Practical Manual

Crop Physiology

(PPY 121)

Prepared By----

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Crop Physiology

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Practical No.-1

Objective: The objective of studies is to gain a detailed understanding of the organization and function of cellular organelles and structures.

Cell: The cell (from Latin *cella*, meaning "small room") is the basic structural, functional, and biological unit of all known living organisms. A cell is the smallest unit of life. Cells are often called the "building blocks of life". The study of cells is called cell biology. The term cell was first used by the English botanist Robert Hooke in 1665, to describe the individual units of the honeycomb-like structure in cork under compound microscope. Plants are multicellular organisms composed of millions of cells with specialized function. All plant cells have the same basic eukaryotic organization. Several parts of a plant cell can easily be observed under a light microscope, e.g., cell wall, plasma membrane, vacuole, plastids and nucleus (Fig. 1). Due to their relatively smaller size, mitochondria cannot easily be seen with a light microscope but they can be made visible under light microscope by staining with special chemicals.

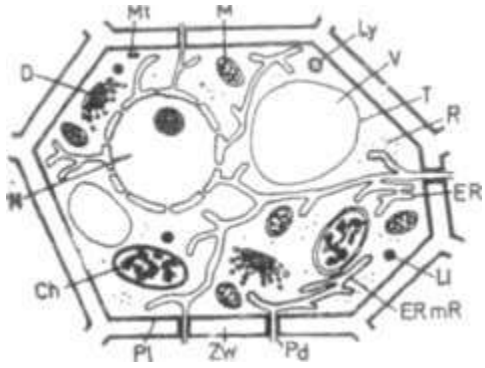


Fig. 1. Schematic diagram of a typical young plant cell. Ch = chloroplast. D = dictyosome, ER = endo¬plasmic reticulum, ERmR = endoplasmic reticulum with ribosomes, Li = Lipid bodies, Ly = Lysosomes. M = mitochondria, Mt = microtubule N = nucleus, Pd = plasmodesmata, PI = plasmalemma, R = ribosomes, T= tonoplast, V = vacuole, Zw = Cell wall.

Material Required:

Leaves of *Funaria hygrometrica***,** *Spinacia oleracea* (spinach), scales of onion bulb (*Allium cepa*), tomato fruit (*Lycopersicum esculentum*), flowers of roses (*Rosa sp.),* pansy (*Viola*

tricolor), potato tuber (*Solanum tuberosum*).

Carmine-alum solution: Dissolve l g carmine + 10 g alum (K_2SO4 . $Al_2(S0_4)_2$. 24 H_2O) in 200 ml distilled water by warming and filter the solution.

Aceto-carmine solution: Boil 4-5 g carmine in 100 ml of 50% acetic acid and filter after cooling

Iodine solution: Dissolve 0.5-1.0 g of potassium iodide (KI) in 100 ml of water followed by dissolving 1 g of iodine crystals

Rhodamine B solution: dissolve 0.10 mg rhodamine B in 100 ml of tap water

Movement of protoplasm: Remove few hair cells from the leaves, leaf petioles of *Cucumber leaves or* tomato and mount them on a glass slide. Put a drop of water and place a coverslip over the material. First observe under the low power of a microscope and select a most suitable cell. Observe under the high power the movement of protoplasm.

Cell wall and nucleus: Cut an onion bulb into four pieces. Cut out a small piece from the interior side of the bulb and peel up carefully an epidermal layer. Place the stripe of epidermal cells on a glass slide and mount on a drop of carmine-alum stain. Allow to stain for about 15 min,. Wash away excess of stain with dilute acids. Nucleus can be stained by applying a drop of aceto-carmine solution. If carefully observed at high power, one can even see nucleioli which are not or only very faintly stained by the stain. In addition to epidermal cells of onion scale, very distinct and big nuclei can also be seen in the hairs (trichome cells) of a cucumber leaf or the epidermal cells of young leaves of *Tradescantia.*

Plastids: Chloroplasts: Mount thin sections of green leaves of maize, spinach on a microscopic slide. Place a cover slip over the material. First observe under the low power and then under high power of a microscope. In maize leaf transverse section, one can observe chloroplast dimorphism, i.e., two different kinds of chloroplasts. Observe the chloroplasts in the meosphyll cells and the bundle sheath cells. Mark the differences between the two types of chloroplasts.

Chromoplasts: Chromoplasts are plastids which contain no chlorophyll pigments. They contain yellow (carotenes, xanthophylls) or red (anthocyanins) pigments and are commonly found in fruits (tomato) and flowers. Make thin sections of the yellow flower petals (e.g.

pansy, roses), red tomato-fruit skins and carrot. Mount them with a drop of water on a glass slide, cover with a coverslip and observe carefully, first under low power and then under high power. Yellow and red coloured round bodies can be distinctly be seen.

Leucoplasts: They are colourless plastids and are found in roots, rhizomes and different storage organs. Peel off lower epidermis from the leaves of *Rhoea* discolor and mount it in a drop of water on a glass" slide. Cover with a cover slip. Focus under the high power a distinct nucleus.

Mitochondria: Prepare few stripe of epidermis from onion scales as described above. Place them in a bottle containing water. Apply suction using an aspirator. Take out the stripes, place them on a glass slide and treat with few drops of rhodamine-B solution for about 30 min. Wash away the excess of stain with tap water. Observe under a phase contrast microscope. Under green light, mitochondria appear as black rounded structures.

Result:

Draw diagrams of a plant cell showing its different parts and their ultrastructure.

Precaution

- Ensure proper fixation of cells to preserve their structures.
- If staining is required, use cell-compatible stains and follow recommended protocols.
- Use the appropriate fixative, and follow recommended fixation times and concentrations to avoid over-fixation or under-fixation.

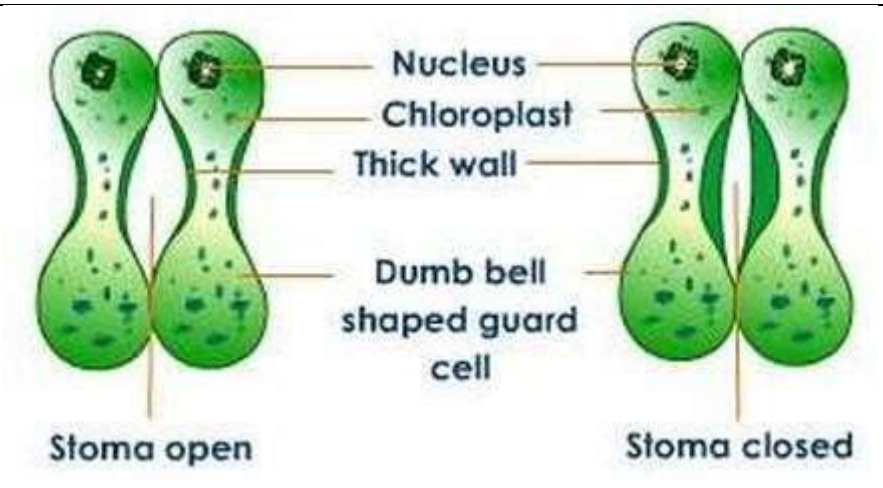
Practical No.-2

Objective: **Clearly identify and characterize the different components of stomata, including the guard cells, subsidiary cells, and stomatal pores**.

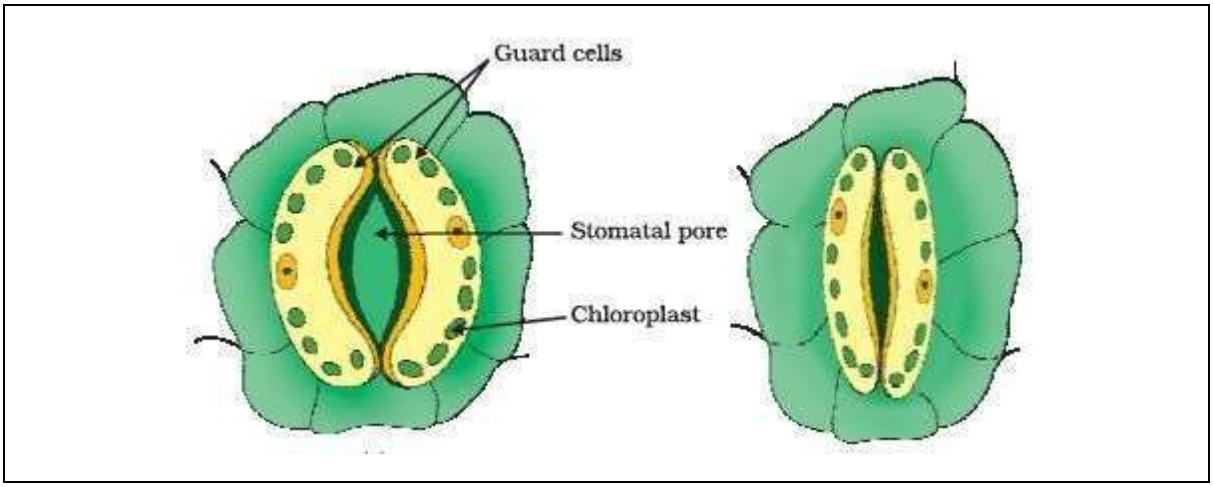
Stomata are tiny pores found in the epidermis of leaf. Size may be ranged from 7/3 µ (Phaseolus vulgris) and 38×8 µ (in Avena sativa). Each stoma is normally surrounded by two specialized eprdermal cells known as Guard cells. The guard cells are kidney shaped in dicotyledonous plants and dumbbell shaped in the members of poaceae family (monocotyledonous plants) and they remain joined at the end.

Guard cells play significant role in opening and closing of stomata and the outer layer guard cells wall is thin and elastic and inside the layer is thick and inelastic due to the presence of a secondary layer of cellulose. Guard cells contains many organelles such as nucleus, vacuole, chloroplast, starch molecules floating under cytoplasmic fluid, central vacuole containing cell sap. Stomata was discovered by Pfeffer & name 'stomata' was given by Malphigii. Stomata cover 1-2% of leaf area. It is minute pore present in soft aerial parts of the plant. Algae, fungi and submerged plants do not possess stomata

DUMBBELL SHAPED IN MONOCOTYLEDONOUS PLANTS



KIDNEY SHAPED IN DICOTYLEDONOUS PLANTS



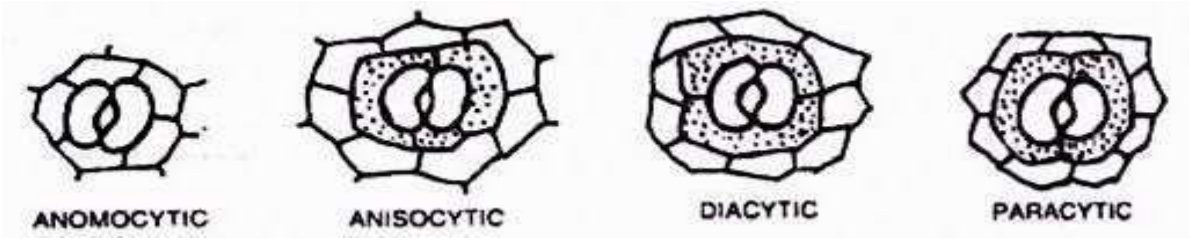
METACALF AND CHALK RECOGNIZED FOUR TYPES OF STOMATA ON THE BASIS OF THEIR STRUCTURE:

a. Anomocytic type:

In these stomata, accessory cells are absent. The guard cells are surrounded by ordinary epidermal cells, e.g., families Ranunculaceae, Cucurbitaceae, Papaveraceae and Malvaceae.

b. Anisocytic type:

In these stomata the guard cells are surrounded by three accessory cells. Of these two are larger whereas one is smaller in size.g., family Brassicaceae.



c. Diacytic type:

In these stomata the guard cells are surrounded by two accessory cells. Their common walls are at right angle to the walls of guard cells, families Caryophyllaceae, Acanthaceae.

d. Paracytic type:

In these stomata the guard cells are also surrounded by two accessory cells, but their common walls are parallel to guard cells, e.g., families Rubiaceae, Fabaceae etc.

DISTRIBUTION AND TYPES OF STOMATA:

Depending upon the distribution and arrangement of stomata in the leaves five categories of stomatal distribution have been recognized in plants.

1. Apple or mulberry (hypostomatic) type:

Stomata are found distributed only on the lower surface of leaves, e.g., apple, peach, mulberry, walnut, etc.

2. Potato type:

Stomata are found distributed more on the lower surface and less on its upper surface, e.g., potato, cabbage, bean, tomato, pea, etc.

3. Oat (amphistomatic) type:

Stomata are found distributed equally upon the two surfaces, e.g. maize, oats, grasses, etc.

4. Water lily (epistomatic) type:

Stomata are found distributed only on the upper surface of leaf, e.g., water lily, Nymphaea and many aquatic plants.

5. Potamogeton (astomatic) type:

Stomata are altogether absent or if present they are vestigeal. e.g., Potamogeton and submerged aquatics.

Materials Required

Fresh leaf, Glycerin, Safranin solution, Forceps, Needle and brush, cover slip, compoundmicroscope, Distilled water, dropper, blade, watch glass, glass slide

Safranin solution: Add 200mg of safranin in 200ml of distilled water and add 5ml of absolute alcohol.

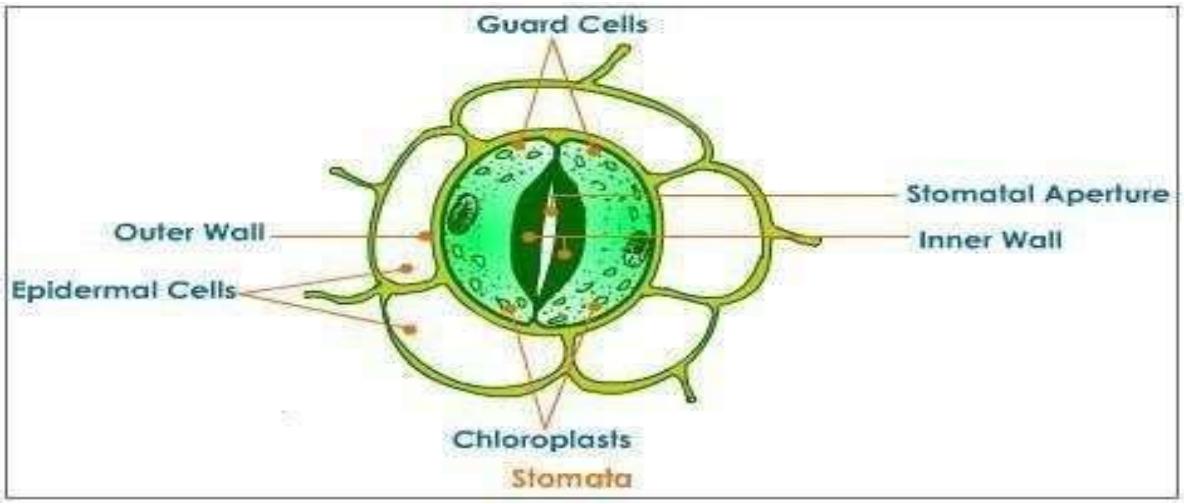
Procedure

- Pluck one fresh leaf of a four-o'clock plant.
- Take two watch glasses and pour some distilled water into the both watch glasses.
- Split the leaf from the four-o'clock plant obliquely.
- Take the peel from the upper surface of the leaf using the forceps.

- Place the peel into a watch glass containing water.
- Take another peel from the lower surface of the leaf using the forceps.
- Place the peel into the other watch glass containing water.
- Using a dropper, take few drops of Safranin solution and put it into the two watchglasses.
- Take two clean glass slides and place the leaf peel on the slides one by one, using abrush.
- Take a blade and cut a small rectangle or square piece from each peel.
- Take some glycerin using a dropper and put one drop of glycerine on both slides.
- Take a cover slip and place it gently on the peel with the help of a needle.
- Take the glass slide and place it under compound microscope.
- Observe under the microscope.
- Count the number of stomata in the peels of both upper and lower epidermis of the leaf appearing in the microscopic field.

Precaution:

- Ensure that plant samples are healthy, free from diseases
- Handle plant tissues and samples with care to avoid physical damage to stomata.



Practical No.-3

Objective: The objective of determining the percentage of water imbibition by gram seeds is to assess the water-absorbing capacity of the seeds under specific conditions.

Principle: A type of diffusion where the water is absorbed by the solid particles called colloids, causing an enormous increase in volume. Seeds and other such materials have almost no water hence they absorb water easily. Water potential gradient between the absorbent and liquid imbibed is essential for imbibition. The substance that imbibes water is called imbibant and the liquid which is imbibed is called adsorbent. The process of imbibition occurs mainly due to the presence of hydrophilic or lyophilic colloids. Water is imbibed through the sub microscopic capillaries present on the surface of the body.

Material required:

Seeds of rice, pigeon pea, groundnut, 50 ml beakers, distilled water, blotting papers and weighing balance, hot water, water of room temperature, cold water, 50 ml beakers, blottingpapers and concentration of different NaCl solution and weighing balance.

PROCEDURE :

a) Take 5 g of each seeds of rice, pigeon pea and groundnut into 50 ml beakers.
Pour equal volume of water in all beakers and allow them for one hour two imbibe.

Later takeout the seeds and place them on blotting paper to wipe out excess water. Then take the weight and weight after imbibition of seeds will give the amount of water imbibed. Record your observation and write the conclusion.

b) Take hot water, water at normal room temperature and cold water in equal volume into three different beakers.
Place 5g of pigeon pea seeds in each beaker and allow them for half an hour to

imbibe. Later take out the seeds and place them on blotting paper to remove the excess coater.

Then take the weight to imbibe seeds. The difference between initial weight and weight after imbibition of seeds will give the amount of water imbibed. Record your observation and write down the conclusion.

c) Take 0.5 molar, 1M, 1.5M and 2M solutions of NaCl into different beakers and place 5g of pigeon pea seeds in each beaker and allow them for half an hour to imbibe. Later, takeout the seed and place them on blotting paper to wipe out the excess water. Then, take weight of imbibe seed. The difference between initial weight and the weight of after imbibition of seeds will give the amount of water imbibed. Record your observation and write down the conclusion.

PRACTICAL NO. UTILITY:

1) The absorption of water by germinating seeds is mostly cause by imbibition.

2) In the young cell before the origin of centriole, vacuole, water absorption is mainly clue to cytoplasmic colloids.

Sr. No.	Name of Seed	Initial weight w_1 (g)	Final weight w_2 (g)	Amount of water w_2- w_1	Remark
1.	Rice				
2.	Pigeon pea				
3.	Groundnut				

Sr. No.	Name of Seed	Initial weight w_1 (g)	Final weight w_2 (g)	Amount of water w_2- w_1	Remark
1.	Pigeon pea				
2.	Pigeon pea				
3.	Pigeon pea				

Sr. No.	Name of Seed	Initial weight w_1 (g)	Final weight w_2 (g)	Amount of water w_2-w_1	Remark
1.	Pigeon pea (0.5 M)				
2.	Pigeon pea (1 M)				
3.	Pigeon pea (1.5 M)				
4.	Pigeon pea (2 M)				

Precaution:

- Ensure a uniform distribution of water over the seeds during the imbibition process. Uneven water distribution may lead to variations in imbibition rates.
- Use of Filtered Water
- Draining Excess Water
- Avoid seed Damage:

Practical No.-4

Objective: The objective of demonstrating the process of osmosis with varying solution concentrations is to illustrate how water moves across a semi-permeable membrane in response to differences in solute concentrations.

Osmosis is a process by which the molecules of a solvent pass from a solution of low concentration to a solution of high concentration through a semi-permeable membrane." There are three different types of solutions:

- Isotonic Solution
- Hypertonic Solution
- Hypotonic Solution

An **isotonic solution** is one that has the same concentration of solutes both inside and outside the cell.

A **hypertonic solution** is one that has a higher solute concentration outside the cell than inside.

A **hypotonic solution** is one that has a higher solute concentration inside the cell than outside.

Osmosis is of two types:

- **Endosmosis**– When a substance is placed in a hypotonic solution, the solvent molecules move inside the cell and the cell becomes turgid or undergoes deplasmolysis. This is known as endosmosis.
- **Exosmosis**– When a substance is placed in a hypertonic solution, the solvent molecules move outside the cell and the cell becomes flaccid or undergoes plasmolysis. This is known as exosmosis.

Material Required: A potato tuber, sugar, beaker, or petridish, knife and water

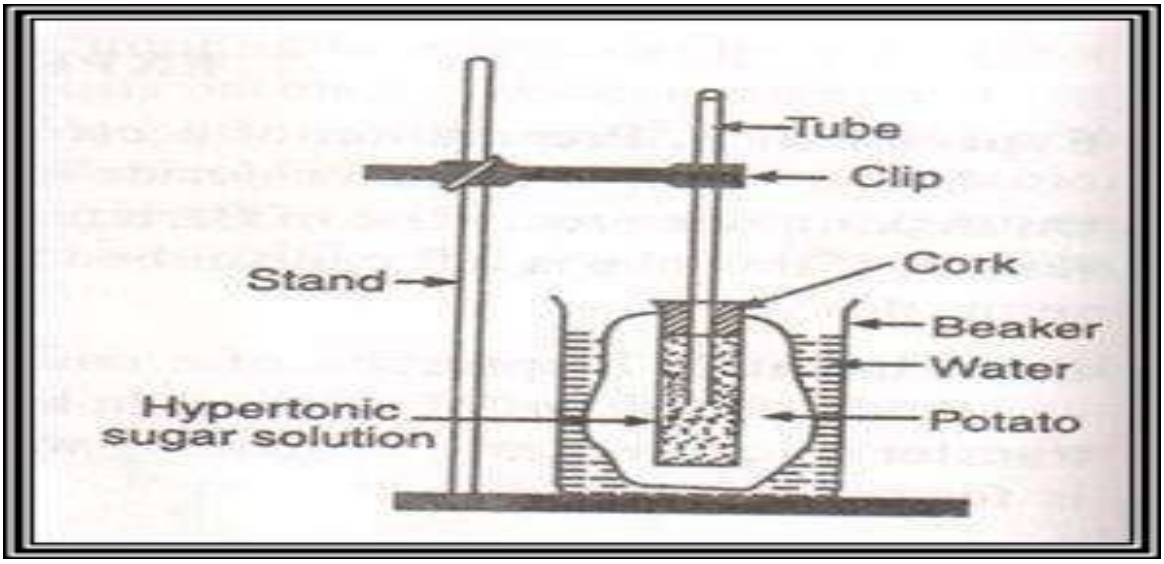
Procedure: Sugar solution are placed in a cuplike cavity made in a peeled potato tuber. It is placed in an upright position in the petridish containing water. The mouth of the cavity is fixed airtight with a glass tube through a cork. The change in the level is recorded and the experiment is kept for a few hours.

Note: In reverse case, i.e., sugar solution in petridish and water in the tuber cavity, the

movement of water would be in reverse direction and exosmosis would occur.

Water begins to enter the cavity of the potato tuber and a rise in the level inside the glass tube is recorded. This entrance of water occurs due to osmosis (endosmosis) through plant membrane. Water enters the cavity because of osmosis, i.e. water molecules can diffuse through the semi-permeable membrane from the region of higher concentration to the region of lower concentration.

(Variation: If potato tuber is killed in the boiling water and protoplasm is denatured, the cytoplasm stops functioning as a semi-permeable membrane. As a result the sugar solution in cavity does not show any changes in the level).



DEMONSTRATION OF OSMOSIS WITH THE HELP OF POTATO OSMOSCOPE

Practical No.-5

Objective: The objective of demonstrating the process of plasmolysis using onion cells is to illustrate the changes that occur in plant cells when exposed to a hypertonic solution. Plasmolysis is a phenomenon where the protoplast (cell contents) shrinks away from the cell wall due to the loss of water.

Theory

Plasmolysis is the process of shrinkage or contraction of the protoplasm of a plant cell as a result of loss of water from the cell. Plasmolysis is one of the results of osmosis and occurs very rarely in nature, but it happens in some extreme conditions. We can induce plasmolysis inthe laboratory by immersing living cell in a strong salt solution or sugar solution to lose waterfrom the cell.

The cell membrane is a semipermeable membrane that separates the interior of all cells from the surrounding environment. The semipermeable membrane allows some particles, ions, or water molecules across the membrane, but blocks others. Water molecules constantly move inside and outside the cell across cell membranes. This free flow of water has the very important consequence of enabling cells to absorb water.

Plasmolysis and deplasmolysis

When a plant cell is immersed in concentrated salt solution (hypertonic solution), water from the cell sap moves out due to exosmosis. Exosmosis is the passage of water from higher water concentration to lower water concentration through a semipermeable membrane.

When a plant cell is placed in concentrated salt solution, water concentration inside the cell isgreater than that which is outside the cell. Therefore, water moves through the cell membraneinto the surrounding medium. Ultimately the protoplasm separate from the cell wall and assumes spherical shape. It is called plasmolysis.

When a plasmolysed cell is placed in a hypotonic solution, (i.e., the solution having solute concentration lower than the cell sap), the water moves into the cell because of

the higher concentration of water outside the cell than in the cell. The cell then swells to become turgid. Itis called deplasmolysis.

If we place living cells in isotonic solution (i.e., both solutions have the same amount of soluteconcentration), there is no net flow of water towards the inside or outside. Here, the water moves in and out of the cell and is in equilibrium, so the cells are said to be flaccid.

Materials-required:

Onion bulb, watch glass, petri-dish, slides, cover-slips, forceps, brush, needles, microscope and20% concentrated sucrose solution.

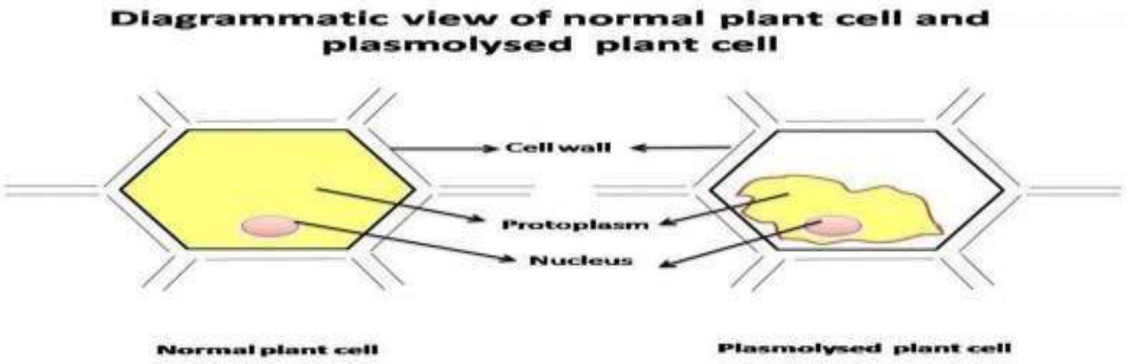
Procedure:

- Take an onion bulb, with the help of forceps pull a thin transparent peel gently.
- Keep this peel in water filled watch glass.
- Transfer the peel gently on a clean slide in a drop of water with the help of a brush andneedle.
- Examine it under high power of a microscope.(40X)
- Observe the individual cells and make a sketch of the cells showing the cell wall and cellmembrane. **(observation 1)**
- With the help of dropper put the sucrose solution on the slide by the sides of cover-slip sothat it reaches the peel under the cover-slip.
- Examine the peel again after 10 mins. **(observation 2)**
- Drain out the concentrated sugar solution from the peel and add few drops of water intothe peel.
- Record observation after 10 minutes. **(observation3)**

	Condition of the cell	Explanation
Observation 1		
Observation 2		
Observation 3		

Inference:

- When the peel of onion is kept in concentrated solution (hypertonic), the protoplasm shrinks as the water starts moving out due to ex osmosis.
- This phenomenon of shrinkage of protoplasm when the cells are kept in a concentratedsolution is known as plasmolysis.
- Further, when the cells are kept in water (hypotonic solution) the protoplasm again regains its original shape due to movement of water into the cells by the process of endosmosis. This phenomenon is called deplasmolysis.



Practical No. 6

Objective: To compare the rate of transpiration between the upper and lower surfaces of a leaf.

Principle: The objective of comparing the rate of transpiration between the upper and lower surfaces of a leaf is to investigate whether there are variations in transpiration rates in different leaf regions.

Transpiration is the process of water movement through a plant and its evaporation into the atmosphere from its aerial parts. In leaves and in young shoots the epidermal layer contains minute microscopic pore like structures called stomata. Transpiration occurs chiefly through the stomata of the leaves. The stomata are mainly concerned with exchange of gases during the process of photosynthesis and respiration. Each stomata has a slit like opening called the stomatal pore, which is surrounded by two special cells called the guard cells. These special cells help to regulate the rate of transpiration by opening and closing the stomata.

Materials Required:

A healthy potted plant, forceps, filter paper strips, wire gauze, 3% cobalt chloride solution, clips, petridish, glass slide.

Procedure:

- Take 3 % cobalt chloride solution from beaker and pour into the Petri dish.
- Take some filter paper strips and dip them in the cobalt chloride solution.
- Keep the strips in the solution for 3-5 minutes. They become pink in colour when wet.
- Remove the strips from the solution using forceps.
- Place the strips on the wire gauze to allow them to dry.
- The filter paper becomes blue in colour on drying.
- Select one healthy leaf and clean the leaf to remove the water droplets using a filterpaper.
- Take the dry pieces of cobalt chloride paper from the wire gauze.

- Place the dried strips of cobalt chloride paper: one on the upper and the other on thelower surface of a leaf of the potted plant.
- Take two glass slides and place one over the upper and the other over the lower sideof the leaf.
- Clip the slides together using binder clips.
- Note the time taken by the cobalt chloride paper to change its blue colour to pink.

Note: Dry cobalt chloride paper that is blue in colour turns pink when it comes in contact withwater. Using this property of cobalt chloride paper we can study the rate of transpiration fromthe two surfaces of a leaf by comparing the loss of water vapour from the two surfaces of the leaf.

Observation:

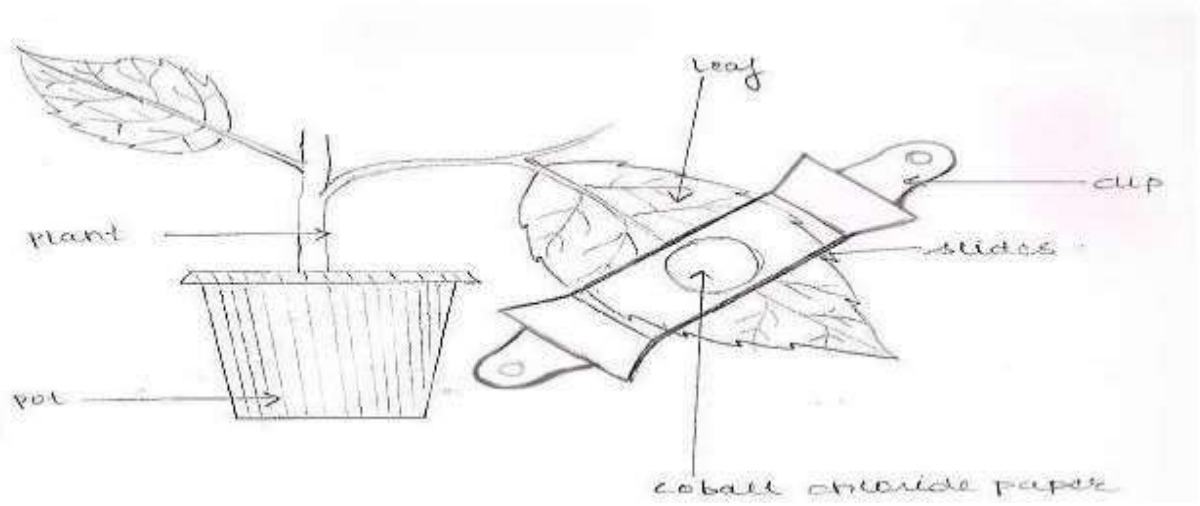
The time taken to change colour of the cobalt chloride paper from blue to pink on the lower leaf surface is less than the upper surface.

Conclusion:

The quick change in the colour of cobalt chloride paper on the lower surfaces indicates higherrate of loss of water vapour from this surface than the upper one.

Precautions:

- Always use a well watered potted plant for the experiment.
- Always handle the dried cobalt paper with dry hands or forceps.
- The leaf surface should not be wet while applying the cobalt chloride strips.


leaf
clip
plant
slides
pot
cobalt chloride paper

Practical No.-7

Objective: The objective of separating chloroplast pigments by paper and thin-layer chromatography is to analyze and identify the individual pigments present in chloroplasts.

Chlorophylls and carotenoids are the main chloroplast pigments.. Most commonchlorophyll types in the vascular plants are chlorophyll a and b. Chlorophyll a is a large molecule with a tetra pyrrole ring and a magnesium ion held in it. Attached to one of the rings is a long insoluble hydrocarbon ring, a 20-carbon phytyl group. Chlorphyll b differs from chlorophyll a in having a -CHO group instead of-a -CH3 on of the pyrrole ring 3. Another class of chloroplast pigments is the carotenoids. Carotenoids are 40-carbon compound. They are the red, orange, or yellow fat-soluble pigments found in chloroplasts. Normally two kinds of carotenoids are found in chloroplasts. They are carotenes and xanthophylls. Xanthophylls contain oxygen in their molecules whereas carotenes do not.Chloroplast pigments can conveniently be extracted from green plant tissues in solventssuch as acetone and separated from one another by paper- and thin layer chromatography (TLC).

Principle: In paper chromatography substances placed on one end of the chromatographic paper get deposited on various zones of the paper when an appropriate solvent runs over to the other end of the paper. The mobility of substances on the paper depends upon their degree of solubility in the solvent system (the mobile phase) and the affinity to the chromatographic papers (stationary phase) which are mostly made of pure cellulose fibre. Flat paper sheets or round paper cylinders may be used for the separation of substances (Fig.). 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 thepaper is immersed in the solvent. The mixtures of substances are loaded at about 2 cm high from the base and the solvent moves up against the gravitational pull. In descending paper 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 paper chromatography is faster than the ascending one.

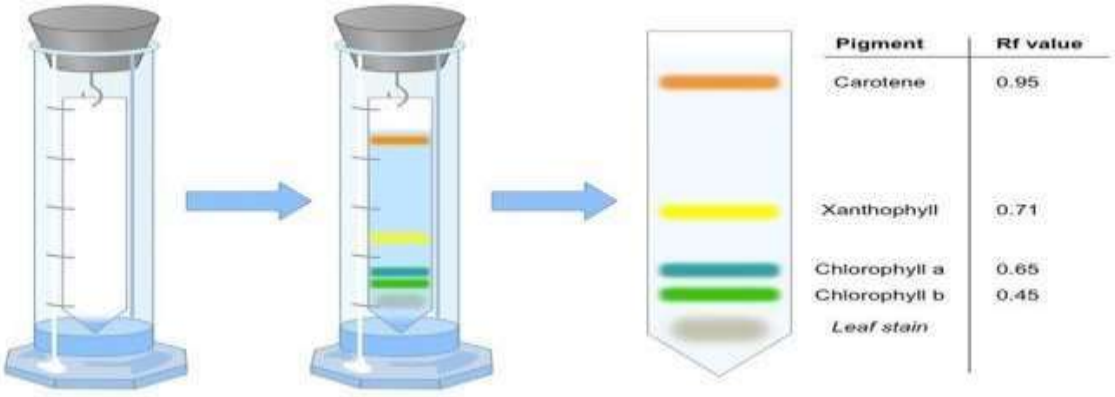
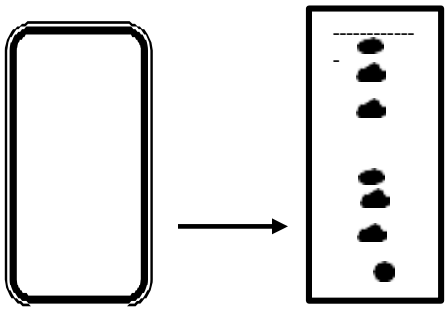


Fig. Separation of chloroplast pigments by paper chromatography (a) and the bands of different pigments (b). (After Molisch and Dobat 1979, modified)

Thin layer chromatography (TLC) is a relatively new technique for the separation of plant pigments. In this method a thin layer of an adsorbent is coated on a glass plate (Fig.) or rod (Fig.)

TCL Plate



Solvent front
Carotene
Chlorophyll a
Chlorophyll b

Lutein
Violaxanthin
Neoxanthin

Solvent

Loading

and the sample is spotted on this coating and developed in a tank containing a small amount of solvent by ascending method. These days glass plates or thin films pre-coated with large varieties of adsorbents are available commercially. Also available in the market are devices used for the precise coating of glass plates with an adsorbent. The significant advantages of this method ever other previous chromatographic methods such as paper chromatography and columns chromatography lie in the fact that TLC method is much 'aster (20 to 40 minutes for most application) and requires very little amount of sample. It is much more sensitive than other methods and the separation is noticeably much sharper. As long as there is a difference in the adsorbtion properties of the fractions involved, this method can be successfully be used to separate any compound by choosing the right combination of adsorbent and solvent.

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 the solvent in a chromatogram:

$$Rf = \frac{\text{distance trailed by the substance}}{\text{distance travelled by the solvent}}$$

Seperation by Paper chromatography:

Materials required:

- Fresh spinach leaves or other fresh green leaves
- 80 % aceton (v/v), carbon tetrachloride (CCI4), petroleum 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 equilibrate 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 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 file 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. The pigments are arranged in the following sequence from top (solvent front) to bottom: orange-yellow carotenes, one or more yellow bands of xanthophylls, blue-green chlorophyll a and yellow-green chlorophyll b. Mark the spots with a pencil since the colours will fade away quickly. Calculate the Rf value of each pigment.

Pigment	Distance travelled by different pigments	Distance travelled by solvent	R_f Value
Carotene			
Xanthophyll			
Chlorophyll a			
Chlorophyll b			

Practical No.-8

Objective: The objective of testing the essentiality of carbon dioxide (CO_2) for photosynthesis is to investigate the role of CO_2 in the process of photosynthesis. This experiment aims to demonstrate that CO_2 is a crucial factor for the synthesis of organic molecules during photosynthesis.

During Photosynthesis, carbon containing compounds are formed from CO_2 in illuminated green cells.

Material Required:

A wide mouth glass bottle, KOH tablets or solution, splitted cork, destarched potted plant, tripod stand, Iodine solution, alcohol.

Procedure:

1) Firstly, the plant is destarched by keeping it in dark for about 48 hours.

2) Insert one half leaf in wide mouth bottle containing a few tablets of KOH or bottle filled with one-third (1/3) of volume with the solution of KOH (Caustic potash) through splitted cork (lead should not tough KOH solution or tablets).

3) Make the connection air tight.

4) The half leaf present inside the bottle will release the CO2 but it is absorbed by KOH solution or tablets, when this apparatus is placed in the sunlight for few hours.

5) Remove the leaf and test it for the presence of starch with iodine. After killing it in boiling water and decolourising with alcohol.

6) The area of leaf outside of the bottle turns blue because of presence of starch in this region. The region of the leaf which was within cork piece and that which was inside the bottle do not show any colour change, because of absence of starch in those regions.

7) The outer portion of leaf shows presence of starch because it gets light and CO2 both and photosynthesis takes place. Middle portion does not get light whereas inner portion does not get CO2. Both failed to perform photosynthesis. In the inner portion starch is

not formed due to absence of CO2 which is absorbed by KOH, thereby checking the photosynthesis.

8) Considering the above facts, it becomes evident that CO_2 is essential for photosynthesis.

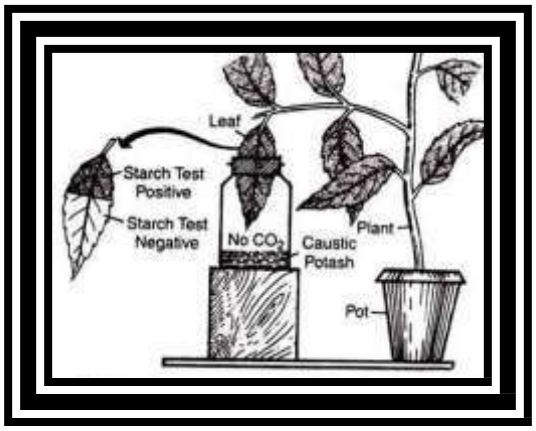


Fig. CO_2 IS NECESSARY FOR PHOTOSYNTHESIS

Practical No.-9

Objective: The objective of measuring photosynthetic CO2 assimilation by an Infra-Red Gas Analyzer (IRGA) is to quantitatively assess the rate of carbon dioxide uptake during photosynthesis. IRGA is a sophisticated instrument that allows real-time, precise measurement of gas concentrations, making it a valuable tool for studying photosynthetic processes.

An infrared gas analyzer is used to measure the quantity of various gas. The amount of gas is determined by amount of a particular frequency of light absorbed by the gas when the light is passed through the gas. Different molecules in the air absorb different frequencies of light, measuring the absorbed frequency clearly gives the relation to the amount of particular gas in the air.

There are dispersion and non-dispersion types of infrared analyzers. Dispersion infrared analyzers are used in laboratories as spectrophotometers; **non-dispersion infrared analyzers** (NDIR) are used for continuous measurement in industrial applications.

Principle of Operation:

The absorption spectrum of infrared radiation absorbed by a gas, is unique to the type of gas, In every absorption-type, optical analyzer, the fundamental equation relating photon absorption to substance concentration is the Beer-Lambert Law (sometimes called the Lambert-Beer Law):

$$A = abc = \log\left(\frac{I_0}{I}\right)$$

Where,

A = Absorbance

a = Extinction coefficient for photon-absorbing substance(s)

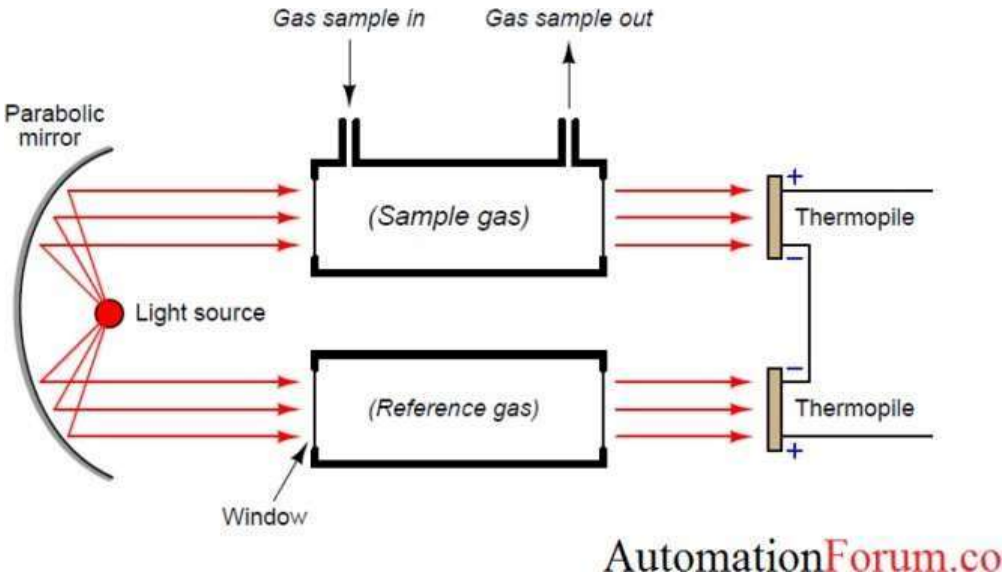
b = Path length of light traveling through the sample

c = Concentration of a photon-absorbing substance in the sample

I0 = Intensity of source (incident) light

I = Intensity of received light after passing through the sample

Construction and Working:



There is two tubes in the analyzer one is filled with reference gas and other with the sample gas or process gas which absorbs light. The reference gas is usually gases like nitrogen which will not absorb light.

The incident light (infrared) is split into two parallel beams by the upper mirror. One beam is used for the measurement and the other is used as the reference.

The reference light beam passes through the reference cell, which is filled with air or N2 , and is then reflected off the bottom mirror onto a semiconductor detector. The measurement light beam passes through the measurement cell, and is reflected onto a semiconductor detector in the same fashion as the reference light.

The component to be measured in the gas sample flowing through the measurement cell absorbs some of the measurement light, thus reducing the strength of the light relative to

the strength of the reference light. The two light beams are alternately cut off from the detector by a semi- circular rotary sector. This allows the detector to convert the difference in the strengths of the measurement and reference light beams into an alternating electrical signal that represents the concentration of the gas being measured.

Advantages:

- Gas molecule doesn't interact directly with the gas.
- Non-destructive analysis.
- Infrared gas analysers are standard detectors for the measurement of gas in any given environment.
- Monitoring emission levels over longer periods of time.

Disadvantages:

- A simple measurement becomes a complex measurement
- Cost of measuring the gas normalization parameter is greater than the cost of primary dust measurement

Practical No.-10

Objective: The objective of estimating relative water content (RWC) is to assess the water status of plant tissues. RWC provides valuable information about the hydration level and overall health of plant cells. This measurement is particularly useful in understanding how plants respond to water availability and stress.

Relative water content (RWC) is probably defined as it is the most appropriate measure of plant water status in terms of the physiological consequence of cellular water deficit. Water potential as an estimate of the energy status of plant water is useful in dealing with water transport in the soil-plant-atmosphere continuum.

The concept of relative water content as a measure of water status of plant has been found useful in water stress experiments. Because of the difficulties in using fresh and dry weight as a base, investigations of leaf water content as percentage of turgid water content is estimated. Estimating RWC is an appropriate method to assess the plant water status. By the way it can be used as an index to screen the plants for drought tolerant. RWC is mainly based on turgid weight.

Function of turgidity

1. Cell elongation - Ultimately influences the leaf area development and light absorption
2. Opening of stomata - Ultimately influences the gas exchange and photosynthesis
3. 5 to 10% reduction of RWC reduced the photosynthetic efficiency by 40 to 60% in crop plants.

Principle:

The technique consists essentially in comparing the water content of leaf tissue when fresh leaf sampled with the fully turgid water content and expressing the results on percentage basis.

Material required:

Plant sample, Hot air oven, weighing balance

Procedure (BARRS AND WEATHERLEY, 1950):

- The selection of leaf tissue and time of sampling are equally important in expressing reliable results.
- Normally third leaf (physiologically functional) from the top is selected for relative water content estimation.
- Take physiologically functional leaf samples of different crops and make 50 uniform leaf discs. Record the fresh weight (Fw).
- Float the leaf discs in water for one hour to attain full turgid and after take out the leaf discs from water and wipeout the water droplets sticking on the leaf surface by using filter paper.
- Immediately record the turgid weight (Tw) after an hour of floating.
- Transfer the leaf discs to a butter paper cover and then keeps the cover in hot air oven at 80°C for 48 hours. Record the dry weight (Dw).
- Calculate the RWC by using following formula:

$$RWC = \frac{Fw - Dw}{Tw - Dw} \times 100$$

Where,

Fw - Fresh weight of leaf sample

Tw - Turgid weight of leaf sample

Dw - Dry weight of leaf sample

Water Saturation Deficit (WSD) = 100 — RWC

The floating duration or time to obtain full turgidity varies with the crop species, and the following duration has been found to be the optimum requirement.

Cotton and castor - 4 hours

Tobacco - 3 hours

Eucalyptus - 2 hours

Groundnut - 1 hour

PURPOSE OF ESTIMATION:

1. To assess drought tolerant capacity of crop plants-More RWC during drought gives better tolerance.
2. To assess photosynthetic efficiency of crop plants-More RWC gives high photosynthetic efficiency by cell elongation and stomata open.