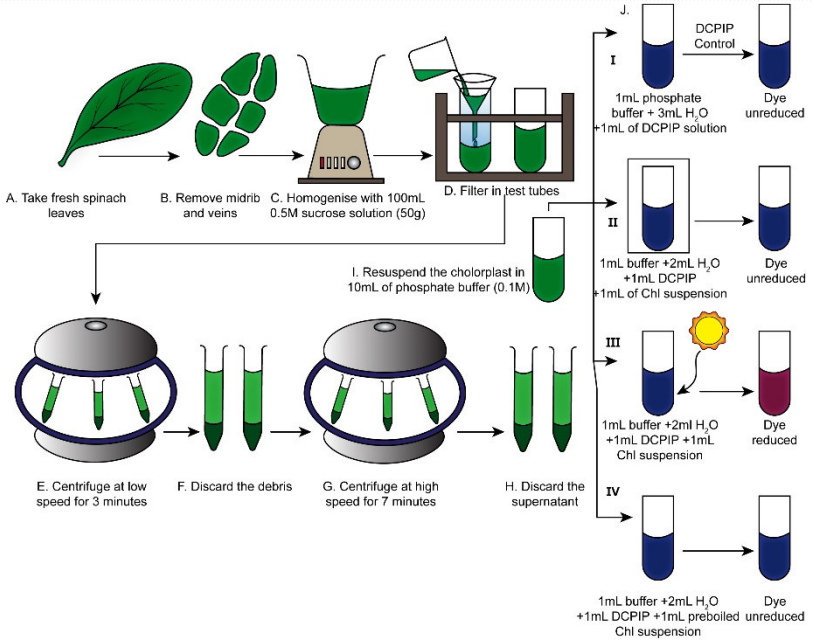


Fig.4.1: Pictoral depiction of the protocol for Hill reaction.

4.5 OBSERVATIONS

Test Tube	Spectrophotometer Reading		
I	0 minOD/T	5 min OD/T	10 min OD/ T
II	-	-	-
III	-	-	-

Plot a graph between Time (X-axis) and Optical Density –OD(Y axis).

4.6 RESULTS

The test tube I will show maximum reduction of dye while test tubes II and III will show very low or no reduction of dye.

4.7 PRECAUTIONS

1. Homogenization should be carried out carefully so that chlorophyll does not get mechanically crushed and denatured.
2. All equipments and reagents should be pre-chilled.
3. Only fresh leaves should be used.
4. All reagents should be freshly prepared.
5. Healthy spinach leaves must be frozen and their petioles and mid veins removed.

EXPERIMENT 5

TO DEMONSTRATE THE ACTIVITY OF CATALASE AND STUDY THE EFFECT OF pH AND ENZYME CONCENTRATION

Structure

5.1 INTRODUCTION

All the living cells carry out huge variety of biochemical reactions, yet they are able to rapidly construct very large and complicated molecules, or regulate the flow of materials through complex metabolic pathways with absolute precision and accuracy. Enzymes make this possible without any error. Enzymes are biological catalysts which make possible the conversion of substrate molecules to products, but they themselves are not permanently changed by the reaction .Cells contain thousands of enzymes, each one catalyzing a particular reaction or specific for a particular reaction.

The most remarkable characteristics of enzymes are their catalytic power and specificity. Catalytic activity takes place at a particular site on the enzyme which is called **active site**. Enzymes accelerate reactions by factors of as much as a million or more without affecting its equilibrium position.

In the present experiment, you will study the effect of two parameters, viz., pH and enzyme concentration on the activity of the enzyme *catalase*.

5.2 MATERIALS REQUIRED

- Potato tuber (*Solanum tuberosum*)
- 3% H_2O_2 , standard buffer solution (of pH 1.0,4.0,7.0,9.0 and 12.0), Distilled water

- Y-shaped apparatus, burettes (2), beaker, measuring cylinder.
- Clamp stands (2)

5.3 PRINCIPLE

Photosynthesis results in splitting of water (photolysis) into protons and hydroxyl ions during light reaction.

$$H_2O \longrightarrow H^+ + OH^-$$

H^+ is utilized in forming NADPH which is used in Calvin Cycle. The OH^- undergoes reaction to form hydrogen peroxide ($OH^- + OH^- + 2e^- \longrightarrow H_2O_2$) which is highly toxic and may cause degradation of membranes and generates free radicals. ***Catalase*** enzyme scavenges or removes H_2O_2 by breakingit into water and oxygen.

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

Catalase is an important enzyme for photosynthetic cells (It can be obtained from green leaves and also from potato tubers).

5.4 PROCEDURE AND OBSERVATIONS

1. Activity of *catalase* enzyme

- For testing the activity of *catalase* enzyme, you must take 5mL of *catalase* enzyme extract and add 2mL of 3% H_2O_2 in a test tube.
- In another lot boiled potatoes extract should be taken.
- Observe the reaction as follows:

Observation Table

S. No.	Nature of Enzyme	Observations		Inference
		Initial	Final	
1.	*Active*	No froth	Froth	Froth formation because of evolution of O_2 gas.
2.	*Denatured* (Boiled potato extract)	No froth	No froth	No froth because O_2 gas is not evolved as the enzyme is killed.
3.	*Control* (No potato extract)	No froth	No froth	No froth because reaction mixture lacks the enzyme.

2. Effect of pH on enzyme activity

i) Set up the experiment as shown in the figure.

ii) Fill the burettes with water.

iii) Weigh approximately 5g of potato tuber and add 1-2 mL of water in pestle and mortar and crush it. Keep the potato extract on one side of the Y–shaped apparatus.

iv) Add 5mL of 3% H_2O_2 and 5mL of buffer of pH 1.0 on the other side. Connect the apparatus to the burette.

v) Note down the initial level of water in the burette towards left side.

vi) Mix the contents of both the sides of apparatus and keep it for about 5 minutes. Record final level of water in the burette. Repeat the same procedure for other pH ranges, i.e. 4.0, 7.0, 9.0 and 12.0.

vii) Plot a graph with pH (X-axis) against the amount of water displaced in the burette (Y-axis).

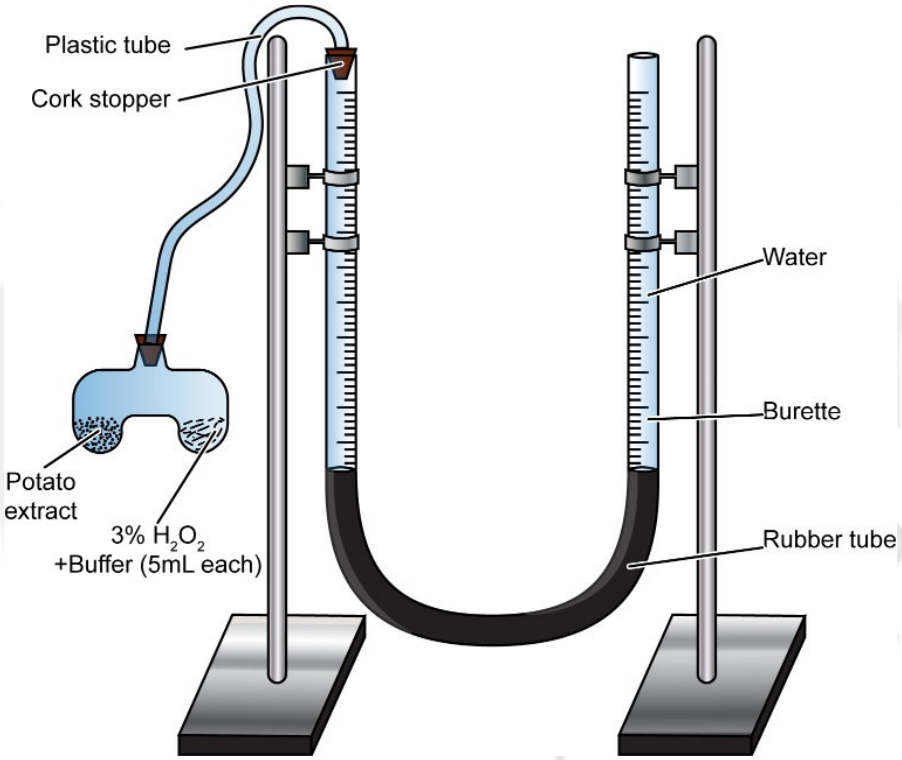


Fig 5.1: Estimation of *catalase* activity by Y –shaped apparatus.

ObservationTable

a) Effect of pH

S. No.	pH of buffer solution (5 mL)	Substrate (5 mL)	Enzyme extract (5g)	Burette reading		Volume of water displaced (mL)
				Initial (mL)	Final (mL)	
1.	1.0	3 % H_2O_2	Potato tuber			
2.	4.0	3 % H_2O_2	,,			
3.	7.0	3 % H_2O_2	,,			
4.	9.0	3 % H_2O_2	,,			
5.	12.0	3 % H_2O_2	,,			

b) Effect of enzyme concentration

Prepare a crude enzyme extract as in the above experiment. Make similar enzyme extracts. Dilute the stock solution to 4%, 3%, and 1% by pipetting out accurate amounts of enzyme and buffer of pH 7.0.

S. No.	Substrate (5 mL)	Enzyme Extract	Buffer pH 7.0	Burette reading		Volume of water displaced (mL)
				Initial (mL)	Final (mL)	
1.	3 % H_2O_2	1 mL	4 mL			
2.	3 % H_2O_2	2 mL	3 mL			
3.	3 % H_2O_2	3 mL	2 mL			
4.	3 % H_2O_2	4 mL	1 mL			
5.	3 % H_2O_2	5 mL	0 mL			

5.5 RESULTS

The amount of water displaced in the burette indicates the amount of O_2 evolved which in turn depends upon the *catalase* activity. Discuss your results at every pH and find out how much water was displaced. Plot a graph between pH (X-axis) and the amount of water displaced (Yaxis) to show the pH at which maximum activity is recorded.

Likewise, plot a graph between enzyme concentration (X-axis) and the amount of water displaced (Y-axis) to record the pattern of *catalase* activity as influenced by its concentration. Rate of reaction increases with an increase in the enzyme concentration. However, after a certain level the reaction rate becomes constant due to lack of substrate for the active sites.

5.6 PRECAUTIONS

1. Buffer solution and enzyme extract should be freshly prepared
2. Reaction time should be constant for all readings.
3. Initial level of water must be recorded before mixing of contents in the Y-shaped apparatus.

EXPERIMENT 6

TO STUDY THE EFFECT OF LIGHT INTENSITY AND BICARBONATE CONCENTRATION ON O_2 EVOLUTION IN PHOTOSYNTHESIS

Structure

6.1 INTRODUCTION

Photosynthesis is an anabolic, endergonic, oxido-reductive process and can be defined as the formation of carbohydrates from CO_2 and H_2O by illuminated green (chlorophyll containing) cells with O_2 and H_2O being the by-products.

$$6CO_2 + 12H_2O \xrightarrow[\text{Chlorophyll}]{\text{Light}} \underset{\text{Carbohydrate}}{C_2H_{12}O_6} + 6O_2 + 6H_2O$$

Various external factors affecting the process of photosynthesis are: light, CO_2, O_2, temperature, and amount of available water. Since photosynthesis is controlled by many factors which interact, it is also governed by the **Law of Limiting Factors** (Blackman).

According to the concept of three cardinal points introduced by Sachs (1880), there is minimum, optimum and maximum for each factor in relation to photosynthesis.

For example, any species has a minimum temperature below which no photosynthesis takes place, an optimum temperature at which the highest rate takes place, and a maximum temperature above which photosynthesis ceases altogether.

6.2 MATERIALS REQUIRED

Plant material : Fresh twigs of *Hydrilla verticillata;* Family-Hydrocharitaceae

Chemicals : Filtered pond water, sodium bicarbonate

Glass apparatus : Measuring cylinder (100 mL), glass rod, beaker, pipette, a large petri dish.

Miscellaneous : Meter rod, blade, thread, stop watch, taly counter, lux meter, table lamps, electric balance, graph paper.

6.3 PRINCIPLE

Effect of light*:* Light affects photosynthesis through its intensity, quality and duration. A direct relationship between the rate of photosynthesis and light intensity is shown at the lower light intensities. As intensity of light is increased, beyond a certain level, there is a decline in the photosynthetic rate because of some other limiting factor or the destructive effects of high light intensity (photo-oxidation). Also, the point of saturation may be achieved, after which the rate of photosynthesis will remain constant.

Effect of CO_2 concentration*:* The amount of CO_2 in air, although small, is relatively constant. It provides a steady and adequate supply of carbon dioxide to the plant world. With an increase in the concentration of CO_2 there is an increase in the rate of photosynthesis at the lower levels of CO_2 concentration. A decline in rate is noticed at higher concentrations, as it may inhibit respiration and also cause cell damage.

Bubble-counting method is used for measurement of photosynthesis in which the rate of evolution of bubbles by a submerged aquatic plant is considered to be proportional to the rate of apparent photosynthesis.

6.4 PROCEDURE

1. Cut a healthy or actively growing twig of *Hydrilla,* about 15 cm length (with apex intact), under water. An oblique cut must be given to the stem.
2. Place it in a measuring cylinder containing 100 mL of filtered pond water.
3. With the help of glass rod, tie the twig loosely so that when placed in water it stays submerged under the water. The cut end of the twig should face upwards whereas the intact shoot apex downwards.
4. When the twig starts producing bubbles uniformly, count the number of bubbles given out from the cut end at an interval of one minute, using a taly counter. Repeat counting at least thrice.

a) Effect of Light Intensity

Repeat the bubble counting under different intensities of light achieved by varying the distance between the light source and the plant material.

Allow the plant to remain in each intensity of light for 5 minutes before starting the count. Record the light intensity using lux meter.

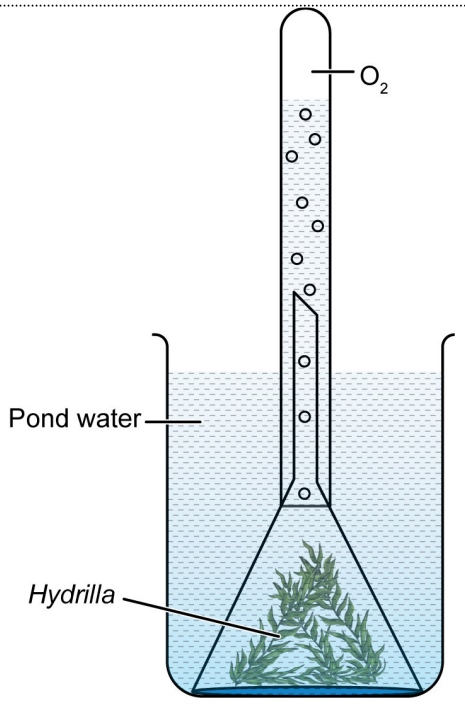


Fig.6.1: Experimental setup for studying effect of light and CO_2 on photosynthesis.

6.5 OBSERVATIONS

Observation Table

S. No.	Environmental Factor	Light intensity (Lux)	Oxygen bubbles per minute	Mean
1.	Diffuse Sunlight		i) ii) iii)	
2.	Diffuse Sunlight + 1 Lamp		i) ii) iii)	
3.	Bright Sunlight + 2 Lamps		i) ii) iii)	

Graph : Plot your recorded observations (Light Intensity -X-axis) *vs* Number of bubbles evolved per minute -Y-axis) on a graph paper.

b) Effect of CO_2 concentration

$$NaHCO_3 \rightleftharpoons Na^+ + HCO_3^-$$

$$H_2O \rightarrow H^+ + OH^-$$

$$Na^+ + OH^- \rightarrow NaOH$$

$$H^+ + HCO_3^- \rightarrow \underset{\text{Carbonic acid}}{H_2CO_3} \rightarrow \underset{\text{Both act as reactants in photosynthesis}}{H_2O + CO_2}$$

The set up for this experiment is the same as explained in 6.4a. Effect of different concentrations of $NaHCO_3$ (source of CO_2) on the rate of photosynthesis is studied at a constant light intensity.
Dissolve 0.01 g $NaHCO_3$ in 100 mL of filtered pond water (Sol.A). Wait for 5 minutes and record the number of oxygen bubbles evolved from the cut end of *Hydrilla* twig per minute (3 concordant readings). Take out 5 mL of solution A, and add 0.09 g of $NaHCO_3$. Dissolve and then put the solution back into A. This is now the solution B. Wait for 5 minutes and record the number of oxygen bubbles evolved from the cut end of *Hydrilla* twig per minute 3 concordant readings). Similarly to prepare solution C, take out 5 mL of solution B, add 0.9 g of $NaHCO_3$ dissolve and then put the solution back into B. Record the observations as done for solutions A and B. Sodium bicarbonate powder is not added directly into the flask as it will not disperse uniformly.

Sol. A : 0.01% - 0.01 g dissolved per 100 ml of pond water

Sol. B : 0.1% - 0.09 g + Sol. A (0.1 g/100 ml)

Sol. C : 1% - 0.9 g + Sol. B (1 g/100 ml)

Fixed Environmental Factor = Diffuse Sunlight + 1 Lamp (at a distance of 30cm)

S. No.	Concentration of $NaHCO_3$	O_2 bubbles evolved per minute	Mean
1.	0.01 %	i) ii) iii)	
2.	0.1 %	i) ii) iii)	
3.	1 %	i) ii) iii)	

Graph: Plot your recorded observations ($NaHCO_3$ concentration (X-axis) *vs* number of bubbles evolved per minute (Y-axis) on a graph paper.

6.6 RESULTS

With an increase in light intensity, the rate of photosynthesis goes on increasing till some other factor (CO_2 or temperature) becomes limiting. Very high light intensity results in decline in photosynthetic rate.

With an increase in CO_2 concentration, the rate of photosynthesis increases till some other factor (light/temperature) becomes limiting. Also, higher CO_2 concentrations are toxic to a plant.

6.7 PRECAUTIONS

1. *Hydrilla* twig should be healthy, dark green, injury-free and with intact shoot apex.
2. Oblique cut should be given to the twig, under water.
3. Twig should not touch the side walls of the cylinder and can be supported by using glass rod.
4. Same twig should be used throughout the experiment.
5. The twig should be allowed to remain in each light intensity for 3-5 minutes before starting the bubble count.
6. The twig should be allowed to remain in each $NaHCO_3$ concentration for 3-5 minutes before starting the bubble count.
7. A sufficient quantity of $NaHCO_3$ (approx 0.1 g/100 ml) should be added to the filtered pond water so that CO_2 does not become a limiting factor. It is desirable not to add different concentrations of $NaHCO_3$ directly into the solution in the cylinder but should be first dissolved in 5 ml of solution withdrawn from the cylinder, using a pipette and then added back.

EXPERIMENT 7

TO COMPARE THE RATE OF RESPIRATION IN ANY TWO PARTS OF A PLANT

Structure

7.1 INTRODUCTION

We all know that during photosynthesis, light energy is converted into chemical energy which is stored in carbohydrate molecules in form of glucose and starch. Living organisms use this energy for other activities by oxidising these molecules into simple ones i.e., carbon dioxide and water. This reaction is known as respiration. You can define respiration as a process by which living cells break down complex high energy molecules into simple low energy molecules, CO_2 and H_2O, releasing the energy trapped within the chemical bonds. This energy is made available for various activities through an intermediate compound known as adenosine triphosphate (ATP).

7.2 MATERIALS REQUIRED

- Whole plant of *Ageratum conizoides*
- Brine solution (saturated solution of NaCl); KOH pellets
- Buchner's flasks (two sets), measuring cylinder (1000ml), beakers (250) ignition tubes
- Pestle and mortar, electric balance, clamp standand black paper.

7.3 PRINCIPLE

You know that cellular respiration undergoes a series of independent pathways through which carbohydrates and some other molecules are oxidized for the purpose of retrieving the energy stored in photosynthetic products.

The reaction which takes place in respiration may be summed up as follows:

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

The rate of respiration depends upon amount of protoplasm and its state of activity. Younger cells have highest protoplasmic activity because of active growing regions while spores and dry seeds or drying leaves have low respiration rates. Environmental conditions also affect the rate of respiration

7.4 PROCEDURE

1. First weigh equal amounts (7-10 g) of two organs of the same plant : leaves and roots.
2. Make two set-ups consisting of Buchner's flask with a rubber cork, an ignition tube which contains a thick paste of KOH pellets and a beaker containing brine solution as shown in Fig 7.1.
3. Place roots and leaves in a separate Buchner's flask. Now take the flask with green leaves and make the apparatus air tight, while the side tube is immersed in the measuring cylinder containing brine solution. Cover this flask with black paper to avoid photosynthesis.
4. In both set-ups suspend the side tube in a 250 ml beaker containing brine solution. Note the initial level of brine solution and then record the rise in level in the setup at 5.10,15 min intervals respectively. Repeat the procedure with roots of the plant.

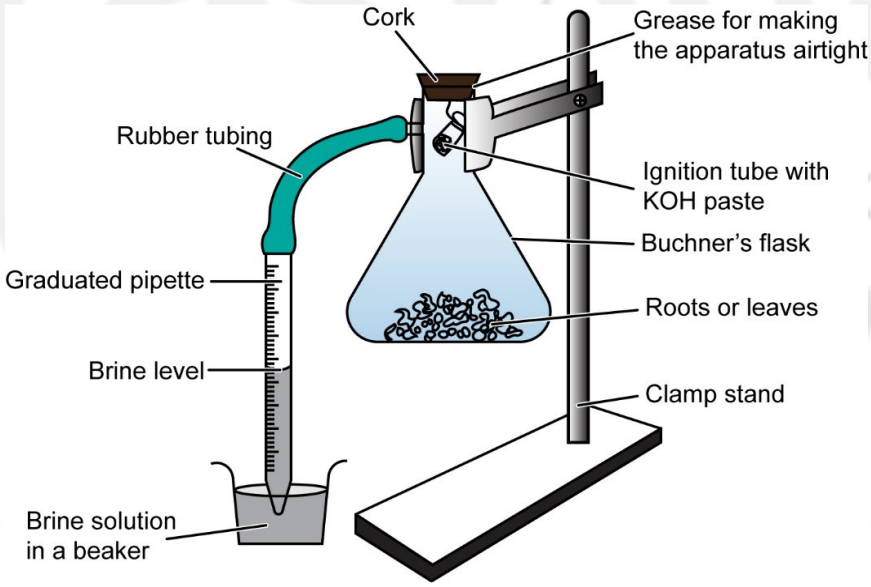


Fig 7.1: Setup to estimate respiration in plant parts.

7.5 OBSERVATIONS

Observation Table

Plant material	Rise in the level of brine solution				Rate of respiration d\t (mL)
	Initial	5min	10 min	15 min	
Roots					
Leaves					

7.6 RESULTS

The CO_2 released during the respiration of roots and leaves is absorbed by KOH in the ignition tube and a vacuum is created in the flask causing brine level to rise. The rise in the level of brine in the side tube after 5, 10 and 15 minutes can be calculated by the formula as given. Compare the values obtained from leaves and roots and see which organ respires more.

7.7 PRECAUTIONS

- You have to put same amount of plant material in both the sets.
- You should keep the ignition tube open throughout the experiment.
- Keep the graduated side tube untouched by the wall of the beaker.
- The apparatus should be vertical and air tight.
- Do not wash plant material before starting the experiment

EXPERIMENT 8

TO SEPARATE AMINO ACIDS BY PAPER CHROMATOGRAPH

Structure

8.1 INTRODUCTION

Living organisms contain thousands of different molecules in a single cell. If we want to study a single molecule it is necessary to separate it from rest of them. Chromatography is one of the most effective and widely used techniques for separation and identification of biomolecules. Different kinds of chromatography techniques have been evolved and improved in recent years.

In this exercise you will learn how to separate amino acids from a given mixture by paper chromatography.

Objectives

After performing this exercise you should be able to:

- separate amino acids in a given mixture
- learn chromatography technique
- use the paper chromatography technique for separation of leaf pigments.

8.2 MATERIALS REQUIRED

- Amino acids, ninhydrin (0.2 % in acetone)
- Organic solvent (butanol:aceticacid:distilled water ::3:1:1)
- Chromatography jar with a lid, glass plate, capillary tube or microsyringe
- Whatman paper (No.1), atomizer, hair dryer, oven, pencil.

8.3 PRINCIPLE

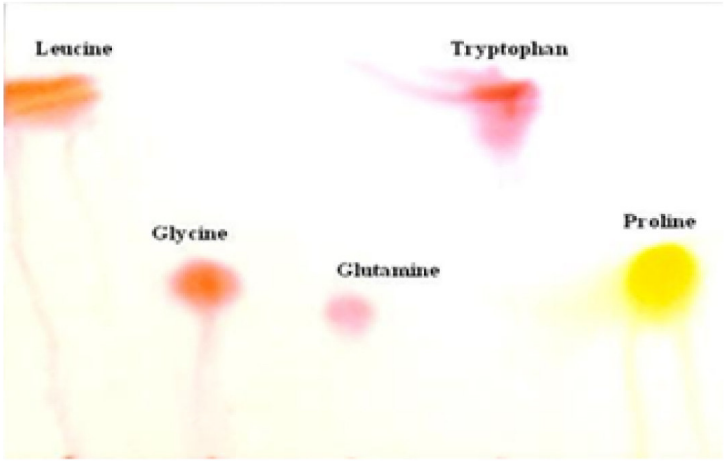
The solute separation is based on liquid – liquid partitioning in this technique. The principle of paper chromatography technique is based on the difference in solute partition between two immiscible phases – a **stationary** aqueous phase which is strongly bound to the cellulose fibers of the paper, and **mobile** organic phase passing through the paper through capillary action. The more soluble a solute in the mobile phase, the farther the molecules will travel along the paper. The migration rate of a substance may be expressed according to its **Rf** (Resolution front/Relative front) value.

Rf is more or less constant for a compound provided the solvent system, solute concentration, temperature and pH are carefully controlled.

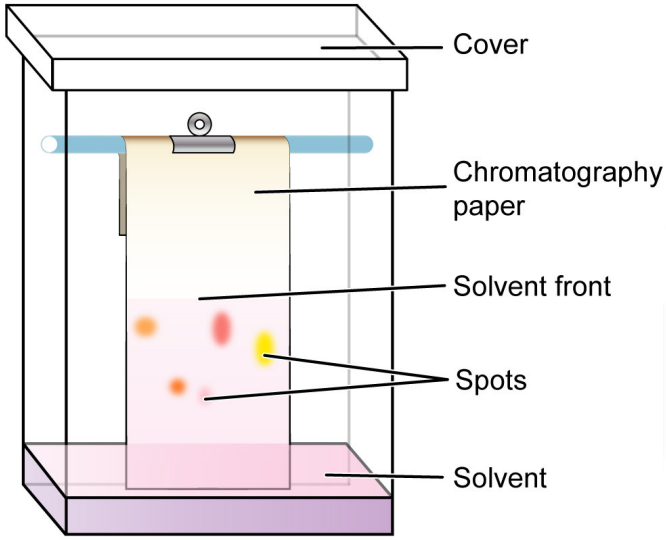
$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

8.4 PROCEDURE

1. Cut a sheet of Whatman no.1 paper to a convenient size. Draw a line 2.5 cm with the help of a pencil from the edge of the paper.
2. Now load the sample using fine capillary tubes, keeping the diameter of each spot as small as possible. This process should be repeated 5 to 6 times. After each application you should dry the first spot with the help of a hair –dryer.
3. In the mean time you should **pre-saturate** the jar in which you are going to run the chromatographic paper with the vapours of organic solvent by closing the lid of the jar so that the jar is saturated with the vapours of the solvent.
4. Now lower the chromatography paper into the solvent to place the strip in the centre of the jar. Take a thick square size paper, a few inches bigger in size than the mouth of the jar. Fold it and place the Whatman 1 filter paper strip between the folds perpendicular to the line of fold. Pin them together with a paper clip. Open the folded side. The strip can be hanged in the jar keeping the thicker paper above the mouth. You may use some other device to hang the paper. Make sure that its lower edge is dipped in the solvent layer but the spot remains well above it. The paper should not touch the walls of the jar.
5. Leave the jar undisturbed. Note the solvent front from time to time and allow the solvent to run about 2/3rd of the length of the paper.
6. Remove the paper from the jar and immediately mark the position of the **solvent front** with the help of a pencil.
7. Dry the chromatogram with hair-dryer. Now spray the paper with the ninhydrin solution using an atomizer.
8. Dry the paper for 5 min at room temperature followed by at 100°C in an oven for 2-3 min.



(a)



(b)

Fig.8.1: a) fully developed chromatogram showing various amino acids; b) Experimental setup for descending paper chromatography.

8.5 OBSERVATIONS

Observation table

Solvent Front = cm

Distance travelled by amino acid	Distance travelled by solvent(s)	$R_{f\ value}$
A		A/S
B		B/S
C		C/S

8.6 RESULTS

Trace the chromatogram and show the amino acidswhich appears in blue, purple or yellow spots (use pencil).

Make an outline of the spots visible on the chromatogram. Measure the distance from baseline to the centre of each spot. Calculate the Rf for each amino acid.

8.7 PRECAUTIONS

1. Hold the chromatogram from an edge only so as to avoid finger marks.
2. Spots should be kept small for maximum resolution.
3. The jar must be saturated with solvent. Therefore, do not leave it without lid except while you hang chromatogram.
4. Do not disturb the apparatus once you have set it.
5. Always prepare the ninhydrin solution fresh.
6. Avoid contact with ninhydrin (preferably use a ventilated chamber).
7. You must mark the solvent front with pencil while the paper is wet.
8. Chromatography paper strip must always be cut in the machine direction.