PRACTICAL MANUAL

PLANT PHYSIOLOGY

FBT-111 3(2+1)

Dr. Ashutosh Srivastava
Dr. Ashutosh Singh

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College of Agriculture
Rani Lakshmi Bai Central Agricultural University
Jhansi-284003, Uttar Pradesh

COURSE- PLANT PHYSIOLOGY FBT 111 3(2+1)

Preparation of solutions. C3 and C4 leaf anatomy. Estimation of transpiration using porometer. Estimation of photosynthesis using IRGA. Extraction and estimation of chlorophyll in plants. Estimation of stomatal index. Demonstration of plasmolysis. Estimation of water potential in plants using Plant water status console. Estimation of leaf area of plants. Plant growth analysis – RGR, NAR, and LAR specific leaf area and leaf weight ratio LAI CGR – LAD etc… Measurement of moisture stress tolerance parameters in trees - membrane stability, chlorophyll stability, proline content, wax and cuticle thickness. Measurement of relative water content, leaf water potential, osmotic potential. Measurements of stomatal resistance/stomatal conductance under varying stress condition. Observation on tree architecture of important species.

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CONTENTS

Experiment No. 01

Objective: To prepare various solutions

Exercise 1. Prepare 0.1 N solution of NaOH

Material **Required:**

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Procedure: ……

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Calculations: ……

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Exercise 2. Prepare 0.2 M solution of NaCl

Material **Required:**

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Procedure: ……

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Calculations: ……

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Exercise 3. Prepare 10 ppm solution of $CuSO_4$

Material Required:

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Procedure: ……..…

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Calculations: ……

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Experiment No. 02

Objective: To study the leaf anatomy of C3 and C4 plants

Leaf anatomy of C3 plant

Material Required:

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Procedure: ……

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Observations: Observe the slide and write characteristic features:

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Draw well labelled diagram of leaf anatomy of C3 plant

Leaf anatomy of C4 plant

Material Required:

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Procedure: ……

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Observations: Observe the slide and write characteristic features:

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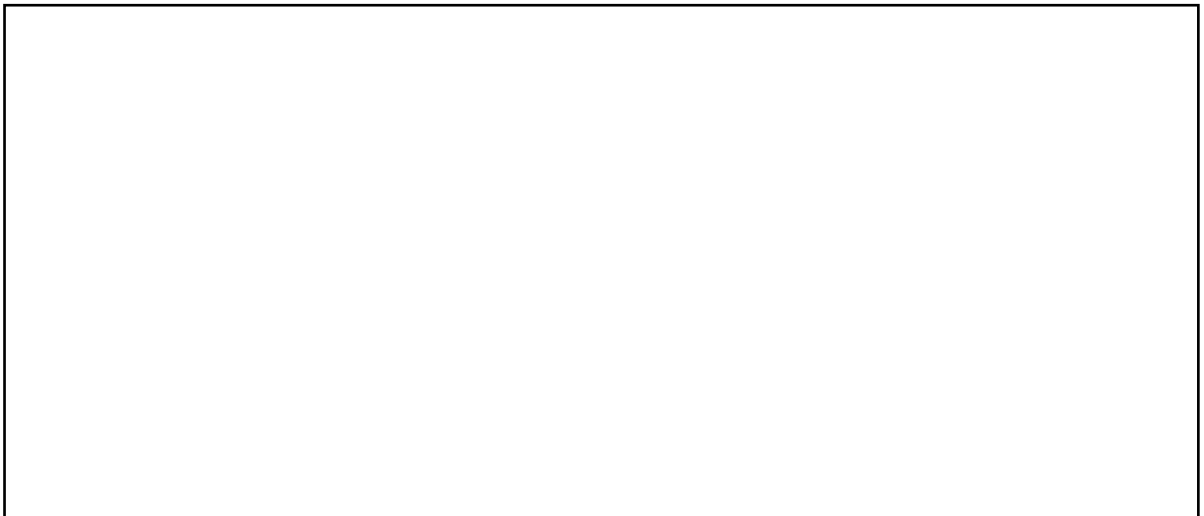
Draw well labelled diagram of leaf anatomy of C4 plant



Experiment No. 03

Objective: To study the phenomenon of plasmolysis

Material Required:

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Procedure: ……

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Observations (Observe the extent of plasmolysis in the graded solutions and record your observations in the table)

Treatment	Condition of the cell	Stage of plasmolysis
Observation 'A' (Control)		
Observation 'B' (10% SS)		
Observation 'C' (20 % SS)		

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Inference: ……..

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Experiment No. 04

Objective: To measure the water potential by weight/ Gravimetric method

Material Required:

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Procedure: ……..……

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Observations: When the points are connected, intercept at the abscissa represents the water potential of tissue, with zero weight gain or loss. It indicates the solution that had the same water potential as that of the tissue at the state of the experiment. So, the water potential of the tissue must be equal to that of the solution.

CALCULATIONS: Calculate the water potential (ψ) using the following formula: ψ= -miRT

Where,

m = Molarity of the solution	I = Ionization constant (1.0 for sucrose)
R = Roul's gas constant 90.083 litre bar/mole degree)	T = Absolute temperature (°C + 273)

From the graph, determine the sucrose concentration at which no change in weight occurred. Calculate the ψ for this solution. This value equals to the water potential of the tissue.

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Experiment No. 05

Objective: To measure the water potential by Chardakov's Dye method/Falling Drop method

Material Required:

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Procedure: ……

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Observations: If the drop rises, the solution in test series has increased in density due to uptake of water by the plant tissue. Conversely, If the drop falls, it means that solution in test series has been diluted and decreased in density. Accordingly, when the density of test and control solution is similar, the drop will neither rises nor falls, but will diffuse into the solution uniformity. At this point the water potential of the tissue and solution is equal.

Record the observations and determine the water potential of the tissue.

Sucrose Solution (M)		Pattern of movement of control solution	Nature of Sucrose solution
Control Series	Test Series		
0.15	0.15		
0.20	0.20		
0.25	0.25		
0.30	0.30		
0.35	0.35		
0.40	0.40		
0.45	0.45		
0.50	0.50		

Note: If movement of control solution is rising then hypotonic, falling then hypertonic and diffuse then isotonic

Observe the point grading concentration where the drop of the control solution when placed in corresponding test solution showed diffusion. It indicates that the water potential of the tissue is equal to the osmotic potential of sucrose solution.

Experiment No. 06

Objective: To study the structure and distribution of stomata in monocot and

Material Required: ……..……

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Procedure: ……..….

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Observations: The epidermal cells are visible. These are irregular in outline and have no intercellular spaces. Many small pores (stomata) are seen scattered among the epidermal cells. Each pore is guarded by two bean shaped guard cells, each containing chloroplasts and a nucleus.

S. No.	Name of the plant	Type of plant	Shape of guard cells	Distribution of stomata	
				Adaxial	Abaxial
1					
2					
3					
4					
5					

Draw Well-Labelled Diagram of Monocot and Dicot stomata:

Experiment No. 07

Objective: To prepare temporary slide of leaf peel to show stomatal density and stomatal index

Material Required:

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Procedure: ……..……

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Observations: Calculate the number of stomata for mm^2 of area for upper and lower surface of leaves.

Plant species	Replicate	No of stomata cm^2		No. of epidermal cell	
		Upper	Lower	Upper	Lower
Sample A	1				
	2				
	3				
	Mean				
Sample B	1				
	2				
	3				
	Mean				

Calculation: ……

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Experiment No. 08

Objective: To demonstrate the rate of transpiration by Ganong's potometer

Material Required:

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Procedure: ……

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Observations: While the transpiration takes place, air bubble moves in the tube from one end to another end. After it has reached the reservoir, open it to push the air bubble back and then note its movement again. The time taken for the bubble to move from end to another end represents the relative rate of transpiration.

Condition	Time taken in minutes	Initial reading of bubble	Final reading of bubble	Distance travel by bubble
Sunlight				
Shade				
Dark				

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Experiment No. 09

Objective: To calculate leaf area of plants by various method

1. Graphic method

Material **Required:**

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Procedure: ……

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Calculations: Count the squares of estimated leaves and calculate the leaf area in cm^2.

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2. Dry weight method

Material **Required:**

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Procedure: ……

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Calculations:

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3. Linear method

Material **Required:**

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Procedure: ……..……

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Calculations: ……..….

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Experiment No. 10

Objective: To study parameters of plant growth analysis

Exercise 1: Leaf area of bitter gourd at 15, 30 and 45 DAS are given in the following table. Calculate LAI, if the spacing is 30 cm x 10 cm.

Leaf area (cm^2/plant)		
15 DAS	30 DAS	45 DAS
45.37	361.84	475.82

Calculations:

LAI at 15 DAS

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LAI at 30 DAS

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LAI at 45 DAS

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Exercise 2: Plant dry weight and leaf area of bitter gourd at 15, 30 and 45 DAS are given in the following table. Calculate the LAR.

15 DAS		30 DAS		45 DAS	
Leaf area (cm^2/plant)	Plant dry weight (g/plant)	Leaf area (cm^2/plant)	Plant dry weight (g/plant)	Leaf area (cm^2/plant)	Plant dry weight (g/plant)
47.37	0.27	361.84	2.16	475.82	4.71

Calculations:

LAR at 15 DAS ……

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LAR at 30 DAS ……

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LAR at 45 DAS ……

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Exercise 3: Leaf area index of bitter gourd at 15, 30 and 45 DAS are given in the following table. Calculate the LAD.

Leaf Area Index		
15 DAS	30 DAS	45 DAS
0.15	1.21	1.59

Calculations:

LAD between 15-30 DAS: ……

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LAD between 30-45 DAS: ……

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Exercise 4: Leaf dry weight and leaf area of bitter gourd at 15, 30 and 45 DAS are given in the following table. Calculate the SLA.

15 DAS		30 DAS		45 DAS	
Leaf area (cm^2/plant)	Leaf dry weight (g/plant)	Leaf area (cm^2/plant)	Leaf dry weight (g/plant)	Leaf area (cm^2/plant)	Leaf dry weight (g/plant)
45.37	0.16	361.84	1.22	475.82	1.71

Calculations:

SLA at 15 DAS:……

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SLA at 30 DAS:.....

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SLA at 45 DAS:.....

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Exercise 5: Leaf dry weight and leaf area of bitter gourd at 15, 30 and 45 DAS are given in the following table. Calculate the SLW.

15 DAS		30 DAS		45 DAS	
Leaf area (cm^2/plant)	Leaf dry weight (g/plant)	Leaf area (cm^2/plant)	Leaf dry weight (g/plant)	Leaf area (cm^2/plant)	Leaf dry weight (g/plant)
45.37	0.16	361.84	1.22	475.82	1.71

Calculations:

SLW at 15 DAS:.....

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SLW at 30 DAS:.....

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SLW at 45 DAS:.....

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Exercise 6: Leaf area and leaf dry weight of bitter gourd at 15, 30 and 45 DAS are given in the following table. Calculate the NAR.

15 DAS		30 DAS		45 DAS	
Leaf area (cm^2/plant)	leaf dry weight (g/plant)	Leaf area (cm^2/plant)	Leaf dry weight (g/plant)	Leaf area (cm^2/plant)	Leaf dry weight (g/plant)
45.37	0.16	361.84	1.22	475.82	1.71

Calculations:

NAR between 15-30 DAS: .....

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NAR between 30-45 DAS: ……

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Exercise 7: Plant dry weights of bitter gourd at 15, 30 and 45 DAS are given in the following table. Calculate the RGR.

Plant dry weight (g/plant)		
15 DAS	30 DAS	45 DAS
0.27	2.16	4.71

Calculations:

RGR between 15-30 DAS:

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RGR between 30-45 DAS: ……

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Exercise 8: Plant dry weights of bitter gourd at 15, 30 and 45 DAS are given in the following table. Calculate the AGR.

Plant dry weight (g/plant)		
15 DAS	30 DAS	45 DAS
0.27	2.16	4.71

Calculations:

AGR between 15-30 DAS:

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AGR between 30-45 DAS: ……

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Exercise 9: Plant dry weights of bitter gourd at 15, 30 and 45 DAS are given in the following table. Calculate the CGR, if spacing is 30 cm x 10 cm

Plant dry weight (g/plant)		
15 DAS	30 DAS	45 DAS
0.27	2.16	4.71

Calculations:

CGR between 15-30 DAS:

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CGR between 30-45 DAS: ……

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Exercise 10: Calculate the harvest index of the wheat if grain yield is 4000 kg/ha and biomass is 9000 kg/ha.

Calculations: ……

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Experiment No. 11

Objective: To determine cell membrane stability in plant tissue

Material Required:

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Procedure: ……

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Calculations: ……

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Experiment No. 12

Objective: To detect the presence of depositions of wax and cutin

(A) To detect the presence of cutin in a given leaf sample

Material Required:

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Procedure: ……

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Observations:……

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(B) To detect the presence of cutin in a given leaf sample

Material Required:

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Procedure: ……

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Observations:……

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Experiment No. 13

Objective: To measure the stomatal resistance/stomatal conductance under varying stress condition

Material Required:

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Procedure: ……

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Observations:

Plant Species	Conditions	stomatal conductance (mmol $m^{-2}s^{-1}$)

Precautions: ……..…….

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Experiment No. 14

Objective: Extraction and estimation of chlorophyll pigment in plant tissues

Material Required:

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Procedure: ……

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Formula for calculation: The spectrophotometer is calibrated by using 80% acetone as blank sample.

$$\text{Chlorophyll a} = 12.7\,(A663) - 2.69\,(A\,645) \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll b} = 22.9\,(A\,645) - 4.69\,(A\,663) \times \frac{V}{1000 \times W}$$

$$\text{Total chlorophyll} = \frac{A652 \times 1000}{34.5} \times \frac{V}{1000 \times W}$$

Where A= Optical density

V= Final volume of leaf sample (25 ml)

W= Weight of leaf tissue (in gm i.e. 025 g)

The chlorophyll content of the leaf sample is expressed as **mg/ g** of fresh leaf

Calculation: ……

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Experiment No. 15

Objective: Separation of photosynthetic pigments by paper chromatography

Material Required:

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Glassware & Equipment:

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Procedure: ……

Pigment Extraction:

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Separation of Pigments: ……

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Observation: Observe the separation of pigments on the chromatography paper. The pigments are arranged in the following sequence from top (solvent front) to bottom-

Pigments	Colour
carotenes	Orange-yellow
xanthophylls	one or more yellow band
chlorophyll a	blue-green
chlorophyll b	yellow-green

Mark the spots with a pencil since the colours fade away quickly. Calculate the Rf value of each pigment.

Calculation: ……

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Experiment No. 16

Objective: Measurement of relative water content (RWC)

Material Required:

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Procedure: ……

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Observations:

Sample	Fresh weight (g)	Turgid weight (g)	Dry weight (g)	RWC (%)
A				
B				

Calculation: ……..

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Precautions: ……

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Experiment No. 17

Objective: Measuring Photosynthesis by Infra-Red Gas Analyser (IRGA)

Gas exchange measurements: Measuring gas exchange is the most commonly utilized technique at present for commercial and research purposes in order to measure photosynthesis of individual leaves, whole plants or plant canopy. Gas exchange measurements provide direct measure of the net rate of photosynthetic carbon assimilation. Main advantages of gas exchange measurements: instantaneous, non-destructive, direct. CO_2 exchange systems use enclosure methods, where the leaf in closed in a transparent chamber. The rate of CO_2 fixed by the leaf enclosed is determined by measuring the change in the CO_2 concentration of the air flowing across the chamber. Because ambient atmospheric CO_2 concentration is only 0.04 % (400 ppm), it is difficult to measure photosynthetic CO_2 uptake and sensitive sensors are needed.

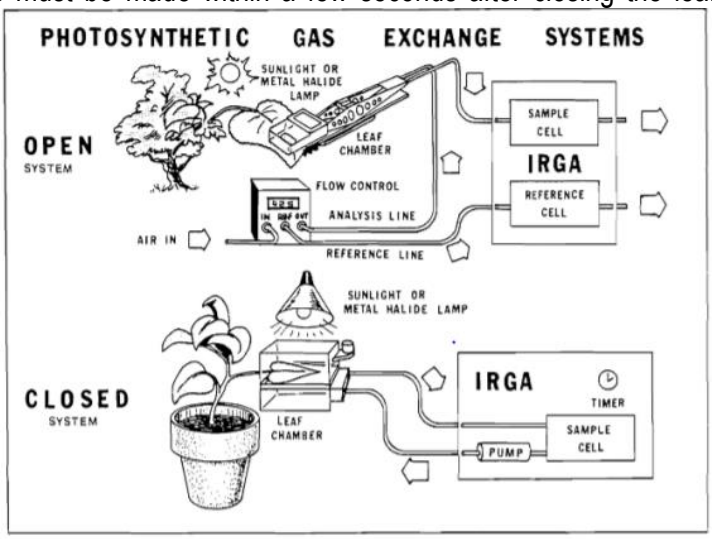
Infrared gas analysis: Heteroatomic gas molecules absorb radiation at specific infrared (IR) wavebands, each gas having a characteristic absorption spectrum. Infrared gas analyzers (IRGAs) measure the reduction in transmission of IR wavebands caused by the presence of CO_2 between the radiation source and a detector. The reduction in transmission is a functio n of the concentration of CO_2. The only gas normally present in the air with an absorption spectrum overlapping that of CO_2 is water vapour. Since water vapour is usually present in the air at much higher concentrations than CO_2,

this interference is significant, but may be overcome simply by drying the air or measuring H_2O concentration by another IRGA.

PHOTOSYNTHESIS GAS EXCHANGE SYSTEMS: General parts of a gas exchange system:

- leaf chamber
- flow meter
- means of generating and controlling air flow over the leaf

Open versus closed systems: In closed systems the signal from the sample cell is compared to the zero-gas reference signal to provide an absolute measurement of CO_2 concentration. A leaf is enclosed in a chamber, sealed to avoid gas exchange with the atmosphere, and the rate at which the CO_2 and H_2O concentration changes in the chamber are monitored. Major disadvantages of closed IRGA system: • Photosynthesis measurements must be made within a few seconds after closing the leaf chamber (once the leaf is sealed in the chamber CO_2 concentration in the leaf chamber is continually decreases and water vapour increases). The operator has limited control over environmental conditions within the chamber.



Open systems are configured to allow air from a single source to enter both the analysis and reference lines. Air is continuously passed through the leaf chamber (to maintain CO_2 in at fixed concentration) and measurements of photosynthesis and transpiration are based on the differences in CO_2 and H_2O in an air stream that is flowing into the leaf cuvette (reference cell) compared to the air stream flowing out of it (sample cell). The rate of CO_2 uptake is used to assess the rate of photosynthetic carbon assimilation, while the rate of water loss is used to assess the rate of transpiration (counted on a leaf area basis).

Following parameters can be measured with IRGA

- measurements of net photosynthetic rate and transpiration
- indirect assessment of stomatal conductance and many other variables.
- Instantaneous gas flux measurements
- Photosynthesis light response curves
- A/Ci curves: response of CO2 uptake to intercellular mole fraction of CO2
- Daily courses of gas exchange

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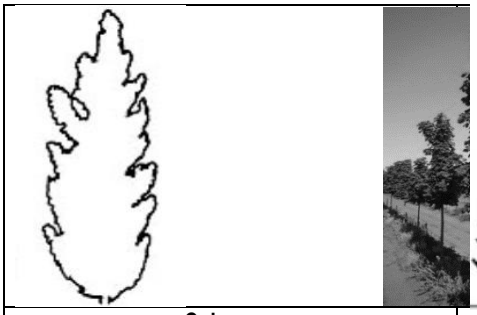
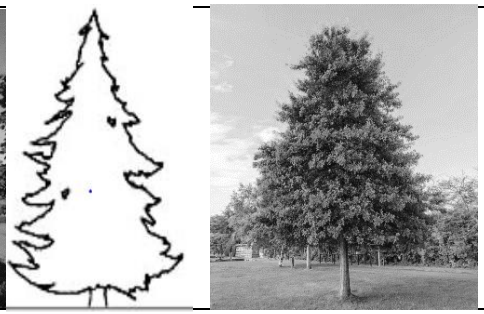
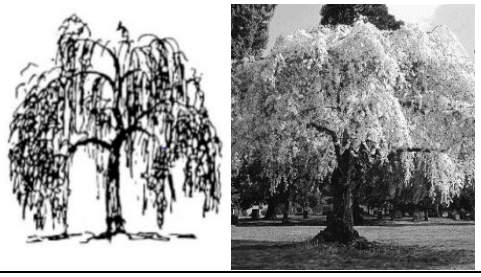
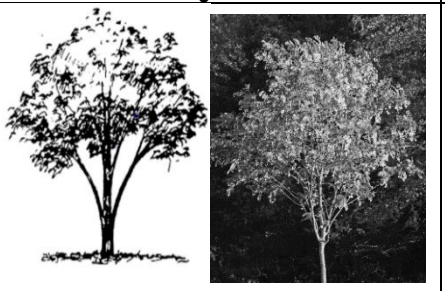
Experiment No. 18

Objective: Observation on tree architecture of important species

Crown architecture of trees is the manner in which the foliage parts of trees are positioned in various microenvironments. Crown architecture is crucial for light capture and for the distribution of light to each particular photosynthetic unit of the crown. Tree crown architecture can be represented with 'models' which delineate the basic growth strategies that determine the successive architectural phases. The forms and morphogenesis of trees are far more variable in the tropics than in the temperate regions.

There are different types of tree architecture on the basis of vegetative meristem, growth habit of stem and their branches and their arrangement.

	
Columnar	**Pin Oak**
	
Mango Tree	**Weeping**
	
Yellow Wood	**Red Maple**

Columnar trees: Columnar trees are tall and very thin, with upright branches. Their vertical shape adds height and also provides great screening without taking up much room in the landscape. Eg. Columnar apple - *Malus spp*., Columnar peach - *Prunus persica* 'Crimson Rocket'

Pyramidal or Conical shaped trees: Christmas trees as the classic pyramidal tree, but the shape applies to deciduous trees as well. Pyramidal trees are wider at the bottom, with a main center trunk and horizontal branches. The branches may start at ground level or higher up the trunk. Conical trees are similar but are usually slenderer and bullet-shaped. These trees are very dramatic and need space to reach their full width.

Vase shaped trees: Vase shaped trees are just what they sound like: a central trunk that branches into an upright, arching shape that's widest at the top. Vase-shaped trees are graceful and perfect for lining walkways, because they offer both shade and headroom.

Round or Oval shaped trees: These trees are upright, with a central strong trunk that branches into a dense round or oval-shaped crown. They make strong shade and may have such dense foliage that the branches are concealed.

Spreading or open shaped trees: These trees have an open, irregular shape that may be wider than it is tall. Some are towering skyline trees, and others are smaller, bushy specimen trees. Eg. Cherry, mango, sapota.

Weeping shaped trees: A good choice for specimens are trees with a weeping shape, where the branches droop down and sweep the ground. Eg. Weeping cherry - *Prunus subhirtella* 'Pendula'

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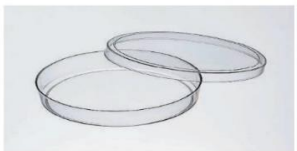
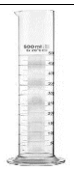
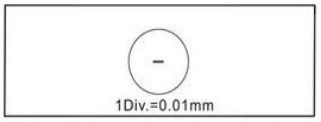
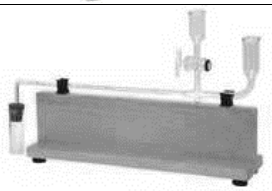
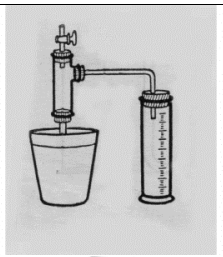
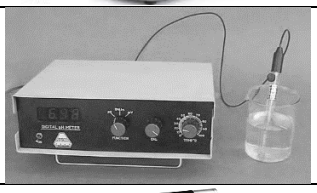
APPENDICES

IDENTIFICATION OF EQUIPMENTS/GLASSWARES

	Equipment's /glassware's	
1.	**Petri-plate/Petri-dish** It is small shallow dish of thin glass or plastic with a loose cover used especially for cultures in bacteriology. It is also commonly used as temporary receptacles for viewing samples, especially plant tissue or specimen under microscope.	
2.	**Pestle & Mortar** The mortar is a like bowl, typically made of hard wood, metal, ceramic, or hard stone, such as granite. The pestle is a heavy and blunt club-shaped object. The substance to be ground, which may be wet or dry, is placed in the mortar, where the pestle is pressed and rotated onto it until the desired texture is achieved	

3.	**Measuring Cylinder** A graduated cylinder, measuring cylinder or mixing cylinder is a common piece of laboratory equipment used to measure the volume of a liquid. It has a narrow cylindrical shape. Each marked line on the graduated cylinder represents the amount of liquid that has been measured.	
4.	**Spatula** Spatula is a broad spoon like end at one side and flat end at another side made of steel, it is used to lift or take chemical materials for the laboratory purpose	
5.	**Scalpel** A scalpel, is a small and extremely sharp bladed instrument like knife used for anatomical dissection or cutting of tissue/specimens. Scalpels may be single-use disposable or re-usable	
6.	**Cork Borer** Cork borer is a metal tool for cutting a hole in a plant tissue material like potato or any other materials for experiment purpose. It is usually come in a set of nested sizes along with a solid pin for pushing the removed cork (or rubber) out of the borer. The individual borer is a hollow tube, tapered at the edge, generally with some kind of handle at the other end.	
7.	**Stage Micrometer** A Stage Micrometer is simply a microscope slide with a finely divided scale marked on the surface. The scale is of a known true length and is used for the calibration of optical systems with eyepiece graticule patterns. It is a glass slide on which a 1 mm scale is accurately ruled into 10 equal divisions of 1/10 mm. Each division is subdivided into 10, so that each small division equals 1/100 mm (10 µm).	 
8.	**Centrifuge** Centrifuge is a laboratory equipment, driven by a motor, which spins liquid samples at high speed. There are various types of centrifuges, depending on the size and the sample capacity. Laboratory centrifuges work by the sedimentation principle, where the centrifugal acceleration is used to separate substances of greater and lesser density	
9.	**Hot Air Oven** Hot air ovens are electrical devices which use dry heat to sterilize. Generally, they can be operated from 50 to 300 °C, using a thermostat to control the temperature.	
10.	**Ganong's Potometer** It consists of a graduated tube dipped into the beaker containing water. The graduated tube is connected with a vertical arm bearing a cork on its mouth. The cork contains one hole through which a twig is inserted in the water of the vertical arm. Vertical arm is also attached with a stop cork connected with a water reservoir. It is used for measure the rate of transpiration	
11.	**Ganong's Respirometer** Respirometer is a apparatus used to measure the rate of respiration of a living substance/tissue by measuring its rate of exchange of oxygen and/or carbon dioxide.	

12.	**Root Pressure Manometer** Manometers are used to measure the pressures at which water is forced into the xylem. If a root pressure manometer 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.	
13.	**Water Bath** A water bath is laboratory equipment made from a container filled with heated water. It is used to incubate samples in water at a constant temperature at defined time. It is also used to enable certain chemical reactions to occur at high temperature.	
14.	**Compound light Microscope** A compound microscope is an upright microscope that uses two sets of lenses (a compound lens system) to obtain higher magnification than a stereo microscope. Compound microscopes typically provide magnification in the range of 40x-1000x. Compound microscopes are used to view small samples that cannot be identified with the naked eye. These samples are typically placed on a slide under the microscope	
15.	**pH Meter** pH meter is electric device used to measure hydrogen-ion activity (acidity or alkalinity) in solution. Fundamentally, a pH meter consists of a voltmeter attached to a pH-responsive electrode and a reference (unvarying) electrode.	
16.	**Infra-red Gas Analyser (IRGA)** A Photosynthesis system is design for the non-destructive measurement of photosynthetic rates in the field. Type of analysis possible- • CO_2 assimilation rates, • Stomatal conductance, • Carboxylation and light use efficiencies • CO_2 and light compensation points • PAR(photosynthetically active radiation)	

PREPARATION OF STANDARD SOLUTIONS

A standard solution contains a known weight of the substance is known volume of solvent.

1. **Percent (%) solution:** It contains in 100 ml of solvent, particular grams of a solute which is denoted by the percentage. The percentage of a solution can be expressed in three way:

   Weight per unit weight (w/w)- A 1% w/w has 1 g of solute and 99 g of solvent to make 100g of solution.
   Weight per unit volume (w/v)- A 1% of w/v solution contains 1 g of solute in 100 ml of solution.
   Volume by volume (v/v)- A 1% of v/v solution contains 1 ml of solute in 100 ml of solution.

   E.g., for preparing 10% NaCl, 10 g of the salt is dissolved in water and the volume is made to 100 ml. 10% Glycerol will contain 10 ml Glycerol and 90 ml water.

2. **Parts per million (ppm) solution:** 1 ppm solution is prepared by dissolving 1 mg of solute to 1 litre of solvent. For solutions 1 ml is diluted to 1 litre.

3. **Normal solution:** Normal solution contains in 1 litre of solution, one-gram equivalent of dissolved substance. One-gram equivalent of a substance corresponds to its molecular weight expressed in grams divided by its velency, e.g. 1 N Na OH contains 40 g NaOH in 1 litre. 1 N HCl may be prepared as follows:

   36.5 g of HCl per litr5e make a normal solution. Converting weight into volume = 36.5/1.16 = 31.4 ml. (Sp. Gravity of HCL = 1.16). The purity of HCl is 26 to 28%, hence (100/28) x 31.4 = 112.1 ml. Approximately 113 ml HCl dissolved per litre will give strength equal to 1 N.

4. **Molar solution:** A molar solution of a compound is defined as one mole of that compound per litre. 1 mole/1=molecular weight in grams/litre of solution, e.g., a solution of 0.1 M H_2SO_4, molecular weight 98 contains 9.80 g H_2SO_4, in 1 litre of solution. When 95% H_2SO_4, is available, the required 0.1 M is prepared as follows:

   95 g H_2SO_4, is contained in 100 g 95% H_2SO_4,
   9.8 g H_2SO_4, is contained in x g 95% H_2SO_4,
   Then x = (9.8 x 100)/ 95 = 10.315 g.
   Converting weight into volume = 10.315/1.84 specific gravity of H_2SO_4, = 5.605

   Thus 5.605 ml of 95% H_2SO_4, is diluted to 1 litre with water to obtain 0.1 M H_2SO_4,

Dilution of stock solutions: The stock solution of 1000 ppm can be diluted further to the required concentrations.

Suppose the required concentration is 25 ppm.

Stock concentration/ Required concentration =1000/25 = 40

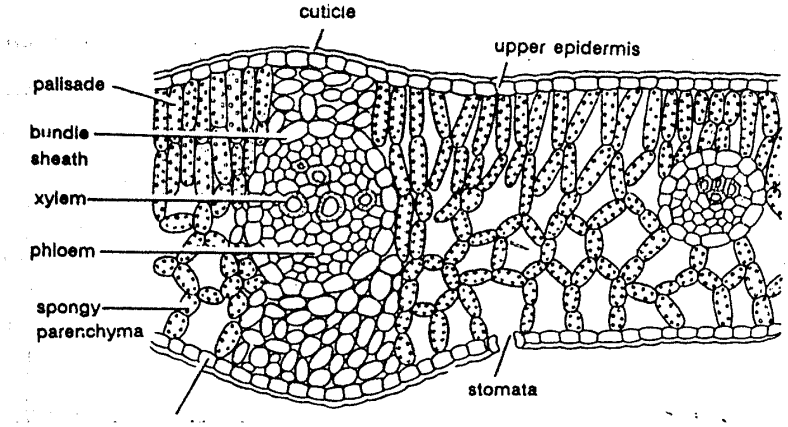
Therefore, to dilute 1000 ppm stock to 25 ppm solution, dilute 1 ml of the stock solution to 40 ml with water.

STUDY THE LEAF ANATOMY OF C3 PLANT AND C4 PLANTS

C3 plants are called temperate or cool-season plants. They reduce (fix) CO2 directly by the enzyme ribulose bisphosphate carboxylase (RUBPcase) in the chloroplast. The reaction between CO2 and ribulose bisphophate, a phosphorylated 5-carbon sugar, forms two molecules of a 3-carbon acid. This 3-carbon acid is called 3-phosphoglyceric acid and that's why the plants using this reaction are called C3 plants. Examples wheat, rye, oats, beans, rice, and potatoes etc.

MATERIAL REQUIRED Permanent slide, Microscope

OBSERVATIONS:



1. Epidermis: The leaf shows distinct upper and lower epidermal layers. The upper epidermis layer is made of single layer of compactly arranged cells. Stomata are completely absent. The lower epidermis is also single layer of cutinized cells. Stomata occur throughout the surface.

2. Mesophyll: It is differentiated into palisade and spongy parenchyma.

3. Palisade tissue is located just below the upper epidermis. The cells are radially elongated with numerous

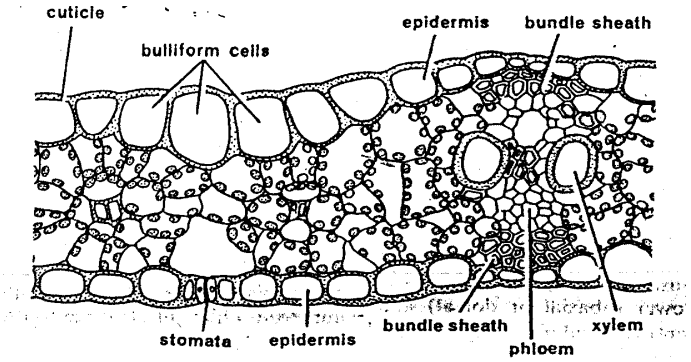
chloroplast close to the wall.

4. Spongy parenchyma forms the rest of the mesophyll. It is situated near the lower epidermis. Large intercellular spaces occur in between spongy parenchymatous cells.

5. Vascular bundles: Each vascular bundle consists of xylem, placed towards the upper epidermis and phloem situated toward the lower epidermis. Each vascular bundle is conjoint, collateral and closed. The vascular bundle is surrounded by a parenchymatous bundle sheath. Parenchyma and often collenchyma cells occur on both upper and lower sides of vascular bundle and reach up to the epidermis. This tissue is Known as bundle sheath extension.

LEAF ANATOMY OF C4 PLANTS

C4 plants are often called tropical or warm season plants. They reduce carbon dioxide captured during photosynthesis to useable components by first converting carbon dioxide to oxaloatcetate, a 4-carbon acid. This is the reason these plants are referred to as C_4 plants. Photosynthesis then continues in much the same way as C_3 plants. This type of photosynthesis in highly efficient and little fixed CO_2 is lost through photorespiration. Examples Maize, sugarcane, amaranth, sorghum etc.



MATERIAL REQUIRED: Permanent slide, Microscope

OBSERVATIONS:

1. Epidermis: The leaf is bound by thickly cuticularized epidermis on both sides. Stomata are found in lower as well as upper epidermis.

2. Mesophyll: This tissue is located between the two epidermal layers. It is composed of spongy parenchyma of varied shapes and sizes, leaving large intercellular spaces.

3. Vascular bundles: The vascular bundles differ in their size. Each vascular bundle is conjoint, collateral and closed. Tightly packed thick-walled bundle sheath cells surround the bundle. A patch of sclerenchyma occurs on both ends of each vascular which extends up to the epidermis on their respective sides. The bundle sheath cells are arranged like a wreath around the bundle. This is called Kranz anatomy because kranz means wreath. Large number of chloroplasts is present in the bundle sheath cells. Xylem is located towards the upper side while phloem is present in lower side.

PHENOMENON OF PLASMOLYSIS

Plasmolysis is the shrinkage of the protoplast of cell from its 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.

When we place a living cell in a solution with an osmotic potential identical to that of its own cell sap (an isotonic solution), the appearance of cell remains normal in every respect. If the water solution of surrounding is less negative than that of cell sap (hypotonic) then water enters cell and if more negative than that of cell sap (hypertonic) then cell shrink. If we immerse epidermal tissue from leaves of plants in a hypertonic solution of sucrose/salt, we can observe the plasmalemma pulling away from the cell wall because there will be a net movement of water out of the cell into the external solution.

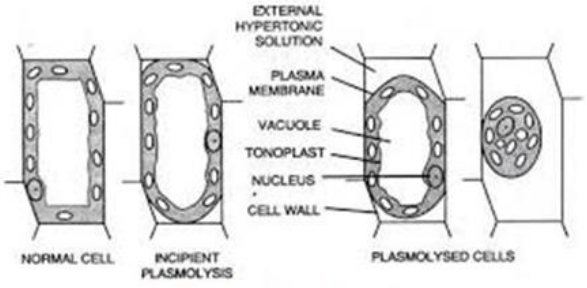


Fig. Stages of plasmolysis

MATERIAL REQUIRED: *Tradescantia* leaf/ Onion bulb, salt solution of different concentration (10 to 20%), glass slides, cover slips, microscope.

PROCEDURE:

- Peel off a small segment from the lower epidermis of *tradescantia* leaf or onion bulb.
- Keep this peel gently on a clean glass slide in a drop of water with the help of a brush and needle and mount with a cover slip. (Stain with safranin if you are taking peel of onion bulb).
- Observe under the microscope. Mark this observation as 'A'.

- Take another two glass slides and mount fully turgid cells of *tradescantia* leaf/ onion bulb peeling in a drop of salt solution of different concentrations (10 & 20 %).
- Allow it stand for 10 minutes. Observe each preparation under microscope. Mark these observations as B and C respectively, in order of their increasing concentrations.

Inference: When the peel of onion is kept in concentrated solution (hypertonic), the protoplasm shrinks as the water starts moving out due to exosmosis.

MEASUREMENT OF WATER POTENTIAL BY WEIGHT/GRAVIMETRIC METHOD

Water potential is defined as the difference between the free energy statuses of water in a system to that of free energy of pure water. Water potential is denoted by the Greek letter "Psi' (Ψ). Water potential is expressed either in bars or mega pascals (1 MPa = 10 bars). Water potential is diagnostic tool that enables the plant scientist to assign a precise value to the water status in plant cells and tissue. Absolute values of water potential are not measured. Instead measurement is made of the difference between the water potential in a system under investigation and that in a reference state. The reference state is pure liquid water at the same temperature and same atmospheric pressure as the system under investigation. Water potential in the reference stat is arbitrarily assigned a value of 0 bar. Water potential in a plant tissue is always less than 0 bar and hence a negative number. Water potential is experimentally determined by the following methods.

1. Gravimetric method
2. Chardakov's method
3. Pressure bomb method
4. Vapour pressure or thermocouple method or Psychrometer

GRAVIMETRIC METHOD

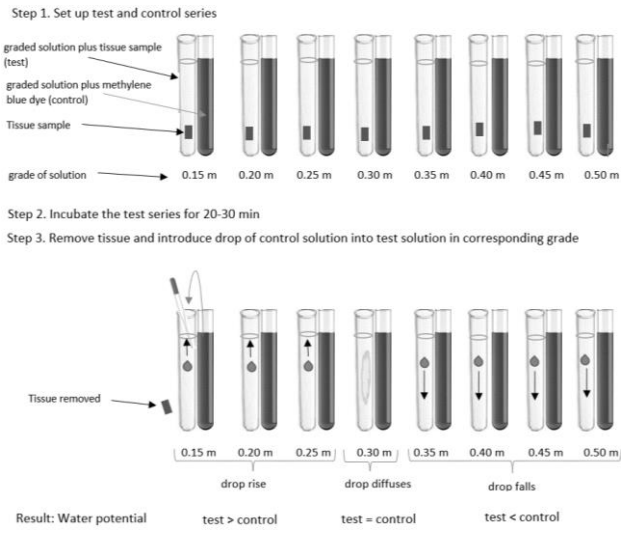
MATERIAL REQUIRED: Potato, sucrose or mannitol, distilled water, analytical balance, test tube, cork borer, blade, filter paper

PROCEDURE: Take 11 test tubes and prepare different concentration of molar sucrose solutions of 0.1 M, 0.2M, 0.3M, 0.4M, 0.5M, 0.6M, 0.7M, 0.8M, 0.9M and 1.0M with control water.

- Take potato and take out 12 potato cylindrical sections with the help cork borer and cut each of at least 2-3 cm long in uniform length with razor blade.
- Weigh each potato cylinder with analytical balance to the nearest milligram.
- After weighing, each potato cylinder place in each of the series of known concentration of sucrose solutions.
- After incubation of 1.5 -2.0 hours remove the potato cylinder and blot them gently on filter paper and weigh them again.
- The weighing should be done in chronological order, in which they were initially placed.
- Note down the molal concentration of sucrose solution, at which there is neither loss nor gain in potato cylinder weight.
- Then plot a graph following the changes in weight against sucrose concentration.

CHARDAKOV'S DYE METHOD OR FALLING DROP METHOD

Russian scientist V.S. Chardakov devised this method in 1948. It is a simple and efficient method of determining the test solution in which no change in concentration occurs. It can be often used in the field. Chardakov's dye method is based on the change in density of the solution, the drop sinks or rises as a result of absorbed water from the tissue water potential of the tissue is determined.



MATERIAL REQUIRED: Plant tissue (potato or leaf), test tube, sucrose, methylene blue indicator, dropper, measuring cylinder and water

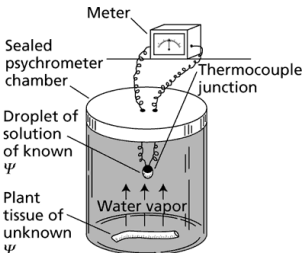
PROCEDURE:

- Prepare 1 M stock solution of sucrose and from this, make graded series of sucrose solution ranging from 0.15 to .50 molar in increments of 0.5 molality) in eight separate test tubes and label this series of test tubes as 'control series'.
- Then set up another series of same graded sucrose solution in eight test tubes and label this as 'test series'.
- Now cut the plant tissue of equal size and transfer into each test tube of test series.
- Add a pinch of methylene blue powder in each test tube of control series. (note- plant tissue is not added in control series and dye does not appreciably change the osmotic potential).
- After the tissue incubated for 20-30 minutes, a small drop from the respective control series solution is introduced one

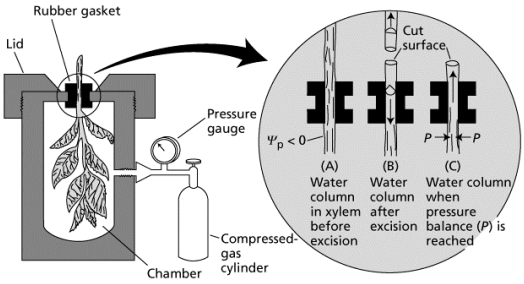
inch below the surface of its corresponding test series solution and slowly release the drop of control solution.

VAPOUR EQUILIBRIUM (THERMOCOUPLE PSYCHROMETER) METHOD

Psychrometer is based on the fact that the vapour pressure of water is lowered as its water potential is reduced. Psychrometers measure the water vapour pressure of a solution or plant tissue, on the basis of principle that evaporation of water from a surface cools the surface.

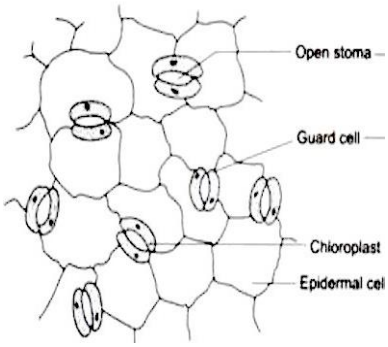


PRESSURE CHAMBER METHOD: This is advanced apparatus for measuring of water potential of plant tissue by the use of the pressure chamber. The pressure bomb is a device that is used to determine the plant moisture stress and water potential of leafy shoot. The shoot is placed in a chamber with the cut end protruding through an airtight hole. Pressure is increase within the chamber and the water column with in the twigs are forced back to the cut end surface. The pressure in the chamber is then recorded.



STOMATA IN MONOCOT AND DICOT LEAVES

INTRODUCTION: Stomata are small openings found widely scattered on the epidermis of leaves and young stems. They are mostly found on the lower surface of a dicot leaf and on both the surfaces of a monocot leaf. Stomata regulate the exchange of gases and water vapour between the atmosphere and leaves. Stomata are surrounded by two distinct epidermal cells called guard cell. Guard cells are various types but most common are kidney shaped or bean shaped in dicot and dumb-bell shaped in monocot. Stomata can be classified on the basis of their distribution on the leaf surfaces. These are of following types:



Kidney shaped stomata in

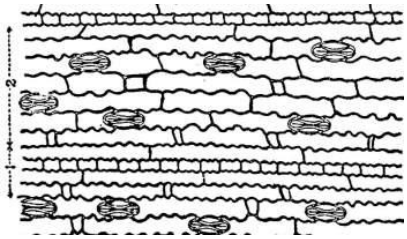
Apple type: When the stomata are present only on the lower surface of the leaf, the condition is known as hypostomatous. e.g. Apple, Peach, Mulberry, and Walnut.

Potato type: When the stomata are found more on the lower surface than the upper surface. e.g. Potato, Tomato, and Pea.

Oat type: When the stomata are found equally on the both surfaces, the condition is known as amphistomatous. e.g. Oat, Maize, and Grasses.

Water lily type: When the stomata are found only on the upper surface. e.g. Water lily.

Potamogeton type: When the stomata are absent or non-functional. e.g. Potamogeton.



Dumb-bell shaped stomata in monocot leaf

MATERIAL REQUIRED: Fresh leaf Tradescantia or Bryophyllum, forceps, needles, watch glasses, glass slides, a dropper, coverslips, a brush, blotting paper, safranin, glycerin and a compound microscope.

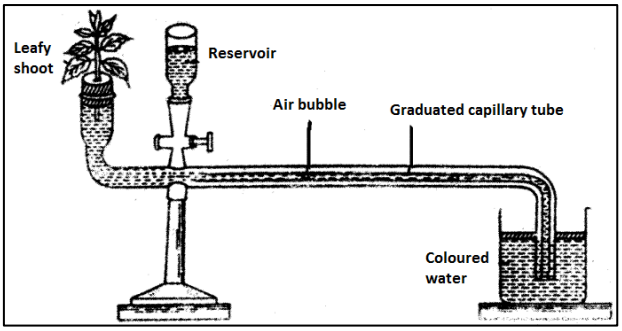
PROCEDURE:

- Remove a healthy leaf from the potted plant.
- 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.
- Put a few drops of safranin stain in a watch glass
- After 2-3 minutes take out the peel and place it on a clean glass slide.
- Put a drop of glycerin over the peel and place a clean coverslip gently over it with the help of a needle
- Remove the excess stain and glycerin with the help of blotting paper.
- Observe the slide under the low-power and high-power magnifications of the compound microscope.

OBSERVATIONS AND RESULTS: The epidermal cells are visible. These are irregular in outline and have no intercellular spaces. Many small pores (stomata) are seen scattered among the epidermal cells. Each pore is guarded by two bean shaped guard cells, each containing chloroplasts and a nucleus.

TRANSPIRATION BY GANONG'S POTOMETER

Ganong's potometer: It consists of a graduated tube dipped into the beaker containing water. The graduated tube is connected with a vertical arm bearing a cork on its mouth. The cork contains one hole through which a twig is inserted in the water of the vertical arm. Vertical arm is also attached with a stop cork connected with a water reservoir.



The loss of water in the vapour form, from the exposed parts of a plant is termed as transpiration. It is generally of four types, viz. stomatal, cuticular, lenticular and bark. Stomatal transpiration is most extensive and accounts for 50-97% of total transpiration from a plant.

MATERIAL REQUIRED: Ganong's potometer, fresh leafy twig, water, beaker, Paraffin wax

PROCEDURE:

- Fill the apparatus with water through the water reservoir by opening the stopcock connected with the water reservoir.
- Cut the fresh twig under water and Insert a freshly cut twig in the water of the vertical arm through the hole of the cork.
- Make all the joints air-tight by applying grease or paraffin wax.
- Dip the other bend end of the graduated tube into a beaker containing water colored with eosin/safranin
- Now introduce an air bubble in the graduated tube by the lifting the tube for a moment and dip into water again and keep the whole apparatus in sunlight.
- Note the initial and final readings of the bubble in given time in different conditions like sunlight, shade, darkness and by placing the plant in front of a fan in sunlight.

LEAF PEEL TO SHOW STOMATAL DENSITY AND STOMATAL INDEX

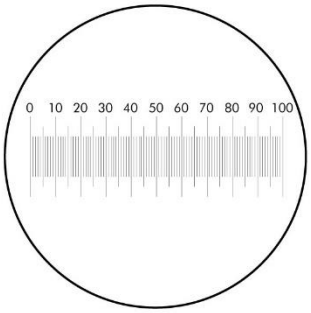
Stoma (plural-stomata) is a minute epidermal opening covered by two kidney shaped guard cells in dicot leaves. These guard cells, in turn, are surrounded by epidermal (subsidiary) cells. Stomata perform the functions of gaseous exchange and transpiration in plants. The nature of the stomata, as well as, the stomatal index and stomatal number are important diagnostic characteristics of dicot leaves. Stomatal number is defined as the average number of stomata per mm^2 of epidermis of the leaf. The actual number of stomata per sq mm may vary for the leaves of the different plant grown in different climatic conditions. Stomatal index is the percentage which the number of stomata form to the total number of epidermal cells present in microscopic view field.

MATERIAL REQUIRED: Fresh leaf of *Tradescantia* or *Bryophyllum* plant, forceps, needles, watch glasses, glass slides, a dropper, coverslips, a brush, blotting paper, safranin, glycerine and a compound microscope.

PROCEDURE:

- Take healthy leaf from the potted plant.
- 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.
- Place the peel on a clean glass slide and Put a few drops safranin stain for staining the epidermal peel.
- Put a drop of glycerin over the peel and place a clean coverslip gently over it with the help of a needle.
- Remove the excess stain and glycerine with the help of blotting paper.
- Observe the slide under the low-power and high-power magnifications of the compound microscope.
- Now count the number of stomata present in microscopic view field can be recorded for calculating the stomatal density that can be expressed in terms of number of stomata/mm^2 and stomatal index by using the formula, stomatal index (%) = (S/S+E) x 100 where, S and E are the number of stomata and epidermal cells respectively in microscopic view field.
- Calculate the diameter of view field by ocular scale. Now the area of circle under microscopic view field can be calculated by following formula: πr^2 where, r is the radius of the circle (view field) i.e., ½ of the diameter of circle.

CALIBRATION OF OCULAR AND STAGE MICROMETER



STAGE MICROMETER: It is a glass slide on which a 1 mm scale is accurately ruled into 10 equal divisions of 1/10 mm. Each division is subdivided into 10, so that each small division equals 1/100 mm (10 µm)

OCULAR MICROMETER: It is a glass disc which can be inserted into the eye piece of the microscope. The disc has a scale, 1 cm long divided into 100 equal spaces. Every tenth space is numbered. Calibration is done by comparing ocular with stage micrometer.

ROCEDURE FOR CALIBRATION:

1. Place the stage micrometer (SM) on the stage of the microscope and focus it at a given magnification e.g. 40X.
2. Place the ocular micrometer (OM) in one of the eye piece of microscope. Align the scale of OM with that of SM. While observing through the eye piece.
3. Count the division of OM coinciding with division of SM.
   For example:
   4 division of OM = 45 division of SM i.e. 450 µm
   1 division of OM = 11.25 µm
4. Following the above three steps calibrate the ocular scale for other magnification also i.e. 60 X, 100 X etc.
5. Once the ocular scale is calibrated for different or required magnifications, SM can be removed and slide having sample material may be placed for observation and measurement.

ESTIMATION OF LEAF AREA OF PLANTS BY VARIOUS METHOD

Leaf is assigned as one of the important organs of plant system and further development of plant depends upon the persistence of leaves. Physiologically, leaf area constitutes the main photosynthetic surface and supplies most of the photosynthates required by the seed, fruit or any storage organs. So, the estimation of leaf area is an essential integral part of classical growth analysis and is often important in physiological reasoning of variations in crop productivity. For the estimation of leaf area, several methods have been developed.

1. Graphic method
2. Dry weight method
3. Linear method
4. Leaf Area Meter

1. Graphic method

This involves the use of graph papers for the estimation of only smaller leaves or irregular shaped leaves (e.g. Castor, papaya etc.). So, this type of method cannot be used for estimating the leaf area for all types of leaves. For estimating the leaf area, the outline of the leaf is drawn on a graph paper and the number of full squares, half squares and quarter squares are counted and added. The leaf area is expressed as cm^2 per leaf.

- **Requirements:** Fresh leaves of various species, graph paper, scale, pencil
- **Procedure:** Place the leaf whose area is to be measured on graph paper and draw its outline. Remove the leaf and mark the squares (cm) within the outline of the leaf as:
  Complete square (A) = 1 cm^2
  Half the square (B) = 0.5 cm^2
  More than half the square (C) = 1 cm^2
  Less than half the square (D)=zero cm^2
  Calculate the area of the leaf as : A + B + C + D = X cm^2

2. Dry weight method

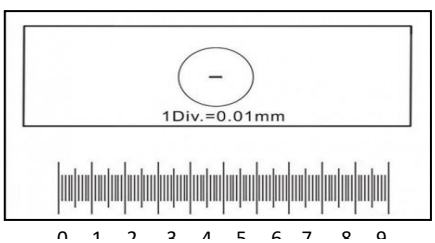
The leaf area (L1) occupied by known dry weight (W1) of the single leaf can be found out adopting any one of the methods. This forms the basis for calculating the whole plant leaf area (L2) based on the total dry weight (W2) by using the given formula.

Total leaf area (L2) = $\frac{L1}{W1}$ x W2

Where, L2 - Total leaf area; W2 - Total leaf weight (dry)
L1- Single leaf area,
W1 – Single leaf weight (dry)

3. Linear method

This method is relatively simple, time saving and non-destructive method for estimating the leaf area of regular shaped leaves (e.g. mango, wheat etc.). Montgomery (1911) studied the statistically defined mathematical relationship between the linear dimensions of the leaf area and proposed the following formula.



Leaf Area (A) = K x L x B

Where,

A = leaf area per leaf

L = maximum length of the leaf

B = maximum breadth of the leaf

K = leaf area constant (**0.75** is a constant evolved after experimentation).

The value of leaf area constant (K) is the ratio between actual leaf area and apparent leaf area and is always less than 1.

4. Leaf Area Meter (Electronic Method)

Leaf Area Meter is used for estimating leaf area of all types of leaves. This method is also termed as direct method. But the leaves should be removed or detached from the plants and fed into to the area meter. The estimation can be done only in the laboratory. In the area meter, fluorescent light source, mirror and scanning camera and a conveyor belt are provided. Initially zero is set. When a leaf is placed in the conveyor belt it moves along with conveyor belt and when the leaf comes close to scanning camera, it reflects the image of leaf on the mirror and the reading is measured digitally. Area of leaf is expressed in cm^2.

PLANT GROWTH ANALYSIS

Growth is a characteristic of life. It is the foremost symbolization of life in action. Growth continues till the end of an organism. It is expressed as height, weight (size), volume, number and area. Growth is always phenomenal. If growth stops vertically it may commence horizontally. If leaf expansion stops, it starts gaining in number and volume. Growth rate is quantified mathematically in terms of time as in cases of other vital processes of plants and this gives us a valuable information documenting growth as influenced by various abiotic, biotic, edaphic and seasonal factors. Growth analysis can be made at individual plant level or of plant communities.

Analysis of Individual plant growth, generally made at the early stage includes relative and absolute growth rate, net assimilation rate, leaf area ratio, specific leaf area, specific leaf weight and allometry (shoot/root ratio). Parameters used in growth analysis of plant communities includes leaf area index, leaf area duration and crop growth rate.

The technique of growth analysis is advantageous to plant scientists as it helps

1. To find out the relationship between photosynthetic production and rate of increase in dry matter.
2. To investigate the ecological phenomenon and competition between different species.
3. To predict the effect of agronomic manipulation.

Parameters of Growth Analysis

1. Leaf Area Index (LAI): Leaf area index (LAI) expresses the ratio of leaf surface to the ground area occupied by the plant. LAI is the measure of available photosynthetic surface per unit land area.

$$LAI = \frac{Leaf\ Area\ of\ Plant}{Leaf\ area\ occupied\ by\ a\ plant}$$

2. Leaf Area Ratio (LAR): The term leaf area ratio was suggested by Redford (1967). It is defined as the ratio of area of the leaf to the total plant biomass per plant. It is measure of leafiness or photosynthetic surface relative to respiratory mass. It is expressed in terms of $cm^2.g^{-1}$

$$LAR = \frac{Leaf\ Area\ of\ Plant}{Total\ dry\ weight\ of\ plant}$$

3. Leaf Area Duration (LAD):

It is ability of the plant to maintain the green leaves per unit area of the land over a period of time. It reflects the vitality of leaves and an opportunity for assimilation. LAD is the integral of leaf area index over a growth period and expressed in **days**.

$$LAR = \frac{LAI_1 + LAI_2}{2} x\ (t_2 - t_1)$$

Where

, LAI_1 = Leaf area index at time t_1 LAI_2 = Leaf area index at time t_2

4. Specific Leaf Area (SLA): Specific leaf area is the ratio of leaf area to its dry weight. It is measure of relative spread of leaves. It is expressed in $cm^2.day^{-1}$

$$SLA = \frac{\text{Leaf Area}}{\text{Leaf dry weight}}$$

5. Specific Leaf Weight (SLW): Specific leaf weight is the ratio of leaf dry weight to leaf area. It indicates the leaf thickness and density and it is expressed as $g.cm^{-2}$

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

6. Net Assimilation Rate (NAR): NAR is a measure of the amount of photosynthetic product going in to plant material. It is the rate of increase of leaf by dry weight per unit area of leaf per unit time. It is expressed in $g.m^{-2}$ (leaf area). day^{-1}

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

Where, L_1 & W_1 = Leaf area and dry weight of the plant respectively at time t_1
L_2 & W_2 = Leaf area and dry weight of the plant respectively at time t_2

7. Relative growth Rate (RGR): It is rate of increase of dry weight per unit weight already present per unit time. Or RGR express the dry weight increase in a time interval in relation to the initial weight. It is expressed in $g.g^{-1}.day^{-1}$

$$RGR = \frac{(l_n W_2 - l_n W_1)}{(t_2 - t_1)}$$

Where, W_1 and W_2 are plant dry weight at time t_1 and t_2 respectively.

8. Absolute growth Rate (AGR): It expresses the dry weight increase per unit time and is expressed in g/plant/day.

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

Where, W_1 and W_2 are plant dry weight at time t_1 and t_2 respectively.

9. Crop growth Rate (AGR): It is the rate of increase of dry weight per unit land area per unit time. CGR is a simple and important aid of agricultural productivity. It is expressed in g. m^{-2} (land area). day^{-1}

$$AGR = \frac{(W_2 - W_1)}{(t_2 - t_1) \times S}$$

Where, W_1 and W_2 are plant dry weight (g) at time t_1 and t_2 respectively.
S is land area (m^2) over which dry matter was recorded

10. Harvest Index (HI): It reflects the production of assimilate distribution between economic yield and total biomass yield.

$$HI\ (\%) = \frac{\text{Economic Yield}}{\text{Plant Biomass (above ground)}}\ x\ 100$$

CELL MEMBRANE STABILITY IN PLANT TISSUE

The environmental stresses in plants affects cellular membranes, which disturb its function partly or totally. The cellular membrane disfunction due to abiotic stress like (heat, drought, salinity and chilling) can be expressed in increase permeability and leakage of ions which can be radially measured by the efflux of electrolytes. Hence, the estimation of membrane dysfunction under stress by measuring cellular electrolyte leakage from affected leaf tissue into an aqueous medium is finding a growing use as a membrane stability (CMS) and as a screen for stress resistance.

REQUIREMENTS: Leaf samples, double distilled water. Conductivity meter, water bath, electrical balance, test tubes

PROCEDURE:

- Take 100 mg fresh leaf tissue and wash thoroughly under running tap water followed by washing with double distilled water.
- Cut the leaves of1-2 cm length or discs and transfer these leaf discs in test tubes containing 10 ml distilled water.
- Now heat the test tubes containing leaf discs in boiling water bath at 40^0C for 30 minutes.
- Measure the electrical conductivity (C1) by conductivity meter.
- Subsequently, keep the same samples in boiling water bath at 100^0C for 10 minutes and take the electrical conductivity (C2). The CMS can be calculated as: CMS = [1-(C1/C2)] x 100

TO DETECT THE PRESENCE OF DEPOSITIONS OF WAX AND CUTIN

A) To detect the presence of cutin in a given leaf sample.

REQUIREMENTS: Microscope, slides, leaves of *Ficus*, *Nerium*, potassium hydroxide (KOH) solution

PROCEDURE:

- Cut a cross section of leaf (*Ficus* and *Nerium*) and place the section in drop of water on clean slide.
- Treat the section with potassium hydroxide solution.
- Observe the colour of outmost deposits on epidermis under the microscope.

B) To detect the presence of suberin in the given material.

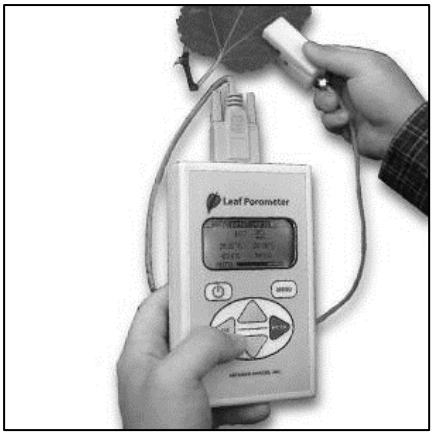
REQUIREMENTS: Microscope, slide, natural cork, alcoholic sudan IV dye, 50% alcohol, glycerine.

PROCEDURE:

- Cut the thin sections of natural cork. Incubate the section in alcoholic sudan IV dye for 20 minutes.
- Wash off the excess stain with 50% alcohol.
- Transfer the section to water and mount in glycerine on the slide**.**
- Observe the colour under microscope.

MEASUREMENTS OF STOMATAL CONDUCTANCE UNDER VARYING STRESS CONDITION.

Stomatal conductance estimates the rate of gas exchange (i.e., carbon dioxide uptake) and transpiration (i.e., water loss) through the leaf stomata as determined by the degree of stomatal aperture (and therefore the physical resistances to the movement of gases between the air and the interior of the leaf). Hence, it is a function of the density, size and degree of opening of the stomata; with more open stomata allowing greater conductance, and consequently indicating that photosynthesis and transpiration rates are potentially higher. The handheld porometer provides rapid measurement of leaf stomatal conductance under varying environmental conditions.



Hand-held Leaf Porometer

Measurement Procedure:

- Choose a flag leaf that is clean, dry, free of disease and receiving sunlight to the adaxial surface.
- Place the leaf into the chamber at the mid-point of the leaf and ensure that the selected area of the leaf completely covers the aperture of the sensor.
- To start taking measurements press 'ENTER'. Once the readings have equilibrated press 'ENTER' again to hold the reading. The reading can then either be recorded manually or saved to the instrument.
- Typical values for irrigated conditions are: 300-700 mmol $m^{-2}s^{-1}$; and for mildly water stressed conditions are: 80-300 mmol $m^{-2}s^{-1}$.

Advice on taking measurements: Measurements should be made on the youngest fully emerged leaf receiving sunlight; typically, the flag leaf once fully expanded. Be sure to select leaves which are exposed to the sun, and not those in the shadow or shade as these will have very different readings to those leaves in the sun. The leaves must be clean, dry, intact, green, with no sign of disease or damage. Measurements are typically made on the upper (adaxial) surface of the leaf.

CHLOROPHYLL PIGMENT IN PLANT TISSUES

Chlorophyll pigment is a large molecule with a tetra pyrrol ring and a magnesium ion held in it. Attached to one of the rings is a long insoluble hydrocarbon ring, a 20-carbon phytol
group. Chlorophyll b has a -CHO group in the third carbon of second pyrrol ring instead of -CH3 group as in the case of chlorophyll a.
Chlorophyll a and chlorophyll b have typical absorption spectra of solar radiation. Maximum peak of chlorophyll a is observed in blue violet (429 nm) and in red region (660 nm) while the chlorophyll b absorbs at 453 nm and 642 nm. Chlorophyll a is usually blue green and chlorophyll b is yellow green in colour. The formula for the chlorophyll a molecule is $C_{55}H_{72}O_5N_4Mg$ and chlorophyll b molecule is $C_{55}H_{70}O_6N_4Mg$.
MATERIALS REQUIRED: Fresh leaf, 80 % acetone, distilled water, balance, spectrophotometer, mortar and pestle
PROCEDURE:

- Take 250 mg of leaf sample and macerated with 10ml of 80% acetone using a pestle and mortar
- Extract or slurry is centrifuged at 3000 rpm for 10 minutes.
- The supernatant solution is transferred into a 25ml test tube and made up to 20ml using 80% acetone.
- The color intensity of the green pigment is read at 645nm, 663nm and 652nm for chlorophyll a, chlorophyll b and total chlorophyll content respectively using spectrophotometer against the solvent (80% acetone) blank.

SEPARATION OF PHOTOSYNTHETIC PIGMENTS BY PAPER CHROMATOGRAPHY

In paper chromatography, substances placed on one end of the chromatographic paper get deposited on various zones of paper when an appropriate solvent runs over to the other end of the paper. The mobility of substance on the paper depends on the degree of solubility in the solvent system (mobile phase) and the affinity to the chromatography paper (stationary phase), which are mostly made up of pure cellulose fibre. Flat paper sheet or round paper cylinders may be used for the separation of substances. Paper chromatography can be distinguished into two types, the ascending chromatography and the descending chromatography. In ascending chromatography, the solvent is placed at the bottom and the paper is hung in such a way that the lower end of the paper is immersed in the solvent. The mixture of substances is loaded at about 2 cm high from the base and the solvent moves up against the gravitational force. In descending chromatography, the solvent is placed on the upper side and the substances are loaded near the upper end of the paper. Which is dipped in the solvent. The solvent migrates down the paper by gravitational pull. Due to the gravitational pull, descending chromatography is faster than ascending chromatography.

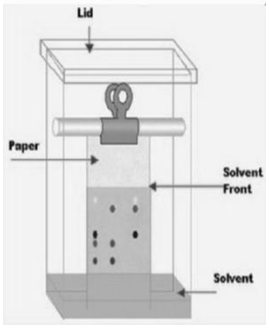
The compounds on the chromatogram can be identified on the basis of their diagnostic feature, the ratio of fronts' (Rf)

values. Rf is the ratio of the distance travelled by the substance to the distance travelled by the solvent in a chromatogram.

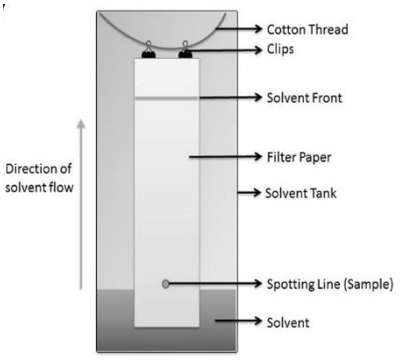
$$Rf = \frac{Distance\ travelled\ by\ the\ substance}{Distance\ travelled\ by\ the\ solvent}$$

MATERIALS REQUIRED: Fresh spinach leaves, 80% acetone (v/v), petroleum ether, benzene, filter paper, chromatography paper, scale, paper clips

GLASSWARE AND EQUIPMENT: Mortar and pestle, Buchner funnel, chromatography jar, support rods, capillary tube, hair dryer



Ascending Paper



PROCEDURE:

Pigment extraction

- Take 250 mg of fresh leaf material in a mortar and add 10 ml of 80% acetone and grind with pestle.
- Filter resulting green-coloured slurry by using Buchner funnel containing a layer of Whatman No.1 filter paper.

Separation of pigments

- Pour a mixture of petroleum ether (95%) and acetone (100:12, v/v) or benzene – acetone (85:15 v/v) in a chromatographic jar to depth of about 2 cm.
- Now, cut a strip of chromatographic paper (Whatman No.1) to desired size (usually 5"x2") and draw a pencil line about 2 cm away from the bottom of strip
- With the help of a glass capillary tube 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 apply the next drops.
- Repeat the application of drops until the marks are dark green
- Now, hang the paper in the chromatography jar with lower end dipped in the solvent but make sure the loading spots just above from upper layer of solvent.
- Remove the paper when the solvent has moved up to the top of the paper. Allow the paper to dry.

MEASUREMENT OF RELATIVE WATER CONTENT

The relative water content (RWC) is a useful indicator of the status of water balance of a plant essentially because it expresses the absolute amount of water, which the plant requires to reach artificial full saturation. Thus, there are a relationship between RWC and water potential. It estimates the current water content of the samples leaf tissue relative to the maximal water content it can hold at full turgidity. This relation varies significantly according to nature and age of plant material. The RWC express the water content in per cent at a given time as related to the water content at full turgor:

$$RWC = \frac{fresh\ weight - dry\ weight}{turgidity\ weight - dry\ weight}\ x\ 100$$

MATERIAL REQUIRED: Fresh leaf material, petri plates, distilled water, scissor, polythene bags, electronic balance, hot air oven

PROCEDURE:

- Collect the leaf sample; usually fully expended top most leaf is preferable.
- Immediately after sampling place the sample in a polythene bag and seal properly to minimize water loss from the leaf and Sample should reach the lab as soon as possible.
- Cut 5-10 leaf discs of around 1.5 cm in diameter or take several leaflets (in smaller composite leaves) depending upon the plant species.
- Weight the sample quickly to record the fresh weight.
- Hydrate the sample to full turgidity by floating on de-ionized water in closed petri-dish for 4 hours at normal room temperature.
- After 4 hours take out the sample and remove any surfaces moisture quickly and lightly with tissue paper and immediately weigh to obtain fully turgid weight.
- Dry the samples in a hot air oven at 70◦C for 48 hours
- Weight the dry weight of the samples after proper drying.

PRECAUTIONS:

- Avoid large veins and mid-rib of leaf tissue
- Excess water should blotted from the leaf surface before recording its turgid weight

- We should not take samples of different ages, different exposures or collected at different time of day.