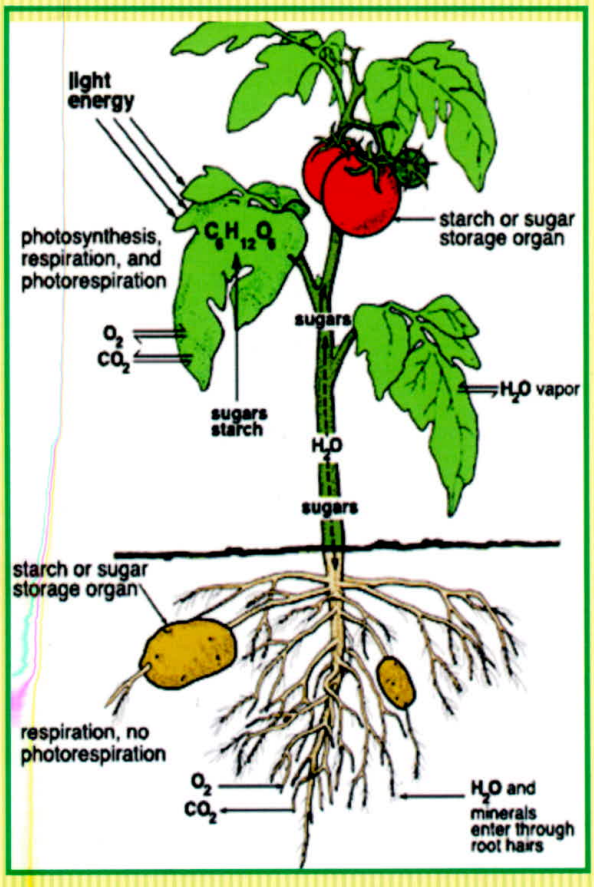
Pub. No. PPHY_AB_MAPs/P.MI./2017/01

PRACTICAL MANUAL ON FUNDAMENTALS OF CROP PHYSIOLOGY (ACP 5121)



Prepared by

V.B. KURUWANSHI

S.P. TIWARI

ARTI GUHEY

PRATIBHA KATIYAR

DEPARTMENT OF PLANT PHYSIOLOGY

COLLEGE OF AGRICULTURE

INDIRA GANDHI KRISHI VISHWAVIDYALAYA

RAIPUR, CHHATTISGARH

Pub. No. PPHY_AB_MAPs/P.MI./2017/01

PRACTICAL MANUAL
ON
FUNDAMENTALS OF CROP PHYSIOLOGY
(ACP 5121)

Prepared by
V.B. KURUWANSHI
S.P. TIWARI
ARTI GUHEY
PRATIBHA KATIYAR

DEPARTMENT OF PLANT PHYSIOLOGY
COLLEGE OF AGRICULTURE
INDIRA GANDHI KRISHI VISHVAVIDYALAYA
RAIPUR, CHHATTISGARH

Citation: Kuruwanshi, V.B., Tiwari, S.P., Guhey, Arti and Katiyar, Pratibha. 2017. Fundamentals of Crop Physiology, Practical manual/01 Published by IGKV, Raipur. pp. 51.

Inspired by:
Dr. S. K. Patil, Hon'ble Vice Chancellor
Indira Gandhi KrishiVishwavidyalaya, Raipur (C.G.)

Compiled and Edited by:
Dr. V. B. Kuruwanshi, Scientist
Dr. S. P. Tiwari, Assistant Professor
Dr. Arti Guhey, Professor & Head
Dr. Pratibha Katiyar, Professor
Department of Plant Physiology,
College of Agriculture, Raipur (C.G.)

Course No. : ACP 5121

Course title : Fundamentals of Crop Physiology

Published by:
Dean, College of Agriculture, IGKV, Raipur (C.G.)

Processed by:
Ramprasad Kujur, Astt. Professor
College of Agriculture, IGKV, Raipur

Publication Year: 2017

DIRECTORATE OF RESEARCH SERVICES
संचालनालय अनुसंधान सेवायें

IGKV

INDIRA GANDHI KRISHI VISWAVIDYALAYA, RAIPUR - 492012 (C.G.)
इंदिरा गांधी कृषि विश्वविद्यालय, रायपुर - 492012 (छ.ग.)

Ph. : (O) 91-771-2443035
Mob. : 094255-25597
Fax : 91-771-2443035
E-mail: drs_igkvr@yahoo.com
ssrao1959@yahoo.com

डॉ. एस. एस. राव
संचालक अनुसंधान
Dr. S.S. Rao
Director of Research

S.No. DRS/2403
Date: 16/11/17

//MESSAGE//

Plant Physiology is more like a rule of this dictum. The teaching in Agriculture and Plant Sciences need to be more practically orientated for researchers and students. However, no standard method or techniques are being used by them due to compendium on the techniques. In view of this, a Practical Manual on Fundamentals of Crop Physiology has been planned as per the syllabus of 5th Dean Committee and makes available the standard methods for Undergraduate students. This manual provide basic information about various physiological process, methods and techniques of Plant Physiology. The methods have been described in a simple way just like "DO IT YOURSELF".

I compliment Dr. V.B. Kuruwanshi, Dr. S.P. Tiwari, Dr. Arti Guhey and Dr. Pratibha Katiyar, Department of Plant Physiology for their efforts for preparation of present manual. I am optimistic that Undergraduate students and young teachers shall find this manual useful as an overview of the basic and applied aspects of Plant Physiology.

(S.S. Rao)

इंदिरा गांधी कृषि विश्वविद्यालय
INDIRA GANDHI KRISHI VISHWAVIDYALAYA
कृषि महाविद्यालय, रायपुर 492012 (छ.ग.)
COLLEGE OF AGRICULTURE, RAIPUR-492012 (C.G.)

Dr. O.P. Kashyap
Dean
College of Agriculture, Raipur

Phone : 0771-2970217 (O)
Mobile No. : 94242-14917, 83494-97814
Email: deanagriraipur@gmail.com

No. /CoA/Dean/2017-18/ 2185 Raipur, Dated: 16/11/2017

MESSAGE

Plant Physiology is a fast developing subject and it needs to be more practically orientated for researchers and students. In view of this, a Practical Manual on **Fundamentals of Crop Physiology** has been prepared as per the syllabus of 5th Deans Committee and makes available the standard methods for Undergraduate students. This manual will serve as ready reference in the laboratory or class room and help to solve many problems of methods and techniques of Plant Physiology.

I compliment Dr. V.B. Kuruwanshi, Dr. S.P. Tiwari, Dr. Arti Guhey and Dr. Pratibha Katiyar, Department of Plant Physiology, Agricultural Biochemistry, Medicinal and Aromatic Plants, for their efforts for present manual. It will be useful for Undergraduate students, Plant Physiologist and other Plant science specialists.

(O.P. Kashyap)

CONTENTS

PRACTICAL NO. 1

Object: Preparation of various types of solutions, Molar, Molal, Normal, ppm, Per cent etc.

Solution

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 to give 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 standard solution.

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 form the true solutions in water.

Molar Solution

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

The SI units for molar concentration are mol/m^3. 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 $C_6H_{12}O_6$ 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.

6 x 12 = 72

12 x 1 = 12

6 x 16 = 96

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

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

Accurately weight 18 g 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 by following 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 water to 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 SI 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: HCl 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 HCl is also same as normality.

Gram molecular weight

The weight of a substance in grams equal to its atomic weight units. The gram molecular weight of any substance contains of 6.02×10^{23} molecules of that substance (Avogadro's number).

Example: The molecular weight of CO_2 is 12+32=44. The gram molecular weight of CO_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 10^6.

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. 1 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.* 1 milligram per megatonne. 1 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 **ppmv**, 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^3 (*e.g.,* µg or mg of particulates per cubic metre of ambient air). This expression eliminates the need to take 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 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 weak base 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.

PRACTICAL NO. 2

Object: To study of Plant Cells

Plant cells are eukaryotic cells *i.e.*, the DNA in a plant cell is enclosed within the nucleus. The most important distinctive structure of plant cell is the presence of the cell wall outside the cell membrane. It forms the outer lining of the cell. The cell wall mostly constitutes of cellulose and its main function is providing support and rigidity. Plants cells also contain many membrane bound cellular structures. These organelles carry out specific functions necessary for survival and normal operation of the cells. There are a wide range of operations like producing hormones, enzymes, and all metabolic activities of the cell.

The following **Table 2.1** of main function of cell organelles is a list of short summary information for each organelle.

A. Membrane bound organelles

No.	Organelle type	Main Function
1.	**Nucleus**	• "Control Center" of the cell. • Contains the cell's DNA (genetic information) in the form of genes. **Nucleic Acids** • Sequestration and replication of DNA. • Transcription and modification of RNA **Nucleoli** • Biosynthesis of ribosomal RNA (rRNA) and production (assembly) of ribosomes.
2.	**Rough Endoplasmic Reticulum (RER)**	• Consists of many interconnected membranous sacs called cisternae, onto whose external surface ribosomes are attached (distinguishing RER from SER on electron micrographs). **Ribosomes** Produce polypeptides that are then either ... • Inserted into the RER membrane or moved into the lumen (central region) of the cisternae, or moved to the Golgi complex and probably onwards from there. **In lumen of cisternae** Produce proteins that are then either • Retained within vesicles, or secreted from the cell (*via* secretory vesicles - see below).
3	**Smooth Endoplasmic**	• Consists of many interconnected membranous sacs called cisternae (without ribosomes).

	Reticulum (SER)	• Many enzymes are either attached to the surface of the SER or located within its cisternae. Chemical reactions within the SER vary with the type and location of cells. *e.g.* • Helps with protein folding and transport of synthesized proteins • **Glycosylation:** Which involves the attachment of oligosaccharides.
4	**Mitochondria**	• The main function of mitochondria in aerobic cells is the production of energy by synthesis of ATP. However, mitochondria also have many other functions, including *e.g.* Processing and storage of calcium ions (Ca^{2+}). • Apoptosis, *i.e.* the process of programmed cell death • Regulation of cellular metabolism
5	**Chloroplasts**	• Chloroplasts are the sites of photosynthesis within plant cells.
6	**Golgi Apparatus**	• The Golgi apparatus modifies, sorts and packages macromolecules for delivery to other organelles or secretion from the cell *via* exocytosis - see (9.) below.
7	**Lysosomes**	• Lysosomes (tiny sacs containing enzymes) are the main sites of intracellular digestion. They enable the cell to make use of nutrients. Their functions can be listed as: • **Autophagy:** Digestion of materials from within the cell. • **Heterophagy:** Digestion of materials originating from outside the cell. • **Biosynthesis:** Recycling unwanted products of chemical reactions to process materials received from outside the cell.
8	**Peroxisomes** (also called "microbodies" - smaller than lysosomes and contain specific enzymes)	• Similar to (but smaller than) lysosomes, the metabolic functions of peroxisomes include: • These structures are involved in plant processes such as photorespiration. • Breakdown of fatty acids by beta-oxidation
9	**Secretory vesicles** (sometimes called simply "vesicles")	• Transport and delivery of their contents (*e.g.* molecules such as hormones or neurotransmitters) either into or out of the cell, in both cases via the cell membrane.
10	**Vacuole**	• Helps maintain turgor pressure (turgidity) inside the cell: which pushes the plasma membrane against the cell wall. Plants need turgidity to maintain rigidity.

Non-Membranous bound organelles

No.	Organelle Type	Main Function
1.	**Ribosomes**	• Ribosomes interpret cellular information from the nucleus and synthesize proteins. • There are different types of ribosomes *e.g.* 80S (eukaryotic), 70S (prokaryotic).
2	**Microfilament**	• Actin has a contractile function in muscle cells. • In non-muscle cells actin microfilaments form part of a web-like layer (called the cell cortex) located immediately below the cell's plasma membrane. This structure helps to define the shape of the cell including the structure of any microvilli. They also facilitate movement of certain particles and structures *e.g.* macrophages, fibroblasts and nerve growth cones.
3	**Microtubules** (Formed from tubulin)	• As the main "building blocks" forming the cytoskeleton: the cell's framework within which all components of the cell are held in position or allowed restricted movement. • Movement of materials and structures within cells *e.g.* Help form the mitotic spindle during the "prophase" part of cell division by mitosis.

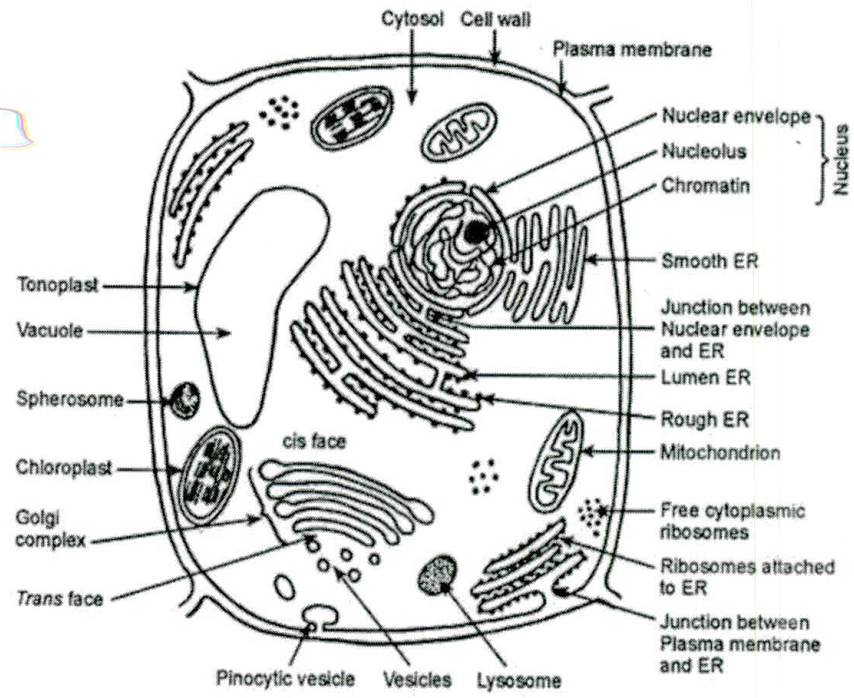


Fig. 2.1. Ultra structure of the typical plant cell

PRACTICAL NO. 03

Object: To study the Fractionation of Cellular Components

Cell fractionation

Cell fractionation is a procedure for rupturing cells, separation and suspension of cell constituents in isotonic medium in order to study their structure, chemical composition and function.

Cell fractionation involves three steps: Extraction, Homogenization and Centrifugation.

1. Extraction

It is the first step toward isolating any sub-cellular structures. In order to maintain the biological activity of organelles and bio-molecules, they must be extracted in mild conditions called cell-free systems. For these, the cells or tissues are suspended in a solution of appropriate pH and salt content, usually isotonic sucrose (0.25 mol/L) at 0-40°C.

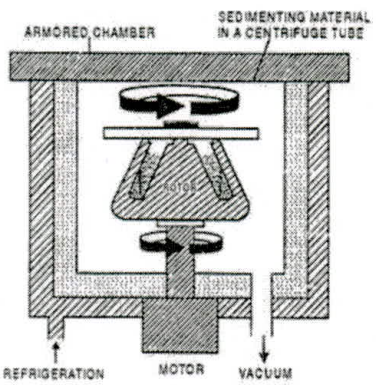


Fig.3.1. A preparative ultra-centrifuge

2. Homogenization

The suspended cells are then disrupted by the process of homogenization. It is usually done by:

(i) Grinding

(ii) High Pressure (French Press or Nitrogen Bomb)

(iii) Osmotic shock

(iv) Sonication (ultrasonic vibrations)

Grinding is done by pestle and mortar or potter homogenizer (a high-speed blender). The later consists of two cylinders separated by a narrow gap. The shearing force produced by the movement of cylinders causes the rupture of ceils. Ultrasonic waves are produced by piezoelectric crystal. They are transmitted to a steel rod placed in the suspension containing cells. Ultrasonic waves produce vibrations which rupture the cells. The liquid containing suspension of cell organelles and ether constituents is called homogenate. Sugar or sucrose solution preserves the cell organelles and prevents their clumping.

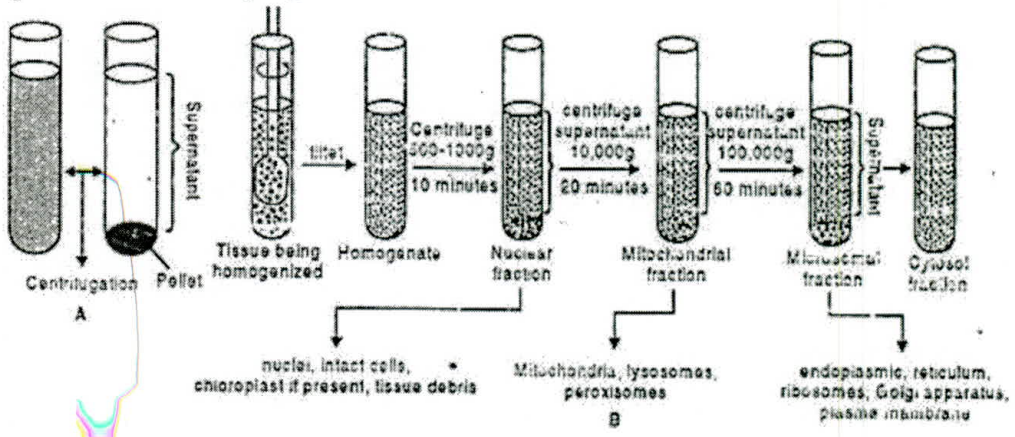


Fig.3.2. Cells fractionation (A) Appearance of a sample in centrifuge tube before and after centrifugation (B) Different steps of differential velocity centrifugation

3.Centrifugation

The separation (fractionation) of various components of the homogenate is carried out by a series of centrifugation in an instrument called preparative ultracentrifuge. The ultracentrifuge has a metal rotor containing cylindrical holes to accommodate centrifuge tubes and a motor that_spin the rotor at high speed to generate centrifugal forces.

The ultracentrifuge rotate at speeds up to 80,000 rpm (rpm= rotations per minute) and generates a gravitational pull of about 500,000 g, so that even small molecules like t-RNA, enzymes can sediment and separate from other components. The chamber of ultracentrifuge is kept in a high vacuum to reduce friction, prevent heating and maintain the sample at 0-4°C. During centrifugation, the rate at which each component settle down depends on its size and shape and described in terms of sedimentation coefficient or Svedberg unit or S-value, where IS = 1 x 10^{-13} second.

The standard cell fractionation technique involves following methods:

(a) Differential velocity centrifugation (Velocity sedimentation or Rate zonal centrifugation)

It is the first step of cell fractionation by which various sub-cellular organelles are separated based on differences in their size. The homogenate in first filtered to remove unbroken cell clumps and collected in a centrifuge tube. The filtered homogenate when centrifuged in a series of steps at successively greater speeds, each step yields a pellet and a supernatant. The supernatant of each step is removed to a fresh tube for centrifugation. For instance, at low speed (600g. for: 10 min) nuclear fraction or pellet will sediment at medium speed (15,000g x 5 min) mitochondria fraction sediment and at high speed (80,000 g. x 5 min.) micro-somal fraction sediment. The final supernatant is soluble fraction or cytosol.

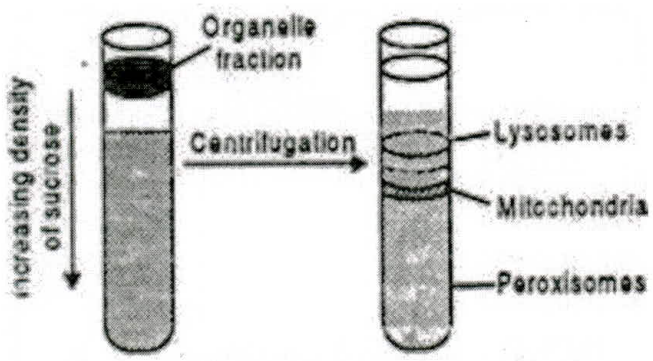


Fig.3.3. Purification of organelles by density gradient centrifugation

(b) Equilibrium Density-gradient centrifugation (Equilibrium sedimentation):

The organelle fractions (pallets) obtained in velocity centrifugation is purified by equilibrium density-gradient centrifugation. In this method organelles are separated by their density not by their size.

The impure organelle fraction is layered on the top of a gradient solution, *e.g.* sucrose solution or glycerol solution. The solution is more concentrated (dense) at the bottom of the centrifuge tube, and decreases in concentration gradually towards the top. The tube when centrifuged at high speed the various organelles migrate to an equilibrium position where their density is equal to the density of the medium.

PRACTICAL NO. 04

Object: To study the Structure and Distribution of Stomata

Stomata are pores formed between two specialized epidermal cells, the guard cells, which are found on the surface of aerial parts of higher plants (leaves). They control the gas exchange between the leaf and the ambient air. Their main function is to facilitate the entry of CO_2 into the leaf for photosynthesis and loss of water vapour for evaporative cooling of leaves.

A. Graminaceous stomata (Monocot leaves)

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. Generally dumbbell shaped guard cells open and close faster and probably consume less energy in the process, than sausage shaped guard cells. So at least the grass family plants have an advantage over the dicots. The difference is due to the operation of so-called potassium shuttle between the guard cell and its subsidiary cell underlying the opening and closing process. Generally the types of stomata are Types I (Canna), II (Palm), III (Wheat, Cyprus) and IV (Onion).

B. Dicot leaves

Each stoma (singular) is formed of two kidney or bean shaped guard. Actually these guard cells are modified epidermal cells and because of their kidney shape they leave a pore in between them. The guard cells which form the stoma are surrounded by some complementary cells. The guard cells along with the supporting cells form a stomatal complex structure. Generally the types of stomata are Anomocytic (*Cucerbites*), Anisocytic (*Brassica*), Paracytic (pulses crops) and Diacytic (*Ocimum*).

Categories of stomatal distribution in plants

Five categories of stomatal distribution have been recognized in plants which is given below-

1. **Apple or mulberry type:** Stomata are found distributed only on the under surface of leaves. *e.g.* apple, peach, mulberry, walnut etc. Such leaves are *hypostomatic type*.
2. **Potato type:** Stomata are found distributed more upon the lower surface (*multistomatic*) and less on its upper surface (*paucitomatic*). *e.g.* potato,

cabbage, bean, tomato, pea etc. Such leaves are *amphistomatic* and *anisostomatic* type.

3. **Oat type:** Stomata are found distributed equally upon the two surfaces. *e.g.* maize, oats, grass etc. Such leaves are *amphistomatic* and *isostomatic* type.
4. **Wild lily type:** Stomata are found distributed upon the upper epidermis. *e.g.* Water lily, *Nymphaea* and many aquatic plants. Such leaves are *epistomatic* type.
5. **Potamogeton type:** Stomata are altogether absent or if present they are vestigial, e.g. *Potamogeton* and submerged aquatics.

Von Mohl (1856) prepared a stomatal clock and observed that stomata open in daylight and close at night. Loftfield has classified three main groups in accordance with their daily movement.

i. ***Alfalfa type*:** The stomata remain open throughout the day and closed all nightly. *e.g.* Pea, bean, mustard etc.
ii. ***Potato type:*** The stomata close for a few hours in the evening. *e.g. Allium*, cabbage etc.
iii. ***Barley type:*** The stomata open only for a few hours in a day. *e.g.* Barley and other cereals.

Table. 4.1: Distribution of stomata in different plants

Name of plants	Number of stomata per cm^2	
	Upper surface	**Lower surface**
Sunflower (*Helianthus annus*)	58	156
Tomato (*Lycopersicum esculentum*)	12	130
Rajmah (*Phaseolus vulgaris*)	40	281
Potato (*Solanum tuberosum*)	51	161
Maize (*Zea mays*)	52	68
Oat (*Avena sativa*)	40	43

PRACTICAL NO. 05

Object: To Study Arrangement of Stomata in Isobilateral and Dorsi Ventral Leaves

Material Required

Isobilateral and dorsi ventral leaves, forceps, Petridish, watch glass, beaker, brush, needle, saffranine, glycerine *etc.*

Procedure

1. Take an isobilateral leaf, peel off a small piece of the lower epidermis and put it in water in watch glass.
2. Take out the peel from the water and place it on a slide and add 2-3 drops of saffranine to stain it.
3. Remove the excess stain with the help of a filter paper and put a drop of glycerine on the peel.
4. Gently place a cover slip on the peel and examine under microscope (high power).
5. Count the number of stomata per focus. Repeat viewing in different fields and obtain a mean value.
6. Find the area of the field of the microscope by measuring the diameter with the calibrated slide or a transparent ruler.
7. The required area is equal to πr^2 (where r is the radius of the field of view and π=3.142).
8. The number of stomata per square millimeter can then be calculated.
9. Similarly, from the other side of the leaf, the stomata count can be made.
10. Repeat the process for dorsi ventral leaf.

Precautions

1. The curling of the peel should be avoided.
2. Always use brush to transfer the peel from watch glass to the slide.

PRACTICAL NO. 06

Object: To study the Counting of Stomatal Frequency

Stomatal frequency is defined as the number of stomata per unit leaf area (no.mm^{-2}).

A. Impression method

Material required

Leaf of different species, xylene, thermocol pieces and Microscope

Procedure

1. Dissolve the thermocol pieces in xylene to make a thin paste.
2. Smear this paste on both the surfaces of the leaf and allow it to dry.
3. Peel off the dry layer of the thermocol paste and observe under a microscope using high power objective lens (40x)
4. Count the number of stomata and other epidermal cells in each microscopic field.
5. Tabulate the data and compute the stomata frequency.

B. Facsimile method

Material required

Leaf sample, Camel hair brush, Fevicol, Steel spatula, Cello tape. Forceps, Micro-slides, Cover slips and Microscope

Procedure

Take the leaf sample and clean its dust with camel hair brush and water. Apply a uniform thin layer of fevicol or very light coloured good quality nail polish of with steel spatula. Allow the adhesive film to dry and then apply good quality colourless and transparent from left to right. The tape contains surface replica of the leaf sample. Place the tape directly on a clean and dry slide and observe under microscope directly. Count the number of stomata and epidermal cells per unit area.

Table. 6.1. Stomatal frequency of different species

Species	Stomatal frequency (mm^{-2})		Guard cell length (µm)
	Abaxial	Adaxial	
Herbaceous plants			
Dicots			
Sunflower	175	120	32
Tobacco	190	50	31
Broad bean	75	65	46
Monocots			
Maize	108	98	43
Barley	85	70	30
Wheat	40	50	53
Tree species			
Pine oak	909	00	10
Black oak	405	00	50
European lime	307	00	12

PRACTICAL NO. 07

Object: Demonstration of Imbibition and Imbibition Pressure

The adsorption of water by hydrophilic colloids is known as imbibition. Imbibition of water increases the volume of the imbibant due to which pressure is created which is known as imbibitional pressure.

A. Imbibition

Material required

Dry gelatin pieces, water, blotting paper

Procedure

Dried gelatin pieces of about 2 cm square are placed in water for a few hours and then dried on blotting paper. They are measured again. An increase in the area of gelatin pieces is observed due to imbibition of water.

B. Imbibition pressure

Material required

Dry pea seeds, test tubes, water, cork and thread.

Procedure

1. Fill a test tube with dry pea seeds and water.
2. Close the mouth of the tube with cork and fasten with thread.
3. Make sure that cork is not getting displaced as a result of the pressure built inside the test tube following imbibition.

Results

Within few hours, enough pressure will be built inside the imbibing seeds to break open the test tube.

PRACTICAL NO. 08

Object: Demonstration of Osmosis with help of Potato Osmoscope

The phenomenon whereby, when a solution is separated from a weaker one by a semipermeable membrane, the weaker solution diffuses through the membrane into the stronger solution in an effect to equalize the strength of the two solutions is called osmosis.

Material required

A large potato tuber, sugar solution, knife, petri dish, water, capillary tube 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

1. Give a flat cut to one end of the potato tuber.
2. On another end of tuber, make a hollow cavity slightly more than half of its diameter.
3. Remove the skin of the tuber.
4. Place the tuber on its flat cut end in a petri-dish half full of water.
5. Fill half of the cavity of the potato tuber with sugar solution.
6. By inserting a pin in the wall of the tuber mark the initial level of the solution.
7. 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).
8. 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.

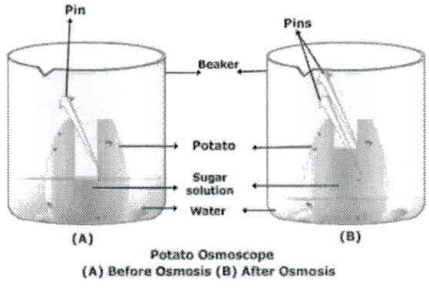


Fig. 8.1. Potato Osmoscope

PRACTICAL NO. 09

Object: Demonstration of Plasmolysis Process

Material required

Leaf of *Trodescantia,* Sugar solution, Pipette, Petridishes, Slides, Cover slips and Water

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 phenomenon of deplasmoylsis has been exhibited by plasmolysed cells when they are kept in a water or hypotonic solution.

Procedure

1. Peel the lower epidermis of *Trodescantia* leaf.
2. Divide the leaf into small strips.
3. Place these strips in different concentrations of sugar solution (0.1, 0.2, 0.3, 0.4 M) as well as in fresh water (control).
4. With help of microscope observe the changes in the cells.
5. 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.
6. Count the number of cells under the microscope.

Precautions

1. Sugar solution concentration must be correctly prepared.
2. Peelings should be done from lower epidermis of leaf very carefully.
3. Mount the peeling in glycerin carefully and avoid air bubbles and folding of peeling.

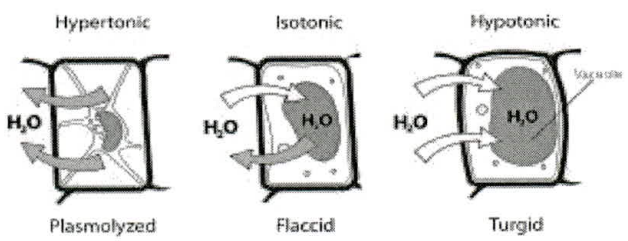


Fig. 9.1.Various stages in plasmolysis

PRACTICAL NO. 10

Object: Measurement of the Root Pressure in Plants by Manometer

Material required

A potted plant of Balsam or *Bryophyllum,* manometer with tube, stand, rubber tube, knife and thread.

Principle

There is rise in mercury level in the manometer is 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

1. Fully saturated a potted plant of Balsom *or Bryophyllum* with water and keep it overnight.
2. Next morning, cut its stem a few inches above the base with a sharp knife.
3. Attach the cut end of the stem to a manometer fixed to a stand through a rubber tube and threads.
4. Keep it at a moist and shady place for few hours.
5. Measure the initial level of water in the glass tubes.
6. Observe rise in the mercury level in the manometer 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.

Precautions

1. The plant selected should be succulent.
2. Stem should be cut under water to avoid the entrance of air bubbles in xylem vessels.
3. Rubber tubing should be fixed *c*arefully.
4. All connection should be made air tight with wax.

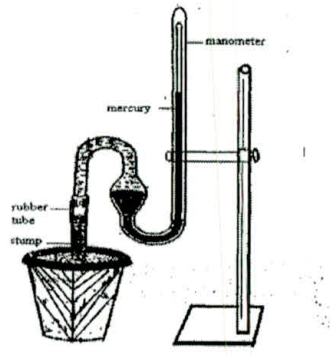


Fig. 10.1. Measurement of root pressure

PRACTICAL NO. 11

Object: Measurement of Transpiration Rate

Stephan hales (1727) in his *vegetable staticks* first describe a method of demonstration transpiration in plants. The leaves of a well-watered plant were closed in transparent bell jar and water vapours condensing on the inner sides of the jar were seen. Hales devised a variety of method for measuring the rate of transpiration. Some of the methods are described here:

a) By weighing potted plants

A potted herbaceous plant is taken. It is well watered and the pot including soil surface is well covered with a waterproof material to prevent evaporation of soil. The pot is kept over a top pan balance and loss of weight over a definite time is noted **(Fig 11.1a)**.It will be reasonable to employ this method of transpiration measurement over short periods only. If the measurements are continued for a few days, the increase in plant weight may occur due to growth.

b) By Ganong's potometer

With Ganong's potometer (meaning drink measurer), the rate of water loss from a cut plant or shoot can be measured assuming that the rate of water absorption is equal to the rate of transpiration. There are several types of potometers. In one type, there is a water reservoir to which the plant or its parts are fitted **(Fig 11.1b)**. To this reservoir a glass capillary tube of known diameter is attached. A scale is also attached to the glass tube. An air bubble is introduced in the capillary tube and its movement is recorded with the help of scale. From the diameter of the tube, the amount water absorbed (hence amount transpired) can be calculated.

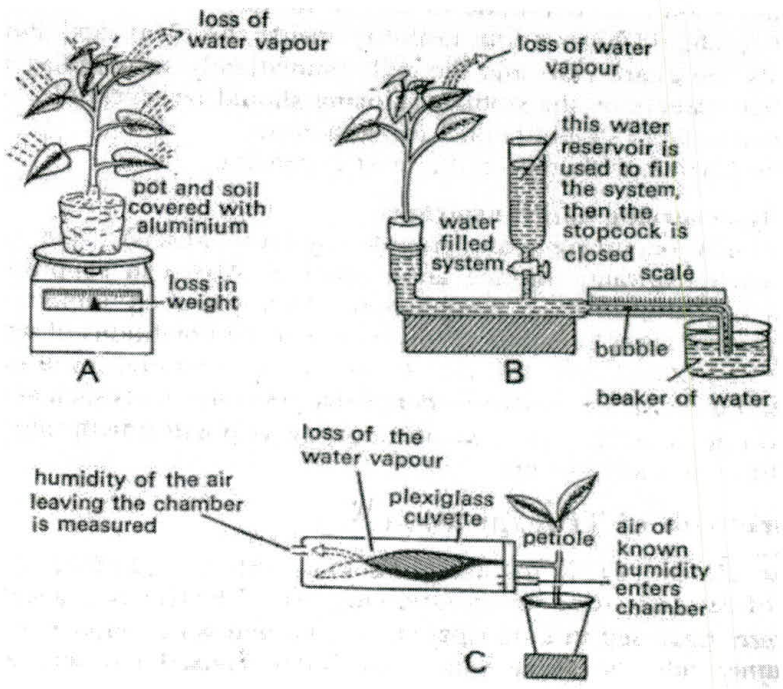


Fig 11.1. (a): By weighing a plotted plant (b) By a Ganong's potometer (c) By measuring humidity in the air

c) By measuring the humidity of air

This method can be used for measuring the rate of transpiration from single leaf. The leaf is enclosed in a Plexiglass (plastic glass) chamber and sealed. Air is passed through the chamber at a definite flow rate. The humidity of the air before entering the chamber and after leaving the chamber is measured **(Fig 11.1c)**. The amount of water transpired can be calculated by finding out the difference in humidity. Different methods are available for measuring the humidity of the air. In a simpler method, humidity is measured by collecting it through hygroscopic substances such as P_2O_5 or $CaCl_2$. The attached leaves are enclosed in the chamber which is also contains small tubes containing weighed amount of P_2O_5 or $CaCl_2$. In a control chamber, similar tubes are placed but no leaf is enclosed. The tubes are weighed after a definite time and the amount of water transpired by leaf is calculated.

PRACTICAL NO. 12

Object: Measurement of Transpiration rate 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.

Material required

A well-watered potted plant, Bell jar, a rubber sheet or oilcloth, a glass sheet and Grease or Vaseline

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.

Procedure

1. Select a small well-watered plant.
2. Cover the external soil surface of the pot and its soil thoroughly with oil cloth or polythene bag.
3. Weigh the whole pot and place it on glass plate.
4. Invert a dry bell jar over the pot.
5. Keep it for few hours and observe the bell jar.
6. 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.
7. Weigh of the pot again to know the loss in weight due to transpiration.

Precautions

1. The bell jar should be air tight.
2. The pot should be well watered.
3. The pot surface and soil must be covered carefully to avoid evaporation losses.

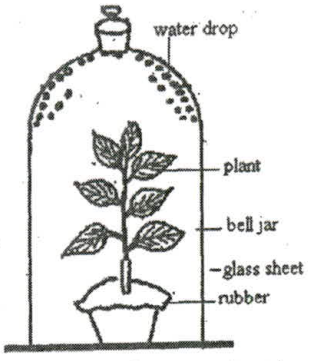


Fig.12.1. Demonstration of transpiration

PRACTICAL NO. 13

Object: Separation of Photosynthetic Pigments through Paper Chromatography

The chloroplast pigments (chlorophyll *a,* chlorophyll *b*, carotenes and xanthophylls) are situated on the thylakoid membrane of the chloroplasts. These pigments participate in the photochemical reactions of photosynthesis.

Material Required

Fresh spinach leaves or other fresh green leaves, 80 % acetone (v/v), carbon tetrachloride, petrolium ether, benzene, Mortar and pestle, filter paper, Buchner funnel, chromatography jar, chromatography paper, support rods, glass tubings drawn to a fine tubing, paper clips, hair dryer, ruler.

Procedure

Pigment extraction: Grind 500 mg of small cut pieces of fresh leaf material in 20 ml of 80 % acetone for about 5 min in a clean mortar. Carefully transfer the resulting green liquid to a Buchner funnel containing a layer of Whatman no. 1 filter paper. Filter the extract using suction.

Separation of pigments: Pour carbon tetrachloride or a mixture of petroleum ether and benzene (9:1, v/v) in a chromatographic jar to a depth of about 2 cm. Allow the internal atmosphere in the jar to equilibriate for few hours. Cut chromatography paper (Whatman no.l paper) to a desired size and draw a pencil line about 2 cm away from the bottom. With the help of a glass tubing drawn to a fine tip spot two or three points about 3 cm apart from one another with pigment extract. Allow each pigment drop to dry completely before applying the next drops. Drying may be hastened by using a hair dryer. Repeat the application of drops until the marks are deep green. (The number of drops required will depend upon the concentration of pigment in the extract. Normally, 5-10 drops may be required). Hang the paper in the chromatography jar with the lower end dipping in the solvent. Close the jar. Remove the paper when the solvent has moved up to the top of the paper. Allow the paper to dry.

Results

Observe the separation of pigments on the paper and record the results as indicated in **Table 13.1**. Mark the spots with a pencil since the colours will fade away quickly. Calculate the R_f value of each pigment.

The compounds on the chromatogram can be identified on the basis of their diagnostic feature, the ratio of fronts (R_f) values. R_f is the ratio of the distance travelled by the substance to the distance travelled by the the solvent in a chromatogram:

$$R_f = \frac{\text{Distance from origin to pigment}}{\text{Distance from origin to solvent front}}$$

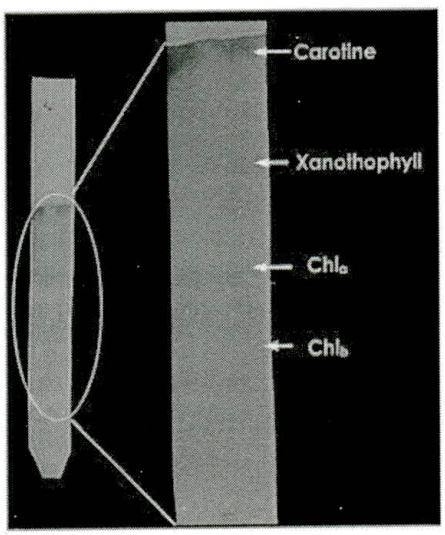


Fig 13.1: Paper Chromatography

Table.13.1: Identification of pigments alongwith their characterstics range of colours.

Spot/Band	Distance travelled	R_f value	Colour	Identification
Solvent front	9.1			
Band 1	9.0	0.989	Orange Yellow	Carotene
Band 2	1.7	0.187	Yellow	Xanthophyll
Band 3	0.9	0.099	Bluish green	Chlorophyll *a*
Band 4	0.4	0.044	Yellowish green	Chlorophyll *b*

PRACTICAL NO. 14

Object: To Demonstrate that Oxygen is liberated in the Process of Photosynthesis

Material Required

Beaker, aquatic *hydrilla* plant, test tube, funnel, water and pond water

Principle

Photosynthesis is the process by which CO_2 of the air is converted into the organic matter of the green plants with the aid of energy of light. Oxygen is by product of photosynthesis which is evolved in the presence of water, light and chlorophyll.

Procedure

1. Place some fresh twigs of aquatic *hydrilla* plant under inverted funnel in beaker filled with water, keeping the cut ends facing towards the tube of a funnel
2. Place a test tube filled with water upside down over the tube of the funnel partially dipped in the water of the beaker.
3. Keep the apparatus in bright sunlight.
4. The air bubbles start emerging out from the cut ends of *hydrilla* plant which are collected at the top of the test tube.
5. Remove the test tube when sufficient gas is collected.
6. Test it for presence of oxygen by taking glowing splinter to it by introducing pyrogallol (pyrogaffic acid), which absorbs this gas and the tube gets refilled with water.

Precautions

1. Don't injure the plant except at one place, where it is cut.
2. The apparatus should be kept in bright light.

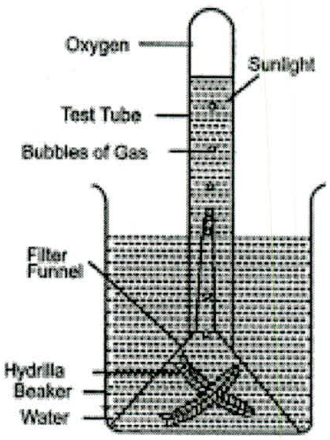


Fig 14.1: Demonstration of photosynthesis

PRACTICAL NO. 15

Object: Estimation of Chlorophyll Content

Chlorophyll can be regarded as true representation of health of green plants. This presumption is based on the face that chlorophyll is the most commonly and immensely influenced by biotic and abiotic stress conditions in addition to events like pathogenic invasion, pollution, mineral deficiencies as well as mineral toxicity. This is the reason that estimation of chlorophyll is considered to be one of the most important parameters in plant physiological studies.

(a) Acetone method

Chlorophyll is soluble in acetone. When the sample is macerated in acetone, chlorophyll gets dissolved in it. The optical density of the extract is measured at 663 and 645 nm wavelengths using a spectrophotometer or colorimeter because at these wavelengths, maximum absorption of chlorophyll 'a' and 'b' takes place, respectively. The amount of chlorophyll 'a', 'b' and total chlorophyll are determined using the formulae given by Arnon (1949).

Chlorophyll 'a' = $[(12.7 \times A_{663}) - (2.69 \times A_{645})] \times V/1000 \times W$

Chlorophyll 'b' = $[(22.9 \times A_{645}) - (4.68 \times A_{663})] \times V/1000 \times W$

Total Chlorophyll = $[(8.02 \times A_{663}) + (20.2 \times A_{645})] \times V/1000 \times W$

The value, so obtained, are expressed as mg chlorophyll per gram fresh weight of sample, where;

A = Absorption at given wavelength,

V = Total volume of sample in extraction medium and

W = Weight of sample (g)

Material Required

(i) Acetone (80%), (ii) Pestle and Mortar, (iii) Funnel, (iv) Whatman Filter Paper No.1, (v) Volumetric flasks (25 ml), (vi) Measuring cylinders (50, 100 ml), (vii) Pipettes, (viii) Test tubes, (ix) Spectrophotometer/Colorimeter, (x) Microbalance, (xi) Plant material.

Procedure

Take 200 mg freshly cut material
(leaf pieces devoid of mid rib)

↓

Grind in a pestle with mortar (use 10 ml 80% acetone)

↓

Filter the homogenate in a volumetric flask (25 ml)
(using Whatman Filter Paper No.1)

↓

Washout the homogenate 2-3 times with 5 ml of
80% acetone each time

↓

Make the final volume of the filtrate up to
25 ml with 80% acetone

↓

Take the filtrate in a cuvette (3/4 volume) and record its
absorbance at 663 and 645 nm separately using
Spectrophotometer/ Colorimeter (keep 80% acetone as blank)

↓

Calculate the amount of chlorophyll (with help of formulae given below)

Precautions

(i) Extraction should be carried out at subdued light and low temperature because light influences pigments.

(ii) 2-3 washings are necessary to ensure maximum extraction of chlorophyll pigments.

(b) By SPAD or Chlorophyll Meter

It is a sample and non-destructive method of chlorophyll estimation. It can be used in the laboratory as well as in the field of standing crop **(Fig 15.1).**

Principle

SPAD (Soil and Plant Analytical Development) is a simple diagnostic tool. We get readings in terms of SPAD values that indicate relative chlorophyll content. It is based upon a unique linear relationship between SPAD and leaf area based nitrogen concentration in plants. However, it requires standardization/calibration for each crop because this relationship varies with crop growth stage and variety.

Fig 15.1 : SPAD or Chlorophyll meter

Procedure

(i) Switch on the instrument and let it warm up for about 10-15 minutes.

(ii) Calibrate the instrument for accuracy check using a special disc provided with the instrument.

(iii) As soon as the 'beep' sound is over, put a fully expanded leaf into it in such a way that only the leaf portion between the lamina and the mid-rib remains inside.

(iv) Note down the reading. To check a reading, take another reading on the same leaf.

(v) Use 'average' button to calculate the average of readings taken automatically.

(vi) Close until you hear 'beep'.

Precautions

(i) If you want to take reading of a detached leaf in the laboratory, care should be taken so that leaf does not wilt.

(ii) While working in the field, you should shield the leaf (sample) from direct sunlight.

PRACTICAL NO. 16

Object: Measurement of Respiration Rate

Respiration rate, irrespective of plant parts (*viz.* leaves, fruits or seeds), is considered to be an important parameter in plant physiological studies. Respiratory gas exchange is just reserve on opposite to that of photosynthesis and as the later is an energy building phenomenon where ATP is stored in the photosynthates produced; respiration involves breakdown of photosynthates for release of energy to be utilized for the performance of other activities of life. On the basis of respiration a climacteric fruit can well be distinguished from a non-climacteric one as the former reveals a characteristic 'Climacteric Bust' in respiration, which is completely lacking in the later.

There are few instruments used for the measurement of respiration in plants *viz.*

(a) Infra-Red Gas Analyzer

It is generally used for the measurement of photosynthesis. However, it can also be used for measuring respiration provided:

1. The sample chamber (containing leaves) is covered with dark black cloths; so that no light reaches the leaf surface and photosynthesis may be avoided; and
2. Necessary tubing connections are made depending upon the 'closed' or 'open' type of measurement mode, as required.

Here, we measure the rate of evolution of CO_2, Which is taken to be the rate of respiration.

(b) Respirometer

Basically, it is an Infra-Red Gas Analyzer, however, its CO_2 measurement range varies from 0-50,000 ppm ($\mu mol\ mol^{-1}$) or even higher. Hence, it can be conveniently used for the measurement of respiration of large amount of sample such as fruits of big size etc. Secondly, this instrument is equipped with an 'Auto Zero' device which allows for fast warm up, adaptation to changing ambient conditions and excellent stability for the CO_2 signal **(Fig 16.1)**.

Principle

Gases with di-atomic molecules such as CO_2 strongly absorb photon in the infra-red range. For CO_2, one region of strong absorption is 4.26 microns. A source emitting strongly at this wavelength is a light bulb. If this is positioned at one end of a tube and at the other end is placed a sensor that is sensitive to photon at 4.26 micron,

we have a simple infra red analyzer. As carbon dioxide is passed down the sample cell, it absorbs some of the infra-red radiation and the sensor reading decreases.

Respirometer is consists of the following parts: (1) Electronic housing with air supply fan (2) Air supply chimney, (3) Sample chamber (4) Environmental Gas Monitor for CO_2 (EGM-4)

There are two gas ports on the top of the EGM-4; 'Gas in' and 'Gas out'. The sampling line is fitted to 'Gas in' and 'Gas out' line is left open to atmosphere to allow the sample air to exhaust without restriction **(open system)**. For **'closed system'**, one gas line of the sample chamber is connected to the 'Gas in' port and the other to the 'Gas out' port. On the rear panel, there is an absorber column. It contains soda lime. When air passes through this column, it removes all of the CO_2 from the air stream. The 'Auto Zero' facility, built into the EGM-4, periodically switches the flow of the gas from the analyzer through this column to check the analyzer zero. This routine ensures long term stability and accuracy of the CO_2 analyzer. Soda lime turns green to brown when exhausted. It should be removed with fresh stock periodically. The EMG-4 has one connector used for transferring stored data to a PC or Laptop computer. It has a 2x16 character backlit LCD display. The keypad consists of 12 keys, each having specific function. The system is supplied with an AC adaptor/ charger for charging the internal 12V lead acid battery.

Assembly

The air supply chimney is pushed into the air supply fan and a 3-pin electrical connector (from the Mass Flow Meter) is connected to the Electronic Housing. The exhaust pipe is attached to the sample chamber and the later is pushed on to the port next to the air supply fan. The sample chamber is also connected with the Electronic Housing using a 6-pin connector. The electronic housing is finally connected with the EGM-4.

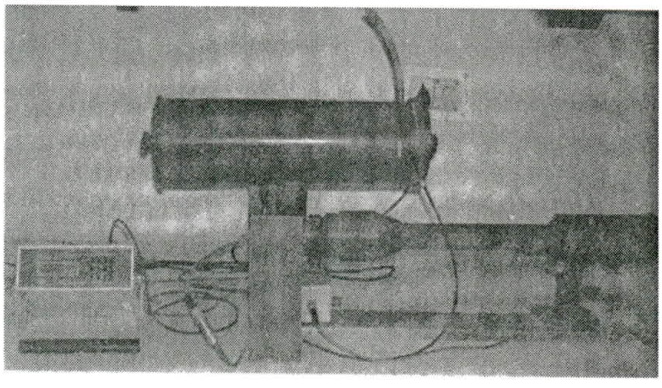
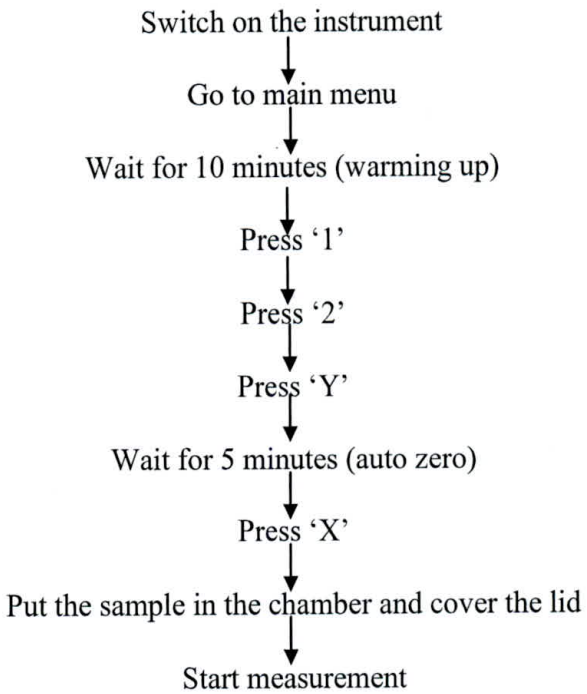


Fig 16.1: Respirometer

Operation

Air is pushed through the sample chamber by the air supply fan and flow rate is controlled by the mass flow meter. Rotating the flow control knob on the electronic housing, the flow rate in the chamber is adjusted.

The air flow rate should be adjusted to give 20-30 ppm differentials. Thereafter.



The abbreviated form of digital output appears as follows:

Rnnnnn Dnnnn Tnn

A+/- nnnn Fnnnnn

Where,

R = Reference CO_2

D = Differential CO_2

T = Chamber temperature

A = Assimilation rate

F = Flow rate (ml/min.)

Measurement is automatically corrected for temperature and pressure.

Results are given in µmol m^{-2} s^{-1} for CO_2

To convert it into g CO_2 m^{-2} h^{-1}, the values should be multiplied by 0.1584.

PRACTICAL NO. 17

Object: Diagnosis of Nutrient Deficiencies for Mineral Nutrient through Rapid Tissue Test

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.13.1: 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 and lamina	Leaf blade	Leaf blade
Cotton	Petiole	Petiole	Petiole
Papaya	Petiole	Petiole	Petiole
Tomato	Petiole	Petiole	Petiole
Banana	Lamina	Lamina	Lamina

NITROGEN

Reagents

0.1% of Diphenylamine in conc. sulphuric acid

Procedure

Small bits of leaf or petiole are taken in a petridish and a drop of 0.1% diphenylamine is added. The development of blue colour indicated the presence of nitrate nitrogen. Depending on the intensity of blue colour the nutritional status 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

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

Preparation of plant extract

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

POTASSIUM

Reagents

35% sodium cobalt nitrite

50% Glycerine

Isopropyl alcohol

Procedure

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

Clear reddish brown	-	In sufficient
Deep canary yellow turbidity	-	Sufficient

CALCIUM

Reagents

50% Glycerine

Ammonium oxalate

Procedure

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

Colourless	-	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

Concentration Nitric acid

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

Colourless - 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 concentration 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% tetramethyl diamino diphenyl methane

Procedure

To finely chopped leaf bits, added 2 ml 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

PRACTICAL NO. 18

Object: Estimation of Relative Water Content (RWC)

Principle

Plant leaves generally have lower (more negative) water potential than pure water; hence, they osmotically absorb water and become turgid. A measure of this property is the Relative Water Content (RWC) which expresses the leaf water content (%) of the turgid leaf water content. It is calculated by the formula:

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

As similar parameter is the Water Saturation Deficit (WSD) that is computed by the formula:

$$\text{WSD (\%)} = \frac{\text{Turgid weight (g) - Fresh weight (g)}}{\text{Turgid weight (g) - Oven dry weight (g)}} \times 100$$

The water is absorbed till the existence of a Water Saturation Deficit (WSD) (*i.e.* till the tissue becomes fully turgid and stops absorbing water) so that RWC + WSD = 100% or WSD = 100- RWC (Kramer, 1983).

Material required

Leaf tissue, deioniesed water, Petri dishes, balance etc.

Procedure

Excise the leaf tissue (or any other tissue) which RWC is to be determined. Weigh the tissue and now dip the tissue in deionised water in covered Petri dishes or any other suitable container for 4-5 hrs at room temperature till the tissue is fully saturated in a perfectly humid environment. Remove the tissue, blot it to remove surface water and weigh to get the turgid weight. Now dry the tissue to a constant weight in an oven at 65-70^{0}C for 72 hrs and weigh it to record the dry weight. Determine the RWC from the recorded parameters **(Table.18.1)** as described as above:

Table 18.1: Calculation of Relative Water Content (RWC)

Tissue	Fresh weight (g)	Turgid wt. (g)	Dry weight (g)	RWC %
1.				
2.				
3.				

PRACTICAL NO. 19

Object: Measurement of Photosynthetic CO_2 assimilation by Infra Red Gas Analyzer (IRGA)

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 airtight chamber and the CO_2 fluxes are determined by measuring the CO_2 concentration changes in the chamber atmosphere **(Fig 19.1)**.

Principle of IRGA

Infra-Red Gas Analyzers (IRGA) are used for the measurement of a wide range of Heteroatomic gas molecules including CO_2, H_2O, NH_3, CO, SO_2, N_2O, NO and gaseous hydrocarbons like CH_3. Heteroatomic molecules have characteristic absorption spectrum in the infrared region. Therefore, absorption of radiation by a specific Heteroatomic molecule is directly proportional to its concentration in an air sample.

The major absorption peak of CO_2 is at 4.25 μm with secondary peaks at 2.66, 2.77 and 14.99 μm. The only heteroatomic molecule normally present in the air with an absorption spectrum overlapping with that of 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 **(Fig 19.2)**.

(1) 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 constantly depleting CO_2 concentrations around the leaf. Further, since the leaves transpire simultaneously there will be a constant build up of humidity in the chamber. Increase in humidity alters the vapour pressure difference (VPD) between the leaf and its ambient air. Small changes in VPD have been shown to significantly alter the stomatal opening and hence the gas

exchange rates. These disadvantage are overcome in the open system of measurements.

(2) Open system

Here the leaf is clamped in a chamber through which ambient air is passed continuously. The change in CO_2 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_2 than the air entering it. The difference between the CO_2 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_2 concentration. Further, by altering the flow rate of dry air into the leaf chamber, it is also possible to maintain a specific relative humidity in the chamber and hence the gas exchange parameters can be measured at a constant set of conditions. This renders the comparison more realistic and acceptable.

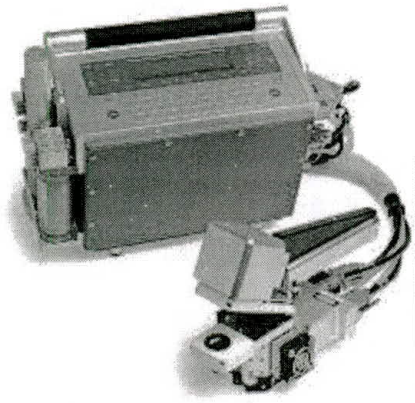


Fig 19.1. IRGA

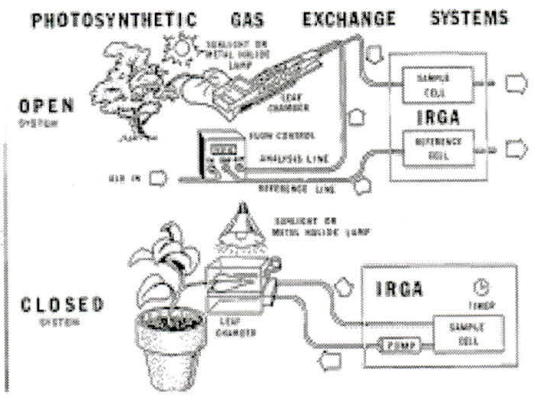


Fig 19.2. Closed System & Open System

PRACTICAL NO. 20

Object: To study the Seed Viability Test

Seed viability is defined as the degree to which seed is alive, metabolically active and possess all the enzymes capable of catalyzing metabolic reactions needed for germination and seedling growth.

Seeds must be evaluated for quality before planting. The routine test for seed quality *i.e.,* the germination test is a very lengthy process, so this necessitated the search for a quick and effective test to predict seed viability, which helps in timely processing and marketing of seeds. This has become very useful and relevant in the fast expanding seed trade and marketing of costly seeds.

Methods to determine seed viability

Tetrazolium test (TZ test)

Principle

The principle of TZ test is based on the response of all living cells of seeds, which can reduce a colourless salt solution of 2,3, 5-triphenyl tetrazolium chloride (TTC) or bromide into a red coloured compound called formazan. The reduction of TTC takes place in the seeds by a group of dehydrogenase enzymes. Since the reaction takes place within the respiring cells and the formazan is non-diffusible, a clear topography of living and non-living tissues is developed.

Materials required

Blades (preferably) single edged, dissecting needle, petridish, beaker, watch glasses, magnifying glass, towel papers, filter paper, forceps, dispensing bottle, medicine dropper, tetrazolium salt and weighing balance.

I. Preparation of chemical solutions

A. Tetrazolium solution

Several concentrations of 2, 3, 5-triphenyl tetrazolium chloride (TTC) or bromide are prepared. The concentrations of the solutions used for intact seeds are 1%, whereas, for those seeds whose embryo is bisected either 0.1 or 0.5% is used. To prepare 1.0% solution of TZ, dissolve 1 g TZ salt in 100 ml distilled water and then prepare 0.1 and 0.5% concentration by diluting with distilled water. The pH of the solution should be approximately 7.0 and should be stored in dark of in an amber coloured bottle to prevent photo-deterioration. For best staining, the pH of the

solution should not very between 6.0 to 8.0. If pH of water is not in the neutral range, The TZ salt should be dissolved in a phosphate buffer, which is prepared as under:

Solution 1

Dissolve 9.078 g of $KH_2 PO_4$ in 1000 ml distilled H_2O

Solution 2

Dissolve 11.876 of $Na_2 HPO_4.2H_2O$ in 1000 ml distilled H_2O

Take 400 ml solution 1 and 600 ml solution 2 and mix them. In one liter of buffer solution prepared above dissolve 10g of TZ salt. Thus, the tetrazolium solution of pH 7.0 and 1% concentration is obtained.

B. Lactophenol solution

This solution is used for clearing the grass seeds after staining so that the embryo is visible through lemma and palea. The lactophenol solution is prepared as under:

Mix following in the given ratios

(a) 20 parts lactic acid

(b) 20 parts phenol

(c) 40 parts glycerin

(d) 20 parts water

The solution is used with medical dropper and stored under ambient conditions.

II. Preparation of seed for testing

Conditioning

Before staining, conditioning of seed is required. The seeds that are not readily permeable to water must be made permeable by alteration of the seed coat. The moistening of living tissue promotes the activation of enzymes, advancement of embryo activities and improvement of staining.

Table. 20.1. Type of conditioning for different seeds

S.No.	Type of conditioning	Nature of seeds
1.	No moistening or additional preparation	Small seeded legumes with permeable seed coat
2.	Removal of seed coat	Dicot seeds
3.	Piercing, puncturing or cutting of seed coats	Small seeded crops
4.	Longitudinal cut through mid section of the of the embryo and endosperm	Monocots

Procedure

After conditioning wheat seed in water for 8 h, give a longitudinal cut through the mid section of the embryo and endosperm. Discard one part of the cut seed and put the other half in 0.1% TZ solution. Keep the petri plate in a dark place for 2 hrs at room temperature (20^0C to 45^0C). Higher temperature (>45^0C) is not recommended. When short staining tests are desired, then place the test sample in germinator at 30-35^0C. Generally the staining is done twice as fast, when the temperature by 10^0C in the range of 20-40^0C.

Staining

After staining is complete, interpret the results. The seed viability index of monocot and dicot seeds are evaluated based on following observations.

Monocot seeds staining: Various organs may stain as:

(a) Growing tips of the embryonic axis especially plumule.

(b) Point of attachment of the embryo to scutellum.

(c) Region of seminal root emergence

Dicot seed staining: Various organs may stain

(a) Radicle and hypocotyls development

(b) Cotyledons

(c) Plumule region

Proper staining of above seedling parts indicates that seed are viable, while improper staining in these indicate non-germinable or abnormal seeds.

Precautions

(a) Seed sample should be completely covered with TZ solution.

(b) The staining should be done in dark.

(c) The prolonged exposure to strong light oxidizes the testing solution and results in abnormal tissue colour.

PRACTICAL NO. 21

Object: To study the Seed Vigour Test by Colorimetric Method

Seed vigour is defined as those properties of seed, which determine the potential for rapid and uniform emergence and development of normal seedling under wide range of conditions.

Material required

Spectrophotometer, centrifuge, balance, grinding mill, cuvette, centrifuge tubes, TZ solution, acetone and oven.

Procedure

1. Grind 1 g seed in mill with No. 20 screen sieve.
2. Weigh out 200 mg ground seed and put it in centrifuge tube.
3. Add 1.5-5.0 ml of 1% TTC solution (2,3,5-Triphenyl tetrozolium chloride) of pH 7.0 and shake.
4. Incubate the mixture for 2 hours at 35^0C.
5. Centrifuge the mixture for 3 minutes at 10,000 rpm. Pour off TTC solution.
6. Add 5 to 10 ml acetone to the test tube to extract formazan.
7. Keep the centrifuge for 3 minutes at 10000 rpm.
8. Decant acetone solution and read absorbance (OD) at 480nm/ 520nm.

Interpretation

Higher OD will indicate more viability of seeds.

Precaution

Incubation temperature should be maintained at 35 ± 1^0 C

PRACTICAL NO. 22

Object: To study the Seed Germination Test

The emergence from the embryo and development of those essential structures, which indicate the ability of seed type tested to develop into normal plant under favourable environment. (ISTA)

Working sample

Germination test shall be made with seeds from the pure seed fraction of a purity test. Four hundred seeds in replicate of 100, 50 or 25 seeds are taken at random from pure seed, spaced uniformly and adequately apart on the moist substrate. Multigerm seed unit are not broken up for germination test but are tested as though they were single seed.

Test conditions

Permissible substrate, temperature, light conditions and special treatment for dormant seeds are described in the **(Table 22.1)** prescribed for a particular species.

A. Germination methods using paper

1. **TP (Top of paper):** In this method, the seeds are placed directly on one or more layers of moist filter paper in petridishes. These petridishes are covered with lid and placed in the germinator cabinet. The relative humidity in the cabinet must then be maintained as close to saturation prevent drying.
2. **BP (Between papers):** In this method, the seeds are germinated between two layers of paper. This may be achieved by loosely covering the seeds with an additional layer of paper or by placing the seed in rolled towel paper. The rolls of towel paper are then placed inside the germinator in an upright position.
3. **PP (Pleated paper):** The seeds are placed in a pleated, accordion-like paper strip with 50 pleats, usually two to a pleat. The pleated strips kept in boxes are directly in a 'wet' cabinet with a flat strip wrapped around the pleated paper to ensure uniform moisture conditions. This method may be used as alternative where TP or BP are prescribed.

B. Germination methods using sand

1. **TS (Top of sand):** The seeds are pressed into the surface of sand.
2. **S (in sand):** The seeds are planted on a leveled layer of moist sand and covered with 10-20 mm of uncompressed sand depending on the size of the seed. The bottom layer of sand is loosened by raking before sowing to ensure good aeration.

Germination (%)

The germination percentage indicates the proportion of seeds which have produced seedlings during the specific period.

Table.22.1. Permissible substrate, temperature and duration for germination test

Crop	Substrate	Temperature (^{0}C)	Days for	
			First count	Final count
Cereals				
Paddy	S, BP, TP	20-30, 25	5	14
Wheat	S, BP, TP	20	4	8
Maize	BP, S	20-30, 25	4	7
Sorghum	BP, TP	20-30, 25	4	10
Barley	BP, S	20	4	7
Oil seeds				
Groundnut	BP, S	20-30, 25	5	10
Soybean	BP, S	20-30, 25	5	8
Sunflower	BP, S	20-30, 25, 20	4	10
Rape & Mustard	TP	20, 20-30	5	7
Sesame	TP	20-30	3	6
Linseed	TP, BP	20-30, 25, 20	3	7
Castor	BP, S	20-30	7	14
Pulses				
Chickpea	BP, S	20-30, 25	5	8
Redgram	BP, S	30	4	6
Greengram	BP, S	20-30, 25	5	8
Blackgram	S, BP	20-30, 25	4	7
Peas	BP, S	20	5	8
Lentil	BP, S	20	5	10
Fiber crops				
Cotton	BP, S	20-30	4	12
Jute	TP, TP	30	3	5

S= Sand boxes, used for bold seed, BP= Between towel paper, used for medium and bold seeds, TP= Top of paper, used for small seeds.

PRACTICAL NO. 23

Object: Bioassay of Plant Hormones

Growth regulators define as organic compounds synthesized in one part of a plant transported to another where at very low concentration they cause physilogical response. There are five major groups of growth regulators namely, the Auxin, Gibberellins, Cytokinins, Abscissic acid (ABA) and Ethylene.

(A) Wheat coleoptiles straight growth bioassay for Auxin

Principle

The physiological basis for straight growth test is the stimulation of cell elongation by auxins. The increase in length of coleoptiles will be propotional to the auxin levels in the medium. The increase in length is then compared with that of the coleoptiles floated over buffer.

Material Required

Solutions and buffers, wheat seed, sandy trays, a sharp razor blade, a scale to measure the length of coleoptiles.

Solutions and buffers used for the experiment

Acetate buffer (pH 5.6): (Sodium acetate:acetic acid)

- *Solution A:* Prepare 0.2 M sodium acetate.$3H_2O$ by dissolving 2.772 g and make up the volume to 100 ml.
- *Solution B:* Dilute 1.2 ml of of glacial acetic acid to 100 ml with distilled water to obtain 0.2 M acetic acid.To 91 ml of solution A add 9 ml of solution B to prepare 100 ml of acetate buffer of pH 5.6. Use this buffer to prepare the GA solutions as well as for the exptraction of the enzyme from the soaked seed.

Procedure

1. Germinate wheat seeds on moist filter paper and transfer them on to sand or on vermiculite. Allow the seedlings to grow a temperature of 24-26^{0}C under high humid conditions for a period of 73 to 98 hrs in darkness.
2. When the colioptiles have attained a length of 20 to 30 mm, detop the coleoptiles in darkness and allow the auxins to diffuse down in 4 to 5 hrs.
3. From the top portion of the coleoptiles (just below the emerged primary leaf) cut 5 mm long segments using a sharp razor blade.

4. Place five coleopltiles sements in each of the different concentrations of auxin ranging from 10^{-7} to 10^{-4} M (the different auxins concentrations can be serially diluted from a 10^{-3} M stock solution). Similarly place coleoptiles segments in the test solution.
5. Float 5 coleoptile segments on the buffer used for preparing the auxins solutions to serve as control.
6. Measure the length of the segments after 24 hrs and compare with that of the coleoptiles floated over the buffer.
7. Calculate the percentage incerase in length over control and plot the values against the respective auxins concentrations (you may also plot the absolute values of the length of the coleoptiles against the auxins concentrations).
8. Similarly, incubate coleoptiles in test solution and measure the increase in length.
9. Compare the increase with the standard curve and estimate the levels of IAA in the test solution.

(B) Dwarf rice seedling elongation bioassay for Gibberellin

Principle

Dwarf rice, Tan-ginbozu, lacks the capacity to synthesize the specific GA (GA_1) necessary for stem elongation. The stem of these seedlings respond to the external supply of GA and elongate as their normal counterparts. The extent of elongation of dwarf seedlings will therefore the proportional to the concentration of GA supplied.

Material Required

Tan-ginbozu (Dwarf rice) seed, filter paper, buffers and solutions (See below for procedure), 25 ml beaker, humid chambers, scale to measure the length, Acetate buffer (pH 5.6): (Sodium acetate:acetic acid).

- *Solution A:* Prepare 0.2 M sodium acetate.$3H_2O$ by dissolving 2.772 g and make up the volume to 100 ml.
- *Solution B:* Dilute 1.2 ml of of glacial acetic acid to 100 ml with distilled water to obtain 0.2 M acetic acid. To 91 ml of solution A add 9 ml of solution B to prepare 100 ml of acetate buffer of pH 5.6. Use this buffer to prepare the GA solutions as well as for the exptraction of the enzyme from the soaked seed.

- *GA stock solution (10^{-3} M):* Prepare a 10^{-3} M GA stock solution of GA using of acetate buffer. Weigh 34.2 mg of GA_3 and dissolve it in a little amount of alcohal and make up the volume to 100 ml with buffer.
- *Agar gels:* Weigh 1 g of Agar and mix it in 100 ml of distilled water. Boil the mixture until the agar completely dissolves in water. When the agar solution is cooled it gets solidified to form a gel. Appropiate aliquate of GA stock solution was added to the agar before solidification to obtain agar gels with different concentrations of GA. Similarly, the test solution was added to the agar solution after appropiate dilutions.

Procedure

1. Germinate Tan-ginbozu seeds on moist filter paper paper in darkness.
2. Pour the agar solutions with different concentrations of GA ranging from 10^{-4} to 10^{-10} M into 20 ml beakers and allow solidifying.
3. Place a few germinated seeds on each of these agar beakers. Keep the beakers in a humid chamber at room and allow the seedlings to grow for a week to 10 days.
4. Measure the length of each of the seedling in all the beakers and estimate the average stem length in cm.
5. Maintain a set of seedling place on agar gels without any GA to serve as control.
6. Plot the length of the seedlings as afunction of their respective GA concentrations.

(C) Retardation of leaf senescence bioassay for Cytokinin

Cytokinins are substitute amino acid purines that promote cell division in callus tissuse in presence of optimum concentration of auxins. Apart from this function, cytokinins have several others formative effect. All biologically occurring cytokinins are trans forms and the most commonly occurring form of cytokinin is trans Zeatin riboside (t-ZR).

Procedure

The equal size of pieces of barley, oat, tobacco or any other leaf material *i.e.* excised leaf sections or discs are kept on test solution (with different concentration of kinetin) moistened filter papers for 48 hrs and then chlorophyll retention or loss prevented by cytokinin is recorded.

Table 23.1: Based on formative effect of cytokinins various bioassay are developed.

Bioassay	Min. Detectable level	Range
Tobacco callus bioassay	5×10^{-8} M	5×10^{-9} M – 10^{-7}
Carrot explants bioassay	5×10^{-9} M	5×10^{-9} M – 10^{-7}
Radish cotyledon bioassay	5×10^{-8} M	5×10^{-8} M – 10^{-6}
Cucumber cotyledon bioassay	5×10^{-7} M	5×10^{-8} M – 10^{-6}
Barley root growth bioassay	5×10^{-8} M	5×10^{-8} M – 10^{-5}

Precautions

The cucumber bioassay is generally not specific to cytokinins alone. High concetrations of GA can also elicit a similar response of cotylendons expansion. However, a concentration of 25 to 50 mM of K^+ is necessary for the cotyledon expansion. A very high level of syngergistic effect is also seen between K^+ and cytokinins in cotyledon expansion. Therefore, it is necessary to eliminate K^+ from the assay medium or the control set also should have the same concentration of K^+ as that of the test solution.

a. Keep a control where the cotyledons are kept in the buffer without cytokinins.
b. Carefully blot the cotyledons to dryness and record their fresh weight. Compute the per cent increase in fresh weight over the control value.
c. Tabulate and plot the results and write comments.

(D) Bioassay of Abscissic acid (ABA)

ABA is a general plant growth inhibitor. The method of ABA estimation in leaves by using High Performance Liquid Chromatography (HPLC).

Sample collection

Fully expanded leaves should be collected in butter paper bags and kept in ice box. The samples are preserved in liquid nitrogen for further analysis.

Extraction

Take leaf sample (2-5 g) in a flask and pour so much volume of containing 1% acetic acid that the sample dips into it completely

↓

Incubate it overnight at 4^0C

↓

Filter the extract through a filter paper (Whatman No.4)

↓

The extraction should be repeated twice or thrice (*i.e.* repeat the above steps 2-3 times, each time use the residue on the filter paper for extraction

↓

Evaporated acetone from the total volume of extract using a rotary evaporator at 40-50^0C till some residue is left on the bottom

↓

Preserve the residue in a cool and dark place

Sample preparation

Take out the flask with the residue (sample) as mentioned above

↓

To it, add 2 ml distilled water having 1% acetic acid (v/v) and sonicate thoroughly

↓

Transfer the content of the flask into sample vials

↓

Make the volume in each sample vial up to 5 ml with distilled water containing 1% acetic acid (v/v)

↓

Store the sample vials for assay using HPLC

The analysis of ABA using HPLC are maintained as follows:

Column	: Reverse phase C_{18} column having particle size equal to 5µm
Detector	: UV detector
Wavelength	: 265nm
Flow rate	: 1.5 ml/min
Calibration curve	: Using 10 ppm solution of ABA in 95% ethyle alcohal

Solvents

A : 1% acetic acid in distilled water (HPCL grade) v/v

B : 1% acetic acid in methyl alcohal (HPCL grade) v/v

Both the solvents are filtered through millipore nylon filter (13µm) with the help of vacuum pump solvent filter kit.

Assay

Filter the standard solution of ABA through nylon filter (0.45 µm pore size) and inject 10 µm of this solution into HPLC

↓

Record the peak which generally appears after 30-50 seconds

↓

Inject 10 µm of the sample into the HPLC and record the peak area

The ABA content in the sample may be calculated using the equation given below:

$$\text{ABA content (ng)} = \frac{\text{Peak area (cm}^2\text{) of sample x Amount of ABA in 10 µl solution (ng)}}{\text{Peak area (cm}^2\text{) of satndard solution x Fresh weight of the leaf sample (g)}}$$

(E) Bioassay of Ethylene

Ethylene is one of the plant hormones which are extensively quantified using physico-chemical techniques such as the Gas chromatography.

Sample collection

1. Put mango fruits (known weight) in a cylinder or jar.
2. Seal the mouth with a gasket.
3. Incubate for at least 2-4 hrs at room temperature.
4. Withdraw the gas sample (ethylene) using a hypodermic syringe for assay. A known volume of gas should be assayed.

Ideal operating conditions for gas chromatograph

- Carrier gas : Hydrogen/Nitrogen mixture
- Flow rate : 20-30 ml/min.
- Gas for detector : Hydrogen and air
- Column : Porapak-Q 80/100 mesh packed
- Oven/coumn temperature : 60^0C
- Injector temperature : 110^0C
- Detector temperature : 85^0C
- Detector (to be used) : FID (Flame Ionization Detector)
- Retention time for ethylene : 1.3 min.

Procedure

The carrier gas from a cylinder is passed through flow regulator to an injection port, where it picks up the sample for analysis. The carrier gas + sample mixture then passes through the coloumn in thermostatic oven where the component of the mixture are separated. The area of the peak depends upon the amount of substance present, the detector efficiency and the degree of amplification used. If the latter factor is hold constant, the recorded peak area is a direct measure of the amount of substance present in the sample. Prior to assay of sample, the instrument must be calibrated with a known volume of standard ethylene gas. For calculating the amount of ethylene produced per gram of sample per unit time.