PRACTICAL MANUAL

Course Title : **FUNDAMENTALS OF CROP PHYSIOLOGY**

Credits : **2 (1+1)**

Course No. : **BOT - 121**

Course : **B.Sc. (Hons.) Agriculture**

Semester : **II Semester (New)**

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2018

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CERTIFICATE

This is to certify that Shri / Miss Shiv Kotkar.

Reg.No. 2018AGKW79 a student of II[nd] **(New)** Semester, **B.Sc. (Hons.) Agriculture** has completed all the exercises successfully for the Course : **Fundamentals of Crop Physiology,** Course No. : **BOT - 121**, during Summer Semester 2018- 2019.

Place : Kanchanwadi.

Date :

COURSE TEACHER

PRACTICAL MANUAL

Course No. : BOT-121 **Credits : 2 (1+1)**

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INDEX

EXERCISE NO. 1 Date:

STUDY OF PLANT CELL

Experiment 1: To study plant cell

Principle: All the vital activities of a plant are due to that remarkable living substance known since the time of Von Mohl (1846) by the name of **protoplasm**, which makes up the body of the living plants. The protoplasm is not uniformly distributed in the body of the plant but is organised into small units called **protoplasts.** Each such unit is bounded on the outside by a more or less firm wall called the **cell wall,** which together with the enclosed protoplast constitute a **cell.**

Materials Required:

Onion bulb, glycerine, safranin, weak iodine solution, dropper, forceps, distilled water, needle, brush, glass slides, cover slips, compound microscope, blotting paper, watch glasses.

Procedure:

a) Strip off epidermis in the form of peelings from an inner scale of an onion bulb with the help of forcep. Pour some distilled water into a watch glass.

b) Mount the epidermis in the watch glass containing distilled water.

c) Add few drops of safranin solution into another watch glass by using a dropper or brush and transfer the epidermal peels in it. Allow it to remain in the Safranin solution for 30 seconds, so that the peels are stained.

d) Take some peels from the Safranin solution using the brush and place in the watch glass containing distilled water.

e) Put 2-3 drops of glycerine at the center of a dry glass slide and place one piece of peel on the slide containing glycerine.

f) Take a cover slip and place it gently on the peel with the aid of a needle.Remove the extra glycerine using a piece of blotting paper.

g) Place this glass side on the stage of the compound microscope and examine under high power.

h) Study the form and arrangement of the cells. Make out the cell wall and the semi-transparent protoplasm and vacuoles.

i) Add a drop of weak iodine solution to the mount and make out the nucleus. Hairs from gourd or *Luffa sp.* may be similarly examined.

Observations

- There are a large number of regularly shaped cells lying side by side and each cell has a distinct cell wall.
- A distinct nucleus is present on the periphery of each cell.
- Lightly stained cytoplasm is observed in each cell.
- A large vacuole is present at the centre of each cell, and is surrounded by the cytoplasm.

Conclusion

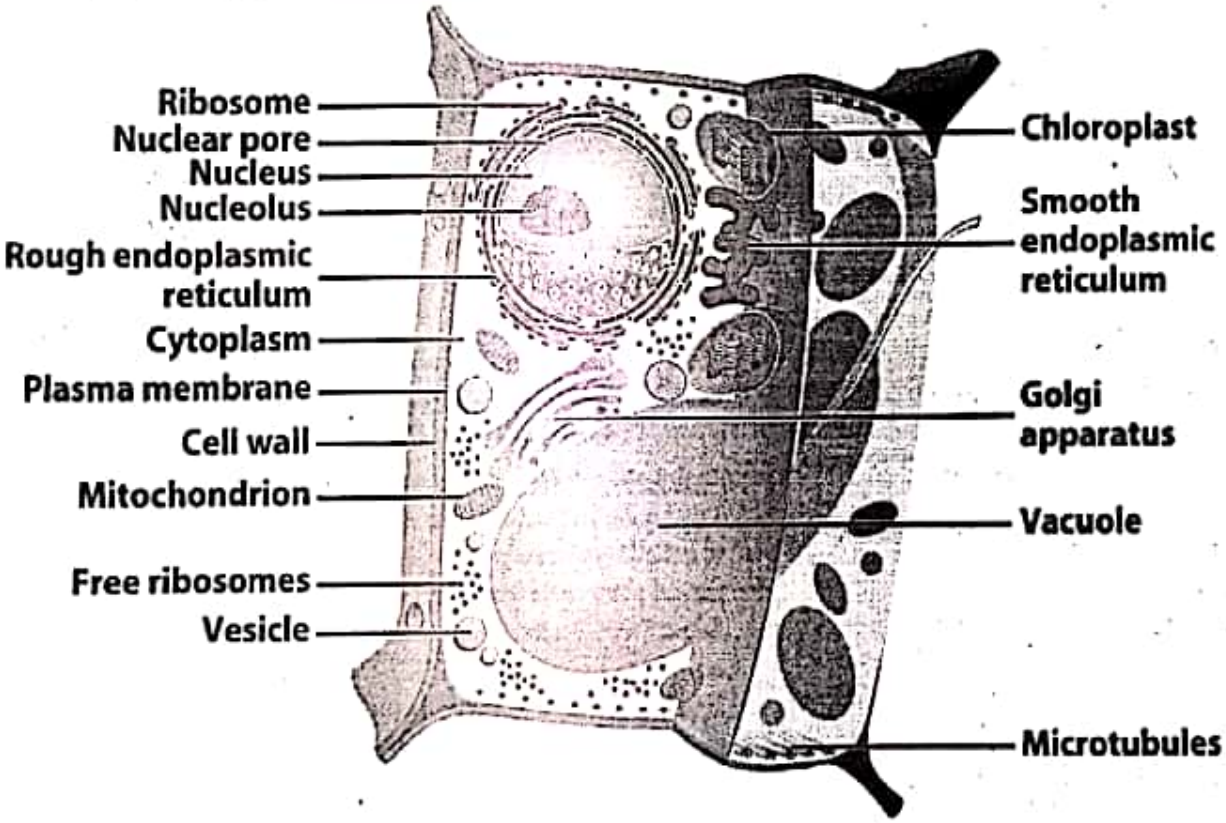
Experiment 2: To study the plastids Date :

a) **Chloroplast:** Strip off epidermis of a young leaf of Tradescantia or Hydrilla or moss plant and observe the chloroplasts filling the cells. Note their form and size.

b) **Chromoplast:** Cut thin sections of carrot root near the outer skin or peel the pericarp of capsicum (red chillies) fruit. Examine these under the microscope and study the shape and size of the chromoplast.

Draw a diagram of a plant cell and with the help of a diagram describe the structure of a plant cell.

Representative Plant Cell:



Above: Diagram of the structure of a plant cell

Note: The diagram above is a general plant cell.. The structures are not necessarily drawn to scale but in enough detail to aid recognition and to help students re-draw this diagram by hand to include in study notes or homework.

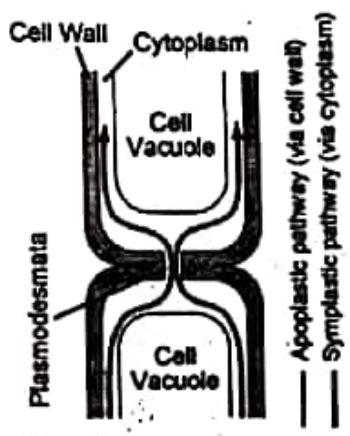
The structure of plant cells has similarities and differences compared with the structure of animal cells. The following table lists the parts of plant cells shown in the diagram above with brief notes about each of the structures types of organelles in plant cells.

Organelles in Plant Cells and other parts of plant cells incl. e.g. the cell wall, plasma membrane, and cytoplasm can be divided into those in the **outer-layer of the cell** and those **inside the cell.**

Outer-layer of the cell: 1. Cell Wall, 2. Plasma membrane (also called the **plasma lemma** and/or **cell surface membrane), 3. Plasmodesmata**

Inside the cell: 4. Cytoplasm, 5. Vacuole, 6. Cell Nucleus, 7. Nucleolus (inside the nucleus), **8. Nuclear Membrane** (enclosing the nucleus), **9. Rough Endoplasmic Reticulum (RER), 10. Smooth Endoplasmic Reticulum (SER), 11. Mitochondrion** (*pl.*) the singular form is "mitochondria", **12. Chloroplasts, 13. Golgy body** (also called the **Golgi Complex**) and/or the **Golgi Apparatus), 14. Microtubules, 15. Ribosomes.**

Each of the 15 items listed above are described with brief details:

Part of Plant Cell:	Notes:
Outer-layer of cell:	
1. Cell Wall 	**Plant cells have cell walls** - as compared with <u>animal cells</u> which do <u>not</u> have cell walls, and <u>prokaryotic cells</u> (bacteria) which do have cell walls but they are of a different construction than those of plant cells. <u>Function(s) of plant cell walls</u>: The main functions of plant cell walls are mechanical. Plant cell walls form part of a transport system called the **apoplast system** via which water and some solutes can pass through plant tissue via **apoplastic pathways** (along / through cell walls) and **symplastic pathways** (i.e. through the cytoplasm of a series of adjacent cells). <u>Structure of plant cell walls</u>: The most important chemical composition of a plant cell wall is **cellulose.** Long cellulose <u>molecules</u> grouped into bundles called **microfibrils** are twisted into rope-like **macrofibrils.** Sometimes there is also another layer component of cell wall e.g. **lignin** - which gives strength, e.g. strengthens wood (xylem cells) in trees. **suberin** - which helps prevent water from penetrating, e.g. suberin in mangroves minimize salt intake from their habitat.
2. Plasmamembrane (also called the plasmalemma and/or the cell surface membrane)	<u>Functions of the plasma membrane</u>: As a differentially-permeable surface, the plasma membrane controls movement of solutes in and out of the cell. Synthesis and assembly of cell wall components <u>Structure of the plasma membrane</u>: The plasma membrane is flexible enough to move closer to or away from the cell wall - according to changes in the water content of the cytoplasm within the cell.
3. Plasmodesmata	Plasmodesmata are tiny strands of <u>cytoplasm</u> that pass through pores in plant cell walls, forming "connections" or "pathways" between adjacent cells. Specifically, plasmodesmata form the **symplast pathway** for the movement of water and solutes through plant structures (see diagram, above-left). These cell-cell connections are especially

important for the survival of plant cells during conditions of drought.

Inside the Cell:

4. Cytoplasm

The cytoplasm is the part of a plant cell that includes all the contents of the cell within the cell membrane but outside of the nucleus of the cell. It therefore includes the **cytosol** (i.e. the semi-fluid part of a cell's cytoplasm - as shown shaded pale green in the diagram above) as well as the plant cell organelles incl. mitochondria, chloroplasts, etc.. Also located within the cytoplasm is the cytoskeleton, which is a network of fibres whose function is to provide mechanical support to the cell, including helping to maintain the cell's shape. In short, cytoplasm consists mainly of water and contains enzymes, salts, organelles, and various organic molecules. It has a clear appearance (i.e. in colour) and a gel-like texture. The cytoplasm helps to move materials around the cell and also dissolves cellular waste.

5. Vacuole

A cell's vacuole can occupy a large proportion of the total volume of the cell - e.g. 90% of the volume of some mature plant cells. Each vacuole is enclosed by a vacuolar membrane called the **tonoplast**.

Contents of the vacuole:

Cell sap, which is a solution of salts, sugars and organic acids.

Enzymes needed for recycling components of cells, e.g. chloroplasts.

Anthocyanins are sometimes present in cell vacuoles. These are chemical pigments responsible for some of the (non-green) colours of flowers, e.g. reds, blues, purples.

Functions of the vacuole:

Helps maintain turgor pressure (turgidity) inside the cell. This pressure pushes the plasma membrane against the cell wall. Plants need turgidity to maintain rigidity.

6. Cell Nucleus

The nucleus is the "*control center*" of a eukaryotic cell (i.e. plant cells and animal cells - but not bacterial cells, which do not have a membrane-bound cell nucleus).

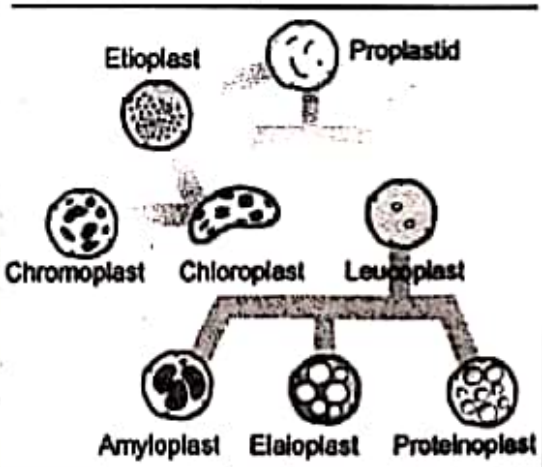
Functions of the cell nucleus:

The cell nucleus controls the activity of the cell by regulating protein synthesis within the cell.

Structure of the cell nucleus:

Each cell nucleus is surrounded (one could equally say "enclosed") by a nuclear membrane that is also known as the "nuclear envelope". The contents of the nucleus - so, inside the nuclear membrane - includes DNA (genetic material) in the form of genes and a nucleolus.

7. **Nucleolus** (inside the nucleus)	The nucleolus is located within the nucleus and is the site of synthesis of: transfer RNA ribosomal RNA ribosomal subunits
8. **Nuclear Membrane** (enclosing the nucleus)	The nuclear membrane is also known as the **nuclear envelope** and encloses the contents of the nucleus of the cell - separating the contents of the nucleus from the rest of the cell. Nuclear pores in the nuclear membrane enable various substances, such as nutrients and waste products, to pass into and out of the nucleus.
9. **Rough Endoplasmic Reticulum (RER)**	Rough endoplasmic reticulum is the site of **protein synthesis** (which takes place within the ribosomes attached to the surface of the RER) as well as storage of proteins and preparation for secretion of those proteins.
10. **Smooth Endoplasmic Reticulum (SER)**	Smooth endoplasmic reticulum is the site of lipid synthesis and secretion within cells.
11. **Mitochondrion** (*pl.*) the singular form is "*mitochondria*"	Mitochondria are structures found in both plant and animal cells. They are bounded by double membranes, the inner of which is folded inwards, forming projections (called cristae), hence the representation of mitochondria in diagrams e.g. as above. Their function of mitochondria is **energy production.** Mitochondria contain enzyme systems needed to synthesize adenosine triphosphate (ATP) by oxidative phosphorylation. The quantity of mitochondria within cells varies with the type of cell. In the case of plant cells, mitochondria may be particularly abundant in sieve tube cells (also called *sieve tube members*), root epidermal cells and dividing meristematic cells. Read more about mitochondria.
12. **Chloroplasts**	Chloroplasts are the sites of photosynthesis within plant cells. Chloroplasts are very important parts of plant cells. Some cells include up to 50 chloroplasts. The number of chloroplasts per cell varies according to the type of cell and its



function. They are plentiful in leaf cells that receive sunlight - as opposed to root cells that do not receive light.

Chloroplasts are a type of **plastid**. There are also other types of plastids (not all of which are present in all plant cells but all of which are derived from **proplastids**).

See the **diagram of plastids** on the right.

13. **Golgi Body** (also called the **Golgi Complex**and/or the **Golgi Apparatus**)

The Golgi apparatus of a cell is sometimes called the "*post office*" of the cell or is more generally described as a "*packaging organelle*" because it plays a role in transporting proteins. It's structure and appearance takes the form of a stack of tiny pancake-like shapes, each of which is enclosed by a single membrane and contains fluid and biochemicals such as proteins, sugars and enzymes.

Functions of the Golgi Apparatus:

Modifies some newly-synthesized biomolecules before storing them in granules, sometimes called **vesicles** - ready for transport later.

Forms lysosomes - which are tiny sacs filled with enzymes that enable the cell to utilize its nutrients, so are sometimes described as "*cell digestion machines*". Lysosomes also destroy the cell after it has died.

Transports the proteins produced in the ER: After a protein has been synthesized in the ER, a transition vesicle (or "sac") is formed then floats through the cytoplasm to the Golgi apparatus, into which it is absorbed. After processing the molecules inside the sac, a secretory vesicle is formed and released into the cytoplasm, moves to the cell membrane, then releases the molecules from the cell.

14. **Microtubules**

Functions of microtubules:

facilitate addition of cellulose to cell wall form the spindles and cell plates of dividing cells play a role in **cytoplasmic streaming** (i.e. moving the fluid cytoplasm within the cell) e.g. to/from chloroplasts.

Structure of microtubules:

Microtubules are hollow rope-like structures composed of the protein tubulin. They can be as long* as 25μm (25 micrometres = 25 x 10^{-6} m) and have a diameter of approx 25 nm (25 nanometres = 25 x 10^{-9} m). See scientific numbers for more about these units. * some sources state up to several mm (millimetres) long.

15. **Ribosomes**

Ribosomes take part in the synthesis of some proteins by catalyzing the formation of those proteins from individual amino acids (using messenger RNA as a template). Examples of proteins catalyzed by ribosomes include glycoproteins, lysosome proteins, membrane proteins and some organelle proteins.

In general, ribosomes can be either "free" (in the cytoplasm of the cell, but not within the nucleus or membrane-bound organelles) or "membrane-bound", which in the cases of plant and animal cells means attached to the rough endoplasmic reticulum (RER) - hence the black dots shown in the diagram above, distinguishing the RER from the smooth endoplasmic reticulum (SER). An individual ribosome could be "membrane-bound" - while it is making one protein, then "free" while making a different protein. Some diagrams of plant cells include "free" ribosomes while others only show ribosomes attached to endoplasmic reticulum, forming RER.

Exercise No: 02

Study of Imbibition

Imbibition. The adsorption of water by hydrophilic colloids is known as imbibition.

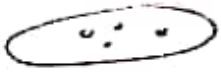
procedure:

1) Take handful of pea / gram wheat / Rice & groundnut seed & dry them in oven at 130°F (40°C) for 4 hrs

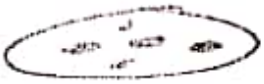
2) Take 10 gm of each pea / gram, wheat & ground accurately

3) each type of seed is then taken in a separate glass bottle & equal volume of water is poured in each so

Groundnut



Gram



wheat.

4) keep the bottles at 38°C for about 4 hrs. Remove the sample from water & dry them thoroughly with blotting paper & record the increase in weight

EXERCISE NO. 2 Date:

STUDY OF IMBIBITION

DEMONSTRATION OF IMBIBITION OF WATER BY DIFFERENT TYPES OF SEEDS

Imbibition: The adsorption of water by hydrophilic colloids is known as imbibition. It is a physical process in which certain substances like hydrophilic colloids absorb water and increase in size e.g. wooden doors do not open and close easily during rainy season, germination of seed and pulses put in water for sprouting.

The substance which absorbs water is calld imbibant and water as imbibing liquid. When imbibition takes place volume of imbibant is increased while volume of imbibing liquid is decreased. But their total volume is more as compared to final volume because some liquid is held in inter molecular space and the kinetic energy of the molecule is given out as heat. Imbibition of water increases the volume of the imbibant due to which pressure is created which is known as imbibitional pressure. Different types of organic substances have different imbibing capacities. Proteins have a very high imbibing capacity, starch less and cellulose least . That is why proteinaceous gram seeds swell more on imbibition than starchy wheat seeds.

Requirements: Pea or gram, wheat or rice and groundnut seed, distilled water, glass bottle with stopper, blotting paper, weight box, balance, oven etc.

Procedure: Take handful of pea or gram, wheat or rice and groundnut seed and dry them in oven at 103^0 F (40^0C) for 4 hours. Take 10 grams of each pea or gram, wheat or rice and groundnut seed accurately. Each type of seed is then taken in a separate glass bottle and equal volume of water is poured in each so that the seeds are completely immersed. Put the stopper on the bottles to prevent the evaporation.

Keep the bottles at 38^0C for about 4 hours. Remove the samples from the water and dry them thoroughly with blotting paper and record the increase in weight. The percentage increase in weight by each type of seed is determined and plotted graphically.

Draw the figure of the experiment, record the observations and write conclusions.

Practical utility: Imbibition is utilised for preparing pulses for the sprouting purposes or germination of any type of seed.

EXERCISE NO. 3. **Date:**

STUDY OF OSMOSIS

Osmosis:

The diffusion of solvent molecules into the solution through a semi permeable membrane is called as osmosis (sometimes called as *Osmotic diffusion*). In case there are two solutions of different concentration separated by the semi permeable membrane, the diffusion of solvent will take place from the less concentrated solution into the more concentrated solution till both the solutions attain equal concentration.

During osmosis, the movement of solvent molecules takes place from the solution whose osmotic pressure is lower (i.e less concentrated as hypotonic) into the solution whose osmotic pressure is higher (i.e, more concentrated as hypertonic). Osmotic diffusion of solvent molecules will not take place if the two solutions separated by the semipermeable membrane are of equal concentration having equal *Osmotic pressures* (i.e., they are isotonic). In plant cells, plasma membrane and tonoplant act as selectively permeable or differentially permeable membrane.

The Importance of Osmosis in Plants:

i) In the absorption of water by plants
ii) Cell to cell movement of water occurs throughout the plant body
iii) The rigidity of plant organs is maintained

Experiment No. 1) Study of process of osmosis in a simple osmometer

Materials: 10% sugar solution, a long stemmed thistle funnel, animal bladder or parchment paper, thread, scissors, stand, beaker, colored water, glass marking pencil and rubber solution

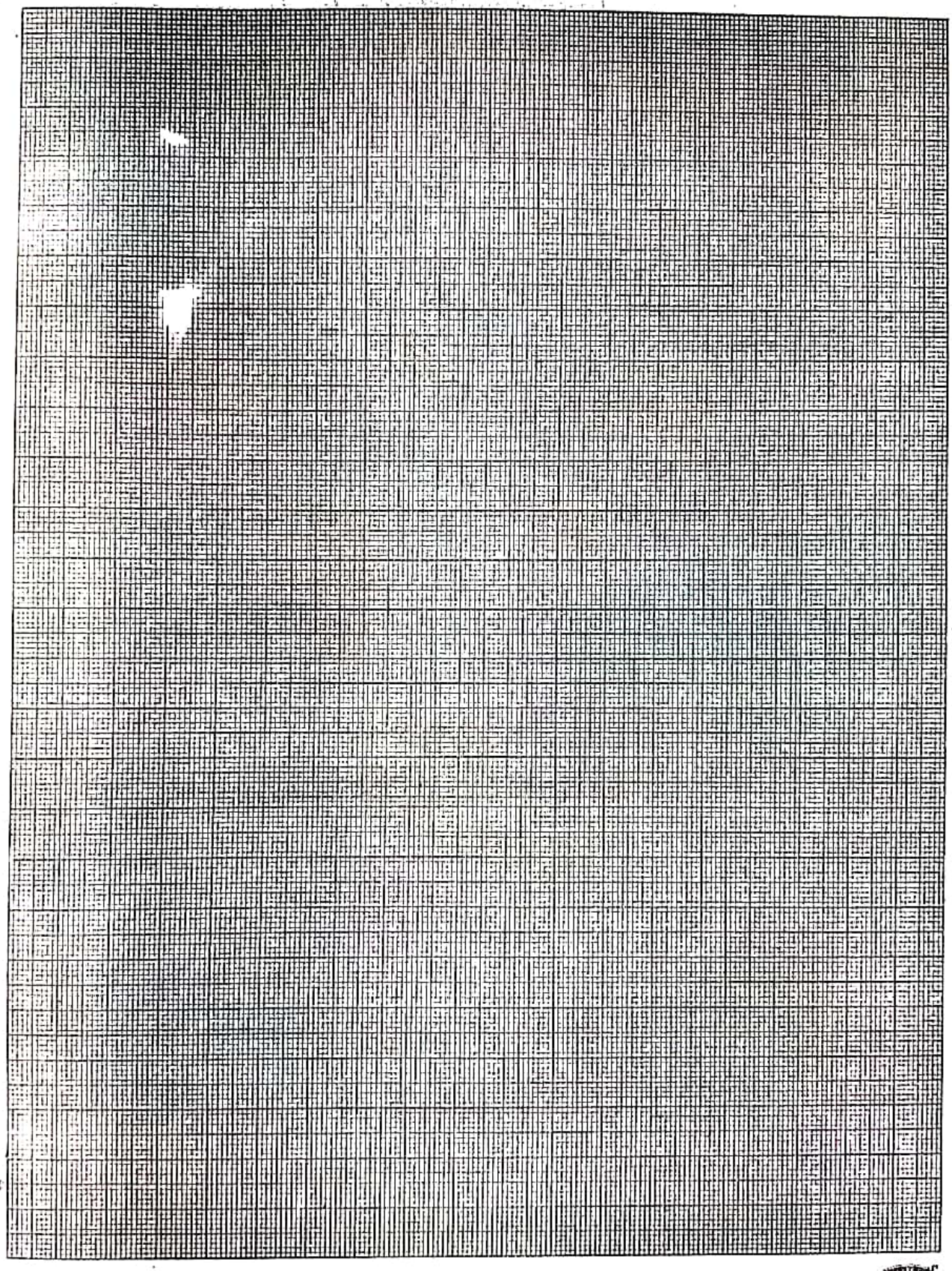
Principle:

A semi-permeable membrane having of very small sized pores allows only water to pass through the pores but prevent the movement of solute across them. The animal bladder or parchment paper acts as a semi-permeable membrane.

Procedure

i) Cover the thistle funnel mouth with an animal bladder or parchment paper (semi-permeable membrane) with the help of a thread.
ii) With the help of scissors, remove free edges of the bladder as close to the thread as possible.
iii) Seal the free margin of the bladder with the help of rubber solution.

Name: Std.: Roll No.: Date.:



Observation:

Sr. No.	Name of crop	Initial weight of seed sample (gm)	Final weight of seed sample (gm)	Difference in wt. (gm)	Amount of water Imbibed per g seed	Percentage increase in weight by each type of seed
1	Pea or gram	10				
2	Wheat or rice	10				
3	groundnut	10				

Discussion:

When dry seeds are put in water then water is absorbed by the seeds mainly by imbibition owing to the colloidal nature of the protoplasm of seeds.

As a result seeds become turgid and osmotically active and absorption of water is then regulated by osmotic process. The rate and amount of absorption by different types of seeds vary because of their different protoplasmic constituents which control their imbibing capacity.

Results of the present experiment show that the percentage of absorption of water is maximum in case of proteinacous seeds and minimum in case of fatty seeds. Proteins and polypeptides are hydrophilic colloids which have strong affinity for water. Cellulose and starch also absorb water strongly to the surfaces of these hydrophilic colloids.

N.B.

a) Imbibition of intact seeds leads to considerable error due to formation of water layer in the air space between the endosperm and the seed coat. In order to determine the imbibitions of water by intact seeds, amount of imbibitions without seed coat is to be added to the amount of imbibitions by seed coat only.

b) Effect of seed coat on imbibitions can be studied by setting experiment with seeds coat and without seed coat.

c) Q_{10} of absorption of water can be studied by allowing the seeds to imbibe water at room temperature, 10^0 C below and above it.

Conclusion:

iv) Fill the thistle funnel with 10% sugar solution to about 1/3rd neck height and mark the level as 'A'by means of glass marking pencil.

v) Dip the covered end of the thistle funnel in a beaker containing colored water. Support it in its position by means of a stand.

vi) After few hours, the sugar solution slowly will become colored and its level in the vertical neck of the funnel will rise up steadily to point 'B'.

vii) Taste the water of solution in funnel. It will no longer be sweet.

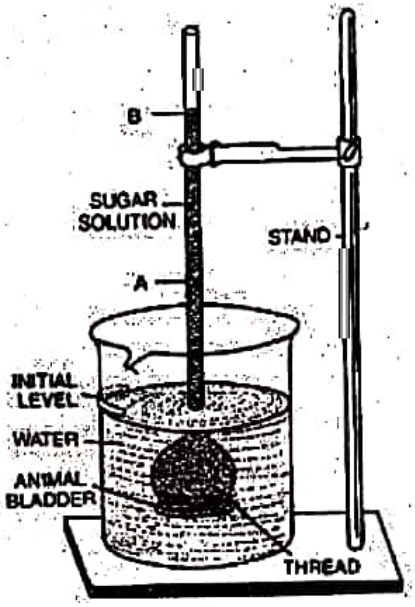


Fig. 11.7. Thistle funnel experiment to demonstrate osmosis.

Demonstration of osmosis

Precautions:

i) The membrane should attach with the thistle funnel properly. The edges must be properly sealed. The thread should be tied carefully so as not to rupture the membrane.

ii) The sugar solution must be of sufficiently higher concentrations.

iii) The initial level of sugar solution should be marked only after dipping the mouth of thistle funnel inside water of the beaker.

iv) Pour sugar solution in the thistle funnel in such a way as not to leave any air bubble.

v) Support and fix the thistle funnel firmly in its vertical position by means of a stand.

Results: The rise of sugar solution in thistle funnel can only be due to the entry of water into it through the animal bladder. But no sugar has gone out into the water of the beaker as it does not taste sweet.

The experiment, therefore, proves that:

i) Animal bladder, egg membrane, parchment paper is a semi permeable membrane because it allows only water to pass through it.

ii) Sugar solution is osmotically active solution and can absorb water when it is separated from it by a semi permeable membrane.

iii) Water diffuses into a solution when the two are separated by a semi permeable membrane. The phenomenon is called osmosis.

Experiment No. 2) Study of process of osmosis by potato osmoscope

Materials: A large potato tuber, 20% sugar solution, knife or scalpel, petri dishes or beakers, water, capillary tube, two pins and marking pencil

Principle:

A semi-permeable membrane having very small sized pores allows only water to pass through the pores but prevent the movement of solute across them. The tuber wall acts as a semi-permeable membrane.

Procedure:

i) Take a large size potato tuber. Give a flat cut to one end of the potato tuber.

ii) On another end of tuber, make a hollow cavity slightly more than half of its diameter.

iii) Remove the skin of the tuber because skin of the tuber is impermeable to water.

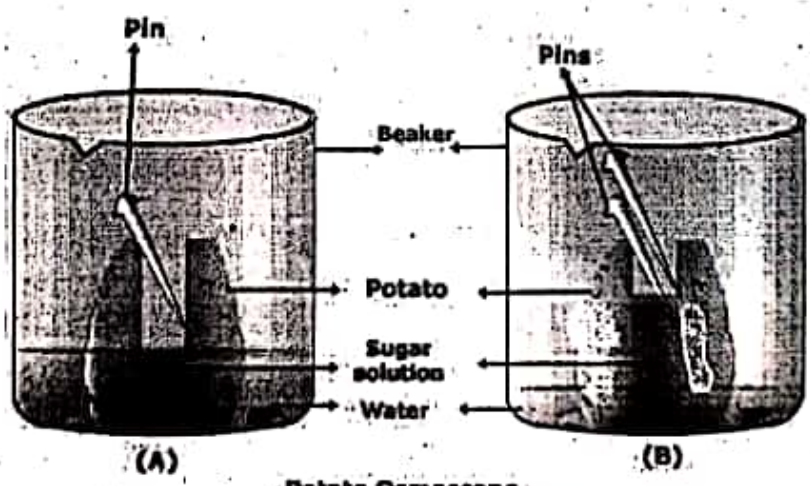
iv) Place the tuber on its flat cut end in a petri-dish half full of water or in a beaker.

v) Fill half of the cavity of the potato tuber with 20% sugar solution.

vi) By inserting a pin in the wall of the tuber mark the initial level of the solution.

vii) After few hours, the level of sugar solution is found to increase as water enters in the cavity. It is due to inward diffusion of water (endosmosis). Mark this reading also with another pin.

viii) Repeat the experiment after killing a potato tuber in boiling water. The protoplasm is denatured and the cytoplasm does not function as membrane. Thus there is no change in the level of sugar solution in the cavity.



Potato Osmoscope
(A) Before Osmosis (B) After Osmosis

Precautions:

i) The cavity of potato must be larger to pour sufficient amount of sugar solution. The cavity should be deep so as to leave only a thin layer of tissue at the base.

ii) Peel off the skin of the tuber from the base and the sides; make the base flat so as to keep the tuber flat in the dish or beaker.

iii) The initial level of sugar solution should be marked carefully.

iv) The water level in the Petri dish or beaker should be enough to dip a major portion of potato tuber.

v) Sugar solution should have a higher osmotic concentration as compared to cell sap of the tuber cells.

Results: The rise in the level of sugar solution in the cavity of the potato tuber indicates that the solution has absorbed water from the petri dish or beaker. The two are separated from each other by a large number of cells of the tuber.

The entry of water into the sugar solution, therefore, proves that:

i) Sugar solution is osmotically active solution.

ii) The cytoplasm of the cells of the tuber that lie between the sugar solution and the water of the petri dish or beaker act as a single semi permeable membrane.

iii) Water enters the sugar solution when it is separated from it by semi permeable membrane. This process is called osmosis.

Experiment No. 3) Demonstration of Endosmosis and Exosmosis:

Materials: A few grapes and raisins with intact stalks, water, petri dishes and 10% salt solution etc.

Procedure: Place a few raisins in water for about 5 – 6 hours. Raisins will swell up. The swelling can be due to the absorption of water from the petri dish. In another petri dish place a few fresh grapes (or swollen raisins) and pour 10% salt solution into the dish. After a few hours the grapes will shrivel which can be possible only when they have lost water to the salt solution (Fig. 11.9)

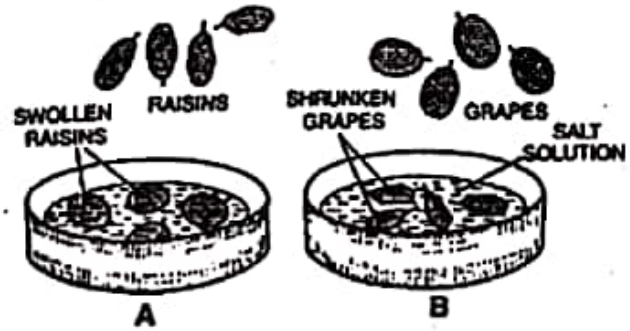


Fig. 11.9. Experiment to demonstrate endosmosis in raisins and exosmosis in grapes.

Draw the figure of the experiment, record the observations and draw the conclusions.

Practical utility: The process of plasmolysis is used for salting and drying of the fish, preparing the pickles etc.

Question: Why the cytoplasm shrinking occurs in higher concentration of sugar solution?

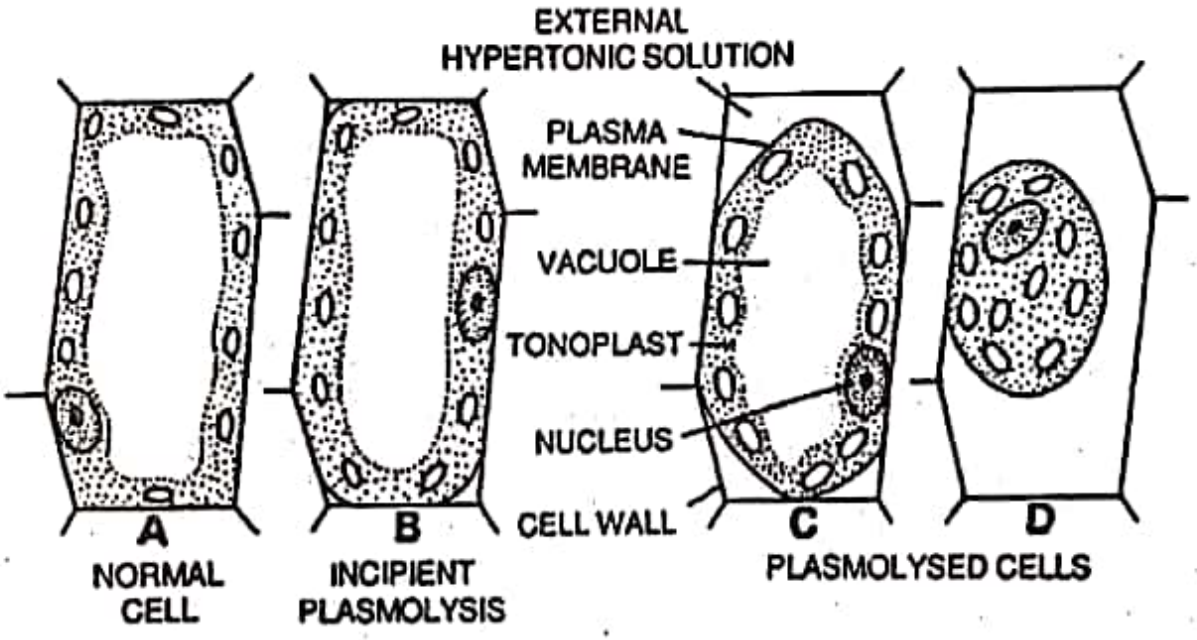


Fig. 2.9. Plasmolysis. Stages in plasmolysis of a plant cell.

Observations and conclusion:

Questions: Draw the figure of the experiment, record observations and write conclusion.

EXERCISE NO. 4. Date:

STUDY OF PLASMOLYSIS

Principle:

Plasmolysis is the shrinkage *of* the protoplast of a cell from its cell wall under the influence of a hypertonic solution whereas the swelling up of a plasmolysed protoplast under the influence of a hypotonic solution or water is called deplasmolysis. The phenomenon of plasmolysis has been exhibited by cells when they are kept in hypertonic solutions. The phe nenon of deplasmoylsis has been exhibited by plasmolysed cells when they are kept in a water or hypotonic solution.

When a plant cell or tissue is placed in a hypertonic solution water comes out from the cell sap into the outer solution by exosmosis and the protoplasm begins to shrink or contract. The protoplasm separate from the cell wall and assumes a spherical form this phenomenon is called **plasmolysis**. Incipient plasmolysis is stage where protoplasm begins to contract from the cell wall or A stage at which there is just separation of cytoplasm at certain points that stage is called as **incipient plasmolysis.** If a plasmolysed cell is placed in water, the process of endosmosis take place. Water enters into the cell sap, the cell becomes turgid and the protoplasm again assumes its normal shape and position. This phenomenon is called **deplasmolysis.**

Materials: Sugar, petri dishes, water, microscope, slides, cover slips, Tradescantia leaf etc.

Proc dure:

i) Prepare the series of sugar solution starting from 0.1, 0.2, 0.3 and 0.4 M

ii) Take the red coloured epidermal peel of the lower surface of *Trodescantia* leaf.

iii) Divide the leaf peelings into small strips.

iv) Place one strip each in different concentrations of sugar solution (0.1, 0.2, 0.3, 0.4 M) as well as in fresh water (control) for half an hour.

v) Remove the strips and with the help of microscope observe the changes in the cells i.e. stages of plasmolysis.

vi) The protoplast of the peelings kept in lower concentration (0.1 M) or in water remains homogenously distributed. Whereas, the protoplast in the peelings kept in higher concentrations will shrink.

vii) Count the number of cells under the microscope.

Precautions:

i) Sugar solution concentration must be correctly prepared.

ii) Peelings should be done from lower epidermis of leaf very carefully.

iii) Mount the peeling in glycerin carefully and avoid air bubbles and folding of peelings.

Results: In the first case the raisins have absorbed water from the outside due to the presence of higher concentration of solute in them. This is an example of endosmosis. In the second case, the grapes have lost water to the salt solution because salt solution is more concentratrd than the sap present in grapes. Therefore, it is an example of exosmosis.

Precautions:

(i) Grapes and raisins should be with intact stalks.

(ii) The solution for exosmosis must be stronger than sap concentration of grapes

Questions: Draw the figures of all the experiments, record observations and write conclusion.

EXERCISE NO. 6 Date:

DEMONSTRATION AND MEASUREMENT OF RATE OF TRANSPIRATION

Transpiration is defined as the loss of water from the living tissues of aerial parts of the plant in the form of water vapour. It is often said as a 'necessary evil' because it is a vital and unavoidable phenomenon of plants. Transpiration is regarded as a boon to the plants for many of the following reasons.

1) It affects the diffusion pressure deficit thereby indirectly helps in the diffusion of water through the cell
2) It affects the absorption of water and minerals by roots
3) Role in movement of water i.e. ascent of sap
4) It plays an important role in the translocation of food from one part to the other part of the plant.
5) It maintains leaf temperature
6) It brings about the opening and closing of stomata which indirectly influence the process of photosynthesis and respiration

6.1 Demonstration of transpiration by Bell Jar Method:

The water drops come out in the form of water vapors by the aerial parts of the plant during transpiration and consequently condense on the inner surface of the bell jar. A decrease in the weight of the pot is also due to the loss of water from the aerial portions of plants during transpiration.

6.1.1 Materials: A well-watered potted plant, glass sheet, bell jar, oil cloth or rubber sheeting, vaseline or grease and water etc.

Principle:

Bell Jar Method of transpiration is based on the principle that the water vapors can be seen in the form of water droplets if a transpiring plant is observed thoroughly.

1.1. 2 Procedure:

1) Select a small well-watered potted plant.
2) Cover the external soil surface of the pot and its soil thoroughly with oil cloth or rubber sheeting to check evaporation .
3) Weigh the whole pot and place it on a glass sheet.
4) Invert a dry bell jar over the pot.
5) Apply vaseline at the rim of the bell jar to make the apparatus airtight
6) Keep the whole set for 1 to 2 hours in sunlight.

v) Measure the initial level of water in the glass tubes.

vi) Observe rise in the mercury level in the manomemeter due to the pressure created by water exuded from the cut end of the stem on account of root pressure generated due to osmotic entry of water in the roots. The pressure is read on the graduated scale.

Results:

The rise in the level of mercury in the glass tubing is due to pumping of sap by root. The phenomenon is called root pressure. The same is read directly by manometer. A pressure of up to 5 atm. has been recorded by this method.

Precautions:

i) The plant selected should be succulent.

ii) Stem should be cut under water to avoid the entrance of air bubbles in xylem vessels.

iii) Rubber tubing should be *fixed* carefully.

iv) All connection should be made air tight with wax.

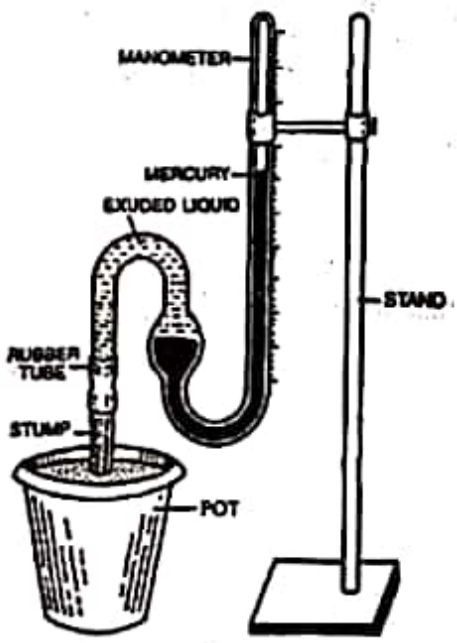


Fig. 11.25. Demonstration of root pressure.

Measurement of root pressure

Questions: Draw the figure of the experiment, record observations and write conclusion.

Conclusions:

EXERCISE NO. 5. Date:

DEMONSTRATION AND MEASUREMENT OF ROOT PRESSURE

Root pressure is 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 under the following conditions:

i. When the soil moisture level is high at night.

ii. When transpiration is low during the day.

When transpiration is high, xylem sap is usually under tension, rather than under pressure, due to transpiration 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 transport of mineral nutrient ions into the root xylem. Without transpiration to carry the ions up the stem, they 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 about 7 meters, and the tallest trees are over 100 meters tall.

Measurement of the root pressure in plants by manometer:

Materials: A potted plant of Balsam, Petunia or *Bryophyllum* or any other herbaceous plant, manometer with tube, stand, rubber tube, knife, thread and wax etc.

Principle:

There is rise in mercury level in the manometer due to the pressure created by water exudates from the cut end of the stem on account of root pressure generated due to entry of water by osmosis in root system.

Procedure:

i) Take a potted plant of Balsom *or Bryophyllum* that has been well watered the previous day and keep it overnight.

ii) Next morning, cut its stem a few inches above the base with a sharp knife.

iii) Attach the cut end of the stem (stump) to a manometer fixed to a stand through a rubber tube and threads.

iv) Keep it at a moist and shady place for few hours.

6.2.1.4 Procedure:

1) Take a Farmers / Ganong's potometer.
2) Cut a leafy branch of transpiring plant under water and fit it to the potometer with the help of rubber cork in such a way that the lower end of the branch should be under water.
3) Fill the whole apparatus completely with water using the reservoir.
4) Make all the joints of the apparatus air tight with the help of wax or grease or plaster of paris.
5) Introduce an air bubble in the graduated arm (capillary tube) with a pipette and mark the initial positions of air bubble i.e. adjust is to zero mark with the help of stop cock.
6) Dip the lower end of the capillary tube in water contained in a beaker.
7) Keep the whole apparatus in sunlight. Transpiration will occur in the plant kept in sunlight and the air bubble will move towards twig.
8) Record the distance (ℓ) travelled by the air bubble in a definite interval of time. Repeat the observations and calculate the average distance travelled by the air bubble. The experiment may be repeated by driving the air bubble back to zero mark by turning the stopper of the water reservoir. As transpiration proceeds, the air bubble will move along the capillary tube and gives a measure of the rate of transpiration.
9) Measure the total area of leaves of a twig.
10) Calculate the rate of transpiration in terms of ml of water transpired/hour/unit leaf area by using following formula; $V=\pi r^2 \ell$

Where,

V = Amount of water transpired in ml

π = 22 /7 or 3.14

r = radius of the graduated tube (bent tube)

ℓ = length of the tube or distance travelled by the air bubble

The rate of transpiration is determined in terms of ml/hour/unit area.

6.2.1.5 Observations:

Sr. No. of	Distance traveled by the air bubble (ℓ)	Amount of water transpired (V)
1		
2		
3		
4		
5		

1.1. 3 Observations:

1.1.4 Conclusions:

6.2 Measurement of rate of transpiration by potometric method:

This experiment is based on the principle that the amount of water absorbed is almost equal to that of water transpired in a given period and the difference between the two is negligible. Transpiration rate can be measured by different methods viz., Potometric method, Gravimetric method and Cobalt chloride paper method. The details of these methods are described below.

6.2.1 Potometric method :

This method is indirect and consists in measuring the amount of water absorbed by the plant to compensate for that lost by transpiration. There are two types of potometers:

6.2.1.1 Ganong's potometer :

It consists of a horizontal graduated capillary bent tube or with an attached scale. This tube is connected to two vertical tubes, of which one vertical tube is covered with one holed cork stopper and another vertical tube is provided with a reservoir having stop cock. The other end of the horizontal arm is bent downwards and is dipped in a beaker containing water.

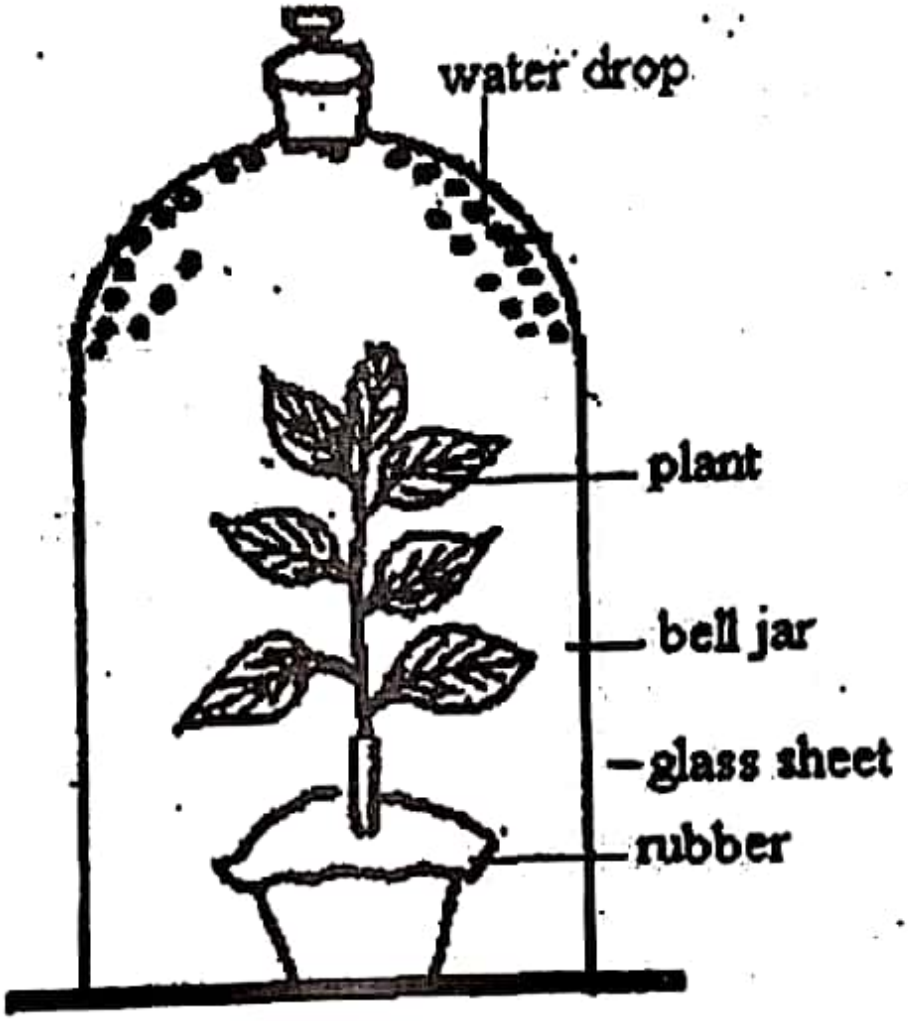
6.2.1.2 Farmer's potometer:

It consists of a wide mouthed bottle fitted with a rubber cork pierced with three holes, the middle one being just large enough to admit of the passage of a leafy branch. Into one of the other holes is fitted a thistle funnel provided with a stop cock to serve as a water reservoir and into the third, a bent tube of narrow bore is passed, so that its one end is flush with the inner surface of the cork. The other end of the tube is dipped in a beaker containing full of water. The rate of movement of the air bubble can be measured by fixing a scale to the bent (capillary) tube.

6.2.1.3 Materials: Ganong's potometer or Farmer's potometer, transpiring plant twig (leafy branch), beaker, pipette, water, wax, grease or plaster of paris and stop watch.

7) Observe the inner surface of the bell jar for appearance of water drops. The bell jar becomes misty after sometime and its inner walls contain drops of water that may flow down the sides of the bell jar.

8) Weigh of the pot again to know the loss in weight due to transpiration.



Demonstration of transpiration

Precautions:

i) The bell jar should be air tight.

ii) The pot should be well watered.

iii) Bell jar should be made up of glass to penetrate light.

Iv) The pot surface and soil must be covered carefully to avoid evaporation losses.

8) After the experiment, remove all the leaves from each pot and calculate the total leaf area of the plant.
9) Compute the amount of water loss per unit leaf area per unit time.

6.2.2.3 Observations :

Sr. No.	Name of the Plant	Initial weight (W_1)	Final weight (W_2)	Difference in the weight (W_1-W_2)	Total leaf area of plant (cm^2)	Amount of water loss ($g\ cm^{-2}\ hr^{-1}$)
1	Bean					
2	Sunflower					
3	Tomato					
4	Maize					

6.2.2.4 Conclusions:

Questions: Draw the figure of the experiment, record observations and write conclusion.

6.2.3 Measurement of rate of transpiration by Cobalt Chloride paper method:

The cobalt chloride method can be used for measuring only the relative rates of transpiration of different plants. This method although rarely used today in studies on relatiive transpiration rates, does have historical significance and contributed to our knowledge of transpiration process.

Principle: The cobalt chloride method makes use of difference in colour of Cobalt chloride in dry and moist conditions. Anhydrous cobalt chloride is blue in colour but turns pink when moist. The principle behind using cobalt chloride paper for measuring the transpiration is the time required to change the colour of cobalt chloride paper.

Precautions:

i) The shoot must be cut under water to avoid blocking of vessel by air.

ii) Apparatus should be air-tight.

iii) The twigs must have sufficient leaves.

6.2.1.6 Conclusions:

Questions: 1. Draw the figure of the experiment, record observations and write conclusions.

2. Why the air bubble in capillary tube moves upward?

6.2.2 Gravimetric method :

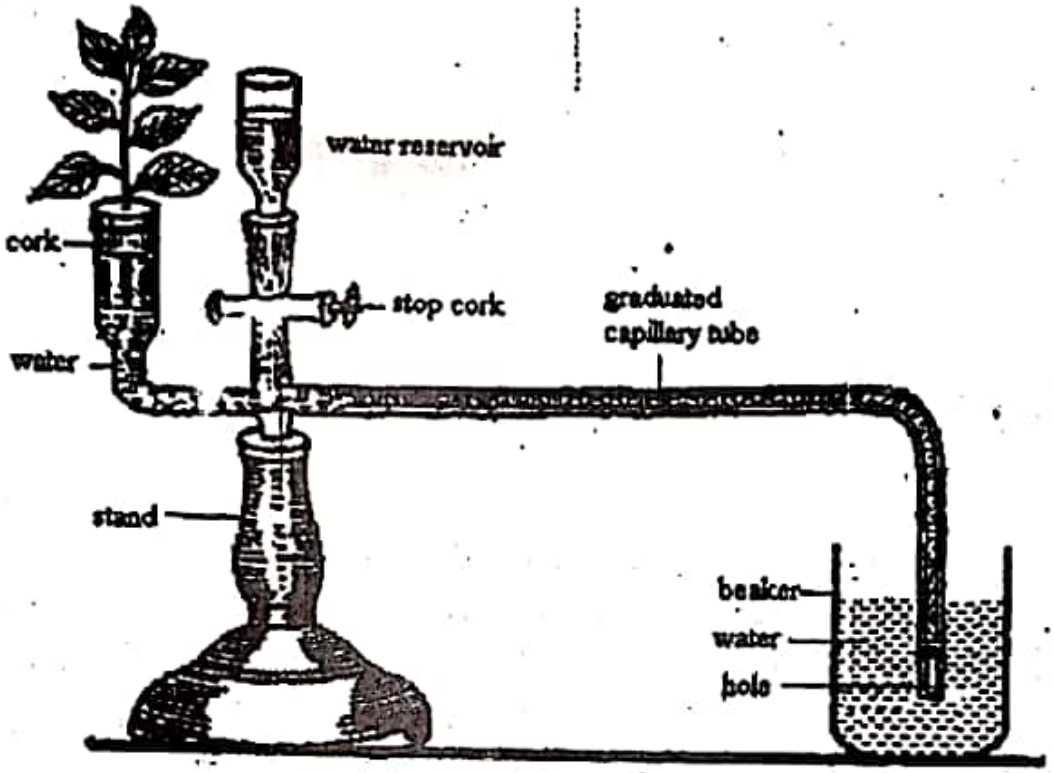
This method is restricted to the use of small potted plants and it is suitable for studying the influence of various environmental factors on the relative rates of transpiration in small plants. It consists in weighing the plant together with the pot at intervals and finding out the loss in weight due to transpiration. However, this method cannot be used for the study of transpiration rates of trees and of plants growing under natural field conditions.

6.2.2.1 Materials:

Four potted plants (bean, sunflower, tomato and maize), oil cloth or aluminium foil, rubber band, and wax.

6.2.2.2 Procedure:

1) Take four different potted plants and water them
2) Allow the pots to stand until all the excess of water is drained off
3) Cover each pot completely with a large piece of oil cloth or aluminum foil to eliminate the evaporation from the soil surface.
4) Immediately record the initial weight of all the pots. Let it be 'W_1'
5) Keep the pots for one hour in sunlight
6) After one hour, weigh each pot, which gives the final weight. Let it be 'W_2'
7) Find out the difference in the weights of the pots by subtracting each weight obtained from initial weight of respective pot.



Ganong's potometer

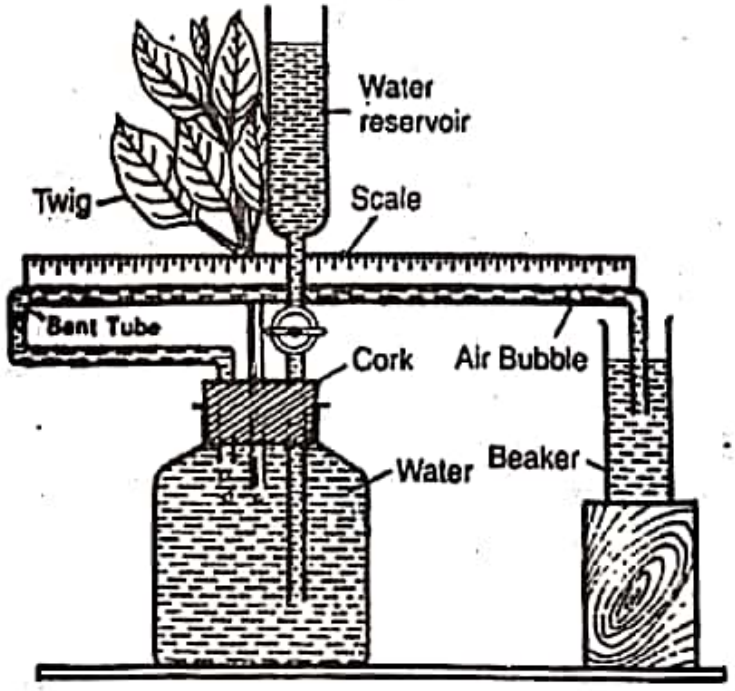
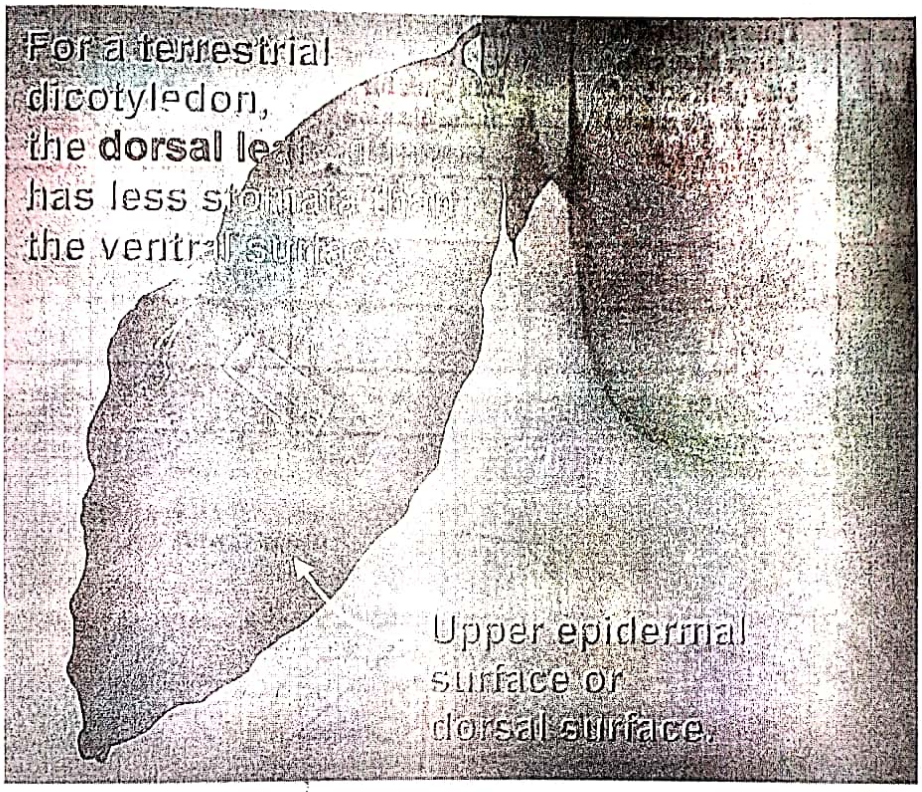


Fig. 30. Farmer's potometer.



6.2.3.3 Observations :

Name of the Plant	Time required to change blue colur into pink on leaf surface (Seconds)	Time required to change blue colour into pink for standardization (seconds)	Transpiration Index (TI)	Rate of Transpiration (g / disc / hr.)

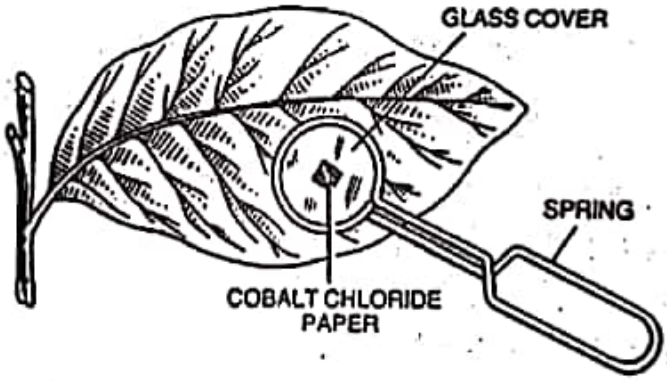


Fig. 2.23. Transpiration. Demonstration of phenomenon. (Cobaltchloride method).

Note: Above diagram is just indicative, students will have to draw a diagram comprising potted plant along with Cobalt Chloride filter discs on both the surfaces of transpiring leaf fitted with the help of glass slides and clips or U pins or rubber bands.

Questions: 1.Draw the figure of the experiment, record observations and write conclusion.

2. Why the Cobalt Chloride paper turn pink in colour?

6.2.3.1 Materials: Potted plant, cobalt chloride ($CoC1_2$), HCl, fitter paper, beaker, water, glass slides, clips, stop watch, forceps, oven, desiccators and anhydrous calcium chloride.

6.2.3.2 Procedure:

1) Prepare 3% cobalt chloride solution and slightly acidify with few drops of HCl
2) Soak the circular discs of filter paper (about 1 cm diameter) in cobalt chloride solution
3) Treat about 150 such circular discs of filter paper in the same manner
4) Remove the discs of fitter paper from the solution and dry them thoroughly in hot air oven at 40-50 ^{0} C
5) When the discs turn into blue colour, remove them and preserve in the desiccator containing anhydrous calcium chloride
6) Remove the discs from the desiccator using forceps
7) Place the discs on both the surfaces of transpiring leaf of potted plant with the help of glass slides and clips
8) Note down the time required to change the blue colour of cobalt chloride fitter paper discs into pink
9) In a similar manner, remove some other discs from the desicator and expose them to free water evaporating surface and note down the time required to change blue colour into pink (This indicates the cobalt chloride paper standardization)
10) Determine the relative rate of transpiration for the plant i.e. calculate the transpiration index (TI) by the following formula

$$\text{Transpiration Index (TI)} = \frac{\text{Time taken for cobalt chloride paper standardization (S)}}{\text{Time taken for cobalt chloride paper colour change on leaf (S)}}$$

11) Calculate the rate of transpiration with the help of following formula:

$$\text{Grams of water transpired/disc/hr} = \frac{3600 \times X}{T}$$

Where, T = Time required in seconds to change blue colour into pink.

X = Factor for Cobalt Chloride disc (in grams)

Calculation of X factor: Select 100 Cobalt Chloride discs and record the oven dry weight in grams, keep the discs in 80 to 90 % humidity for 3 to 4 hours and record the weight (grams). Calculate the difference in weight for one disc i.e. X (factor)

Table 7.1: The number of stomata in plants:

Plants	Number/ epiderm (mm^2)	
	The upper surface	The lower surface
Wheat	33	14
Maize	52	68
Oat	25	23
Sun flower	58	156
Tomato	12	130
Bean	40	281
Apple	00	400
Lotus	46	00

Objective: To study the stomatal distribution on the upper and lower leaf surfaces and to calculate the stomatal index.

Stomata are minute pores found on the epidermis of leaves and young shoots of plants that are used to control exchange of gases. The pore is surrounded by a pair of specialized cells called the guard cells that are responsible in regulating the size of the opening.

Water is released through the stomata into the atmosphere in the form of water vapour through the process called transpiration. Besides this, the exchange of oxygen and carbon dioxide in the leaf also occurs through the stomata.

Distribution of Stomata:

Distribution of stomata varies between monocots and dicots, between plant species, and between the underside and top side of the leaves on a plant.

Stomata are found more on plant surfaces thriving under higher light, lower atmospheric carbon dioxide concentrations and in moist environments.

Usually the lower surface of a dicot leaf has a greater number of stomata while in a monocot leaf they are more or less equal on both surfaces. In most of the floating plants, stomata are found only on the upper epidermis.

single motion (A little practice is required to gauge the proper amount of fluid needed to brush). –

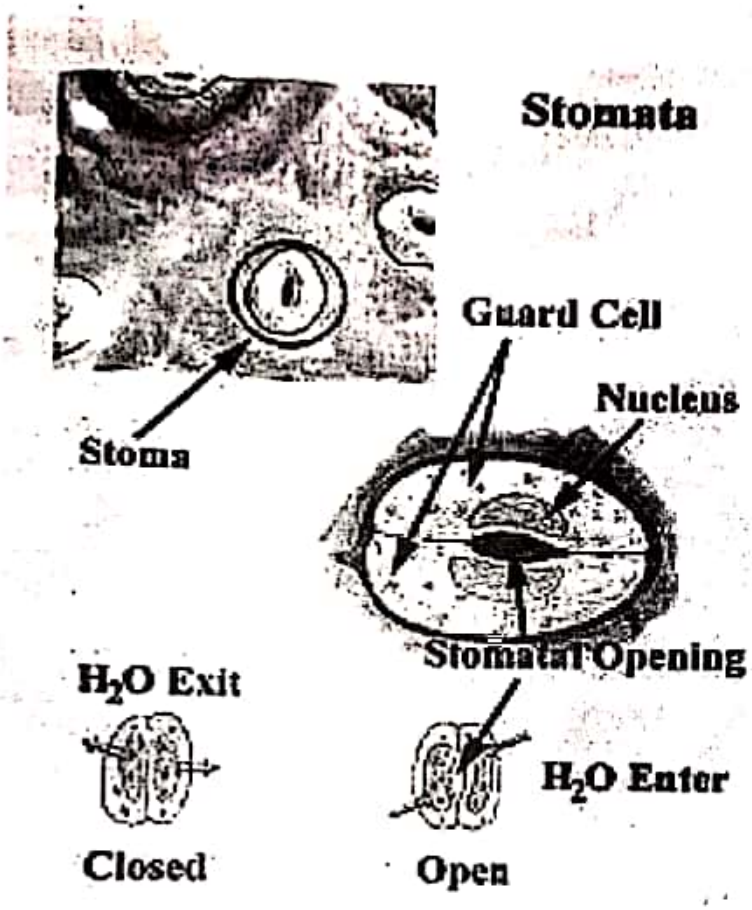


Fig. 7.1: Stomata with guard cells

After a few seconds when the fluid becomes firm and replica is ready to be removed, a small piece of double adhesive celluloid tape is to be secured to a glass slide. The exposed sticky surface is then placed over the replica and with slight pressure remove the film from the leaf surface. Mount the replica for viewing as an imprint on reverse side of the image of leaf surface.

Using the calibrated grid, the number of stomata and epidermal cells can be counted.

Take a circular piece of paper of the size of eyepiece with a rectangular cut in the centre. Insert paper in an eye piece. The microscopic field now appears rectangular. Scan small area of leaf using low power objective.

Count number of epidermal cells (E) and stomata (S) in a specified field.

EXERCISE NO. 7

Date:

Experiment 1: STUDY OF STRUCTURE AND DISTRIBUTION OF STOMATA

Stomata are specialized organs with an opening bordered by two epidermal cells called guard cells. Stomata sometimes occur only on the bottom of the leaf but often found on both top and bottom.

Only through these pores or the stomatal openings, the following processes take place.

i) Gaseous exchange mechanisms, *viz.*, CO_2 intake for photosynthesis and O_2 intake for respiration

ii) Transpiration for regulation of water

iii) Inhibition of pollutants intake into the leaf

Stomatal movement:

The mechanism by which the opening and closing of the stomatal pore is by direct response to increase or decrease in the osmotic potential of the guard cells. The changes in the water potentials that result from the osmosis causes water to move in or out of the guard cells. If the water moves into the guard cells, the cells become turgid and cells expand the stoma is open, when the guard cells are flaccid; stoma is closed.

Anatomy of stoma:

Stoma consists of two guard cells. The cellulose micro-fibrils making up the wall of the guard cell are arranged radially rather longitudinally. This orientation of cellulose micro fibril is termed radial micellation. In addition, the guard cell wall adjacent to the stomatal pore is thicker than the outer wall, this helps in the formation of pore.

Estimation of stomatal index and stomatal frequency:

Replica method

Replica method was developed by Wolf (1979). Simple method, gives useful information not only on stomatal index but also its frequency per unit leaf area. This method can be employed in most of the crops and can have useful data on leaf anatomy. If leaf lamina is much waxy and hairy it is difficult to measure stomata and epidermal cells.

Materials required: Leaf sample, blue / red correction fluid, double adhesive celluloid tape, slides, ocular micrometer, stage micrometer, microscope, thermocol, xylene.

Procedure

Collect the leaf samples to be used. The applicator brush attached to correction fluid is dipped into the fluid. The brush is stroked across the area to be replicated with a

Experiment 2: Determination of the pore size and pore area of stomata

Theory: Stomata are the passage for the exchange of water vapour and gases like CO_2, O_2 between the intercellular spaces of leaf and atmosphere. The rate of transpiration and gaseous exchange mainly depend on the pore area of leaf. The pore size and pore area of stomata can be measured by ocular micrometer.

Materials required:

1. Stage micrometer, 2. Ocular micrometer, 3. Compound microscope
4. Slide and cover slips, 5. Fresh leaf, 6. Safranin etc.

Procedure:

1. Peel off a strip of epidermis of a leaf that has been fully exposed to light for several hours. Stain and mount on a slide and put a cover slip on it.
2. Determine the area of high power field of the microscope with the help of a stage micrometer.
3. Observe the slide under high power magnification and measure the width and the length of stomatal pores of the film with the help of a ocular micrometer.
4. Record the observed data in table.

Calculation:

i) The pore area (PA)= $\frac{L \times B}{4} \times K$

Where,

L= Length of stomatal pore, B= Width or breadth of stomatal pore

K= Constant (0.9 for dicotyledons and 0.66 for monocotyledons)

ii) % pore area = $\frac{\text{Total pore area of stomata present in high power field}}{\text{Area of high power field}} \times 100$

Result:

STOMATAL FREQUENCY:

Mount the stage micrometer on the stage of microscope, stage micrometer is divided into 100 equal divisions. Each division is of 10 μ. Coincide the stage division with rectangle /square that is in the eye piece. Calculate the area of the rectangle /square in μ^2 using low power objectives.

Count the number of stomata with in the area of rectangle. Half cut stomata may also be counted.

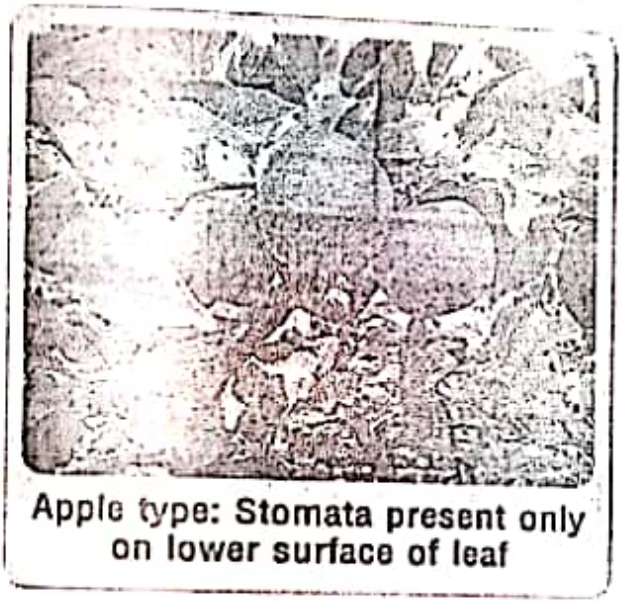
Compute the stomatal frequency in mm^2 per unit leaf area.

Note: The material used for replica can be silicon rubber, negative and several dissolved compounds. A transparent finger nail polish (collodion) can be used to get impressions. Dissolved compounds such as thermocole can also be used.

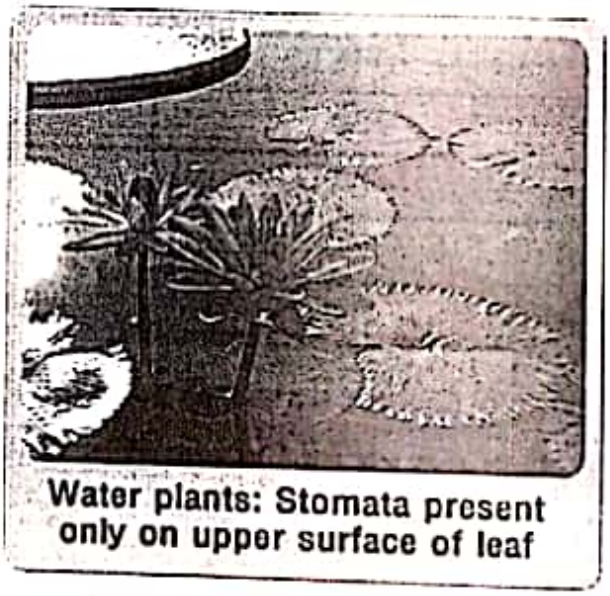
Results:

Monocot plants: Stomata present equally on both surfaces of leaf

Dicot plants: Stomata present more on lower surface of leaf



Apple type: Stomata present only on lower surface of leaf



Water plants: Stomata present only on upper surface of leaf

Calculation of Stomatal Index:

The distribution of stomata on the upper and lower surfaces of the leaf can be studied by removing the peels of the leaf from the upper and lower surfaces and observing the same under a microscope.

The count of the the number of stomata and epidermal cells in the microscopic field is taken and the stomatal index of each surface of the leaf can be calculated using the following formula:

$$\text{Stomatal Index} = \frac{\text{No: of Stomata}}{\text{No: of Stomata + No: of epidermal cells}} \times 100$$

This is the proportion of stomata to the epidermal cells. This has relevance in studies on plant water relations.

9) The Relative water Content technique Consist in Comparing in Initial & forgund water Content on % basis

∴ Calculate RWC

Given: fresh wt = 0.612 gm
turgid wt = 0.620 gm
dry wt = 0.082 gm

$$\therefore RWC\% = \frac{\text{Fresh wt} - \text{Dry wt}}{\text{Turgid wt} - \text{Dry wt}} \times 100$$

$$= \frac{0.53}{0.538} \times 100$$

$$= 98.5\%$$

∴ RWC is 98.5%

EXERCISE NO. 8

Date:

ESTIMATION OF RELATIVE WATER CONTENT OF LEAF TISSUE

RWC is one of the most dependable and easy technique for measuring the water status of a tissue. The relative water content technique consists in comparing the initial and turgid water content on a percentage basis. Relative leaf water content (RLWC) gives an idea about water status of plant i.e. whether the plant is under water stress or not. It also gives information about drought tolerance of crop. The RLWC was primarily estimated with whole leaf method but most workers were unable to obtain high values. The procedure given by Barrs and Weatherly (1962) is explained below:

Procedure:

The selection of leaf tissue and time of sampling are equally important in expressing reliable results. Normally, a leaf that is physiologically functional (third leaf from top) is selected for RLWC estimation. It's fresh weight is immediately recorded after it is excised. The leaf samples can be brought to the laboratory after careful sealing in double walled thermocol chamber or plastic bag in order to prevent water loss and fresh weight is recorded. The petiole may be detached keeping only leaf lamina.

Secondly, keep these leaf samples floating on water under diffused light to get turgid weight for 4 to 6 hours depending upon the degree of imbibition. After thorough blotting record the turgid weight. Finally, the same leaf is kept in an oven at 75° C for assessing dry weight. The dry weight is recorded after obtaining a constant oven dry weight. Further the values are plugged in the following formula :

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

The RWC value so calculated doesn't attain the limit of 98% and therefore, they further reported disc method to have better and accurate estimate of RWC.

Collect leaf as stated above by removing 3rd leaf from top (physiologically most active) of maize / sorghum / sunflower plant. Twenty leaf discs of 1 cm. diameter are taken with a closed punch specially designed for this kind of work. This facilitates rapid ejection of the leaf discs. While cutting leaf discs, they should be cut midway between base and tip of leaf blade by excluding the midrib. Record fresh weight of all twenty discs immediately after cutting and keep them floating in a closed petri dish containing distilled water for 4 to 6 hours at constant temperature under diffused light. The extra water from leaf discs may

Table 7.2: Length, width and Pore area of leaf sample:

Sl. No.	Length	Width	Pore area
Average			

Draw diagram of all the experiments and record observations.

Conclusion: (Also describe the importance of the studying the stomatal size at different varietal or situations)

Materials:

Spinach leaves, chromatography chamber, mortar and pestle, ether, acetone, scissor, pencil, capillary tube, or fine micropipette, spatula, scale, Whatman No.1 filter paper, watch glasses, thread, stapler etc.

Procedure

1. Take a few freshly plucked healthy green spinach leaves.
2. Using scissors cut the spinach leaves into small pieces and let them fall into the mortar.
3. Grind the spinach leaves in a mortar using pestle with 80% acetone.
4. Place the extract into a watch glass using a spatula.
5. Take a long strip of Whatman No.1 filter paper having a narrow notch at one end of the strip.
6. Take a pencil and a scale and draw a horizontal line with a pencil about 2-3 cm away from the tip of the notch.
7. Put a drop of the pigment extract in the middle of the line about 3 cm away from the base of the strip with the help of a capillary tube or a fine micropipette. Allow the drop to dry and repeat till four or five drops are placed on the paper. Care should be taken to avoid over spreading of the extract.
8. Prepare solvent mixture of petroleum ether and acetone in the ratio of 100:12. Take the chromatographic chamber and pour ether acetone solvent in it.
9. Fold one end of the filter paper strip and staple it. Using a thread, hang the filter paper strip in the chromatographic chamber.
10. The loading spot should remain about 1 cm above the solvent level. Leave the chromatographic chamber undisturbed for some time.
11. We can observe, as the solvent moves through the paper, it spreads the different pigments of the mixture to various distances.
12. When the solvent rises about 3/4th up the strip, remove the strip carefully and let it dry.

Observation:

The dried chromatographic paper strip shows four distinct paper bands. Different pigments can be identified by their colors. Carotene moves faster comparing to other pigments viz. chlorophyll 'a', Chlorophyll 'b', and xanthophyll. Identify the pigments by their colours and by calculating their *Rf* values. To find out the value of *Rf* for each pigment, measure the distance travelled by it from the base to the point of origin (loaded spot) and also the distance travelled by the solvent. Record the colour of pigment band and its *Rf* value in a tabular form as shown below.

EXERCISE NO. 9

Date :

A) EXTRACTION AND SEPARATION OF PHOTOSYNTHETIC PIGMENTS –PAPER CHROMATOGRAPHIC METHOD:

Object : To extract and separate the photosynthetic pigments from the plant tissue by paper chromatography.

Introduction: Photosynthetic plants convert light energy from the sun to chemical food energy. During photosynthesis, molecules referred to as pigments are used to capture light energy. Pigments are chemical compounds which reflect only certain wavelengths of visible light. Plant leaves contain four primary pigments: chlorophyll 'a' (dark green), chlorophyll 'b' (yellowish-green), xanthophylls (yellow) and carotenoids (orange).

To separate and visualize the four primary pigments of green plants, we can use a simple technique called chromatography.

What is Chromatography?

Chromatography is a technique used to separate molecules on the basis of differences in size, shape, mass, charge, solubility and adsorption properties. The term chromatography is derived from Greek words Chroma-colour and Graphe-write. There are many types of chromatography: paper chromatography, column chromatography, thin layer chromatography and partition chromatography. These techniques involve the interaction between three components: the mixture to be separated, a solid phase and a solvent.

Principle: The technique of chromatography is used for separating and identifying substances present in a mixture. The substances get separated because of their different affinities for a stationary phase and their differential solubility in moving phase. In paper chromatography the hydrated cellulose molecules of the paper constitute the stationary phase and the mobile phase is a liquid. The separation of compounds in a mixture is dependent upon the partition, between the mobile and stationary phases. The rate of movement of the different substances in the mixture is determined by their partition coefficient. Hence some compounds will move faster while others move at a slower rate and get separated. Hence compounds are identified by their distance travelled by the organic compounds (d) and the distance travelled by the solvent (D), from the point of origin $Rf = d/D$. The values thus obtained are compared with standard values.

be removed by blotting paper before weighing to determine their turgid weight. Take dry weight after drying the leaf discs in hot air oven at 70°C till constant weight is observed. Calculate the percent RLWC by using above formula and write the conclusions.

Observations: Hypothetical example is given below; replace the values by recording actual observations and calculate RWC.

Plant Part	Fresh wt. (g)	Turgid wt. (g)	Dry weight (g)	Relative Water Content (%)
Leaf	0.512	0.520	0.062	98.2
Leaf discs				

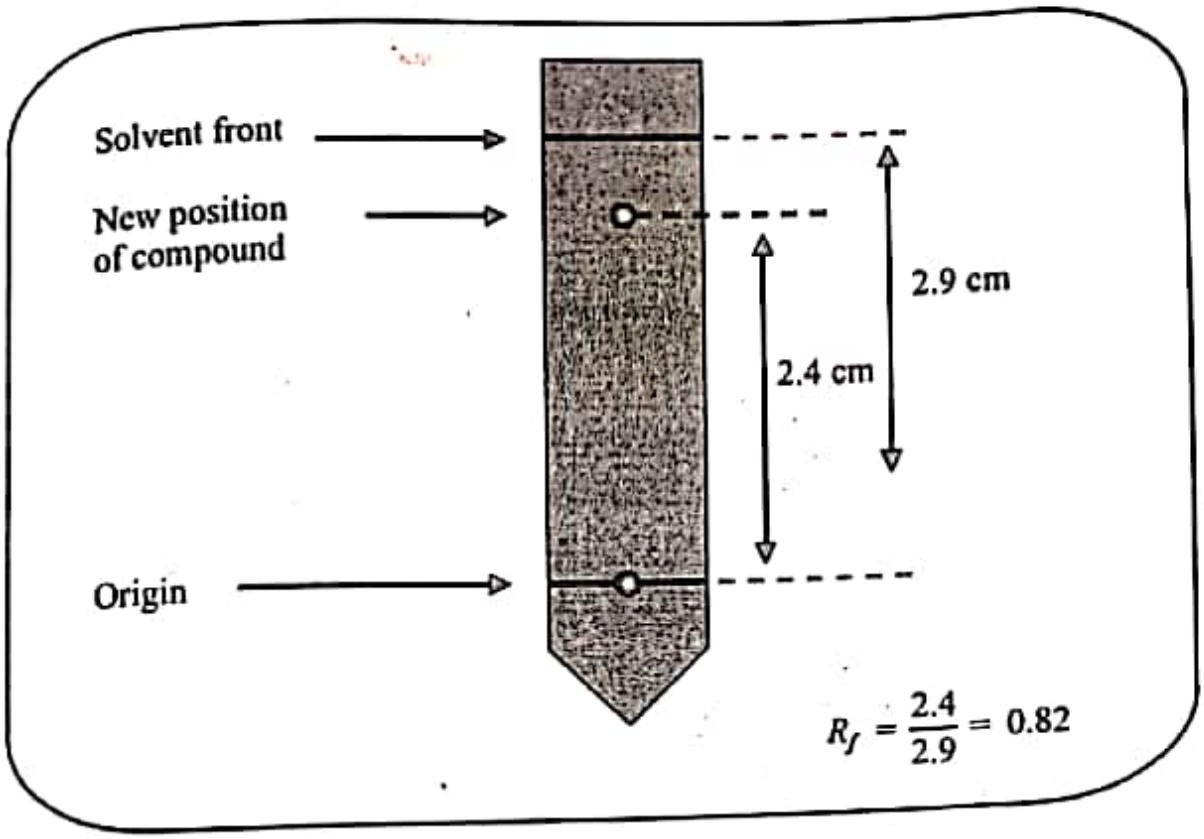
Calculation:

$$R.W.C. = \frac{0.512 - 0.062}{0.520 - 0.062}$$

$$= 98.2\ \%$$

Result : The R.W.C. of the given leaf sample is 98.2 %

Conclusions:



Diagrammatic example that demonstrates R*f* value:

B) Extraction and separation of photosynthetic pigments by solvent extraction method:

Extraction theory:

Solvent extraction is a process of purification involving the use of two solvents or solutions that are immiscible with one another. The main use is to employ extraction as a means of isolating chemicals that are present in plant (natural products). It is extensively used in organic chemistry as a way of purifying reaction mixtures after a laboratory experiment. It is based on the differential solubility of compounds in water and an immiscible organic solvent.

Extraction and separation of plant pigments:

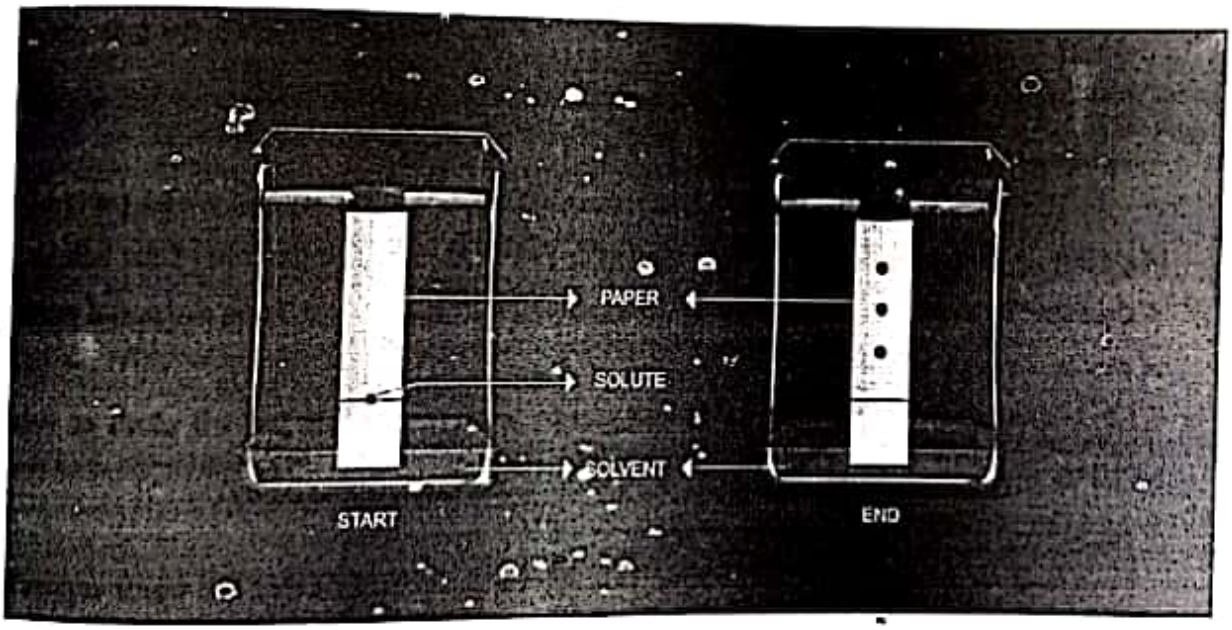
It is based on their solubility and partitioning in different immiscible organic solvents. This could be done by using different organic solvents in sequence for separation of pigments by separating funnel.

RESULT:

Rf value of Chlorophyll 'b' =
Rf value of Chlorophyll 'a' =
Rf value of Xanthophyll =
Rf value of Carotene =

How does paper chromatography work?

In paper chromatography, the mixture is spotted onto the paper, dried and the solvent is allowed to flow along the sheet by capillary attraction. As the solvent slowly moves through the paper, the different compounds of the mixture separate into different coloured spots. The paper is dried and the position of different compounds is visualized. The principle behind the paper chromatography is that the most soluble substances move further on the filter paper than the least soluble substances. Different plant pigments can be separated by using the technique of paper chromatography.



What is Retention Factor or *Rf* value?

Retention factor or *Rf* value is applied in chromatography to make the technique more scientific than a mere analysis. The retention factor or *Rf* is defined as the distance travelled by the compound divided by the distance travelled by the solvent.

Rf = (Distance travelled by the compound)/ (Distance travelled by the solvent)

Sr. No.	Name of the pigment	Colour of the pigment	Rf value Rf= d/D
1.	Chlorophyll 'b'	Light green	
2.	Chlorophyll 'a'	Dark green	
3.	Xanthophyll	Light yellow	
4.	Carotene	Orange yellow	

Calculations:

Rf value of the each pigment spot can be calculated by the equation;

Rf value = Distance travelled by the pigment (d) / Distance travelled by the solvent (D)

Measure the distance of each pigment band from the loading spot and also the distance travelled by the solvent. Calculate the *Rf* value using the equation and record the values in the table.

Result:

The topmost orange yellow band of pigments in the separation corresponds to carotene. The yellowish band appearing below it indicates the xanthophylls. The third from above dark green band represents chlorophyll 'a'. The lowermost yellowish green band is that of chlorophyll 'b'

Sr. No.	*Rf* value of the pigment	D/d	Rf value Rf= d/D
1.	Chlorophyll 'b'		
2.	Chlorophyll 'a'		
3.	Xanthophyll		
4.	Carotene		

Conclusion:

There are 4 bands separated on the paper, namely chlorophyll 'b', at the bottom light green in colour, chlorophyll 'a' in dark green colour, xanthophyll in light yellow colour and carotene as dark yellow colour band at the top.

EXERCISE NO. 10

Date:

METHODS OF MEASURING RATE OF PHOTOSYNTHESIS

Some of the methods are deduced from the classic summary equation of photosynthesis taking into account the initial and final components and the energetic changes in the system i.e., $CO_2 + 2H_2O + 472.8$ KJ (112.3 K Cal) $\rightarrow CH_2O + O_2 + H_2O$. There are nine possible ways of measuring photosynthesis, which are described below.

10.1 CHANGE IN ENERGY:

The most exact criterion of photosynthetic rate is the direct determination of energetic changes in the intact closed photosynthesising system. This is the only procedure for the absolute evaluation of photosynthetic efficiency and the yield of energy transformation. It requires (i) an exact knowledge of the amount of radiant energy absorbed by the photosynthesizing system (ii) the portion of energy converted to sensible heat (appearing as a change in the temperature of the system), and (iii) the amount reradiated or dissipated as latent heat of vaporization of water.

Measurements of this type are very difficult since it requires exact laboratory experiments such as a special and very sensitive calorimeter. For example, the calorimeter used by Arnold (1949) was made up of a tiny silver cup containing a leaf disc or algal suspension. The crop was supported by number of fine wire thermocouples, the other ends of which were connected to a copper ring in thermal contact with an aluminium block so that the system was totally closed. PAR was allowed into the assimilating tissue or suspension through a quarter window. Temperature differences between the cup and the block were annealed by passing a measured current through the thermocouples and thus heating or cooling the cup. The temperature changes thus measured indicate the exact energetic changes in the whole system and in the irradiated plant material.

10.2 CONSUMPTION OF WATER:

It is very difficult to determine the consumption of water in the photosynthetic processes. To do this, would require the use of deuterated or tritiated water and the physiological behaviour of which is different from that of normal water. This is the main reason why water uptake has not been used to measure the rate of photosynthesis.

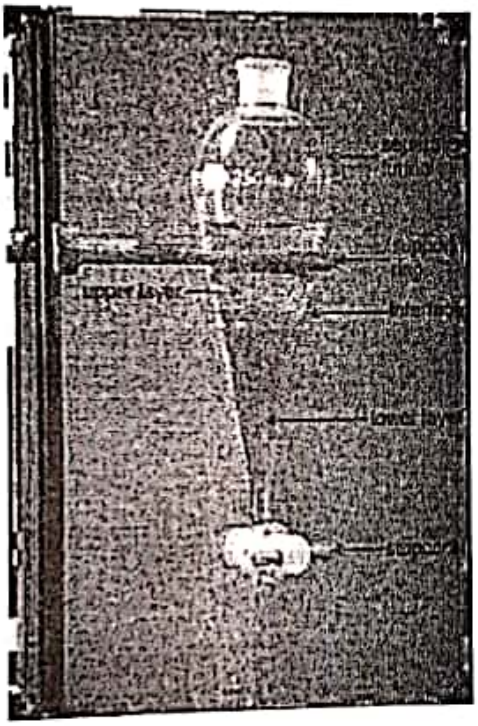
10.3 OXYGEN EFFLUX:

The amount of O_2 produced reflects the rate at which water is split in photosynthesis and hence the activity of photosystem II and of the whole process. The

13. Collect the lower methanolic- KOH fraction (green layer) in a test tube that contains chl.b and collect upper ether fraction (yellow layer) in another test tube that contains xanthophylls.

Observation to be recorded:

Separate all the major plant pigments from different plant species and observe the relative content of pigments in all the species.



Separating funnel:

Draw diagram of all the experiments and record observations.

Conclusions:

Materials : Acetone (80%), Petroleum ether, Methanolic – KOH (30%), Ethyl ether, Methyl alcohol (92%), Distilled water, fresh leaves, Separating funnel, Beakers, Measuring cylinder, Balance, Razor blade, Filter paper (Whatman No. 1) , Mortar and pestle etc.

Procedure:

1. Weigh 3 g of fresh leaf tissue and macerate in mortar with pestle by using 50 ml of 80% acetone,
2. Filter the extract through Whatman No. 1 filter paper. The filtrate contains an extract of Chlorophyll 'a', Chlorophyll 'b', carotene and xanthophylls.
3. Take 40 ml of filtrate in separating funnel and add 60 ml of petroleum ether. The funnel is then gently rotated and 60ml of distilled water is added to form two distinct layers after rotating the funnel. Distilled water is poured gently down the side of the funnel to avoid the formation of emulsion. Discard the lower acetone water layer retaining the upper petroleum ether fraction.
4. Wash twice with water discarding the lower water fraction each time.
5. Add 50 ml of 92% methyl alcohol to petroleum ether fraction and rotate the funnel gently to allow to form two separate layers viz., the upper petroleum ether layer (contains chl 'a' and carotene) and lower alcohol fraction.
6. Collect the lower methyl alcohol fraction in a beaker that contains chl. 'b' and xanthophylls. Methyl alcohol is poisonous and therefore, its fumes should be avoided.
7. Add 15 ml of 30 % methanolic – KOH solution to petroleum ether fraction (Which is retained in separating funnel) and shake gently. Further, add 30 ml of distilled water and allow it to form two layers.
8. Collect the lower petroleum ether fraction (Orange layer) in a test tube that contains carotenes and collect the upper methanolic- KOH from separating funnel (blue green layer) in another test tube that contains chl. 'a'
9. Wash the separating funnel clearly with water and take methyl alcohol fraction (which was collected in beaker) in the separating funnel.
10. Add 50 ml of ethyl ether and shake gently and add 10 ml of distilled water to form two separate layers.
11. Discard the lower methyl alcohol fraction and retain the upper ether fraction that contains chlorophyll 'b' and xanthophylls.
12. Add 15 ml of 30% methanolic- KOH solution to the ether fraction and shake the contents for 10 minutes. Add 30 ml of distilled water to form two layers.

a) Problem to be studied and the plant material used.
b) Required sensitivity, accuracy and response time to the measurement of either instantaneous or integrated photosynthesis.
c) Measurement of photosynthetic rate under natural or controlled environmental conditions.

10.10.2 Availability of the equipment:

Only a few methods of studying photosynthesis such as, growth analysis procedures require no complex equipments. The equipments for measuring biological process usually have two functions.

a)Measurement of physical or chemical changes that characterise the processes and serve as basis for assessing its kinetic parameters.

b) Enclosure of the experimental material during the measurement in an environment that keeps the external conditions constant or their changes definable.

10.10.3 Laboriousness of the method :

Some methods produce a small number of exact data (gas exchange systems with IRGA), while with other methods a large number of less exact measurements can be made in the same period. Labouriousness of the method is not necessarily a sign of its exactness or perfection.

MEASUREMENT OF RATE OF PHOTOSYNTHESIS BY DRY WEIGHT METHOD

Principle:

The rate of photosynthesis can be measured by direct and indirect methods. In direct method Oxygen evolved or CO_2 consumed is measured. While in indirect method increase in plant growth is measured for a given period which takes place as a result of photosynthesis. By drying plant sample in the beginning and at the end of given period, difference in dry weight can be obtained and rate of photosynthesis per unit time can be calculated. In indirect method the rate can be determined by I) Sach's method ii) Field method.

1. Sach's dry weight method :

Requirement:

Young growing plant of sunflower, Ganong's leaf punch, balance, oven etc.

formed (or energy fixed) is not uniquely related to the amount of CO_2 bound, but varies according to the chemical composition and energy content of the products. For example, 1 g CO_2 may be transformed into 0.4 g fat with energy content of 37.7 KJ g^{-1} (4.2 K cal g^{-1}). Alternatively, 1 g CO_2 may be transformed into 0.62 g starch with an energy content 17.6 KJ g^{-1} (4.2 K cal g^{-1}). Proteins are intermediate i.e. , 1 g CO_2 produces 0.5 g protein. Thus, diverse amounts of photosynthates result from the assimilation of the same amount of CO_2. Unfortunately, this method has not yet been a common procedure. The increase in total photosynthates in a leaf or sample of isolated tissues may be directly measured with $^{14}CO_2$

10.7 ACCUMULATION OF ENERGY :

The accumulation of energy in the tissue may be determined as an increase in the heat of combustion resulting from the production of organic matter in photosynthesis. Comparability of results obtained by this procedure and their conversion to gas exchange data is limited because of the different energy contents of leaves of various plant species.

10.8 FORMATION OF ENERGY RICH INTERMEDIATES :

The determination of simultaneous changes in the ATP and NADPH contents of assimilating tissues is not practical because of the small concentrations of these substances present and also their participation in metabolic processes other than photosynthesis. It is only possible to measure such changes with isolated chloroplasts than with whole plant tissues or leaves.

10.9 PROPERTIES OF THE PHOTOCHEMICAL APPARATUS:

It includes mainly the absorption, transfer and fluorescence of oxidised and reduced forms of photosynthetic pigments and other participating compounds in the photosynthetic electron transport chain (P-700, cytochromes, PC, PQ, ferredoxin, etc.). It is also possible to measure the rate of electron transport through PS –II, PS –I, whole chain electron transport with the use of suitable acceptors and donors. Like the measurements in method 9.8, these things are possible only with the isolated chloroplasts or membranes and are related with the rate of photosynthesis.

10.10 CHOICE OF METHODS:

The choice of methods is largely governed by the following:

10.10.1 Aim of the study:

In choosing a method, the following inter – related aspects may be taken into account.

stoichiometry of photosynthesis is that the net amount of O_2 evolved is equivalent to the net amount of CO_2 fixed. The photosynthesis quotient i.e. $+ O_2 / -CO_2$, is generally equal to 1. 0. Hence, preference for the determination of O_2 or CO_2 is largely governed by the technical considerations and by the type of plant to be studied.

O_2 determination is most common with submerged plants. The solubility of O_2 in water is very low, and O_2 determination is not complicated by different equilibrium states accompanying dissolution as in the case with CO_2 dissolved in water or solution. In land plants, O_2 production is determined less often, largely because of high concentration of O_2 in air (20.99% by volume). As a consequence, very sensitive methods are required to determine the small changes in O_2 concentration, relative to that in air, which results from photosynthesis. More recently, zirconium oxide oxygen analysers with adequate resolution are useful with intact leaves and plants have become available.

10.4 INFLUX OF CARBONDIOXIDE:

CO_2 influx is a measure of photosynthetic rate on a molecular basis equal to or similar to the efflux of O_2. This method is most frequently used with higher plants. The most frequently used instrument for the determination of CO_2 exchange by intact plants is the Infra Red Gas Analyser (IRGA) and measurements with this are adequately exact.

10.5 DRY MATER ACCUMULATION:

Dry matter accumulation and increase in the assimilatory surface area of a plant community are the basis of method used to determine the production of the entire stand in the natural environment. Using the procedures of growth analysis, these methods yield the estimates of net assimilation rate (NAR) from the relations between the accumulation of photosynthate and growth of the plant. NAR provides an integrated response of a large number of processes which differ in their individual response to the varying environment. Simultaneous measurements on a large amount of tissue or number of treatments are possible. These methods do not need expensive apparatus but are laborious and time consuming. They are very useful for measurements in large plant stands, where it is possible to sample and destroy some of the plants and repeat the samplings throughout the growing season. This uses simplest procedures and gives integrated estimates of net photosynthesis of the whole plant over periods of days or weeks.

10.6 ACCUMULATION OF PRODUCTS:

The amount of photosynthates produced is a measure of the photosynthesis similar in some respects to the amounts of CO_2 absorbed. However, the quantity of products

Observations:

Sr.No.	Observation	Sunflower leaf discs	Sorghum Plants
1	Initial dry weight	0.51 mg	0.61 mg
2	Final dry weight	0.91 mg	0.93 mg
3	Interval of sampling	10 Day	
4	Number of plants / leaf discs harvested		

Rate of photosynthesis (mg) Day

Draw diagram of Ganongs leaf punch.

Question: What are the errors observed in calculating the rate of photosynthesis by dry weight difference method?

Rate of photosynthesis (mg/pay) sunflower = Final Dry wt - Initial Dry wt / Interval of Sampling

$= \frac{0.91 - 0.91}{10}$

$= \frac{040}{10} = 0.04$

Rate of photosynthesis (mg/day) sorghum $= \frac{0.93 - 0.61}{10}$

$= \frac{0.32}{10} = 0.032$

Rate of photosynthesis = final dry wt - Initial dry wt / Interval of sampling x no of plant

Sunflower $= \frac{0.91 \times 0.51}{10 \times 5}$

$= \frac{032}{50}$

$= 0.0064$

Procedure:

Select young leaf of sunflower plant. Remove the definite number of leaf discs (5-10) from half part of the leaf with the Ganong's leaf punch. Dry them at 40^0 C in the oven for about 24 hours. Then record their weight accurately in milligrams. The plant with the other leaf halves intact is then exposed to light. After a period of 10 days illumination remove same number of leaf discs from the attached i.e. intact half of the leaf and dry them in same way and record the dry weight. From these observations calculate the rate of photosynthesis using following formula.

$$\text{Rate of photosynthesis (mg/day)} = \frac{\text{Final dry weight} - \text{initial dry weight}}{\text{Interval of sampling}}$$

Limitations:

This method takes in to account increase in thickness of leaf due to photosynthesis and not increase in size of leaf. Secondly it does not give rate of photosynthesis for entire plant.

2. Field method:

Requirement: Sorghum crop, oven, sample (brown paper) bags, balance etc.

Procedure:

Raise a sorghum crop. When it is about 30 days old harvest 5 plants at random. Separate them into leaves and stem and put in sample (brown paper) bags. Dry them in oven, initially at 90^0 C for 4 hours and then at 65^0 C till constant weight is obtained. Then weigh the sample and record its weight. Repeat the same procedure after 10 days interval and calculate the rate of photosynthesis by above formula and divide it by number of plants harvested so that rate of photosynthesis (g/day/plant) will be obtained.

$$\text{Rate of photosynthesis (mg/day)} = \frac{\text{Final dry weight} - \text{initial dry weight}}{\text{Interval of sampling} \times \text{No. of plants harvested}}$$

Practical utility: The crop having more rate of photosynthesis indicates that it's growth will be more.

11.2 Measurement of CO_2 produced during root respiration:

11.2.1 Materials: Plant seedlings, conical flasks, burette, 1 N $Ba(OH)_2$, 0.1 N HCl and Phenolphthalein indicator.

11.2.2 Procedure:

1. Take known amount of $Ba(OH)_2$ solution in the conical flask
2. Place the roots of growing seedling in $Ba(OH)_2$ solution for one hour
3. Remove the seedlings from $Ba(OH)_2$ solution and record the root weight
4. Titrate the $Ba(OH)_2$ solution against 0.1 N HCl by using phenolphthelene indicator and note down the reading (designate it as 'X' cc)
5. Titrate $Ba(OH)_2$ solution (blank) against 0.1 N HCl by using phenolphthelene indicator (designate it as 'Y' cc)
6. Calculate the amount of CO_2 produced per g. of root weight per hour by the following formula.

$$\text{mg } CO_2 \text{ produced / g / hour} = \frac{Z \times 0.1 \times 22.4}{W \times A}$$

Where,

$Z = Y - X$

W = Weight of the root

A = time taken (min)

11.2.3 Observations:

11.2.4 Conclusions:

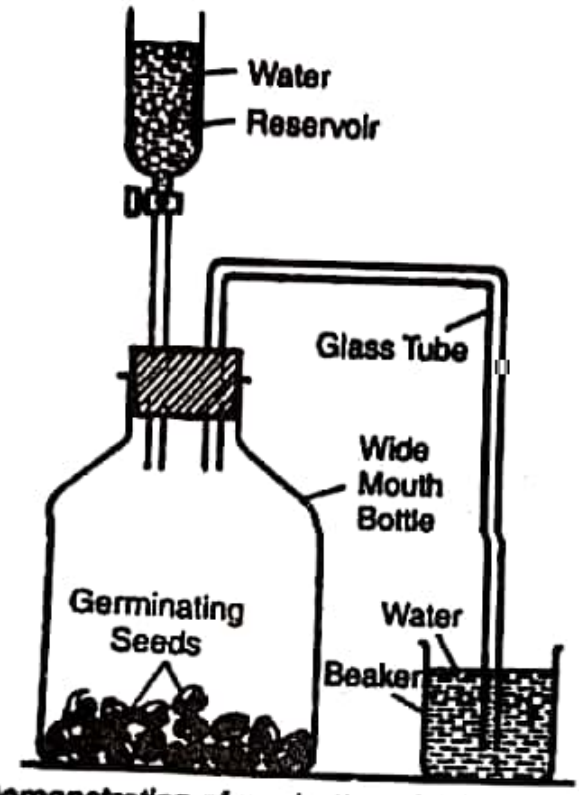


Fig. 43. Demonstration of production of CO_2 during respiration.

Note: Above diagram is just indicative, students will have to draw a diagram comprising conical flask with side spout connected by a rubber tube instead of glass bottle and glass bent tube.

11.1.3 Observations: After some time the air bubbles come out and the lime water becomes milky.

11.1.4 Results: Lime water turns milky due to the carbon dioxide evolved during the process of germination of seeds. When water is poured by opening the stopcock of water reservoir, it drives out the air through the rubber tube connected to spout and as the air passes through the lime water, the latter turns milky due to the fact that the air contains carbon dioxide.

11.1.5 Conclusions:

EXERCISE NO. 11

Date:

MEASUREMENT OF RESPIRATION

Respiration is an oxidation and reduction process in which respirable substrates such as carbohydrates, proteins, fats, etc; are oxidised to CO_2 and O_2 absorbed is reduced to water with the release of energy.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 674K.Cal \text{ (Energy)}$$

11.1 Demonstration of CO_2 release during respiration:

11.1.1 Materials : Conical flask with side spout, Rubber cork, Rubber tube, dropping funnel, stop cock , Pinch cock, Water, Flower buds or germinating seeds, Beaker, Lime water and Plaster of Paris.

11.1.2 Procedure:

1) Take a conical flask with a side spout and connect a rubber tube to the spout and another end of the tube is dipped in a beaker containing water and attach pinch cock to the rubber tube.
2) Place some flower buds or germinating seeds in conical flask and close the mouth of flask with rubber cork
3) Introduce the dropping funnel (water reservoir fitted with a stop cock) into the flask through the opening of rubber cork.
4) Make the apparatus airtight with plaster of paris
5) Fill up the dropping funnel completely with water
6) After 24 hours, water containing beaker is replaced by lime water beaker then release the stop cock and allow the water to run down from dropping funnel into the flask (respiratory chamber). Simultaneously, release the pinch cock attached to the rubber tube.
7) Record the observations.

Exercise 12-13

Nutno-physioloay

Q1) Define Neutrophysioloay

It deals with study nutrient & there role in growth a health & deceases of plant.

Q2) classification of mineral element.

i) Major element = C, H, O

ii) Macro element = N, P, K, Ca, S, Mg, Fe.

iii) Micro element = Mn, Cu, Zn, S, Mo, Cl

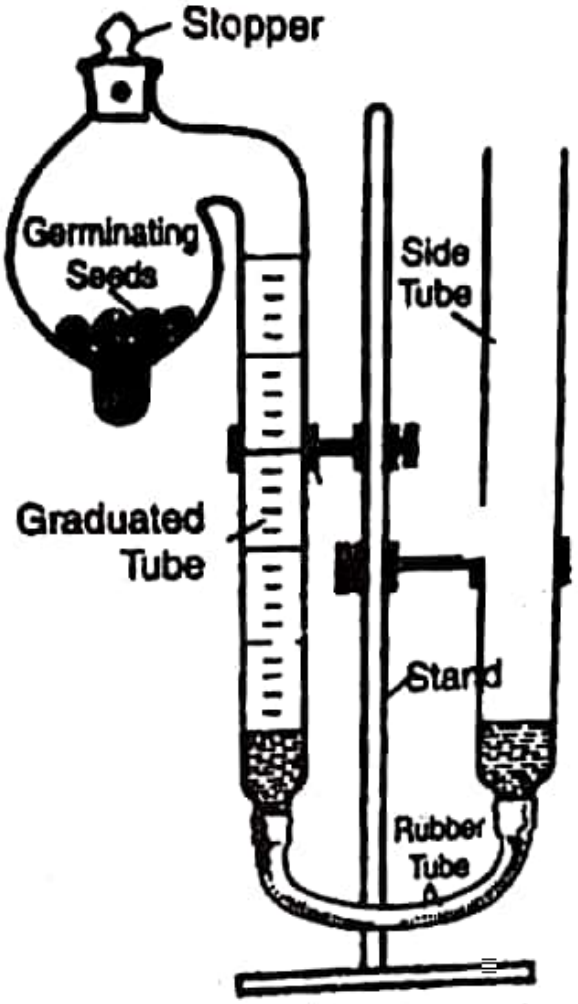


Fig. 51. Ganong's respirometer.

11.3.3 Observations:

11.3.4 Conclusions:

Practical utility: The R.Q. values gives idea about nature of seeds.

Draw diagram of all the experiments and record observations.

Question: What is the role of NaOH and pyrogallol solutions?

11.3 MEASUREMENT OF RATE OF RESPIRATION AND DETERMINATION OF R.Q. WITH THE HELP OF GANONG'S RESPIROMETER IN DIFFERENT SEEDS

Principle:

The respiratory quotient is the ratio of volume of CO_2 released to the volume of O_2 absorbed simultaneously in the respiratory process by a given weight of the tissue in a given period of time at standard temp. and pressure. The value of the R.Q. depends upon the nature of respiratory substance utilised in respiration. The R.Q. value of carbohydrates may be one or unity, that of fats and protein may be less than one, while that of organic acid it is more than one.

11.3.1 Requirement: Ganong's respirometer, germinating jowar seeds, stands, beaker, mercury, NaOH, Pyrogallol solution, measuring cylinder, scissors.

11.3.2 Procedure:

Germinate few jowar seeds for about 2 to 3 days. Then measure 20 cc of seeds by water displacement method. Take a dry and clean respirometer. Fix it to retort stand. Put 20 cc of germinating seeds carefully in it. Connect the free end of granduated tube to glass tube by means of rubber tube.Pour the mercury in glass tube. Raise or lower glass tube so that mercury level in graduated tube reaches to 100 cc mark. Adjust the mercury level accurately at 100 cc mark. Stopper the side bulb.

After 24 hours, prepare solutions of NaOH and pyrogallol. First place the beaker of NaOH solution below experimental setup and dip the mid portion of rubber tube in it. Cut the rubber tube carefully in the NaOH solution with the help of scissor. Note the mercury comes down and its space is occupied by NaOH solution in the graduated tube. This level gives the volume of CO_2 absorbed by the NaOH (V_1).

Close the cut end of rubber tube of respirometer with the fingers and remove it from NaOH solution and dip it quickly in pyrogallol solution. Note that pyrogallol solution rise in the graduated tube and NaOH solution comes down. When steady level reaches, record the volume of pyrogallol solution above the final level of NaoH solution. This gives the amount of oxygen absorbed by pyrogallol solution. From that calculate the volume of oxygen absorbed by seeds during respiration.

Then calculate the respiratory ratio:

$$R.Q. = \frac{CO_2}{O_2} = \frac{V_1}{V_2}$$

iii) Yellowing of leaves or chlorosis (Interveinal chlorosis)
iv) Stunted growth
v) Mottling of leaf (irregular spotted surface)
vi) Abnormal curling of leaves
vii) Pre-mature drying and withering of plant parts

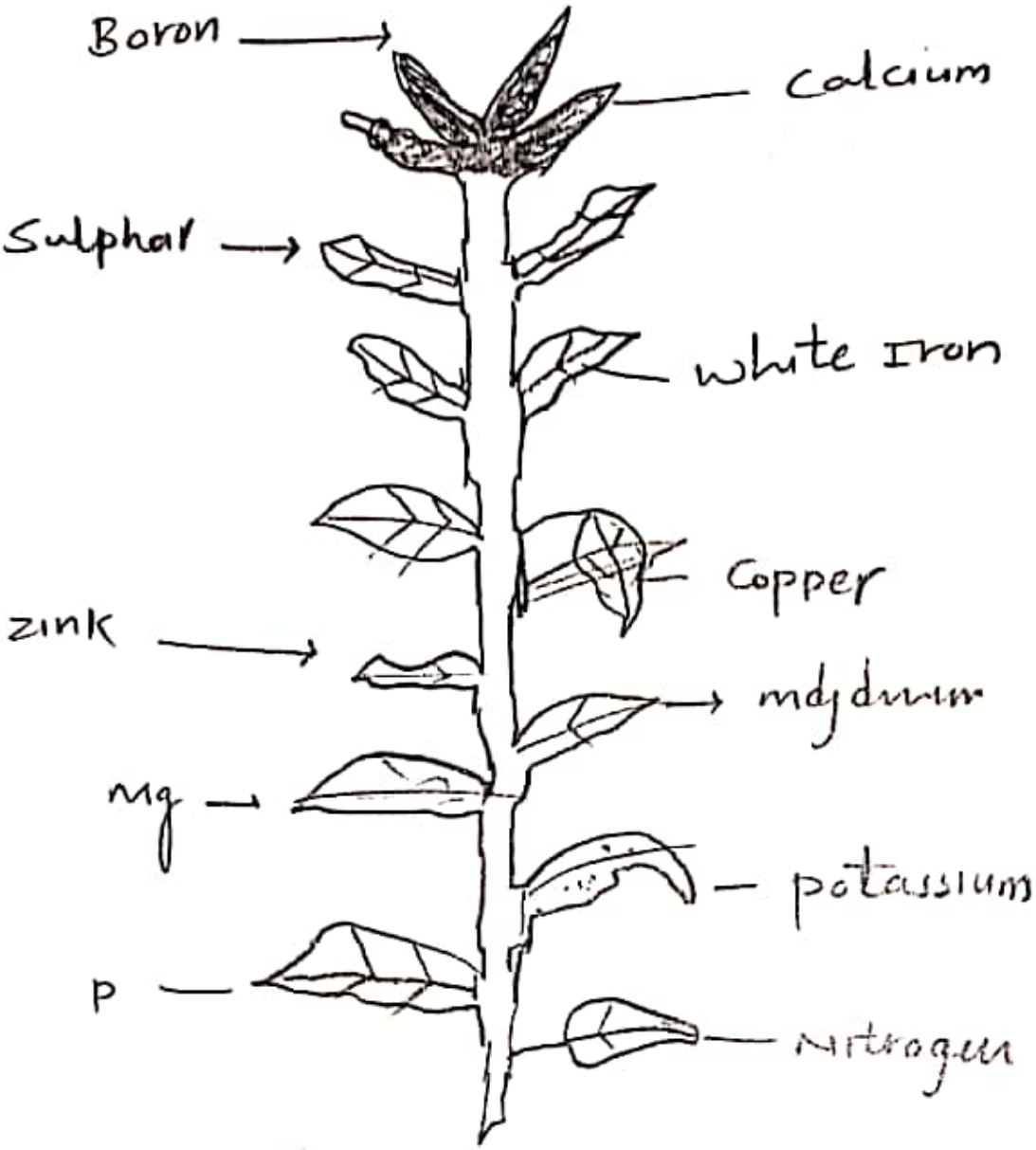
Experiment 1: Rapid tissue test for nutrients

The crop growth and productivity is conditioned by many factors of which, the nutrient status (Content) of plant parts such as leaf, stem, etc play a critical role. Moreover the leaf and stem are considered as the indicator parts of plants for assessing the nutrients content of plant. Each crop plant requires the essential element at a specific concentration at different growth stages and it is known as 'critical level'. When the nutrients content of plant depletes below the critical level the plants may exhibit some symptoms. The requirement or otherwise the availability of nutrients can be assessed by i) plant diagnosis ii) soil analysis and iii) plant analysis by two methods a) by qualitative test and b) by quantitative estimation. Based on the plant or soil tests, the required nutrients can be applied for crops to sustain the growth and rectify the deficiency disorders. The rapid tissue test would pave way for rectifying the nutritional problems for quick recovery; however the quantitative estimation of both plant and soil for nutrients concentration will be more useful and economic for applying fertilizers either as basal or foliar and would be the long term strategy to cope up with nutritional problems.

On dry weight basis, the normal healthy cultivated crop plant will have the foliar concentration of essential elements. Nevertheless it will vary depends up on the variety, type of soil, growth stage and other environmental and cultural operations.

Nitrogen	: 1.0 to 3.0 %	Iron	: 20 to 100 ppm
Phosphorus	: 0.05 to 1.0 %	Zinc	: 15 to 50 ppm
Potassium	: 0.8 to 1.2 %	Manganese	: 2.0 to 10 ppm
Calcium	: 0.3 to 0.6 %	Copper	: 10 to 20 ppm
Magnesium	: 0.2 to 0.4 %	Boron	: 5 to 15 ppm
Sulphur	: 0.2 to 0.3 %	Molybdenum	: 0.5 to 5.0 ppm

For rapid tissue test to assess the nutrient status, different parts of plant should be taken as indicator tissue and some of the representative crops are furnished below:



Deficiency systems of Leaves

EXERCISE NO. 12 & 13

Date:

NUTRIO-PHYSIOLOGY

The mineral elements are very much essential for the successful growth and development of a majority of the plants. All the essential elements except carbon, hydrogen and oxygen are taken by the green plants from the soil and water. Whereas, C, H, O are taken from the soil and atmosphere. Out of 105 elements existed in earth crust only 16 elements are essentially required by the plants for growth and development without which plant can not complete its life cycle.

Arnon's criteria for essentiality of nutrients:

1. Plant will be unable to grow normally and complete its life cycle in the absence of elements
2. The elements are specific and cannot be replaced by other elements
3. The elements play direct role in plant metabolism

Classification of mineral elements:

1. Major elements : C, H, O
2. Macro elements : N, P, K, Ca, Mg, S and Fe
3. Micro/trace elements: Mn, Cu, Zn, B, Mo, Cl.

General roles/functions of essential elements:

1. Elements provide basic structure to the plants and they are constituents of organic material (C, H, O, S)
2. Trigger and control mechanisms: by controlling osmotic potential, membrane permeability, electro-potential and conductance (K, Mg, Ca, Cl, Na)
3. Structural influences : by binding to organic molecules particularly enzymes and thus altering their conformation (K, Ca, Mg, Mn) involved in accepting the electrons and thus many catalyse or polarize reactive groups (Mg, Ca, Mn, Fe, Cu, Zn).
4. Redox reactions : These ions are essential components of prosthetic groups, which bring about electron transfer (Cu, Fe, Co, Mn).

Identification of deficiency symptoms:

Deficiency symptoms are identified on the basis of;

i) Region of occurrence (older leaves or younger leaves)

ii) Presence of necrotic or dead spots

A tea spoonful of freshly chapped leaf bits are taken in a test tube and 10 ml of ammonium molybdate reagent is added and kept for few minutes. After shaking, a pinch of stannous chloride is added. Colour development is observed.

Dark blue : Sufficient Phosphorus
Bluish green : Slightly deficient Phosphorus
No colour : Highly deficient Phosphorus

3. Potassium

Reagent: (1) Sodium cobalt nitrate reagent, (2) Ethyl alcohol (95%).

Take 5 gm cobalt nitrate and mix with 30 gm of sodium nitrate in 80ml of distilled water. To this, 5ml of glacial acetic acid is added. The volume is made up to 100 ml distilled water. Dilute reagent prepared (5 ml) with 15 mg sodium nitrate to 100 ml using distilled water.

Finally cut leaf bits are taken in a test tube and 10 ml diluted reagent is added and shaken vigorously for few minutes and kept for 5 minutes. Then add 5 ml of ethyl alcohol reagent, allowed to stand for 3 minutes. The solution is observed for the formation of turbidity.

No turbidity : Deficiency of Potassium
Slightly turbidity : Moderate deficiency
High turbidity : Sufficient Potassium

4. Calcium

Morgan's Reagent: 30 ml of glacial acetic acid and 100 grams of sodium acetate are dissolved in a little of distilled water

Procedure: 0.5 g of finely cut plant material is taken into a glass vial (both of healthy plant and deficient plant in different vials) and 5 ml of Morgan's reagent is added. After allowing it to stand for 15 minutes, 2 ml of glycerin and 5 ml of 10% ammonium oxalate is added and the solution is shaken for 2 minutes. The turbidity resembling after 15 minutes indicate the amounts of calcium in normal plant tissue.

5. Magnesium

Reagents:

(1) 5% pure sucrose solution
(2) 2% Hydroxylamine hydrochloride
(3) Titan yellow
(4) Sodium hydroxide

150 mg of Titan yellow is dissolved in 75 ml of 95% ethyl alcohol and 25 ml distilled water. This solution is stored in darkness.

Crops	Nutrients					
	N	P	K	Ca	Mg	S
Cereals	Stem/Midrib	Leaf blade	Leaf blade	Leaf lamina	Leaf lamina	Leaf blade
Pulses	Petiole	Leaf blade	Leaf blade	Leaf lamina	Leaf lamina	Leaf blade
Oil seeds	Petiole	Leaf blade	Leaf blade	Leaf lamina	Leaf lamina	Leaf blade
Cotton	Petiole	Petiole	Petiole	Petiole	Petiole	Petiole
Banana	Leaf lamina	Leaf lamina	Leaf lamina	Leaf lamina	Leaf lamina	Leaf lamina
Papaya	Petiole	Petiole	Petiole	Petiole	Petiole	Petiole
Vegetables	Petiole, Leaf blade	Petiole Leaf blade	Petiole, Leaf blade	Petiole, Leaf blade	Petiole, Leaf blade	Petiole, Leaf blade

Fruit trees

Either leaf blade/mid rib/leaf lamina can be taken.

Ornamentals, Tea, coffee, etc.,

The leaf blade should be taken.

Micronutrients:

The leaf lamina/ leaf blade/ mid rib portion of leaf can be taken.

Procedure for tissue test

1. Nitrogen

Reagent: 1-% diphenylamine in conc. sulphuric acid.

Small bits of leaf or petiole are taken in a petridish and a drop of 1% diphenylamine is added. The development of blue colour indicated the presence of nitrate – nitrogen. The degree of colouration indicates the amount of nitrogen present in that leaf.

Dark blue : Sufficient Nitrogen

Light blue : Slightly deficient Nitrogen

No colour : Highly deficient Nitrogen

2. Phosphorous

Reagents: (1) Ammonium molybdate solution, (2) Stannous chloride powder.

Eight gm ammonium molybdate is dissolved in 100 ml of distilled water. To this, add 126 ml of conc. Hydrochloric acid (HCl) and volume is made up to 300 ml with distilled water. 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 using distilled water.

procedure of tissue test

1) Nitrogen

1:1 Conc HCl.

Amount of N_2 present in leaf

Dark blue → sufficient N_2
Light blue → Difficient N_2
No Colour → High Difficient N_2.

2) phosphorous

Colour darkoap observed

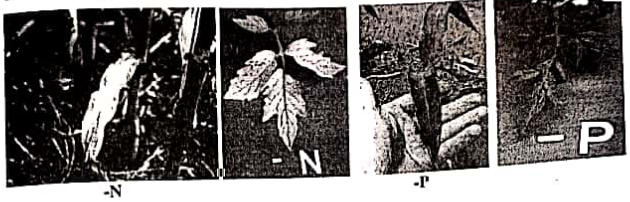
Dark blue → sufficient phosphorous
Blush green → slightly Difficied phos.
No colour → Highly difficied phos

3) potassium

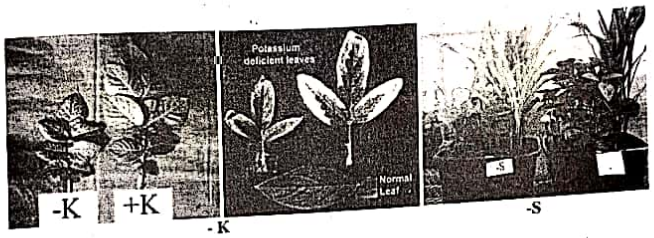
Soln is observe for the formation of turbidity

No turgidity → Diticiency of K
slightly tur → Moderate diticiency
High turgidy → Sufficient K

Observation: After 1-2 months of treatment imposture the visual symptoms of treated and control plants were compared.



-N -P



- K -S

- Ca -S

- Mg

Table-12.2: Nutrient composition for test solutions:

-N	Use KCl and CaCl in place of KNO_3 and $CaNO_3$. and NaH_2PO_4 in place of $NH_4H_2PO_4$
-P	Use $CaNO_3$ in place of $NH_4H_2PO_4$
-K	Use Na_2NO_3 in place of KNO_3
-Ca	Use Na_2NO_3 in place of $CaNO_3$
-Mg	Use Na_2SO_4. $7H_2O$ in place of $MgSO_4$. $7H_2O$
-S	Use $MgCO_3$, $Cu\ CO_3$, $Zn\ CO_3$ and $Fe\ CO_3$ in place of $MgSO_4$. $7H_2O$, $CuSO_4$ .5 H_2O, $Zn\ SO_4$ .7 H_2O and $FeSO_4$.$7H_2O$ respectively
-B	Do not use H_3BO_3

Procedure:

1. Coarse sand were first collected, wash repeatedly with tap water, dilute mineral acid and rain or distilled water to remove all organic matter and nutrient if any present in the sand.
2. The sands were then sundried and filled up in 3 equal size pots which were marked as A, B and C.
3. Seeds of indicator/ typical plant were collected and soak in water for few hours.
4. Two seeds were sown in each pot after surface sterilization.
5. Two sets of nutrient solutions were prepared, one with all nutrients as complete solution for control plant and another with deficient of one particular element as test solution for treated plants.
6. The sand of pot A was continuously kept moisten with control solution (diluted 10 times with distilled water) and considered as control pot.
7. Likewise the sand of pot B and C were also continuously kept moisten with diluted test solution and marked as treated pots.
8. The growth and development of the plants were critically observed and utmost care was taken for them.
9. After few days of sowing the seeds one seedling was removed keeping the healthy one in each pot.

Procedure

To a tea spoonful of finely cut material, following reagents are added in sequence. One ml of 5 % sucrose solution, 1 ml of 2 % Hydroxylamine hydrochloride and 1 ml of Titan Yellow. Finally solution was made alkaline with 2 ml of 10% NaoH. Red colour indicates the presence of magnesium and yellow colour indicates absence or traces of Magnesium.

6. Iron

Finely cut leaf materials (0.5g) are taken into a glass vial and 1ml of con. Hcl is added in it. After 15 minutes, 10ml of distilled water and 2-3 drops of con HNO_3 are added. 10 ml of this solution is pipetted out into a specimen tube after 2 minutes and 5ml of 20% ammonium thiocynate is added and stirred. Further, 2 ml of amyl alcohol is added, shaked well and allowed to stand for few minutes. The intensity of red colour in amyl alcohol layer indicates the quantity of iron.

Experiment 2: Studies on of deficiency and toxicity symptoms of macro elements in the plant

Theory: Quantitative estimation of any mineral element in the soil and plant parts helps to diagnose nutrient deficiency or toxicity. Likewise visual observations of specific symptoms also give indication about deficiency or toxicity of a particular nutrient. Leaves are the best indicators and therefore, foliar diagnosis has been extensively used in this purpose. Indicator plants which produce typical symptom quickly under deficient or excess condition greatly help in nutrient management system.

Table-12.1: Nutrient composition for complete solution. (Arnon and Hogland, 1940 nutrient solution):

Salt	Concentration
KNO_3	1.02 g/L
$Ca(NO_3)_2$	0.492 g/L
$NH_4H_2PO_4$	0.23 g/L
$MgSO_4.7H_2O$	0.49 g/L
H_3BO_3	2.86 mg/L
$MnCl_2.4H_2O$	1.81 mg/L
$CuSO_4.5H_2O$	0.08 mg/L
$Zn SO_4.7 H_2O$	0.22 mg/L

Table 13.2: Preparation of Hoagland solution from stock solution (ml/litre) for the development of deficiency symptoms

Compound	Complete sol.	N	P	K	Ca	Mg	S	Fe	Zn	Mn	B	Cu	Mo
KNO_3	6 ml	-	6 ml	-	6	6	6	6	6	6	6	6	6
$Ca(NO_3)_2.4H_2O$	4	-	4	4	-	4	4	4	4	4	4	4	4
$NH_4H_2PO_4$	2	-	-	2	2	2	2	2	2	2	2	2	2
$MgSO_4.7H_2O$	1	1 ml	1	1	1	-	-	1	1	1	1	1	1
$NaNO_3$	-	-	-	6	8	-	-	-	-	-	-	-	1
$MgCl_2$	-	-	-	-	-	-	1	-	-	-	-	-	-
Na_2SO_4	-	-	-	-	-	1	-	-	-	-	-	-	-
$CaCl_2$	-	4	-	-	-	-	-	-	-	-	-	-	-
KCl	-	6	-	-	-	-	-	-	-	-	-	-	-
NaH_2PO_4	-	2	-	-	-	-	-	-	-	-	-	-	-
NH_4Cl	-	-	2	-	-	-	-	-	-	-	-	-	-
Micronutrient													
KCl	1	1	1	-	1	1	1	1	1	1	1	1	1
H_3BO_3	1	1	1	1	1	1	1	1	1	1	-	1	1
$MnSO_4.7H_2O$	1	1	1	1	1	1	-	1	1	-	1	1	1
$ZnSO_4.7H_2O$	1	1	1	1	1	1	-	1	-	1	1	1	1
$CuSO_4.5H_2O$	1	1	1	1	1	1	-	1	1	1	1	1	1
H_2MoO_4	1	1	1	1	1	1	-	1	1	1	1	1	-
Fe citrate	1	1	1	1	1	1	1	-	1	1	1	1	1

Table 13.1. Preparation of stock solution:

Chemical	Formula	Amount (g/litre)	Concentration of the solution (Molar)
A. Ammonium acid	$NH_4H_2PO_4$	23	0.20
B. Ammonium nitrate	NH_4NO_3	40	0.50
C. Calcium nitrate	$Ca(NO_3)_2$	189	1.15
D. Calcium chloride	$CaCl_2$	29	0.26
E. Magnesium chloride	$MgCl_2.6H_2O$	41	0.20
F. Magnesium nitrate	$Mg(NO_3)_2.6H_2O$	51	0.20
G. Magnesium sulphate	$MgSO_4.7H_2O$	99	0.40
H. Potassium acid phosphate	KH_2PO_4	27	0.20
I. Potassium nitrate	KNO_3	121	1.20
J. Potassium sulphate	K_2SO_4	87	0.50
K. Ferric chloride	$FeCl_3.6H_2O$	10	0.04
L. Micronutrient stock solution elements mixed in one litre of distilled water			Molarity ($X10^{-2}$)
Boric acid	H_3BO_3	0.72	1.2
Copper chloride	$CuCl_2.H_2O$	0.02	0.012
Manganese chloride	$MnCl_2.4H_2O$	0.45	0.230
Zinc chloride	$ZnCl_2$	0.06	0.044
Molybdic acid	$H_2MoO_4.H_2O$	0.01	0.006

M. Fe EDTA (Iron complex of ethylene diamine tetra acetic acid) : Dissolve 1340 mg. EDTA in 500 ml of distilled water and heat. While still hot add 990 mg $FeSO_47H_2O$ and stir vigorously.

Experiment 3: Studies on of deficiency symptoms of macro and micro elements in the crop plants

In order to observe the deficiency symptoms of mineral elements in crop plants, the plants are often grown in artificial nutrient media to which a solution of all the essential elements are added except for the element being tested. At optimum levels, the available elements tend to enhance the biochemical reactions for which they are essential and inhibit reactions that require the deficient element leading to particular symptoms in plants.

Materials: Seeds of bean or tomato or sunflower, aqueous stock solution (Hoagland solution) in glass bottles (Table 13.1), volumetric flasks, Pipettes, measuring jars, earthen pots, sand, HCl, distilled water.

Procedure:

1. Prepare the required amount of stock solution (given in Table 13.1) in one liter of volumetric flask. Use only one chemical per container.
2. Select the pots, which are for knowing the deficiency symptoms of elements and one for adding whole stock solution.
3. Fill up the pots up to ¾ of its height with pure sterilize sand and fix a glass tube in it at one side to facilitate addition of water and nutrient solution.
4. Select six well developed seeds and sow them ¼ inch deep into the pot containing sand.
5. Moisten the sand with distilled water and cover the surface with filter paper. Keep the filter paper moist to create humidity inside the pot for rapid germination.
6. Remove the filter paper after the seeds have sprouted well. Continue adding small amount of distilled water till the seedlings get well established (about 7 to 10 days old).
7. Thin out the seedlings so that, only three seedlings should be retained in the pot.
8. Pipette exact quantity of nutrient solution from each stock solution (as given in Table 13.2) and add to the pot through glass tube. Then add required amount of distilled water. One pot is kept for adding whole stock solution.
9. Drain off the solution at least once in a week from the pot through drain by adding distilled water to the pot.
10. Plug the drainage hole and add nutrient solution as before.
11. Make weekly observations and take note on the appearance of the plants and record the observations after three and five weeks of growth.

Observation to be recorded:

Treatments	No. of days taken for ripening
Ethrel (5 ppm)	
Ethrel (10 ppm)	
Control	

Practical utility: By applying ethylene we can enhance the rate of ripening in fruits like apple, oranges, banana and limes, while by inhibiting the production of ethylene we can delay ripening when required.

Question: Why ethylene treated fruits ripe earlier?

EXERCISE NO. 14 Date:

STUDIES ON EFFECT OF ETHYLENE ON FRUIT RIPENING

Principle: Ethylene the hormone promotes the ripening of some fruits:

In everyday usage, the term fruit ripening refers to the changes in fruit that make it ready to eat. Such changes typically include softening due to the enzymatic breakdown of the cell walls, starch hydrolysis, sugar accumulation, and the disappearance of organic acids and phenolic compounds, including tannins.

Because of their importance in agriculture, the vast majority of studies on fruit ripening have focused on edible fruits. Ethylene has long been recognized as the hormone that accelerates the ripening of edible fruits. Exposure of such fruits to ethylene hastens the processes associated with ripening, and a dramatic increase in ethylene production accompanies the initiation of ripening. However, surveys of a wide range of fruits have shown that not all of them respond to ethylene.

All fruits that ripen in response to ethylene exhibit a characteristic respiratory rise called a *climacteric* before the ripening phase. Such fruits also show a rise of ethylene production immediately before the respiratory rise. Apples, bananas, avocados, and tomatoes are examples of climacteric fruits. In contrast, fruits such as citrus fruits and grapes do not exhibit the respiration and ethylene production rise and are called *nonclimacteric* fruits. In climacteric fruits, treatment with ethylene induces the fruit to produce additional ethylene, a response that can be described as autocatalytic. Low temperature and low O_2 inhibit ethylene production and fruit ripening.

Requirements: Ethrel (5 ppm and 10 ppm), bunches of unripened matured banana fruits, polyethylene sheets or bags, atomizer etc.

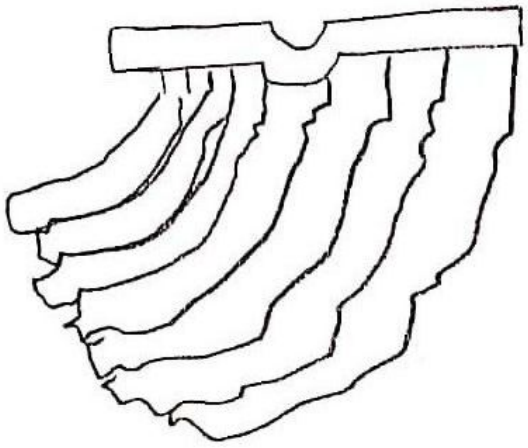
Procedure:

1. Take three uniform bunches of unripened matured banana fruits
2. Spray one bunch with 5 ppm Ethrel and second bunch with 100 ppm Ethrel
3. Keep a third bunch without spray of Ethrel (control)
4. Cover all the bunches with polythene sheets or bags
5. Keep them for 6 to 7 days and observe the period required for ripening as well as development of uniform yellow colour etc.

Exercise 14

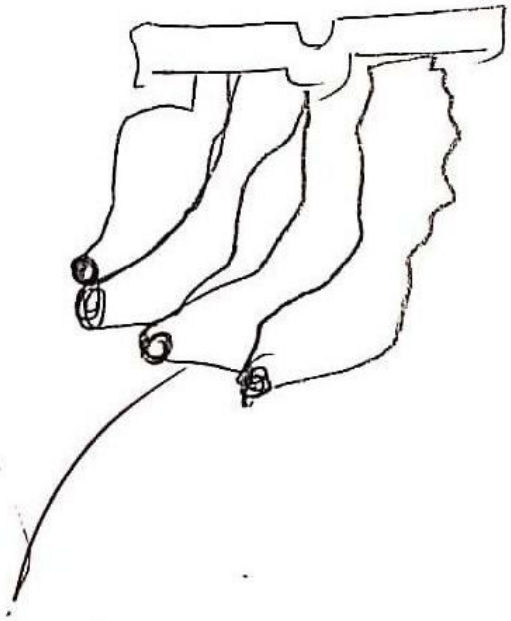
Studies on effect of etylene on fruit Ripenny.

eg Bunch of Bañanas



Bunch of banana without ethylene spray.

Bananas



with ethylene spray.

(B) DEMONSTRATION OF EFFECT OF OSMOTIC PRESSURE (POTENTIAL) ON SEED GERMINATION

Principle: The embryo lies dormant in the seed but when the later is supplied with water the embryo becomes active , tends to grow and later on develops into a seedling. The process by which the dormant embryo awakes up ,grow out of seed coat and establishes itself as a seedling is known as germination. A very negative water potential (Ψw) exists in the dry seed. Therefore when such seeds are placed in pure water a stiff Ψw gradient is established and water is imbibed resulting germination of seeds.

For pure water the Ψw is the highest and is assumed as zero, since in biological system pure water is not found so the Ψw of all biological system is always –ve. The Ψw of a solution depends on its salt concentration. If a solution is highly concentrated due to the presence of salt, its Ψw is decreased which inhibits imbibition and thereby germination. The lack of water absorption by a plant growing in saline water is an example of physiological dryness.

Requirements: Sodium chloride, gram seed, distilled water, petri dishes, blotting paper, weight box, balance, oven etc.

Procedure:

1. Prepare100cc each of 0.5M, 1M, 2M, and 4M solutions of NaCl using distilled water (approximately O.P. of the solutions is 19, 38, 72 and 140 atm. respectively).
2. Gram seeds were surface sterilized by dipping the seeds in 0.1% $HgCl_2$ solution for one minute followed by repeated washing with tap water & finally with distilled water.
3. Fifty (50) number of healthy, uniform sized seeds were placed in five glass Petri dishes lined with blotting papers of which four were moistened uniformly with the above mentioned salt solutions and one in the distilled water for the entire period of investigation.
4. The Petri dishes were covered and kept in room temperature. Wet the petri dishes with respective solution when required.
5. Record the germination at 48 hours interval and express in percentage

Observation:

Sr. No.	Concentration of NaCl solution	O.P. of solution	No. of seeds sown	No. of seeds germinated	Shoot length (cm)	Root length (cm)
1	Distilled water	0	50			
2	0.5 M	19	50			
3	1.0 M	38	50			
4	2.0 M	72	50			
5	4.0 M	140	50			

4. Put only distilled water in one petri dish as a control.
5. Place the petri dishes under laboratory conditions. Wet the petri dishes with respective solution when required.
6. Record the germination at 48 hours interval and express in percentage
7. After 7 days after the treatment measure the length of radical and plumule.

Observations to be recorded:

Plant species: Any one C_3 and C_4 crop each as per availability of seeds

Water potential (bars)	Germination (%)	% over control	Radical length (cm)	% over control	Plumule length (cm)	% over control
0 (Control)						
-3						
-6						
-9						
-12						
-15						

Calculations:

Calculate the germination percentage, index and seedling vigour as under:

$$\text{(i) Germination \%} = \frac{\text{No. of seeds germinated}}{\text{No. of seeds sown}} \times 100$$

$$\text{(ii) Germination Index} = \frac{\text{Mean germination \%}}{\text{No. of days taken}}$$

(iii) Seedling Vigour = Mean germination x Seedling Length (Root length + Shoot length)

Question: Why the germination decreases in PEG and mannitol solutions?

Conclusion:

EXERCISE NO. 15

Date:

(A) DEMONSTRATION OF EFFECT OF MOISTURE STRESS ON SEED GERMINATION, GERMINATION INDEX AND SEEDLING VIGOUR INDEX

Growth and development of crop plants influenced by environmental factors like temperature, water, minerals *etc.* These factors should be provided at the optimum levels to give maximum yield potential. Any deviation from the optimal condition leads to apparent changes in physiological process prevailing for a longer period it leads to injury *i.e.*, strain. Moisture stress refers to the situation where transpiration exceeds the absorption such that plant experiences a stress or otherwise non-availability of water for various physiological functions in term it affect the plant growth and development at various stages. Water stress affects germination percentage. If the amount of moisture in the soil is less and dissolved salts are somewhat constant then it decreases water potential (Ψw). Therefore, if the water potential of soil solution is lower than that of seed, it cannot absorb water and cannot germinate.

Materials: Seed of both C_3 and C_4 plants, mannitol, PEG-6000, petri dishes, filter paper, beakers, distilled water, balance etc.

Procedure:

1. Prepare the following concentration (bars) of mannitol or PEG-6000 as per the details given in table.

Water potential (bars)	Mannitol (g) in 100 ml of distilled water	PEG-6000 (g) in 100 ml of distilled water
0 (control)	-	-
-3	2.148	7.78
-6	4.296	15.55
-9	6.444	23.33
-12	8.592	31.10
-15	10.740	38.88

2. Take healthy seeds in two sets in each crop and treat the seeds with $HgCl_2$.
3. Take 6 petri dishes in two sets each for mannitol and PEG-6000. Put 50 or 100 seeds on the filter paper on petri dishes and moist the filter paper with the respective concentrations of either mannitol or PEG-6000.

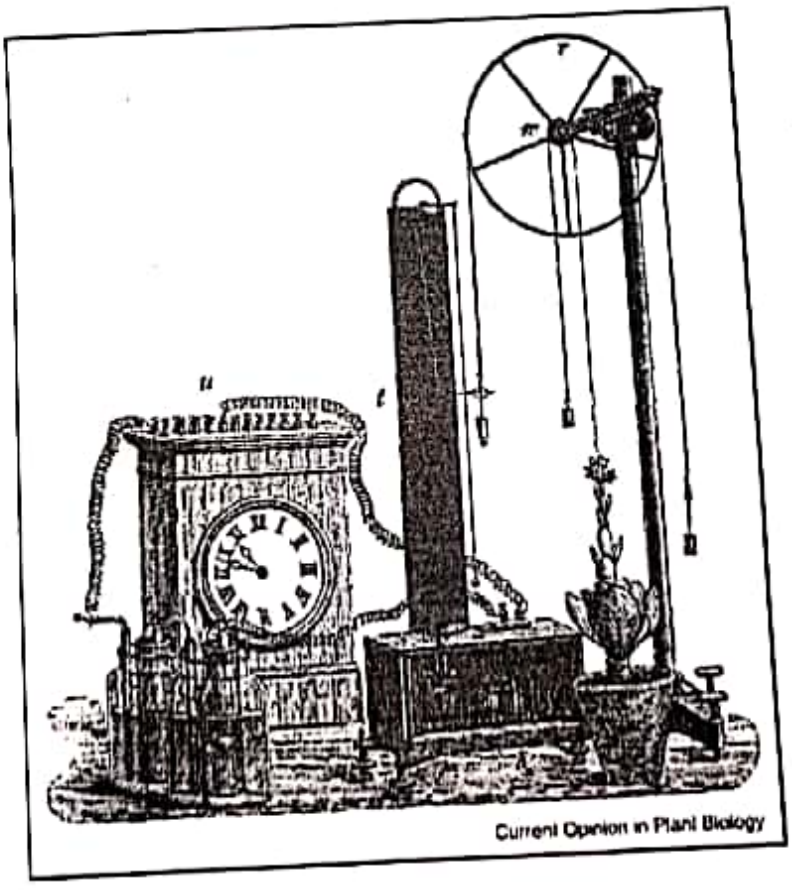
Experiment 2. Measurement of vegetative growth by Pfeffers automatic auxanometer

Materials: Young potted plant, Pfeffers automatic auxanometer, thread, weight etc.

Procedure:

1. A thread attached to the stem tip of a young potted plant is passed over the small wheel of a compound pulley which is fixed on the large wheel and accurately centered about the same axis.
2. A thin needle is attached to another thread which is passed over the large wheel and is made to draw upon the smoked surface of a paper fastened around a cylindrical drum.
3. The string is kept stretched by a counter balancing weight.
4. The drum is rotated slowly upon its axis by a clock-work, so that the indicator (needle) traces a line along its surface.
5. If there is no growth this line is horizontal. When growth occurs the indicator moves downwards and traces a spiral on the smoked paper.
6. The rate at which the drum revolves being known the rate of elongation can be easily calculated. The actual elongation is magnified in the record in the ratio between the radii of the large and small wheels. The perpendicular distance between two adjoining marks indicates the hourly growth.

Draw a diagram of both the experiments and record observations.



$$\text{Magnification (X)} = \frac{\text{Length of pointer x 2}}{\text{Diameter of pulley}}$$

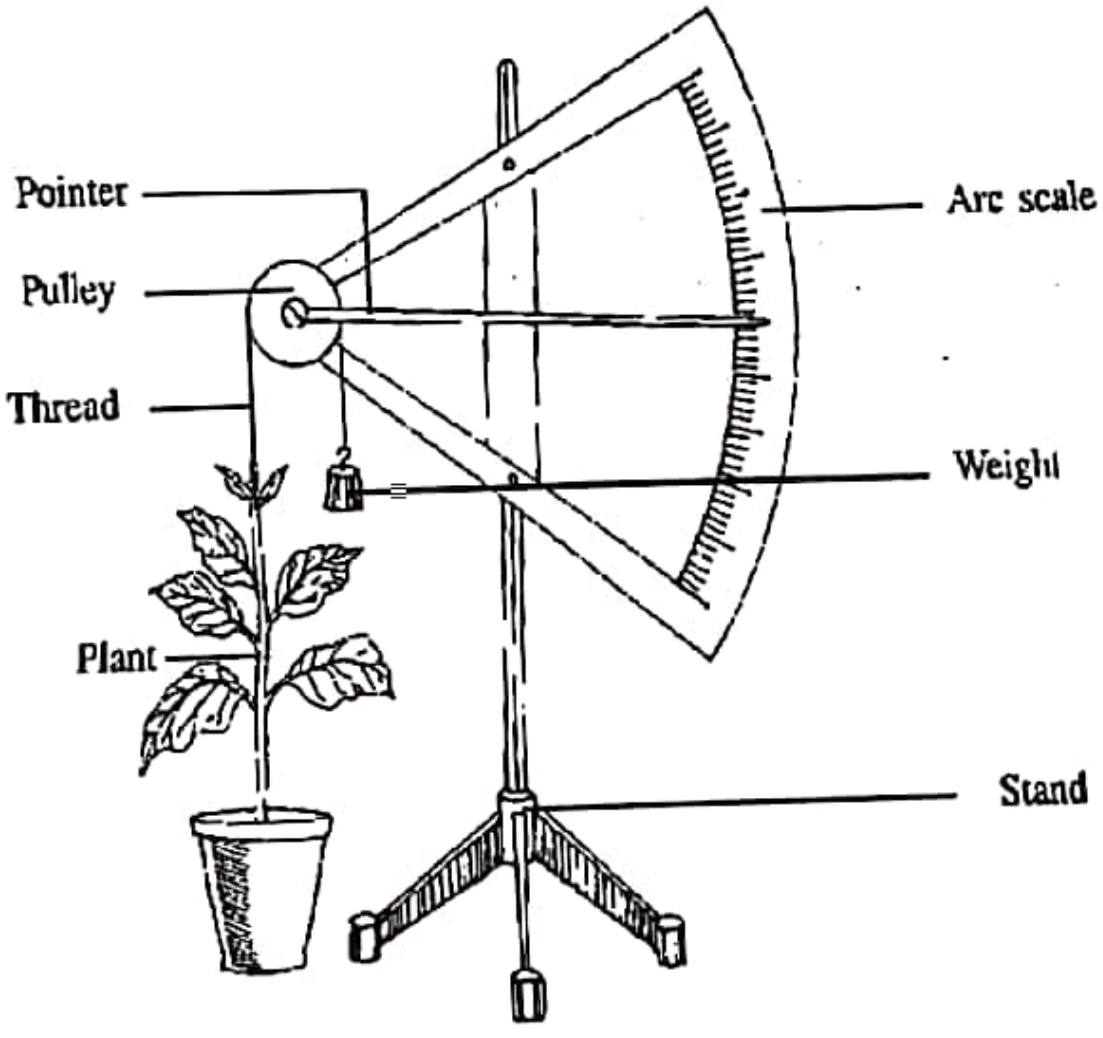
6. Calculate the growth of young growing potted plant by following formula:

$$\text{Growth} = \frac{\text{Distance travelled by the indicator}}{\text{X}}$$

Precautions:

i. Attach thread to the growing point of the plant very carefully.

ii. The potted plant should be healthy



Arc Auxanometer

EXERCISE NO. 16 **Date:**

MEASUREMENT OF PLANT GROWTH

Principle: Growth may be defined as a vital process which brings about a permanent and irreversible change in any plant or its part in respect to its size, form, weight and volume. (or any dimension of an organism). Growth can be measured by a variety of parameters as follows:

A. Fresh Weight B. Dry Weight C. Length D. Area

Commonly employed methods for measuring the growth in plants:

1. Direct method: Increase in length with scale.
2. Horizontal microscope
3. Auxanometers :
   i) Arc auxanometer or auxograph or arc indicator
   ii) Pfeffers automatic auxanometer
4. Bose's Crescograph
5. Space-marker disc

Experiment 1. Measurement of vegetative growth by Arc auxanometer

Materials: Young potted plant, Arc auxanometer, thread, weight etc.

Procedure:

1) In the arc auxanometer, a thread fixed (tied) to the stem tip of a potted plant is passed over a pulley to which is attached a long needle (pointer) which moves freely over a graduated arc.
2) The thread is kept tight by means of a weight tied to its free end. Note the initial reading on the graduated scale on the arc where pointer or indicator or needle rests.
3) The whole experiment is kept for one or two days and observed.
4) As the stem elongates due to growth, the weight moves the thread down. This moves the pulley and along with it the needle. The movement of the needle is read on the scale on the arc.
5) The growth is indicated in a magnified way. The magnification depends upon the diameter of the pulley and the length of the pointer (needle). For example if the diameter of the pulley is 4 inches and the length of the pointer is 20 inches, the magnification indicated on the arc will be ten times. Hence, for measuring the growth by arc auxanometer first measure the length of the pointer and diameter of the pulley and then calculate magnification (x) by following formula.

Suggested Readings:

SR	Name of Book	Author	Publisher
1	A Text Book Plant Physiology*	Dr. V. Verma	Emkay Publisher, Delhi-110 051
2	Plant Physiology*	S. N. Pandey& B. K. Sinha	Vikas Publishing House, New Delhi-110 014
3	Practical Plant Physiology*1967	Amar Singh	Kalyani Publisher, Ludhiana
4	Plant Physiology*2005	C. P. Malik	Kalyani Publisher, Ludhiana
5	Plant Physiology@	K. N. Dhumal, T. N. More and M. R. Munnali	Niraliprakashan, Pune
6	Plant Physiology	Robert M. Devlin & Francis H. Witham	CBS Publisher & Distributors, Delhi-110 032
7	Plant Physiology@	H. S. Shrivastava	Rustogi Publications, Meerut-250 002
8	Crop Physiology*	C. N. Chore, S. R. Ghadekar& R. K. Patil	Agromet Publisher, Nagpur-440 010
9	Plant physiology 2005@	S. Mukharji and A. K. Ghosh	New central book agency, Kolkatta
10	Plant physiology*2010	Taiz&Zeiger, E	Sinaurasso.Inc,USA
11	Introductory Plant physiology* 2013	G. Roy Noggle& George friz	PHI learning pvt ltd, N. Delhi
12	A Text Book Plant Physiology* 2005	c. P. Malik & A. K. Srivastava	Kalyani publisher, Ludhiyana
13	Plant Physiology@1993	S. Chandra Datta	Wiley Eastern ltd, Daryaganj, N. Delhi
14	Experiment in Plant Physiology –A Lab. Manual * 1998	DayanandBajracharya	Narosa publishing house, panchshil park, N. Delhi
15	Plant Physiology – fundamentals & applications @2005	Arvindkumar& S. S. Purohit	Agrobios (India), Jodhpur
16	Modern Plant physiology 2007@	R. K. Sinha	Narosa publishing house, panchshil park, N. Delhi

*Text book & practical book

@Reference book

Lecture	Topic	Weightage (%)
9	Dark reaction- C_3, C_4 and CAM plants factors affecting photosynthesis, Photorespiration	5
10	Respiration- Definition, types, glycolsis TCA cycle and electron transport chain	10
11	Fat metabolism- fatty acid synthesis and break down	5
12	Plant Growth Regulators, Definition, types , physiological role and Agricultural uses of PGRS.	10
13	Growth : Definition, types of growth, measurement of growth, growth analysis	5
14-15	Physiological aspects of growth and development of important cereals, pulses and oil seed crops	15
16	Photoperiodism- Definition, types, SDP, LDP and Day neutral plants- Induction a flowering and florigene concept	5
	Total	100

b) Practical

Experiment	Topic
1	Study of plant cell
2	Study of imbibitions
3	Study of osmosis
4	Study of plasmolysis
5	Study of root pressure
6	Measurement of rate of transpiration
7	Study of structure and distribution of stomata
8	Estimation of relative water content of tissue
9	Study of separation of photosynthetic pigment through paper chromatography
10	Measurement of rate of photosynthesis by different methods
11	Study of respiration and respiratory quotient
12	Rapid tissue tests for macro-elements
13	Rapid tissue tests for micro-elements
14	Study of use of PGR in fruit ripening
15	Effect of osmotic pressure on seed germination.
16	Measurement of Plant growth.

Syllabus

Course :	BOT 121	Credit:	2(1+1)	Semester-II
Course title:	Fundamentals of Crop Physiology			

Syllabus

Theory

Introduction to Crop Physiology and its importance in Agriculture; Plant cell: an Overview; Diffusion and osmosis; Absorption of water, transpiration and Stomatal Physiology; Mineral nutrition of Plants: Functions and deficiency symptoms of nutrients, nutrient uptake mechanisms; Photosynthesis: Light and Dark reactions, C3, C4 and CAM plants; Respiration: Glycolysis, TCA cycle and electron transport chain; Fat Metabolism: Fatty acid synthesis and Breakdown; Plant growth regulators: Physiological roles and agricultural uses, Physiological aspects of growth and development of major crops: Growth analysis, Role of Physiological growth parameters in crop productivity.

Practical

Study of plant cells, structure and distribution of stomata, imbibitions, osmosis, plasmolysis, measurement of root pressure, rate of transpiration, Separation of photosynthetic pigments through paper chromatography, Rate of transpiration, photosynthesis, respiration, tissue test for mineral nutrients, estimation of relative water content, Measurement of photosynthetic CO_2 assimilation by Infra Red Gas Analyser (IRGA).

Teaching Schedule

a) Theory

Lecture	Topic	Weightage (%)
1	Introduction to Crop Physiology and its importance in Agriculture	5
2	Plant cell- structure, cell organelles and their role	5
3-4	Absorption of water and path of water. Ascent of sap and theories of ascent of sap	10
5	Transpiration- Definition, types , structure of stomata, physiology of stomata, factors affecting transpiration, Water use efficiency & factors affecting W.U. E.	5
6	Mineral nutrition of plants. Classification of mineral element, criteria of essentiality. General and specific role of mineral element and deficiency symptoms, mechanism of mineral element uptake.	10
7-8	Photosynthesis : Definition pigment involved, structure of chloroplast, light reaction- Photolysis of water, Emersion effect, Cyclic and non cyclic electron transfer, Significance of light reaction.	10