The Neotia University

Practical Manual on Crop Physiology

Credits: 3 (1+2)
Subject code: CC-AGL211 CC-AGP211
Semester: Second


Compiled by: Mr. Pragun Pal
Teaching Associate
School of Agriculture and Allied Sciences
Neotia University

LIST OF EXPERIMENTS

Sl. No	Particulars of experiment	Page No.	Date	Signature	Remark
1.	Preparation of different types of solutions				
2.	Study of Plant Cell				
3.	Structure and Distribution of Stomata				
4.	Study of Imbibition				
5.	Study of Osmosis				
6.	Demonstration on Plasmolysis and Deplasmolysis				
7.	Measurement of Root Pressure				
8.	Separation of Photosynthetic Pigments Through Paper Chromatography				
9.	Measurement of Leaf area and different growth and yield parameters				
10.	Determination of Rate of Transpiration				
11.	Study of Rate of Photosynthesis				
12.	Study of Respiration				
13.	Tissue Test for mineral nutrients				
14.	Estimation of Relative Water Content of Leaf				
15.	Measurement of light interception and light extinction coefficient				
16.	Measurement of Photosynthetic CO_2 Assimilation by Infra-Red Gas Analyzer(IRGA)				

PRACTICAL NO. 1

Title: Preparation of various types of solutions,

Solution is a homogenous mixture of the single phase containing one or more of the chemicals and species on the molecular scale.

Solvent and Solute

Solvent is a substance capable of dissolving another substance, the solute togive rise to solution.

Standard solution

A solution containing known quantity (weight or volume) of substance dissolved in known quantity (weight or volume) of solvent is called a standardsolution.

True solution

A true solution is a homogenous solution in which the solute particle have diameter less than 10-7 i.e. the solute particles are of molecular dimensions. The particles are invisible even under powerful microscope.

Example: Sodium chloride in water is a true solution. Most ionic compounds formthe true solutions in water.

Molar Solution Molar solution is defined as that solution which contains one gram molecular weight of substance (ie, solute) in a total solution volume of exactly a litre (ie, 1000 ml). The symbol used for molar solution is M.

The SI units for molar concentration are molam. However, most chemical literature traditionally uses mol/dm^3, or mol dm^{-3}, which is the same as mol/l.Example: Preparation of 100 ml 1.0 M stock of Glucose

Gram molecular weight of glucose

The chemical formula of glucose is C_6, H12, O6, and the atomic weights of C, H and O are 1 and 16 respectively. Therefore, molecular weight of $C_6H_{12}O_6$, is calculated as following.

6x12=72

12 x1=12

6 x 16-96

Molecular weight of $C_6H_{12}O_6$ - 180.0

Gram molecular weight of $C_6H_{12}O_6$ =180.0 gm

Accurately weight 18 gm of glucose and dissolve it in a little quantity of water in a 100 ml volumetric flask Shake the solution until the solute is dissolved completely and then make up the final volume up to 100 ml. This is the 100 ml stock solution of 1M concentration. This may be used as stock for further preparation of diluted concentrations.

Preparation of dilute concentrations from any stock solution

Using the stock solution, different diluted concentration can be prepared byfollowing the simple equation.

$M \times X = M_1 \times V_1$

Where, M_1= Molarity of the required solution

V_1= Total volume of the required solution M=Molarity of the stock solution

X=Total volume of stock solution to be diluted by adding distilled water

Example: How would you prepare 50 ml of 0.25 M solution from a stock of 0.5M?Solution

Using the formula $M \times X = M_1 \times V_1$

M=0.5

X=?

M_1= 0.25

V_1 =50

Therefore, the equation will be $0.5 \times X = 0.25 \times 50$

Therefore 25 ml of stock solution is to be diluted to 50 ml with distilled waterto obtain 0.25 M solution.

Molal solution

When a solution contains a gram molecular weight of a substance per kilogram of the solvent solution is said to be one molal solution. Molal also is not a valid S.I. expression. The symbol for molal is m.

The SI unit for molality is mol/kg. In a dilute aqueous solution near room temperature and standard atmospheric pressure, molarity and molality will be very similar in value. This is because 1 kg of water roughly corresponds to a volume of 1 L at these conditions and because the solution is dilute, the addition of the solute makes a negligible impact on the volume of the solution. However, in all other conditions, this is usually not the case.

Normal solution

A normal solution contains one gram equivalent weight of the substance per liter of solution.Normal solution is denoted by symbol N.

Example: HCI react with NaOH on a 1:1 basis -1 equivalent of HCl for one equivalent of NaOH. The equivalent weight is the same as the molar weight. So the molar concentration of HCI is also same as normality.

Gram molecular weight

The weight of a substance grams equal to its atomic weight units. The gram molecular weight of any substance

contains of 6.02 x 10 molecules of that substance(Avogadro's number).

Example: The molecular weight of CO_2 is 12-32-44. The gram molecular weight ofCO_2 will be 44 grams.

Gram equivalent weight

The weight of an element in grams which combines with or displaces from a compound 8 gram of oxygen or 1.008 grams of hydrogen or 35.5 grams of chloride.

Parts per million (ppm) solution

Parts per million (ppm) denotes the amount of a given substance in a total amount of 1,000,000 regardless of the units of measure used as long as they are the same, e.g. 1milligram per kilogram. 1 part in 10%.

Example: One mg of a substance (i.e., solute) dissolved in water (i.e., solvent) to make final volume of the solution exactly a litre is a one-ppm solution.

On volume/volume basis, 1 ml of a liquid (substance) per litre of solution.makes a 1000 ppm concentration.

Parts per trillion (ppt) solution

Parts per trillion (ppt) denotes the amount of a given substance in a total amount of 1,000,000,000,000 regardless of the units of measure as long as they are the same, e.g. 1 milligram per kilotonne. I part in 10^{12}.

Parts per quadrillion (ppq) solution

Part per quadrillion (ppq) denotes the amount of a given substance in a total amount of 1,000,000,000,000,000 regardless of the units of measure as long as they are the same, e.g. I milligram per megatonne. I part in 10^{15}.

In atmospheric chemistry and in air pollution regulations, the parts per notation is commonly expressed with a v following, such as ppmy, to indicate parts per million by volume. This works fine for gas concentration (e.g., ppmv of carbon dioxide in the ambient air) but for concentrations of non-gaseous substances such as aerosols, cloud droplets, and particulate matter in the ambient air, the concentrations are commonly expressed as $\mu g/m^3$ or mg/ m^2 (e.g., ug or mg of particulates per cubic metre of ambient air). This expression eliminates the need totake into account the impact of temperature and pressure on the density and hence weight of the gas.

Per cent solution (Weight/Volume)

When one gram of substance is dissolved in water and the volume is t made up to 100 ml, it makes one per cent solution.

Example: If 15 gram of solute is present in 100 ml, it is a 5% solution (w/v).

Buffer solution

A solution that contains a weak acid and its salt (e.g. acetic acid and sodium acetate) or a weakbase and its salt resists changes in hydrogen ion concentration when small amounts a strong acid or base are added to it. There are buffer solutions.

Buffering solutions are universally present in living plant cells. Enzymes function within narrow pH ranges. Any large increase or decrease in hydrogen ion concentration is resisted by buffer solutions and kept within limits where enzyme can function properly.

Suggested links:

https://youtu.be/A2YyIo8vSCA

https://youtu.be/6vWC-Zp48cA

EXPERIMENT NO: 02
STUDY OF PLANT CELL

PRINCIPLE

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

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

Plant cell anatomy

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

1. The Cell Wall

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

2. Cell membrane

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

3. Nucleus:

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

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

Nuclear membrane: Nuclear membrane is perforated with holes called nucleo pore that allows proteins and nucleic acids.

4. Plastids

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

i. Leucoplasts

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

ii. Chloroplasts

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

iii. Chromoplasts

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

5. Central Vacuole

It occupies around thirty per cent of the cell's volume in a mature plant cell. Tonoplast is amembranethatsurroundscentralvacuole.Thevitalfunctionofcentralvacuoleapartfromstorage is to sustain turgid pressure against the cell wall. The central vacuole consists of cell sap. It is a mixture of salts, enzymes, and other substances.

6. Golgi apparatus

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

7. Ribosome's

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

8. Mitochondria

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

9. Lysosome

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

MATERIALS REQUIRED:

Compound microscope, forceps, tweezers, droppers, clean microscopic slides, cover slips, onions, iodine, distilled water

PROCEDURE

1. Take a small piece of onion and peel off the membrane from the underside by the forceps.
2. Lay the membrane flat on the surface of a clean glass slide and then add one drop of distilled water onto the specimen. It is important to make sure that the membrane is absolutely flat.
3. Lower a thin glass cover slip over the slide carefully by a pin. Make sure that there is no air bubbles trapped in the slide.
4. Configure your microscope following the user's guidance, making sure the lowest power objective lens, such as 4X or 10x, is in line with the optical tube, and the microscope light is turned on.
5. Place the prepared slide onto the stage of the microscope and adjust accordingly.
6. Look from the side of the microscope, lower the tube using the coarse focus knob until the end of the objective lens is just above the cover glass. Be careful and don't crack the glass cover.
7. Observe through the eyepiece lens and adjust the fine adjustment knob until the image comes into

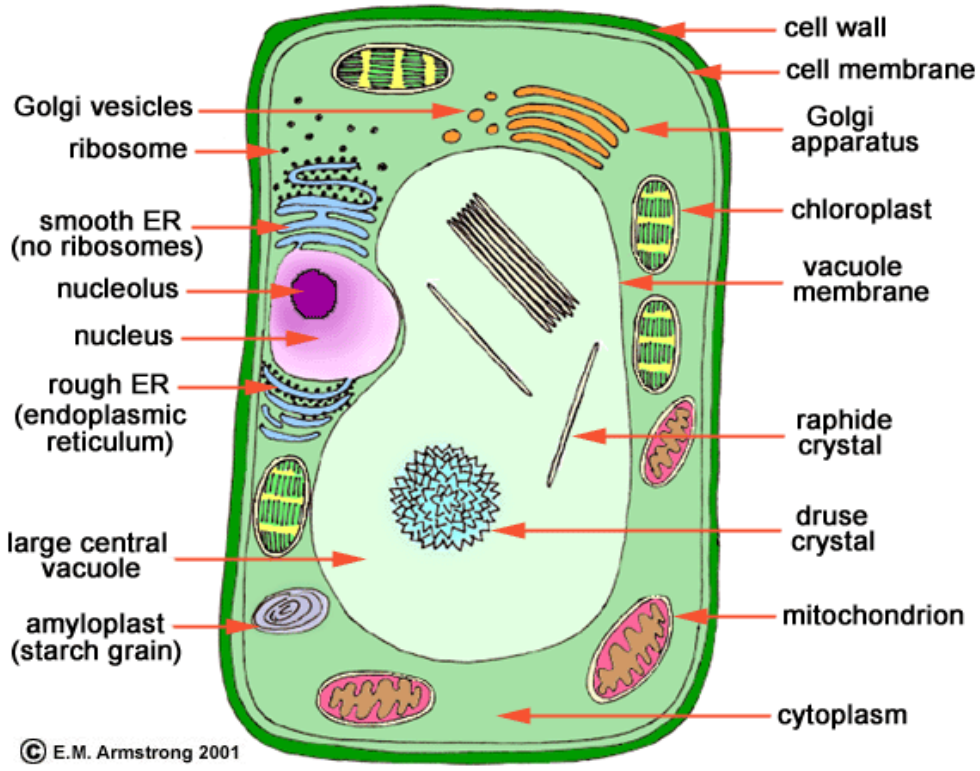
focus.

8. Swap the objective lens to high powered one, such as 40×lens, so that you can see the cells at higher magnification.

9. Record the cell shape and the nucleus and cell wall in each cell.

10. Stain the slide with iodine instead of distilled water.

1I. Repeat steps 1-9.



OBSERVATION:

CONCLUSION:

SUGGESTED LINKS:

https://youtu.be/jWDkJKcw8IQ

EXPERIMENT: 03
STUDY OF STRUCTURE AND DISTRIBUTION OF STOMATA

PRINCIPLE

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

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

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

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

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

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

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

MATERIALS REQUIRED

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

PROCEDURE

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

6. Remove the excess stain and glycerin with the help of blotting paper.

7. Observe the slide under the low-power and high-power magnifications of the compound microscope.

OBSERVATION

1. The epidermal cells are visible. These are irregular in outline and have no intercellular spaces.

2. Many small pores (stomata) are seen scattered among the epidermal cells.

3. Each pore is guarded by two bean-shaped guard cells, each containing chloroplasts and a nucleus.

4. The inner concave boundary of each guard cell is thick, whereas its outer boundary is thin.

5. The stomata may be open or closed. The guard cells regulate the opening and closing of the stomata.

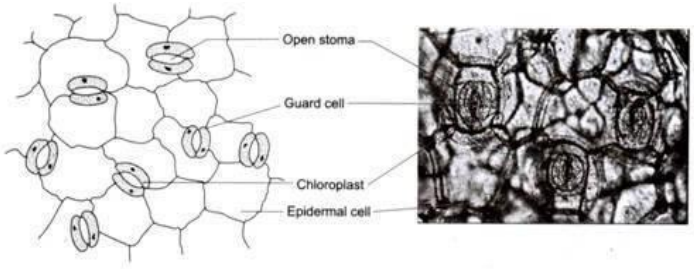
RESULT

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

CONCLUSION:

PRECAUTIONS

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



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

SUGGESTED LINKS:

https://youtu.be/JSTqhuCUyl4

EXPERIMENT NO: 04

STUDY OF IMBIBITION

PRINCIPLE

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

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

MATERIALS REQUIRED

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

PROCEDURE

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

OBSERVATION

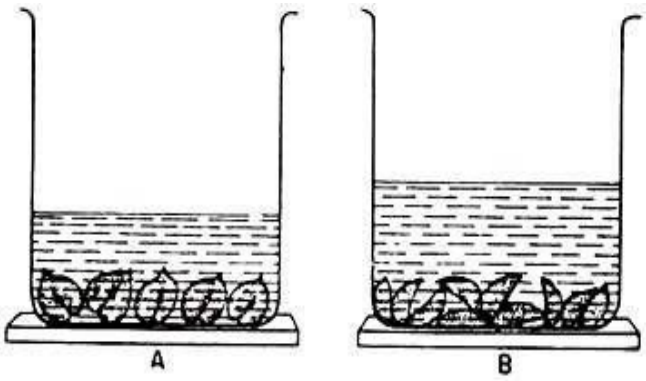


Fig. 3.5 : Absorption of water by seeds : (A) seeds with coat and (B) seeds without coat

RESULT

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

CONCLUSION

SUGGESTED LINKS:

https://youtu.be/T5FhDDZzRMs

EXPERIMENTNO: 05

STUDY OF OSMOSIS

PRINCIPLE

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

Permeability of Membrane :

Semipermeable:- Such membranes permit the movement of solvent molecules through them but prevent the movement of solute particles eg:-egg membrane, parchment membrane and tonoplast

Selectively permeable membrane:-These are normally semipermeable but allow selective passage of solutes through them eg:-plasmalemma

METERIALSREQUIRED

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

PROCEDURE

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

OBSERVATION

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

Mark the level of solution when it stops to move.

RESULT

CONCLUSION:

SUGGESTED LINKS:

https://youtu.be/uixn83fA5_Q

EXPERIMENTNO: 06

DETERMINATION OF PLASMOLYSIS AND DEPLASMOLYSIS

PRINCIPLE

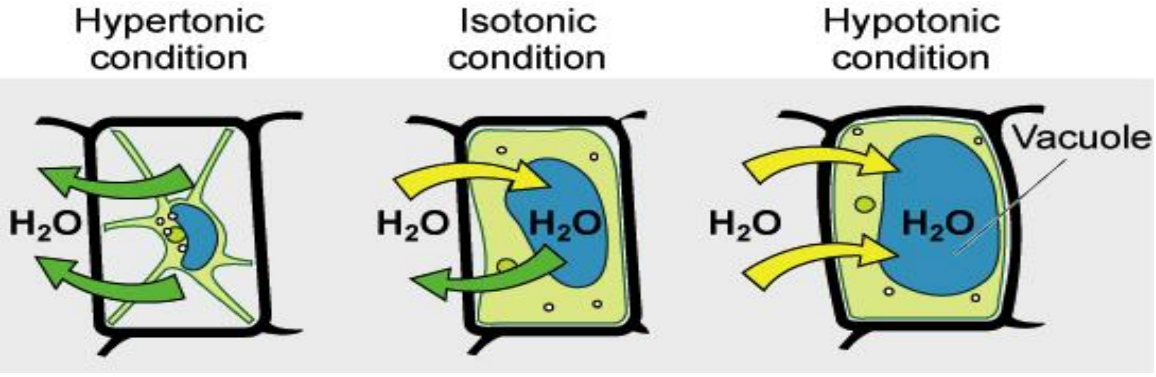
In normal condition the protoplasm is highly pressed against the cell wall. If this plant cell or tissue is placed in hypertonic solution, water comes out from the cell sap into the outer solution due to ex-osmosis and the protoplasm begins to contract from the cell wall. The process is called as plasmolysis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

There are three basic types of solution:

Isotonic solutions have the same water concentration on both sides of the cell membrane. Blood is isotonic.

Hypertonic solutions have less water (and more solute such as salt or sugar) than a cell. Seawater is hypertonic. If you place an animal or a plant cell in a hypertonic solution, the cell shrinks, because it loses water (water moves from a higher concentration inside the cell to a lower concentration outside).

Hypotonic solutions have more water than a cell. Tap water and pure water are hypotonic. A single plant cell placed in a hypotonic solution will fill up with water and become turgid.. Plant cells have a cell wall around the outside than stops them from bursting, so a plant cell will swell up in a hypotonic solution, but will not burst.



METERIALS REQUIRED

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

PROCEDURE

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

Phase-2: The leaf peel was then immersed in 0.5M sucrose solution taken in a watch glass for15 min to allow plasmolysis. After which the peel was taken over a glass slide, add 0.5M sucrose solution and observed under microscope.

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

OBSERVATION

RESULT

CONCLUSION

SUGGESTED LINNKNKS:

https://youtu.be/VPwLN6U1spk

EXPERIMENTNO: 07

MEASUREMENT OF ROOT PRESSURE

PRINCIPLE

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

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

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

MATERIALS REQUIRED

Potted plant with stem cut, clamp, manometer

PROCEDURE

1. Take a regularly watered potted plant and cut the stem portion 1cm above the ground level.
2. Then connect the glass tube of manometer with the cut stem.
3. Take care while joining tube and stem being bound tightly, water cannot escape the tube.
4. Mark the level of mercury (M1) in manometer.
5. Keep your arrangement aside for 2 to3 hours.
6. Then observe and mark the final mercury level (M2) in the manometer.
7. The difference between M2 and M1indicates the root pressure of the selected plant.

OBSERVATION

RESULT

CONCLUSION

SUGGESTED LINKS;

https://youtu.be/6KTc0rRHGJ0

EXPERIMENTNO: 08

SEPARATIONS OF PHOTOSYNTHETIC PIGMENTS THROUGH PAPER CHROMATOGRAPHY

PRINCIPLE

Paper chromatography is a useful technique in the separation and identification of different plant pigments. In this technique, the mixture containing the pigments to be separated is first applied as a spot or a line to the paper about 1.5 cm from the bottom edge of the paper. The paper is then placed in a container with the tip of the paper touching the solvent. Solvent is absorbed by the paper and moves up the paper by capillary action. As the solvent crosses the area containing plant pigment extract, the pigments dissolve in and move with the solvent. The solvent carries the dissolved pigments as it moves up the paper. The pigments are carried along at different rates because they are not equally soluble. Therefore, the less soluble pigments will move slower up the paper than the more soluble pigments. This is known as developing Chromatogram. Paper Chromatography is useful for identifying unknown compounds often used in crime scene investigations to match ink, lipstick, or colored fibers. This set-up shows two different pen inks.
Rf. equation:

Rf. =distance traveled by compound / distance traveled by solvent

MATERIALS REQUIRED

Chromatography paper, Acetone solution, Beaker, Pencil, chromatography chamber

PROCEDURE

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

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

ANALYSIS:

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

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

CONCLUSION

EXPERIMENT - 09

MEASUREMENT OF LEAF AREA AND DIFFERENT GROWTH AND YIELD PARAMETERS

PRINCIPLE

Growth analysis can be used to account for growth in terms that have functional or structural significance. The type of growth analysis requires measurement of plant biomass and assimilatory area (leaf area) and methods of computing certain parameters that describe growth. The growth parameters that are commonly used in agricultural research are given below.

1. Leaf Area

This is the area of photosynthetic surface produced by the individual plant over a period of interval of time and expressed in $cm^2 plant^{-1}$.

2. Leaf Area Index (LAI)

Williams (1946) proposed the term, Leaf Area Index (LAI). It is the ratio of the leaf of the crop to the ground area over a period of interval of time. The value of LAI should be optimum at the maximum ground cover area at which crop canopy receives maximum solar radiation and hence, the TDMA will be high.

$$LAI = \frac{\text{Total leaf area of a plant}}{\text{Ground area occupied by the plant}}$$

3. Leaf Area Ratio (LAR)

The term, Leaf Area Ratio (LAR) was suggested by Radford (1967), expresses the ratio between the areas of leaf laminate the total plant biomass or the LAR reflects the Leafiness of a plant or amount of leaf area formed per unit of biomass and expressed in $cm^{-2} g^{-1}$ of plant dry weight.

$$LAR = \frac{\text{Leaf area per plant}}{\text{Plant dry weight}}$$

4. Leaf Weight Ratio (LWR)

It was coined by (Kvet*etal.*, 1971) Leaf weight ratio is expressed as the dry weight of leaves to whole plant dry weight and is expressed in g g^{-1}.

$$LWR = \frac{\text{Leaf dry weight}}{\text{Plant dry weight}}$$

5. Leaf Area Duration (LAD)

To correlate dry matter yield with LAI, Power *et al.* (1967) integrated the LAI with time and called as Leaf Area Duration. LAD takes into account, both the duration and extent of photosynthetic tissue of the crop canopy. The LAD is expressed in days.

$$LAD = \frac{L_1+L_2}{(t_2 - t_1)} \times 2$$

L_1=LAI at the first stage

L_2=LAI at the second stage,(t_2-t_1) =Time interval in days

6. Specific Leaf Area(SLA)

Specific leaf area is a measure of the leaf area of the plant to leaf dry weight and expressed in cm^2g^{-1} as proposed by Kvet *et al.* (1971).

$$SLA = \frac{\text{Leaf area}}{\text{Leaf weight}}$$

Hence, if the SLA is high, the photosynthesizing surface will be high. However no relationship with yield could be expected.

7. Specific Leaf Weight (SLW)

It is a measure of leaf weight per unit leaf area. Hence, it is a ratio expressed as gcm^{-2} and the term was suggested by Pearce *et al.* (1968). More SLW/unit leaf area indicates more biomass and a positive relationship with yield can be expected.

$$SLW = \frac{\text{Leaf weight}}{\text{Leaf area}}$$

8. Absolute Growth Rate (AGR)

AGR is the function of amount of growing material present and is influenced by the environment. It gives Absolute values of biomass between two intervals. It is mainly used for a single plant or single plant organ e.g. Leaf growth, plant weight etc.

$$AGR = \frac{h_2 - h_1}{{}^{1}t_2 - t_1} \quad cm \ day^{-}$$

Where, h1and h2 are the plant height at $t_1$and t_2 times respectively.

9. Net Assimilation Rate (NAR)

The term, NAR was used by Williams (1946). NAR is defined as dry matter increment per unit leaf area or per unit leaf dry weight per unit of time. The NAR is a measure of the average photo synthetic efficiency of leaves in a crop community.

$$NAR = \frac{(W_2 - W_1)}{(t_2 - t_1)} \times \frac{(\log_e L_2 - \log_e L_1)}{(L_2 - L_1)}$$

Where, $W_1$and W_2 is dry weight of whole plant at time t_1 and t_2 respectively

L_1 and $L_2$are leaf weights or leaf area at t_1 and t_2 respectively

t_1–t_2 is time interval in days

NAR is expressed as the grams of dry weight increase per unit dry weight or area per unit time (g $g^{-1}day^{-1}$).

10. Relative Growth Rate (RGR)

The term was coined by Williams (1946). Relative Growth Rate (RGR) expresses the total plant dry weight increase in a time interval in relation to the initial weight or Dry matter increment per unit biomass per unit time or grams of dry weight increase per gram of dry weight and expressed as unit dry weight/ unit dry weight/ unit time(g $g^{-1}day^{-1}$)

$$RGR = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}$$

Where, W1 and W_2 are whole plant dry weight at t_1 and t_2 respectively

t_1 and t_2 are time interval in days

11. Crop Growth Rate (CGR)

The method was suggested by Watson (1956). The CGR explains the dry matter accumulated per unit land area per unit time (g $m^{-2}day^{-1}$)

$$CGR = \frac{(W_2 - W_1)}{\rho\ (t_2 - t_1)}$$

Where, W1and $W_2$are whole plants dry weight at timet_1 –t_2 respectively

ρ is the ground area on which $W_1$and$W_2$are recorded.

CGR of specie are usually closely related to interception of solar radiation

12. Harvest Index

The harvest index is expressed as the percent ratio between the economic yield and total biological yield and was suggested by Nichi Porovich (1951).

$$HI = \frac{\text{Economic yield}}{\text{Total biological yield}} \times 100$$

SUGGESTED LINKS:

https://youtu.be/YdXqUWZY0lY

EXPERIMENT NO-10
DETERMINATION OF RATE OF TRANSPIRATION

PRINCIPLE

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

MATERIALS REQUIRED

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

PROCEDURE

At first, area of the leaf was drawn over a graph paper with the help of pencil and rubber. After which, the terminal part of the petiole was cut obliquely with the help of blade to allow more surface area for observation of water. The conical flask was then filled with water and covered with a polythene sheet having a hole in the center for the end of the petiole. The polythene sheet was tied with the help of rubber band. The leaf petiole was inserted into the conical flask through the hole and the weight of the conical flask along with the leaf was taken in the electrical balance (W1gm) the whole set was then kept under bright source of light for one hour to allow transpiration. After which, final weight of the set was taken (W2 gm). The rate of transpiration was expressed as loss of watering m/sq.cm of leaf area/hour.

OBSERVATIONS

The following traits were observed, measured and recorded:

No. of big squares:

No. of medium squares:

No. of small squares:

Total area of leaf:

Initial weight of setup:

Final weight of setup:

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

CALCULATION

RESULT

CONCLUSION

SUGGESTED LINKS:

https://youtu.be/gXocZZDDPaw

EXPERIMENTNO: 11

STUDY OF RATE OF PHOTOSYNTHESIS

PRINCIPLE

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

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

MATERIALS REQUIRED

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

PROCEDURE

1. Fill the beaker with distilled water up to2/3 mark.
2. Take some fresh and healthy Hydrilla plants and cut their ends and tie them loosely with a thread.
3. Insert the cut ends inside the neck of the funnel with a jet.
4. Place the funnel inside the beaker in such a manner that all plants remain inside the funnel.
5. Add a few ml of 0.1% $KHCO_3$ soln. for dissolved CO_2 source.
6. Invert a graduated test tube filled with water over the neck of the funnel so that the jet of the funnel remains inside the tube in vertical position.
7. Place the whole set-up under bright light and keep the graduated tube erect with stand and clamp, if necessary.
8. Allow the experiment to continue for about 20minutes.Then record the evolution of air bubble (O_2gas) inside the tube after passing through jet for 5 or10 minutes.

OBSERVATION

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

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

RESULT

CONCLUSION

SUGGESTED LINKS:

https://youtu.be/gXocZZDDPaw

EXPERIMENTNO: 12

STUDY OF RESPIRATION

PRINCIPLE

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

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

PROCEDURE

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

(i) Respiroscope which consists essentially of as tout vertical tube which is bent into a bulb at the one endor,

(ii) With the help of a long-necked round-bottomed flask fitted with a centrally-bored cork at them out through which passes a glass tube.

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

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

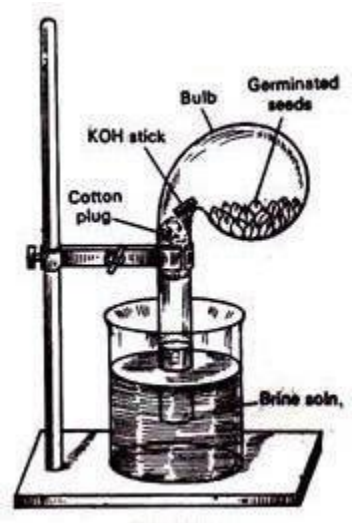
A few caustic potash (KOH) pellets are introduced in the bent portion of the Respiroscope or in the long neck of the round bottom flask and kept in position with loosely held cotton wool Care should be taken that respiratory materials and KOH pellets do not come in contact. Precautions should be taken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

OBSERVATION

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

INTERFERENCE

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



RESULT

CONCLUSION

SUGGESTED LINKS:

https://youtu.be/gXocZZDDPaw

EXPERIMENT: 13
DIAGNOSIS OF NUTRIENT DEFICIENCIES FOR MINERAL NUTRIENT THROUGH RAPID TISSUE TEST

PRINCIPLE

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

There are two types of plant analysis.

1. Tissue testing 2. Whole plant analysis

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

Table: Specified part of the different crop for nutritional status

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

NITROGEN

Reagents

0.1%of Diphenylaminein Conc. Sulphuric Acid

PROCEDURE

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

Dark Blue-Sufficient

Light Blue - Slightly deficient

No Colour- Highly Deficient

PHOSPHORUS

Reagents

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

PROCEDURE

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

Dark Blue- Rich in Phosphorus, Light Blue-Moderately, Sufficient Green or Bluish Green-Deficient, No Colour or Yellow-Highly deficient

POTASSIUM, CALCIUM, MAGNESIUM and CHLORIDE

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

Morgan's reagent

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

Preparation of plant extract

Take 4g of plant sample and add15 ml of Morgan’s regent. Add pinch of Darco and filter through muslin cloth. This extract could be subsequently used for detection.

POTASSIUM

Reagents

35%Sodiumcobaltnitrite50%Glycerine

Isopropyl alcohol

PROCEDURE

2 ml of Morgan’s reagent extract is taken in a test tube and to this added 0.2 ml of sodium cobaltnitrite.1mlof50%glycerineand2mlofisopropylalcoholandobservedforcolourdevelopment.

Clear reddish brown-Insufficient

Deep canary yellow turbidity-Sufficient

CALCIUM

Reagents

50% Glycerin Ammonium oxalate

PROCEDURE

To 2 ml of Morgan’s reagent extract, added 2ml of 50% glycerin and 5 ml of saturated ammonium oxalate.

Colorless-Insufficient

Greenish white turbidity- Sufficient

MAGNESIUM

0.15% Titan yellow

2%Hydroxylamine hydrochloride

5% Sucrose

10% Sodium hydroxide

PROCEDURE

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

observed for colour development.

Straw Yellow- Insufficient

Salmon pink colour-Sufficient

CHLORIDE

N/50 silver nitrate Concentrated Nitric acid.

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

Colorless – Insufficient White turbidity-Sufficient

IRON

Reagents

Concentrated sulphuric acid concentrated nitric acid

20% Ammonium thiocyanate in amyl alcohol

PROCEDURE

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

Brick red –Sufficient, Faint colour-Deficient

MANGANESE

Sensitive test for deficient leaves Reagents

Saturated solution of Potassium periodate

1% Tetra methyl diamino diphenyl methane

PROCEDURE

To finely chopped leaf bits, added 2ml of potassium periodate and 0.4 ml of 1% tetramethyl diamino diphenyl methane reagent. The contents were shaken vigorously and observed for colour development.

Pale Blue-Insufficient, Deep Blue–Sufficient

RESULT

CONCLUSION

SUGGESTED LINKS:

https://youtu.be/gXocZZDDPaw

EXPERIMENT NO.14

MEASUREMENT OF RELATIVE WATER CONTENT OF LEAF

PRINCIPLE

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

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

MATERIALS REQUIREMENTS

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

PROCEDURE

Measurements of RWC were performed on leaves collected from either one-year old potted seedlings, or from branches of mature trees. Leaves were always collected from the mid section of either branches or seedlings, in order to minimize age effects. At first leaves were cut into small pieces (1cm x 1cm). Around one gram of leaf sample was weighed for each which is considered as fresh weight. Subsequently, they were immersed in double distilled water for 4hours to absorb water inside a closed Petri dish. After which, the leaves were removed from water, surface water was removed by blotting paper and turgid weight of each sample was recorded. The leaves were then dried at 80 ± 1^0C in a hot air oven for 48 hours and dry weight was recorded. Using the formula relative water content (RWC) of leaf samples were determined and expressed in percent.

OBSERVATION

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

CALCULATION

RESULT

CONCLUSION

SUGGESTED LINKS:

https://youtu.be/gXocZZDDPaw

EXPERIMENT-15

MEASUREMENT OF LIGHT INTERCEPTION AND LIGHT EXTINCTION COEFFICIENT

PRINCIPLE:

The amount of solar radiation absorbed by a tree canopy varies with canopy shape, leaf area, leaf distribution, position of the sun, and fractions of sunlight that are direct and diffuse. Penetration of light into the canopy is decreased by clumping of foliage. Hence, in modeling light penetration into tree crowns a distinction often is made between random models, based on the assumption that leaves are randomly dispersed in the canopy. Light interception character of a plant is very important for its productivity.

MATERIALS REQUIRED

Plant samples (Tree, Shrubs), Digital Luxmeter

PROCEDURE:

1. Select orchard or field crops. Several plants should be selected in case of field crops for observation.
2. With the help of luxmeter light penetration is measured in different part of the crop canopy.(Top, middle, base)
3. Take several measurement in different areas of the field

CALCULATION:

Light extinction coefficient

It is the ratio of light intercepted by crop between the top and bottom of crop canopy to the LAI.

$$K = \frac{\log_e I / I_o}{LAI}$$

Where, I_o and I are the light intensity at top and bottom of a population with LAI

Light Transmission Ratio (LTR)

It is expressed as the ratio of quantum of light intercepted by crop canopy at top to the

bottom. Light intensity is expressed in K lux or Wm^{-2}

$$LTR = I / I_o$$

Where, I: light intercepted at the bottom of the crop canopy I_o:

light intercepted at the top of the crop canopy

CONCLUSION:

SUGGESTED LINKS:

https://youtu.be/gXocZZDDPaw

EXPERIMENT: 16

MEASUREMENT OF PHOTOSYNTHETIC CO_2 ASSIMILATION BY INFRA-RED GAS ANALYZER (IRGA)

PRINCIPLE:

In all the gas exchange equipments designed to measure the photosynthetic rates, the Infra-Red Gas Analyzer (IRGA) is a major component and the primary role of this component is to measure the CO_2 concentration. The IRGA is very sensitive to detect even a change of 1 ppm of CO_2. A leaf or a plant is enclosed in an air tight chamber and the CO; fluxes are determined by measuring the CO_2concentration changes in the chamber atmosphere.

Infra-Red Gas Analyzers (IRGA) is used for the measurement of a wide range ofHeteroatomicgasmoleculesincludingCO_2, H2O, NH3, CO, SO2, N2O, NO and gaseous hydrocarbons like CH_3. Heteroatomic molecules have characteristic absorption spectrum in the infra red region. Therefore, absorption of radiation by a specific Heteroatomic molecule is directly proportional to its concentration in an air sample.

The major absorption peak of CO_2 is at 4.25µm with secondary peaks at 2.66, 2.77 and 14.99 µm. The only Heteroatomic molecules normally present in the air with an absorption spectrum overlapping with that of CO_2 is water vapour. Both molecules absorb IR radiation in the 2.7 µm range. However, this interference does not pose any serious problem at 4.25 µm range.

Measurement of Photosynthesis

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

Closed system

In this system, a leaf is clamped in a leaf chamber and air is circulated around the leaf repeatedly. The decrease in CO_2 concentration over time is measured to determine the photosynthetic rate. The major disadvantage in this system is that the measurements of photosynthetic rates are done under constantlydepletingCO_2concentrationsaroundtheleaf.Further, since the leaves transpire

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

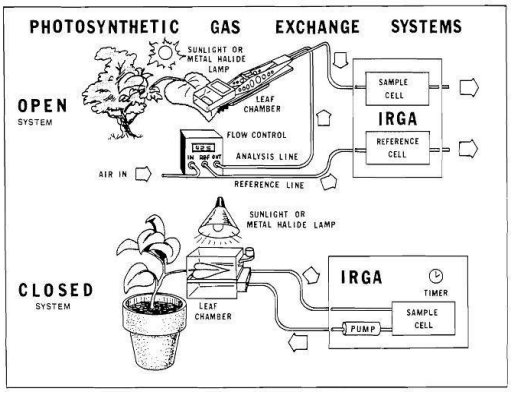
Open system

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

The major advantage in the open system is that, the measurement of photosynthetic rates can be achieved at a constant CO_2concentration. Further, by altering the flow rate of dry air into the leaf chamber, it is also possible to maintain a specific relative humidity in the chamber and hence the gas exchange parameters can be measured at a constant set of conditions. This renders the comparison more realistic and acceptable.

MATERIALS REQUIRED

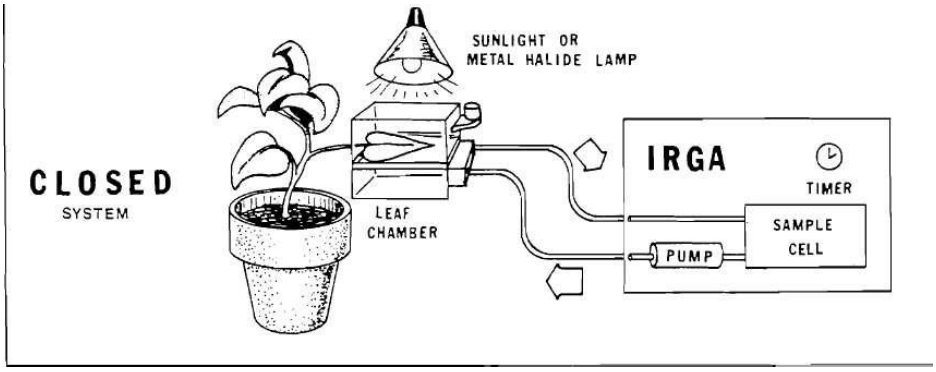
Plant samples of different crops and varieties, Infra-Red Gas Analyzers (IRGA)



PROCEDURE

- The portable photosynthesis system is a portable IRGA and is to operate as an open system to measure the gas exchange parameters.
- It consists of separate IRGAs to measure CO_2 and H_2O vapour concentrations, an internal air supply unit and the necessary software for the computation of gas exchange parameters.
- Ci 340 uses for independent infrared gas analyzers, 2 each for CO_2 and H_2O. One pair of CO_2 and H_2O analyzers defined as reference measures the CO_2 and water vapour concentration in the ambient air that is sent into leaf chamber.
- Similarly second pair, the analysis chambers measures the CO_2 and water vapour concentrations in the air that is coming from the leaf chamber.
- The difference between the reference and the analysis IRGAs are computed.
- Physiological efficiency of green gram genotypes under moisture stress conditions was measured in laboratory.
- A leaf is clamped to the leaf chamber.
- The leaf chamber is provided with suitable pads to clamp an area of 2.5 cm^2 under airtight conditions.
- Separate tubing is provided to send and withdraw air from the leaf chamber.
- These tubes are connected to either of the reference or analysis IRGA for the determination of gas concentrations.
- A quantum sensor is placed inside the leaf chambers transparent cover to measure the actual light intensity in PAR range at the leaf surface.
- Blue and red LED (light emitting diode) is fixed on the top of the leaf chamber.
- The LEDs emit light in the PAR region and the intensity of which can be fixed and controlled at a required level.
- The light source is capable of providing the photosynthetically active radiation in the energy range of 0 to 2000 μ mole m^{-2} s^{-1}.
- A CO_2 cartridge normally carrying 8g of pure CO_2 in liquid form is used to get the requisite CO_2 concentration in the leaf chamber.
- The system mixes the ambient air with the CO_2 to obtain the requisite concentration in the leaf chamber.

- The path of the ambient air is provided with 2 scrubbers to remove moisture (drierite used as a desiccant) and CO_2 (soda lime to remove CO_2)



IRGA WORKING PROCEDURE:

1. First charge the batteries one day prior to record data using IRGA.
2. Load the charged batteries first.
3. Connect the CO_2 tube to the inlet of the instrument.
4. All screws of this instrument must be in tight fitting.
5. Connect the CO_2 tube in a proper way. Connect this tube very tightly otherwise it shows leak (-ppm) in display.
6. The 2nd edge of this tube was kept in empty thermocol box and enclosed for uniform entry of air into the tube.
7. Switch "ON" the instrument.
8. Displays shows –
    A. Welcome to loading open system.
    B. Starting net working.
    C. It shows the fluorescence + WUE X m1 – press "enter".
    D. Is the chamber IRGA connected Y/S – Yes – press "Y".
9. Open the IRGA leaf chamber one time and close it.
10. Select 'New measurements' press (F4).
11. In display select 'open log file' press (F1).
    A. Give file name and press "enter".
    B. Next – gives sub file name and press "enter".

C. Give date and press “enter”.

12. Next – CO_2 matching.

A. Select Match (F5)

B. Wait up to we get equal values of reference CO_2 and sample CO_2.

C. If we need close matching press ‘Match IRGA’ (F5) after that press “exit” (F1).

13. In display set the rows - m, n, c and 9.

A. If we want ‘m row’ – press ‘m alphabet’.

B. If we want ‘n row’ – press ‘n alphabet’.

C. If we want ‘c row’ – press ‘c alphabet’ it is already exist.

D. If we want ‘9 rows’ – press ‘9 number’.

14. In this condition wait for 15-20 min. for warming of instrument (before inserting the leaf in IRGA chamber).

15. Leaf should not fold in IRGA chamber. If leaf get folds it shows negative readings. Leaf should not have any moisture and dust before inserting leaf.

16. Insert the leaf in IRGA chamber.

A. Give the ‘Dark pulse’ (F3).

B. Press ‘zero’ getting ‘zero’ row.

C. Before going to next step, see the ‘F’ value must be stable and df/dt value is <5.

D. Select DO Fo Fm – (F3).

17. Select row no: 9: press ‘Actinic On’ (F4).

18. Select row no: 8: press ‘Define Actinic’ (F3).

A. It shows ‘Actinic Definition – press “enter”.

B. Type 1000 (PAR value 1000) press “enter”.

19. Select ‘zero’ row.

A. Before going to next step, see them, ‘F’ value must be stable and df/dt value is <5

B. Select DO FsFoFm – (F4).

20. If we want fluorescence value select ‘O’ alphabet and note down the Fv’/Fm’ value.

21. Now note down the IRGA readings (photosynthetic rate, transpiration rate, stomatal conductance).

22. Before taking next reading ‘Actinic is in OFF’ (F4). Do as above for taking every next reading.

23. Time taken for each reading is 10-20 min.

24. After taking of readings IRGA chamber must be in open conditions (loose the screw).

25. Replace the fluorescence chamber foam (white foam) at the time of entire damage.

PARAMETERS RECORDED FROM IRGA:

1. Photosynthetic rate (photo): µmole CO_2 m^2/sec.

2. Stomatal conductance (cond): mole H_2O m^2/sec.

3. Transpiration rate (Trmmol): m. mole H_2O m^2/sec.

4. Intercellular CO_2 concentration (Ci): µmole CO_2 $mole^{-1}$.

5. Chlorophyll fluorescence (Fv'/ Fm' values) Where, Fv'= Variable fluorescence; Fm'= Maximum fluorescence.

RESULTS:

S.No	Crops	Varieties	Photosynthetic rate (photo): µmole CO_2 m^2/sec.
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

CONCLUSION:

SUGGESTED LINKS:

https://youtu.be/gXocZZDDPa