B.Sc. BOTANY LAB MANUAL

5th Semester

Prepared By
Biological Science Dept.
Botany

MIDNAPORE CITY COLLEGE

B.SC (Honours) in Botany (CBCS)

C11P: Reproductive Biology of Angiosperms

Study the slide showing T.s. of young anther.

Observations:

The section shows following characters.

1. The section appears slightly lobed.

2. The outermost is a single layered epidermis. The cells are cuticillarised.

3. At four corners of the anther, the derivatives formed as a result of archesporial cells are present.

4. Of these, wall layers are situated below the epidermis and mass of sporogenous cells near the centre of the lobe.

5. The epidermis is followed by a layer or two of parenchymatous wall layers. The innermost wall layer is called tapetum. It is nutritive in function.

Study the slide showing T.S. of mature anther.

Observations

It shows following characters.

1. An organized anther is four chambered in a transection.

2. The wall consists of an outer epidermis, an endothecium, one to three middle layers and an innermost tapetum.

3. The tapetum at maturity is multinucleate and contains dense cytoplasm which is finally used up by the developing microspores.

4. Prior to dehiscence, the tapetum and also the middle layers degenerate. The cells of the endothecium are radially elongated and exhibit, characteristic fibrous thickenings.

5. The microspores or pollen grains, are at first arranged in tetrads, (as a result of reduction division of the microspore mother cell). Later, these separate and occur as individual pollen grains, dispersed throughout the chamber. Each shows characteristic shape, size and structure.

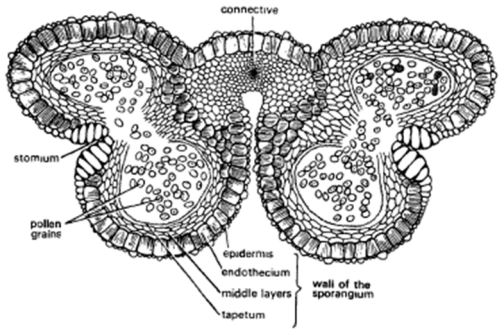


Fig. 2. Anther : T.s. organized anther.

Preparation of pollen grains for study

Following arc the steps in the preparation of slides for pollen study.

Collection of material

1. The polliniferous material (anthers) is collected fresh or herbarium sheets can also be used.

2. The anthers arc picked by a clean forceps.

Preparation of material

1. The anthers are tapped by needles or glass rod on a clean slide to obtain a mass of pollen grains.

2. This mass of pollen grains is picked up by the flat end of the forceps and transferred to the centre

of another clean microscopic slide.

Pre-treatment

1. A drop of alcohol is added to the pollen grains. This releascs oily and resinous substances in the form of a ring around pollen. Upto 3 or 4 drops of alcohol can be used.

2. The ring is wiped clean with cotton moistened with alcohol.

Mounting

1. A small pellet of glycerine jelly prestained with methyl green is taken. It is placed over the mass of pollen grains. Coverslip is also placed over the pellet.

2. A small piece of paraffin wax (melting point 60-70^{0}C) is placed close to the coverslip.

3. Both, jelly and wax are warmed over the flame of the spirit lamp in such a way that while the jelly spreads a little, the remaining vacant space below the coverslip is occupied by wax.

Acetolysis of Pollen Grains

This method is used to study exine ornamentation in detail. Acetolysis removes all nonsporopollenin components of the pollen (the contents, intine and components present in and on the pollen wall) leaving only the exine intact. Special Requirements A cetolysis mixture: acetic anhydride and concentrated sulphuric acid (9:1). Add the acid slowly to acetic anhydride in the fume hood; as it results in exothermic reaction, the container gets hot. Low-speed centrifuge. Glacial acetic acid.

Procedure:

1. Collect suffi cient amount of pollen grains (2–4 mg) and suspend them in about 10 ml of acetolysis mixture taken in a test tube. 2. H eat the suspension to boiling in a water bath for 1–2 min. Remove the tube and allow it to cool. 3. T ransfer pollen suspension into a centrifuge tube. Remove acetolysis mixture by low- speed centrifugation (about 2,000 rpm).

4. Add about 5 ml of glacial acetic acid to the centrifuge tube. Mix pollen pellet and remove acetic acid by centrifugation. 5. Add about 5 ml of water to centrifuge tube and mix pollen pellet in water. 6. Mount pollen grains on a slide in water or 10 % glycerol and observe under the microscope. They can also be mounted in glycerin jelly as semi-permanent

preparations for later observations and also as reference slides.

Tetrazolium Test for Pollen Viability

One of the simple methods used in earlier studies to assess pollen viability has been the tetrazolium test (TTC test). TTC test is based on the demonstration of the activity of dehydrogenases (an important group of enzymes in the respiratory cycle) in the pollen cytoplasm. When pollen grains are mounted in a solution of colourless soluble tetrazolium salt, dehydrogenases of the pollen, if active, reduce colourless soluble tetrazolium salt to a reddish insoluble substance called formazan. Formazan accumulates in the pollen cytoplasm and gives reddish colour to pollen. 2,3,5-triphenyl tetrazolium chloride and nitroblue tetrazolium are the most commonly used tetrazolium salts.

Special Requirements: 2,3,5-triphenyltetrazolium chloride (0.2–0.5 % solution prepared in a suitable concentration of sucrose solution to prevent bursting of pollen). TTC solution needs to be stored in a brown bottle as it undergoes photo-oxidation. It can be stored under refrigeration for a couple of weeks. Simple humidity chamber.

Procedure: 1. Mount pollen grains in TTC solution and lower a cover glass.

2. Incubate the slide in a humidity chamber in dark (such as table draw) at laboratory temperature for 30–60 min.

3. After the incubation period, observe the slide under the microscope and score pollen grains that have turned red as viable. Oxygen inhibits reduction of TTC; it is better to score pollen grains from the central part of the cover glass. cover glass may show variable degree of red colouration due to higher oxygen availability.

4. Calculate percent of viable pollen grains in the sample.

Germination of pollen grains

Materials

Anthers of *Antirhinum* (snap dragon), *Catharanthus roseus* (Periwinkle; Sada bahar) , *Papaver somniferum* (Poppy; Afim) or any other easily available plant; sugar, boron, cavity slides, cover slips, microscope, water, etc.

Procedure

1. Prepare 15% sugar solution by dissolving 1.5 gm sugar in 100 ml of water.

2. Add a pinch of boron to sugar solution.

3. Clean the cavity slide and place a drop of this solution in the cavity.

4. Remove mature anthers from fresh tlowers.

Crush them on a slide. Collect the pollen grains with a brush from the crushed anthers. Dust the brush free of anthers in the cavity fIlled with solution.

5. Place a cover slip over the cavity.

6. Allow the slide to remain as such for a few hours or overnight.

7. Remove the coverslip slowly and gradually. Mount the coverslip on a fresh and clean slide in a drop of safranin. The lower side of the coverslip with germinated pollen grains should be in contact with safranin.

8. Observe the slide.

Observations

The following characters are observed.

1. Numerous germinated pollen grains are seen.

2. A pollen grain has a distinct ornamented exine with germ pores.

3. Intine lies internal to exine. It is thin and uniform.

4. Intine forms a pollen tube that comes out through one of the germ pores.

5. Pollen tube shows a vegetative nucleus and two small male gametes.

OVULE

Ovule is defined as integumented megasporangium. It encloses embryo sac which is

the female gametophyte of angiosperms. Following are the types of ovules.

Types of ovules

(1) Atropous or Orthotropous. The ovule is straight, so that the micropyle lies on the same vertical axis with the funicle and chalaza, e.g., *Polygonum, Piper,* etc .

(2) Anatropous. In this type, the body of the ovule becomes completely inverted, so that the micropyle and hilum come to lie very close to each other. The micropyle and the chalaza lie on the same vertical axis but not funicle, e.g., *Helianthus,* Castor, etc.

(3) Campylotropous. When the ovule is curved in such a way, so that the micropyle and chalaza donot lie on the same straight line, it is called campylotropous, e.g. Pea, Mustard, etc.

(4) Hemianatropous. In this type, the nucellus and integuments lie more or less at right angles to the funicle, e.g. *Ranunculus,* etc.

(5) Amphitropous. When the curvature of the ovule is so much pronounced that the embryo sac bends like a horse-shoe, the ovule is called amphitropous, e.g. Poppy, etc.

(6) Circinotropous. In this type, the nucellar protuberance is at first in the same line as the axis, but the rapid growth on one side makes it anatropous. The curvature continues till the ovule has turned over completely with the micropylar end again pointing upward, e.g. *Opzmtia,* etc.

Study the slide showing L.s. of anatropous ovule.

Observations

The following characters are observed.

1. Aoatropous ovule is most common among angiosperms.
2. The ovule is a rounded structure attached to the placenta by a stalk, the funicle. The place of attachment of funicle to the body of the ovule is known as hilum.
3. The basal region of the ovule, where from integuments arise, is known as chalaza.
4. In anatropous ovules, the funicle extends above, along the body of the ovule to form a ridge, known as raphe.
5. The ovule consists of integuments, nucellus and embryo sac.
6. Integuments which number between one (unitegmic) or two (bitegmic) surround the nucellus. These extend well beyond the nucellus to form a narrow opening called micropyle.
7. Nucellus lies below the integuments. If it is massive, ovules are called crassinucellate and if scanty, these are called tenuinucellate. (initegmic ovules are crassinucellate and bitegmic ovules are tenuinucellate).
8. Enveloped by nucellus is the female gametophyte or embryo sac. A typical embryo sac shows an egg apparatus consisting of an egg and two synergids towards micropyle. In the centre are 2 polar nuclei and 3 antipodals are present at the chalazal end.

L.s. of ovule showing archesporial cell

Study the slide showing archesporial cell in longitudinal section of ovule.

Observations

The slide shows following characters.

1. The ovule is attached to the placenta with its stalk called funicle.
2. The ovule is poorly differentiated and shows only integuments and nucellus.
3. Archesporial cell originates from nucellar hypodermis.
4. It is identified by its large size, dense cytoplasm and prominent nucleus.
5. Archesporial initial may directly behave as megaspore mother cell or it may cut off some parietal tissue.

L.s. of ovule showing megaspore tetrad

Study the slide of longitudinal section of ovule showing megaspore tetrad.

Observations:

It shows following characters.

1. The ovule is attached to the placenta by its funicle.
2. The ovule consists of integuments and the nucellus.
3. Outermost are two integuments which cover the ovule.
4. In the nucellus, a few cells below the nucellar epidermis lies a linear tetrad of megaspore.

5. The lowermost or chalazal megaspore is functional while the three upper or micropylar megaspores are non-functional and, therefore, degenerate.

6. The functional megaspore'is large in size. The protoplasm is dense and the nucleus is prominent.

L. s. of ovule showing binucleate embryo sac

Study the slide of longitudinal section of ovule showing binucleate embryo sac.

Observations

The slide shows following characters.

1. The ovule is attached to the placenta by a stalk called funicle.

2. The ovule is made of integuments and the nucellus.

J. Outermost part of the ovule is made of two integuments.

4. Inner to the integuments lies nucellus.

5. A few layers below the nucellar epidermis, binucleate embryo sac is situated.

6. At the top of the sac, three degenerating megaspores can still be seen.

7. The embryo sac has two nuclei, one at each pole, separated by a large vacuole.

L.s. of ovule showing 4 nucleate embryo sac

Study the slide of longitudinal section of ovule showing 4- nucleate embryo sac.

Observations

The slide shows following characters.

1. The ovule consists of stalk and the body.

2. The body of the ovule is made of integuments and the nucellus.

3. The outer covering of the ovule is made of two integuments.

4. Nucellus is situated inner to the integuments.

5. A few layers below the nucellar epidermis, 4-nucleate embryo sac is present.

6. The embryo sac shows four nuclei, out of which two are located at the micropylar end and the rest two at the chalazal end.

7. The nuclei at two ends are separated by large vacuole in the centre.

L.s. of ovule showing 8-nucleate polygonum type of embryo sac

Study the slide of longitudinal section of ovule showing 8- nucleate Polygonum type of embryo sac.

Observations:

Following characters are observed.

1. The ovule shows stalk and the body.
2. The body consists of integuments, nucellus and the embryo sac.
3. There are two integuments which form the outermost covering of the ovule.
4. A small amount of nucellus is present between the integuments and the embryo sac.
5. Embryo sac is present deep into the tissue of nucellus.

6. Organised 8-nucleate Polygonum-type embryo sac has an egg apparatus, two polar nuclei and
three antipodals.
7. Egg apparatus is situated at the micropylar end. It consists of centrally placed egg cell with two
synergids, one on each side of the egg.
8. An egg cell has a large vacuole towards its micropylar end while synergids have a small vacuole toward its chalazal end.
9. Each syneTgid ha:; a beak-like structure on its lateral side and filiform apparatus at its micropylar end.
10. 'IWo polar nuclei are located in the centre of the embryo sac. These later fuse to form the secondary nucleus.
11. Three antipodal cells are located at the chalazal end. These degenerate soon, either before or
just after fertilization.
12. Since this embryo sac develops from a single megaspore, it is known as monosporic, 8-nucleate Polygonum-type embryo sac.

L.s. of ovule showing nuclear endosperm

Study the slide of longitudinal section of ovule and observe the endosperm.

Observations

It shows following characters.
1. The ovule is made of stalk and the body.
2. The body consists of integuments, nucellus, embryo and the endosperm.
3. There are two integuments which form the outermost covering of ovule.
4. A small amount of nucellus lies inner to integuments.
5. The major and the central part of ovule is occupied by a large amount of endosperm. It surrounds a small embryo present near the micropylar end.
6. The endosperm is nuclear endosperm. In this type the primary endosperm nucleus divides amitotically to form many free nuclei. The division of the nucleus is not followed by wall formation. This type of division is also known as free nuclear division.
7. Many free nuclei formed by this method lie towards the periphery in the cytoplasm.

Dissection of embryo

Seeds of mustard, *Petunia* tomata, etc. are used to dissect out the embryo.
The following is the procedure.
1. Place the seed on the stage of dissecting microscope or binocular.
2. Locate the micropyle which appears like a small opening.
3 Remove the seed coat from this point, carefully with the help two sharply pointed needles.
4. Once the seed coat is removed, embryo could be seen clearly between the cotyledons.
5. Place this embryo on a slide in a drop of water and study the structure.

To remove mature dicot embryo

Crucifer embryo is typical of dicotyledons.

Capsella bursa- pastoris is the commonest example. However, mustard seeds would equally be useful.Remove the seed coat while observing the seed under the dissecting microscope. A curved embryo can be easily seen and separated. Mount the embryo

in glycerine and study.

Observations

It shows following characters.

1. The embryo consists of embryonal axis with two large lateral cotyledons. The cotyledons cover a small plumule (shoot), which is terminal position.
2. At the other end is the swollen suspensor.
3. The portion of embryonal axis which is above the level of cotyledons is called epicotyl and the portion below the level of cotyledons is called the hypocotyl.
4. The epicotyl forms the plumule (emhryonic shoot) and lower end of hypocotyl forms the radical (embryonic root).
5. In the hypocotyl region, the central cells become somewhat elongated and form the procambial strand.
6. The pronounced curvature of the cotyledons is due to their own enlargement and also of the hypocotyl.

L.s. of ovule showing 8-nucleate polygonum type of embryo sac

Study the slide of longitudinal section of ovule showing 8- nucleate Polygonum type of embryo sac.

Observations

Following characters are observed.

1. The ovule shows stalk and the body.
2. The body consists of integuments, nucellus andthe embryo sac.
3. There are two integuments which form the outermost covering of the ovule.
4. A small amount of nucellus is present between the integuments and the embryo sac.
5. Embryo sac is present deep into the tissue of nucellus.
6. Organised 8-nucleate Polygonum-type embryo sac has an egg apparatus, two polar nuclei and three antipodals.
7. Egg apparatus is situated at the micropylar end. It consists of centrally placed egg cell with two synergids, one on each side of the egg.
8. An egg cell has a large vacuole towards its micropylar end while synergids have a small vacuole toward its chalazal end.
9. Each syneTgid ha:; a beak-like structure on its lateral side and filiform apparatus at its micropylar end.

C12T: Plant Physiology

1. Determination of water potential of given tissue (potato tuber) by weight method

Material –Potato tubes, Potato peelers, Knives, ruler, petri-dishes and sucrose solution of 0.2M, 0.4M, 0.6M and 0.8M.

Procedure-

- Prepare 1 molar solution of sucrose.
- Now prepare the rest of the solutions as following.

Serial dilution table for preparation of final sucrose concentration

SL.NO.	Amount of distilled water to be added in ml	Amount of sucrose solution to be added in ml	Final sucrose concentration
1	8	2	0.2M
2	6	4	0.4M
3	4	6	0.6M
4	2	8	0.8
5	0	10	1M

- Take 4 petri-dishes and label them 1,2,3,4 and 5.
- With the help of knife take 5 potato strips of the size of 3 cm x 0.5 cm x 0.5 cm .
- Record the initial weight and length.
- Place each piece in petri-dishes labeled 1, 2, 3,4 and 5 containing 0.2M, 0.4M, 0.6M, 0.8M and 1M sucrose solution in each.
- Cover the petri-dishes and keep them aside.
- After 30 minutes, take out the piece from the petri-dish 1, dry it on a filter paper and measure it and weigh it.
- Repeat the procedure with the pieces kept in petri-dishes2, 3,4 and 5.
- Record the length and weight of the pieces in a tabular form.

OBSERVATION: Table showing change in the size and mass of the potato tissue

Sucrose solution	At the start		After 30 minutes		Change in	
	Length	weight	Length	weight	Length	weight
0.2M						
0.4M						
0.6M						
0.8M						
1M						

2. Determination of osmotic potential of plant cell sap by Plasmolytic method.

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 in the laboratory by immersing living cell in a strong salt solution or sugar solution to lose water from 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 is greater than that which is outside the cell. Therefore, water moves through the cell membrane into 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. It is called deplasmolysis.

If we place living cells in isotonic solution (i.e., both solutions have the same amount of solute concentration), 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 and 20% concentrated sucrose solution.

Procedure

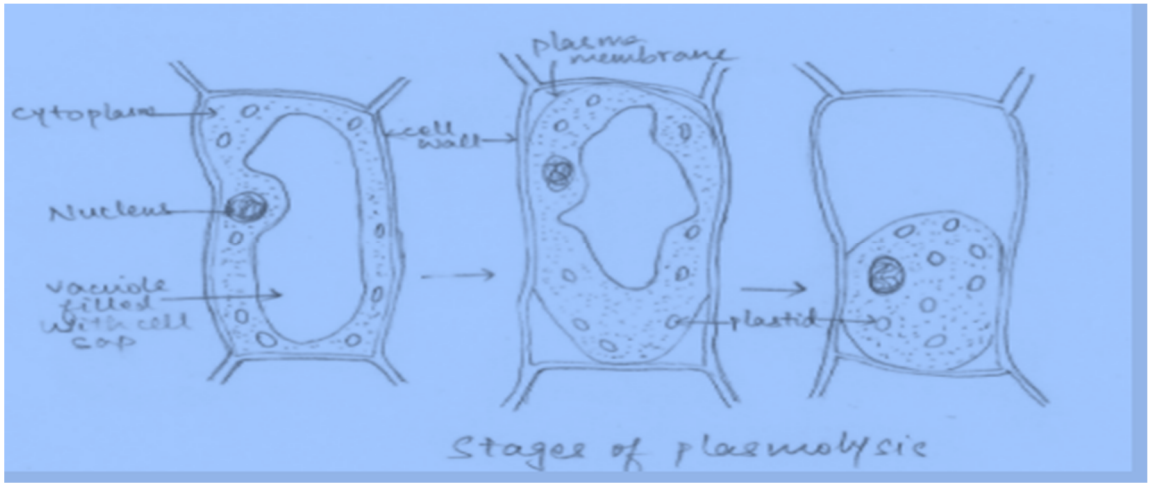
- Take an onion bulb, with the help of forceps pull a thin transparent peel gently.
- Keep this peel in water filled watch glass.
- Transfer the peel gently on a clean slide in a drop of water with the help of a brush and needle.
- Examine it under high power of a microscope.(40X)
- Observe the individual cells and make a sketch of the cells showing the cell wall and cell membrane. (observation 1)
- With the help of dropper put the sucrose solution on the slide by the sides of cover-slip so that 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 into the peel.
- Observe the cells again after 10 mins.(observation 3)

Observation table:

Observation	Condition of the cell	Explanation
1		
2		
3		

Inference:

- When the peel of onion is kept in concentrated solution (hypertonic), the protoplasm shrinks as the water starts moving out due to exosmosis.
- This phenomenon of shrinkage of protoplasm when the cells are kept in a concentrated solution 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.



Diagrammatic view of normal plant cell and plasmolysed plant cell

3. Study of the effect of wind velocity and light on the rate of transpiration in excised twig/leaf.

Principle- Transpiration is the process by means of which excess water present in plant tissue eliminates in the form of water vapour through the aerial parts of plants like twig or leaf etc. The stomata constitute the main organ for land plants. The rate of transpiration depends on the different factor such as light, wind, humidity etc**.**

Requirements-

- Plant material-A twige of Ficus leaf .
- Glass Ware- Conical flask -2 pcs. 250 ml.
- Glass rod- 2pcs.
- Chemical – Oil .
- Instruments- Digital weight machine
- Graph paper, Pencil, Thread, blotting paper etc**.**

Method- Two conical flasks were filled with distilled water upto their neck. Two petiolate leaves were cut under water from the twig .Then immediately inserted into flask, their petioles remain under water. Now oil was poured to cover the upper surface of water to check the evaporation. The whole sets were weighted and one set was allowed to stay in open air with the sufficient light and another set was kept in dark condition for two hours. After two hours two sets were reweighted. The difference between two weights is the amount of water loss. The total transpiration areas are measured by a graph paper method.

Result- The rate of the transpiration under air with the sufficient light = mg/sq./hr.

Dark condition = mg/sq./hr.

Precaution-

- The leaf should be fresh and clean.
- Oil should cover the whole upper surface of water.
- All weight should be taken carefully.
- Only petiole should be under water.
- When blowing of wind started having chance of leaf fall from the set, it should be checked.

Discussion- The rate of the transpiration is directly depends on the velocity of the wind and light. The rate of the transpiration is increased due to the effect of wind velocity and light. As the wind flows, it accelerates the transpiration from the surface of the leaf compare to dark condition of set.

Condition of set	Initial wt. in grams	Final wt. in grams	Difference of wt. in grams	Total area in sq.	Time	Rate of transpiration
In wind velocity and light						
Dark condition						

4. Calculation of stomatal index and stomatal frequency from the two surfaces of leaves of a mesophyte and xerophytes.

Theory-Stomatal frequency can be defined as the number of stomata present per unit area of a leaf. Determination of stomatal frequency and the total area of stomata covered in a leaf are the essential prerequisite to assess the rate of water loss through stomata. Several environmental and genetical factors affect stomatal frequency. The following environmental factors change the stomatal frequency- water availability, carbon dioxide concentration, temperature and light intensity. Water stress results in a greater stomatal frequency. Plants growing in wet soil with high humidity have lower frequency than the plants growing in dry soil with low humidity. Stomatal frequency is reduced in polluted atmosphere, e.g. Trifolium partense. Light intensity has marked effect on stomatal frequency. In low light intensity frequency reduces. Stomatal frequency becomes higher when a plant grows in full sunlight.

Stomatal frequency is not constant within a plant. Highest frequencies are often found in those leaves that occur on the top. Even within a leaf there exists variation in frequency. As for example the grass leaf has lowest frequency at the tip. The highest frequency is observed at the point of insertion of the leaf where the cells are still developing and smallest.

Because the stomatal frequencies often vary Salisbury (1928) proposed the term ‘stomatal index’. Stomatal index relates the number of stomata to number of epidermal cells.

Stomatal index is the percentage which the number of stomata form to the total number of epidermal cells, each stoma being counted as one cell.

Stomatal index is calculated in the following way:

Stomatal index= S x 100/E+ S, {Where S= Number of stomata per unit area.

E= Number of epidermal cells in the same unit area}

Requirements:

- Leaves of Ficus and Nerium sp.
- Compound microscope
- Camera lucida
- Drawing board
- Slides
- Cover slips
- Forceps
- Spirit lamp
- Small watch glass
- Blade
- Cello tape
- Drawing sheet
- Pencil
- Chloral hydrate solution.

Procedure: Take a mature leaf. If the leaf is small, the whole leaf may be taken and if the leaf is large cut 5mm square pieces from the middle portion between the lamina and midrib.

- Sometimes the epidermis can be easily peeled off in thick leaves by breaking it into pieces by sheering action. Separate the epidermis and treat with chloral hydrate.
- Cut a number of 5mm pieces from the the middle portion between the lamina and midrib.
- Boil with chloral hydrate in a test tube placed in a water bath. The epidermis separates out. Carefully place the epidermis on a slide with the help of a brush along with 1 – 2 drops of chloral hydrate ;cool and then place a cover glass.

Tracing of cells:

- Draw a square of about 8 – 10 cm square on a drawing sheet or any unit area.
- Place the prepared slide on the stage of the microscope
- Focus epidermal cells and the stomata first with 10 x 10 and later focus with 10 x40 or 10 x 20
- With the help of camera lucida, trace the stomata and the epidermal cells in the square.
- Trace epidermal cells and the stomata outside the square for completion on two adjacent sides of the square for counting purpose.
- Number the complete epidermal cells and stomata within the square.
- Then continue numbering the cells that are more than half on two adjacent sides

5. To calculate the area of an open stoma and percentage of leaf area open through stomata in a mesophyte and xerophyte (both surfaces).

Material Required:

Leaves of Ficus and Nerium, forceps, Petridish, watch glass, beaker, brush, needle, safranine, glycerin etc.

Procedure:

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

Inference:

In the mesophytic leaf, the no. of stomata is more in the lower epidermis, and only few stomata are present in the upper epidermis. However, in xerophytic leaf have a few sunken stomata in the epidermis.

Precautions:

- The curling of the peel should be avoided.
- Always use brush to transfer the peel from watch glass to the slide.
- Excess of glycerin should be removed by using blotting paper

Table-1 **Standardization of Microscpoe**

Nature of object	**No. of observation**	**Observation**		**Value of one ocular division**	**Mean value of 1 ocular division**
		No of ocular division	**No of stage division**		
High power	1.			1 ocu. =	
	2.			1 ocu. = 1 ocu =	
	3.				

Table-2 **Number of stomata per field**

Position of leaf suface	**No of stomata on lower surface**		**Mean of stomata on lower surface**	**Final mean of stomata on lower surface**	**No of stomata on upper surface**		**Mean of stomata on upper surface**	**Final mean of stomata on upper surface**
	No. of observation	No of stomata			No of obser.	No of stomata		
Apical	1.				1			
					2.			
	2.				3 .			
	3.							
Middle	1.				1.			
	2.				2.			
	3.				3.			
Basal	1.				1.			
	2.				2.			
					3			
	3.							

Table-3 **Length and breadth of individual stomata**

Position of leaf	**Length of stomata in ocu. division**				**Mean length**	**Breadth of stomata in ocu. division**				**Mean breadth**
	Obs. no	Reading in ocu.	Mean	1 ocu= ?		Obs. no	Reading in ocu.	Mean	1 ocu=?	
Basal	1. 2. 3.					1. 2. 3.				
Middle	1. 2. 3.					1. 2. 3.				
Apical	1. 2. 3.					1. 2. 3.				

Table-4 **Diameter of microscopic field**

Observation no	**Microscopic vision/Stage division**	**Mean value=A**
1	2r=X, r=X/2	
2	2r=Y, r=Y/2	
3	2r=Z, r=Z/2	

OR, We know, 1 stage division =0.01 mm or 0.001 cm.

So, radius of microscopic field =A X O.OOI cm.

Area of Microscopic field is $\boldsymbol{\pi r^2}$ =3.14x A X O.OOI x A X O.OOI $\mathbf{cm^2}$

3.14x A X O.OOI x A X O.OOI $\mathbf{cm^2}$ **area of microscopic field contain……P….**

1 ‘’ ‘’ ‘’ ’P/ 3.14x A X O.OOI x A X O.OOI = Area of a stoma= $\boldsymbol{\pi}$ **X L X B/4**

6. Demonstration experiments:

Suction due to transpiration (Transpiration pull)

Principle: Transpiration refers to loss of water in vapour form from the aerial parts of plants. Transpiration develops a pull/upward suction force because of the presence of a continuous water column (formed due to cohesive and adhesive properties) that starts from the mesophyll cells of leaves (from where water is lost) to the root hairs (from where water enters the plants). This is called transpiration pull. This force is responsible for the absorption and upward conduction of water and minerals in the stem.

Requirement: A healthy branch of a shrub or a tree (20-25 cm long), beaker (100mL) or a glass tube (15 cm long and 0.5 cm diameter), a rubber tube (8-10 cm long and 0.5 cm diameter), 50ml beaker, eosin/methylene blue/blue ink, scalpel/knife, grease or petroleum jelly, tray, stand with clamp.

Procedure

• Insert a graduated 1 mL pipette to one end of a rubber tube so that it fits tightly.

• Select a twig of appropriate diameter and size and make an oblique cut with a knife taking care not to rip off the bark. Moisten the cut edge with water.

• Fill the rubber tube and pipette with water by suction.

• Insert the twig into the other end of the rubber tube and tie a thread to make it air tight.

• Fix the set up with the help of burette stand and clamp as shown in the Fig.

• Take a glass tube/beaker and fill it with coloured water (using eosin/methylene blue/blue ink) and keep the nozzle of the pipette in it.

• Note the level of coloured water in pipette. Keep the setup in sunlight/under a fan for an hour and note the final level of coloured water in the pipette.

Observation: Initially when the experiment is set up, a continuous column of water is observed in the pipette. After an hour coloured water rises in the pipette.

Discussion A strong suction force was developed in the water column due to transpiration from leaves. First a water potential gradient was created between the stomata and the spongy mesophyll. As a result of transpiration through stomata, water from the mesophyll moved into the stomata. A similar water potential gradient was built up sequentially from mesophyll cells - leaf veins- leaf midrib xylem - petiole xylem - stem xylem - water column in the experiment. The intense suction pressure generated sucked water against gravity. In nature this suction force is transmitted into the root xylem and root hair, which enables plants to absorb water from the soil