

Practical Manual
B. Sc. (Hons.) Agriculture
Fundamentals of Crop Physiology
Course No.: ASPH1201
Credit: 3(2+1)
Semester: 2nd



Centurion
UNIVERSITY

*Shaping Lives...
Empowering Communities...*

Department of Crop Physiology and Biochemistry
M. S. Swaminathan School of Agriculture
Centurion University of Technology and Management, Paralakhemundi
www.cutm.ac.in

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Certificate

Certified that this is a bonafide record of practical work done by

Mr./ Ms. _____

Regd. No. _____ in B. Sc. (Hons.) Agriculture degree

programme, Course No. ASPH1201 entitled “Fundamentals of Crop

Physiology” during 2nd semester of the academic year 2022-23.

Date

Faculty In charge

Evaluation System
Fundamentals of Crop Physiology
Course No.: ASPH1201
Credit: 3(2+1)

Sl. No.	Marks distribution*	Total marks	Marks obtained
1	Attendance	6	
2	Assignment	5	
3	Class test	5	
4	Viva	5	
5	Record	4	

***NB: Marks distribution will vary department wise**

Signature of Faculty In-charge

List of Experiments

No of Expt.	Particulars of experiment	Page No.	Date	Signature	Remark
1	Study of Plant Cell				
2	Preparation of Different type of Solution				
3	Study of Structure and Distribution of Stomata				
4	Study of Imbibition				
5	Study of Osmosis				
6	Demonstration on Plasmolysis and Deplasmolysis				
7	Measurement of Root Pressure				
8	Determination of Rate of Transpiration				
9	Separation of Photosynthetic Pigments Through Paper Chromatography				
10	Determination of Chlorophyll content of given leaf sample and estimation of Absorption Spectrum of Chlorophyll				
11	Study of Rate of Photosynthesis				
12	Study of Respiration				
13	Measurement of Relative Water Content of Leaf				
14	Diagnosis of Nutrient Deficiencies for Mineral Nutrient Through Rapid Tissue Test				
15	Measurement of Photosynthetic CO ₂ Assimilation by Infra-Red Gas Analyzer (IRGA)				

EXPERIMENT NO: 01
STUDY OF PLANT CELL

PRINCIPLE

Plant cells are eukaryotic cells that vary in several fundamental factors from other eukaryotic organisms. Both plant and animal cells contain nucleus along with similar organelles. One of the distinctive aspects of a plant cell is the presence of a cell wall outside the cell membrane. They are rectangular and comparatively larger than animal cells.

Even though plant and animal cells are eukaryotic and share a few cell organelles, plant cells are quite distinct when compared to animal cells as they fulfill different functions. Some of these differences are obvious when the cells are examined under an electron microscope.

Plant cell anatomy

Just like an organ in an animal, plant cells have various components known as cell organelles that perform various tasks and function to sustain itself. These organelles include:

1. The Cell Wall

It is a rigid layer which is composed of cellulose, glycoproteins, lignin, pectin, and hemicelluloses. It is located outside the cell membrane. It comprises proteins, polysaccharides, and cellulose. The primary function of the cell wall is to protect and provide structural support to the cell. The plant cell wall is also involved in protecting the cell against mechanical stress and to provide form and structure to the cell. It also filters the molecules passing into and outside the cell. The formation of the cell wall is guided by microtubules. It consists of three layers i.e., primary cell wall, secondary cell wall, and the middle lamella. The primary cell wall is formed by cellulose laid down by enzymes.

2. Cell membrane

It is the semi-permeable membrane that is present within the cell wall. It is composed of a thin layer of protein and fat. The cell membrane plays an important role in regulating the entry and exit of specific substances within the cell. For instance, cell membrane keeps toxins from entering inside, while nutrients and essential minerals are transported across.

3. Nucleus:

The nucleus is a membrane-bound structure that is present only in eukaryotic cells. The vital function of a nucleus is to store DNA or hereditary information required for cell division, metabolism, and growth.

Nucleolus: It manufactures cell's protein-producing structures and ribosomes.

Nuclear membrane: Nuclear membrane is perforated with holes called nucleopore that allows proteins and nucleic acids

4. Plastids

They are membrane-bound organelles that have their own DNA. They are necessary to store starch, to carry out the process of photosynthesis. It is also used in the synthesis of many molecules which form the cellular building blocks. Some of the vital types of plastids and their functions are stated below:

i. Leucoplasts

They are found in non-photosynthetic tissues of plants. They are used for the storage of protein, lipid, and starch.

ii. Chloroplasts

It is an elongated organelle enclosed by phospholipid membrane. The shape of the chloroplast is disk-shaped and the stroma is the fluid within the chloroplast that comprises a circular DNA. Each chloroplast contains a green colored pigment called chlorophyll required for the process of photosynthesis. The chlorophyll absorbs light energy from the sun and uses it to transform carbon dioxide and water into glucose.

iii. Chromoplasts

They are heterogeneous, colored plastids organelle which is responsible for pigment synthesis and for storage in photosynthetic eukaryotic organisms. Chromoplasts have red, orange and yellow colored pigments which provide color to all ripened fruits and flowers.

5. Central Vacuole

It occupies around thirty per cent of the cell's volume in a mature plant cell. Tonoplast is a membrane that surrounds central vacuole. The vital function of central vacuole apart from storage is to sustain turgid pressure against the cell wall. The central vacuole consists of cell sap. It is a mixture of salts, enzymes, and other substances.

6. Golgi Apparatus

They are found in all eukaryotic cells and are involved in distributing synthesized macromolecules to various parts of the cell.

7. Ribosome's

They are the smallest membrane-bound organelle which comprises RNA and protein. They are the sites for protein synthesis hence they are also referred to as the protein factories of the cell.

8. Mitochondria

They are the double-membrane organelles found in the cytoplasm of all eukaryotic cells. They provide energy by breaking down carbohydrate and sugar molecules hence they are also referred to as the "Powerhouse of the cell."

9. Lysosome

Lysosomes are called as suicidal bags as they hold digestive enzymes in an enclosed membrane. They perform the function of cellular waste disposal by digesting worn-out organelles, food particles and foreign bodies in the cell.

EXPERIMENT NO: 02

PREPARATION OF DIFFERENT TYPE OF SOLUTION

Theory

Preparing different types of solutions is a fundamental laboratory skill that is required in many scientific disciplines. In this practical we are going to learn about the principle and preparation of Molar solution, Normal solution, Molal Solution, Percentage solution and ppm solution.

Requirements

- I. Different chemical
- II. Distilled water
- III. Balance
- IV. Beaker
- V. Stirring rod
- VI. Funnel
- VII. Volumetric flask

Molar Solution:

Principle:

A molar solution is a solution in which the concentration of the solute is expressed in moles per liter (mol/L). The molarity of a solution is defined as the number of moles of solute present in one liter of solution.

Example: Prepare 1 liter of 0.1 Molar solution of sodium chloride.

Given: Molecular weight of sodium chloride = 58.44 g/mol, Volume of solution = 1 liter, Desired molarity = 0.1 M.

Procedure:

Weigh 5.84 grams of sodium chloride using a balance. Add the sodium chloride to a clean, dry beaker. Add a small amount of water to dissolve the sodium chloride. Stir the mixture until the solute is completely dissolved. Transfer the solution to a volumetric flask using a funnel. Add distilled water to the volumetric flask up to the 1-liter mark while stirring the mixture. Once the solution is completely mixed, adjust the volume to the mark on the flask with distilled water.

Mix the solution thoroughly by inverting the flask several times.

Normal Solution:

Principle:

A normal solution is a solution in which the concentration of the solute is expressed as the number of equivalents of solute present per liter of solution. The normality of a solution is the number of equivalents of solute in one liter of solution.

Example: Prepare 1 liter of 0.1 Normal solution of hydrochloric acid (HCl).

Given: Molecular weight of HCl = 36.46 g/mol, Volume of solution = 1 liter, Desired normality = 0.1 N, HCl is a monoprotic acid.

Procedure:

Weigh 3.646 grams of hydrochloric acid using a balance. Add the HCl to a clean, dry beaker. Add a small amount of water to dissolve the HCl. Stir the mixture until the solute is completely dissolved. Transfer the solution to a volumetric flask using a funnel. Add distilled water to the volumetric flask up to the 1-liter mark while stirring the mixture. Once the solution is completely mixed, adjust the volume to the mark on the flask with distilled water. Mix the solution thoroughly by inverting the flask several times.

Molal Solution:

Principle:

A molal solution is a solution in which the concentration of the solute is expressed as the number of moles of solute present per kilogram of solvent. The molality of a solution is the number of moles of solute in one kilogram of solvent.

Example: Prepare 500 mL of 0.1 Molal solution of glucose ($C_6H_{12}O_6$).

Given: Molecular weight of glucose = 180.16 g/mol, Mass of solvent = 500 g, Desired molality = 0.1 m.

Procedure:

Weigh 9.008 grams of glucose using a balance. Calculate the number of moles of glucose present in 9.008 grams of glucose by dividing the mass by its molecular weight. Number of moles of glucose = $9.008 \text{ g} / 180.16 \text{ g/mol} = 0.05 \text{ mol}$. Weigh 500 grams of solvent using a balance. Add the glucose to a clean, dry beaker. Add a small amount of solvent to dissolve the glucose. Stir the mixture until the solute is completely dissolved. Add distilled water to the beaker to make up the desired amount of solvent while stirring the mixture. Transfer the solution to a volumetric flask using a funnel. Mix the solution thoroughly by inverting the flask several times.

Percentage Solution:**Principle:**

A percentage solution is a solution in which the concentration of the solute is expressed as the percentage of the total solution. The percentage can be by weight, by volume, or by mass/volume.

Example: Prepare 250 mL of 5% w/v solution of sodium chloride (NaCl).

Given: Weight of NaCl = 5% of total weight, Total volume of solution = 250 mL.

Procedure:

Weigh 12.5 grams of NaCl using a balance. Add the NaCl to a clean, dry beaker. Add a small amount of distilled water to dissolve the NaCl. Stir the mixture until the solute is completely dissolved. Add distilled water to the beaker to make up the desired volume of solution while stirring the mixture. Transfer the solution to a volumetric flask using a funnel. Add distilled water to the volumetric flask up to the 250 mL mark while stirring the mixture. Once the solution is completely mixed, adjust the volume to the mark on the flask with distilled water. Mix the solution thoroughly by inverting the flask several times.

Parts per Million (ppm)

Principle:

Parts per million (ppm) is a unit of concentration that expresses the number of parts of a substance per million parts of solution or mixture. It is commonly used to express the concentration of small amounts of impurities or contaminants in a larger sample.

Example

Prepare a 1 liter solution of 100 ppm (parts per million) NaCl (sodium chloride):

Weigh out 0.1 g of NaCl using a precision balance. Transfer the weighed NaCl into the 1 liter volumetric flask. Add distilled or deionized water to the flask, filling it up to the mark on the neck of the flask. Close the volumetric flask with a stopper or a plastic cap, and mix the contents thoroughly by inverting the flask several times or using a magnetic stirrer. The resulting solution will be a 100 ppm NaCl solution.

Conclusion

EXPERIMENT: 03

STUDY OF STRUCTURE AND DISTRIBUTION OF STOMATA

PRINCIPLE

Stomata is a pore, found in the epidermis of leaves, stems, and other organs, that facilitates gas exchange. The pore is bordered by a pair of specialized parenchyma cells known as guard cells that are responsible for regulating the size of the stomatal opening.

The term is usually used collectively to refer to the entire stomatal complex, consisting of the paired guard cells and the pore itself, which is referred to as the stomatal aperture. Air enters the plant through these openings by gaseous diffusion, and contains carbon dioxide and oxygen, which are used in photosynthesis and respiration, respectively. Oxygen produced as a by-product of photosynthesis diffuses out to the atmosphere through these same openings. Also, water vapor diffuses through the stomata into the atmosphere in a process called transpiration.

Stomata are present in the sporophyte generation of all land plant groups except liverworts. In vascular plants the number, size and distribution of stomata varies widely. Dicotyledons usually have more stomata on the lower surface of the leaves than the upper surface. Monocotyledons such as onion, oat and maize may have about the same number of stomata on both leaf surfaces. In plants with floating leaves, stomata may be found only on the upper epidermis and submerged leaves may lack stomata entirely. Most tree species have stomata only on the lower leaf surface. Leaves with stomata on both the upper and lower leaf are called amphistomatous leaves; leaves with stomata only on the lower surface are hypostomatous, and leaves with stomata only on the upper surface are epistomatous or hypostomatous. Size varies across species, with end-to-end lengths ranging from 10 to 80 μm and width ranging from a few to 50 μm . Depending upon the distribution and arrangement of stomata in the leaves five categories of stomatal distribution have been recognized in plants.

1. **Apple or mulberry (hypostomatic) type:** Stomata are found distributed only on the lower surface of leaves, e.g. Apple, Peach, Mulberry, Walnut, etc.
2. **Potato type:** Stomata are found distributed more on the lower surface and less on its upper surface, e.g. Potato, Cabbage, Bean, Tomato, Pea, etc.

3. **Oat (amphistomatic) type:** Stomata are found distributed equally upon the two surfaces, e.g. Maize, Oats, Grasses, etc.
4. **Water lily (epistomatic) type:** Stomata are found distributed only on the upper surface of leaf, e.g. Water lily, Nymphaea and many aquatic plants.
5. **Potamogeton (astomatic) type:** Stomata are altogether absent or if present they are vestigial. e.g. Potamogeton and submerged aquatics

Loftfield (1921) classified the stomata into four types, depending upon the periods of opening and closing.

- 1) **Alfalfa type:** The stomata remain open throughout the day but close during night, e.g. Pea, Bean, Mustard, Cucumber, Sunflower, Radish, Turnip, Apple, Grape.
- 2) **Potato type:** The stomata close only for a few hours in the evening, otherwise they remain open throughout the day and night e.g. Allium, Cabbage, Tulip, Banana.
- 3) **Barley type:** The stomata open only for a few hours in day time, otherwise they remain close throughout the day and night e.g. Cereals
- 4) **Equisetum type:** The stomata remain always open throughout the day and night e.g. Amphibious plant or emergent hydrophytes.

MATERIALS REQUIRED

A potted Tradescantia or Bryophyllum plant, forceps, needles, watch glasses, glass slides, a dropper, cover slips, a brush, blotting paper, safranin, glycerin and a compound microscope

PROCEDURE

1. Remove a healthy leaf from the potted plant.
2. Remove a part of the peel from the lower surface of the leaf. You can do this by folding the leaf over and gently pulling the peel apart using forceps. Keep the peel in a watch glass containing water.
3. Put a few drops of safranin stain in a watch glass.
4. After 2-3 minutes take out the peel and place it on a clean glass slide.
5. Put a drop of glycerin over the peel and place a clean coverslip gently over it with the help of a needle.

6. Remove the excess stain and glycerin with the help of blotting paper.
7. Observe the slide under the low-power and high-power magnifications of the compound microscope.

OBSERVATION

1. The epidermal cells are visible. These are irregular in outline and have no intercellular spaces.
2. Many small pores (stomata) are seen scattered among the epidermal cells.
3. Each pore is guarded by two bean-shaped guard cells, each containing chloroplasts and a nucleus.
4. The inner concave boundary of each guard cell is thick, whereas its outer boundary is thin.
5. The stomata may be open or closed. The guard cells regulate the opening and closing of the stomata.

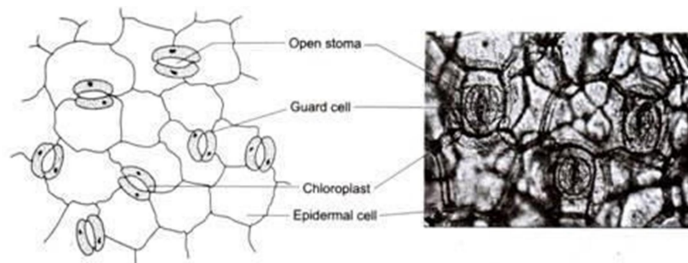
RESULT

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

CONCLUSION

PRECAUTIONS

1. Cut the peel to a proper size and avoid folding it.
2. Always place the peel at the centre of the slide and hold the slide at the edges.
3. Do not overstrain or under strain the peel.
4. Always handle the peel with a brush as a needle may damage the cells.
5. Take care to prevent the peel from drying by using glycerin.
6. Place the coverslip gently, avoiding any air bubbles.
7. Remove excess stain and glycerin with a blotting paper.



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

EXPERIMENT NO: 04

STUDY OF IMBIBITION

PRINCIPLE

Process of 'imbibition' is considered as a special type of diffusion since the net movement of water is along a diffusion gradient. Dry seeds, when placed in water show a noticeable swelling resulting in an increase in volume and weight of the seeds. The imbibition properties of seeds or dry plant materials are due to the presence of colloidal materials.

The rate and extent of the process is affected primarily by temperature, the nature of colloidal substances present that regulates the osmotic balance, nature of imbibants and also the seed coat impermeability. The seed coat acts as a permeability barrier as tested in terms of water absorption efficiency in seed with or without seed coat.

MATERIALS REQUIRED

1. Dry Gram or Pea seeds.
2. Beaker, Glass rod, Balance with weight box, Blotting paper, Forceps, Graph paper, Pencils etc.

PROCEDURE

1. Place 5 g seeds with seed coat in a 50 ml beaker. In another 50 ml beaker place 5 g of gram seeds without seed coat.
2. Put 30 ml distilled water in both the beakers and then keep both of them at room temperature i.e. $29 \pm 1^\circ\text{C}$.
3. After 1 hour remove seeds from both the experimental sets and blot off their surface water by blotting paper.
4. Then weigh the seeds separately.
5. Finally, tabulate the initial and final weight and % of water absorption in the two sets and plot the data graphically to denote the effect of seed coat on water absorption efficiency by dry seeds due to imbibitional properties.
6. Repeat each experimental set mentioned above.

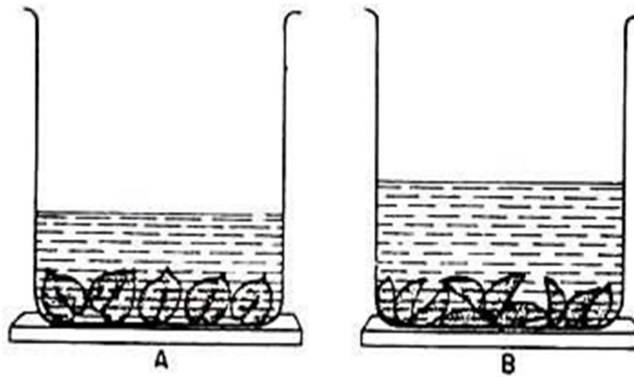


Fig. 3.5 : Absorption of water by seeds : (A) seeds with coat and (B) seeds without coat

RESULT

Experimental condition	No. of observation	Initial seed weight (g)	Final seed weight (g)	Difference (g)	% of water absorption (mean)
Seeds with seed coat	1.				
	2.				
	3.				
Seeds without seed coat	1.				
	2.				
	3.				

CONCLUSION

EXPERIMENT NO: 05
STUDY OF OSMOSIS

PRINCIPLE

Osmosis is the spontaneous net movement of solvent molecules through a selectively permeable membrane into a region of higher solute concentration, in the direction that tends to equalize the solute concentrations on the two sides. It may also be used to describe a physical process in which any solvent moves across a selectively permeable membrane (permeable to the solvent, but not the solute) separating two solutions of different concentrations. Osmosis can be made to do work. Osmotic pressure is defined as the external pressure required to be applied so that there is no net movement of solvent across the membrane. Osmotic pressure is a colligative property, meaning that the osmotic pressure depends on the molar concentration of the solute but not on its identity.

MATERIALS REQUIRED

Petri-dish, water, potato, sugar solution, cork and capillary tube

PROCEDURE

1. Take a potato tuber, remove its outer covering from one end and cut the same end flat.
2. Scoop out a cavity from the other end of the tuber running almost up to the bottom.
3. Fill the cavity with the sugar solution and fit an airtight cork fitted with a capillary tube on the upper end of the cavity.
4. Place the capillary fitted potato tuber in the water- filled petri-dish.
5. Mark the solution level in the tube and watch the experiment for some time.

OBSERVATION

After some time the level of the solution in the tube increases

Mark the level of solution when it stops to move.

RESULT

CONCLUSION

EXPERIMENT NO: 06

DETERMINATION OF PLASMOLYSIS AND DEPLASMOLYSIS

PRINCIPLE

In normal condition the protoplasm is highly pressed against the cell wall. If this plant cell or tissue is placed in hypertonic solution, water comes out from the cell sap into the outer solution due to ex-osmosis and the protoplasm begins to contract from the cell wall. The process is called as plasmolysis and the cell or tissue is referred as plasmolysed cell or tissue respectively. On the other hand if a plasmolysed cell is placed in water the process of endosmosis takes place and the protoplasm again assumes its normal shape and position. This phenomenon is called as Deplasmolysis. In the present experiment, plasmolysis and deplasmolysis were demonstrated with the help of Rhoeo discolor leaf tissue particularly from the lower surface of the leaf, where cell sap is purple in colour due to presence of anthocyanin pigment.

MATERIALS REQUIRED

Rhoeo-discolor leaf, Watch glass, slide and cover slip, forceps, 0.5M sucrose solution, distilled water, scale, pencil and rubber.

PROCEDURE

Phase-1: Peel the lower surface of Rhoeo discolor leaf which was taken in a watch glass. The leaf peel was mounted over a glass slide added distilled water then cover with cover slip and watched under microscope.

Phase-2: The leaf peel was then immersed in 0.5M sucrose solution taken in a watch glass for 15 min to allow plasmolysis. After which the peel was taken over a glass slide, add 0.5M sucrose solution and observed under microscope.

Phase-3: The plasmolysed leaf peel was then immersed in distilled water taken in a separate watch glass for 15 min to allow deplasmolysis, the leaf was then mounted over a glass slide added distilled water this time, covered with cover slip as per normal practice and observed under microscope.

OBSERVATION

RESULT

CONCLUSION

EXPERIMENT NO: 07

MEASUREMENT OF ROOT PRESSURE

PRINCIPLE

Root pressure is the transverse osmotic pressure within the cells of a root system that causes sap to rise through a plant stem to the leaves.

Root pressure occurs in the xylem of some vascular plants when the soil moisture level is high either at night or when transpiration is low during the day. When transpiration is high, xylem sap is usually under tension, rather than under pressure, due to transpirational pull. At night in some plants, root pressure causes guttation or exudation of drops of xylem sap from the tips or edges of leaves. Root pressure is studied by removing the shoot of a plant near the soil level. Xylem sap will exude from the cut stem for hours or days due to root pressure. If a pressure gauge is attached to the cut stem, the root pressure can be measured.

Root pressure is caused by active distribution of mineral nutrient ions into the root xylem. In the absence of transpiration ions accumulate in the root xylem and lower the water potential. Water then diffuses from the soil into the root xylem due to osmosis. Root pressure is caused by this accumulation of water in the xylem pushing on the rigid cells. Root pressure provides a force, which pushes water up the stem, but it is not enough to account for the movement of water to leaves at the top of the tallest trees. The maximum root pressure measured in some plants can raise water only to 6.87 meters, and the tallest trees are over 100 meters tall.

MATERIALS REQUIRED

Potted plant with stem cut, rubber tube, glass tube, clamp.

PROCEDURE

1. Take a regularly watered potted plant and cut the stem portion 1 cm above the ground level.
2. Then connect a glass tube by means of a strong rubber tube.
3. The size of glass tube should be equal to the size of the stem.
4. Take care while joining tube and stem being bound tightly, water cannot escape the tube.
5. Now pour some water in the glass tube until water level can be seen above the rubber tube.
6. Mark the level of water (M1) in tube.
7. Keep your arrangement aside for 2 to 3 hours.
8. Then observe and mark the water level (M2) in the tube.

9. The difference between M2 and M1 indicates the level of water raised.

OBSERVATION

RESULT

CONCLUSION

EXPERIMENT NO- 08

DETERMINATION OF RATE OF TRANSPIRATION

PRINCIPLE

Transpiration is a physiological process of plants by which excess water is released in the form of water vapor from the plants through stomata, epidermis and cuticle.

MATERIALS REQUIRED

Leaf with long petiole, conical flask filled with water, polythene sheet, rubber band or cotton thread, graph paper, pencil, blade and balance

PROCEDURE

At first, area of the leaf was drawn over a graph paper with the help of pencil and rubber. After which, the terminal part of the petiole was cut obliquely with the help of blade to allow more surface area for observation of water. The conical flask was then filled with water and covered with a polythene sheet having a hole in the center for the end of the petiole. The polythene sheet was tied with the help of rubber band. The leaf petiole was inserted into the conical flask through the hole and the weight of the conical flask along with the leaf was taken in the electrical balance (W_1 gm) the whole set was then kept under bright source of light for one hour to allow transpiration. After which, final weight of the set was taken (W_2 gm). The rate of transpiration was expressed as loss of water in gm/sq.cm of leaf area/hour.

OBSERVATIONS

The following traits were observed, measured and recorded:

No. of big squares:

No. of medium squares:

No. of small squares:

Total area of leaf:

Initial weight of setup:

Final weight of setup:

$$\text{Rate of transpiration} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Area of the leaf}} \quad \text{g/ cm}^2/\text{hour}$$

CALCULATION

RESULT

CONCLUSION

EXPERIMENT NO: 09

SEPARATION OF PHOTOSYNTHETIC PIGMENTS THROUGH PAPER CHROMATOGRAPHY

PRINCIPLE

Paper chromatography is a useful technique in the separation and identification of different plant pigments. In this technique, the mixture containing the pigments to be separated is first applied as a spot or a line to the paper about 1.5 cm from the bottom edge of the paper. The paper is then placed in a container with the tip of the paper touching the solvent. Solvent is absorbed by the paper and moves up the paper by capillary action. As the solvent crosses the area containing plant pigment extract, the pigments dissolve in and move with the solvent. The solvent carries the dissolved pigments as it moves up the paper. The pigments are carried along at different rates because they are not equally soluble. Therefore, the less soluble pigments will move slower up the paper than the more soluble pigments. This is known as developing a Chromatogram.

Paper Chromatography is useful for identifying unknown compounds often used in crime scene investigations to match ink, lipstick, or colored fibers. This set-up shows two different pen inks.

Rf. equation:

$R_f = \text{distance traveled by compound} / \text{distance traveled by solvent}$

MATERIALS REQUIRED

Chromatography paper, Acetone solution, Beaker, Pencil

PROCEDURE

1. Cut a strip of coffee filter (or filter paper). Draw a horizontal line with a pencil (not pen) about half an inch from the bottom. Place a spinach leaf on the line and roll a penny over it so that you get a line of green pigment on the filter. Using a different part of the leaf, roll the penny again over the same line. Repeat this process until the line is fairly dark.
2. Put about an inch of acetone in the beaker (isopropyl alcohol will also work.)
3. Tape the top of the coffee filter strip to a pencil and balance the pencil across the top of the beaker. See the image below for the set-up.
4. It is very important that the bottom of the filter strip is in the acetone, but the green spot is not in the liquid. If the acetone touches the spot directly, the pigment will just dissolve away.

5. Observe what happens to the liquid in the beaker and the spot on the filter paper. Results will take about 20 minutes.

ANALYSIS:

Assign a band number for each pigment band - you should see greens, yellows, oranges...etc.

Band colour	Plant pigments	Distance (mm)	Rf. (use formula)
Yellow to yellow orange	Carotene		
Yellow	Xanthophyll		
Bright green to Blue green	Chlorophyll a		
Yellow green to Olive green	Chlorophyll b		

CONCLUSION

EXPERIMENT NO: 10

DETERMINATION OF CHLOROPHYLL CONTENT OF GIVEN LEAF SAMPLE AND ESTIMATION OF ABSORPTION SPECTRUM OF CHLOROPHYLL

Principle:

Chlorophyll is a green pigment present in plant leaves that plays a critical role in photosynthesis. Chlorophyll extraction involves grinding the plant material to release the chlorophyll from the cell walls, and then using an organic solvent such as acetone to extract the pigments. The pigments can be separated and quantified by measuring their absorbance at specific wavelengths using a spectrophotometer.

Absorption spectrum of chlorophyll is an important technique used to understand the light absorption properties of chlorophyll.

Materials required:

Fresh plant tissue (leaves or stems)

Acetone (80% v/v)

Mortar and pestle

Volumetric flask(25 ml)

Whatmann's No-1 filter paper

Spectrophotometer

Quartz cuvettes

Extraction Procedure:

Collect fresh plant tissue and rinse with distilled water to remove any debris or contaminants. Grind the plant tissue using a mortar and pestle to obtain a homogenous sample. Add approximately 10 ml of 80% acetone to the sample, and grind the mixture until the tissue is completely macerated. Filter the content into a 25 ml volumetric flask using Whatmann's No-1 filter paper. Rinse the mortar and

pestle with additional 80% acetone, and transfer the rinse to the same volumetric flask and make up the volume to 25 ml.

Procedure for determination of chlorophyll content

Use a blank solution of 80% acetone to calibrate the spectrophotometer and zero the readings. Measure the absorbance of the extract at two wavelengths - 660 nm and 645 nm to calculate chlorophyll a and b content.

Calculation

Calculate the chlorophyll content using the following formulas

$$\text{Chl. a (mg/g)} = \frac{(12.7 * A_{660} - 2.69 * A_{645}) * V}{1000 * W}$$

$$\text{Chl. b (mg/g)} = \frac{(22.9 * A_{645} - 4.68 * A_{660}) * V}{1000 * W}$$

$$\text{Total Chlorophyll (mg/g)} = \text{Chl. a} + \text{Chl. b}$$

Here

A₆₆₀- Absorbance at 660 nm

A₆₄₅- Absorbance at 645 nm

V- Final volume of extract in ml

W- Weight of sample taken in gm

Procedure for estimation of Absorption Spectrum of Chlorophyll

Use a blank solution of the solvent to calibrate the spectrophotometer and zero the readings. Measure the absorbance of the extract at different wavelengths in the visible spectrum (400-700 nm) in 20 nm interval. Plot the absorbance readings against the corresponding wavelengths to obtain the absorption spectrum of chlorophyll.

Conclusion

EXPERIMENT NO: 11

STUDY OF RATE OF PHOTOSYNTHESIS

PRINCIPLE

Photosynthesis is a combination of biophysical and biochemical processes during which solar energy is captured and converted into chemical energy which is contained in the molecules of organic compounds. The central role of this process is in the energy cycle of life. Chemically, this process involves the uptake of CO_2 which, in turn, gets converted into organic compounds, and oxygen is evolved.

The process can be measured usually by measuring the volume of CO_2 consumed, or influence of CO_2 conc. on the rate of photosynthesis, or volume of O_2 evolved, or total amount of dry mass/grain formed. The whole process is principally dependent on light, O_2 , temperature and water supply from the surrounding environment.

MATERIALS REQUIRED

1. Beaker (1L), graduated test tube, funnel fitted with jet etc.
2. 0.1% KHCO_3 solution, dist. water
3. Hydrilla plants
4. Thread, blade, stand with clamp, etc.

PROCEDURE

1. Fill the beaker with distilled water up to 2/3 mark.
2. Take some fresh and healthy Hydrilla plants and cut their ends and tie them loosely with a thread.
3. Insert the cut ends inside the neck of the funnel with a jet.
4. Place the funnel inside the beaker in such a manner that all plants remain inside the funnel.
5. Add a few ml of 0.1% KHCO_3 soln. for dissolved CO_2 source.
6. Invert a graduated test tube filled with water over the neck of the funnel so that the jet of the funnel remains inside the tube in vertical position.
7. Place the whole set-up under bright light and keep the graduated tube erect with stand and clamp, if necessary.
8. Allow the experiment to continue for about 20 minutes. Then record the evolution of air bubble (O_2 gas) inside the tube after passing through jet for 5 or 10 minutes.

OBSERVATION

It is observed that evolution of bubbles from the cut ends of the plants takes place in the set-up exposed to light.

Observation	No. of bubbles formed per 5 min	Mean value
1		
2		
3		
4		
5		

RESULT

CONCLUSION

EXPERIMENT NO: 12

STUDY OF RESPIRATION

PRINCIPLE

Cellular respiration is a set of metabolic reactions and processes that take place in the cells of organisms to convert biochemical energy from nutrients into adenosine triphosphate (ATP), and then release waste products. The reactions involved in respiration are catabolic reactions, which break large molecules into smaller ones, releasing energy in the process, as weak so-called "high-energy" bonds are replaced by stronger bonds in the products. Respiration is one of the keyways a cell releases chemical energy to fuel cellular activity. Cellular respiration is considered an exothermic redox reaction which releases heat. The overall reaction occurs in a series of biochemical steps, most of which are redox reactions themselves. Although cellular respiration is technically a combustion reaction, it clearly does not resemble one when it occurs in a living cell because of the slow release of energy from the series of reactions.

Nutrients that are commonly used by animal and plant cells in respiration include sugar, amino acids and fatty acids, and the most common oxidizing agent (electron acceptor) is molecular oxygen (O_2). The chemical energy stored in ATP (its third phosphate group is weakly bonded to the rest of the molecule and is cheaply broken allowing stronger bonds to form, thereby transferring energy for use by the cell) can then be used to drive processes requiring energy, including biosynthesis, locomotion or transportation of molecules across cell membranes.

PROCEDURE

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

- (i) Respiroscope which consists essentially of a stout vertical tube which is bent into a bulb at the one end or,
- (ii) With the help of a long-necked round-bottomed flask fitted with a centrally-bored cork at the mouth through which passes a glass tube.

The Respiroscope or the inverted flask is fixed vertically to a stand and a few germinating gram seeds or flower petals are placed in the bulb of the respective or in the inverted flask plugged

with cotton at the base the vertical tube of the Respiroscope or inverted flask is dipped just below the surface of water or mercury in a beaker.

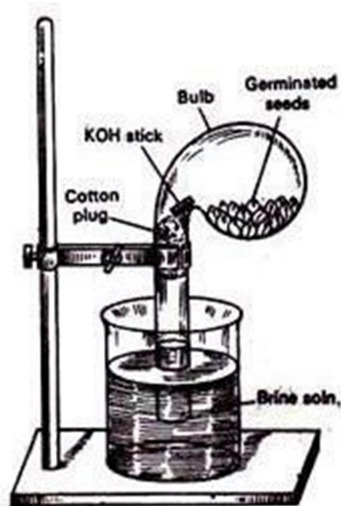
A few caustic potash (KOH) pellets are introduced in the bent portion of the Respiroscope or in the long neck of the round bottom flask and kept in position with loosely held cotton wool. Care should be taken that respiratory materials and KOH pellets do not come in contact. Precautions should be taken that the free end of the tube does not touch the bottom of the water or mercury trough. Fittings must be air-tight to avoid any leakage.

OBSERVATION

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

INTERFERENCE

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



RESULT

EXPERIMENT NO. 13

MEASUREMENT OF RELATIVE WATER CONTENT OF LEAF

PRINCIPLE

Major mass of plant cell is composed of water. Approximately 80 to 90% mass of growing plant tissues including leaf is composed of water. Measurement of relative water content (RWC) in leaf tissues are commonly used to assess the water status of plants. Leaf water status is intimately related to several leaf physiological activities, such as leaf turgor, growth, stomatal conductance, transpiration, photosynthesis and respiration. Deficiency of water creates adverse effects on metabolic processes that hinder growth and development of plants. Relative water content (RWC) of leaf can be measured employing the following formula.

$$\text{RWC (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

REQUIREMENTS

Leaf sample, Scissor, Balance, Beaker, Petri dish, Brown paper packet, Blotting paper, Distilled water, Hot air oven

PROCEDURE

Measurements of RWC were performed on leaves collected from either one-year old potted seedlings, or from branches of mature trees. Leaves were always collected from the mid section of either branches or seedlings, in order to minimize age effects. At first leaves were cut into small pieces (1cm x 1cm). Around one gram of leaf sample was weighed for each which is considered as fresh weight. Subsequently, they were immersed in double distilled water for 4 hours to absorb water inside a closed petri dish. After which, the leaves were removed from water, surface water was removed by blotting paper and turgid weight of each sample was recorded. The leaves were then dried at $80 \pm 1^{\circ}\text{C}$ in a hot air oven for 48 hours and dry weight was recorded. Using the formula relative water content (RWC) of leaf samples were determined and expressed in per cent.

OBSERVATION

Sample Content	Fresh weight (g)	Turgid weight (g)	Dry weight(g)	Relative Water%
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A

B

CALCULATION

RESULT

CONCLUSION

EXPERIMENT: 14

DIAGNOSIS OF NUTRIENT DEFICIENCIES FOR MINERAL NUTRIENT THROUGH RAPID TISSUE TEST

PRINCIPLE

Plant tissue analysis could directly reflect the nutrient status or nutrient requirement of plants themselves. In recent year, probably as a result of advances in knowledge and understanding of the role and function of nutrient elements, new approaches to diagnosis are being developed which differ in principle from plant analytical techniques. There are two types of plant analysis for confirmation of different symptoms and also for assessing the nutrient status at particular stage of the plant.

There are two types of plant analysis.

1. Tissue testing
2. Whole plant analysis

Tissue testing is done usually with fresh leaves of the plant in the field itself whereas the total plant analysis is performed in the laboratory. These plant analysis methods are based on assumptions that the particular element is an indicator of the supply of that particular nutrient. The whole plant analysis methods involve elaborate equipment and a lot of chemicals and cannot be performed in the field itself. However, tissue testing is done in the field itself and also very rapid. The test is made with fresh plant saps and very useful in quick diagnosis of the needs of growing plant. In the test, the sap from the cell is tested for unassimilated N and K. Test for Fe, Ca and Mg are also used frequently in variety of crops. In general it is necessary to test that specified part of the plant which will give the best indication of the nutritional status.

Table: Specified part of the different crop for nutritional status

CROP	NITROGEN	PHOSPHORUS	POTASSIUM
Maize	Stem, Midribs	Leaf blade	Leaf blade
Soybean	Petiole	Leaf blade	Petiole
`Black gram	Petiole & Lamina	Leaf blade	Leaf blade
Cotton	Petiole	Petiole	Petiole
Papaya	Petiole	Petiole	Petiole
Tomato	Petiole	Petiole	Petiole
Banana	Lamina	Lamina	Lamina

NITROGEN

Reagents

0.1% of Diphenylamine in conc. sulphuric acid

PROCEDURE

Small bits of leaf or petiole are taken in a petridish and a drop of 0.1% diphenylamine is added. The development of blue colour indicated the presence of nitrate nitrogen. Depending on the intensity of blue colour the nutritional status maybe diagnosed sufficient or not sufficient.

Dark Blue - Sufficient

Light Blue - Slightly deficient

No Colour - Highly Deficient

PHOSPHORUS

Reagents

Ammonium molybdate reagent

8 g of ammonium molybdate is dissolved in 200 ml of distilled water. To this solution, added a mixture of 126 ml conc. hydrochloric acid and 74 ml of distilled water slowly by constant stirring. This stock solution is kept in an amber coloured bottle and at the time of use, it is taken and diluted in the ratio of 1:4 with distilled water.

PROCEDURE

Small fine bits of the plant material are taken in a test tube and 10 ml of diluted reagent is added and shaken continuously for a minute. To this added a pinch of stannous chloride powder. The contents were mixed thoroughly and observed for colour development.

Dark Blue- Rich in Phosphorus

Light Blue-Moderately Sufficient

Green or Bluish Green-Deficient

No Colour or Yellow-Highly deficient

POTASSIUM, CALCIUM, MAGNESIUM and CHLORIDE

With Morgan's reagent, the following elements would be detected as soluble potassium, calcium, magnesium and chloride.

Morgan's reagent

10 g of sodium acetate is dissolved in 30 ml of glacial acetic acid (pH 4.8) is used for the extraction.

Preparation of plant extract

Take 4 g of plant sample and add 15 ml of Morgan's reagent. Add pinch of Darco and filter through muslin cloth. This extract could be subsequently used for detection.

POTASSIUM

Reagents

35% Sodium cobalt nitrite

50% Glycerine

Isopropyl alcohol

PROCEDURE

2 ml of Morgan's reagent extract is taken in a test tube and to this added 0.2 ml of sodium cobalt nitrite. 1 ml of 50% glycerine and 2 ml of isopropyl alcohol and observed for colour development.

Clear reddish brown- Insufficient

Deep canary yellow turbidity- Sufficient

CALCIUM

Reagents

50% Glycerin

Ammonium oxalate

PROCEDURE

To 2 ml of morgan's reagent extract, added 2 ml of 50% glycerine and 5 ml of saturated ammonium oxalate.

Colorless- Insufficient

Greenish white turbidity- Sufficient

MAGNESIUM

0.15% Titan yellow

2% Hydroxylamine hydrochloride

5% Sucrose

10% Sodium hydroxide

PROCEDURE

2 ml of Morgan's reagent extract is taken in a test tube. To this 2 ml of Titan yellow, 0.5 ml of hydroxylamine hydrochloride, 0.5 ml of sucrose and 2 ml of 10% sodium hydroxide were added and observed for colour development.

Straw Yellow- Insufficient

Salmon pink colour- Sufficient

CHLORIDE

N/50 silver nitrate

Concentrated Nitric acid.

To 2 ml of morgan's reagent extract, 2 ml of N/50 AgNO_3 and 3 drops of concentrated nitric acid were added and kept for colour development.

Colorless - Insufficient

White turbidity-Sufficient

IRON

Reagents

concentrated sulphuric acid

concentrated nitric acid

20% Ammonium thiocyanate in amyl alcohol

PROCEDURE

0.5 g of the material to be tested is taken in a test tube and added 1 ml of conc. sulphuric acid and allowed to stand for 15 minutes. After that, 10 ml of distilled water and 2-3 drops of concentrated nitric acid are added. After 2 minutes, 10 ml of this solution is taken and 5 ml of 20% Ammonium thiocyanate solution is added and observed for colour development.

Brick red -Sufficient

Faint colour- Deficient

MANGANESE

Sensitive test for deficient leaves

Reagents

Saturated solution of Potassium periodate

1% tetramethyldiaminodiphenyl methane

PROCEDURE

To finely chopped leaf bits, added 2 ml of potassium periodate and 0.4 ml of 1% tetramethyldiaminodiphenyl methane reagent.

The contents were shaken vigorously and observed for colour development.

Pale Blue - Insufficient

Deep Blue – Sufficient

RESULT

CONCLUSION

EXPERIMENT: 15

MEASUREMENT OF PHOTOSYNTHETIC CO₂ ASSIMILATION BY INFRA-RED GAS ANALYZER (IRGA)

PRINCIPLE:

In all the gas exchange equipments designed to measure the photosynthetic rates, the Infra-Red Gas Analyzer (IRGA) is a major component and the primary role of this component is to measure the CO₂ concentration. The IRGA is very sensitive to detect even a change of 1 ppm of CO₂. A leaf or a plant is enclosed in an air tight chamber and the CO₂ fluxes are determined by measuring the CO₂ concentration changes in the chamber atmosphere.

Infra-Red Gas Analyzers (IRGA) are used for the measurement of a wide range of Heteroatomic gas molecules including CO₂, H₂O, NH₃, CO, SO₂, N₂O, NO and gaseous hydrocarbons like CH₄. Heteroatomic molecules have characteristic absorption spectrum in the infrared region. Therefore, absorption of radiation by a specific Heteroatomic molecule is directly proportional to its concentration in an air sample.

The major absorption peak of CO₂ is at 4.25 μm with secondary peaks at 2.66, 2.77 and 14.99 μm. The only heteroatomic molecule normally present in the air with an absorption spectrum overlapping with that of CO₂ is water vapour. Both molecules absorb IR radiation in the 2.7 μm range. However, this interference does not pose any serious problem at 4.25 μm range.

Measurement of Photosynthesis

Two different kinds of photosynthesis systems (IRGA) are generally used for the measurement of photosynthetic rates i.e. closed system and open system.

Closed system

In this system, a leaf is clamped in a leaf chamber and air is circulated around the leaf repeatedly. The decrease in CO₂ concentration over time is measured to determine the photosynthetic rate. The major disadvantage in this system is that the measurements of photosynthetic rates are done under constantly depleting CO₂ concentrations around the leaf. Further, since the leaves transpire

simultaneously there will be a constant build up of humidity in the chamber. Increase in humidity alters the vapour pressure difference (VPD) between the leaf and its ambient air. Small changes in VPD have been shown to significantly alter the stomatal opening and hence the gas exchange rates. These disadvantages are overcome in the open system measurements.

Open system

Here the leaf is clamped in a chamber through which ambient air is passed continuously. The change in CO₂ concentration before the air enters the leaf chamber and in the air leaving the leaf chamber is determined to compute the photosynthetic rates. If photosynthetic rate is high, then the air leaving the leaf chamber will have less CO₂, than the air entering it. The difference between the CO₂ concentrations at a given flow rate is determined to compute the photosynthetic rate.

The major advantage in the open system is that, the measurement of photosynthetic rates can be achieved at a constant CO₂ concentration. Further, by altering the flow rate of dry air into the leaf chamber, it is also possible to maintain a specific relative humidity in the chamber and hence the gas exchange parameters can be measured at a constant set of conditions. This renders the comparison more realistic and acceptable.

