MSCBOT-510 (L)

M. Sc. II Semester
LABORATORY PRACTICAL II

DEPARTMENT OF BOTANY
SCHOOL OF SCIENCES
UTTARAKHAND OPEN UNIVERSITY

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BLOCK-1 CELL BIOLOGY OF PLANTS

UNIT-1 IDENTIFICATION OF DIFFERENT STAGES OF MITOSIS AND STUDY OF MORPHOLOGY OF METAPHASE CHROMOSOMES USING ONION ROOT MERISTEMS

1.1 Objectives

1.2 Introduction

1.3 Identification of different stages of mitosis

1.4 Study of morphology of metaphase chromosomes using onion root meristem

1.5 Summary

1.6 Glossary

1.7 Self Assessment Questions

1.8 References

1.9 Suggested Readings

1.10 Terminal Questions

1.1 OBJECTIVES

After reading this unit students will be able-

- To understand the process and different stages of mitosis
- To visualize different phases of mitosis

1.2 INTRODUCTION

Every cell has a tendency to pass its genetic information to the next generation to maintain their gene pool and for that purpose each cell increases its number (Robert, 2005; Carter and Fankhauser, 2010). The process by which a parent cell divides into two or more daughter cells is called cell division (Lodish, 1999). We all know cell division is a small component of the cell cycle.

Cell division is of two types, mitotic cell division and meiotic cell division. The mitotic cell division occurs in somatic cells and the meiotic cell division is found in reproductive cells.

Mitotic cell division is the process of nuclear division in which duplicated chromosomes are faithfully separated from one another, producing two nuclei, each with a complete copy of all the chromosomes present in the original cell. It is a process where the numbers of chromosome are reduced so that cells formed contain only one member of each pair of homologous chromosomes.

Cell cycle can generally be divided into two phases: M-phase and Interphase. Interphase is the largest part of the cell division. Interphase is the part in which the cell prepares for the M-phase or division phase. This is the most important phase in the cell cycle which includes diverse activities and these activities are the basic requirement for the next mitotic phase. Interphase is primarily divided into three phases: G_1 phase, S phase and G_2 phase. S phase is the period during which DNA synthesis and replication occurs. G_1 is the gap period between the point where the mitosis ends and DNA replication starts and G_2 is the gap period between the point where the DNA replication ends and the M-phase starts. These phases also have certain check points which make sure that all the necessary requirements for the M-phase are complete and the whole cell cycle is strictly regulated.

Each cell division whether it is mitosis or meiosis can further be divided into two types; karyokinesis and cytokinesis. Karyokinesis is the division of the nuclear material which is

completed during the prophase, pro-metaphase, metaphase, and anaphase stages. Cytokinesis is the division of the cytoplasm which occurs during the last stage of cell division known as telophase.

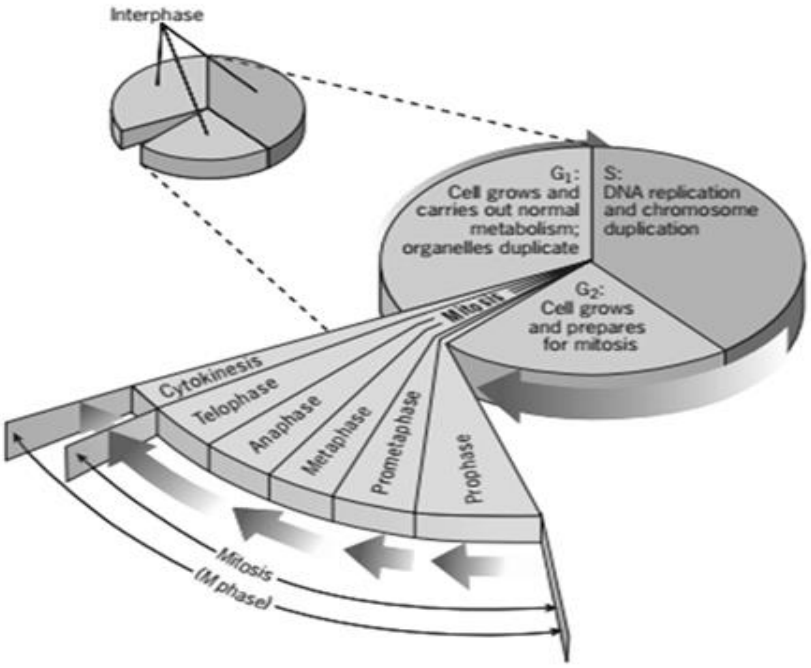


Fig.1.1. An overview of the eukaryotic cell cycle

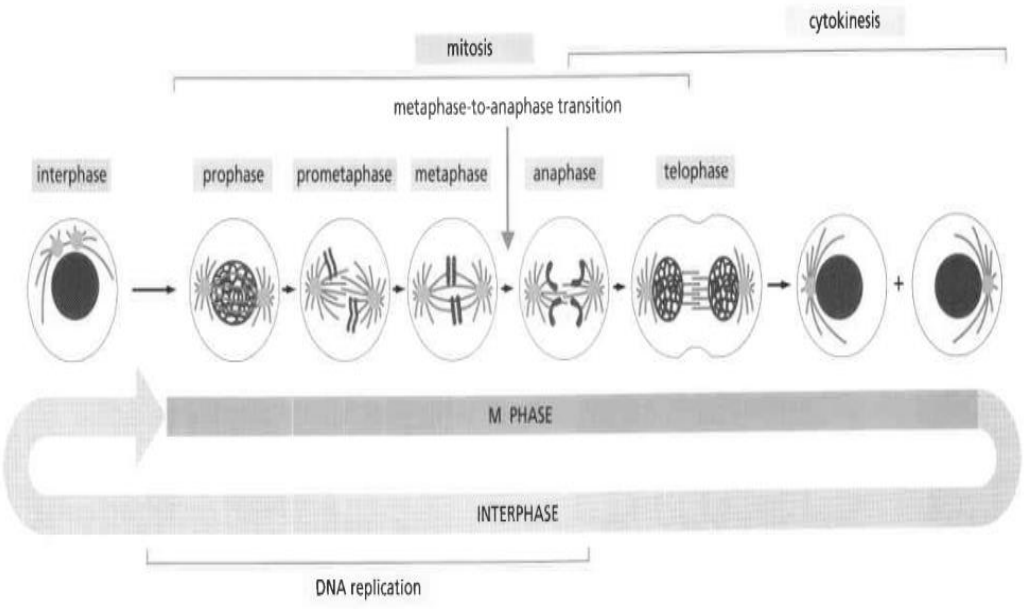


Fig.1.2. The events of eukaryotic cell division as seen under a microscope

The process of Mitosis is divided into four stages called as Prophase, Metaphase, Anaphase and Telophase. These stages are mentioned below in brief:

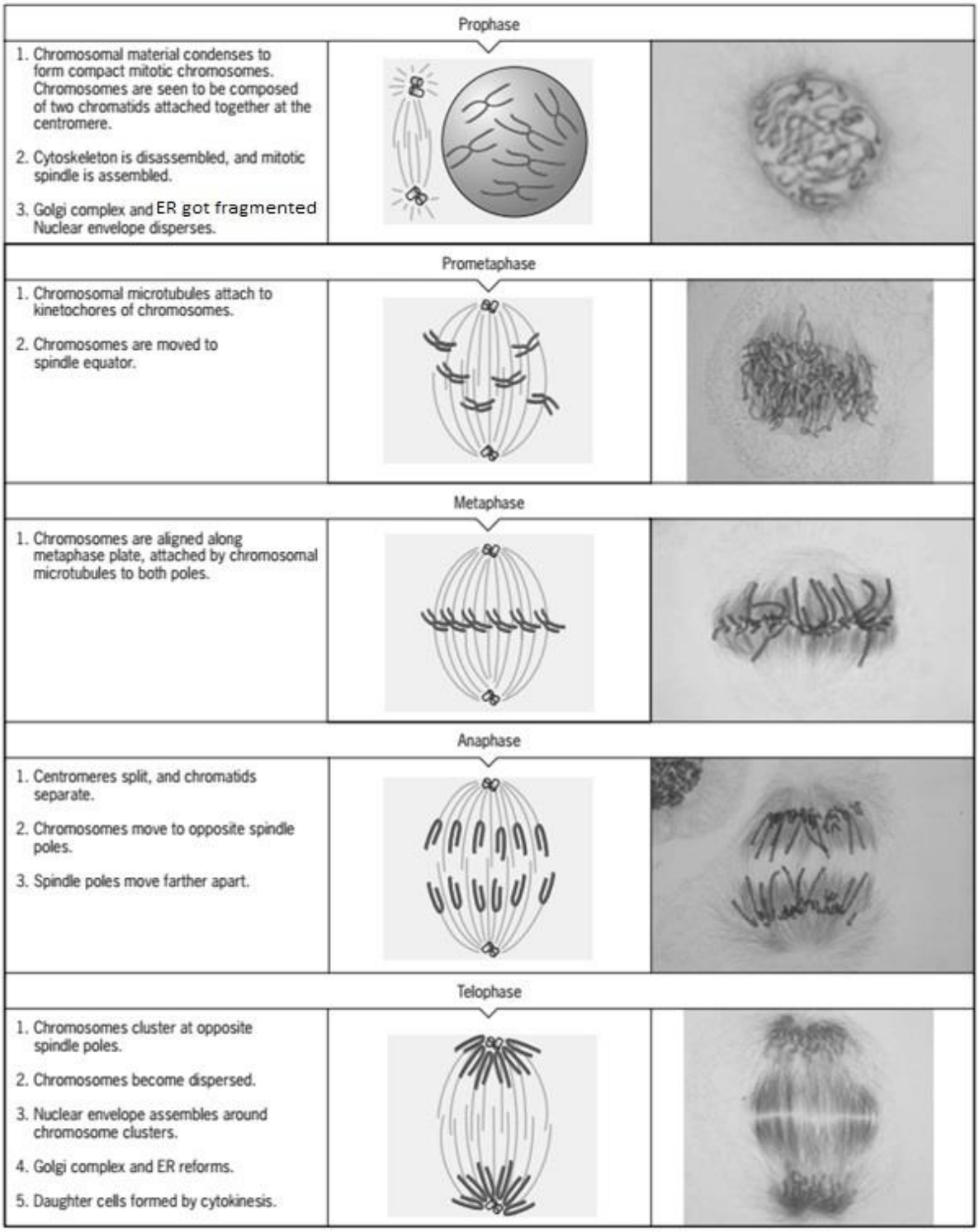


Fig.1.3. The stages of mitosis in an animal cell (left drawings) and a plant cell (right micro-photographs)

Prophase: During this stage, the chromosomes super coil, condense and become visible for first time during the cell cycle. The spindle fibers start forming. Its nuclear membrane starts disintegrating.

Prometaphase: Begins abruptly with the breakdown of the nuclear envelop. Chromosomes can now attach to spindle microtubules via their kinetochores and undergo active movement.

Metaphase: During this stage, the spindle fibers reach and attach to centromere of each sister chromatid. The chromosomes align along the center plane of the cell. The nuclear membrane disintegrates completely.

Anaphase: During this stage, the centromeres start splitting and the sister chromatids begin to migrating towards the opposite poles of the cell.

Telophase: During this stage, the chromosomes are clustered on the either end of the cell. The nuclear membrane starts reforming. The cell plate (new cell wall) starts to form between the two daughter nuclei. This is followed by cytokinesis.

1.3 IDENTIFICATION OF DIFFERENT STAGES OF MITOSIS

a) Requirements

1. Onion plant with root
2. Feulgen stain
3. 1 N HCl
4. Scissors
5. Forceps
6. Razor blade
7. Pipette
8. 1.5 ml plastic tubes
9. Dissection probe with wooden back
10. Microscopic slides and cover slips
11. Water bath
12. Light Microscope

b) Methodology

1. Take the onion plant with newly sprouted roots and cut two root tips using scissors and transfer them into a plastic microfuge tube.
2. Fill 2/3 of the tube with 1N HCl using a dropper.
3. Place the tube in a 60°C water bath and incubate the tube for 12- 15 minutes.
4. Remove the tube from the water bath after the incubation.
5. Discard the HCl from the tube using a pipette to the running tap water.
6. Add some drops of distilled water into the tube and rinse the root. Then remove the water from the plastic tube using the pipette. (Rinse the roots at least three times).
7. After the washing step add 2-3 drops of Feulgen stain into the tube with root tips and incubate the roots for 12-15 minutes. (During the incubation, the very tip of the root will begin to turn red as the DNA stains the numerous small actively dividing cells at the time).
8. After incubation remove the stain using a pipette.
9. Again rinse the root tips with distilled water. (Rinse the roots at least three times).
10. Transfer a root tip from the tube to the centre of the microscopic slide and add a drop of water over it.
11. Take a razor blade and cut most of the unstained part of the root.
12. Cover the root tip with a cover slip and then carefully push down on the cover slide with the wooden end of a dissecting probe. (Push hard, but do not twist or push the cover slide sideways). The root tip should spread out to a diameter of about 0.5- 1cm.
13. Observe it under a compound microscope in 10× objective. Scan and narrow down to a region containing dividing cells and switch to 40× for a better view.

c) An alternative methodology

1. Cut the tip 5 to 8 mm from the freshly sprouted root. Discard the rest of the root.
2. Place the cut tip on a clean microscope slide.
3. Add 2-3 drops of acetocarmine stain to the slide.
4. Warm the slide gently and intermittently over the alcohol lamp for about one minute. (Do not allow the slide to get hot to the touch. Do not let the root dry out).
5. Cover the slide with a cover slip or lens paper.
6. Squash the slide with your thumb using a firm and even pressure. (Avoid squashing with such force that the cover slip breaks or slides).

7. Observe it under a compound microscope in 10× objective. Scan and narrow down to a region containing dividing cells and switch to 40× for a better view.

1.4 STUDY OF MORPHOLOGY OF METAPHASE CHROMOSOMES USING ONION ROOT MERISTEM

To study the chromosomes is difficult because the cell cycle is a very rapid process, and the chromosomes can be visualized only for a few minutes during the cell cycle. This is the only time period when the study of chromosome morphology must be completed (Karp, 2010; Fankhause, 2010; Alberts et al., 2008). Maximum condensation of chromosomes is achieved during only a short phase of this cycle, and is known as the metaphase. For the convenience of studying the metaphase chromosomes a method is required to halt the cell division at metaphase.

There are some known drugs available in market which inhibit the formation of the spindle, arrest cell division at metaphase as well as cause further chromosome condensation. This inhibition results in the formation of super-condensed chromosomes scattered in the cytoplasm. This state is often termed as an "exploded metaphase" or "colchicine metaphase" (c-mitosis). Some drugs which are being used frequently for this purpose are colchicine, 8-hydroxyquinolin, and paradichlorobenzene. Treatment of *Allium* with concentrated solution of colchicine for long periods of time results in polyploidy in chromosomes.

Onion root tips are placed in dilute colchicine solutions (0.01%, 0.05%, 0.1%, or 1.0% solutions) for five to ten minutes and then taken out and left for thirty minutes. Then squash the roots immediately and prepare the slides. After thirty minutes colchicine metaphases can be observed. To avoid polyploidy, lower concentrations of colchicine (e.g., 0.05%) should be used, and treatment should be terminated within four hours after initiation.

Apart from colchicine, 8-hydroxyquinolin and 0.002 molar solution of oxyquinolin (for three to six hours at 180 °C) are an effective mitotic inhibitor in preparing cells for chromosome analysis. The roots may then be squashed immediately, or killed and fixed for later use after the treatment. A wide variety of stains is available to analyze a chromosome and one of the most used stain is acetocarmine. The first advantage to select acetocarmine over any other stain is that it can be applied directly to the living tissues without any pre-conditioning such as killing and fixing. The second advantage is that it completes the requirements of both a stain and a fixing agent. Acetocarmine also has one disadvantage of staining the cytoplasm in many cells. This can be

overcome by fixation of the root tip cells with 1:3 acid alcohol for twelve hours before attempting to stain the tissue.

The staining of the chromosomes can be enhanced by adding iron to the stain and this can be easily achieved by teasing the stained tissue with the help of an iron dissecting needle. The iron of the needle reacts with the acetic acid, causing the stain to darken.

1.5 SUMMARY

The process of cell division (M-phase of the cell cycle) consists of nuclear division (mitosis) followed by cytoplasmic division (cytokinesis). The nuclear division is mediated by a microtubule-based mitotic spindle, which separates the chromosomes, while the cytoplasmic division is mediated by an actin-filament-based contractile ring. Mitosis is largely organized by the microtubule asters that form around each of the two centrosomes produced when the centrosome duplicates. Centrosome duplication begins during the S and G2 phases of the cell cycle, and the duplicated centrosomes separate and move to opposite sides of the nucleus at the onset of M phase to form the two poles of the mitotic spindle. Large membrane-bounded organelles, such as the Golgi apparatus and the endoplasmic reticulum, break up into many smaller fragments during M phase, which ensures their even distribution into daughter cells during cytokinesis.

1.6 GLOSSARY

Acetocarmine stain: Carmine is a basic dye that is prepared from the insect *Coccus cacti*. Dissolve 10 g carmine (Fisher C579-25) in 1 L of 45% glacial acetic acid, add boileezers, and reflux for 24 h. Filter into dark bottles and store at 4°C. This solution can be stored for a long time. Staining can be intensified by adding ferric chloride ($FeCl_2 \cdot 6H_2O$) by adding 5 mL of a 10 % ferric chloride solution per 100 mL of % acetocarmine.

Centromere: Constricted region of a mitotic chromosome that holds sister chromatids together. Also the site on the DNA where the kinetochore forms that captures microtubules from the mitotic spindles.

Colchicine: *It* is an alkaloid derived from the plant autumn crocus (*Colchicum autumnale*). It inhibits microtubule polymerization and thus assembly of the mitotic spindle, demonstrates the presence of another checkpoint in the cell cycle. When colchicine is added to cultured cells, the cells enter mitosis and arrest with condensed chromosomes.

Compound microscope: A compound microscope is an instrument that is used to view magnified images of small objects on a glass slide. It can achieve higher levels of magnification than stereo or other low power microscopes and reduce chromatic aberration.

DNA replication: Process by which a copy of a DNA molecule is made.

Feulgen stain: Feulgen stain is a staining technique discovered by Robert Feulgen and used in histology to identify chromosomal material or DNA in cell specimens. It is darkly stained. It depends on acid hydrolysis of DNA, therefore fixating agents using strong acids should be avoided.

Kinetochores: Complex structure formed from proteins on a mitotic chromosome to which microtubules attach. Plays an active part in the movement of chromosomes to the poles.

Light Microscope: A light microscope is an instrument that uses visible light and magnifying lenses to examine small objects not visible to the naked eye, or in finer detail than the naked eye allows.

Microtubules: Long hollow cylindrical structure composed of the protein tubulin. It is one of the three major classes of filaments of the cytoskeleton.

Pipette: A *pipette* is a laboratory equipment commonly used in chemistry, biology and medicine *to* transfer a measured volume of liquid, often as a media dispenser.

Polyploidy: It is the heritable condition of possessing more than two complete sets of chromosomes.

Sister chromatids: Tightly linked pair of chromosomes that arise from chromosome duplication during S phase. They separate during M phase and segregate into different daughter cells.

Spindle fibers: *Spindle fibers* form a protein structure that divides the genetic material in a cell. The *spindle* is necessary to equally divide the chromosomes in a parental cell into two daughter cells during both types of nuclear divisions: mitosis and meiosis. During mitosis, the *spindle fibers* are called the mitotic *spindle*.

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 over a long period of time. All water baths have a digital or an analogue interface to allow users to set a desired temperature.

1.7 SELF ASSESSMENT QUESTIONS

1.7.1 Multiple Choice Questions:

1. Out of the following which is a DNA specific stain used for viewing mitosis in onion root tip cells?

(a) Nigrosin stain (b) Phloroglucinol stain

(c) Carbol fuchsin stain (d) Feulgen stain

2. How many chromosomes are there in onion cells?

(a) Twenty (b) Six

(c) Seven (d) Eight

3. What should be the concentration of HCl used for softening of onion root tip cells before staining?

(a) 1 N (b) 0.1 N

(c) 0.01 N (d) 0.001 N

3. Which plant's root tip is most commonly used for viewing mitosis in labs?

(a) Onion (b) Potato

(c) Wheat (d) Rice

4. Out of the following which is the actively growing part of plant?

(a) Leaves (b) Bark

(c) Root tip (d) Flower bud

5. The term mitosis was first given by

(a) C. Nageli (b) W. Waldeyer

(c) Walther Flemming (d) Alexander Flemming

1.7.1 Answer Key: 1-(d), 2-(d), 3-(a), 4(a), 5-(c), 6-(c)

1.8 REFERENCES

- Alberts B, Johnson A, Lewis J, Raff M, Roberts K and Walter P (2008) *Molecular Biology of the Cell*. 5th ed. Garland Science, New York.
- Brooker RJ (2005) *Genetics: Analysis and Principles*. McGraw-Hill Publishing, New York.
- Carter S and Fankhauser D. B (2010) *Mitosis, Meiosis and Genetic.* Genetics
- Fankhauser D.B (2008) *Chromosomes in Root Tips.* University of Cincinnati Clermont College.
- https://vlab.amrita.edu/?sub=3&brch=188&sim=1102&cnt=3

- Karp G. (2010) *Cell and Molecular Biology: Concepts and Experiments*. John Wiley and Sons, Inc.
- Lodish H, Harvey, Berk A, Zipursky L, Matsudaira P, Baltimore D and Darnell J (1999) *Molecular Cell Biology*. WH Freeman and Company, New York.

1.9 SUGGESTED READINGS

- Bruce A, Johnson A, Lewis J, Raff M, Roberts K, Walter P (2008) *Molecular Biology of the Cell*. Garland Science, Taylor and Francis, New York.
- Harvey L, Berk A, Zipursky SL, Matsudaira P, Baltimore D, Darnell J (2000) Molecular Cell Biology. WH Freeman and Company, New York.
- Karp G. (2010) *Cell and Molecular Biology: Concepts and Experiments*. John Wiley and Sons, Inc.

1.10 TERMINAL QUESTIONS

1.10.1 Short answer type questions

1. What is a chromosome?
2. How to prepare acetocarmine dye?
3. What is metaphase?
4. What is metaphase chromosome?
5. What is mitosis and in what type of cells it occurs?

1.10.2 Long answer type questions

1. Explain cell cycle in detail?
2. State the difference between DNA, chromatin and chromosome?

UNIT-2 UNIT-2-IDENTIFICATION OF DIFFERENT STAGES OF MEIOSIS FROM SUITABLE PLANT MATERIAL (ONION BUDS)

2.1 Objectives

2.2 Introduction

2.3 Identification of different stages of meiosis from suitable plant material

2.4 Summary

2.5 Glossary

2.6 Self Assessment Questions

2.7 References

2.8 Suggested Readings

2.9 Terminal Questions

2.1 OBJECTIVES

After reading this unit students will be able-

- To identify various cells undergoing different stages of meiosis in onion flower buds.

2.2 INTRODUCTION

The process of producing an offspring through sexual reproduction comprises of the association of two cells with haploid set of chromosomes. On the association of these two cells (or fertilization) chromosome number doubles so this doubling of chromosome number is compensated by an equivalent reduction in chromosome number at a stage prior to formation of the gametes (Alberts et al., 2008; Carter and Fankhauser, 2010; Fankhauser 2008). This is done by a type of cell division called meiosis. The term meiosis is coined in 1905 from the Greek word meaning "reduction." The meiosis type cell division assures the production of a haploid phase during the whole life cycle, and fertilization makes sure a diploid phase (Brooker, 2005). In the absence of meiosis there would be no reduction in the chromosome number only the chromosome number would double with each generation, and in these circumstances sexual reproduction would be impossible to achieve.

During meiosis, in contrast, the four chromatids of a pair of replicated homologous chromosomes are distributed among four daughter nuclei (Karp 2010; Lodish et al., 1999). Meiosis accomplishes this feat by incorporating two sequential divisions without an intervening round of DNA replication (Table 2.1). In the first meiotic division, each chromosome (consisting of two chromatids) is separated from its homologue. As a result, each daughter cell contains only one member of each pair of homologous chromosomes. For this to occur, homologous chromosomes are paired during prophase of the first meiotic division (prophase I, Table 2.1) by an elaborate process that has no counterpart in mitosis (Snustad and Simmons, 2012). As they are paired, homologous chromosomes engage in a process of genetic recombination that produces chromosomes with new combinations of maternal and paternal alleles (metaphase I, Table 2.1). In the second meiotic division, the two chromatids of each chromosome are separated from one another (anaphase II, Table 2.1).

All the stages of meiosis are being described here separately:

Interphase

Before undergoing in meiosis-I, each cell will remain in an interphase, during which the genetic materials are duplicated due to active DNA replication.

Prophase-I

This stage is comparatively complex and can be characterized by the production in the chromosome number followed by the no separation of chromatids. Prophase is the longest phase and has 5 stages: leptotene, zygotene, pachytene, diplotene and diakinesis.

Leptotene (Leptos = slender, tene = band or thread)

The nuclear membrane and the nucleolus cannot be observed conspicuously. Chromosomes appear as long thread like structure interwoven together and uniformly distributed. Chromosomes display a beaded appearance and are called chromomeres. Ends of chromosomes are drawn toward nuclear membrane near the centriole. In some plants, chromosomes may form synthetic knots.

Zygotene (Zygon = paired)

The characteristic feature of this stage is the pairing of the homologous chromosomes with one another, gene by gene, over the entire length of the chromosomes. The pairing of the homologous chromosomes is called synapsis. Each pair of homologous chromosomes is known as bivalent.

Pachytene (pachy = thick)

Each paired chromosomes get condensed and look shorter and thicker than in earlier sub-stages and splits into 2 sister chromatids except at the region of the centromere. As a result of the longitudinal division of each homologous chromosome into chromatids, there are 4 groups of chromatids in the nucleus, this configuration is called tetrads. Crossing over take place in this stage.

Diplotene (diplos = double)

In this stage a movement of homologous chromosomes can be seen except at the regions where they are attached. The regions of attachment are called as chiasmata, they are the site of exchange of parts between two homologous chromosomes. Chiasmata appear to move towards the ends of the synapsed chromosomes in the process of terminalization.

Diakinesis (Dia = opposite, kinesis = movement or seperation)

The chromosomes begin to coil, and appear shorter and thicker in comparison to earlier stages. Terminalization is completed. The nucleolus detaches from the nucleolar organizer and

disappears completely. The nuclear membrane starts to disintegrate and spindle formation is in progress.

Metaphase-I

The bivalents (pair of homologous chromosomes) orient themselves at random on the equatorial plate. The centromere of each chromosome of a terminalized tetrad is directed toward the opposite poles. The chromosomal microtubular spindle fibers remain attached with the centromeres and homologous chromosomes ready to separate.

Anaphase-I

It is characterized by the separation of whole chromosomes of each homologous pair (tetrad), so that each pole of the dividing cell receives either a paternal or maternal longitudinally double chromosome of each tetrad. This ensures a change in chromosome number from diploid to monoploid or haploid in the resultant reorganized daughter nuclei.

Telophase-I

The chromosomes may persist for a time in the condensed state, the nucleolus and nuclear membrane may be reconstituted, and cytokinesis may also occur to produce 2 haploid cells.

Metaphase-II

Metaphase-II is of very short duration. The chromosomes rearrange in the equatorial plate. The centromeres lie in the equator, while the arms are directed toward the poles. The centromeres divide and separate into 2 daughter chromosomes.

Anaphase-II

Daughter chromosomes start migrating toward the opposite poles and the movement is brought about by the action of spindle fibers.

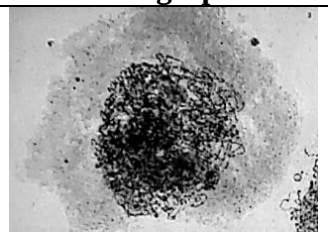
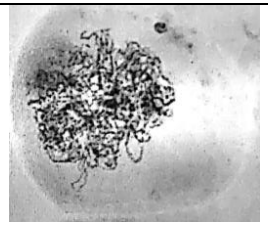
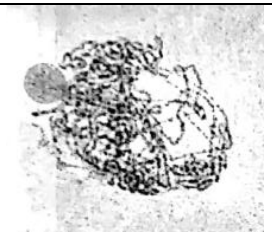
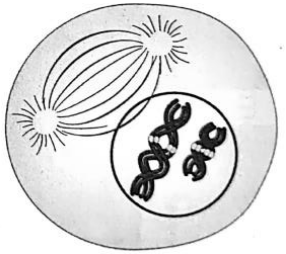
Telophase-II

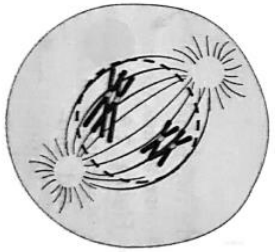
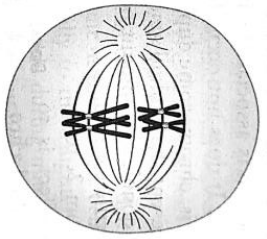
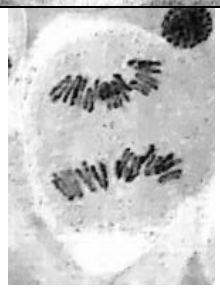
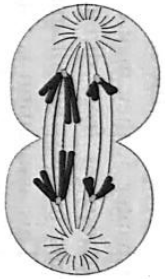
The chromosomes uncoil after reaching the opposite poles and become less distinct. The nuclear membrane and nucleolus reappear, resulting in the formation of 4 daughter nuclei, which are haploid.

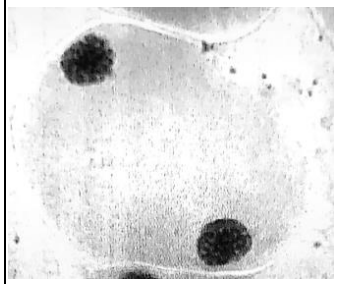
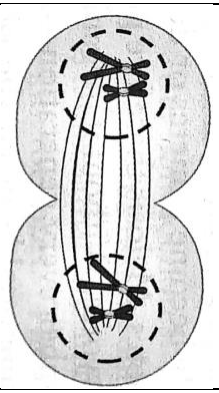
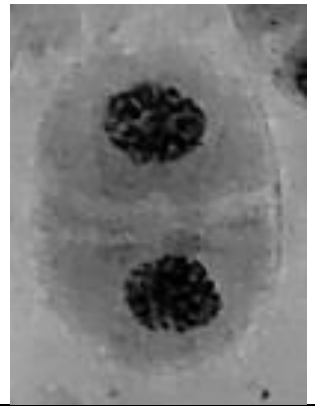
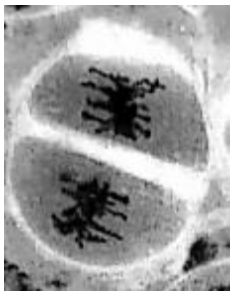
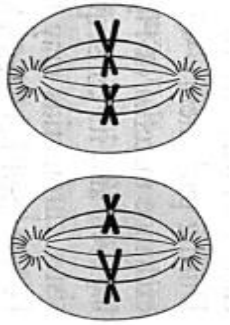
Cytokinesis

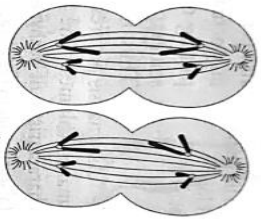
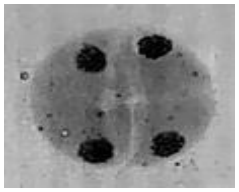
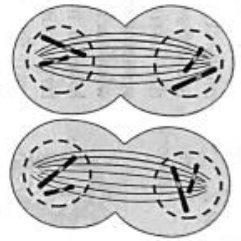
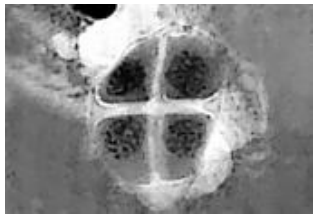
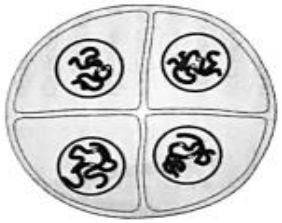
This separates each nucleus from the others. The cell wall is formed and 4 haploid cells are produced.

Table-2.1: Showing different stages of meiosis including key features, photographs and diagrams

S. No.	Stages of Division			Key Features	Photographs	Diagrams
1	**MEIOSIS I**	**PROPHASE I**	LEPTOTENE	Chromosomes, each consisting of two sister chromatids, begin to condense.		
2			ZYGOTENE	Homologous chromosomes begin to pair.		
3			PACHYTENE	Homologous chromosomes are fully paired.		
4			DIPLOTENE	Homologous chromosomes separate, except at chiasmata.		

5			DIAKINESIS	Paired chromosomes condense further and become attached to spindle fibers.		
6		METAPHASE 1		Paired chromosomes align on the equatorial plane in the cell.		
7		ANAPHASE 1		Homologous chromosomes disjoin and move to opposite poles of the cell.		

8		TELOPHASE 1	Chromosome movement is completed and new nuclei being to form.		
9	**MEIOSIS II**	PROPHASE II	Chromosomes, each consisting of two sister chromatids, condense and become attached to spindle fibers.		
10		METAPHASE II	Chromosomes align on the equatorial plane in each cell.		

11		ANAPHASE II	Sister chromatids disjoin and move to opposite poles in each cell.		
12		TELOPHASE II	Chromosomes decondense and new nuclei begin to form.		
13	Cytokinesis		The haploid daughter cells are separated by cytoplasmic membranes.		

2.3 IDENTIFICATION OF DIFFERENT STAGES OF MEIOSIS FROM SUITABLE PLANT MATERIAL (ONION BUDS)

Requirements:

1. Onion flower buds
2. Acetocarmine stain
3. Glass slides
4. Cover slips
5. Blotting paper

Procedure:

1. Select appropriate flower buds of different sizes from the inflorescence.
2. Fix them in Carnoy's fluid, which is used as fixative.
3. Take a preserved flower bud and place it on a glass slide.
4. Separate the anthers and discard the other parts of the bud.
5. Put 1 or 2 drops of acetocarmine stain and squash the anthers.
6. Leave the material in the stain for 5 minutes.
7. Place a cover slip over them and tap it gently with a needle or pencil.
8. Warm it slightly over the flame of a spirit lamp.
9. Put a piece of blotting paper on the cover slip and apply uniform pressure with the thumb.
10. Observe the slide under the microscope for different meiotic stages.

2.4 SUMMARY

1. In sexually reproducing organisms gametes are produced as a result of meiosis. Therefore, cell division in reproductive cells is called meiosis.
2. This two phase process divides the chromosome of diploid germ cell, generating four haploid gametes. (These two phases are meiosis-I and meiosis-II).
3. During Prophase-I of meiosis-I the nuclear envelope begins to breakdown and nuclear chromatin starts to condense into individual chromosome made up of two sister chromatids.
4. Prophase-I passes to various sub stages: Laptotene, Zygotene, Pachytene, Diplotene and Diakinesis.
5. During Metaphase-I pairs of homologous chromosomes (called tetrads) move along their microtubule attachments, so they are lined up along the metaphase plate.

6. During Anaphase-I attachment between homologous chromosomes breakdown and kinetochores pull the homologous chromosomes towards opposite poles.
7. The final stages of meiosis-I are Telophase-I and cytokinesis during which cells split apart forming two daughter cells.
8. The first phase of Meiosis-II is prophase-II during which the nuclear envelope breaks down and spindles reform.
9. In Metaphase-II, the chromosome aligns along the metaphase plate.
10. In Anaphase-II sister chromatids (considered individual chromosome after chromosome separation) move towards opposite poles of meiotic spindle.
11. In the final stage of meiosis-II, chromosome reaches the poles, the spindle breaks down and nuclear envelope reforms.
12. Cytokinesis produces four haploids (the haploid daughter cells) from the original diploid cell.

2.5 GLOSSARY

Carnoy's fluid: Carnoy's solution is a fixative composed of 60% ethanol, 30% chloroform and 10% glacial acetic acid, 1gm of ferric chloride.

Diploid: Containing both members of each pair of homologous chromosomes, as exemplified by most somatic cells. Diploid cells are produced from diploid parental cells during mitosis.

Fertilization: Fusion of haploid gametes, egg and sperm, to form the diploid zygote.

Generation: All of the offsprings that are at the same stage of descent from a common ancestor: Mother and daughters represent two **generations**.

Genetic recombination: Reshuffling of the genes on chromosomes that occurs as a result of breakage and reunion of segments of homologous chromosomes.

Haploid: Containing only one member of each pair of homologous chromosomes. Haploid cells are produced during meiosis, as exemplified by sperm.

Homologous chromosomes: Paired chromosomes of diploid cells, each carrying one of the two copies of the genetic material carried by that chromosome.

Inflorescence: An *inflorescence* is categorized on the basis of the arrangement of flowers on a main axis and by the timing of its flowering (determinate and indeterminate).

Reproduction: Reproduction is the biological process by which new individual organisms "offspring" are produced from their "parents".

2.6 SELF ASSESSMENT QUESTIONS

2.6.1 Multiple Choice Questions:

1. In meiosis reduction division occurs during

(a) Meiosis-II (b) Meiosis-I

(c) Meiosis-I and meiosis-II both (d) None of the above

2. 'Synapsis' occurs during

(a) Leptotene of prophase-I (b) Metaphase-I

(c) Zygotene of prophase-I (d) Pachytene of prophase-I

3. During 'pachytene' which activity occurs?

(a) Alignment of homologous chromosomes (b) Chiasmata formation

(c) Crossing over between homologous chromosomes (d) Formation of synaptonemal complex

4. Which one of the following statements is incorrect?

(a) Chiasmata are the product of crossing over

(b) Chiasmata form during diplotene phase

(c) Contraction of chromosome is maximum and rapid during pachytene

(d) Chiasmata move towards the ends and terminalize during diakinesis

5. During Metaphase-I which one of the activities takes place?

(a) Chromosome separation

(b) Homologous centromere moving towards opposite poles

(c) Chromosome alignment at the equator

(d) Chiasmata disappear completely

6. A diploid cell produces _____ cells by meiotic division

(a) Four diploid daughter cells (b) Two diploid daughter cells

(c) Two haploid and two diploid daughter cells (d) Four haploid daughter cells

7. During Prophase-II spindle reformation occurs with the help of

(a) Microtubules (b) Centromere

(c) Endoplasmic reticulum (d) None of these

8. Which one of the following is longest meiotic phase?

(a) Prophase-I (b) Metaphase-I

(c) Metaphase-II (d) Prophase-II

9. Separation of sister chromatids occurs during

(a) Metaphase-II (b) Anaphase-II

(c) Anaphase-I (d) Metaphase-I

10. Separation of homologous chromosomes occur during

(a) Anaphase-I (b) Metaphase-II

(c) Metaphase-I (d) Prophase-II

2.6.1Answer Key: 1-(b), 2-(c), 3-(c), 4-(c), 5-(c), 6-(d), 7-(a), 8-(a), 9-(b), 10-(a)

2.7 REFERENCES

- Alberts B, Johnson A, Lewis J, Raff M, Roberts K and Walter P (2008) *Molecular Biology of the Cell*. 5th ed. Garland Science, New York.
- Brooker RJ (2005) *Genetics: Analysis and Principles*. McGraw-Hill Publishing, New York.,
- Carter JLS, Fankhauser DB (2010) Mitosis, Meiosis and Genetics.
- Fankhauser D.B (2008) *Chromosomes in Root Tips.* University of Cincinnati Clermont College.
- Karp G. (2010) *Cell and Molecular Biology: Concepts and Experiments*. John Wiley and Sons, Inc.
- Lodish H, Harvey, Berk A, Zipursky L, Matsudaira P, Baltimore D and Darnell J (1999) *Molecular Cell Biology*. WH Freeman and Company, New York.
- Snustad DP, Simmons MJ (2012) *Genetics*. Sixth edition, John Wiley and sons Singapore.

2.8 SUGGESTED READINGS

- Alberts B, Johnson A, Lewis J, Raff M, Roberts K and Walter P (2008) *Molecular Biology of the Cell*. 5th ed. Garland Science, New York.
- Karp G. (2010) *Cell and Molecular Biology: Concepts and Experiments*. John Wiley and Sons, Inc.
- Lodish H, Harvey, Berk A, Zipursky L, Matsudaira P, Baltimore D and Darnell J (1999) *Molecular Cell Biology*. WH Freeman and Company, New York.
- Snustad DP, Simmons MJ (2012) *Genetics*. Sixth edition, John Wiley and sons Singapore.

2.9 TERMINAL QUESTIONS

2.9.1 Short answer type questions:

1. Write a brief account of meiosis-I.
2. Write a short note on synaptonemal complex.
3. What are the chiasmata? Mention their significance.

4. Write the activity of Anaphase-I.
5. Write the basic differences between meiosis-I and meiosis-II.
6. What is the importance of meiosis?
7. What is a bivalent?
8. What is the number of daughter cells produced at the end of meiosis?

2.9.2 Long answer type questions:

1. Summarize the events of first meiotic prophase.
2. Describe the complete process of second meiotic division.
3. Difference between meiosis and mitosis. What is the significance of meiosis?

UNIT-3 STUDY OF MITOTIC INDEX FROM SUITABLE PLANT MATERIAL

3.1 Objectives

3.2 Introduction

3.3 Study of mitotic index from suitable plant material

3.4 Summary

3.5 Glossary

3.6 Self Assessment Questions

3.7 References

3.8 Suggested Readings

3.9 Terminal Questions

3.1 OBJECTIVES

After reading this unit students will be able-

- To calculate the mitotic index from onion root tip

3.2 INTRODUCTION

The growth and development of every organism depends on the capacity of its cells to divide and multiply (Campbell, 1983). But not all cells have the same amount of capacity of division. Some cells take part in the process of cell division very actively and on the other hand some cells lose the capacity to divide as they mature or divide only rarely. For example, as plant roots grow, cells near the tip of the root, in the apical meristem, divide rapidly to push the root through the soil. The root cap detects the pull of gravity and directs the rapid growth of cells near the tip.

So, if you want to figure out that in any plant part how many cells are in active division mode and how many have lost the capacity to divide, then you can calculate their Mitotic Index (MI) and you will know the exact amount of dividing cell population.

Mitotic index is defined as a ratio of the total number of dividing cells (n) and the total number of cells (N) in a particular vision field chosen randomly under the microscope. By randomly selecting 5 to 10 such vision fields, you can estimate the Mitotic Index for any given plant material. With the help of Mitotic Index, you can observe that how cells differ in their ability to divide. **The mitotic index provides a measure of the capacity of cells to divide and of the rate of cell division. It is used to identify the sites of growth within a tissue and to determine which cell types are dividing.**

3.3 STUDY OF MITOTIC INDEX FROM SUITABLE PLANT MATERIAL

Outline of Methodology:

A. Prepare a slide of onion root tip as describe in previous chapters.

B. Observe it under the microscope.

C. Randomly select 5-10 vision fields and take their photographs with the help of a camera.

D. Count the total number of cells present in the vision field.

E. Count the number of cells that are actually in dividing state present in the vision field.

F. Put the value in the formula of mitotic index.

All these steps are being described here in detail:

A. Slide preparation of onion root tip: Refer unit 1.

B. Observation under the microscope: Place the prepared slide under the microscope for observation. Take a thorough survey of the whole slide.

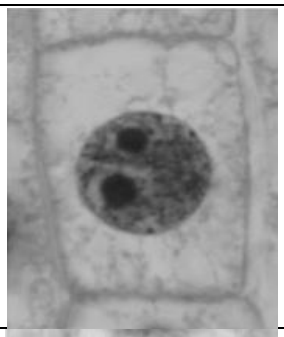
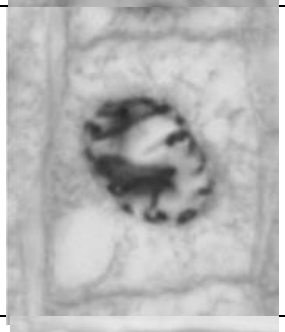
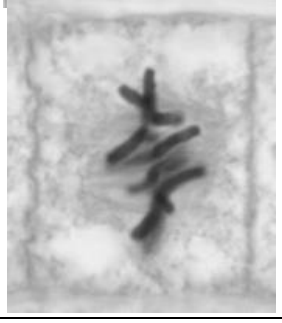
C. Selection of vision field and their photographs: Select some (5-10) random vision fields in the slide and take their 5-10 high quality photographs with a digital camera.

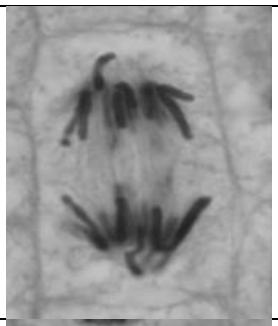
D. Counting the total number of cells present in the vision field: Carefully observe the photographs taken by you and try to count the total number of cells present in the photograph of vision fields (Figure 3.1 and 3.2; Table 3.1).

Identification of different stages of mitosis:

Here we are providing some key features to identify and differentiate the cells that in which stage of mitosis they are:

Table 3.1: identification of the different stages of mitosis division

S. No.	Stage of Division	Key Features	Photograph
1	**Interphase**	One intact nuclear region	
2	**Prophase**	Condensed chromosomes inside a nuclear membrane	
3	**Metaphase**	Condensed chromosomes present along the equator of the cell	

4	**Anaphase**	Two separate clusters of chromosomes at two different poles of the cell	
5	**Telophase**	Two nuclear regions present within a single cell (difficult to see as cytokinesis occurs concurrently)	

A. Count the number of cells that are actually in dividing state present in the vision field: Now count the number of dividing cells that means the cells which are under any stage of mitosis e.g. (Prophase, Metaphase, Anaphase or Telophase).

B. Put the value in the formula of Mitotic index: The formula of Mitotic Index is:

$$Mitotic\ Index\ (MI) = n \div N$$

Where, n = Number of cells undergoing mitosis

N = Total number of cells in the vision field check for visibility

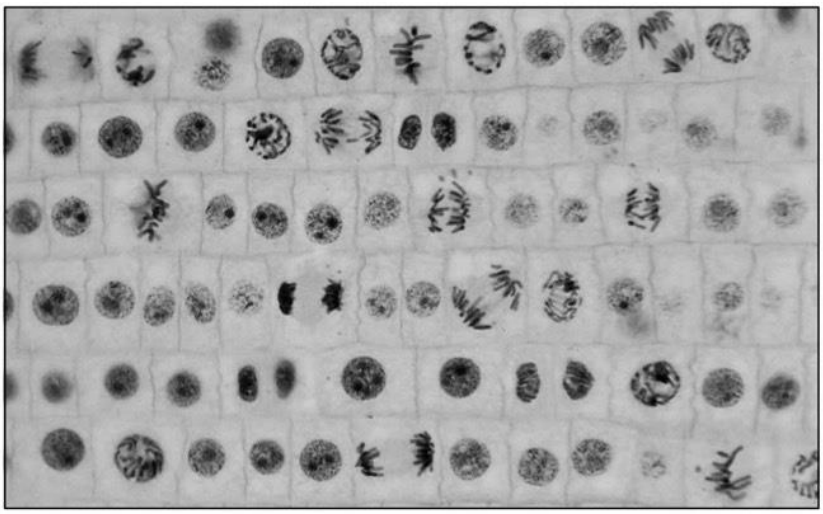


Fig.3.1: A microphotograph showing various cells (with and without visible chromosomes) under microscope

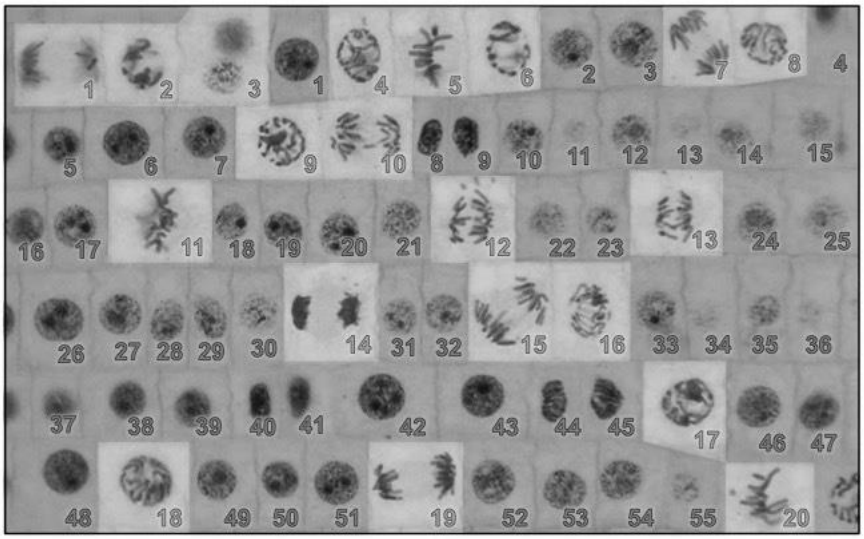


Fig.3.2: A microphotograph showing counts of cells under mitosis and total number of cells under microscope

Cells in mitosis = 20

Total number of cells = 75

Mitotic index = 20/75 = 0.267

3.4 SUMMARY

1. Mitotic index is defined as the ratio between the number of cells in a population undergoing mitosis to the total number of cells in a population.
2. The mitotic index is a measure of cellular proliferation.
3. An elevated mitotic index indicates more cells are dividing.
4. Mitotic index is an important prognostic factor predicting both overall survival and response to chemotherapy in most type of cancers.
5. Mitotic index is used to quantify the differences in cell division when environmental parameters are changed.
6. Mitotic activity (and mitotic index in turn) decreases with increasing distance from the zone of meristematic cells in the root tip.

3.5 GLOSSARY

Cell division: The process by which new cells originate from other living cells.

Chromosomes: Thread like strands that are composed of the nuclear DNA of eukaryotic cells and are the carriers of genetic information.

Development: The process of an individual organism growing organically; a purely biological unfolding of events involved in an organism changing gradually from a simple to a more complex level.

Growth: The increase in size of a cell, organ, or organism. This may occur by cell enlargement or by cell division.

Meristem: A group of plant cells that are capable of dividing indefinitely and whose main function is the production of new growth. They are found at the growing tip of a root or a stem (apical meristem); in the cambium (lateral meristem) and in grasses, also within the stem and leaf sheaths (intercalary meristem).

3.6 SELF ASSESSMENT QUESTIONS

3.6.1 Multiple Choice Questions:

1. Mitotic index considers those cells which are undergoing division in

a) Prophase and metaphase only
b) Prophase, metaphase, anaphase, telophase
c) Metaphase only
d) Metaphase and Anaphase only

2. In which of these cells mitotic index value should be higher

a) Cellular repair of the site of an injury
b) In normal growth of cells
c) In cancer cells
d) In stem cells

3. Mitotic index calculation in respect of plants depict that

a) Mitotic index decreases with increasing distance from root tip
b) Gradual decrease in cell division as it moves from the zone of cell division to zone of cell elongation
c) Both “a” and “ b” are correct
d) Mitotic index of root tip meristematic zone and shoot tip meristematic zone is equally high

4. Mitotic activity increases in plants when

a) Decrease in gravity occurs

b) Increase in gravity occurs

c) Gravity does not affect mitotic activity weather it increases or decreases

d) Gravity is normal

3.6.1 Answer Key: 1-(b), 2-(c), 3-(c), 4-(a)

3.7 REFERENCES

- Campbell RD (1983) *Mitotic Index*. In: Lenhoff HM (ed) Hydra: Research Methods. Springer, Boston, MA
- http://www.tiem.utk.edu/~gross/bioed/webmodules/mitoticindex.htm
- https://ib.bioninja.com.au/standard-level/topic-1-cell-biology/16-cell-division/mitotic-index.html

3.8 SUGGESTED READINGS

- Campbell RD (1983) *Mitotic Index*. In: Lenhoff HM (ed) Hydra: Research Methods. Springer, Boston, MA

3.9 TERMINAL QUESTIONS

3.9.1 Short answer type questions:

1. Write down the brief account on application of mitotic index?
2. Brief account on environmental aspect of mitotic index?

3.9.2 Long answer type questions:

1. What is mitotic index? Explain in detail?
2. What is the significance of mitotic index?

UNIT-4 TECHNIQUES OF PREPARATION OF PERMANENT AND SEMI-PERMANENT SLIDES

4.1 Objectives

4.2 Introduction

4.3 Techniques of preparation of semi-permanent slides

4.4 Techniques of preparation of permanent slides

4.5 Summary

4.6 Glossary

4.7 Self Assessment Questions

4.8 References

4.9 Suggested Readings

4.10 Terminal Questions

4.1 OBJECTIVES

After reading this unit students will be able

- To prepare semi-permanent and permanent slides of different plant materials.

4.2 INTRODUCTION

One of the major difficulties encountered in the anatomical examination of monocot and dicot plants and taxonomic study of microscopic organisms is the frequent absence of reliable type material, particularly of long-established taxa. Most of the early species descriptions are based on only drawings and type specimens have not survived to the present day and it is not ideal situation to be reliable on the drawings for the morphological characters of any organism. Collection and preservation of materials in fixative is helpful but it has its own demerits. The material to be studied might be wrongly named or the name tag can fade away from the bottle and there is a probable risk of drying up of the contents if left neglected.

Material stored from long time works fine for most plant species but they are not suitable for microscopic organisms. Living type cultures are of great value but many materials do not retain their original morphology in cultures which are therefore of no use as type material. The most accessible and easy-to-handle type specimen of any organism is a permanent slide on which the particular type cell or colony can be marked. The location of the slide should then be included with the original description and drawing of the taxon. A collection of good permanent slides of plant material is also a valuable aid to the teaching of any branch of botany. Live cultures should be used as much as possible to study the correct features of any particular species, but there are many growth forms and life cycle stages which cannot be induced in culture and which do not occur naturally in sufficient quantity or at the right time of year to enable them to be demonstrated in the living state. These can be taught in practical classes only with the help of permanent slides. Semi-permanent preparations, such as sealed wet mounts, glycerine mounts are easy to prepare but rarely survive without drying up for more than a few years. The ideal permanent slide is one in which the material is mounted in a natural or artificial resin which will set hard.

There are various methods available to make the permanent or semi-permanent slides but we should not be confused by looking at the variety of methods, we should select a method which is suitable in completing our objective perfectly. The method should be selected keeping in mind that which plant lineage (Angiosperm, Gymnosperm, Pteridophyta, Bryophyta or Algae) or class

of microscopic organisms (Bacteria or Fungi) you are going to study. The other thing which should be taken into consideration while selecting a method to prepare slides is that what do you want to study in a particular material e.g. morphology, anatomy, particular organelle in a cell, chromosomes during cell division, etc. Here are provided some general steps to prepare slides of a material to be examined; these steps may vary depending on the material to be studied.

Broad classification of slide preparation techniques:

The main methods of placing samples onto microscope slides are dry mount, wet mount, section cutting, squash and staining are described here in brief (Fig 4.1):

4.2 Methods

1. Dry mount: It is the most basic technique for slide preparation. In this technique, a thin sliced section of material is mounted on the center of the slide and covered it with a cover slip. This method is ideal for observing airborne particles such as pollens and dust as well as dead matter such as insect and aphid legs or antennae, hair, feathers etc.

Requirements: Object (sample/material) to be observe, blades, forceps, cover slips, glass slides, microscope etc,

Procedure: Simply position a finely cut or sliced section on the center of the slide and place a cover slip over the sample.

Advantages: Rapid preparation of slide.

Disadvantages: Dry mount can be prepared only for those samples which do not need water to live.

Precautions: After staining one should gently blot the slide dry.

2. Wet mount: This is made by introducing a fluid solution on a slide, suspending an object in a solution, and after covering the specimen and the solution with a cover slide.

Requirements: A liquid (e.g. water, brine, glycerin and immersion oil), forceps, pipette, glass slides, cover slips and paper towels etc.

Procedure:

- Place a drop of fluid in the center of the slide
- Position sample on liquid, using forceps
- At an angle, place one side of the cover slip against the slide making contact with outer edge of the liquid drop

- Lower the cover slowly, avoiding air bubbles
- Remove excess water with the paper towel

Advantages:

- Specimen fixation is not necessary.
- This technique of mount is easy and quick to prepare.

Disadvantages: This method provides a transitory window as the liquid will dehydrate and living specimens will die.

Precautions: More water must be added under the cover glass from time to time in order to avoid dryness.

3. Section cutting: It is a step in preparing a slide of biological material by cutting thin sections at suitable plane for microscopic examination of preserved or fresh materials.

Requirements:: Razor, blade, wax, slide, cover slide, slides etc.

Procedure:

- Wash the material with tap water to remove the surface dust particles or microorganism.
- Cut the thin sections of the material.

Advantages: Easy and rapid preparation of slide.

Disadvantages: Does not allow serial cuts, which slows down the process.

Precautions: Exposure of the blade can cause accidents.

4. Squash technique for soft materials

Requirements: *Material to be examined,* a liquid (e.g. water, brine, glycerin and immersion oil), forceps, pipette, glass slides, cover slips and paper towels, microscope etc

Procedure:

- Prepare a wet mount of material
- Place paper towel over the cover slip
- Gently press down, careful not to destroy the sample or break the cover glass
- Squash the sample
- Remove excess water

Advantages: It is rapid and simple method

Disadvantages: Possibility of cell damage is always there in squash while applying pressure.

Precautions: Do not squash over because material could overlap

5. Staining method: Cell staining techniques and preparation depend on the type of stain and analysis used. One or more of the following procedures may be required to prepare a sample.

5.1. Semi permanent and temporary slide: This is very quick method requiring only a few minutes or hours to prepare a slide. However, after examination, the slide is discarded.

Requirements: Razor, blade, wax, cover slide, slides, dyes, alcohol, glycerol etc

Procedure: Following steps are following to get the semi permanent slide.

(1) Fixation: It is the necessary process to kill tissues rapidly by precipitating proteins in fresh plant material. The chemical which used in this process is called fixative. Various fixatives are used in laboratory requiring on the nature of the hardness of the tissues. The most common fixatives are use 70% alcohol and formalin.

(2) Staining: After fixation**,** material needs to stain to emphasize the internal difference between the components of a tissue or organ. Usually single (cotton blue, safranin, fast green) and double staining (safranin and fast green) are use to stain the material depending upon the requirements.

(3) Mounting: Mounting media employed for temporary preparations include water and 1,2,3-propanetriol (glycerol) 30-50% aqueous solution.

Advantages: This is rapid and simple method. These preparations may be needed for a matter of minutes or hours only.

Disadvantages: After examination slides cannot store for the long time.

Precautions:

- Discard the incomplete and oblique sections of the material.
- Tissues should be washed well after fixation.
- If the fixation process is not done or improperly done, tissues may not stain properly and some types of fixative may crystallize out.
- Safranin is to be used to stain only the lignified tissues, over staining can be removed by washing in water.
- Air bubbles must be avoided in slide preparation.

5.2 Permanent slide: If the slide is to be kept for long-term reference, for days or even years, it must be made as a permanent preparation.

Requirements: sample material, water, series of alcohol/formaldehyde, filter paper, slides, cover slips, xylene, DPX or canada balsam, paper towels, microscope etc.
Procedure: *Fixation* and *staining* have already been discussed above. These two steps are the same in both the method of slide preparation.

Dehydration: Removal of water content from the cell is called dehydration. This step should be done gradually until all traces of water are removed from the plant cells. In this process, the section of the plant material get dehydrates with the series of different concentration of alcohol (ethyl alcohol) step by step (30%, 50%, 70% and 90% and end with absolute alcohol).

Clearing: Where the dehydrating agent is immiscible with the mounting medium, it is necessary to introduce an intermediate fluid that is miscible with both. Such a fluid is known as a clearing agent. The main purpose of clearing is to remove all traces of alcohol, thus allowing the tissues to be infiltrated with the Canada balsam or other mountant.
The most common clearing agent use in the plant science laboratory is 1,2-dimethylbenzene (xylene) for small soft tissues. Apart from this clove oil and cedar-wood oil were also use (for thick tissue). Toluene is another good clearing agent but it is a bit costly.

Mounting: Permanent preparations are obtained by enclosing tissues in solid, resiniferous inedia such as Canada balsam, DPX. After the tissues have been cleared, they are mounted in a semi-fluid 1,2-dimethylbenzene (xylene) balsam mixture. The 1,2-dimethylbenzene (xylene) subsequently evaporates and the balsam hardens.

Advantages:

- Best method to preserve the slide for long time.
- Easy to carry the slide from one place to another without damaging the material
- After dehydration the tissues appear opaque and bacterial decay ultimately sets in.

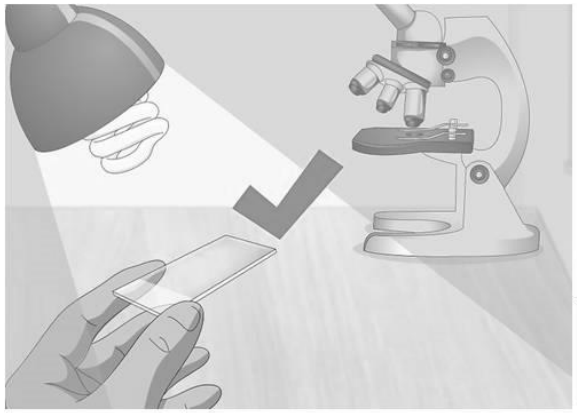
Disadvantage:

- Preparation of permanent slide is a long and time taking process.

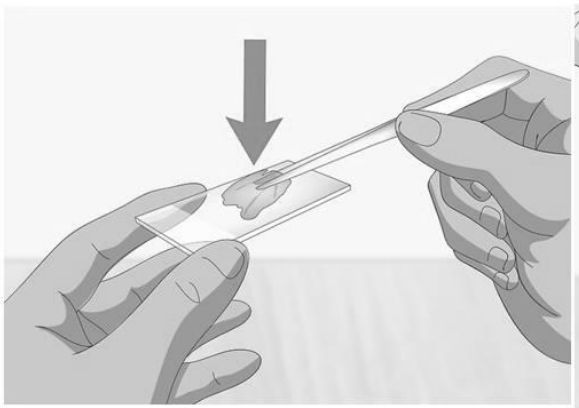
- It is a step by step process. If any step is not done properly, then the whole process has to be done.

Precautions:

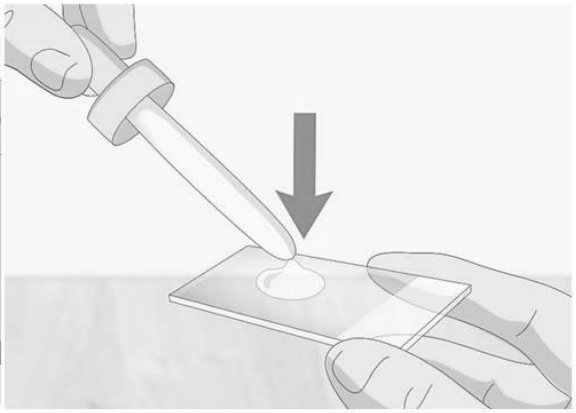
- If dehydration carried out too rapidly, it causes distortion and shrinkage, especially of delicate tissues, by setting up violent diffusion currents.
- Incomplete dehydration is indicated by cloudiness in the clearing agent and the slide should be returned to absolute alcohol.
- Alcohol is a highly flammable material. Adequate ventilation and no naked flames are essential safely precautions.
- Xylene is inflammable and toxic and the drying process is prolonged and this mountant discolours with time.



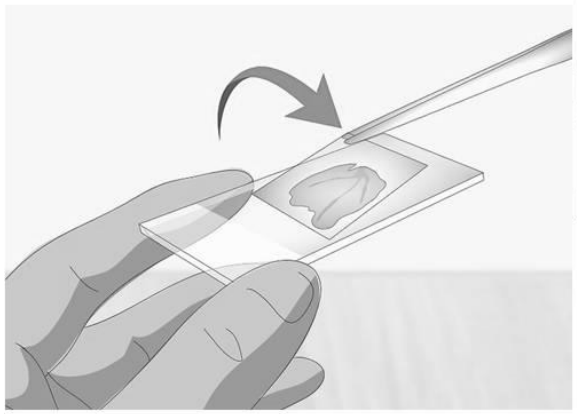
1. Select a clean slide



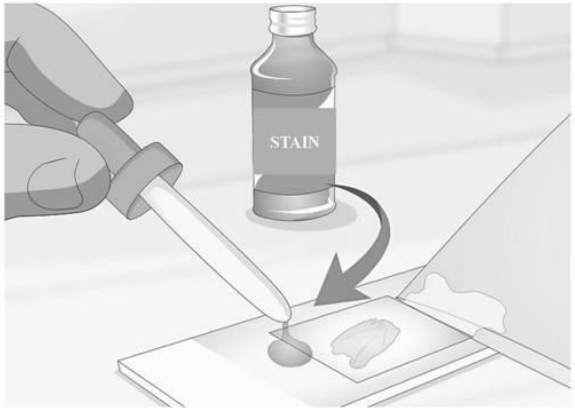
2. Place the sample specimen on the slide



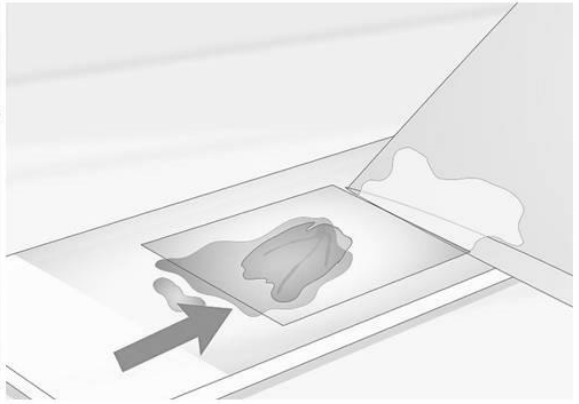
3. Place a drop of water onto your slide



4. Set a cover slip over the sample specimen



5. Place a drop of stain according to the protocol on the other side of the cover slip



6. Wait while the staining agent is drawn under the slide cover

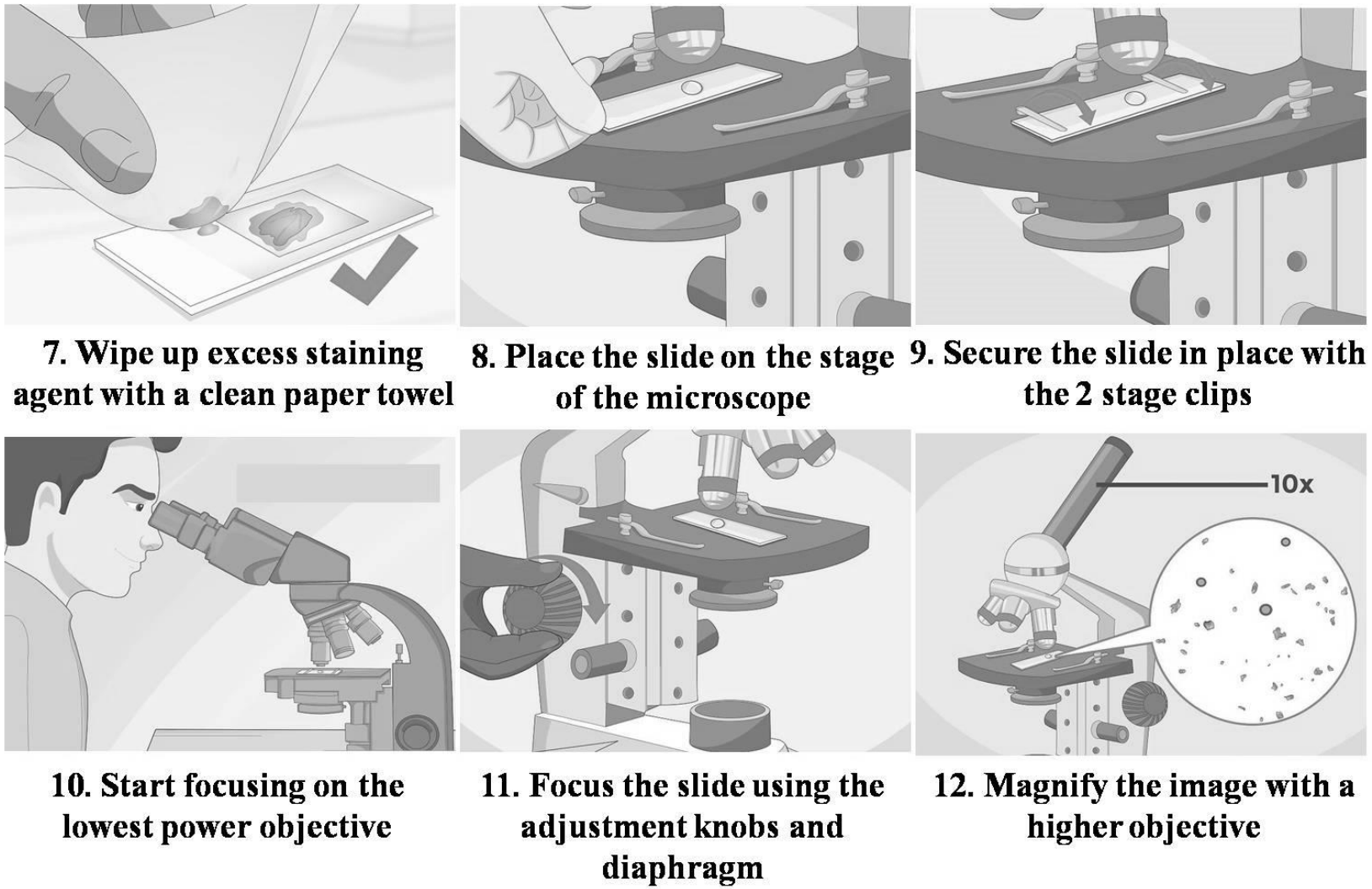


Fig 4.1: Common techniques of preparation of slides

4.3 *TECHNIQUES OF PREPARATION OF SEMI-PERMANENT SLIDES*

For a quick observation and daily laboratory experimentation this method is very handy. There are various kinds of plant materials given to students in various stages of their undergraduate and postgraduate classes, hence to study all those materials there are some techniques are being provided here.

Materials to be examined: Dicot and monocot plants (Stem and root)

Requirements: Sharp razor, brush, dropper, needles, watch glass, microscopic slides, cover-slips, safranin, glycerine and compound microscope.

Procedure:

Preliminary work:

- Take 2-3cm long pieces of the material.
- Hold the material between thumb and first finger of your left hand.
- Hold the razor in the right hand with edge of the blade facing you and handle at right angle.
- Dip the top of the material in water.
- Then start cutting transverse sections as fast as possible in a watch glass containing water.
- Select the thinnest section of the material with the help of a delicate brush.
- Take a clean watch glass with water, transfer thin sections of the material.

After the preliminary work done follow the given steps i.e., fixation, staining and mounting

(1) Fixation: It is the necessary process to kill tissues rapidly by precipitating proteins in fresh plant material. The chemical which used in this process is called fixative. Various fixatives are used in laboratory requiring on the nature of the hardness of the tissues. The most common fixatives are use 70% alcohol and formalin.

(2) Staining: After fixation**,** material needs to stain to emphasize the internal difference between the components of a tissue or organ. Usually single (cotton blue, safranin, fast green) and double staining (safranin and fast green) are use to stain the material depending upon the requirements.

For the staining, put a few drops of stain in the watch glass with water. Leave the material dipped in it for 3-5 minutes. Drain off the excess stain and wash with water if necessary. Put the thinnest stained section in the centre of the slide.

(3) Mounting: Mounting media employed for temporary preparations include water and 1,2,3-propanetriol (glycerol) 30-50% aqueous solution.

For the mount of section, put a drop of glycerine over the material. Cover it with a cover slip with the help of needle at the angle of 45°. Observe the slide under a compound microscope after staining and mounting.

Precautions:

- Safranin is to be used to stain only the lignified tissues, over staining can be removed by washing in water.
- Air bubbles must be avoided in the sections.
- Use only brush to transfer or to handle the sections. Do not use needles for this purpose.
- Discard the incomplete and oblique sections.

After preparing the slides by following the above mentioned methodology, observe it under the microscope. Under the microscope you will identify the material whether it is section of a monocot or dicot plant material, whether it is a section of a stem or a root. To identify the given material you have to find some features in the section placed under the microscope for observation:

Dicot Stem

- Multicellular hairs present on the epidermis.
- Hypodermis collenchymatous.
- Xylem endarch (metaxylem towards periphery and protoxylem towards centre).
- Vascular bundles are arranged in a ring.
- Vascular bundles are conjoint, collateral and open i.e. cambium is present.

Monocot Stem

- Hypodermis is sclerenchymatous.
- Cortex is not differentiated into endodermis and pericycle.
- Vascular bundles are scattered in the ground tissue.
- Vascular bundles are conjoint, collateral and closed.
- Each vascular bundle is surrounded by a bundle sheath.
- Xylem is y-shaped and metaxylem lies towards periphery.

Dicot Root

- Unicellular hairs are present on the epidermis.
- Hypodermis is absent.
- Vascular bundles are radial. Xylem and phloem are present on separate radii.
- Xylem and phloem bundles are less than 6.
- Protoxylem lies towards periphery and metaxylem lies towards centre.

Monocot Root

- Unicellular hairs are present on the epidermis.
- Hypodermis is absent.
- Vascular bundles are radial.
- Xylem and phloem are present on separate radii.
- Xylem or phloem bundles are more than 5.
- Metaxylem lies towards centre.

4.4 *TECHNIQUES OF PREPARATION OF PERMANENT SLIDES*

To prepare a permanent slide we should follow the steps mentioned step below:

Preliminary work:

- Take 2-3cm long pieces of the material.
- Hold the material between thumb and first finger of your left hand.
- Hold the razor in the right hand with edge of the blade facing you and handle at right angle.
- Dip the top of the material in water.
- Then start cutting transverse sections as fast as possible in a watch glass containing water.
- Select the thinnest section of the material with the help of a delicate brush.
- Take a clean watch glass with water, transfer thin sections of the material.

After the preliminary work done following steps are necessary to follow. 1. Stopping metabolic activities or fixing: It is the first and the most significant step in the preparation of permanent slides. This step includes the instant stoppage of all the cellular metabolic activities so that no changes can occur after the death of the cell. The stoppage of all cellular activities can be done with the help of some reagents with very quick reactions. The best reagents for this purpose are: Absolute alcohols, Osmic acid etc.

2. Staining: The process of coloring of various components and parts of a tissue for purpose of clear and absolute differentiation through use of different dyes (stains) is called staining. The nature of dyes may be acidic, basic or neutral. There are various kinds of dyes are in use in different staining methods (Table 4.1 & 4.2).

Table 4.1: Some other fixing and washing agents used in various protocols

Fixing agents	**Washing agents**
Bouin's fluid	70% Alcohol
Mercuric chloride	Iodine + 70% Alcohol
70% Alcohol	-
Acetic acid	50% Alcohol
Formaline (Formaldehyde)	70% Alcohol
Potassium dichromate	Water and 0.12% Chloral hydrate
Osmic acid (Osmium tetra-oxide)	Water

Usually the acidic dyes stain the cytoplasmic part and basic dyes stain the nuclear part. Most stains can be used on fixed, or non-living cells, while only some can be used on living cells; some stains can be used on either living or non-living cells. However, with a nuclear dye cytoplasm may also be stained. For the purpose of undergraduates usually general staining (single staining) is used, which may stain both nucleus and cytoplasm at the same time.

But, where specific stains are used, usually they are used in combinations of two (double staining), three (triple staining), four or even more stains.

a) Single Staining: For the purpose of single staining the dyes used are, fast green/safranin in 70% alcoholic solution or in water.

Procedure:

- Wash the material thoroughly.
- Pass the material through the gradually increasing percentage of the solvent in which stain is made e.g. if the stain is made in alcohol then it should first be passed through 30% alcohol, 50% alcohol and 70% alcohol for at least 3 minutes in each.
- After the saturation of the material with the solvent, put it in the stain for about 3 minutes or till the material becomes dark red (safranin) or dark green (fast green).

- Wash the material with the solvent or with the help of acid alcohol by placing the material for 1-2 minutes in it, and examined under microscope, till desired results are achieved.

Table 4.2: Some general stains used in various staining protocols

Type of stain	Used for plant material/tissue/organism	Stain acquired by part of sample
Safranin (single stain)	Algae	Stains cellular nuclei
Cotton blue + lactophenol	Fungi, yeast, moulds.	Cotton blue an acid dye stains the chitin present in the cell wall of fungi. Lactophenol as mounting fluid.
Crystal violet + Iodine	Gram positive Bacteria	Bacterial cell wall (thick wall of peptidoglycon).
Safranin (as counter stain)	Gram negative bacteria	Bacterial cell wall (PGL is thin).
Safranin + 10% glycerine	Bryophytes.	Epidermis, chlorenchyma stained red. Rhizoids are also stained but little more dark. Glycerine as mounting fluid.
Safranin+ fast green (double staining)	Pteridophytes, gymnosperms, monocot and dicot plant samples.	Thick walled cells get red stain and all the thin-walled cells get the green stain.
Aceto-orcein	Usually used in order to study mitosis in growing root tips	Biological stain for chromosome. Colour of stain changes from a deep red to brownish. (Over time stain precipitates).
Bismarck Brown	Used to stain live cells, cellulose and DNA.	Stains acid mucin to yellow color.
Carmine	Used as staining agent in histology.	Stains glycogen, mucopolysaccharides and cell nuclei red to dark pink.
Coomassie blue	Used to analyze protein (in analytical biochemistry). Commonly used to stain protein in SDS-PAGE gels.	Stains proteins a brilliant blue.
DAPI	Used to detect DNA in plant, bacteria, and virus particles.	A fluorescent nuclear stain that is excited by ultraviolet light, showing blue fluorescence when bound to DNA.
Eosin (Counter stain)	Used in techniques of histology. Counter stain to haematoxylin.	Tissue stained with haematoxylin and eosin shows cytoplasm stained pink orange and nuclei stained darkly, either blue or purple.
Eosin Y	Used for histological analysis of plants, animals.	Stains alkaline cell parts (like cytoplasm) pink.
Ethidium bromide	Used in molecular biology techniques to	Commonly used as florescent tag (nucleic acid stain)

	observe DNA sample in agarose gel.	On the exposure to ultraviolet light, it will perform florescence with an orange color.
Fuchsin	Basic fuchsin used to study vascular bundle of herbaceous plants and the water conducting system of woody plants. Used to study mitochondria as well. Also use to stain bacterial cell.	Leaves red color on vascular tissue components.
Hoechst stains	Two types of fluorescent stains, 33258 and 33342. 33258 suited for subsequent determination of mitotic index of plant cells grown as cell suspensions in liquid medium. Used to observe plant cell protoplasts and plant cell nuclei. 33342 used to observe compact chromatin of apoptotic nuclei to identify replicating cells.	This is a blue florescent dye, which stains DNA and the chromatin material of the cell blue.
Iodine	Used as a starch indicator.	When in solution, starch and iodine turn a dark blue colour.
Leishman's stain	Generally used to differentiate between and identify WBC, malaria parasites, and trypanosomes.	Stains nucleus of WBC blue and blood cells pink.
Malachite green	Used to observe endospore in bacterial population.	Stains endospore with green colour.
Methylene blue	Used to highlight parts of animal, bacteria, and blood tissue specimens.	Stains acidic cell parts (like nucleus) with blue colour.
Neutral/Toluylene red	Used in histology. Used in living cells.	Stains lysosomes red.

b) Double Staining: For the purpose of double staining usually Delafleld's Haematoxylin in distilled water and Eosin in 70% alcohol are used.

Procedure:

- Wash the material thoroughly.
- Put the material in Haematoxylin for 5 minutes or till it turns dark or blackish blue in color.
- After the first staining of the material it is passed through the solvent in which the second stain is prepared e.g. since Eosin is prepared in 70% alcohol the material is passed through 30%, 50% and 70% alcohol and is kept in Eosin for one minute.
- After staining it is just washed through a dip in 90% alcohol (never use 70% alcohol).

Advantages:

- Provides contrast by means of color that reveals structural details undetected in other slide preparation techniques.
- With the help of double staining we can differentiate between different tissue or cellular organization.

Precautions:

- Do not leave the material over stained because it can fool the microscopic observations.
- Carefully focus on washing the material after staining.

3. Dehydration: This process removes water from the cells and replaces it with alcohol, because the clearing agent (e.g. Xylene or Benzene) and mounting agent and its solvent (e.g. Xylene) are soluble in alcohol not in water.

Procedure:

- Place the material firstly in 30%, then in 50%, then in 70% and then in 90% alcohol.
- Finally leave the sample in 100% or absolute alcohol for 3-5 minutes, because if we put the material directly in absolute alcohol it will shrink because of sudden loss of water.

Advantages:

- By dehydrating the sample we can mount our preserved preparation in a precise way.
- By dehydrating a sample of permanent preparation we can keep it for observation purpose for long.

Precautions:

- The material should always be over stained and then wash it until you get your desired color.
- After staining (single and double both) the extra stain is washed off by placing the material in acid alcohol or acid water (depending on the solvent in which the stains were prepared) until you achieve your desired differentiation.
- Always use either staining tube or covered cavity blocks to avoid atmospheric moisture, excessive evaporation of alcohol and reagent and to protect the material.

4. Clearing (De-alcoholization): This step includes the replacement of dehydrating agent (alcohol) by the solvent of mounting medium. This step is called clearing because the clearing agent gives transparency to the cells. Cedar wood oil and Clove oil are considered as best clearing agents but the most commonly used reagent is Xylene. As xylene makes the tissue hard and brittle and also causes its shrinkage so it may be replaced by Cedar wood oil or Clove oil.

Procedure:

- Now place the material in any clearing agent.
- If the clearing agent turns turbid or white, it means that the dehydration is not done perfectly.
- Place the material in absolute alcohol for 5 minutes and then in clearing agent until it becomes transparent.
- Repeat this until the clarity achieved.

Advantage:

- De-alcoholizing agent provides transparency to cell which is a very significant thing in slide preparation.

Precaution:

- Concentration of clearing agent should be taken as per the sample type.

5. Mounting: Now transfer the material onto a drop of mounting medium which is placed in the centre of slide and cover it with a cover slip. Always keep in mind that the mounting medium should be of the same Refractive Index as crown glass (R.I. = 1.5). Common mounting mediums are: Canada balsam dissolved in xylene (1.4 refractive index), DPX, Euparol (1.4 refractive index) etc.

Procedure:

- Put a small drop of Canada balsam in center of slide with the help of glass rod.
- Now transfer the material to this drop with the help of a brush.
- Now place the cover slip on it carefully and avoid creating an air bubble.
- Clean the extra amount of Canada balsam with the help of a blotting paper.

Advantages:

- We can physically protect the material.
- Mounting medium bonds specimen, slide and cover slip together with a clear durable film.

6. Labelling: Now, write your name on one edge of the slide and identification of material on the other edge of the slide and put it under microscope for examination.

4.5 SUMMARY

Material stored from long time works fine for most plant species but they are not suitable for microscopic organisms. Living type cultures are of great value but many materials do not retain their original morphology in cultures which are therefore of no use as type material. To rectify these problem slide preparation of those organisms or part of the organism is a solution. A collection of slides of plant material is also a valuable aid to the teaching of any branch of botany.

There are various methods available to make the permanent or semi-permanent slides but we should not be confused by looking at the variety of methods. We should select a method which is suitable in completing our objective perfectly. The method should be selected keeping in mind that which plant lineage (Angiosperm, Gymnosperm, Pteridophyta, Bryophyta or Algae) or class of microscopic organisms (Bacteria or Fungi) you are going to study. The other thing which should be taken into consideration while selecting a method to prepare slides is that what do you want to study in a particular material e.g. morphology, anatomy, particular organelle in a cell, chromosomes during cell division, etc. Here are provided some general methods of preparation of slides with their advantage, disadvantage and precautions measurements.

Method	**1. Dry mount method**
Used for	Primarily inorganic (e.g. dust particles, dead matter, hair, feathers, pollens).
Requirements	Material to be observed, Blades, brush, needles, forceps, glass slides, cover

	slips.
Procedure	Simply position a finely cut or sliced section or dried pollen cells on the center of the slide and place a cover slip over the sample.
Advantages	Quick preparation.
Limitations	Dry mount can be prepared only for those samples which do not need water to live.
Precautions	After staining one should gently blot the slide dry.
Method	**2. Wet mount method**
Used for	Fresh pollen, living organisms Aquatic materials,
Requirements	A liquid (e.g. water, brine, glycerin and immersion oil), forceps, pipette, brush, needle, glass slides, cover slips and paper towels
Procedure	• Place a drop of fluid in the center of the slide • Position sample on liquid, using forceps, brush and needle. • At an angle, place one side of the cover slip against the slide making contact with outer edge of the liquid drop • Lower the cover slip slowly, avoiding air bubbles • Remove excess water with the paper towel
Advantages	Specimen fixation is not necessary so these mount are quick and easy to prepare.
Limitations	This method provides a transitory window as the liquid will dehydrate and living specimens will die.
Precautions	More water must be added under the cover glass from time to time in order to avoid dryness.
Method	**3. Section cutting method**
Used for	The most common important and frequent method used in plant science.
Requirements	Razor, blade, wax, slide, cover slide, slides etc.
Procedure	• Wash the material with tap water to remove the surface dust particles or microorganism. • Cut the thin sections of the material, cover the section with cover slip. • Examine under microscope.

Advantages	Easy and rapid preparation of slide.
Limitations	Does not allow serial cuts, which slows down the process.
Precautions	Exposure of the blade can cause accidents.
Method	**4. Squash method**
Used for	Soft materials
Requirements	Material to be examined, a liquid (e.g. water, brine, glycerin and immersion oil), forceps, pipette, glass slides, cover slips and paper towels.
Procedure	• Prepare a wet mount of material • Place paper towel over the cover slip • Gently press down, careful not to destroy the sample or break the cover glass • Squash the sample and remove excess water
Advantages	These are rapid and simple
Limitations	Possibility of cell damage is always there in squash. While applying pressure.
Precautions	Do not squash over and over, material could overlap and preparation will get affected.
Method	**5. Staining method:** Cell staining techniques and preparation depend on the type of stain and analysis used. One or more of the following procedures may be required to prepare a sample. Two type of staining method is used for the preparation of slides i.e., (i) Semi permanent or temporary slide and (ii) Permanent slide **(i). Semi permanent and temporary:**
Used for	This is very quick method requiring only a few minutes or hours to prepare a slide. However, after examination, the slide is discarded.
Requirements	Razor, blade, wax, cover slide, slides, dyes, alcohol, glycerol etc.
Procedure	**1.** ***Fixation*****:** It is the necessary process to kill tissues rapidly by precipitating proteins in fresh plant material. The most common fixatives are use 70% alcohol and formalin. **2.** ***Staining*****:** Usually single (cotton blue, safranin, fast green) and double staining (safranin and fast green) are use to stain the material depending

upon the requirements.

3. ***Mounting*:** Mounting media employed for temporary preparations include water and 1,2,3-propanetriol (glycerol) 30-50% aqueous solution.

Advantages This is rapid and simple method. These preparations may be needed for a matter of minutes or hours only.

Limitations After examination slides cannot store for the long time.

Precautions

- Discard the incomplete and oblique sections of the material.
- Tissues should be washed well after fixation.
- If the fixation process is not done or improperly done, tissues may not stain properly and some types of fixative may crystallize out.
- Safranin is to be used to stain only the lignified tissues, over staining can be removed by washing in water.
- Air bubbles must be avoided in slide preparation

(ii) Permanent slide:

Used for If the slide is to be kept for long-term reference, for days or even years, it must be made as a permanent preparation.

Requirements Sample material, water, series of alcohol/formaldehyde, filter paper, slides, cover slips, xylene, DPX or canada balsam, paper towels, microscope etc.

Procedure

1. ***Fixation* and *staining:*** have already been discussed above. These two steps are the same in both the method of slide preparation.
2. ***Dehydration:*** Removal of water content from the cell is called dehydration In this process, material get dehydrates with the series of different concentration of alcohol (ethyl alcohol: 30%, 50%, 70% and 90% and end with absolute alcohol).
3. ***Clearing*:** Clearing is necessary to remove all traces of alcohol. The most common clearing agent use in the plant science laboratory is 1,2-dimethylbenzene (xylene) for small soft tissues, clove oil and cedar-wood oil were use (for thick tissue). Toluene is another good clearing agent but it is a bit costly.
4. ***Mounting*:** Permanent preparations are obtained by enclosing tissues in

	solid, resiniferous inedia such as Canada balsam, DPX. 5. ***Labelling:*** Write the name of the material on the edge of the slide.
Advantages	• Best method to preserve the slide for long time.
Limitations	• Preparation of permanent slide is a long and time taking process.
Precautions	• If dehydration carried out too rapidly, it causes distortion and shrinkage, especially of delicate tissues, by setting up violent diffusion currents. • Incomplete dehydration is indicated by cloudiness in the clearing agent and the slide should be returned to absolute alcohol. • Alcohol is a highly flammable. Xylene is also inflammable and toxic.

4.6 GLOSSARY

De alcoholization: This step includes the replacement of dehydrating agent (alcohol) by the solvent of mounting medium.

Dehydration: Process to remove the water from the cell.

Double staining: Stain the material with two dyes.

Fixative: It is a chemical used in the slide preparation to make the tissues optically differentiated by changing their refractive index value.

Labelling: Notify the slide on one edge it for identification

Mounting: A chemical used to mount microscopic specimens in slide preparations. It dries quickly and preserves stain.

Permanent slide: If the slide is to be kept for long-term reference, for days or even years.

Semi permanent or temporary slide: This is very quick method requiring only a few minutes or hours to prepare a slide. However, after examination, the slide is discarded.

Single staining: Stain the material with a single dye.

Staining: The process of colouring of cell or tissues with the chemical dyes (stains).

4.7 SELF ASSESSMENT QUESTIONS

1. Which type of slide is easiest to prepare?

(a) Dry mount (b) Wet mount

(c) Prepared mount (d) Section mount

2. Name the stain which is commonly used to study plant cells:

(a) Methylene blue (b) Cotton blue

(c) Safranin (d) Acetocarmine

3. Sample dehydaration usually done by:

(a) Xylene (b) Alcohol

(c) Paper towel (d) Formalin

4. Mounting in permanent slide preparation is done by:

(a) Canada balsam (b) Glycerine and xylene

(c) DPX (d) Both a and c both

5. What is the purpose of 'fixation'?

(a) To kill the specimen only.

(b) To place the specimen on slide gently and retain immovable till observation.

(c) To ensure cellular parts survival of specimen using chemicals.

(d) To kill the specimen and to preserve all structural and cellular element near original state as possible.

6. DPX is more significant as mountant because:

(a) It is highly viscous.

(b) It prevents the entry of air bubble, while applying cover slip.

(c) DPX mounting dries quickly and preserves stain.

(d) Both (b) and (c).

7. Cover slip is put on the mounted material on a slide very gently to:

(a) Avoid oozing of stain. (b) Avoid oozing of glycerine.

(c) Avoid entry of air bubbles. (d) Avoid the crushing of mounting material.

8. Temporary mount of a tissue is made in:

(a) Wax (b) Alcohol

(c) Xylene (d) Glycerine

9. Which of the following is one advantage of putting liquid on a specimen as used in wet mount slides?

(a) More permanent slide fixation. (b) Magnification of the specimen.

(c) Flattens the specimen. (d) Kills the specimen.

10. What must be added to a prepared mount slide to permanently preserve and adhere to specimen to the slide and cover slip?

(a) Stains (b) A fixative

(c) Mordant (d) Mounting medium

4.8 REFERENCES

- **Brandham, PE (1970) Techniques for the rapid preparation of permanent slides of microscopic algae.** *Br. phycol.* J. 5(1): 47-50.
- http://www.biologydiscussion.com/zoology/practicals/preparation-of-permanent-slides-zoology/60158
- https://www.microscopemaster.com/microscope-slides.html
- https://biology4isc.weebly.com/slide-preparation.html.
- https://www.wikihow.com/Use-a-Microscope
- https://www.wikihow.com/Prepare-Microscope-Slides
- https://www.biologydiscussion.com/botany/practicals-botany/permanent-slide-preparations-3-methods/54089

4.9 SUGGESTED READINGS

- **Brandham, PE (1970) Techniques for the rapid preparation of permanent slides of microscopic algae.** *Br. phycol.* J. 5(1): 47-50.
- https://www.thoughtco.com/how-to-prepare-microscope-slides-4151127
- https://www.biologydiscussion.com/botany/practicals-botany/permanent-slide-preparations-3-methods/54089

4.10 TERMINAL QUESTIONS

4.10.1Short answers type questions:

1. Give a Brief account of preparing Dry mount semi permanent slide.

2. Write down the significance of dehydration done during permanent slide preparation.
3. Give a brief account of staining.
4. Why do we wash stain after a while of applying it?
5. What could be the disadvantage of air bubble, if they enter inside cover slip over the sample to be observed?

4.10.2 Long Answer type questions:

1. Describe the stepwise procedure of preparing permanent slide.
2. Describe the procedure of preparing semi- permanent slide; also mention account on which they differ from permanent preparations.

BLOCK-2 CYTOGENETICS AND PLANT BREEDING

UNIT-5 DEMONSTRATION OF SEM AND TEM

5.1 Objectives
5.2 Introduction
5.3 Demonstration of TEM
5.4 Demonstration of SEM
5.5 Summary
5.6 Glossary
5.7 Self Assessment Questions
5.8 References
5.9 Suggested Readings
5.10 Terminal Questions

5.1 OBJECTIVES

After reading this unit students will be able to-

- Understand about microscopy.
- Understand the difference between light and electron microscope.
- Understand the functioning of light microscope.
- Understand the functioning of electron microscope.
- Understand the uses and importance of electron microscope in field of botany.
- Understand the difference between TEM and SEM.

5.2 INTRODUCTION

Microscopy is the branch of physics more appropriately optics. In real words, it is not branch but a technical field itself which uses different types of microscopes to view micro objects and ultra structure of samples that cannot be possible to see with the naked or unaided eye. Thus the technical definition of microscopy is "the use of microscope" or "the examination of minute objects by means of a microscope". The instrument or device which is used in microscopy is called microscope. Microscopes are devices that are designed to produce magnified images of small objects with the help of combination of lenses. Thus, the microscope is the scientific use of lenses in form of combinations with their focal lengths. This group of instruments includes not only multiple-lens designs with objectives, condenser and eyepiece, but also single lens very simple hand-held devices such as a magnifying glass.

More than seven hundred years ago, simple glass magnifiers were developed in the form of biconvex lens when Roger Bacon (1267) described a lens for the first time. However, his observation was not pursued immediately thereafter. These may be assumed as first microscopes to be used in which object (or specimen) could be magnified and focused by use of the magnifier placed between the object and the eye. These "simple microscopes" could spread the image on the retina by magnification through increasing the visual angle on the retina. Near the end of sixteenth century (1590) glass polishers; father and son team of Hans and Zacharias Jansen constructed a crude type of simple microscope by placing two lenses together, which permitted them to see minute objects. In 1609-1610, Galileo made the first simple microscope with a focusing device and observed the water flea through his microscope. In 1617-1619 the first

double lens microscope with a single convex objective and ocular appeared. The inventor of which was thought to be the physicist C. Drebbel. This microscope was used to study the cells, plant and animal tissue, and also the minute living organisms. Till then, the name microscope had not been given to this device; the name 'microscope' was first proposed by Faber in 1625. The "simple microscope" reached its highest state of perfection, during sixteenth century when of Anton von Leeuwenhoek a Dutch businessman and microbiologists used lens in systematic way for observation of animalcules. The image produced by such a magnifier, held close to the observer's eye, appears as if it were on the same side of the lens as the object itself. The credit of developing a compound microscope with multiple lenses goes to Robert Hooke of England.

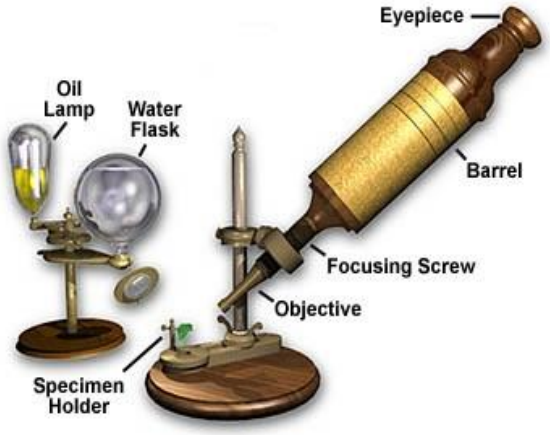


Fig.5.1: Microscope of Robert Hooke sometime in the 1660s

The microscope illustrated in Fig.5.1 is a simple compound microscope fabricated by British microscopist Robert Hooke sometime in the 1660s. The microscope is illuminated by oil lamp and light from the lamp is diffused through the water filled reservoir and then focused onto the specimen.

The early microscopes developed during the 17th and 18th centuries suffered from chromatic and spherical aberration. The chromatic aberrations do not degrade image quality only, but also hamper resolution. Resolution is the ability to distinguish two closely placed objects. Thus magnification and resolution two important concept which determines the ability of microscope.

Magnification: Magnification is not the best measure of a microscope alone. In terms of physics magnification is defined as "a measure of the ability of a lens or other optical instruments to magnify, expressed as the ratio of the size of the image to that of the object". This means ratio of image size and size of objective in actual.

In general magnification image can be calculated using the formula:

$$\text{Magnification} = \frac{\text{Size of the image seen via microscope}}{\text{Size of the image seen via naked eye}}$$

The magnification of a light microscope is calculated by multiplying the magnifying powers of the eyepiece and the objective lens. If eyepiece has a power of 10x as in laboratory microscope and the objective lens of 100x, then the final magnification would be 1000x (10 x 100). It means the image would appear 1000 times larger than it actually or seen by naked eye. Light microscopes generally have three different objective lenses to allow the slide to be viewed in three separate manners. The compound microscopes achieve two-stage magnification where the objective magnifies image projects into the body tube of the microscope; then eyepiece further magnifies the projected image. Thus the total magnification equals to the magnification of the objective multiplied by the magnification of the eyepiece:

Total Magnification = Magnification of objective x Magnification of eyepiece

Resolution: The function of any microscope is to enhance **resolution**. The microscope enlarges the image size of an object and makes possible to view by eye. Because of the enlargement, resolution is often confused with **magnification**, which refers to the size of an image. In general, the greater the magnification, the greater the resolution, but this is not always true. There are several practical limitations of lens design which can result in increased magnification without increased resolution.

The resolution of an optical microscope is defined as the shortest distance between two points on a specimen that can still be distinguished as separate entities. The limit of resolution is the closest distance between two points at which the points still can be distinguished as separate entities.

$$\text{Resolution (r)} = \frac{\lambda}{2\ NA}$$

Resolution (r) = λ/(2NA)

$$\text{Resolution (r)} = \frac{0.61\lambda}{\text{n } sin\alpha}$$

Resolution (r) = 0.61λ/NA

λ= Wavelength of illuminating radiation

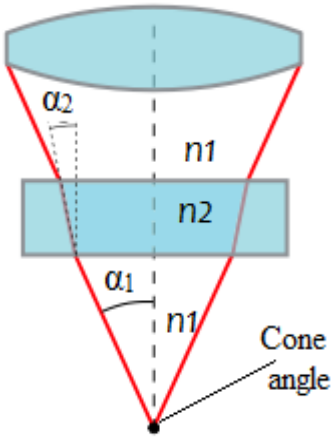
n = Refractive index of medium (For glass lens n=1.5)

n sin α = Numerical aperture (NA)

α= Angle of aperture

For better microscope the resolution should be minimum and for minimum resolution we can decrease the magnitude of λ by means of using low wavelength radiation.

Numerical aperture (NA): Numerical aperture is the function of light collecting ability of microscope. The numerical aperture of a lens is dependent upon two parameters, the angle of the incidence of light onto the lens, and the refractive index (n) of the glass of which the lens is composed. The angle of incidence is also known as the cone angle and 1/2 of this value is designated by the symbol α. The quantity "n sin α" is called is called numerical aperture (NA) and sin α is the sine of semi-angle of aperture of the objective.



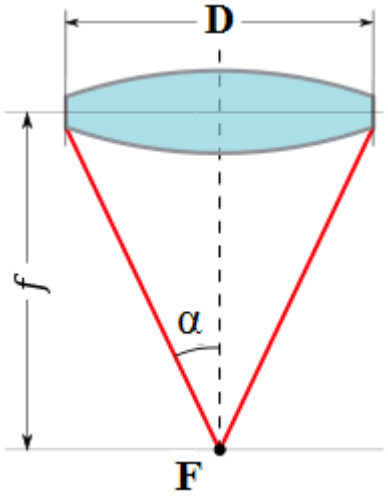


Fig.5.2: Cone Angle and numerical aperture (NA) **Fig.5.3:** Numerical aperture of a lens

According to Snell's law the numerical aperture remains the same: n sin α_1 = n sin α_2

Resolving Power (RP): The ability to distinguish the closely placed point objects called resolving power. Resolving power is inversely proportional to limit of resolution. Consequently, most microscopists today use resolution rather than limit of resolution to measure the quality of their lenses. Resolving power can be increased by use of lower wavelength radiation.

$$\text{Resolving Power (RP)} = \frac{1}{R}$$

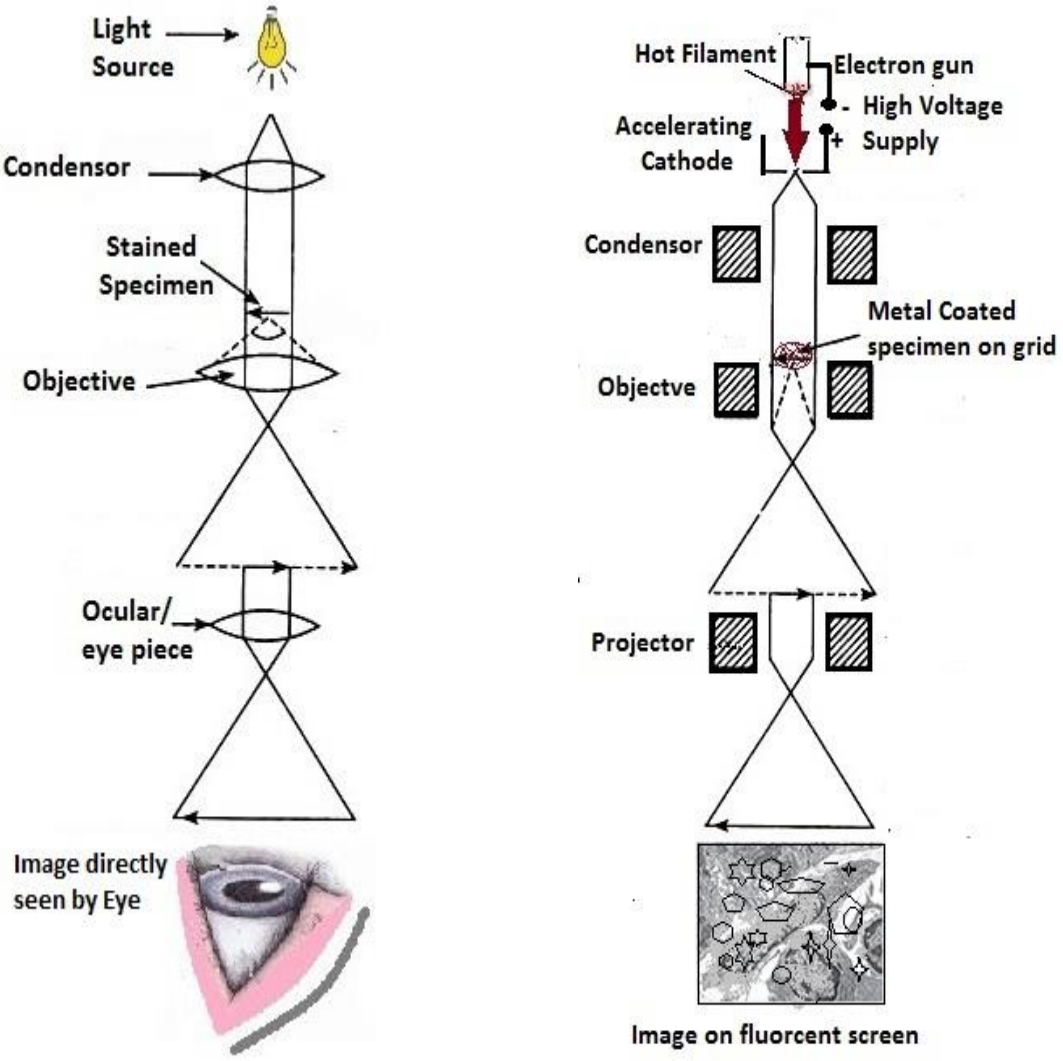
$$\text{Resolving Power (RP)} = \frac{n \sin\alpha}{0.61\lambda}$$

All electron microscopes use electromagnetic and/or electrostatic lenses to control the path of electrons. In 1931 the German engineers Ernst Ruska and Maximillion Knoll succeeded in magnifying and formation of image by use of electrons. This was, in retrospect, the moment of the invention of the electron microscope but the first prototype was actually built by Ruska in 1933 and was capable of resolving to 50 nm. Electron microscopy is used when greatest resolution is required i.e. to see ultrastructure of cells. There are two kinds of electron microscope based on the electrons used in image formation: the transmission electron microscope (TEM) and scanning electron microscope (SEM). In this unit you will study in detail about transmission electron microscope and scanning electron microscope.

Table.1: Difference between Light Microscope and Electron Microscope

S. No.	Particular/ Features	Light Microscope	Electron Microscope
1.	**Source of Illumination**	Light	Beam of electrons
2.	**Medium**	Air	Vacuum
3.	**Lenses**	Glass	Electromagnetic
4.	**Magnification**	≈ 500X -1500X	≈ 1,50,000 X -2,00,000 X
5.	**Specimen seen**	Live or dead.	Only dead or dried specimens are seen.
6.	**Specimen preparation Time**	Usually few minutes to hours.	Takes few days.
7.	**Resolution**	Low ≈ 0.25µm to 0.3µm.	High ≈ 0.001µm (≈ 250 times higher than light microscope.
8.	**Image**	Coloured	Image is black and white

9.	**Radiation**	No risk of radiation.	There is risk of radiation.
10.	**Stain**	Specimens are stained by colored dyes.	Specimens are coated with heavy metals (Pt or Au) in order to reflect electron.
11.	**Image observation**	Directly by eyes through ocular lens.	Image is received in fluorescent screen or photographic plate.
12.	**Uses**	For the study of detailed gross internal structure (Suitable for anatomical observation).	Used in the study of external surface, ultra structure of cell and very small organisms. (Suitable for cellular or molecular observation)
13.	**Cost and Maintenance**	Low price and maintenance costs.	Very expensive to purchase and high maintenance cost.



A. Light Microscope **B. Electron Microscope**

Fig.5.4: Schematic presentation and comparison of light and electron microscopy

5.3 DEMONSTRATION OF TRANSMISSION ELECTRON MICROSCOPE (TEM)

In 1931, two German electrical engineers, Max Knoll and Ernst Ruska, succeeded in using electron beam and electromagnetic lenses in place of light and glass lenses in microscopy thus finally creating the first transmission electron microscope (TEM) for which Ruska was awarded the Nobel Prize for Physics in 1986.

The transmission electron microscope (TEM) operates on the same basic principles as the light microscope but uses electron beam and electromagnetic lenses instead of light beam and glass lenses respectively. The electron beam posseses wave nature and its wavelength is far lesser than light; it makes possible to get a resolution a thousand times better than with a light microscope. If specimen is illuminated with the beam of electrons the better resolution is achieved which was requirement for improvement in the field of microscopy.

Electrons are made to pass through the specimen and the image is formed on the fluorescent screen, either by using the transmitted beam or by using the diffracted beam. The possibility for high magnifications better resolution the TEM proved a valuable instrument in the field of medical, biological and material science.

Transmission electron microscope (TEM), has three principle components or systems and an additional component the vacuum system: (1) an electron gun, (2) the image-producing system (3) the image-recording system. An electron gun (or **electron emitter**) produces the electron beam, which is utilized for image formation. Image-producing system consists of the series of electromagnetic coils which are called which focus the electrons passing through the specimen (Fig.5.4). Image-recording system usually consists of fluorescent screen or CCD (Charged Coupled devices) which produces image from transmitted electrons perceptible to the eye. In addition, a vacuum system, consisting of pumps and their associated gauges and valves, and power supplies are required.

Image formation

The beam of electrons from the electron gun is focused into a small, thin, coherent beam by the use of the condenser lens. This beam is restricted by the condenser aperture, which excludes high angle electrons. The beam then strikes the specimen and parts of it are transmitted depending upon the thickness and electron transparency of the specimen. This transmitted portion is focused by the objective lens into an image on phosphorescent screen or charge coupled device (CCD) camera. Optional objective apertures can be used to enhance the contrast by blocking out high-angle diffracted electrons. The image then passed down the column through the intermediate and projector lenses, is enlarged all the way. The image strikes the phosphorescent screen and light is generated, allowing the user to see the image. The darker areas of the image represent those areas of the sample that fewer electrons are transmitted through while the lighter areas of the image represent those areas of the sample that more electrons were transmitted through.

Sample Preparation

Materials to be observed under an electron microscope require processing to produce an observable image. TEM is a microscopic technique in which an image is formed from the electrons transmitted through the specimen, thus specimen preparation is crucial phase of TEM. In TEM electron beam is passed through inside of an electron microscope under high vacuum in order to enable the electron beam to travel in straight lines. Biological materials contain ample amount of water on evaporation it can cause problem to travel electron beam. Following are steps of specimen preparation in electron microscopy.

Cryofixation: The first stage in specimen preparing is the fixation, one of the most important and critical stages. Fixing specimen under ultra low temperature is known as cryo-fixation. In cryofixation specimen freezed rapidly to liquid nitrogen temperatures or below so that the water forms vitreous (non-crystalline) ice.

Fixation: To prevent further deterioration sample are fixed by use of fixing chemicals. In chemical fixation for electron microscopy, glutaraldehyde ($C_5H_8O_2$) and osmium tetroxide (OsO_4) is often used to crosslink protein molecules and preserve lipids respectively.

Dehydration: Dehydration is the process of removing excess water from the samples. This is done by use of organic solvents such as ethanol or acetone. For SEM specimens it helps in total drying of specimen and for TEM specimens' infiltration with resin and subsequent embedding.

Embedding and Sectioning: Embedding is the process of infiltration of specimen into resin media (such as araldite or LR white resin), which are hardened into a block for subsequent sectioning just like with wax for light microscopy. Then the specimen sectioned into thin slices with the help of ultra-microtome with a glass or diamond knife.

Staining: Staining is done to get contrast among different structures in image. For staining heavy metals like lead and uranium, etc. are used to scatter electrons and to give contrast between different structures in image. Heavy metal staining provides electron density to the sample which results in to interactions of electrons with illuminating electron beam, which provides contrast in the final image.

Freeze-fracture and freeze-etch: For the TEM, it can be rotary-shadowed with evaporated platinum at low angle (typically about 6°) in a high vacuum evaporator. A second coat of carbon, evaporated perpendicular to the average surface plane is generally performed to improve stability

of the replica coating. The specimen is returned to room temperature and pressure, and then the extremely fragile "shadowed" metal replica of the fracture surface is released from the underlying biological material by careful chemical digestion with acids, hypochlorite solution or SDS detergent. The floating replica is thoroughly washed from residual chemicals, carefully picked up on an EM grid, dried then viewed in the TEM.

The freeze fracture technique gives *en face* views of the internal structure and organisation of biological membranes like integral membrane spanning and structure of proteins, lipid bilayer and other membrane features. The sample is now ready for imaging in SEM. According to Bullivant and Ames (1966) and Bullivant *et al.*, (1979) freeze fracture can be undertaken using very simple equipment like a standard vacuum coating unit or a specialized high vacuum freeze-fracture apparatus, with a liquid nitrogen-containing holder for specimens. The cryofixed specimen is then fractured by simply breaking with sharp knife or by using a microtome while maintained at liquid nitrogen temperature.

The specimen may then optionally be etched after fracturing. During etching, water molecules are allowed to sublime from the frozen surface of the fractured specimen, by increasing the temperature to about -95°C for a few minutes. Etching lowers the specimen ice table and exposes the true surfaces of freeze-fractured membranes, thereby revealing membrane surface features of interest. According to Severs and Shotton (1995) the introduction of ultrarapid freezing techniques has made it possible to freeze a wide variety of living tissue sufficiently rapidly to achieve good cryofixation of the surface layer of cells in the absence of chemical fixation and glycerol cryoprotection, enabling their subsequent etching.

Sputter Coating: Sample is coated by an ultra-thin film of gold, gold/palladium, platinum, chromium, etc. under low vacuum condition. This is done to prevent charging of the specimen by the accumulation of static electric fields of the electron irradiation which is the requirement of imaging. It also increases the amount of secondary electrons that can be detected from the surface of the sample in the SEM and therefore increases the signal to noise ratio.

Working

The electron beams are generated by the electron gun and facilitated to fall over the prepared specimen with the help of magnetic condensers/lenses. The specimen is adjusted in between the condensing lens and the objective lens on specimen grid. The electron beam that has been partially transmitted through the very thin specimen carries information about the structure of the

specimen. The incidence beam of electrons is partially transmitted and partially diffracted by specimen. These beams are recombined at the E-wald sphere to form combined phase contrast image. To increase the contrast of the formed image, an amplitude contrast has to be obtained. This can be achieved by using the transmitting only and eliminating diffracted one. The beam is passed through the objective condenser/lens and the aperture. In order to eliminate the diffracted beam, the aperture is adjusted in such a way that the diffracted image would eliminated. The final image formed by transmitted beam of electrons is only which is passed through the projector lens for further magnification. This magnified final image is visualized on fluorescent screen or light sensitive sensor such as a CCD (charge-coupled device) camera. The image detected by the CCD may be displayed in real time on a monitor or computer. Such produced image is called Bright Field Image. Transmission electron microscopes produce two-dimensional, black and white images (Fig.5.4).

5.4 DEMONSTRATION OF SCANNING ELECTRON MICROSCOPE (SEM)

In 1940 German physicist, Manfred von Ardenne, used an another method of TEM, and called it scanning electron microscope (SEM) which uses secondary electrons to produce images. This is also what makes SEM images look three dimensional because rather than projecting the electron signature onto a single flat surface, the SEM measures electrons that travel at all angles and combines it to form an image with variable depth.

SEMs are also more amenable to researchers because the specimen preparation process is less involved. While specimens for TEM require extremely thin slicing and treatment with stains, SEM specimens only need a thin layer of conductive coating such as a gold film before being placed in the vacuum container, a process not even necessary if the electron beams are strong enough. The elimination of destructive preparation techniques has allowed researchers to image larger objects such as insects or small mechanical parts. The images produced show such fine details of everyday objects in new ways that they at times seem alien. The microscope is still expensive and needs to be precisely configured for optimal results, but overall, SEMs are easier to use compared to other forms of electron microscopy. The scanning electron microscope (SEM) is one of the most versatile instruments available for the examination and analysis of the

microstructure morphology and chemical composition characterizations. It is necessary to know the basic principles of light optics in order to understand the fundamentals of electron microscopy.

Image formation in the SEM is dependent on the acquisition of signals produced from the electron beam and specimen interactions which are mainly two types: elastic and inelastic interactions. An elastic interaction causes scattering/deflection of the incident electron by the collision of specimens' atomic nucleus or outer shell electrons. There is negligible loss of energy in such kind of interaction during the collision and by a wide-angle directional change of the scattered electron. Incident electrons that are elastically scattered through an angle of more than 90° are called BSE (backscattered electrons), and used for imaging (Fig.5.5). Detection of BSEs, provide both compositional and topographic information in the SEM. A BSE is defined as one which has undergone a single or multiple scattering events and which escapes from the surface with an energy greater than 50 eV. The elastic collision between an electron and the specimen atomic nucleus causes the electron to bounce back with wide-angle directional change. Roughly 10–50% of the beam electrons are backscattered toward their source, and on an average these electrons retain 60–80% of their initial energy. The lost energy during collision causes excitement of spinning electrons of sample atoms orbit. As a result, the excitation of the specimens' atoms electrons during collision leads to the generation of secondary electrons (SE, Fig.5.5), which can be used in image formation. Secondary electrons are used principally for topographic contrast in the SEM, *i.e.*, for the visualization of surface texture and roughness. The topographical image is dependent on how many of the secondary electrons actually reach the detector. A secondary electron signal can resolve surface structures down to the order of 10 nm or better. Although an equivalent number of secondary electrons might be produced as a result of the specimen primary beam interaction, only those that can reach the detector will contribute to the ultimate image. Secondary electrons that are prevented from reaching the detector will generate shadows or be darker in contrast than those regions that have an unobstructed electron path to the detector. BSEs carry information about features that are deep beneath the surface. In examining relatively flat samples, BSEs can be used to produce a topographical image that differs from that produced by secondary electrons, because some BSEs are blocked by regions of the specimen that secondary electrons might be drawn around. The detector for BSEs differs from that used for secondary electrons in that a biased Faraday cage is not employed to attract

the electrons. In fact the Faraday cage is often biased negatively to repel any secondary electrons from reaching the detector.

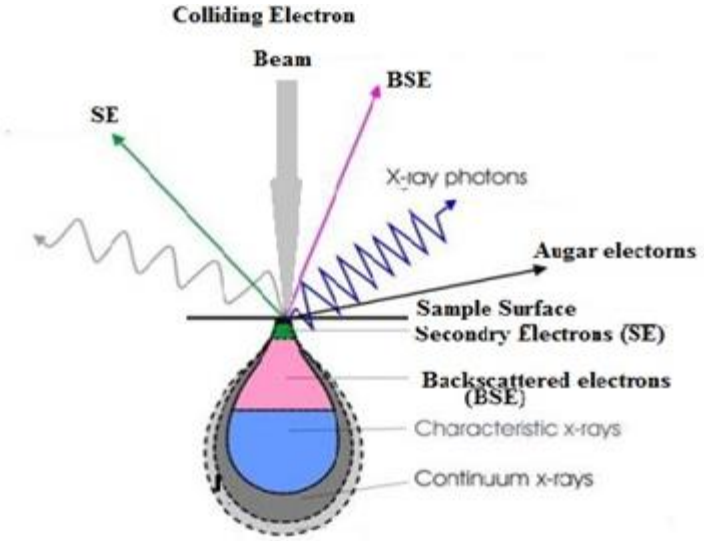


Fig.5.5: Production of SE and BSE

Construction: The SEM instrument is made up of two components: the electronic console and the electron column. The **electronic console** is controlling system that allows instrument adjustments like current, voltage, focusing, magnification, brightness contrast, *etc*.

Electron Column: The electron column generates electron beam under vacuum and condensed to a small diameter by electromagnetic deflection coils or electromagnetic lenses, and scanned across the surface of a specimen in the specimen chamber where secondary electron detector is placed above the specimen grid inside the specimen chamber. The components of the electron column are:

(1) Electron gun: Located at the top of the column where free electrons are generated by thermionic emission from a tungsten filament at high voltage. These thermionic electrons are primarily accelerated toward an anode after where controlled by condenser coils/lenses.

(2) Condenser lenses/coils: After the beam passes the anode it is influenced by two condenser lenses that cause the beam to converge and pass through a focal point. What occurs is that the electron beam is essentially focused down to 1000 times its original size. In conjunction with the selected accelerating voltage the condenser lenses are primarily responsible for determining the intensity of the electron beam when it strikes the specimen (Fig.5.6).

(3) Apertures: apertures are used to reduce and exclude the un-necessary electrons in the lenses or coils. The lens aperture also determines the diameter or spot size of the beam at the specimen and the resolution and depth of field.

(4) Scanning System: Scanning system is formed by back scattered electrons and secondary electrons. The secondary electrons from the specimen are attracted to the detector by a positive charge.

(5) Specimen Chamber: Specimen chamber is located at the lower portion of the column where specimen stage and controls are located.

Vacuum System

The vacuum system provides a smooth media for travel of controlled electron beam which requires that the electronic column be under vacuum at a pressure of at least $5x10^{-5}$ Torr.

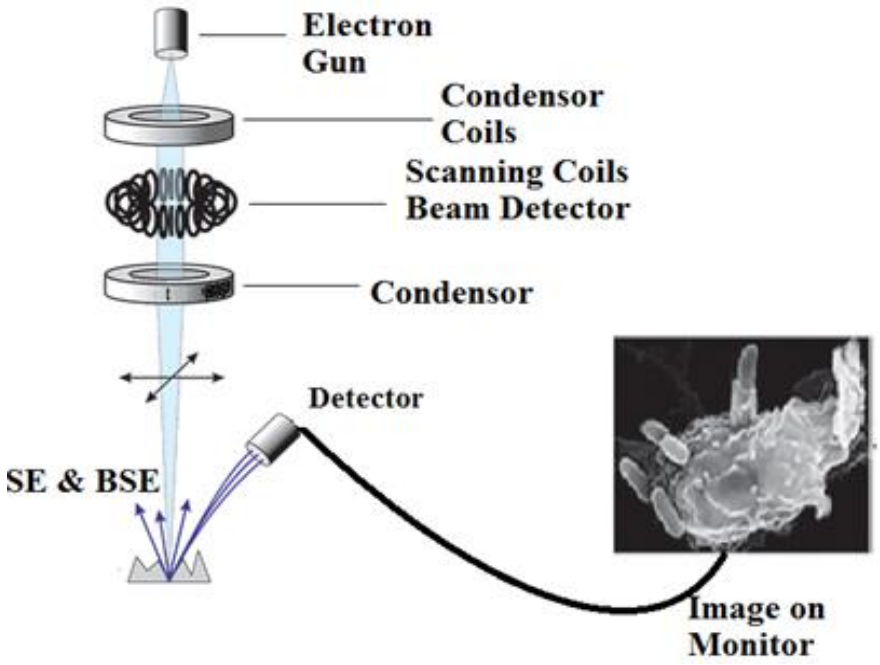


Fig.5.6: Diagram representing functioning of SEM

Fig.5.7: A SEM in Laboratory

(**Courtesy:** https://ares.jsc.nasa.gov/research/laboratories/sem.html)

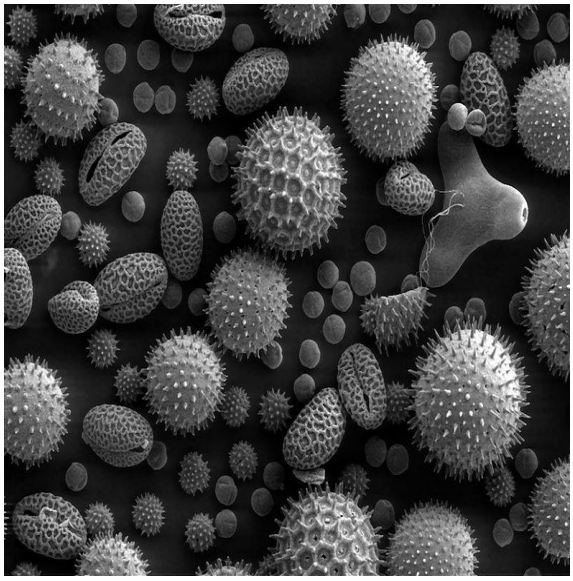


Fig.5.8: An Image formed by SEM

(**Courtesy:** https://en.wikipedia.org/wiki/File:Misc_pollen.jpg)

5.5 SUMMARY

1. Microscopy is the branch of physics more appropriately optics.
2. The technical definition of microscopy is "the use of microscope" or "the examination of minute objects by means of a microscope".
3. The instrument or device which is used in microscopy is called microscope.
4. Microscopes are devices that are designed to produce magnified images of small objects with the help of combination of lenses.
5. More than seven hundred years ago, simple glass magnifiers were developed in the form of biconvex lens when Roger Bacon (1267), described the lens for the first time.
6. These "simple microscopes" could spread the image on the retina by magnification through increasing the visual angle on the retina.
7. Near the end of sixteenth century (1590) glass polishers; father and son team of Hans and Zacharias Jansen constructed a crude type of simple microscope. In 1609-1610 Galileo made the first simple microscope with a focusing device and observed the water flea through his microscope.
8. The name microscope had not been given to this device; the name 'microscope' was first proposed by Faber in 1625.

9. The credit of developing a compound microscope with multiple lenses goes to Robert Hooke of England.
10. The early microscopes developed during the 17th and 18th centuries suffered from chromatic and spherical aberration.
11. Resolution is the ability to distinguish two closely placed objects.
12. Magnification and resolution are two important concepts which determine the ability of microscope.
13. Magnification is not the best measure of a microscope alone.
14. The magnification of a light microscope is calculated by multiplying the magnifying powers of the eyepiece and the objective lens.
15. The function of any microscope is to enhance **resolution**.
16. The resolution of an optical microscope is defined as the shortest distance between two points on a specimen that can still be distinguished as separate entities.
17. The limit of resolution is the closest distance between two points at which the points still can be distinguished as separate entities.
18. For better microscope the resolution should be minimum and for minimum resolution we can decrease the magnitude of λ by means of using low wavelength radiation.
19. Numerical aperture is the function of light collecting ability of microscope.
20. The ability to distinguish the closely placed point objects called resolving power.
21. Resolving power is inversely proportional to limit of resolution.
22. All electron microscopes use electromagnetic and/or electrostatic lenses to control the path of electrons.
23. In 1931 the German engineers Ernst Ruska and Maximillion Knoll succeeded in magnifying and formation of image by use of electrons.
24. There are two kinds of electron microscopes based on the electrons used in image formation: the transmission electron microscope (TEM) and scanning electron microscope (SEM).
25. The transmission electron microscope (TEM) operates on the same basic principles as the light microscope but uses electron beam and electromagnetic lenses.
26. The electron beam posses wave nature and its wavelength is far lesser than light; it makes possible to get a resolution a thousand times better than with a light microscope.

27. Electrons are made to pass through the specimen and the image is formed on the fluorescent screen, either by using the transmitted beam or by using the diffracted beam.
28. **Transmission electron microscope (TEM)**, has three principle components or systems and an additional component the vacuum system: (1) an electron gun, (2) the image-producing system (3) the image-recording system.
29. Materials to be observed under an electron microscope require processing to produce an observable image.
30. The steps of specimen preparation in electron microscopy are **cryofixation, fixation, dehydration, embedding and sectioning, staining.**
31. The freeze fracture technique gives *en face* views of the internal structure and organisation of biological membranes like integral membrane spanning and structure of proteins, lipid bilayer and other membrane features.
32. In 1940 German physicist, Manfred von Ardenne, uses an another method of TEM, and called it Scanning Electron Microscope (**SEM**) which uses secondary electrons to produce images.
33. The scanning electron microscope (SEM) is one of the most versatile instruments available for the examination and analysis of the microstructure morphology and chemical composition characterizations.
34. Image formation in the SEM is dependent on the acquisition of signals produced from the electron beam and specimen interactions which are mainly two types: elastic and inelastic interactions.
35. The SEM instrument is made up of two components: the electronic console and the electron column.
36. The **electronic console** is controlling system that allows instrument adjustments like current, voltage, focusing, magnification, brightness, contrast, *etc.*
37. The electron column generates electron beam under vacuum.
38. Components of the electron column are: **(1) Electron gun (2) Condenser lenses/coils (3) Apertures (4) Scanning System (5) Specimen Chamber**

5.6 GLOSSARY

Biconvex: Convex (curved) on both sides.

CCD (Charged Coupled Device): Sensors/devices used in digital photography to record still and moving images.

Contrast: An obvious difference between two or more objects or organelles in an image.

Cryofixation: Fixation of specimens by rapid freezing at very low temperature,

Dehydration: The process of removal of water from specimens.

Device: A piece of mechanical or electronic equipment used for a particular purpose.

Electron gun: A heavy metal cathode piece or device that produces a narrow stream of electrons on heating.

Electron microscope: A microscope that uses a accelerated electron beam as a source of illumination:

Electron: A negatively charged subatomic particle.

Embedding: The process by which the tissues or the specimens are enclosed in a mass of the embedding medium using a mould.

Fluorescence: Emission of higher wavelength light by absorbing lower wavelength light.

Lens: a piece of glass with curved sides for concentrating or dispersing light rays.

Magnification: Magnification is the process of enlarging the size of object.

Microscopy: Field of science viewing small things.

Resolution: The ability to see two points as two separate points.

Resolving power: Ability of a lens to show two adjacent objects as discrete entities.

Retina: The retina is the nerve layer that lines the back of the eye which senses light.

Specimen: Any object used scientific study or display.

Staining: An auxiliary technique of dying specimen with suitable dye, used in microscopy to enhance contrast in the microscopic image.

Ultramicrotome: A microtome for cutting extremely thin sections for electron microscopy.

Wavelength: the distance between successive crests or troughs of a wave.

5.7 SELF ASESSMENT QUESTIONS

1.6.1 Multiple Choice Questions:

1. The electron microscope deviced by:

(a) Knoll and Ruska (b) Galileo

(c) Robert Hooke (d) Zacharias Jansen

2. Lenses used in electron Microscopy:

(a) Glass (b) Magnetic

(c) Electromagnetic (d) None of above

3. The credit of developing a compound microscope with multiple lenses goes to:

(a) Robert Hooke (b) Zacharias Jansen

(c) Robert Brown (d) Galileo

4. To distinguish the closely placed point objects called:

(a) Magnification (b) Resolution

(c) Resolving power (d) None of these

5. Source of electron beam in electron microscopy is:

(a) Thermions (b) Electron gun

(c) Photo-electrons (d) All

6. Total Magnification is =Magnification of…… x Magnification of eyepiece.

(a) Eyepiece (b) Objective

(c) Projector (d) Condensor

7. Source of illumination in Electron Microscope:

(a) Light (b) X- Rays

(c) Electrons (d) All

8. Specimen type observed under microscope:

(a) Live (b) Dead

(c) Dead hydrated (d) Both live and dead

9. Stain used in electron microscopy:

(a) Heavy metal (b) Liquid nitrogen

(c) Colored dye (d) Carbon

10. Medium of electron beam travel in TEM:

(a) Air (b) Vapor

(c) Dry air (d) Vacuum

11. For better microscope the resolution should be:

(a) Minimum (b) Maximum

(c) Moderate (d) Can't say

12. Fixing specimen under ultra low temperature is known as:

(a) Cryo-fixation (b) Ultra fixation

(c) Shadowing (d) Spur coating

13. Medium of travel of electrons in electron microscopy:

(a) Air (b) Vacuum

(c) Cedar oil (d) Wax

14. ……. specimen used in electron microscope

(a) Live (b) Dead

(c) Both (d) Grilled

15. Use of osmium tetroxide in electron microscopy is for:

(a) Shadowing (b) Staining

(c) Fixing (d) Dehydrator

1.6.2 Fill in the blanks:

(1) The term microscope was given by _____.

(2) There are a _____types of microscopes based on illumination.

(3) Father of microscopy is called to _____.

(4) In electron microscopy _____ lenses are used.

(5) The virtual image of electron microscope formed on _____.

(6) The first compound microscope was crafted by _____.

(7) Back scattered electrons are used for image formation in_____.

(8) Secondary electrons are used for image formation in _____.

(9) Medium of path of travel of electron in electron microscope is_____.

(10) Source of electron beam in electron microscopy is _____.

1.6.3 True or False:

(1) Live specimen can be observed by electron microscope.

(2) Colored image formed by electron microscope.

(3) Glass lenses are used in electron microscopy.

(4) The electron microscope was devised by Knoll and Ruska.

(5) In electron microscopy black and white image is formed on fluorescent screen.

(6) The image can be seen by naked eye directly in electron microscope.

(7) Heavy metal coated specimens used in electron microscopy.

(8) The name 'microscope' was first proposed by Faber in 1625.

(9) The SEM instrument is made up of two components, the electronic console and the electron column.

(10) Dehydration is the process of removing excess water from the samples.

1.6.4 Very short answer questions:

(1) Define microscope.

(2) Who is called father of microscopy?

(3) Who devised electron microscope?

(4) Define electron gun.

(5) Define electromagnetic lens.

(6) Who crafted first compound microscope?

(7) What do you mean by cryofixation?

(8) Define resolving power.

(9) Define magnification.

1.6.1 Answer key: 1-(a), 2-(b), 3-(b), 4-(c), 5-(b), 6-(b), 7-(c), 8-(b), 9-(a), 10-(d), 11-(a), 12-(a), 13-(b), 14-(b), 15-(c).

1.6.2 Answer key: 1- Faber, 2-two, 3-Leeuwenhoek, 4-electromagnetic 5- fluorescent screen, 6- Robert hook, 7- Scanning electron microscope, 8- Scanning electron microscope, 9-Vcuume, 10- electron gun.

1.6.3 Answer key: 1-False, 2-False, 3-False, 4-True, 5-True, 6-False, 7-True, 8-True, 9-True, 10-True.

5.8 REFERENCES

- Broglie, L D 1925. Recherches sur la théorie des quanta (Researches on the quantum theory), Thesis, Paris, 1924, Ann. de Physique 10 (3): 22. (A translation by A.F. Kracklauer 2004) (http://aflb.ensmp.fr/LDB-oeuvres/De_Broglie_Kracklauer.pdf)
- Bullivant, S, and Ames, A 1966. A simple freeze-fracture replication method for electron microscopy. Journal of Cell Biology: 29, 435-447.
- Bullivant, S, Metcalfe, P, and Warne, KP 1979. Fine structure of yeast plasma membrane after freeze fracturing in a simple shielded device. In J.E. Rash and C.S. Hudson, eds. "Freeze Fracture: Methods, Artifacts and Interpretations". 141-147. Raven Press, New York.
- Severs, N J, and Shotton, D M 1995. "Rapid Freezing, Freeze Fracture and Deep Etching." Wiley-Liss, New York.

5.9 SUGGESTED READINGS

- Croft, W J 2006. Under the Microscope: A Brief History of Microscopy; Weiss, R.J., eds.; Series in Popular Science; World Scientific Publishing Co. Singapore, SG.
- Bradbury, S *et al*. 2017. Electron Microscope. Britannica Academic [Online]. http://academic. eb.com
- Bradbury, S *et al*. 2011. Transmission Electron Microscope (TEM). Britannica Academic [Online]. http://academic.eb.com.

- Wilson K, and Walker J 2009. Principles and Techniques of Biochemistry and Molecular Biology. Cambridge University Press.
- Bradbury, S et al. 2016. Scanning Electron Microscope (SEM). Britannica Academic [Online],. http:// academic.eb.com.

5.10 TERMINAL QUESTIONS

5.10.1 Short answer questions:

1. Write short note on magnification.
2. Describe resolution in brief.
3. What do you understand by resolving power?
4. Describe numerical aperture.
5. Differentiate between light and electron microscope.
6. Differentiate between transmission electron microscope and scanning electron microscope.
7. Write short note on secondary electrons.
8. What do you understand by Sputter Coating?
9. Write a note on numerical aperture.
10. Define freeze fracture technique**.**

5.10.2 Long answer questions:

1. Describe electron microscopy in detail.
2. Differentiate between TEM and SEM.
3. Write a detailed note on Transmission Electron Microscopy.
4. Write a note about history of microscopy?
5. Describe about sample preparation in electron microscopy.

UNIT-6 PROBLEMS BASED ON GENETICS

6.1 Objectives
6.2 Introduction
6.3 Problems based on genetics
6.4 Summary
6.5 Glossary
6.6 Self Assessment Questions
6.7 References
6.8 Suggested Readings
6.9 Terminal Questions

6.1 OBJECTIVES

After reading this unit students will be able to:

- Understand about genetic principles.
- Understand the laws of genetics.
- To know about genetic terminology.
- Understand the gene interactions.
- Understand the concept of dominance and incomplete dominance *etc.*
- Understand the major genetic problems.
- To short-out genetic problems.

6.2 INTRODUCTION

Genetics is the branch of science that deals with the study of heredity and variations of inherited characteristics. The passing of traits from one generation (parents) to another generation (offspring) is known as heredity. Heredity and variations are controlled by genes (Factors of Mendel) Genes are located on DNA of chromosome inside the nucleus of a cell and are strung together in such a way that the sequence carries information, which determines various features (phenotypic traits). Thus genetics is the science which studies how traits are passed from generation to generation. Genes are arranged in linear fashion on chromosomes and the position occupied by gene is called locus. Each diploid (2n) organism has a pair of similar chromosomes (one maternal and one paternal) *i.e.,* diploid (2n) organisms generally have two copies of each gene which governs a character.

In the 1866, an Austrian monk Gregor John Mendel postulated theory of inheritance based on his experimental work on garden pea (*Pisum sativum*) and concluded that heredity is the result of discrete units of inheritance called factors. Based on his experimental observation he postulated following principles:

(1) **Principle of paired factors**

(2) **Principle of dominance**

(3) **Principle of segregation= Principle of purity of gametes**

(4) **Principle of independent assortment**

Genetics which follows Mendel's laws known as Mendelian or classical genetics and which doesnot follow Mendelian laws known as non-Mendelian genetics. In this unit you will study problems related to Mendelian and non-Mendelian genetics.

1. **Principles of Paired Factors:** According to this principle genetic characters are controlled by unit factors that exist in pairs in individual organism (diploid). The two factors lie on the two homologous chromosomes at the same locus. They may represent the same *i.e.,* homologous (e.g., TT for pure tall pea plants) or alternate expression *i.e.,* heterozygous (e.g., Tt for hybrid tall pea plants) of the same character. Exceptions of this postulate are haploids (n), polyploids (3n, 4n…) and hemizygous (XY) conditions thus cannot became a law.
2. **Principle of dominance:** If hybridization is made between contrasting traits of a character the F_1 generation shows only one trait is called dominant and another trait that cannot express it known as recessive. The law of dominance is not universally applicable. Exceptions: Codominance, Incomplete dominance, pseudodominance, overdominance.

   (a) Phenotypic and genotypic ratio in case of dominance (Monohybrid Cross):

   A Monohybrid cross is a type of genetic cross between two individuals with homozygous genotypes of a single character or trait, often resulting in an opposite phenotype.

   In a monohybrid cross; A particular character or trait is selected, and the alleles are indicated with certain alphabet characters. The dominant alleles are indicated with upper case letters, whereas the recessive alleles are indicated with lower case letters. The Punnet square is set up by listing the phenotype and genotype of the parents being crossed. The probable combination of the genotypes is written within the Punnet square. The phenotypic and genotypic ratios of the offsprings are determined and written down. The resulting combination is called the F1 generation.

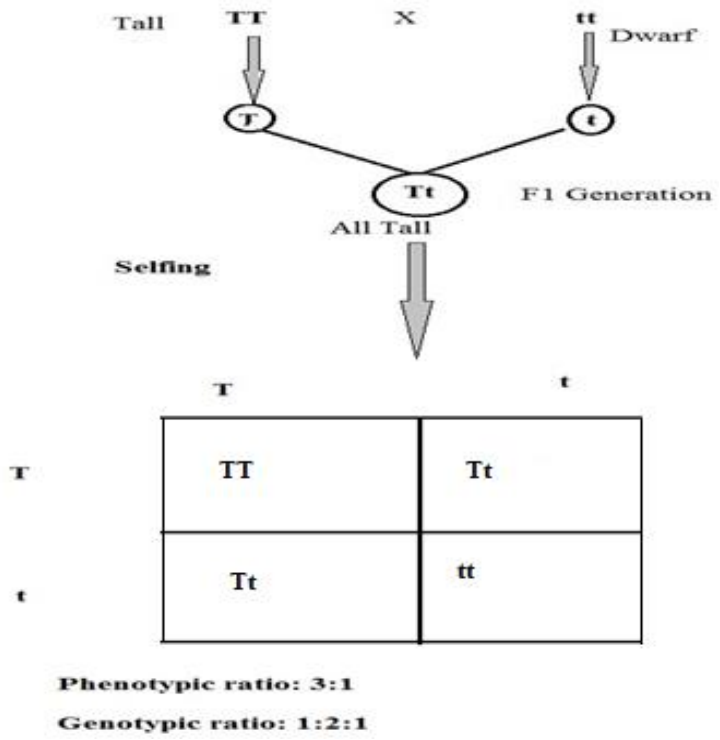


Fig.6.1: An experiment explaining principle of dominance in monohybrid cross

Test Cross Ratio

Phenotypic ratio = 3:1

Genotypic ratio = 1:2:1

(b) Phenotypic and genotypic ratio in case of dominance (Dihybrid Cross):

A dihybrid cross is crossing between two parents that differ in two traits. A hybrid organism is one that is heterozygous, which means that it carries two different alleles at a particular genetic position, or locus. In a dihybrid cross, the parents have different pairs of alleles for each trait under observation. One parent possesses homozygous dominant alleles and the other possesses homozygous recessive alleles. The offspring, or F_1 generation, produced from the genetic cross of such individuals are all heterozygous for the specific traits. This means that all of the F_1 individuals possess a hybrid genotype and express the dominant phenotypes for each trait.

Example: When a pureline round shape and yellow colored seed pea plant **(RRYY)** is crossed with pureline wrinkled shape and green colored seed **(rryy)**, the resulting offspring (**F_1 generation**) are all heterozygous for round shaped and yellow color seeds **(RrYy)**.

On **selfing** offspring (**F_2 generation**) exhibits a 9:3:3:1 phenotypic ratio with respect to variations of seed color and seed shape. This ratio can be predicted by using a Punnett square to reveal the possible outcomes of a genetic cross based on probability. In the F_2 generation, out of the plants **9** have yellow seeds with round shapes, **3** green seeds with round shape, **3** yellow seed color with wrinkled shape and **1** green seed with wrinkled shape. The F_2 progeny produces nine different genotypes with four different phenotypes. It is the inherited genotype that determines the phenotype of the individual. For example, plants with genotypes **(RRYY, RrYY, RRYy, or RrYy)** have round shapes with yellow seeds. Plants with genotypes **(rrYY or rrYy)** have wrinkled shapes and yellow seeds. Plants with genotypes **(RRyy or Rryy)** have round shapes and green seeds, while plants with the genotype **(rryy)** have wrinkled shapes and green seeds.

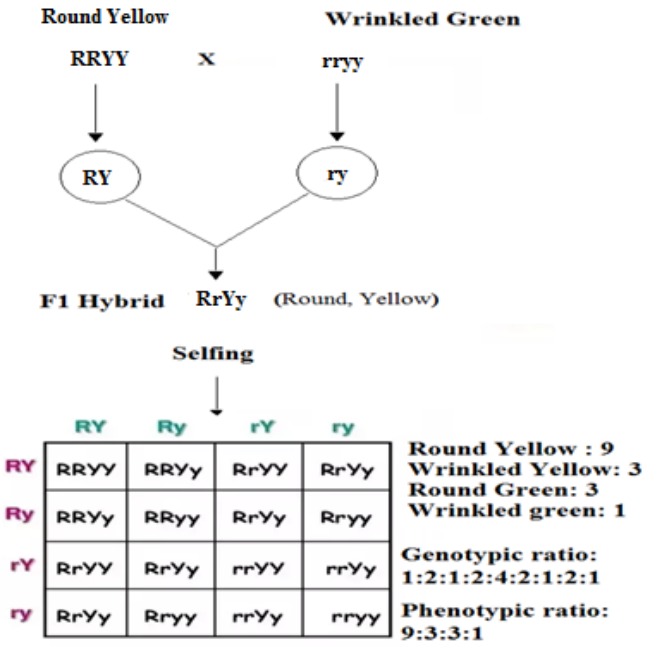


Fig.6.2: Dominance in dihybrid cross

Phenotypic ratio: $(3:1)^2 = (3:1) \times (3:1) = 9:3:3:1$

Genotypic ratio: 1:2:1:2:4:2:1:2:1

The law of dominance is not universally applicable, exceptions are codominance, incomplete dominance etc.

Codominance: Codominance is the genetic phenomenon in which both of the alleles of a gene express themselves in heterozygous condition *i.e.*, neither allele is dominant or recessive and

both get expressed. For example ABO blood groups in human, sickle-cell disease, and coat colour in cattles.

Example: (1) ABO Blood group type: People with this blood type have A and B proteins at the same time. The ABO gene determines what blood type a person has, and everyone has two copies of this gene, one from each parent. There are several combinations of blood types that can result, but when a person has both an A and a B allele, it will lead to blood types visible in the blood, AB.

Parent Alleles	A	B	O
A	AA (Blood group A)	AB (Blood group AB)	AO (Blood group A)
B	AB (Blood group AB)	BB (Blood group B)	BO (Blood group B)
O	AO (Blood group A)	BO (Blood group B)	OO (Blood group O)

Fig.6.3: Co-dominance in case of human blood group

Example: (2) Roan coat colour in Cattle: Cattle which are homozygous for a black coat allele (BB) are black and which are homozygous for a white coat allele (bb) are white, and heterozygous (Bb) cattle appear roan (black patches with white) due to codominance of the black and white coat color alleles (Bb).

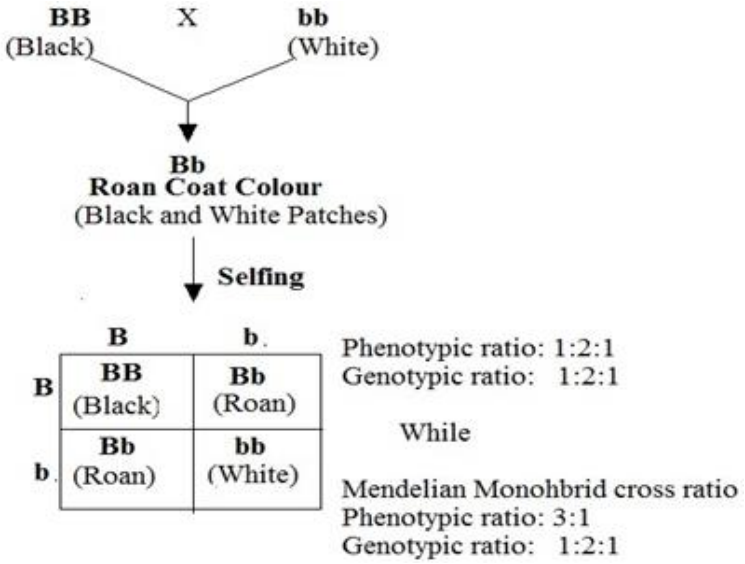


Fig.6.4: Co-dominance in case of coat colour in Cattle

Incomplete Dominance: When the phenotype of F_1 heterozygous genotype lies between the phenotypes of parental homozygous genotypes, is called **incomplete dominance or** partial dominance, or semidominance. Incomplete dominance is when a dominant allele, can not completely mask the effects of a recessive allele, and resulting phenotype shows appearance of blending of both alleles. The genotypic and phenotypic ratio (1:2:1) is same in this case. Eample flower colour in Snap dragon (*Antirrhinum majus*) and 4 O'clock (*Mirabilis jalapa*) plant.

Incomplete Dominance in Snapdragons: As an example, incomplete dominance is seen in red and white flowered snapdragon plants. When red flowered plant is crossed with white flowered plant pink flowered plants are obtained in F_1 generation. When the **F_1** generation consisting of all pink plants is allowed to cross-pollinate, the resulting plants **F_2** generation consist of all three phenotypes **1/4 Red (RR): 2/4 Pink (Rr): 1/4 White (rr)**. The phenotypic ratio is **1:2:1 which is equal to its genotype**. When **F_1** generation is allowed to cross-pollinate with true breeding red plants, the resulting **F_2** plants consist of red and pink phenotypes **1/2 Red (RR): 1/2 Pink (Rr) ratio**. The phenotypic ratio is **1:1**. When the **F_1** generation is allowed to cross-pollinate with true breeding white plants, the resulting **F_2** plants consist of white and pink phenotypes **1/2 White (rr): 1/2 Pink (Rr) ratio**. The phenotypic ratio is **1:1**.

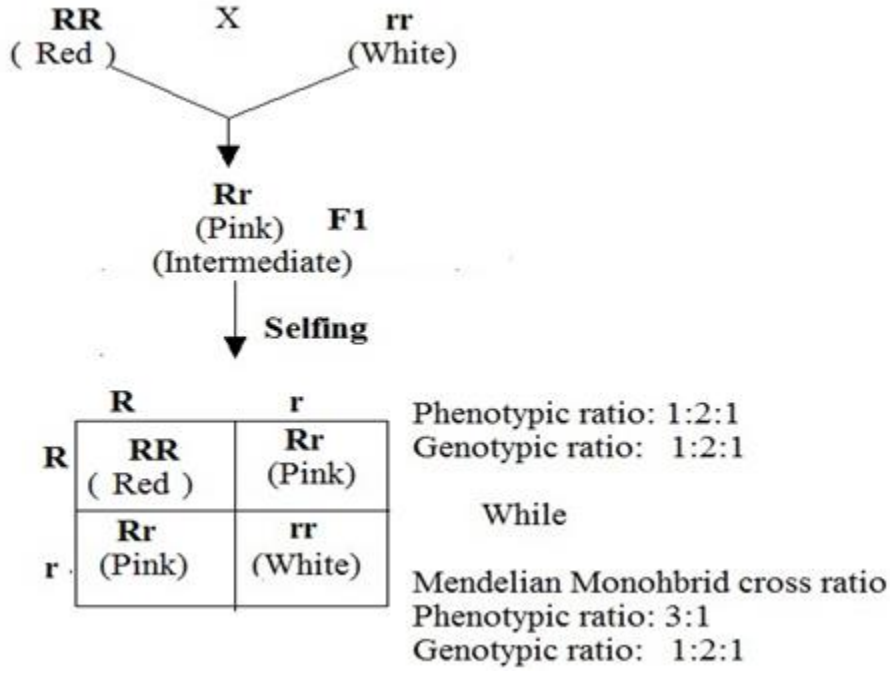


Fig.6.5: Incomplete Dominance in Snapdragons

3. Principle of segregation (Principle of purity of gametes): The Law of Segregation states that every individual organism contains two alleles for each trait, and that these alleles segregate (separate) during meiosis such that each gamete contains only one of the alleles. Alleles **segregate** during **gamete formation,** half of them carry one allele and half carry the other. An offspring thus receives a pair of alleles for a trait by inheriting homologous chromosomes from the parent organisms.

4. Principle of independent assortment: Mendel's **law of independent assortment** states that the alleles of two (or more) different genes assort independently of one another during the course of inheritance. In other words, the allele one gene does not influence the inheritance pattern of the allele of another gene. This is due to Independent behavior of genes at the time of crossing over because

1. They are free to change their position.
2. Random fusion of gametes.
3. Independent orientation of chromosomes during meiotic metaphase I.

For example: Mendelian dihybrid cross in garden Pea (*Pisum sativum*):

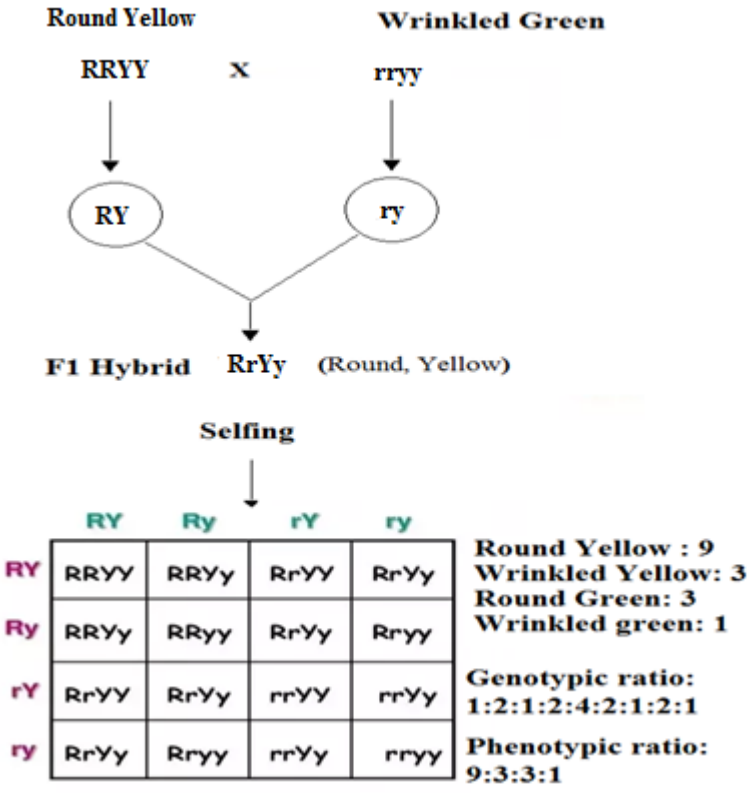


Fig.6.6: Independent assortment in dihybrid cross

Besides the Mendelian principles we should know about some genetic terminology.

Allele: An **allele** is an alternative form of a given gene (Fig.6.7). An allele that produces the same phenotype whether its paired allele is identical or different called dominant allele and represented by uppercase letter (T), an allele that produces its characteristic phenotype only when its paired allele is identical called recessive allele and represented by lowercase letter (t).

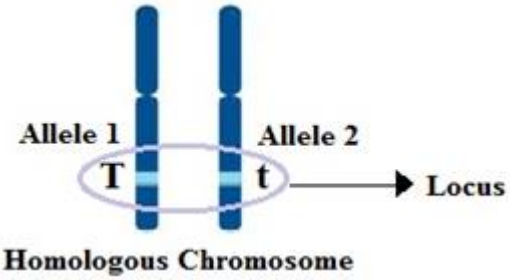


Fig.6.7: Allele on homologous chromosome

Multiple allelism: Three or more kinds of genes which occupy the same locus are referred to as multiple alleles and character governed by more than two alleles called multiple allelism.

Example 1: The ABO system in humans is controlled by three alleles, usually referred to as I^A, I^B, and I^O (I = isohaemagglutinin). I^A and I^B are codominant and produce type A and type B antigens, respectively, which migrate to the surface of red blood cells, while I^O is the recessive allele and produces no antigen. So, the blood groups arising from the different possible genotypes are as follows:

Genotype	*Blood Group*
$I^A I^A$	**A**
$I^A I^0$	**A**
$I^B I^B$	**B**
$I^B I^0$	**B**
$I^A I^B$	**AB**
$I^0 I^0$	**O**

Example 2: Wings of Drosophila: In Drosophila wings are normally long. Two mutations at the same locus cause vestigial (reduced) wings and antlered (less developed) wings. Both vestigial and antlered are alleles of the same wild gene and also of each other and are recessive to the normal gene. If vestigial wings represented by symbol vg, antlered wings by vg^a and the normal allele by + symbol then there are chances of following three types of wing formation in Drosophila:

(i) Long= ++ (+/+)

(ii) Vestigial= vg vg (vg/vg)

(iii) Antlered= vga vg^a (vg^a/vg^a)

Example 3: Coat colour in Rabbit: The coat colour in rabbits is determined by a series of multiple alleles. The normal coat colour is brown. Besides this albino (white) and Himalayan are the mutant races. The Himalayan race is similar to albino but has darker nose, ear, feet and tail. The allele of albino (a) and Himalayan (a^h) occupy the same locus. Both albino and Himalayan are recessive to their normal allele (+). A cross between an albino and Himalayan produces a Himalayan in the F_1 but not intermediate as is case of other multiple alleles.

Self-Sterility in Plants: Kolreuter (1764) described self-sterility in tobacco (*Nicotiana longiflora*). East and Yarnell described that self-sterility is due to series of alleles designated as s_1, s_2, s_3 and s_4 etc. The hybrids S_1/S_2 or S_1/S_3 or S_3/S_4 are self-sterile because pollen grains from these varieties did not develop, but pollens of S_1/S_2 were effective and capable of fertilization with S_3/S_4.

Homozygous and Heterozygous: Organisms can be homozygous or heterozygous for a gene (Fig. 8). **Homozygous** means that the organism has two copies of the same allele for a gene. An organism can be homozygous dominant, if it carries two copies of the same dominant allele (TT), or homozygous recessive, if it carries two copies of the same recessive allele (tt). **Heterozygous** means that an organism has two different alleles of a gene (Tt).

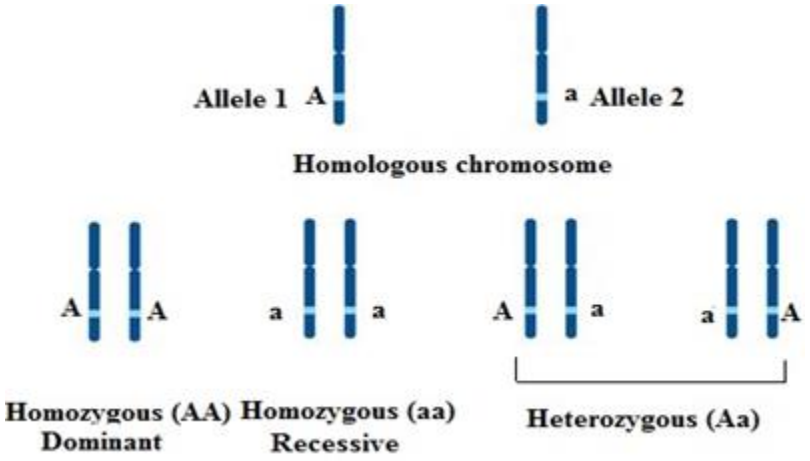


Fig.6.8: Homozygous and heterozygous conditions

Genotype and Phenotype: Genotype is genetic constitution of an organism *i.e.,* full hereditary information. Phenotype is an organism's actual observed properties means external appearance *i.e.,* morphology, development, or behavior. An organism's genotype is a major influencing factor in the development of its phenotype (morphology), but it is not the only one. Even two organisms with identical genotypes normally differ in their phenotypes. The phenotype is the product of genotype and environmental influence. The concept of phenotypic plasticity defines the degree to which an organism's phenotype is determined by its genotype. A high level of plasticity means that environmental factors have a strong influence on the particular phenotype development.

Back Cross, Out cross and test cross: When F_1 individuals are crossed with one of the two parents from which they have been derived, then such a cross is called back cross. Back cross are of two types:- out cross and test cross. When Tt (F_1) is crossed with TT (Homozygous dominant parental), it is called out cross. When Tt (F_1) is crossed with tt (Homozygous recessive parental), it is called test cross. A test cross is a way to explore the genotpye of F_1 individuals. Early use of the test cross was as an experimental mating test used to determine what alleles are present in the genotype. Consequently, a test cross can help to determine whether a dominant phenotype is homozygous or heterozygous for a specific allele.

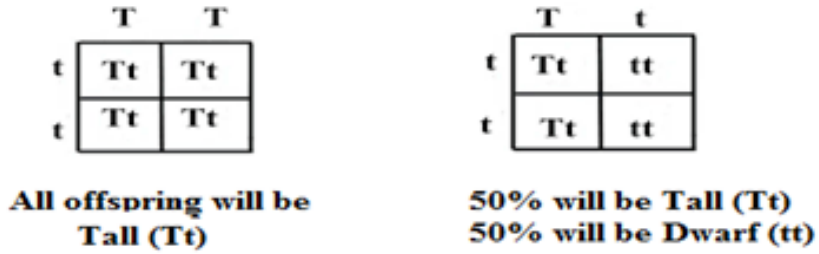


Fig.6.9: Test cross outcomes

Epistasis: Epistasis (Greek=standing upon) is the masking genetic phenomenon when the phenotypic effect of alleles at one gene masks or inhibits the expression of alleles of another gene. The term "epistatis" was first of all used by Bateson (1909). It is the interaction between non allelic genes in which one gene suppresses the expression of other gene. A gene is said to be epistatic when its presence suppresses the effect of a gene at another locus. But when two different genes which are not alleles, both affect the same character in such a way that the expression of one masks, inhibits or suppresses the expression of the other gene, it is called epistasis. The gene that suppresses is said to be epistatic, and the gene which remains obscure is hypostatic.

Monohybrid and dihybrid cross: A monohybrid cross is a breeding experiment conducted between parents, which differ in one specific trait only. Or in other words, the parents are heterozygous (having dissimilar alleles) at only one locus. Over here, one parent has a dominant gene for a specific phenotypic character (e.g. tall trait), while the other has recessive gene for the particular phenotype (*e.g.* dwarf trait).

Contrary to monohybrid cross, parents that differ in two traits breed in a dihybrid cross. To be more precise, the parental organisms are heterozygous for two different traits. In this case,

one parent has dominant genes for two characters (*e.g.* tall plant and red flowers) and the other parent has recessive genes for the same two characters (dwarf plant and white flowers) in the chromosome.

6.3 PROBLEMS BASED ON GENETICS

In this section you will study genetic problem in the form of gene interaction as non-Mendelian genetics. When expression of one gene depends on the presence or absence of another gene in an individual, it is known as gene interaction. The interaction of genes at different loci that affect the same character is called epistasis. The term epistasis was first used by Bateson in 1909 to describe two different genes which affect the same character, one of which masks the expression of other gene. The gene that masks another gene is called epistatic gene, and the gene whose expression is masked is termed as hypostatic gene. Epistasis is also referred to as inter-genic or inter-allelic gene interaction.

The gene interactions have several characteristics as follows:

i. This is an essential feature of gene interaction which always involves two or more genes.

ii. The epistatic genes always affect the expression of one and the same character of an individual.

iii. The phenotype of a gene usually depends upon the presence or absence of epistatic gene. The gene which has masking effect is called epistatic gene and the gene whose effect is masked is known as hypostatic gene.

iv. Epistasis leads to the modification of Mendelian di-hybrid (9:3:3:1) or tri-hybrid ratio (27:9:9:9:3:3:3:1) in F_2 generation.

v. Epistasis is generally governed by dominant genes, but cases of recessive epistasis are also shown.

Mendelian genetics does not explain all kinds of inheritance for which the phenotypic ratios in some cases are different from Mendelian ratios (3:1 for monohybrid, 9:3:3:1 for di-hybrid, 27:9:9:9:3:3:3:1for tri-hybrid in F_2). This is because sometimes a particular allele may be partially or equally dominant to the other or due to existence of more than two alleles or due to lethal alleles. These kinds of genetic interactions between the alleles of a single gene are referred to as allelic or intra- allelic interactions.

Non-allelic or inter-allelic interactions also occur where the development of single character is due to two or more genes affecting the expression of each other in various ways. Thus, the expression of gene is not independent of each other and dependent on the presence or absence of other gene or genes; These kinds of deviations from Mendelian one gene-one trait concept is known as factor hypothesis or gene-interaction. Now epistasis term is used synonymously with almost any type of gene interaction that involves the masking of one gene by another gene. When epistasis is operative (gene interacts) the phenotypic ratio deviates from Mendelian ratio (3:1 for monohybrid, 9:3:3:1 for di-hybrid and 27:9:9:9:3:3:3:1for tri-hybrid) in F_2 generation. The phenomenon of two or more gene affecting expression of each other in various ways in the development of single character of an organism known as gene interaction/epistasis.

Types of gene interactions/epistasis: The two or more genes interact in several manners some common interactions are as follows:

(1) Dominant epistasis (12:3:1)

(2) Recessive epistasis or supplementary gene interaction (9:3:4)

(3) Double recessive/complementary gene interaction (9:7)

(4) Inhibitory gene interaction/dominant recessive epistasis (13:3)

(5) Polymorphic gene interaction (9:6:1)

(6) Duplicate gene interaction/double dominant epistasis (15:1)

(7) Collaborative supplementary/modified gene interaction (9:3:3:1)

(8) Polymeric gene interaction (9:6:1)

1. Dominant epistasis (12:3:1): A genetic phenomenon of non allelic gene interaction in which a dominant gene or a dominant gene pair inhibits or masks the expression of another dominant gene or gene pair.

For example: fruit colour in summer squash

Three types of fruit colours are present in summer squash (*Cucurbita pepo*), *viz.,* white, and yellow and green. White colour is controlled by dominant gene W and yellow colour by dominant gene Y. This white colour gene is dominant over both yellow and green.

The green fruits are produced in recessive condition (wwyy). A cross between plants having white and yellow fruits produced F_1 with white fruits. Inter-mating of F_1 plants produces

plants with white, yellow and green coloured fruits in F_2 in 12 : 3 : 1 ratio (Fig. 10) instead of typical Mendelian dihybrid ratio (9:3:3:1).

W = white non allelic epistatic dominant gene.

Y = yellow hypostatic dominant gene.

y = green recessive gene.

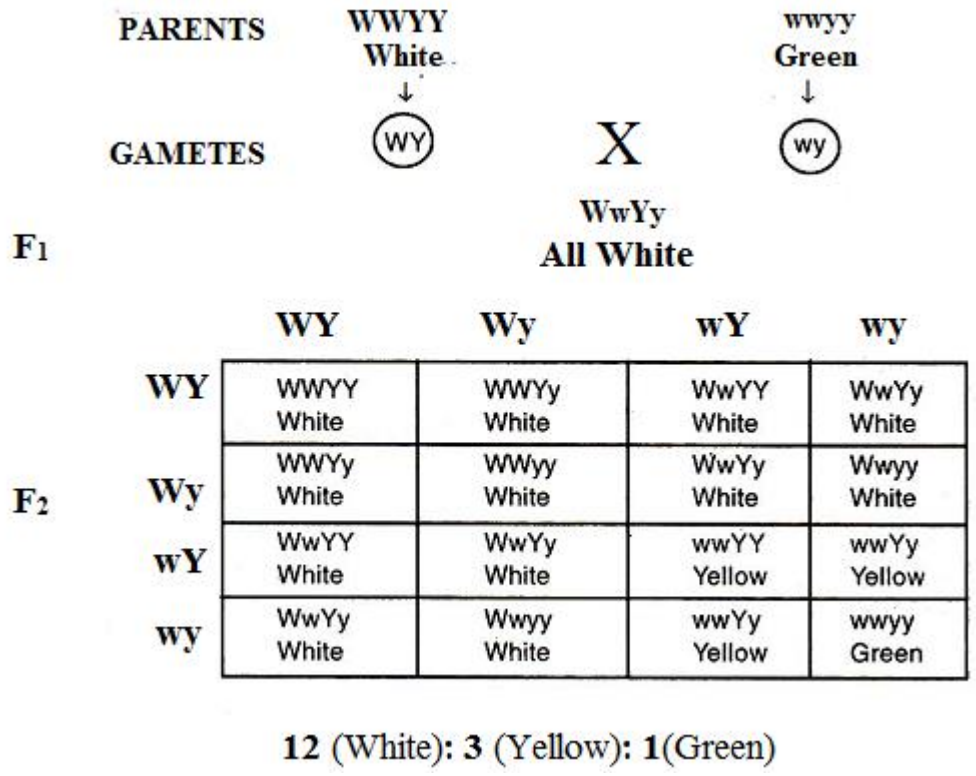


Fig.6.10: Dominant epistasis in summer squash (*Cucurbita pepo*)

2. Recessive epistasis or supplementary gene interaction (9:3:4): A genetic phenomenon of non allelic gene interaction in which a gene in its homozygous recessive state masks the expression of non allelic gene or gene pair.

In supplementary gene action, the dominant allele of one gene is essential for the development of the concerned phenotype, while the other gene modifies the expression of the first gene. For example, the development of grain colour in maize is governed by 2 dominant genes R and P. The dominant allele R is essential for red colour production; homozygous state of the recessive allele (rr) checks the production of red colour. The gene P is unable to produce any colour on its own but it modifies the colour produced by the gene R from red to purple. The recessive allele p has no effect on grain colour.

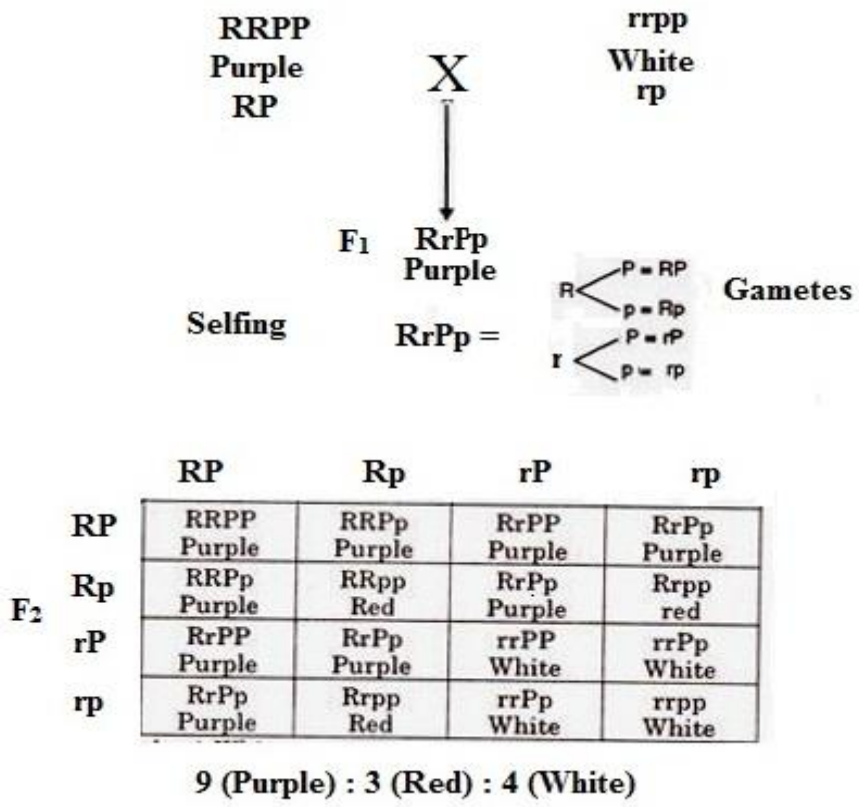


Fig.6.11: Recessive Epistasis or supplementary gene interaction in Maize Grain

3.Double recessive/complementary gene interaction (9:7): A genetic phenomenon of non allelic gene interaction in which homozygous recessive gene inhibits the expression of other gene and *vice versa*.

Or

A genetic phenomenon of non allelic gene interaction in which a gene or gene pair requires the help of dominant non allelic gene interaction. Complete dominance at both gene pairs, but either recessive homozygote is epistatic to the effect of the other gene.

For example: Flower colour in sweet pea (*Lathyrus odoratus*):

For the production of the purple flower colour both dominant C and P (complementary) genes are necessary. Otherwise in the absence of either dominant genes (C or P) the flower colour become white. Thus, C and P genes interact and both are essential for the purple colour expression of flower together. Complementation between two non-allelic genes (C and P) are essential for production of a particular or special phenotype *i.e.*, complementary factor.

P= Purple flower colour

C= Colourless

PPCC= Purple Colour flower

PPcc= Colourless

ppCC= Colourless

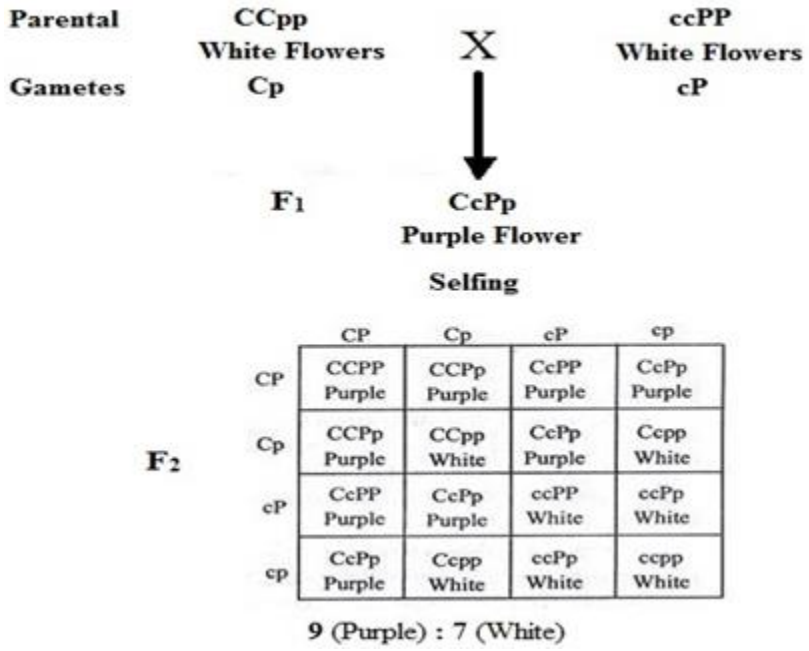


Fig.6.12: Double recessive/Complementary gene interaction in Sweat Pea (*Lathyrus odoratus*)

4. Inhibitory gene interaction (13:3): In **Inhibitory gene interaction** the non-allelic dominant gene inhibits the expression of the other non-allelic dominant gene. A genetical phenomenon in which a gene is recessive for its own but dominant with regard to epistasis. The gene which inhibits the expression of an allele situated at different locus is called as inhibitory gene.

For example: Pigmentation in paddy leaves.

In paddy plants P gene is responsible for deep purple colour. But if I gene is present along with P then expression of purple colour is inhibited and becomes green. Thus in a cross, between green (IIpp) and purple (iiPP), gives all F_1 offspring green but in F_2 progeny, green and purple are obtained in ratio of 13:3 instead of typical 9:3:3:1 Mendelian F_2 ratio.

PPii= Purple

PPII= Green

P= purple but when interacts with I gene than inhibited by it and becomes green.

I= Green but when interacts with P gene than inhibits P gene Make it green.

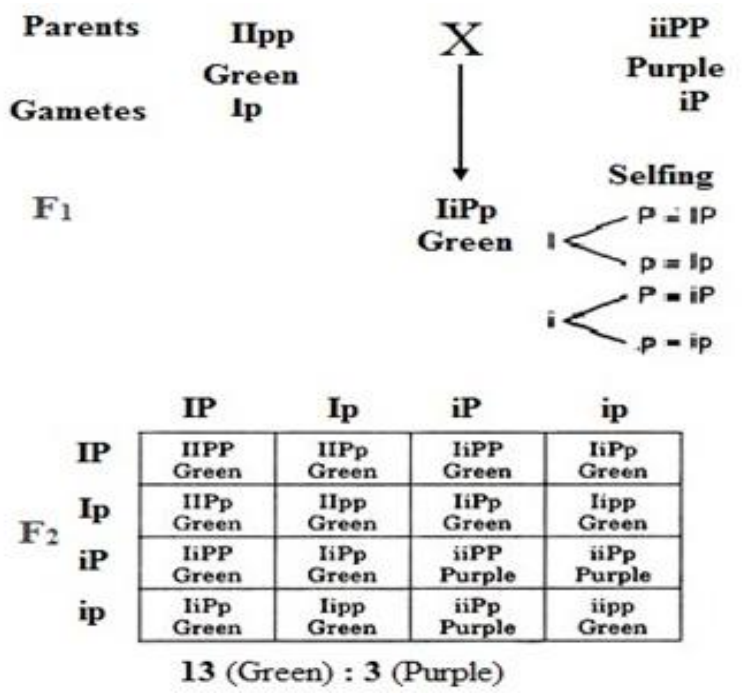


Fig.6.13: Inhibitory gene interaction paddy leaves pigmentation

6. Polymorphic gene interaction (9:6:1): When two or more genes (Allelic and non-allelic) govern any character separately, their effect is equal but when both or all genes are present together, there phenotypic effect is increased or raised as if the effects of the two or more genes were additive or cumulative. In this case both or all genes shows complete dominance. "Additive or cummulative effect of genes present at different loci is called polymerism." Thus, polymeric gene interaction modifies the typical 9:3:3:1 Mendelian ratio in to 9:6:1 ratio.

For example: pericarp colour in Wheat.

$C_1C_1C_2C_2$ = Deep Red

$C_1C_1c_2C_2$ = Light Red

$c_1c_1c_2c_2$ = Brown Colour

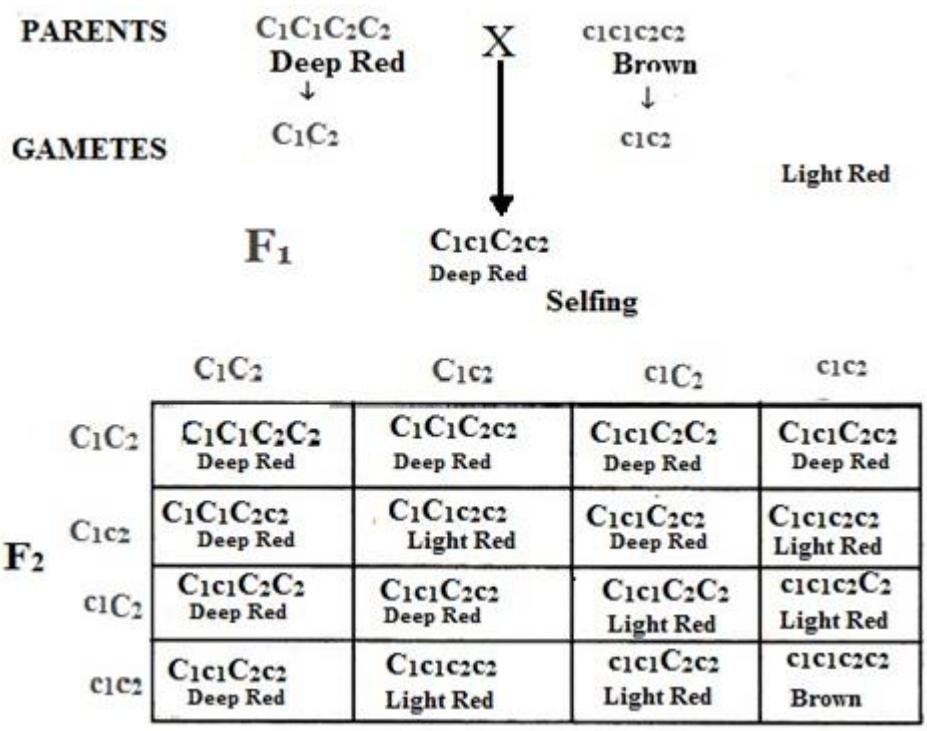


Fig.6.14: Polymorphic Gene interaction in wheat pericarp

7. Collaborative supplementary/modified gene interaction (9:3:3:1): A genetic phenomenon in which two non allelic dominant gene produces the different and independent effect but when they comes together they produces different/new effect.

They are two nonallelic genes which not only are able to produce their own effects independently when present in the dominant state but can also interact to form a new trait.

For example: Comb types in poultry are an example of collaborative supplementary genes, P and R. When homozygous pea combed and homozygous rose combed birds are crossed, all the offspring of F_1 generation have walnut comb. On selfing the walnut combed, F_2 generation comes to have all the four types of combs in the ratio of 9 (walnut): 3 (pea): 3 (rose): 1 (single) (Fig.6.15). This type of gene interaction produces the typical di-hybrid ratio of 9:3:3:1 in F_2 for a single character. Evidently the concerned character is governed by two genes showing complete dominance.

As worked out by Bateson and Punnett (1908), when both dominant alleles are present 'walnut' phenotype appears and when both recessive alleles are present 'single' comb appears. 'Rose' and 'Pea' phenotypes appear due to the presence of different single dominant alleles. If

pea (rrPP) and rose (RRpp) are crossed, F_1 birds showed 'walnut' comb as it has the dominant alleles of both the genes P and R.

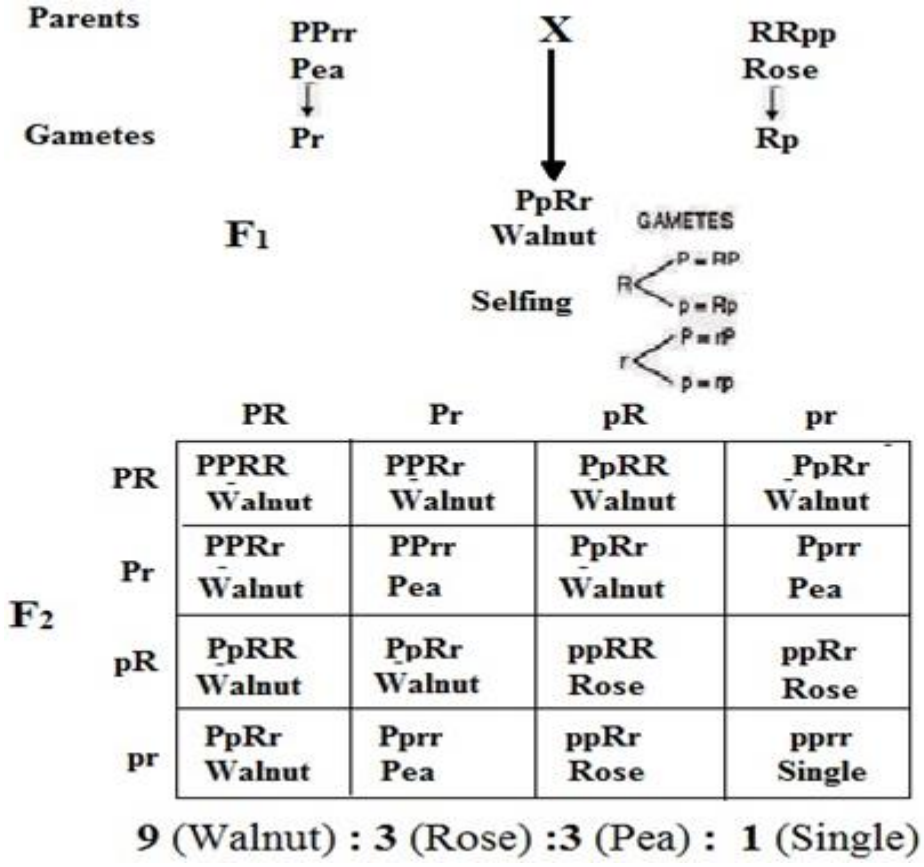


Fig.6.15: Collaborative supplementary/Modified gene interaction in comb types in poultry

6.4 SUMMARY

1. Genetics is the branch of science that deals with the study of heredity and variations of inherited characteristics.
2. Heredity and variations are controlled by genes.
3. Each diploid (2n) organism has a pair of similar chromosomes (one maternal and one paternal) *i.e.*, diploid (2n) organism generally have two copies of each gene which govern a character.
4. In the 1866, an Austrian monk Gregor John Mendel postulated theory of inheritance based on his experimental work on garden pea (*Pisum sativum*) and concluded that heredity is the result of discrete units of inheritance called factors.

5. Genetics which follows Mendels laws is known as Mendelian or classical genetics and which doesnot follow Mendelian laws is known as non-Mendalian genetics.
6. According to Mendelian principle genetic characters are controlled by unit factors that exist in pairs in individual organism (diploid).
7. Mendel postulated four principles: 1. Principle of paired factors 2.Principle of dominance 3.Principle of segregation/Principle of purity of gametes 4. Principle of independent assortment.
8. If hybridization is made between contrasting traits of a character the F_1 generation shows only one trait and is called dominant and one trait that cannot express it is known as recessive.
9. Codominance is the genetic phenomenon in which both of the alleles of a gene express themselves in heterozygous condition.
10. When the phenotype of F_1 heterozygous genotype lies between the phenotypes of parental homozygous genotypes, it is called incomplete dominance.
11. The Law of Segregation states that every individual organism contains two alleles for each trait, and that these alleles segregate (separate) during meiosis such that each gamete contains only one of the alleles.
12. Mendel's law of independent assortment states that the alleles of two (or more) different genes assort independently of one another during the course of inheritance.
13. An **allele** is an alternative form of a given gene.
14. Three or more kinds of genes which occupy the same locus are referred to as multiple alleles and character governed by more than two allele called multiple allelism.
15. Organisms can be homozygous or heterozygous for a gene.
16. **Homozygous** means that the organism has two copies of the same allele for a gene and heterozygous organism has two copies of the different allele for a gene.
17. Genotype is genetic constitution of an organism *i.e.*, full hereditary information. Phenotype is an organism's actual observed properties means external appearance
18. **Epistasis** (Greek=standing upon) is the masking genetic phenomenon when the phenotypic effect of alleles at one gene masks or inhibits the expression of alleles of another gene.
19. The term “epistatis” was first of all used by Bateson (1909).

20. When F_1 individuals are crossed with one of the two parents from which they have been derived, then such a cross is called back cross.
21. Back cross can be of two types: **Out cross and test cross.**
22. When Tt (F_1) is crossed with TT (Homozygous dominant parental), it is called **out cross**.
23. When Tt (F_1) is crossed with tt (Homozygous recessive parental), it is called **test cross**.
24. A test cross is a way to explore the genotpye of F_1 individuals.
25. Non-allelic or inter-allelic interactions also occur where the development of single character is due to two or more genes affecting the expression of each other in various ways called gene interaction.
26. The two or more genes interact in several manners some common interactions are as follows:
    (1) Dominant epistasis (12:3:1)
    (2) Recessive epistasis or supplementary gene interaction (9:3:4)
    (3) Double recessive/complementary gene interaction (9:7)
    (4) Inhibitory gene interaction/dominant recessive epistasis (13:3)
    (5) Polymorphic gene interaction (9:6:1)
    (6) Duplicate gene interaction/double dominant epistasis (15:1)
    (7) Collaborative supplementary/modified gene interaction (9:3:3:1)
    (8) Polymeric gene interaction (9:6:1)

6.5 GLOSSARY

Allele: Alternative form of a gene is called allele.

Back Cross: Backcross is a cross of F_1 hybrid with one of its parents.

Dihybrid cross: A **dihybrid cross** describes a mating experiment between two organisms that are identically hybrid for two traits.

Dominance: An allele or a gene that is expressed in an organism's phenotype, masking the effect of the recessive allele or gene when present.

Epistasis: Epistasis is the interaction between non allelic genes that influences a phenotype.

F_1= The first filial generation of offspring.

F_2= The second filial generation of offspring.

Gene: A unit of heredity which is transferred from a parent to offspring.

Genetics: The scientific study of heredity.

Genotype: Genetic constitution of an organism.

Hemizygous: A condition individual having only one of a given pair of genes.

Heredity: The phenomenon of passing of traits genetically from one generation to another.

Homologous: Having the same or allelic genes with genetic loci usually arranged in the same order homologous chromosomes.

Hybrid: The offspring resulting from the cross between parents of different species.

Monohybrid: A monohybrid cross is a breeding experiment conducted between parents, which differ in one specific trait only.

Offspring: Young born of organisms, produced through reproduction.

Out cross: A backcross of F1 offspring with dominant parent.

Phenotype: The physical or external appearance of an organism as a result of the interaction of its genotype and the environment.

Progeny: A genetic descendant or offspring, Collective offspring progeny.

Pureline: A population having a particular feature that has unchanged through many generations. The organisms are homozygous and are said to **Pureline** or true-breed.

Selfing (Syn= Inbreeding): The union of male and female gametes from same haploid, diploid, or polyploid organism.

Test cross: A backcross of F_1 offspring with recessive parent.

6.6 SELF ASSESSMENT QUESTION

6.6.1 Multiple Choice Questions:

1. Who is called father of genetics:

(a) Mendel (b) Bateson

(c) Punnet (d) Morgan

2. Experimental material of Mendel was:

(a) Pigeon Pea (b) Garden Pea

(c) Sweat Pea (d) None of above

3. ABO blood group type in humans is an example of:

(a) Dominance (b) Codominance

(c) Incomplete dominance (d) Overdominance

4. Typical Mendelian dihybrid ratio in F_2 generation is:

(a) 9:3:4 (b) 12:3:1

(c) 9:3:3:1 (d) 15:1

5. Typical Mendelian dihybrid ratio in F_2 generation is:

(a) 9:3:3:1 (b) 27:9:9:9:3:3:3:1

(c) 9:3:4 (d) 27:9:9:3:3:3:1

6. If hybridization is made between contrasting traits of a character the F1 generation shows only one trait this genetic phenomenon is called.

(a) Epistasis (b) Dominance

(c) Test cross (d) Backcross

7. Alternative form of gene is called:

(a) Phenotype (b) Phenocopy

(c) Allele (d) Genocopy

8. When F_1 individuals are crossed with one of the two parents from which they have been derived, then such a cross is called.

(a) Back cross (b) Test Cross

(c) Out cross (d) All of the above

9. An organism hving two copies of the same allele for a gene is called:

(a) Homozygous (b) Heterozygous

(c) Hemizygous (d) Hybrid

10. When F_1 individuals are crossed with recessive parents then such a cross is called.:

(a) Out cross (b) Reciprocal Cross

(c) Dihybrid cross (d) Test Cross

11. The polymeric gene interaction modifies the typical 9:3:3:1 Mendelian F_2 ratio into:

(a) 9:6:1 (b) 9:3:4

(c) 15:1 (d) 12:3:1

12. A genetic phenomenon of non allelic gene interaction in which a dominant gene or a dominant gene pair inhibits or masks the expression of another dominant gene or gene pair is called

(a) Dominant epistasis (b) Recessive epistasis

(c) Dominance (d) Overdominance

13. The term "epistatis" was first of all used by:

(a) Mendal (b) Bateson

(c) Punnet (d) Kolreuter

14. Self sterility in tobacco is the example of genetic phenomenon.

(a) Epistasis (b) Multiple allelism

(c) Dominance (d) All

15. In case of Double recessive/complementary gene interaction the F_2 ratio becomes:

(a) 12:3:1 (b) 9:6:1

(c) 9:7 (d) 15:1

6.6.2 Fill in the blanks:

(1) The term epistasis was given by _____.

(2) _____is the genetic phenomenon in which both of the alleles of a gene express themselves in heterozygous condition.

(3) The interaction of genes at different loci that affect the same character is called _____.

(4) _____ condition means that an organism has two different alleles of a gene.

(5) Thipolymeric gene interaction modifies the typical 9:3:3:1 Mendelian ratio in to _____.

(6) In _____gene interaction the non-allelic dominant gene inhibits the expression of the other non-allelic dominant gene.

(7) When the phenotype of F_1 heterozygous genotype lies between the phenotypes of parental homozygous genotypes, is called _____dominance.

(8) When F_1 individuals are crossed with one of the two parents from which they have been derived, such a cross is called _____cross.

(9) Alternative form of a gene is called_____.

(10) When F_1 individual is crossed with recessive parent, then such a cross is called _____cross.

6.6.3 True or False:

(1) When F_1 individuals are crossed with one of the two parents from which they have been derived, then such a cross is called test cross.

(2) Mendal coined the term epistasis.

(3) In Epistasiss one allele inhibits the expression of another allele of same gene.

(4) Dominance is allelic while epistasis is non allelic genetic phenomenon.

(5) Self sterility gene is an example of multiple allelism.

(6) A backcross of F_1 offspring with dominant parent is called test cross.

(7) Principle of paired factor was not followed by haploid and polyploidy organisms.

(8) Roan Coat colour in cattle is an example of codominance.

(9) A genetic phenomenon of non allelic gene interaction in which a gene in its homozygous recessive state masks the expression of non allelic gene or gene pair is called recessive epistasis.

(10) Genetic constitution of an organism is called phenotype.

6.6.4 Very short answer questions:

(1) Define genetics.

(2) Who is called father of genetics?

(3) Who coined the term epistasis?

(4) Define allele.

(5) Define dominance.

(6) What is the typical trihybrid ratio in F_2 generation Mendelian inheritance?

(7) What is recessive epistasis?

(8) Describe inheritance pattern of comb in fowl?

(9) What is the F_2 ratio in case of supplementary gene interaction?

(10) Define test cross.

6.6.1 Answer key: 1-(a), 2-(b), 3-(b), 4-(c), 5-(b), 6-(b), 7-(c), 8-(a), 9-(a), 10-(d), 11-(a), 12-(a), 13-(b), 14-(b), 15-(c).

6.6.2 Answer key: 1-Bateson(1909), 2- Codominance, 3- Epistasis, 4- Heterozygous 5- 9:6:1, 6- Inhibitory, 7-Incomplete, 8- Back, 9- Allele, 10- Test.

6.6.3 Answer key: 1-False, 2-False, 3-False, 4-True, 5-True, 6-False, 7-True, 8-True, 9-True, 10-False.

6.7 REFERENCES

- Bateson, W. 1909. Heredity and variation in modern lights. In Darwin and modern science (eds. A. C. Seward), 85-101. Cambridge University Press, Cambridge.
- Bateson, W. and Punnett, R.C. 1908. Experimental studics in the physiology of heredity. Reports to the Evol. Comm. Roy. Soc. Rpt. 4, Poultry, pp. 18-35.
- East, E.M. and Yarnell, S.H. 1929. Studies on self-sterility. VIII. Self-sterility allelomorphs. Genetics 14:455-487.
- Kölreuter, J.G. (1761-1766), Vorläufige Nachricht von inigen das Geschlecht der Pflanzen betreffenden Versuchen und Beobachtungen, nebst Fortsetzungen 1, 2 und 3, Leipzig: in der Gleditschischen Handlung.
- Mendel, G. 1866. Versuche über Plflanzenhybriden. Verhand-lungen des naturforschenden Vereines in Brünn, Bd. IV für das Jahr 1865, Abhandlungen, 3–47. (http://www.esp.org/foundations/genetics/classical/gm-65.pdf).
- Punnett, R. C. 1923 Heredity in poultry. London: Macmillan and Co.

6.8 SUGGESTED READINGS

- Brooker, R. 2015. Genetics: Analysis and Principles (5th Eds.). McGraw-Hill Publishing Company.
- Gardner, E.J., Simmons, M.J. and Snustad, D.P. 2006. Principles of Genetics (8th Ed.). Wiley.

- Klug, W.S., Cummings, M.R., Spencer C. A., Palladino, M.A., Killian, D.2019. Concepts of Genetics (12th Ed.). Pearson.
- Klug, W.S., Cummings, M.R., Spencer C. A., Palladino.2019. Concepts of Genetics (10th Ed.). Pearson.
- Pierce, B. A. 2017.Genetics: A Conceptual Approach (6th Ed.). W.H. Freeman.
- Singh, B.D. 2014. Fundamentals of Genetics. Kalyani Publshers, India.
- Singh, B.D. 2016. Genetics (2nd Ed.). Kalyani Publishers, India.

6.9 TERMINAL QUESTIONS

6.9.1 Short answer type questions:

1. What do you understand by Epistasis?
2. Define dominant epistais with suitable example(s).
3. Differentiate between epistasis and dominance.
4. What is the recessive epistsis, define with example?
5. Define monohybrid and dihybrid cross and what is the typical Mendelian mono- and dihybrid cross ratio in F_2 generation?
6. Describe collaborative gene interaction with sitable examples.
7. Define backcross, testcross and out cross.
8. Define co-dominance with suitable examples.
9. What do you understand by gene interaction describe in brief.
10 Describe complementary gene interaction with example.

6.9.2 Long answer type questions:

1. Bateson and Punnett performed an experiment on sweat pea and concluded that when two white flowered varieties were crossed, F_1 hybrids were all purple coloured but in F_2 generation it segregated in to purple and white colour. Give the parental genotype and describe the type of gene interaction.

2. In a genetic experiment when purple pigmented paddy plant was crossed with green pigmented plant, the F_1 appeared green pigmented. In F_2 the segregation of two types of

pigmentation was as: purple pigment 90, green Pigment 410.What may be the genetic basis for the segregation of pigmentation in F_2. Describe the type of gene interaction in this experiment

3. In summer squash white (W) colour is epistatic to that of an yellow (Y) which is dominant over green colour gene. Find out the fruit colour progeny if following crosses performed and describe the type of gene interaction.

1- WwYy x Wwyy and 2- wwYY x Wwyy.

4. In Fowl dominant gene R gives rose comb and the dominant gene P gives pea comb. When P and R are present together gives walnut. The homozygous recessives of P and R produce single comb. What will be the comb type in the following crosses and describe the type of gene interactin in this case:-

A. RrPp x RrPp
B. rrPP x RrPp
C. Rrpp x RrPp
D. rrPp x RRPp
E. rrpp x Rrpp

5. Define non-Mendelian inheritance. Describe about different kinds of gene interaction with suitable examples.

UNIT-7-IDENTIFICATION OF INDIAN VARIETIES OF IMPORTANT CROPS

7.1 Objectives

7.2 Introduction

7.3 Identification of Indian varieties of important crop

7.3.1 Techniques used in identification of Indian varieties of important crops:

7.3.1.1 Karyotyping

7.3.1.2 Chromosome banding

7.3.1.3 Fluorescent *in situ* hybridization (FISH)

7.3.1.4 Comparative genomic hybridization

7.3.2 Crop Variety

7.3.2.1 Rice

7.3.2.2 Wheat

7.3.2.3 Cotton

7.3.2.4 Sugarcane

7.3.2.5 Tea

7.3.2.6 Coffee

7.4 Summary

7.5 Glossary

7.6 Self Assessment Questions

7.7 References

7.8 Suggested Readings

7.9 Terminal Questions

7.1 OBJECTIVES

To identify the Indian varieties of important crops.

7.2 INTRODUCTION

India produces many crops in the world and most of its population is relied on the crop and agriculture industry. On the basis of seasons, crops are divided into Rabi, Kharif and Zaid. Kharif crops are sown in June-July during monsoon period and harvested in September-october. rice, jowar, bajra, maize, cotton, groundnut, jute, sugercane, turmeric and pulses are the common examples. They need abundant water and hot weather to grow. Rabi crops are sown in October-November and harvested in April-May. Wheat, oat, gram, pea, barley, potato, tomato, onion, oil seeds (mustard, sunflower, sesame, and rapeseed), etc., are some examples. These crops need warm weather to germinate and mature seed but cold weather to grow. Zaid crops are grown in between March-June. They are also called early maturing crops. Cucumbers, Bitter Gourd, Pumpkim, Watermelon, muskmelon, Moong Dal, etc., are some examples.

Also, major crops in India are divided into 4 another categories naming, Food crops which include rice, what, maize, millets and pulses, etc; Cash crops including sugarcane, tobacco, cotton, jute, oilseeds, etc; Plantation crops which has coffee, coconut, tea, rubber, etc; and horticulture crops which contains fruits and vegetables.

Many varieties of these crops have been developed in India by various methods like Plant Breeding. Plant Breeding is a discipline of scientific principle of plant sciences, genetics and cytogenetic. Plant breeding aims to develop genetically superior plants by using and understanding genetics in terms of economic importance for the mankind.

7.3. Identification of Indian Varieties of Important Crops

7.3.1. Technique used in identification of Indian varieties of important crops:

Due to advancement in the field of genetics and plant breeding, there are many approaches to develop new varieties of plants. These varieties belong to the same species and hence do share many morphological and phenotypic similarities. In order to distinguish them from one another, reliable method of identification is needed. Karyotyping, chromosome banding, fluorescent *in situ* hybridization (FISH) and comparative genomic hybridization (CGH) are such techniques.

7.3.1.1. Karyotyping (Fig 7.1)

1. It is the process which involves standard procedures of staining which reveals chromosome characteristic features.
2. It is the process of pairing and chromosome ordering.
3. It can detect coarse genetic changes or changes in chromosome numbers.
4. It is based on the principle of enzymatic digestion of young leaf tissues i.e., shoot tips.
5. The process starts with short term culturing of the specimen derived cells.
6. After sufficient growth and multiplication of the culture, cells are arrested in metaphase by adding colchicines.
7. The colchicine is used in poisoning the mitotic spindles.
8. In the metaphase, chromosomes are evenly and well-distributed which allows accurate counting.
9. Next, cells are treated by hypotonic solution which causes the swelling of nuclei and bursting of the cell.
10. A chemical fixative is added to nuclei and it is then treated with the various stains which reveal structural features of the chromosomes.
11. Karyotypic differentiation is more prominent in the case of root tips than the shoot tips because of longer and less condensed chromosomes in the root tip.
12. It reveals each chromosome as a specifc, unique and constant pattern of alternating dark and light banding regions.
13. While studying the somatic cells of the *Tritium erectum* with the cold treatment method, some chromosomes regions revealed the unique patterns of thin and less intensely stained when compared to the rest of the chromosomes.

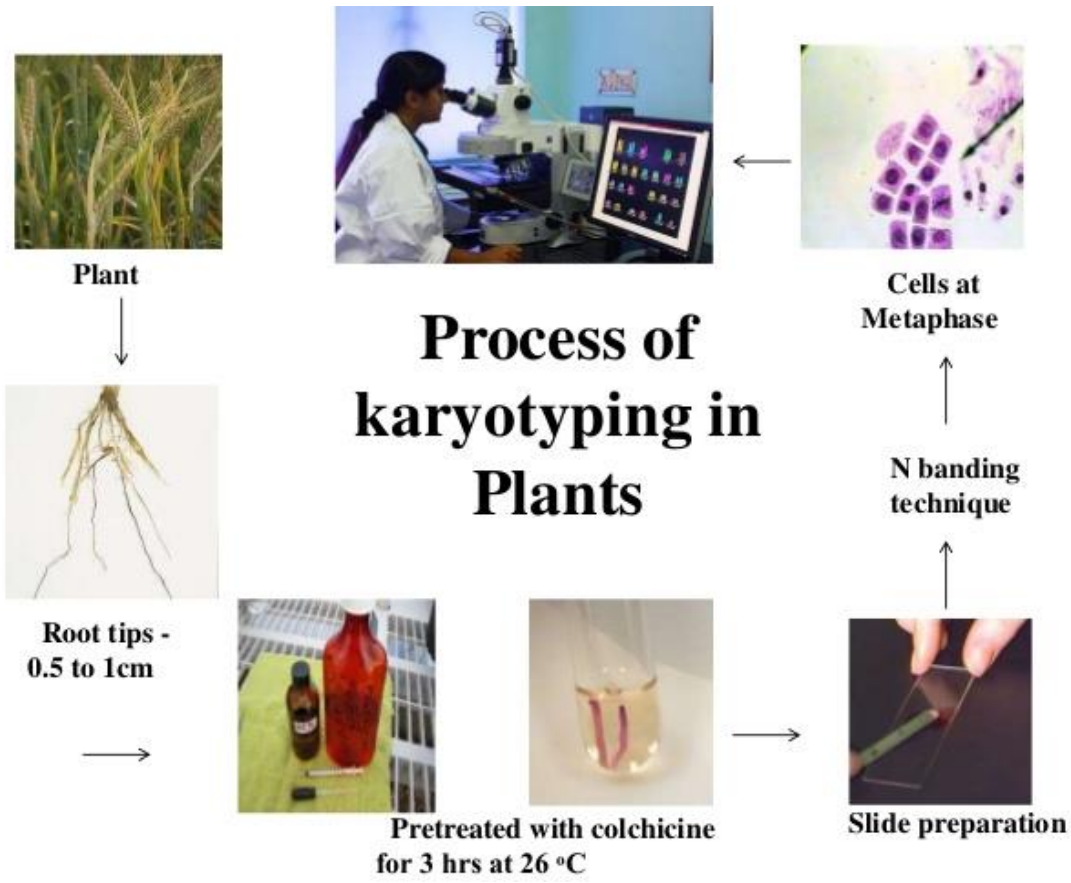


Fig.7.1: The process of Karyotyping in plants (https://www.slideshare.net/shruthibelliappa155/ppt-on-karyotyping-chromosome-banding-and-chromosome-painting).

7.3.1.2. Chromosome Banding

Chromosome banding is referring to the pattern observed comprising of light and dark stain on staining. The bands distinguish various locations in the chromosome. The dark and light bands refer to the adjacent segments present in the chromosome which helps in genomic study and evaluation.

Uses

1. To identify different regions in chromosomes.
2. To differentiate between euchromatin and heterochromatin.
3. To identify abnormal chromosome.
4. To understand various clinical features of chromosomes.
5. To identify different genes and their function.
6. To understand the molecular basis of any genetic disease.
7. To devise strategy to stabilize the unnatural changes in chromosome.

Types of Banding

(i) Q Banding (Fig 7.2 & 7.3)

1. The fluorescent dyes like quinacrine and quinacrine mustard bind preferentially to some certain mitotic chromosomes region.
2. These fluorescent dyes interact with AT base pairs and hence staining is more in the case of AT rich regions.
3. AT rich regions appear as bright bands called the Q bands.
4. These bands help in identifying all the chromosomes and their homologues in most of the species.
5. It does not require any pre-treatment and is the simplest banding method.
6. This staining method can be used in *Cricetulus griseus, Vicia faba* and *Triticum erectum*.
7. It reveals the unique patterns of brightly fluorescent (light) regions alternate to non-fluorescent (dark) regions which were produced in each chromosome.
8. Although, fluorescent bands are not permanent, and for visualization, ultra violet light is needed.
9. This method does stain the chromosome ends and hence its use is considered to limited extent.
10. Q banding is commonly used in *Triticum*, *Scilla*, *Allium*, *Crepis*, *Lilium*, *Secale* and *Vicia*.

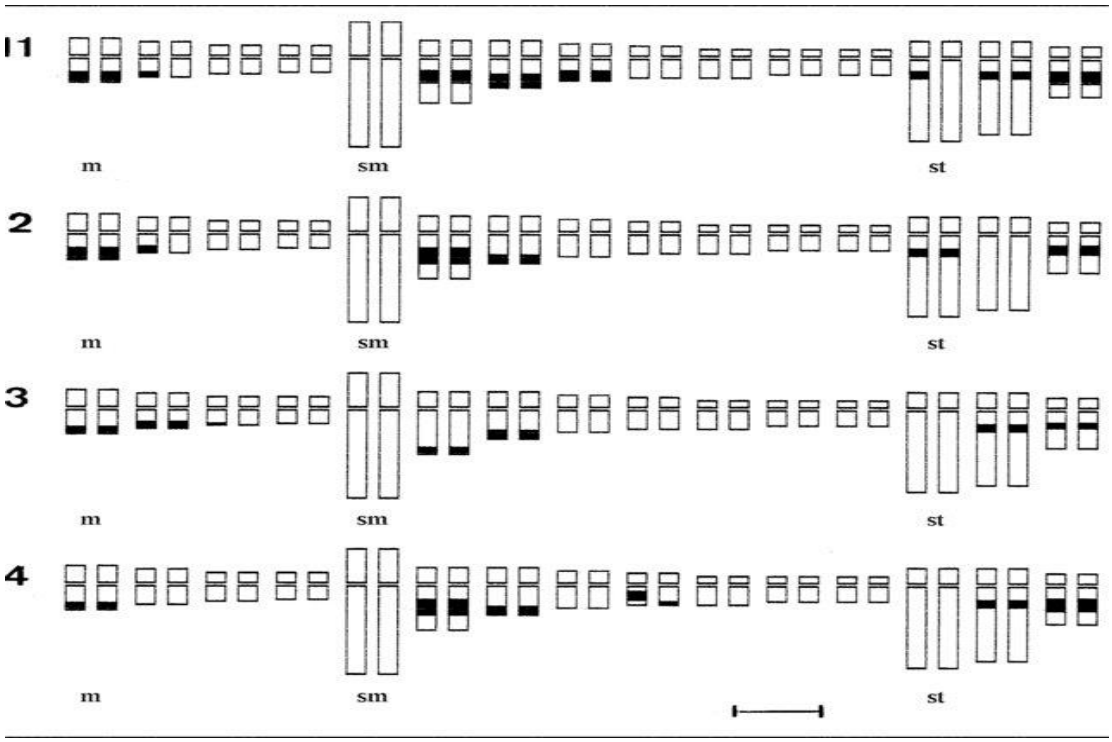


Fig.7.2: Q banding patterns in plant chromosome (Dematteis et al., 2006).

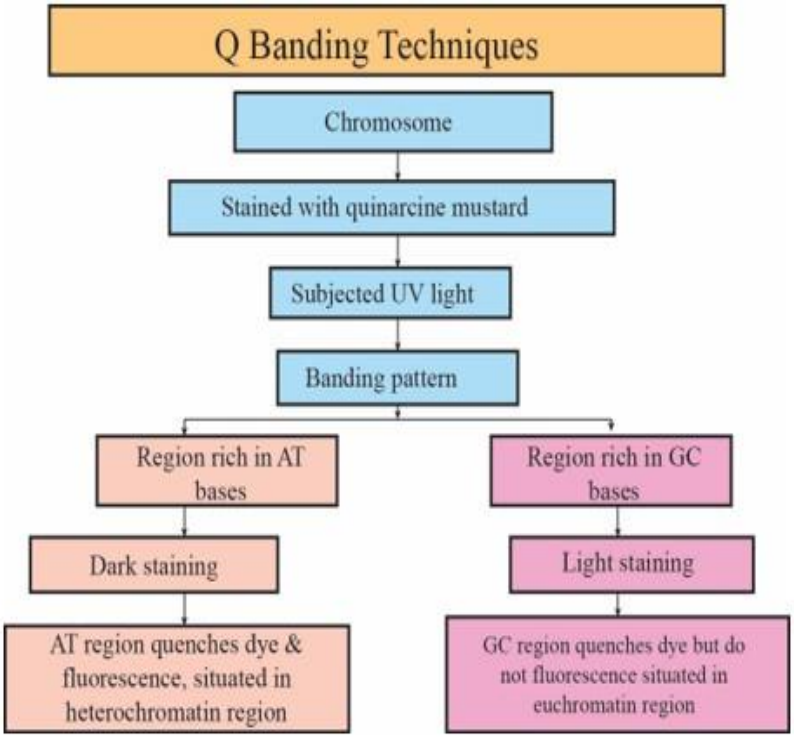


Fig.7.3: Q banding technique in plant chromosome (https://www.chegg.com /learn/biology/introduction -to-biology/banding-pattern).

(ii) G Banding (Gustav Giemsa) (Fig 7.4)

1. Thus technique also reveals each chromosome segment and a unique pattern of bands by the chromosome.
2. The Giemsa stain is used along with trypsin, urease as protease for staining and it yields the banding pattern of normal mitotic chromosomes.
3. The dark regions formed are similar to Q bands and are called G bands.
4. The light regions are similar to the non-fluorescent dark bands which rose by the use of fluorescent dyes.
5. A large number of bands of chromosome are produced in prophase and pro-metaphase.
6. The prophase and metaphase chromosomes contain a basic chromomeric structure which is enhanced due to which G bands are produced.
7. This enhancement is caused by inducing some rearrangement of fibers away from the light bands toward the G bands.
8. In plants, there are few species present which G bands are generated like *Tulipa gesneriana*, *Pinus resinosa* and *Vicia hajsatana*.
9. Tribe like Triticeae fails to produce G bands in the chromosome due to the increased condensation of the plant chromosomes.
10. Improper pre-treatment of plant chromosomes cause alteration in the organization of their chemical constituents which make unresponsive to the procedure of G banding.

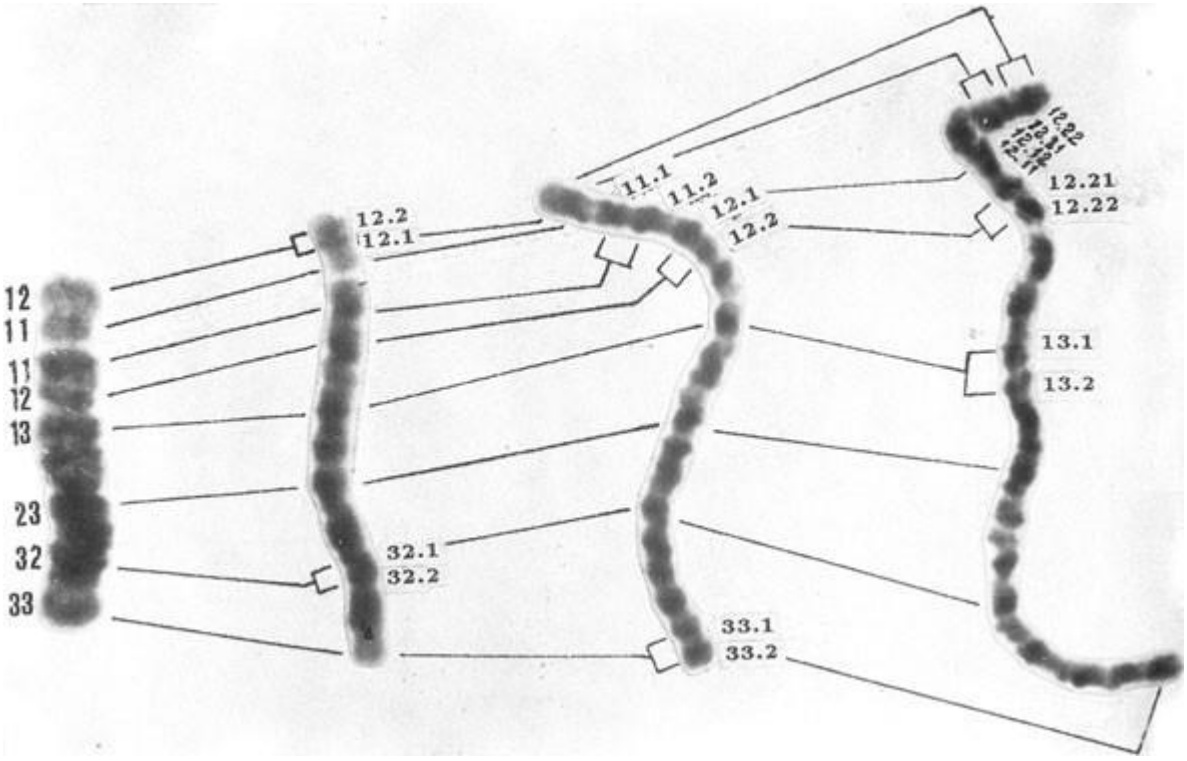


Fig.7.4: G banding chromosome patterns in plants (Chen et al., 1994).

(iii) R Banding

1. R stands for Reverse in R banding.
2. This technique was developed by Dutrillaux and Lejeune in the year 1971.
3. A reverse pattern from the G and Q banding methods is produced by mild denaturation by heat and subsequent staining of chromosomes with Giemsa or a fluorochrome.
4. R bands are produced by GC specific fluorochromes.
5. Dark R bands are produced if chromosomes are stained by Giemsa.
6. The dark R bands produced are similar to light bands produced by G banding technique.
7. When fluorochrome dye like acridine orange or olivomycin is used, bands produced are the reverse of Q banding technique. The R bands are of fluoresce bright green and the faint red color indicates non-R bands.
8. This method is used in detecting structural rearrangements which involve ends of chromosomes or Telomeres stained as T bands.
9. R bands are produced by some plant species only such as *Scilla siberica*, *Vicia faba*, *Alliums* pp.

10. The R banding is produced when DNA and proteins in the G and R bands are denatured selectively under different pH conditions, salt concentrations and temperature.

(iv) C Banding (Fig 7.5)

1. The chromosomes are stained with Giemsa and $Ba(OH)_2$ in the constitutive heterochromatin regions.
2. These regions are called as C bands which are present proximal to the centromeres of all the chromosomes.
3. Constitutive heterochromatin resemble with the satellite DNA, consisting of short, highly repeated base pairs sequences in tandem repeats among one or more regions of nearly all chromosomes of most of the species.
4. The location of C bands is at various sites and also next to centromeres of each chromosomes and next to secondary chromosome constrictions
5. C banding technique is limited as it does not allow recognition of individual chromosomes with accuracy and precision.
6. The C bands are present in many species of *Aegilops*, *Agropyron*, *Elymus*, *Hordeum*, *Secale*, *Triticum*, etc.
7. Some plant species like *Allium crinatum*, *Horden* spp. and *Agropygron elonga* do not reveal C bands next to the centromeres of their chromosomes.
8. Due to this individual chromosomes in the somatic cells can be identified by their C banding patterns.
9. Through this technique, 21 chromosomes are identified in the genome of Chinese Spring and Norim 61 of *T. aestivum*.
10. This method is used in detecting aneuploids translocation and other structural rearrangements and precise physical mapping of genes in the chromosomes.
11. The A and B genomes of *T. turgidum* have been identified which are considered as inconsistent.

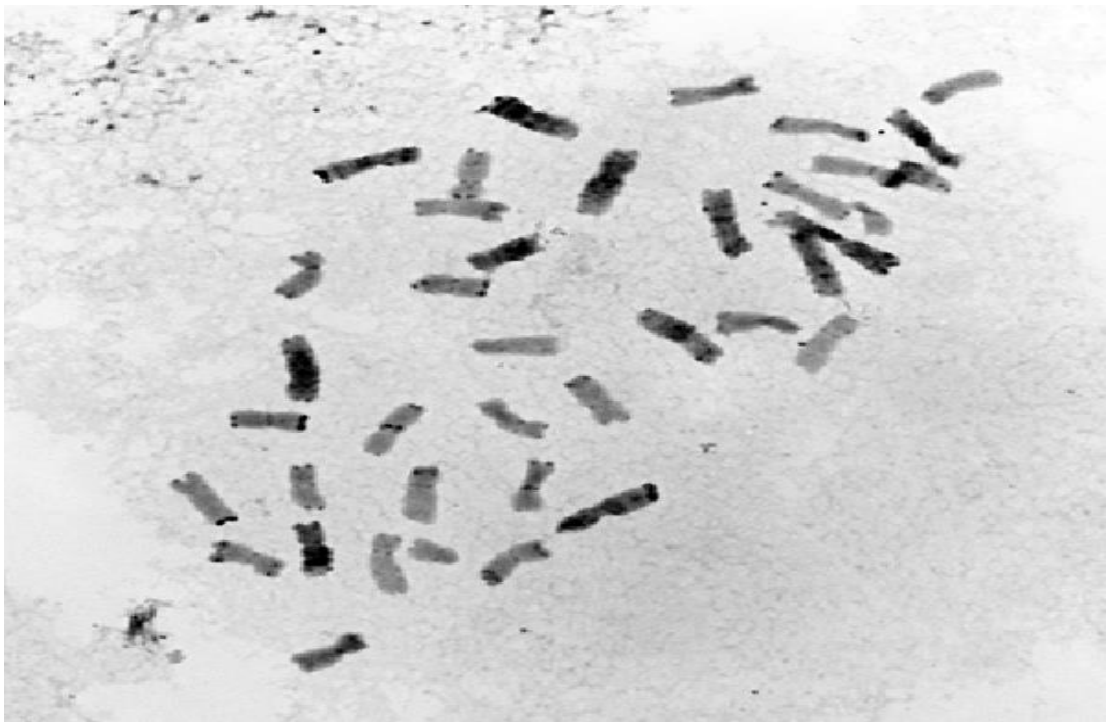


Fig.7.5: C banding patterns in plant chromosome (Jellen, 2016).

(v) N Banding (Fig 7.6)

1. This technique was developed by Matsui and Sasaki in 1973.
2. It stains the NORs in the chromosomes in the mammalian species which are different from the N bands revealed in plant species.
3. The presence of N bands in plant species has some examples such as *Triticum* and *Aegilops* species.
4. 21 chromosomes of common wheat have been identified by N banding patterns.
5. Also, improved N banding protocol revealed the 16 of the 21 chromosomes of common wheat which includes five in the A genome.
6. 14 chromosomes of *Aegilops variabilis* are identified by N banding patterns.
7. The chromosomes have also been identified in barley, rye, lentils and *Elymus* spp. using this technique.
8. This method can identify various types of aneuploids, alien additions and substitution lines and translocation and deletions.
9. Some N bands are unknown and hence cannot be detected by this method as in *T. aestivum*.

10. Many N bands are known to occupy the same position as that of C bands and hence it can be concluded that 2 classes of heterochromatin occur in wheat, rye and other species.
11. Some regions of heterochromatin stains positive for both C and N banding. These are known as C+N bands.
12. C+N bands possess multiple copies of the $(GAA)_n$ $(GAG)_n$ sequences in DNA.

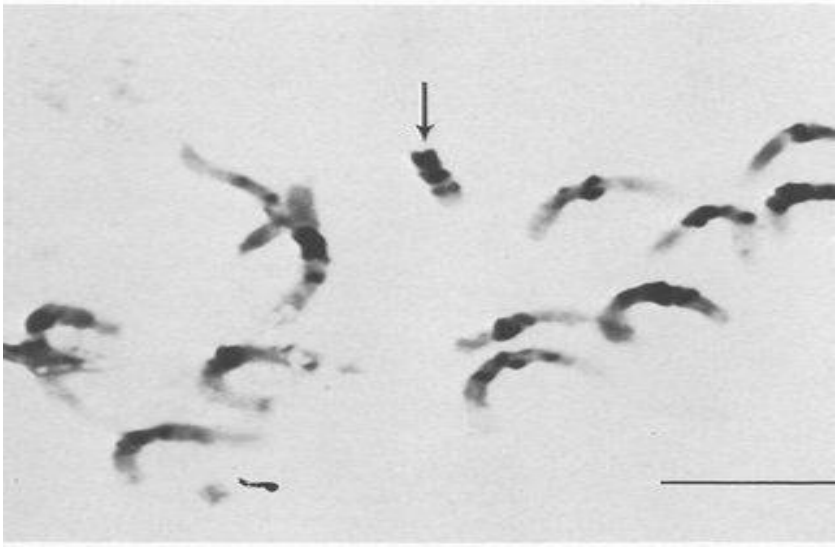


Fig.7.6: N banding patterns in Barley (Singh & Tsuchiya, 1982).

(vi) Analysis (Fig 7.7)

1. A unique banding pattern of chromosome is revealed by this method.
2. It is more reliable staining method for the identification of chromosomes.
3. Five major banding techniques naming, Q, G, R, C and N provide precise cytogenetic and phylogenetic analysis.

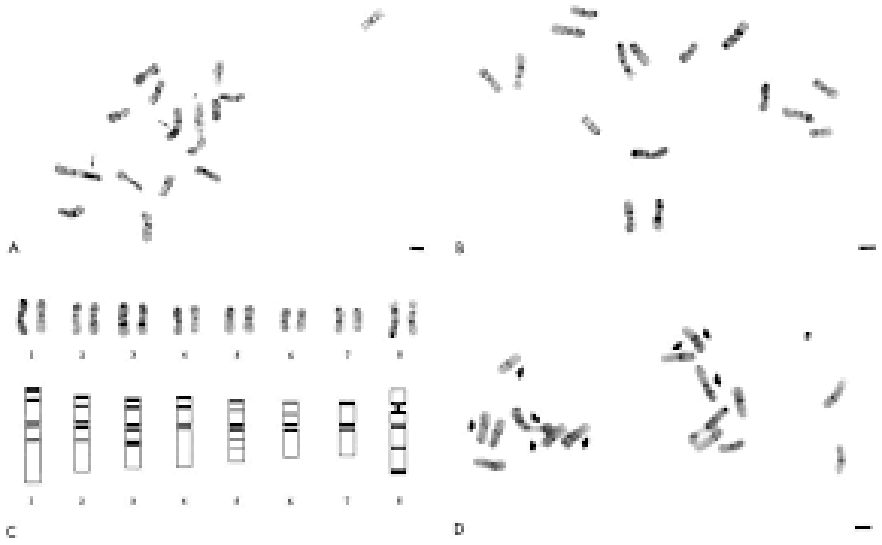


Fig.7.7: Banded Chromosome Patterns of *M. sativa* (Bauchan & Hossain, 1997).

7.3.1.3. Fluorescent *in situ* hybridization (FISH) (Fig 7.8)

1. FISH based karyotyping is a powerful cytogenetic tool to study chromosome organization, behavior and evolution.
2. A probe mixture of centromeric and sub-telomeric satellite repeats, 5 S rDNA and chromosome specific BAC clones is used in this technique.
3. This probe mixture helps in distinguishing all pairs of chromosome like 11 chromosome pairs of the common bean.
4. The gene pools can be used in distinctly identifying several wild relatives and landraces of common bean and other *Phaseolus* species.
5. The genetic evolution of plant can be studied by this method e.g. genus *Phaseolus*.
6. The study in *Phaseolus* genus has revealed that the chromosomal distribution of the centromeric and subtelomeric satellite repeats is stable in common beans but copy number of the repeats was variable.
7. Such variation suggests rapid amplification/reduction of the repeats in specific genomic regions.
8. Although, copy numbers of centromeric repeats can be largely reduced or diverged due to changes in chromosomal distributions due to rapid evolution of centromeric repeats.
9. Due to similar problem, the varied distribution pattern of subtelomeric repeats is also observed.

10. The satellites revealed in FISH-based karyotyping system are active, rapidly evolving, forming genomic features unique to individual common bean accessions and *Phaseolus* species.
11. Similarly, studies have been performed in maize, soybean, and *Brassica* species by using repetitive DNA probes.
12. This method has proven to be very useful in distinguishing the individual chromosomes, and to identify variation in chromosome structure and repeat distributions, both between and within species.

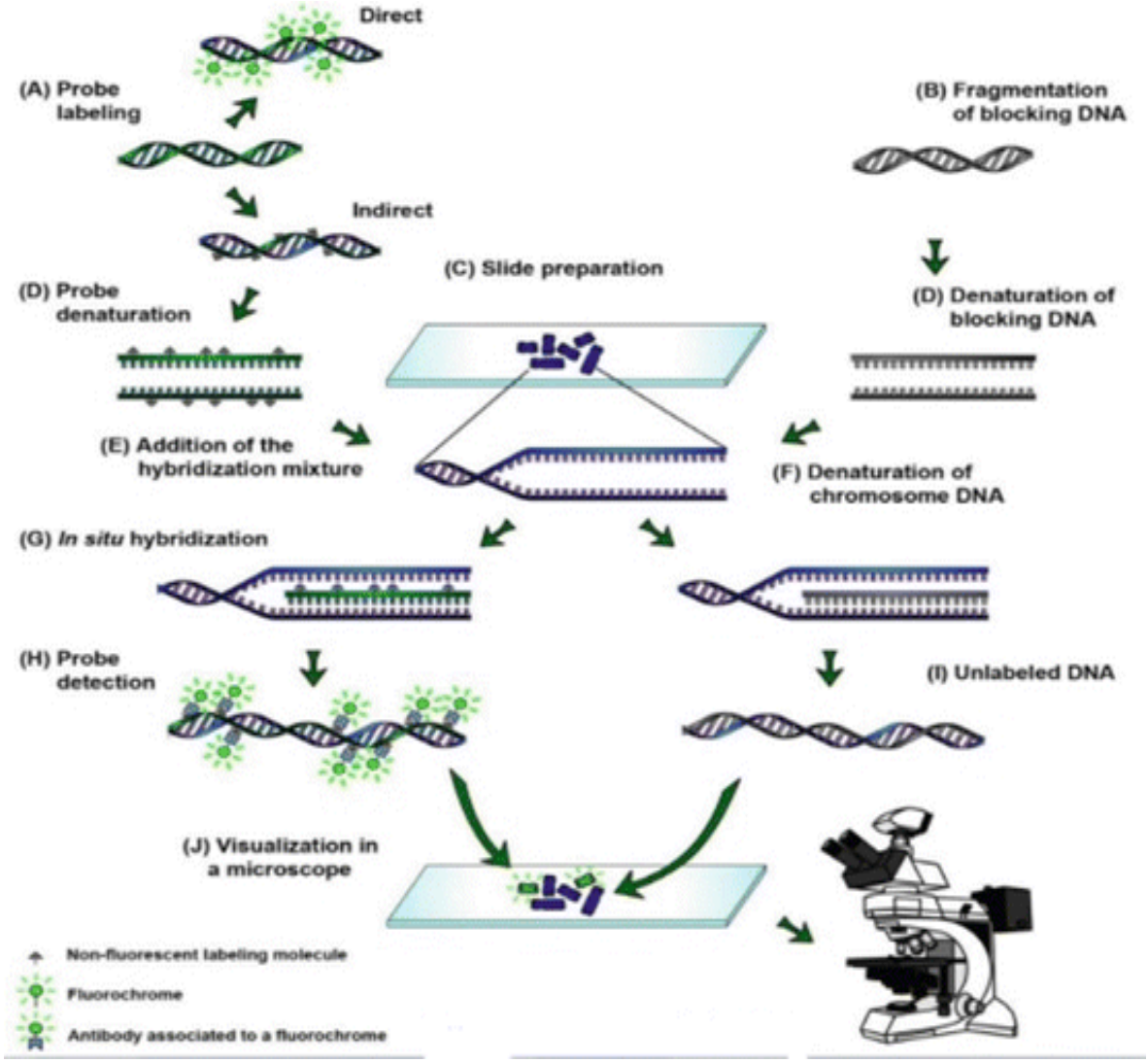


Fig.7.2: The Procedure of Fluorescent in situ hybridization (FISH) (https://www.creative-diagnostics.com/in-situ-hybridization-and-fluorescence-in-situ-hybridization.htm**)**

7.3.1.4. Comparative genomic hybridization (CGH) (Fig 7.9)

1. It is an efficient approach to study entire genomes for variations in DNA copy numbers.
2. Firstly, the total genomic DNA is isolated for the test and reference cell populations.
3. The differential labeling allows following the experimental protocol easily.
4. The DNA is hybridized to allow the binding of sequences at different locations to be easily distinguished.
5. Number of genomes can be compared simultaneously if labels used are suitable.
6. The inclusion of unlabeled Cot-1 DNA in the reaction causes suppression of hybridization of highly repetitive sequences.
7. The physical position is mapped on the chromosomes by using metaphase chromosomes for representing the genome and the location of copy number variations between the test and the reference genomic DNA.
8. DNA microarrays containing elements are directly mapped to the genome sequences.
9. There is a proportional relationship between the relative hybridization intensity of the test and reference signals at a given location with the relative copy number of those sequences in the test and reference genomes.
10. This method can be used to determine the actual copy number associated with a ratio level.
11. Several techniques like ligation-mediated polymerase chain reaction (PCR), degenerate primer PCR using one or several sets of primers and rolling circle amplification are used to amplify the starting material of genomes.
12. For hybridization, cDNAs, selected PCR products and oligonucleotides made by array CGH can be used.
13. The reduced complexity representations of the genome produced by PCR techniques are employed by hybridization with total genomic DNA.
14. Computational analysis of the genome sequence design the array elements which are complementary to the sequences contained in the representation.

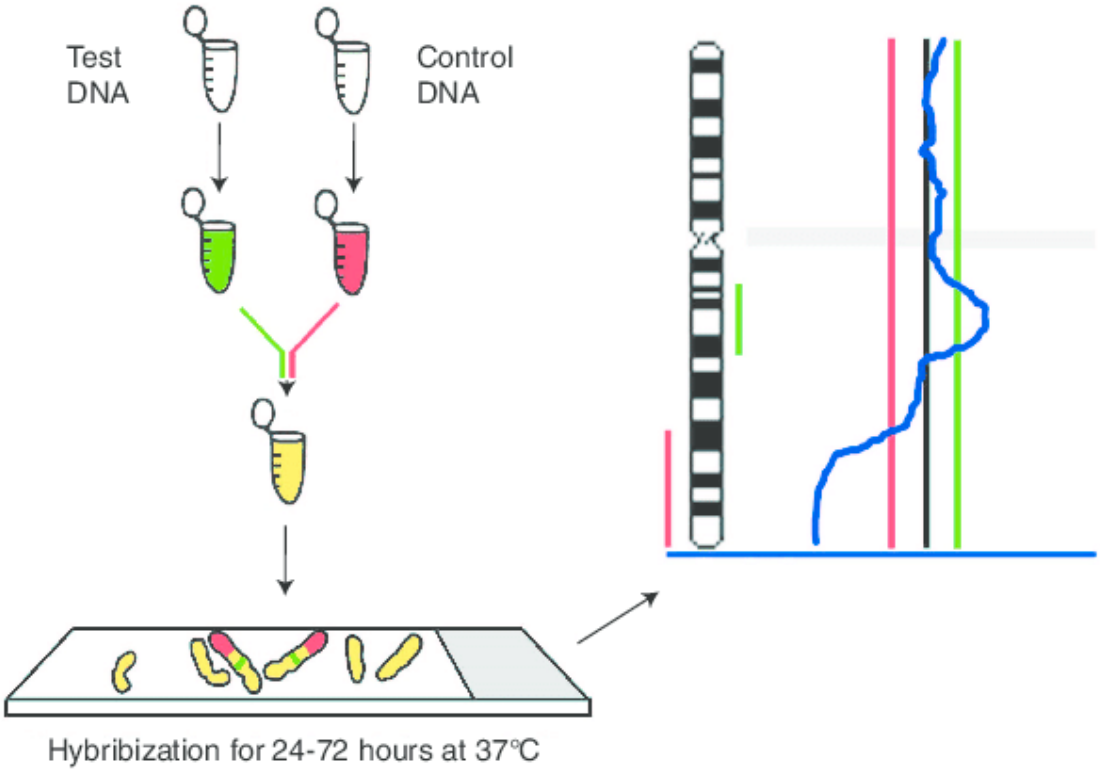


Fig.7.9: Comparative genomic hybridization (CGH) (Dorritie et al., 2004).

7.3.2. Crop Varieties

India is known for its agriculture importance. Many crop varieties, especially after green revolution, have been developed in India. India produces both for its citizens and for the world. Crops like wheat, rice, sugarcane, tea, coffee, cotton, etc. are the important part of Indian agriculture and they are also important from the economic point of view.

7.3.2.1. Rice Varieties

It is one of the most important crops grown in country and is grown throughout the year. It needs atmospheric moisture and rainfall for the irrigation. The rice fields are known as paddy fields and they are flooded by water up to 10-12 cm deep in early stages. It is a predominantly tropical, Kharif crop.

Scientific Classification:

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Monocots

Clade: Commelinids

Order: Poales

Family: Poaceae

Genus: *Oryza*

Habit and Habitat: The tropical climate is optimum for rice varieties to grow. They have the self-supporting growth form which grows in freshwater habitat. The soil can be clay or loamy. The optimum temperature is 24ºC. It covers the one third area of whole India's land. West Bengal, Punjab and Uttar Pradesh are the top producer states of the India. Other states include Tamil Nadu, Andhra Pradesh, Jharkhand, Bihar, Uttarakhand, Chhattisgarh, Odisha, Karnataka, Assam, Maharashtra, Haryana, Gujarat, Madhya Pradesh, Kerala and Kashmir Valley.

Crop Varieties:

1. Inter racial hybridization between *japonicas* and *indicas* give rise to more than 700 hybrid combinations.
2. The *indica* varieties are known for local condition adaptations and tolerance towards diseases and pests whereas *japonicas* are known for high yielding capacity and response.
3. ADT-27 is developed in Tamil Nadu in India.
4. Inter-racial hybridization between semi-dwarf Taiwanese types/derivatives led to the development of Taichung (Native) – I.
5. The two famous varieties developed by this inter racial hybridization are Padma and Jaya.
6. These developed varieties have got high yield potential.
7. The total 123 + 51 varieties were released first from this hybridization.
8. These semi-dwarf varieties are superior as they are efficient grain producer than the tall traditional varieties.

7.3.2.2. Wheat Varieties

It is a Rabi crop and is the 2nd largest crop produced by India in the whole world. T is used as staple food in north and north western India. It is a winter crop as it needs low temperature to grow. The states of Uttar Pradesh, Punjab and Haryana are the top producers of wheat in the country.

Scientific Classification:

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Monocots

Clade: Commelinids

Order: Poales

Family: Poaceae

Subfamily: Pooideae

Supertribe: Triticodae

Tribe: Triticeae

Genus: *Triticum*

Habit and Habitat: The ideal temperature range for wheat to grow is 10-15ºC for sowing and 21-26ºC for harvesting. The rainfall should be less than 100 cm but more than the 75 cm for wheat to thrive. Well drained fertile loamy soil and clayey soil is best for wheat to grow. They are mostly grown in plain areas. The most common habitats are fields, roadsides, areas along railroads, area near grain elevators and open waste areas.

Crop Varieties:

1. Wheat breeding in India was achieved by systematic manner in 1905 at the Imperial Agricultural Research Institute, Pusa, and was developed Pusa4, Pusa6 and Pusa 12.
2. The main Indian varieties of Wheat are VL-832, VL-804, HS-365, HS-240, HD2687, WH-147, PBW-343, WH-896 (d), PDW-233 (d), UP-2338, PBW-502, Shresth (HD 2687), Aditya (HD 2781), HW-1085, NP-200 (di), HW-741.
3. Wheat varieties like PbC518, PbC591, C273, C281 and C286 were developed by pure line selection in indigenous material at Lyallpur, Kanpur, Sabour, Powarkheda, Niphad and Pune.
4. *Rust* resistant varieties like NP783 and NP784 which were resistant to brown rust; NP785 and NP786 which were resistant to all races of yellow rust; NP789 and NP790 which were resistant to black rust were developed in 1935.
5. Also, NP809 resistant to all the three rusts and loose smut was developed.
6. C306 is a tall variety developed by CCSHAU, Hisar for rainfed cultivation in NWPZ during early sixties is still chosen by consumers due to their chapatti quality and other traits.

7.3.2.3. Cotton Varieties

It is a Kharif crop. This fiber crop is also known as 'White gold'. It does not need enough water except at maturity. India holds 3rd rank in worldwide production of cotton. It grows in tropical and subtropical regions. Cotton requires modest rainfall and uniformly high temperature of 21°C to 30°C. The best considered soil for growing cotton is the black soils of Deccan and Malwa plateau, alluvial soils of the Satluj-Ganga plain, red and laterite soils of the peninsular region. Gujarat, Maharashtra and Andhra Pradesh are the major cotton producing states.

Scientific Classification:

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Clade: Rosids

Order: Malvales

Family: Malvaceae

Subfamily: Malvoideae

Tribe: Gossypieae

Genus: *Gossypium*

Habit and Habitat: It grows in tropical and subtropical regions. Cotton requires modest rainfall of 40-100 cm. The uniformly high temperature of 21°C to 30°C is best for cotton to grow. The best considered soil for cotton to grow is the black soils of Deccan and Malwa plateau, alluvial soils of the Satluj-Ganga plain and red and laterite soils of the peninsular region.

Crop Varieties:

1. All four species of cotton are produced in India; *Gossypium arboreum* and *G. herbaceum* (Asian cotton), *G. barbadense* (Egyptian cotton or Sea Island Cotton or Peruvian Cotton or Tanguish Cotton or Quality Cotton) and *G. hirsutum* (American cotton or Upload cotton).
2. *G. arboreum* and *G. herbaceum* are diploid (2n=26) and are indigenous to the old world.
3. *G. hirsutum* contributes to the 90 % of the hybrid cotton production in India.
4. *G. barbadense* and *G. hirsutum* are tetraploid (2n=52) and are considered as New World Cottons.

5. 90 varieties of upland cotton, 3 of Egyptian cotton, 39 of diploid cottons and 43 hybrids have been released for commercial cultivation in different states of India after the inception of All India Coordinated Cotton Improvement Project (AICCIP) in April, 1967.
6. The different states in which cotton varieties released were Punjab, Haryana, Rajasthan and Western U.P.
7. Ludhiana is the main centre and Faridkot is the sub-centre for variety improvement work of cotton.
8. 18 varieties of upland cotton and six of *arboreum* cotton have been released in Punjab naming, LH 900, LH 1556, F846 and F 1378 in *G. hirsutum* and G.27, LD 327 and LD 491 in *G. arboreum*.
9. 'H 4' cotton hybrid was the world's first cotton hybrid developed in India by Dr. C T Patel (Father of hybrid cotton).
10. It has high potential yield and wide adaptability among farmers in Gujarat, Andhra Pradesh, Karnataka, Maharashtra and Madhya Pradesh.
11. The first interspecific hybrid between *G. hirsutum* and *G. barbadense* gave rise to the development of cotton hybrid, "Varalaxmi".
12. It was released in Karnataka, Tamil Nadu, Andhra Pradesh and Maharashtra.
13. Heterosis breeding is also used for developing hybrids in several tetraploid cottons and few in diploid cottons.

7.3.2.4. Sungarcane Varieties

It is considered as cash crop and India is the second largest producer of sugarcane in the world. Sugarcane needs 7-8 months long rainy season. In India, North India and South India are involved in sugarcane production but their properties are different. In north India, sugarcane produced are of sub-tropical varieties and so have low sugar content whereas in south India, tropical varieties and coastal areas hence have high sugar content and high yield.

Scientific Classification:

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Monocots

Clade: Commelinids

Order: Poales

Family: Poaceae

Genus: *Saccharum*

Habit and Habitat: Sugarcane is the perennial grass which belongs to the poaceae family. They grow in the subtropical and tropical regions. The volcanic soil, clayey loamy soil, black cotton soil, red loamy soil, brown loamy soil and alluvial soils of rivers are the best for sugarcane to grow. The rainfall should have range from 75-150 cm. the optimum temperature varies from 20-26^{o} C.

Crop Varieties:

1. CoS 767 sugarcane varieties is not as popular as it deteriorates fast due to increased incidence of GSD and red rot.
2. Co 09022 (Karan-12) sugarcane variety is developed from the open pollinated fluffs of CoLk 8102 (GC).
3. Co 09022 (Karan-12) has high sugar, good quality and free from diseases with low insect-pest incidence.
4. Co 05009 is a mid-late maturing sugarcane variety which is developed by progeny of Co 8353 x Co 62198 in Sugarcane Breeding Institute Regional Centre, Karnal.
5. Co 06030 is a mid-late maturing sugarcane variety which is developed by crossing between the CoC 671 x IG 91-1100.
6. CoC 671 has high sugar variety and the other parent IG 91-1100 is an intergeneric hybrid between CoC 772 and *Erianthus arundinaceus* which helps in maintain high yield and vigour.
7. Co 06027 is mid late maturing sugarcane variety which is developed by the hybridization crossing of CoC 671 x IG 91-1100 and then selection.
8. Again due to the same parents as CoC 671, Co 06027 has high sugar, high yield and vigour.
9. Co 09004 is another sugarcane variety which has high yield, high quality and is again early maturing sugarcane.
10. This variety is developed by the segregating progenies of the cross CoC 671 x CoT 8201.
11. Parent CoC 671 has high sugar variety and act as female parent while CoT 8201 act as male parent, is a high cane tonnage plant.

7.3.2.5. Tea Varieties

It is an evergreen plant. It grows in tropical and subtropical climates. It is not easy to cultivate tea plant as it needs intensive care and laborers to look out. They do not prefer much light and hence grow in shade. India has become 2nd major producer of the world and is 1st on rank as largest consumer of the world.

Scientific Classification:

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Clade: Asterids

Order: Ericales

Family: Theaceae

Genus: *Camellia*

Habit and Habitat: Heavy rainfall of 150-250 cm is required by tree plant although, water logging is not tolerable by roots of tea plant. It requires sloppy areas with well-drained forest loam soil. Tea is grown in 16 states of India among which Assam, West Bengal, Tamil Nadu and Kerala produce 95% of total tea production.

Crop Varieties:

1. Teas are generally diploid (2n=30) but triploid (2n=45) tea plants are also present.
2. The breeding give rise to natural diploids, triploids, tetraploids, aneuploids and polyploidy varieties.
3. Most popular varieties of Tea plant used for commercial purposes are UPASI: BSS-1, UPASI: BSS-2, UPASI: BSS-3 and UPASI: BSS-4.
4. These cultivars are produced by combinations of a common parent TRI 2025 and UPASI-10, UPASI-2, UPASI-9 and UPASI-15.
5. The crossing of parents CR-6017 and UPASI-8 produces UPASI: BSS-5 tea plant hybrid.
6. TISS-1 variety is produced by breeding between UPASI-9 and TRI-2026.

7.3.2.6. Coffee Varieties

A coffee plantation can be found on the gentle to moderate mountainous slopes. They need hot and humid climate to grow. They are generally grown under shady trees. Strong sun shine and

high temperature from 30°C, frost and snowfall, etc., are injurious for coffee cultivation. Moreover, dry weather condition is optimum for plants at the time of ripening of berries. Coffee plantation needs skilled labor.

Scientific Classification:

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Clade: Asterids

Order: Gentianales

Family: Rubiaceae

Tribe: Coffeeae

Genus: *Coffea*

Habit and Habitat: Coffee is generally grown in shade. The two tiers of shade are provided to these plants. Required altitude range varies from 1,000 to 1,500 m above sea level for *arabica* (premier coffee), and 500 to 1,000 m for *robusta* (lower quality). They need well-drained, rich friable loamy soil with humus and mineral conditions, favors high and rich organic matter. Rainfall ranges between the 150-250 cm. Karnataka, Kerala and Tamil Nadu are the major coffee producing states.

Crop Varieties:

1. Coffee arabica has much variability and hybrids in the form of dwarf, semi-dwarf, tall in plant size; erect, spreading, drooping in branching habit; small, medium, bold in fruit size; early, late in fruit ripening; in quality traits and yield potential.
2. Arabica coffee (*Coffea arabica* L) produces superior quality coffee but is susceptible to diseases like leaf rust and pests like white stem borer.
3. 13 selections are produced from the Arabica coffee (*Coffea arabica* L) out of which 7 are used for commercial purposes.
4. These selections have properties like leaf rust resistance, high yielding potential, wide adaptability and superior quality
5. Standard breeding strategies like inter-varietal hybridization followed by pedigree selection in Sln.2 and Sln.3 produces coffee hybrids.

6. The pure line selection in Sln.1, Sln.4 and Sln.8 produces the hybrids
7. Interspecific hybridization followed by backcross breeding in Sln.6, double crosses in Sln.10, multiple crosses in Sln.7.3 and introgressive breeding in Sln.5A, Sln.5B, Sln. 9, Sln.12 and Sln.13 using spontaneous tetraploid interspecific hybrids like Devamachy and Hybrido De Timor (HDT), etc are used to develop coffee hybrids.
8. The S.288 (Sln.1) was the first selection developed by pure line selection from S.26, which is a putative natural hybrid of *C. arabica* and *C. liberica*.
9. The S.288 plants are vigorous growth and manifested resistance to leaf rust race 1 and 2.
10. This selection line is superior to other varieties like 'chiks' and "kents'.
11. Fruits of this selection are round with broad disc and orange yellow to red in color, popularly known as 'golden drops'.
12. This selection is known for its general adaptability and is a moderate yielder (800 to 1000 kg ha-1) but produces high percentage of defective beans (20 to 30%). The liquor quality is rated as Fair Average Quality.
13. **Sln.2 (S.333)** is a hybrid of S.31 and S.22 and has properties like hybrid vigor and resistance to leaf rust.
14. **Although, it does not gain much popularity due to the** high bean abnormalities.
15. **Sln.3 (S.795) is developed by crossing between** S.288 and 'kents' coffee.
16. **This selection is** vigorous and wide spreading with profuse growth and a yield potential of 1500 to 2000 kg ha-1.
17. **They shows resistance towards** leaf rust race 1 and 2 but are susceptible to leaf rust race 7,8,12 and 16.
18. **Sln.4 arises by** composite selection of three Ethiopian *arabica* collections which are Cioccie, Agaro and Tafarikela.
19. Fruits of this selection are relatively small and beans are bluish green, long and bold with 65% 'A' grade and excellent liquor quality.
20. Cioccie and Agaro are resistant to leaf rust races I, II and VIII.
21. Tafarikela is susceptible to rust and exhibits horizontal resistance by showing very less rust build up under field conditions.

22. Sln.5 selection line includes two separate families of cross bred lines involving Devamachy and S.881 which is a wild *arabica* collection from Rume Sudan and Devamachy and S.333.
23. **Sln.11 is derived from** interspecific hybridization between *C. arabica* and *C. eygenioides* and spontaneously obtained by amphiploidy.
24. **Sln.12 also called Cauvery is a** semi-dwarf genotype which is derived from a cross between caturra and HDT.

7.4- SUMMARY

The cytogenetics is a reliable method of studying plant chromosomes and genome. It provides precise information about the physical structure of the chromosomes and location of genes. By using genomics, genetics and cytogenetics; the success rate of plant breeding is increased. There are number of varieties produced through this method which causes the development of better quality, high yield and seed product from the hybrids. There are many methods to study the chromosomal rearrangements, physical structure of chromosome and gene locations such as Karyotyping, Chromosome banding, Fluorescent in situ hybridization (FISH) and Comparative genomic hybridization (CGH). Various variety hybrids of wheat, rice, cotton, sugarcane, tea and coffee are made within India which are used all over the world and has better properties from the wild cultivars.

7.5- GLOSSARY

Accurate- It means correct in all details or exact.

Agriculture- It is the science or practice of farming which includes cultivation of crops and the rearing of animals in order to provide food, wool, and other products.

Aneuploid- It refers to an abnormal number of chromosomes in a haploid set.

Centromeres- It is the region of a chromosome in which the microtubules of the spindle are attached by the kinetochore during cell division.

Chromosome- It is a threadlike structure of nucleic acids and protein which is found in the nucleus of most living cells and carry genetic information in the form of genes.

Constriction- It is the action of making something narrower through pressure or by becoming narrower; tightening.

Denaturation- It is the method of breaking the weak linkages, or bonds of protein, nucleic acids, etc.

Fluorochrome- It is a chemical which fluoresces in the presence of a label in biological research.

Heterochromatin- It is the chromosome material with different density from normal which is usually greater and in which the activity of the genes can be modified or suppressed.

Homologue- It refers to the similarity in the internal or chromosomal structures

Horticulture- It is the science and art of growing fruits, vegetables, flowers, or ornamental plants.

Ligation- It is the process of joining of two molecules or DNA.

Metaphase- It refers to the second stage of cell division which occurs between prophase and anaphase and in which the chromosomes become attached to the spindle fibers.

Mitosis- It is a process of cell division which results in two daughter cells, each having the same number and kind of chromosomes with that of parent nucleus.

Monsoon- Also refer to as rainy season.

Morphology- It means external particular form, shape, or structure.

Phenotype- It is the set of observable characteristics of an individual from outside which results from the interaction of its genotype with the environment.

Plantation- It refers to a land on which crops such as coffee, sugar, and tobacco are grown.

Precision- It refers to the quality, condition, or fact of being exact and accurate.

Prophase- It is the first stage of cell division which occurs before metaphase. The chromosomes are visible as paired chromatids and the nuclear envelope disappears. The first prophase of meiosis includes the reduction division.

Telomeres- It is a region of repetitive nucleotide sequences at each end of a chromosome in which the nucleotide is protected by the end of the chromosome from deterioration or from fusion with neighboring chromosomes.

Translocation- It is the movement of something from one place to another.

7.6- SELF ASSESSMENT QUESTIONS

1. Co 09022 also called _____, has high sugar, good quality and free from diseases with low insect-pest incidence.
2. _____ coffee is susceptible to rust.
3. Inter racial hybridization is the process which involves standard procedures of staining which reveals _____ characteristic features.

4. **Sln.12 also called _____** is derived from a cross between caturra and HDT.
5. _____ is a mid-late maturing sugarcane variety which is developed by crossing between the CoC 671 x IG 91-1100.
6. **Sln.11 is derived from** _____ between C. Arabica and C. *eygenioides*.
7. The japonicas and indicas give rise to more than 700 hybrid combinations by _____.
8. AT rich regions appear as bright bands called the _____.
9. The _____ produced are similar to light bands produced by G banding technique.
10. The fluorochrome dye _____ or _____ is used to produce bands which are the reverse of Q banding technique.
11. The _____ provides the bright green fluorescence.
12. The chromosomes are stained with _____ in the constitutive heterochromatin regions.
13. Unknown _____ are detected by banding technique in T. aestivum.
14. _____ contain the elements which are directly mapped to the genome sequences.

ANSWER TO SELF ASSESSMENT QUESTIONS

1. Karan-12
2. Tafarikela
3. Chromosome
4. **Cauvery**
5. Co 06030
6. Interspecific hybridization
7. Inter racial hybridization
8. Q bands
9. Dark R bands
10. Acridine orange, Olivomycin
11. R bands
12. Giemsa
13. N bands
14. DNA microarrays

7.7- REFERENCES

1. Anamthawat-Jónsson, K. (2004). Preparation of chromosomes from plant leaf meristems for karyotype analysis and *in situ* hybridization. *Methods in Cell Science*, *25*(3-4), 91-95.
2. Banding Pattern. https://www.chegg.com/learn/biology/introduction-to-biology/banding-pattern
3. Bauchan, G. R., & Hossain, M. A. (1997). Karyotypic analysis of C-banded chromosomes of diploid alfalfa: Medicago sativa ssp. caerulea and ssp. falcata and their hybrid. *Journal of Heredity*, *88*(6), 533-537.
4. Cheema, A. K. (2018). Plant breeding its applications and future prospects. *Int. J. Eng. Technol. Sci. Res*, *5*, 88-94.
5. Chen, R., Song, W., Li, X., & An, Z. (1994). Chromosome G-banding in plants by inducing with trypsin and urea. *Cell Research*, *4*(1), 79-87.
6. Chromosome Banding and Karyotyping Ppt. (2020) Kromosom Saints. https://kromosomsaints.blogspot.com/2020/12/chromosome-banding-and-karyotyping-ppt.html
7. Das, S. C., Das, S., & Hazarika, M. (2012). Breeding of the tea plant (*Camellia sinensis*) in India. In *Global Tea Breeding* (pp. 69-124). Springer, Berlin, Heidelberg.
8. Dematteis, M., Fernández, A., & Acosta, A. D. (2006). Heterochromatin variation in Oziroë argentinensis (Hyacinthaceae) revealed by florescent banding. *Caryologia*, *59*(2), 104-111.
9. Dorritie, K., Montagna, C., Difilippantonio, M. J., & Ried, T. (2004). Advanced molecular cytogenetics in human and mouse. *Expert review of molecular diagnostics*, *4*(5), 663-676.
10. Fedak, G., & Kim, N. S. (2008). Tools and methodologies for cytogenetic studies of plant chromosomes. *Cytology and Genetics*, *42*(3), 189-203.
11. Gupta, A., Singh, C., Kumar, V., Tyagi, B. S., Tiwari, V., Chatrath, R., & Singh, G. P. (2018). Wheat varieties notified in India since 1965. *ICAR-Indian Institute of Wheat & Barley Research, Karnal-132001, India*.
12. Iwata-Otsubo, A., Radke, B., Findley, S., Abernathy, B., Vallejos, C. E., & Jackson, S. A. (2016). Fluorescence in situ hybridization (FISH)-based karyotyping reveals rapid

evolution of centromeric and subtelomeric repeats in common bean (Phaseolus vulgaris) and relatives. *G3: Genes, Genomes, Genetics*, *6*(4), 1013-1022.

13. Jellen, E. N. (2016). C-banding of plant chromosomes. In *Plant cytogenetics* (pp. 1-5). Humana Press, New York, NY.
14. Pinkel, D., & Albertson, D. G. (2005). Comparative genomic hybridization. *Annu. Rev. Genomics Hum. Genet.*, *6*, 331-354.
15. Shakoori, A. R. (2017). Fluorescence in situ hybridization (FISH) and its applications. In *Chromosome Structure and Aberrations* (pp. 343-367). Springer, New Delhi.
16. Shukla, S. K., Zubair, A., Awasthi, S. K., & Pathak, A. D. (2018). *Sugarcane varieties identified by AICRP (S) in India*. ICAR-AICRP on Sugarcane. 1-132
17. Singh, P., & Kairon, M. S. (2001). Cotton varieties and hybrids. CICR Technical Bulletin no: 13, 15.
18. Singh, R. J., & Tsuchiya, T. (1982). Identification and designation of telocentric chromosomes in barley by means of Giemsa N-banding technique. *Theoretical and Applied Genetics*, *64*(1), 13-24.
19. https://www.creative-diagnostics.com/in-situ-hybridization-and-fluorescence-in-situ-hybridization.htm

7.8- SUGGESTED READINGS

1. http://apeda.gov.in/apedawebsite/SubHead_Products/Wheat.htm#:~:text=Varieties%3A,di)%2C%20HW%2D741.
2. http://drdpat.bih.nic.in/Rice%20Varieties%20in%20India.htm
3. http://www.businessworld.in/article/India-s-Cotton-Fact-Sheet-2017/21-09-2017-126710/
4. https://indianestates.co.in/development-of-indian-coffee-varieties/
5. https://www.gktoday.in/gk/major-crops-of-india/
6. https://www.patnauniversity.ac.in/e-content/science/botany/l/MScBot12.pdf

7.9- TERMINAL QUESTIONS

1. Describe the different techniques used for studying chromosomes.
2. What is the R Banding technique? Explain.
3. Provide a detail with different coffee varieties in India.
4. Explain the *Triticum* hybrid varieties and their breeding in detail.
5. Explain rice variety found in India.

6. Give a detail account of cotton variety in India.

BLOCK-3 PLANT DEVELOPMENT

UNIT-8-STUDY OF CYTOHISTOLOGICAL ZONES IN THE SHOOT APICAL MERISTEM (SAM) IN SECTIONED AND DOUBLE STAINED SLIDES

8.1-Objectives

8.2-Introduction

8.3- Study of Cytohistological zones in the shoot apical meristem (SAM) in sectioned and double stained slides

8.4-Summary

8.5-Glossary

8.6-Self Assessment Questions

8.7-References

8.8-Suggested Readings

8.9-Terminal Questions

8.1 OBJECTIVES

After reading this unit students will be able to-

- Understand the plant meristematic tissue.
- Understand the different types of meristematic tissues found in plants.
- Understand the sectioning and staining of meristematic tissue.
- Understand the cytohistological zones of shoot apical meristem.

8.2 INTRODUCTION

Meristem is a group of undifferentiated tissue found in plants has a capacity of division. The term meristem was first coined by a Swiss botanist Carl Wilhelm von Nägeli in 1858. The term meristem was mentioned in his book "Contributions to Scientific Botany". It is derived from the Greek word 'merizein' which means 'to divide'. Meristems contain a population of cells with active cell division which serves to constantly replenish the meristem and that differentiates into plant organs and tissues. So a meristmatic tissue is a group of cells that are in continuous state of division or retain their power of division. Meristematic cells have some characteristics like:

- They are generally round, oval or iso-diametric in shape.
- They have compact arrangement *i.e.* without intercellular spaces and with dense cytoplasm.
- They contain large prominent nucleus with small vacuoles or without vacuoles.
- Cells have thin cell wall generally without secondary wall and don't store food material
- The most striking feature is the capacity of division *i.e.,* always in active state of division and divide in a plane.

During postembryonic development, all plant organs are derived from a few meristematic cells found in specialized structures and positions called apical meristems. Apical meristems are the completely undifferentiated meristems, usually positioned at shoot apex and root apex. Apical meristems are employed as synonyms for both root and shoot apical meristem. The shoot apical meristem (SAM) is terminally positioned minute but complex structure that is covered by newly developing lateral organs like leaves or bracts. According to Wardlaw (1957) and Cutter (1965) the shoot apical meristem (SAM) comprises two groups of cells: the initial or source cells and

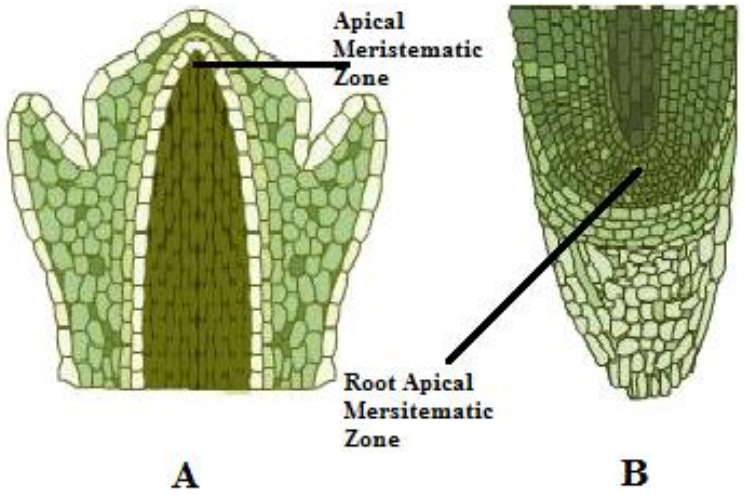


Fig.8.1: Meristematic Tissues in Plants (a) Stem Apex (b) Root Apex

the progenitor cells for tissues and lateral organs. The principal functions of the SAM (shoot apical meristem) is the formation of new cells by division for the growth shoot axis and the initial cells for lateral organs, such as branches, leaves, protective and accessory organs, etc.

The shoot apex is radially symmetrical. There occur great variations in shape and size of the shoot apices among the spermatophytes. Usually the shoot apex in most of the plants is more or less convex. In *Anacharis*, and some grasses it is a narrow cone with a rounded tip. According to Gifford (1950) in some cases,e.g. in *Hibiscus syriacus, Drimys*, etc, it is slightly concave. Clowes (1961) reported the size of *Cycas revoluta* shoot apex at the level of youngest leaf primordium to be 3300 μm in width.

Classification of meristems

There are three types of meristems:

1. **Apical meristems**: The meristems present at the tips of roots and shoot are called apical meristems. Apical meristems are involved in the extension of plant body, continuing for a long period of time at the-apices of roots and stems. The cells derived from apical meristems are grouped into three types (Fig.8.2):

• **Protoderm**: This is the surface layer . Its cells are differentiated into epidermal systems.

- **Procambium:** They are also called provascular tissues. Their cells form different parts of vascular systems at ma urity.
- **Ground meristem:** These cells of these tissues form differ of ground tissues at maturity.

2. **Intercalary meristems:** The meristem situated at the bases of internodes is called intercalary meristem. These are the parts of apical meristem, get separated from apex meristem and play important role in the production of leaves and flower.
3. **Lateral meristems:.** Vascular and cork cambium are the example of lateral meristems. They play an important role in the increase in diameter of stem and root. So they are involved in secondary growth.

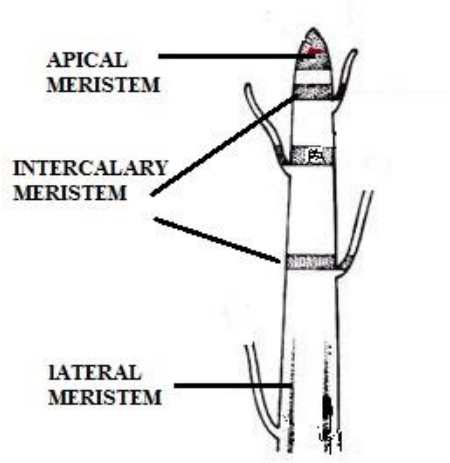


Fig.8.2: Positions of meristems

Organization of cells in Shoot Apical Meristem

There are two concepts about the organization of cells in the .apical meristems.

1. **Histogen theory**: This theory was put forward by Hanstein in 1870. According to this theory the apical meristematic tissues arc divided into three Zones (Fig. 8.3).

(a) Dermatogens: This zone consist the cells of the surface layer. It gives rise to the epidermis.

(b) Periblem: This zone comprise; the cells of the outer region lying under the surface layer. They give rise to the cortex or outer portion of the stem.

(c) Plerome: This zone comprises the central mass of cells. It gives rise to the vascular system and the pith.

2. Tunica-Corpus theory This theory was given by Schmidt in 1924. According to this concept, the dividing cells in the apical meristem are arranged in two zones (Fig.8.4**)**:

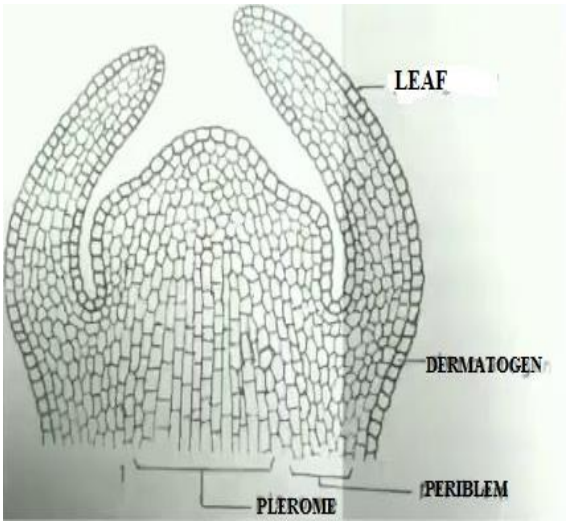


Fig.8.3: Three Histogenic Zones in SAM

Tunica: The outer zone is called tunica composed composed of one or two layers of superficial cells. The epidermis arises from the outer layer of tunica. Single layered tunica is present in monocot plants. Dicots have two layered tunica.

Corpus: The central zone is called corpus which is covered by tunica cells. The cells of corpus divide in all planes. Inner tissues like cortex and vascular tissues are derived from both tunica and corpus.

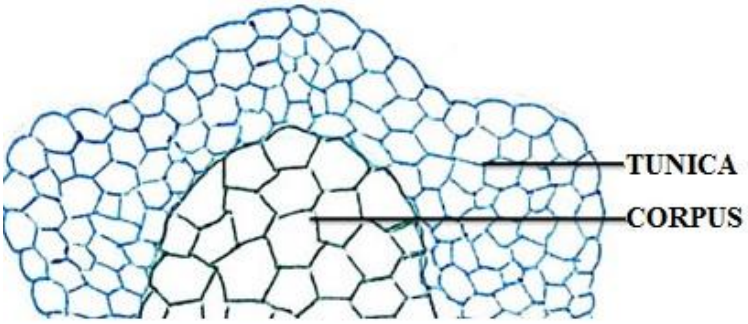


Fig.8.4: Apical Meristem Organization In Tunica And Corpus

3. **Histogenic layer theory**: This theory "as put forward by Dermen in 1868. He proposed the concept of primary histogenic layers. According to him. there are three basic histogenic layers in angiosperms which named them as L-I-L-II.L-III gives rise to vascular tissues and pith region (Fig.8.5**)**.

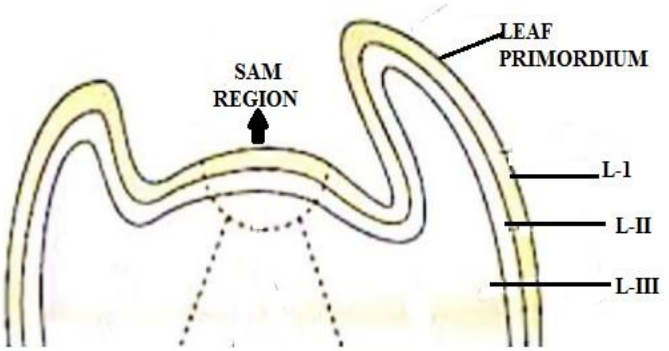


Fig.8.5: Histogenic Layers in Shoot Apex

4. **Cytohistological zonation theory**: This theory was proposed by Foster in 1939. In gymnosperms tunica like layers not found which led Foster to divide shoot apex arganization of *Ginkgo biloba* on the basis of planes of divisions, size of cell and nucleus, differential staining and orientation of cell division. He recognized four interrelated zones.

   (i) Apical Initial group

   (ii) Central Mother Cell Zone

   (iii) Rib Meristem

   (iv) Flank orperipheral meristem (Fig.8.6)

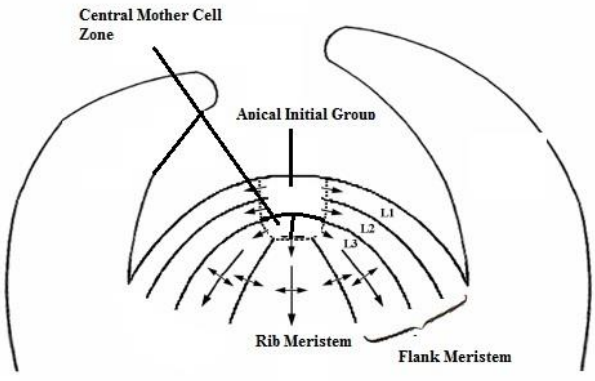


Fig.8.6: L.S. of Shoot meristem of *Ginkgo biloba* showing schematic representation of cytohistological zonation

8.3- STUDY OF CYTOHISTOLOGICAL ZONES IN THE SHOOT APICAL MERISTEM (SAM) IN SECTIONED AND DOUBLE STAINED SLIDES

The cytohistological zones in SAM can be studied by maceration techniques applied with suitable stains. The technique of maceration including the nature of the chemicals used depends mainly on the hardness of the tissues to be macerated. On the nature of tissues Jeffrey's Method or Quick methods can be used in maceration. Jeffrey's method, although quite adequate for all kinds of tissues, is a very slow method. Quick method is suitable for herbaceous material like Cucurbita. In former method the material (dry or fresh) is cut into small slices about 0.5 mm thick. Boil and cool repeatedly until it becomes free of air. Macerate in a solution of equal parts of 10% nitric acid and 10% chromic acid. The material with the solution may be heated in a paraffin bath for woody tissues but not for soft and herbaceous tissues. The material is now stained with any suitable stain, such as eosin or water soluble safranin, mounted in 50% glycerine and observed. For permanent staining, leave the macerated tissue in 1% safranin for 6 hours, rinse thoroughly in water and then dehydrate by rapid addition of hygrobutol. Give two changes of pure hygrobutol and then add a little balsam highly diluted with hygrobutol. Evaporate it down to a mounting consistency and mount on slides. Herbaceous materials, such as Cucurbita stems are sliced into thin pieces and taken in a test tube along with a little 10% or 20% KOH solution, more KOH solution is added, if necessary. When the tissue comes out in shreds, it is washed and stained as before. Siliques (for embryos) or seedlings are fixed in 3% glutaraldehyde, 1.5% acrolein, 1.6% paraformaldehyde and postfixed in 1% osmium tetroxide. Fixed material is dehydrated through an ethanol series and embedded in Spurr's medium. 1 mm sections are cut and stained with 0.5% methylene blue in 0.5% borate. The purple red or blue stained samples are observed under microscope. On observation these traits in root apical meristem and the shoot apical meristem would be seen .The general features of meristematic mells are (a) isodiametric shape(b) thin primary cell wall (c) dense cytoplasm with few tiny vacuoles (e) relatively large nucleus (f) frequent cell divisions (fig. 8.7)

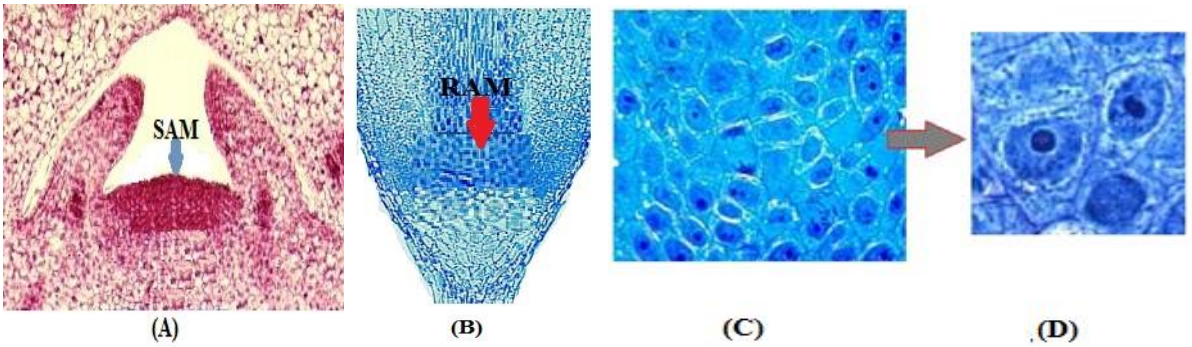


Fig. 8.7: Densely stained cells of: (A) Shoot apical meristem (B) The root apical meristem, (C) Cells from the center of the RAM: dense cytoplasm with clearly visible nuclei (D) Enlarged view of (C).

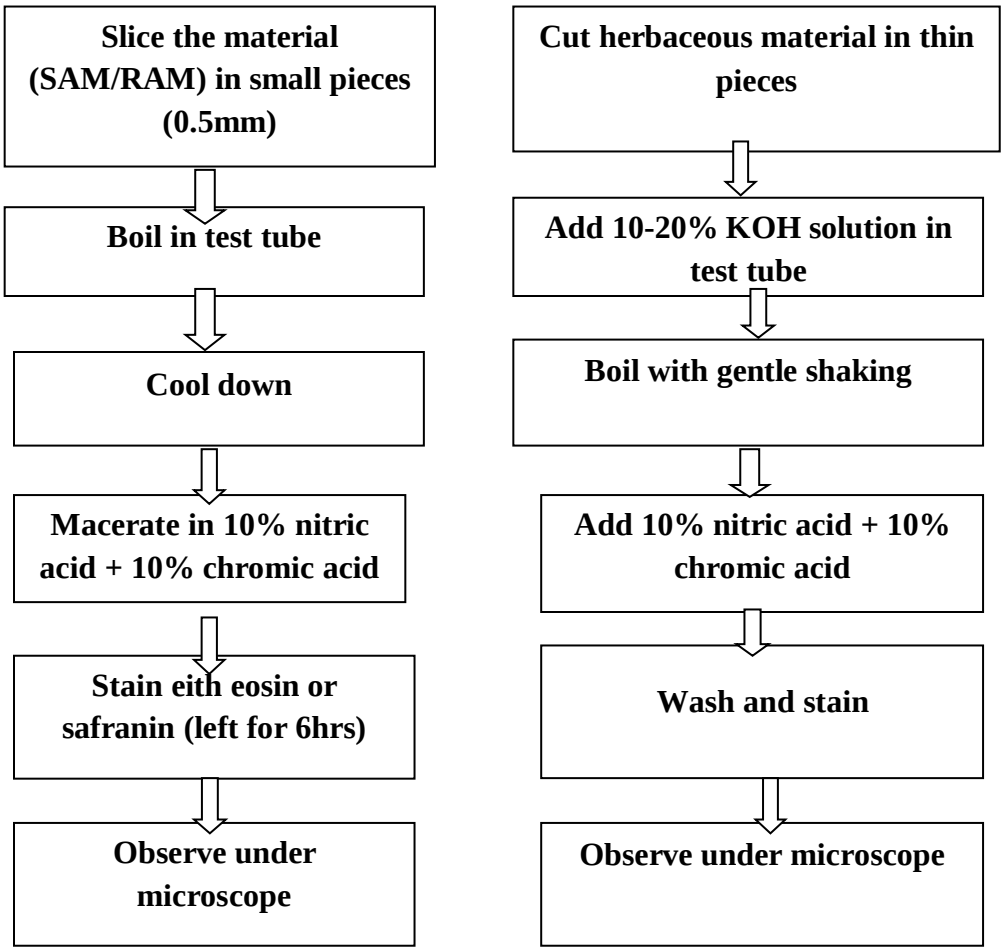


Fig. 8.8: Flow chart of (A) Jeffrey's method (B) Quick Method

The shoot apical meristem generates stem, leaves, and lateral shoot meristems during the entire shoot ontogeny. Vegetative leaves are generated by the meristem in the vegetative developmental phase, while in the reproductive phase either bracts subtending lateral flower primordia or perianth and strictly reproductive organs are formed. Meristem growth is fully characterized by the principal growth rates, directions, volumetric, and aerial growth rates. Growth modelling or sequential *in vivo* methods of meristem observation complemented by growth quantification allow the above growth variables to be estimated. Indirectly, growth is assessed by cell division rates and other cell cycle parameters. Temporal and spatial changes of growth and geometry take place at the meristem during the transition from the vegetative to the reproductive phase. During the vegetative phase, meristem growth is generally indeterminate. In the reproductive phase it is almost always determinate, but the extent of determinacy depends on the inflorescence architecture. In the vegetative phase the central meristem zone is the slowest growing region. The transition from the vegetative to the reproductive phase is accompanied by an increase in mitotic activity in this zone. The more determinate is the meristem growth, the stronger is this mitotic activation. However, regardless of the extent of the activation, in angiosperms the tunica/corpus structure of the meristem is preserved and therefore the mitotic activity of germ line cells remains relatively low. In the case of the thoroughly studied model angiosperm plant *Arabidopsis thaliana*, it is important to recognize that the flower primordium develops in the axil of a rudimentary bract. Another important feature of growth of the inflorescence shoot apical meristem is the heterogeneity of the peripheral zone. Finally, the role of mechanical factors in growth and functioning of the meristem needs further investigation. Shoot apex has apical meristems. The apical meristems of the shoot is more complex than the root. Shoot apex has two types of arrangements:

(a) In lower vascular plants the apical meristem of the shoot is more complex than the shoot apical meristem. The activity of the shoot apcial meristem increases the length of the plant body and also forms leaves and lateral branches (fig.8.9)

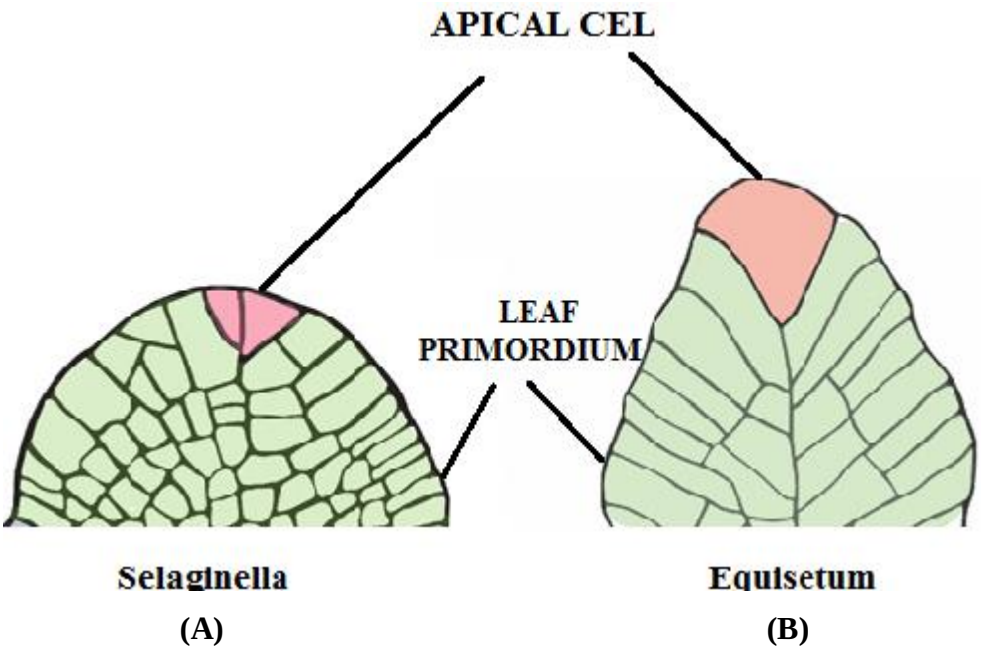


Fig.8.9: Shoot Apical Meristem (SAM) in (A) *Selaginella* and (B) *Equistem* Shoot

(b) In higher vascular plants *i.e.* gymnosperms and angiosperms the shoot apex consists of a set of meristematic cells. These cells differentiate into different zones on the basis of their meristematic activity (fig.8.10).

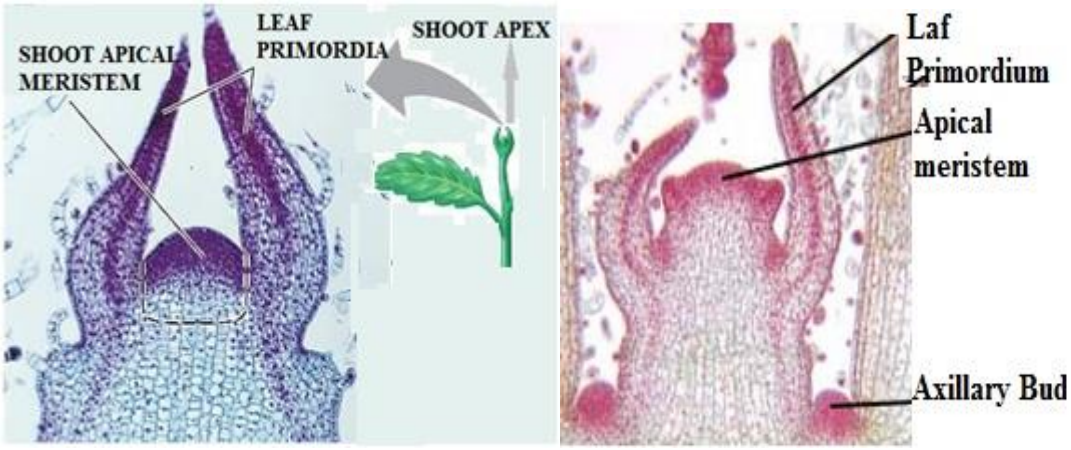


Fig.8.10: Shoot Apical Meristem (SAM) in Higher Vascular Plants

8.4 SUMMARY

1. Meristem is a group of undifferentiated tissue found in plants has a capacity of division.
2. The term meristem was first coined by a Swiss botanist Carl Wilhelm von Nägeli in 1858.

3. Meristem is the tissues which the cells are undifferentiated and has the capacity of division.
4. The term meristem has been derived from Greek word *meristos,* means divisible.
5. Meristems contain a population of cells with active cell division which serves to constantly replenish the meristem and that differentiate into plant organs and tissues.
6. Initially all embryonic cells of an embryo have the capacity to divide and multiply but as the embryo develops into a plant body.
7. During postembryonic development, all plant organs are derived from a few meristematic cells found in specialized structures and positions called apical meristems.
8. Apical meristems are the completely undifferentiated meristems, usually positioned at shoot apex and root apex. A
9. Apical meristems are employed as synonyms for both root and shoot apical meristem.
10. The shoot apical meristem (SAM) is terminally positioned minute but complex structure that is covered by newly developing lateral organs like leaves or bracts.
11. The meristematic activity provides growth and healing in plants.
12. Before the occurrence of any cell division, usually cells become enlarged accompanied with addition of protoplasmic and cell wall material.
13. They are generally round, oval or isodiametric in shape.
14. During postembryonic development, all plant organs derived from a few meristematic cells found in specialized structures and positions are called apical meristems.
15. Apical meristems are the completely undifferentiated meristems, usually positioned at shoot apex and root apex.
16. Apical meristems are employed as synonyms for both shoot and root apical meristem.
17. The shoot apical meristem (SAM) terminally positioned minute but complex structure that covered by newly developing lateral organs like leaves or bracts.
18. There are three types of meristems: apical meristems, intercalary meristems, lateral meristems.
19. The principal functions of the SAM (shoot apical meristem) is the formation of new cells by division for the growth shoot axis and the initial cells for lateral organs, such as branches, leaves, protective and accessory organs etc.

8.5 GLOSSARY

Anticlinal: occurring at right angles to the surface or circumference of a plant organ.

Corpus: The central zone is called corpus which is covered by tunica cells.

Cytohistological: the integrated study of cells and tissues.

Dermatogens: the outer primary meristem of a plant which gives rise to epidermis.

Development: the progressive changes in size, shape, and function during the life of an organism.

Dicot: member of the flowering plants, or angiosperms that has a pair of leaves, or cotyledons, in the embryo of the seed.

Embryo: An embryo is the early stage of development of a multicellular organism.

Epidermis: The epidermis is a single layer of cells that covers the leaves, flowers, roots and stems of plants.

Histogen: a zone or clearly delimited region of primary tissue in plants from which the specific organ are produced.

Internodes: internodes are those intervals between the nodes.

Isodiametric: a cell having a similar diameter in all planes.

Meristem: region of cells capable of division and growth in plants.

Meristos: Greek term used in relation to meristem is 'meristos' which means 'divisible'.

Nodes: nodes are those critical areas from which leaves, branches, and aerial roots grow out from the stem.

Periblem: a primary meristem that gives rise to the cortex and is located between plerome and dermatogens.

Periblem: a primary meristem that gives rise to the cortex and is located between plerome and dermatogens.

Periclinal: Periclinal cell divisions are the ones that occur parallel to the tissue or organ surface.

Plerome: the central core of primary meristem of a plant or plant part that according to the histogen theory gives rise to the stele.

Primordium: an organ, structure, or tissue in the earliest stage of development.

Protoderm: the outer primary meristem of a plant or plant part.

Root apex: the growing tip of the plant shoot.

Shoot apical meristem (SAM): the region in the growing shoot containing meristematic cells.

Spurr's medium: low viscosity embedding medium, introduced in 1969 and used vinyl cyclohexene dioxide (VCD or ERL 4206) as the low viscosity epoxy resin together.

Tissue: A tissue is a group of cells, in close proximity, organized to perform one or more specific functions.

Tunica: the outer layer of cells, which covers tunica.

Vascular: plants with specialized vascular tissue.

8.6 SELF ASESSMENT QUESTIONS

8.6.1 Multiple Choice Questions:

1. Meristem is:

(a) Mature cells (b) Undifferentiated mass of cells

(c) Collenchyma cells (d)All of above

2. Apical meristem found in aerial part of plant tips:

(a)RAM (b) SAM

(c) Both (d) None of above

3. Growing part of plant tips composed of:

(a) Differentiated cells (b) Meristmetic cells

(c) Cambial cells (d) None of above

4. Tunica-Corpus theory given by:

(a) Lamark (b) Hanstein

(c) Buwat (d) Schmidt

5. Dermatogens give rise:

(a) Dermal layer (b) Cortex

(c) Cambium (d) Pith

6. Term meristem has been derived from:

(a) Meristos (b) Mitos

(c) Meritos (d) Merios

7. The meristem situated at the bases of internodes is:

(a)Apical Meristem (b) Intercalary Meristem

(c) Dermatogen (d) Lateral Meristem

8. Histogenic layer theory put forward by.

(a) Dermen (b) Foster

(c) Schmidt (d) Hanstein

9. Corpus is the:

(a) Central Zone (b) Peripheral zone

(c) Both (d) None

10. Protoderm cells are differentiated into:

(a) Vascular system (b) Epidermal systems

(c) Cambium (d) Cortex

11. Tunica-Corpus theory put forward by Schmidt in the year…….:

(a) 1924 (b) 1909

(c) 1960 (d) 1962

12. Histogen theory put forward by Hanstein in the year…….:

(a)1907 (b) 1868

(c) 1960 (d) 1990

13. The cells of tunica divides:

(a) Anticlinally (b) Periclinally

(c) Diagonally (d) All Planess

14. The cells of corpus divides in.

(a) All planes (b) Anticlinally

(c) Periclinally (d) Diagonally

15. Plerome gives rise to:

(a) Dermal system (b) Lateral Branch

(c) Vascular system (d) Leaf Primordia

8.6.2 Fill in the blanks:

(1) The term meristem originated from _____.

(2) _____proposed the Tunica-Corpus Theory.

(3) Histogen theory was put forward by _____.

(4) _____ cells are actively dividing cells.

(5) Plerome gives rise to _____ system.

(6) Dermatogen gives rise to _____ system..

(7) Periblem gives rise to _____ system..

(8) Histogenic layer theory put forward by_____.

(9) The _____activity of cells provides growth and healing in plants.

(10) The _____terminally positioned minute but complex structure that covered by newly developing lateral organs like leaves or bracts.

8.6.3 True or False:

(1) The term meristem originated from Greek term *meristos*.

(2) Peliblem gives rise to epidermal system.

(3) Tunica-Corpus theory was given by Schmidt.

(4) Histogen theory was discarded due to lack of cytological proof.

(5) According to Tunica-Corpus concept, the dividing cells in the apical meristem are arranged in two zones.

(6) The meristem situated at the bases of internodes is Apical Meristem.

(7) The leaf primordia originate in the peripheral part of the apical meristem.

(8) Histogenic layer theory put forward by Dermen in 1947.

(9) The shoot apex consists of a set of meristematic cells in gymnosperms and angiosperms.

(10) Dermatogens consist the cells of the innermost layer and gives rise vascular system.

8.6.4 Very short answer questions:

(1) Define meristems.

(2) Who proposed Tunica-Corpus theory?

(3) Who proposed Histogen theory?

(4) Define corpus.

(5) Define histogenic layers.

(6) What is the significance of periblem in plants?

(7) What is the anticlinal division?

(8) Describe anticlinal and periclinal division?

(9) Describe histogenic layers.

(10) Define plerome.

(11) Define Spurr's media.

8.6.1 Answer keys: 1-b, 2-b, 3-b, 4-d, 5-a, 6-a, 7-b, 8-a, 9-a, 10-b, 11-a, 12-b, 13-a, 14-a, 15-c.

8.6.2 Answer key: 1-Meristos, 2-Schmidt, 3-Hanstein, 4-Meristmetic 5-Vascular, 6- Dermal, 7-Cortical, 8-Foster, 9- meristematic, 10- shoot apical meristem (SAM).

8.6.3 Answer key: 1-True, 2-False, 3-true, 4-True, 5-True, 6-False, 7-True, 8-True, 9-True, 10-False.

8.7 REFERENCES

- Clowes, F.A.L. (1961). *Apical Meristems* (Oxford: Blackwell Scientific Publications)
- Cutter, E.G. (1965). *Recent experimental studies of the shoot apex and shoot morphogenesis*. Bot. Rev. 31, 7-113.
- Gilford, E. M. Jr (1954). *The shoot apex in Angiosperm*." Bot. Rev,20, 477–529.
- Wardlaw, C.W. (1957). *On the organization and reactivity of the shoot apex in vascular plants*. Am. J. Bot. 44, 176-185.

8.8 SUGGESTED READINGS

- Abraham Fahn.1990. *Plant Anatomy*. Oxford : Pergamon : Butterworth-Heinemann.
- Crang, Richard, Lyons-SobaskI, Sheila, Wise, Rober T. 2018. *Plant Anatomy.A Concept-Based Approach to the Structure of Seed Plants*. Springer International Publishing,
- Katherine Esau. 1977. *Anatomy of Seed Plants*, 2nd Edition. A John Wiley & Sons.
- Ray F. Evert. 2006. *Esau's Plant Anatomy Meristems, Cells, and Tissues of the Plant Body: Their Structure, Function, and Development* (Third Edition). A John Wiley & Sons, Inc., Publication.

8.9 TERMINAL QUESTIONS

8.9.1 Short answer questions:

(1) Define meristem.

(2) Write a note on Tunica Corpus theory.

(3) Describe SAM.

(4) What is Histogenic layer theory?

(5) Write a note on types of meristem.

8.9.2 Long answer question:

(1) Give a detailed account of SAM.

(2) Describe different theories put forward on sam.

(3) Write detailed note on tunica corpus theory.

(4) Give a illustrated account on apical meristem.

(5) Describe maceration techniques.

UNIT-9- EXAMINATION IN SHOOT APICES IN A MONOCOT IN T.S. AND L.S. TO UNDERSTAND THE ORIGIN OF LEAF PRIMORDIA.

9.1-Objectives

9.2-Introduction

9.3.1-Study of permanent/temporary slides showing SAM

9.3.1.1- *Carex*

9.3.1.2- *Smilax*

9.3.1.3- *Datura*

9.3.2-Study of permanent/temporary slides showing RAM

9.3.2.1- *Allium*

9.3.2.2- *Nicotiana*

9.3.3-Study of permanent/temporary slides showing the origin of leaf primordial in L.S. of shoot apex

9.3.3.1- *Triticum*

9.3.3.2- *Coleus*

9.3.3.3- *Saccharum*

9.4-Summary

9.5-Glossary

9.6-Self Assessment Questions

9.7-References

9.8-Suggested Readings

9.9-Terminal Questions

9.1 OBJECTIVES

After reading this unit students will be able-

1. To examine the shoot apices in plant species
2. To study the root apices in plant species
3. To observe the anatomy of leaf primordia

9.2 INTRODUCTION

Shoot apex is the growing terminal of the stem. As shoot apex majorly consists of meristem hence can also be called apical meristem. It is positively phototropic and negatively geotropic and has dome shape. Apical meristem has terminal position in shoot apex. The meristematic cells divide anticlinially near the Shoot Apical Meristems (SAMs). Shoot apex is generally protected by young leaves forming a crown like structure lacks the quiescent centre and has differentiated cells. The leaf primordium is present in the terminal part of the shoot.

Root apex, also called as root apical meristem is a portion of small region at the tip of a root in which all cells are meristematic having high ability to division. The repeated division of cells led to development of primary root tissues. Parenchymatic cells comprise the root cap which protects the tip of roots while passing through the soil. Internal meristematic cells called calyptrogens adds the new cells in the place of old ones and small root hairs arise as simple extensions from root apex, increases the surface area for absorption of water and minerals from the soil.

Leaf primordial arises from foliar buttress which transform into axillar phyllopodium. The meristem cells extend laterally. A highly ordered spatial and temporal pattern led to the development of leaf primordial from the flanks of the SAM. As leaf expands, stomata differentiate and gas spaces are formed.

9.3.1- Study of permanent/temporary slides showing shoot apex meristem (SAM)

9.3.1.1- L.S. of shoot apex of *Carex* (Fig 9.1 & 9.2)

1. The superficial tissue made up of rectangular cells forms a layer of tunica which is uniseriate.
2. The central core of meristem called corpus is covered by the tunica mantle of cells.
3. The tunica initials are made up of a group of similarly appeared small group of apical cells and the corpus is made up of two distinct zones.
4. The cells in the region of rib meristem which is the central region of the corpus, are transverse vacuolated and rectangular in shape arranged with their long axis.

5. Elongated cells are present in the longitudinal extent of rib meristem of the species of this genus.
6. The cells in flank meristem are smaller and less vacuolated than the other neighboring cells which are partly vacuolated the rib meristem.
7. The cells of apical dome are large with vacuoles.
8. Few cells are larger in size than the rest and also more vacuolated than others.

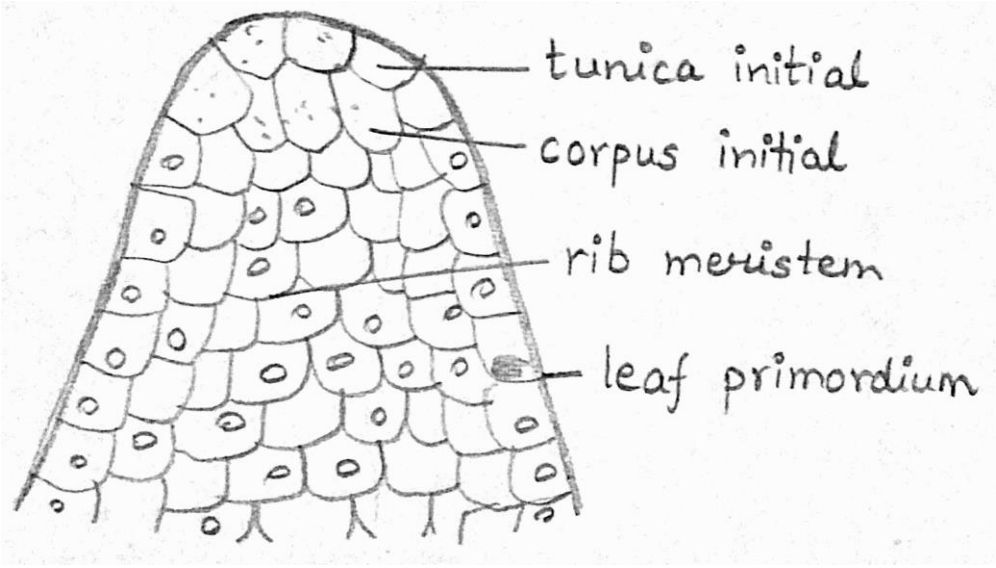


Fig.9.3: Longitudinal sections of growing point of *Carex* shoot.

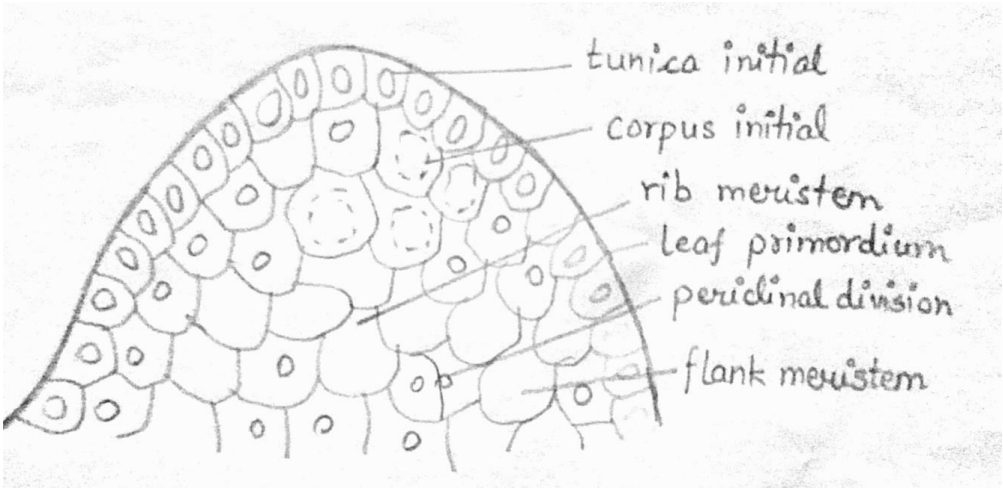


Fig.9.4: Longitudinal section of shoot apex of *Carex hordeistichos*.

9.3.1.2- L.S. of shoot apex of *Smilax* (Fig 9.3 & 9.4)

1. The species of this genus contain biseriate tunica present overlying a homogenous corpus.
2. The cells of the shoot apex are highly stratified.
3. The primary meristem layer made up of meristematic cells is thick and radiates outward below the apical meristem.
4. The meristematic cells are made up of small and nearly isodiametric cells.
5. The species of this genus are said to have true tunica and leaf initiation in subsurface layers.

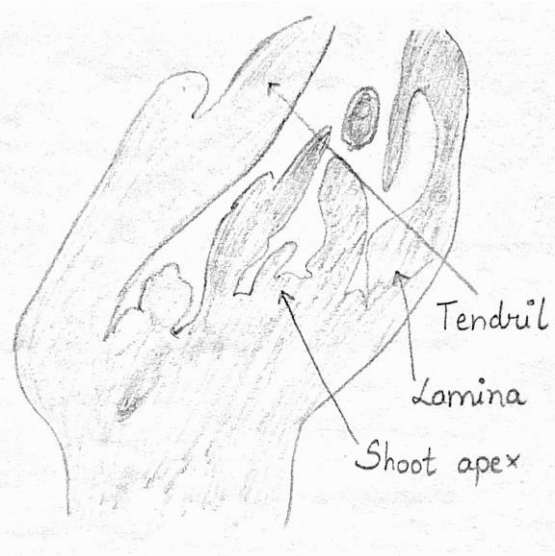


Fig.9.5: Longitudinal section of shoot tip of *Smilax pumila*.

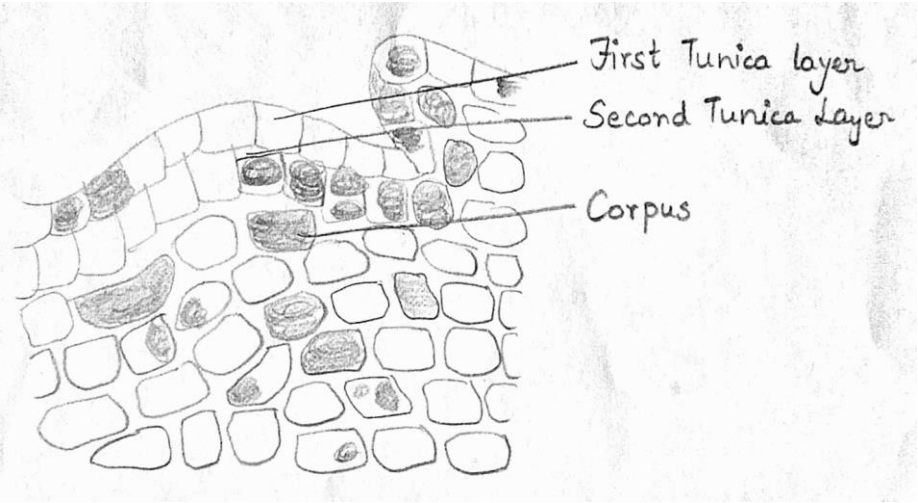


Fig.9.6: Longitudinal section of shoot apical meristem of *Smilax laurifolia*.

9.3.1.3- L.S. of shoot apex of *Datura* (Fig 9.5)

1. The layer of tunica is made up of one or more layers of cells.
2. Three layers of apical meristem are present in which two layers are involved to form tunica.
3. The central tissue of the shoot apex is made by third layer which constitutes to the initial cells of the corpus.
4. The cells in periphery are made up of dividing meristematic cells.
5. The meristematic cells line up the marginal surface of leaf primordium.
6. Large vacuoles are present in the central core region of leaf primordium.
7. The polyploid and diploid cells are used to form the tissue of shoot meristem.

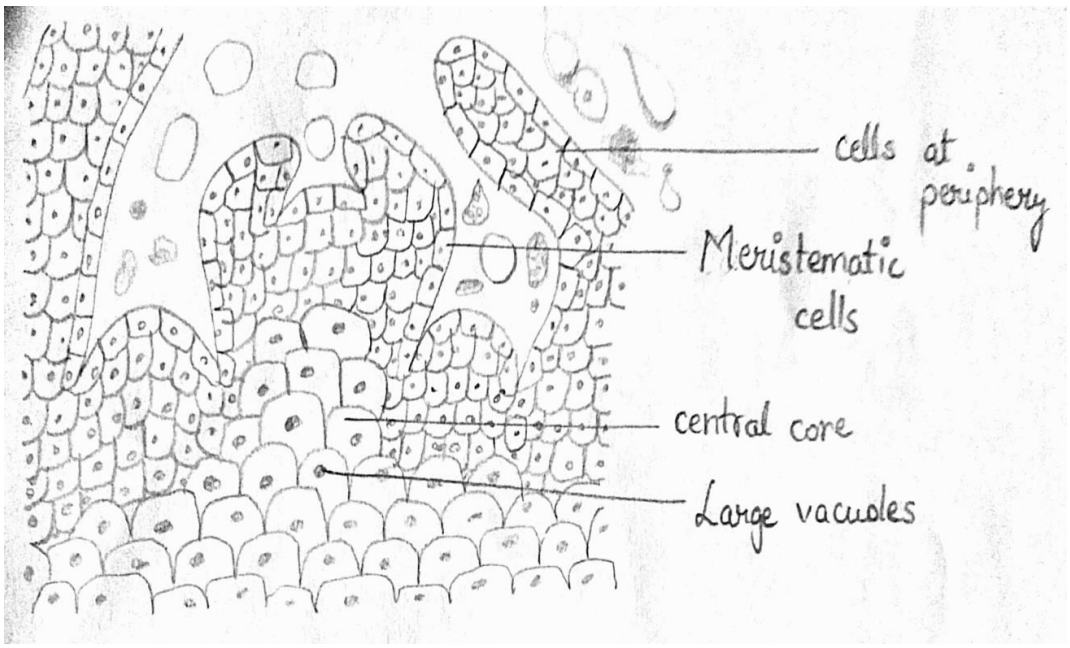


Fig.9.7: Longitudinal section of central core of primordial of *Datura*.

9.3.2- Study of permanent/temporary slides showing root apex meristem (RAM)

9.3.2.1- L.S. of root apex of *Allium* (Fig 9.6 & 9.7)

1. The cells of RAM are divided into different layers of epidermis, exodermis, central cortex and endodermis.
2. The peripheral layer of root apex is the epidermis.
3. The cells made to form one celled thick layer called epidermis and they are closely packed and elongated cells.
4. The cortex is divided into three parts which is comprised of exodermis, central cortex and endodermis.

5. The outermost layer of cortex is called exodermis, whereas the innermost layer is called endodermis whereas the space in between the exodermis and endodermis is occupied by central cortex cells.
6. Casparian bands are present in the anticlinal cells of hypodermis to form exodermis which is either dimorphic or uniform.
7. A dimorphic exodermis is made up of long and short cells whereas a uniform exodermis is formed by uniform-shaped elongated cells.
8. Suberin lamella deposition in long cells acts as plasmodesmata.
9. The region of central cortex is made up of parenchyma cells which are large and loosely arranged with numerous intercellular spaces.
10. The innermost tissue of the root apex is called stele which consists of pericycle, xylem, and phloem and associated parenchyma cells.

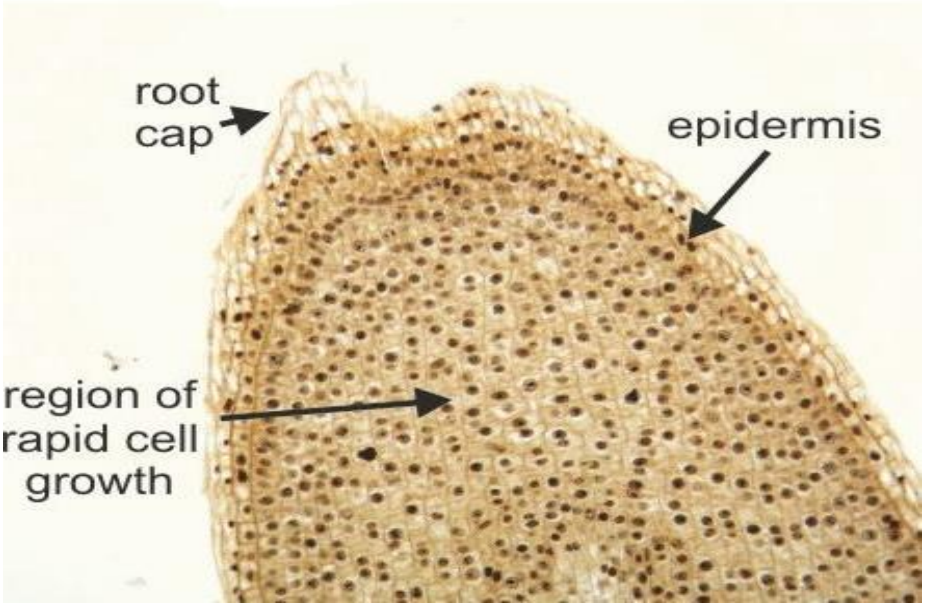


Fig.9.8: The structure of root tip of *Allium* (https://dissectionconnection.com.au /shop/ downloads/labelled-microslides/plant-slides/allium-root-tip-100x/).

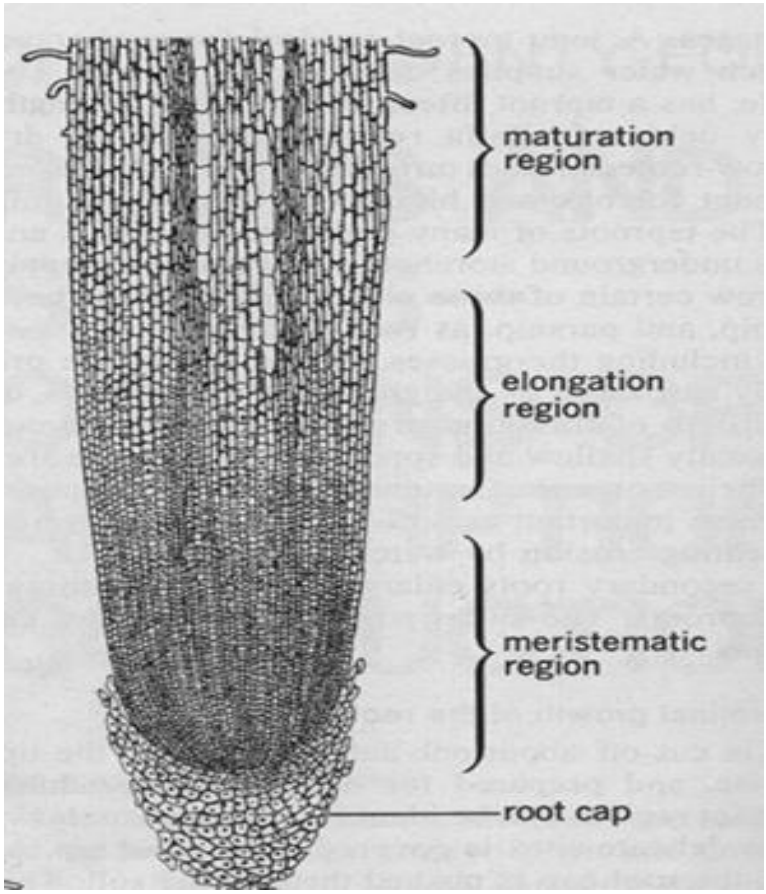


Fig.9.9: Longitudinal section of root apex meristem of *Allium* (http://mrsmorrittscience.weebly.com/9-reactions-of-life-wk1.html).

9.3.2.2- L.S. of root apex of *Nicotiana* (Fig 9.8)

1. The root apex is made up of small group of cells.
2. Different layers or tiers comes together to form the tissue of root apex at the periphery.
3. Four types of initials naming root cap columella initials, the epidermis/peripheral root cap initials, the cortex/endodermis initials and the vascular cylinder initials are present.
4. The initials are made up of three layers or tiers.
5. The lower tier is formed by the root cap, columella and epidermal cells.
6. The middle tier is made up of ground tissue whereas the upper tier is formed by vascular cylinder.

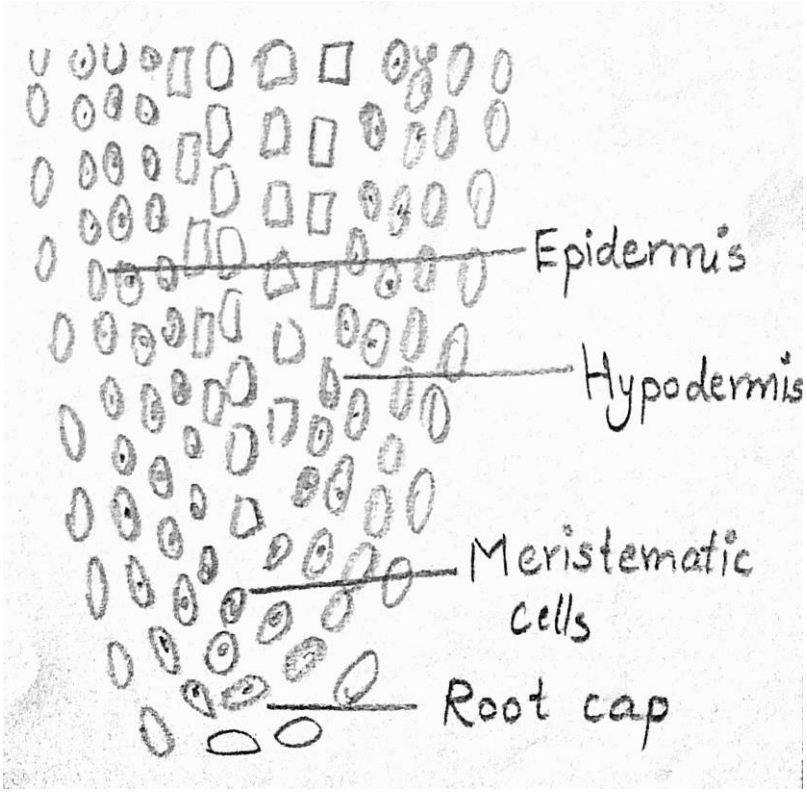


Fig.9.8: The longitudional section of *Nicotiana* root apex meristem.

9.3.3- Study of permanent/temporary slides showing the origin of leaf primordia in L.S. of shoot apex

9.3.3.1- L.S. of leaf primordial of shoot apex of *Triticum* (Fig 9.9)

1. Shoot apex somewhat resembles to convex structure
2. It is slightly elongated with broad bases
3. It is divided into three regions- two outer discrete and virtually self-perpetuating thimble shells of cells (the dermatogens and the hypodermal layer)
4. Uniseriate hypodermal layer is present at the tip of apices
5. Vertical strips of chlorenchyma with small vasuclar bundles are observed in fig. 9.9.
6. Distinguishable hypodermal layer with periclinical breaks
7. Outer Parenchyma sheath is observed
8. New leaf primordium emerged from the periclinal region of dermatogens and hypodermal layer.
9. The entire shoot apex is covered by dermatogen.

10. The uniform isodiametric or only slightly elongated non-vacuolated cells are arranged in no striking arrangement in the central core.
11. At the apical part of the hypodermis, several large cells are arranged.
12. Under the cells of apical part lie small cells with capricious nuclei.
13. There is little distinction in cells at the tip of the apex.

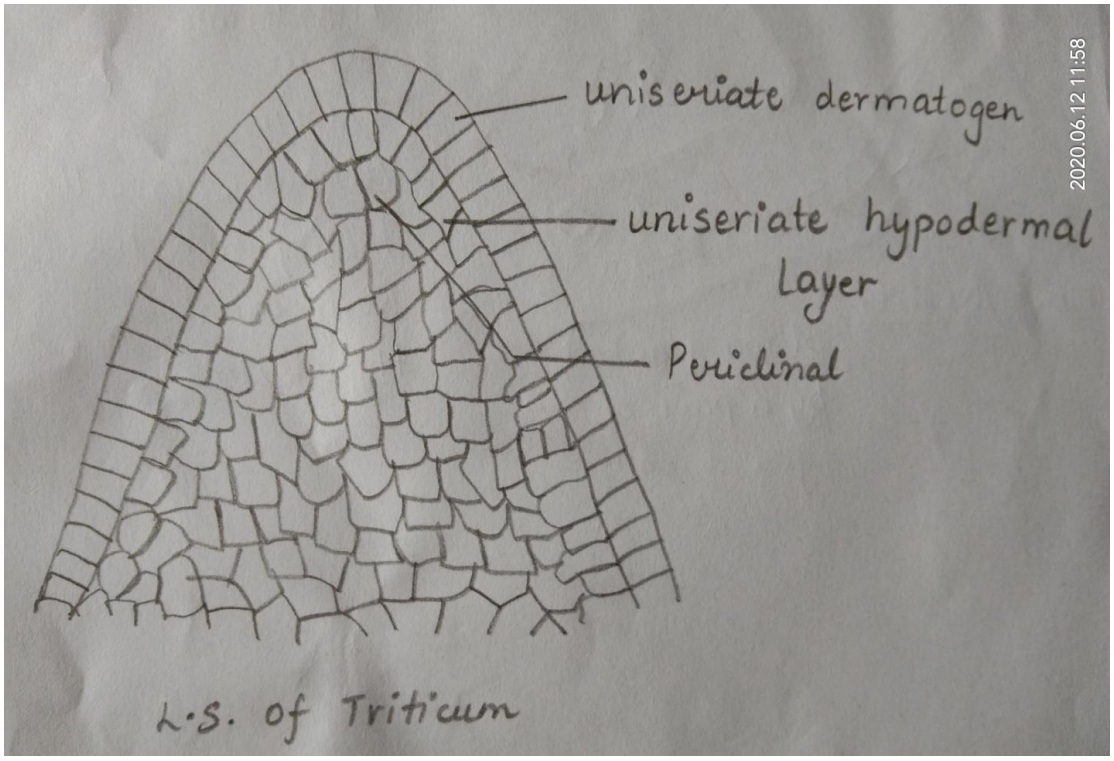


Fig.9.9: L.S. of the *Triticum* leaf primordial.

9.3.3.2- L.S. of leaf primordial of shoot apex of *Coleus* (Fig 9.10 & 9.11)

1. Hairs like structures or projections are called Trichomes, covering the root surface.
2. The centre part of shoot apex is made up of small, closely packed cells known as Shoot Apical Meristem (SAM).
3. Two leaf primordial are present on either side of SAM which later develops into leaves.
4. The centre of leaf primordial is made up of small cells of procambium which later develops into vascular tissue.
5. The SAM is outlined by the protoderm cells.
6. The lignified cambium is present in cells.
7. Distinct xylem cells are present with lignified secondary wall.

8. The contracted protoplast is present is present in the xylem cells and hence it is called as contracted xylem.
9. The pattern of differentiation of contracted xylem is not quite visible but distinction is bit distinguished in distal region to the walled xylem.
10. Vascular cambium is evident due to the distinguished xylem parenchyma.

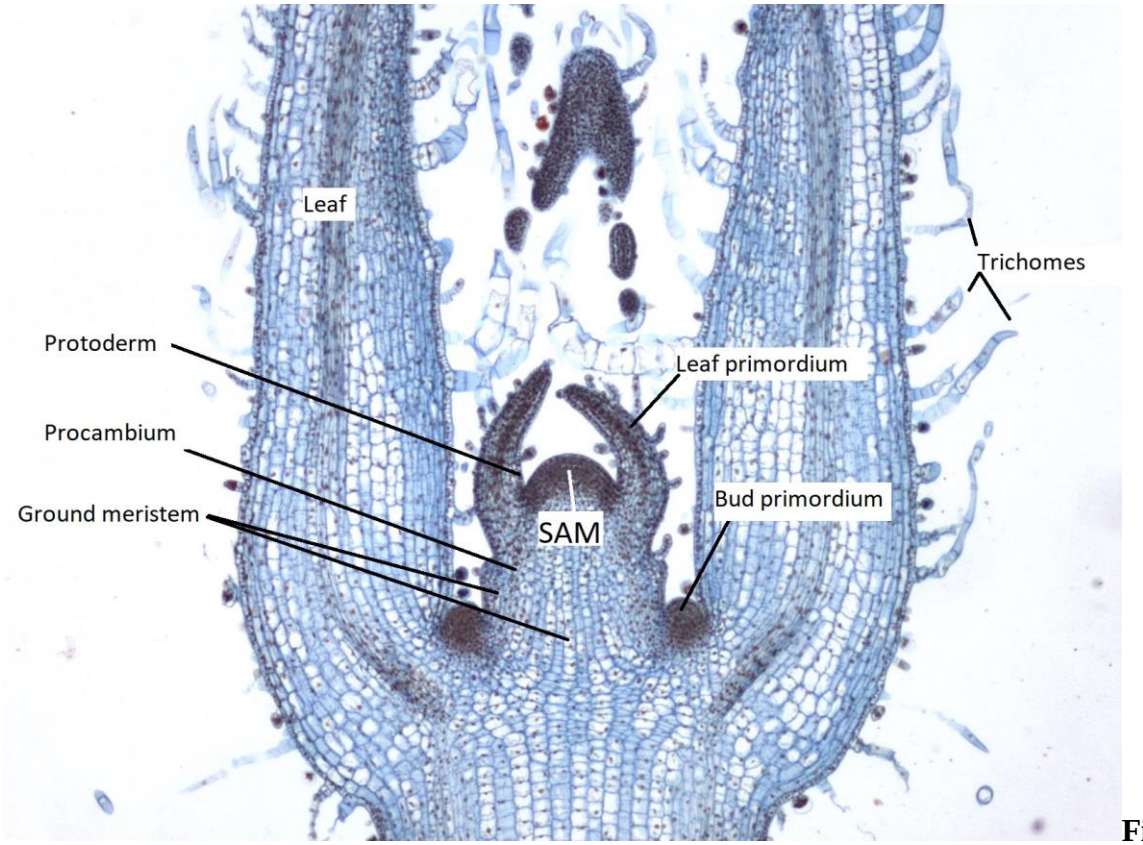


Fig.9.10: The longitudinal section of shoot apex of *Coelus* (https://bio.libretexts.org/Bookshelves/Botany/A_Photographic_Atlas_for_Botany_(Morrow)/12%3A_Stems/12.01%3A_Primary_Growth).

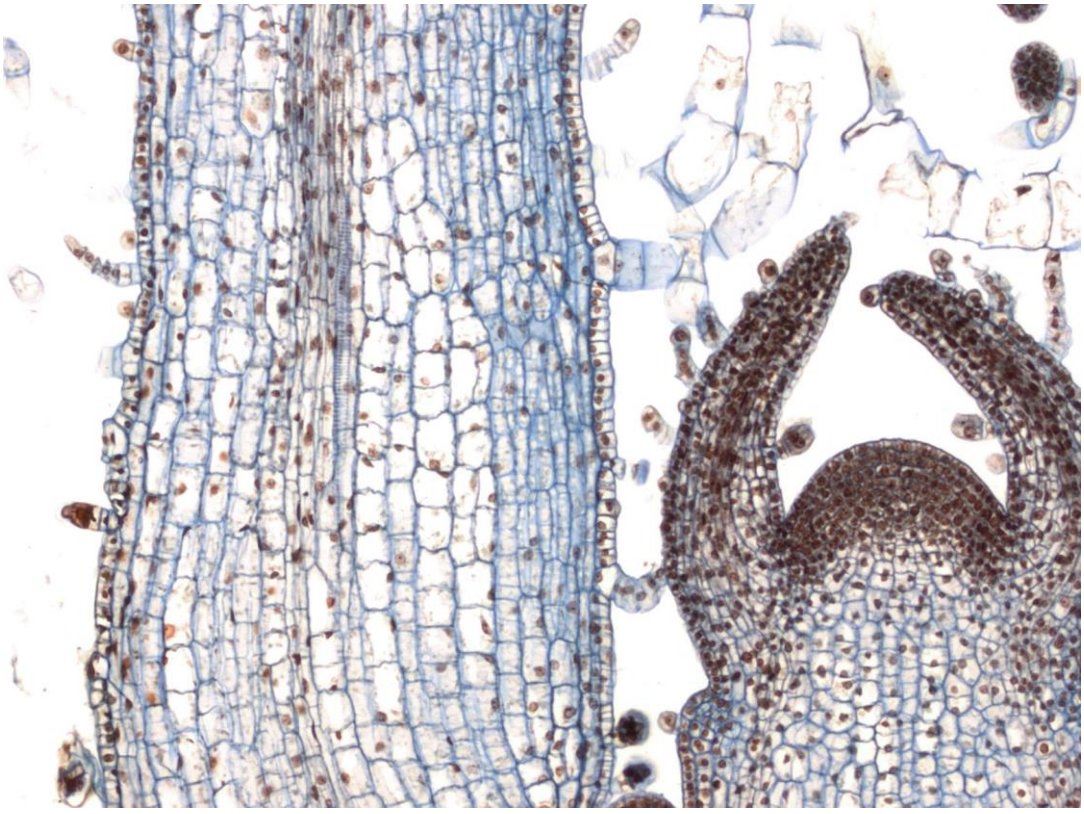


Fig.9.11: The closer view of *Coleus* shoot tip longitudinal section (https://bio.libretexts.org/Bookshelves/Botany/A_Photographic_Atlas_for_Botany_(Morrow)/12%3A_Stems/12.01%3A_Primary_Growth).

9.3.3.3- L.S. of leaf primordial of shoot apex of *Saccharum* (Fig 9.12)

1. The epidermal layer is present in the outermost surface called dermatogen.
2. The epidermis is interrupted by the stomata followed by hypodermis and then to sub-hypodermis.
3. Next to the epidermal layer, ground tissue is present.
4. They hypodermal layer is consisting of the sclerenchyma or sclerified parenchyma cells.
5. The vascular bundles are scattered and embedded in to the ground tissue.
6. They lack endodermal layer and pericycle.
7. The smaller bundles are present in the periphery but the larger bundles are present in the centre.
8. The bundles are collateral and closed, i.e., they lack cambium.
9. The vascular bundles are surrounded by the sclerenchymatous sheath.
10. The protoxylem and metaxylem are placed in the form of Y shape.

11. Phloem is made up of sieve tubes and companion cells.
12. The phloem lacks phloem parenchyma.

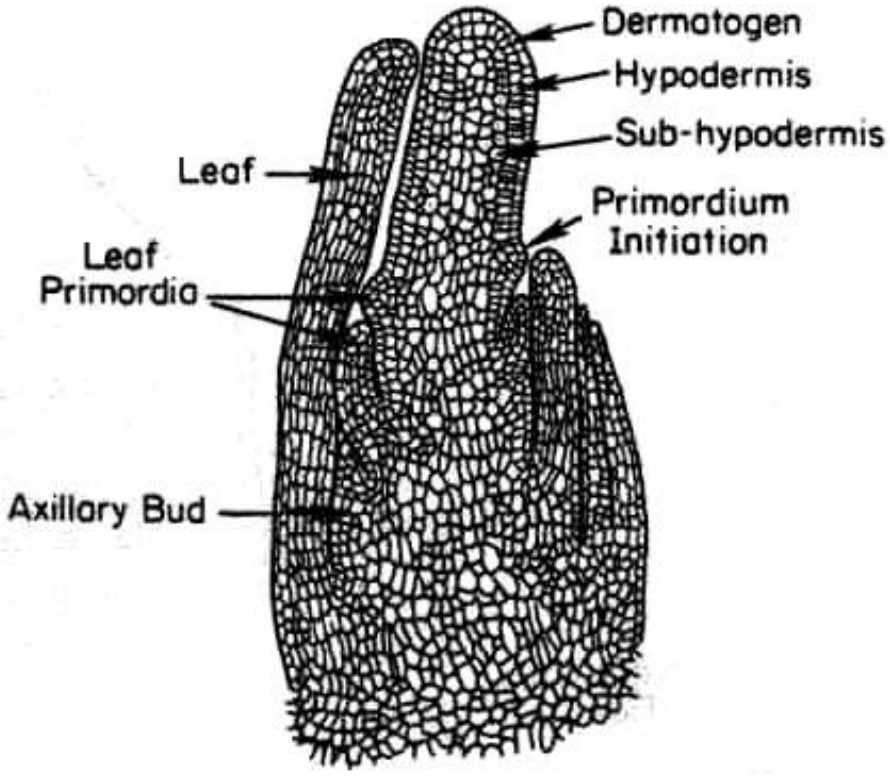


Fig.9.12: L.S. of *Saccharum* leaf primordial.

9.4- SUMMARY

Shoot apex consists of meristematic cells. The parenchymal cells are involved in forming the outermost layer of shoot apex which is called epidermis. Cuticle covers the epidermal surface. The L.S. of shoot apex is distinguished into the regions of tunica, corpus, flank meristem and rib-meristem. The meristematic cells divide anticlinially whereas the cells of tunica region divide periclinally. Trichomes or hairs are generally absent. Epidermis is followed by hypodermis which is made up of sclerenchymatous cells. The vascular bundles contain xylem and phloem but lack cambium.

Root apex has abundant meristematic cells which divide rapidly. The cells at the tip are protected by root cap meristem cells which protects the growing tip. Similar to shoot apex, the outermost

layer is called epidermis which is followed by exodermis, central cortex and endodermis. These different cells are arranged in various ties to form the tissue.

The leaf primordial on the shoot apex has a covering of epidermis made up of meristematic cells. The epidermis is followed by the cells of ground tissue. The vascular bundles are present in abundance and the structure supports the development of stomata structure within them.

9.5- GLOSSARY

Anticlinial- It means occurring at right angles to the surface or circumference of a plant organ an anticlinal pattern of cell walls.

Biseriate- It is a botanical term meaning arranged in two rows.

Capricious- It refers to something which is unpredictable.

Flank- It means be on each or one side of.

Geotropic- It is the directional growth of an organism in response to gravity. Roots display positive geotropism when they grow downwards, while shoots display negative geotropism when they grow upwards. It is also called gravitropism.

Periphery- It refers the outer limits or edge of something.

Perennial- It means living for several years.

Periclinial- It means parallel to the surface of the meristem.

Perpetuate- To be continuing indefinitely.

Phototropic- Phototropism is the growth of an organism in response to a light stimulus.

Thimble- It is a small object with a closed end used for the protection.

Uniseriate- It refers the arrangement (of something) in single row.

9.6.- SELF ASSESSMENT QUESTIONS

1. The vascular bundles of *Saccharum* lack _____.
2. The meristematic cells of shoot meristem divide through _____division.
3. Suberin lamella deposition in long cells of root apex of allium acts as _.
4. The tunica layer of smilax is _____.
5. The tunica region of carex is _____.
6. The entire shoot apex of *triticum* is covered by _____.
7. The central region of shoot meristem of datura is called _____.
8. The peripheral region of the shoot meristem tissue of Datura is called _____.
9. The _____ is formed by inner protoxylem vessel and parenchyma break down in *Zea*.

10. Vascular cambium of coeus is evident due to the distinguished _____.

9.6.1- ANSWER TO THE SELF ASSESSMENT QUESTIONS

1. cambium
2. anticlinial
3. plasmodesmata
4. Biseriate
5. Uniseriate
6. dermatogens
7. corpus
8. tunica
9. xylem parenchyma
10. Sclerenchyma, sclerified parenchyma cells

9.7- REFERENCES

1. *Invasive Plant Compendium. "Canna indica (canna lilly)"* (2014).
2. *Rice Knowledge Bank. "Leptochloa chinensis".* (2015).
3. *www.efloras.org. "Oryza in Flora of China @ efloras.org"* (2016).
4. *www.tropicos.org. "Tropicos | Name – Oryza L."* (2016).
5. [http://wire.ndsu.nodak.edu/] Wheat Information Resource (WIRE)
6. Angiosperm Phylogeny Group. (2009). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society*, *161*(2), 105-121.
7. Bostrack, J. M., & Struckmeyer, B. E. (1967). Effect of gibberellic acid on the growth and anatomy of *Coleus blumei*, *Antirrhinum majus* and *Salvia splendens*. *New Phytologist*, *66*(4), 539-544.
8. Briske, D. D. (1991). Developmental morphology and physiology of grasses. *Grazing management: an ecological perspective*, *85*, 108.
9. Christenhusz, M. J., & Byng, J. W. (2016). The number of known plants species in the world and its annual increase. *Phytotaxa*, *261*(3), 201-217.
10. Cooke, I. (2001). *Gardener's guide to growing cannas*. David & Charles.
11. Ha, C. M., Jun, J. H., & Fletcher, J. C. (2010). Shoot apical meristem form and function. *Current topics in developmental biology*, *91*, 103-140.

12. Heywood, V. H., Moore, D. M., Richardson, I. B. K., & Stearn, W. T. (1993). *Flowering plants of the world* (No. 582.13 F644). Oxford university press.
13. http://www.biologydiscussion.com/plant-tissues/meristematic-tissue/meristematic-tissue-shoot-apex-and-root-apex-plant-cell/13772
14. http://www.biologydiscussion.com/shoot-system/studying-shoot-apex-and-root-apex-with-diagram/34054
15. http://www1.biologie.uni-hamburg.de/b-online/library/webb/BOT410/Stem/Stems/stems-3.htm
16. https://bio.libretexts.org/Bookshelves/Botany/A_Photographic_Atlas_for_Botany_(Morrow)/12%3A_Stems/12.01%3A_Primary_Growth
17. https://dissectionconnection.com.au/shop/downloads/labelled-microslides/plant-slides/allium-root-tip-100x/
18. https://naitc-api.usu.edu/media/uploads/2017/10/04/Anatomy_of_a_Wheat_Plant.pdf
19. https://www.biologydiscussion.com/shootsystem/stems/dicotyledonousstems/dicotyledonous-stem-and-monocotyledonous-stem-plants/69245
20. https://www.easybiologyclass.com/anatomical-difference-between-shoot-apex-and-root-apex/
21. https://www.illinoiswildflowers.info/grasses/plants/wheat.html#:~:text=Typical%20habitats%20of%20such%20plants,until%20perennial%20grasses%20become%20established.
22. https://www.jstage.jst.go.jp/article/pps1998/2/2/2_2_146/_article/-char/ja/
23. Jacobs, W. P., & Morrow, I. B. (1957). A quantitative study of xylem development in the vegetative shoot apex of *Coleus*. *American Journal of Botany*, 823-842.
24. Kellogg, E. A. (2009). The evolutionary history of Ehrhartoideae, Oryzeae, and *Oryza*. *Rice*, *2*(1), 1-14.
25. Khoshoo, T. N., & Guha, I. (1976). Origin and evolution of cultivated *Cannas*. *Glimpses in Plant Research*.
26. Lersten, N. R. (1987). Morphology and anatomy of the wheat plant. In 'Wheat and wheat improvement'.(Ed. EG Heyne) pp. 33–75. *Crop Science Society of America: Madison, WI*. Linnaeus, C. (1753). Carl von (1753). *Species plantarum*, *2*, 782.
27. Martin, B. F., & Tucker, S. C. (1985). Developmental studies in *Smilax* (Liliaceae). I. Organography and the shoot apex. *American Journal of Botany*, *72*(1), 66-74.

28. Opatrná, J., Seidlová, F., & Beneš, K. (1964). The anatomy of the shoot apex of wheat (*Triticutn aestivum* L.) during transition from the vegetative to the reproductive state and the determination of the primordia. *Biologia Plantarum*, *6*(3), 219-225.
29. Pasternak, T., Haser, T., Falk, T., Ronneberger, O., Palme, K., & Otten, L. (2017). A 3D digital atlas of the Nicotiana tabacum root tip and its use to investigate changes in the root apical meristem induced by the *Agrobacterium* 6b oncogene. *The Plant Journal*, *92*(1), 31-42.
30. Rottoli, M., & Castiglioni, E. (2011). Plant offerings from Roman cremations in northern Italy: a review. *Vegetation History and Archaeobotany*, *20*(5), 495-506.
31. Satina, S., Blakeslee, A. F., & Avery, A. G. (1940). Demonstration of the three germ layers in the shoot apex of *Datura* by means of induced polyploidy in periclinal chimeras. *American Journal of Botany*, *27*(10), 895-905.
32. Sharman, B. C. (1983). Developmental anatomy of the inflorescence of bread wheat (*Triticum aestivum* L.) during normal initiation and when affected by 2, 4-D. *Annals of Botany*, *52*(5), 621-639.
33. Soukup, A., Seago Jr, J. L., & Votrubová, O. (2005). Developmental anatomy of the root cortex of the basal monocotyledon, *Acorus calamus* (Acorales, Acoraceae). *Annals of Botany*, *96*(3), 379-385.
34. Stant, M. Y. (1952). The shoot apex of some monocotyledons: I. Structure and development. *Annals of Botany*, *16*(1), 115-129.
35. Tanaka, N. (2001). Taxonomic revision of the family Cannaceae in the New World and Asia. *Makinoa, new series*, *1*, 1-74.
36. Thomas, B., Murphy, D. J., & Murray, B. G. (2016). *Encyclopedia of applied plant sciences*. Academic Press.
37. [USDA, ARS] US Department of Agriculture, Agricultural Research Service. (2011). National Genetic Resources Program, Germplasm Resources Information Network (GRIN) Online Database.
38. Waduwara, I. (2007). *Onion root anatomy and the uptake of sulphate and phosphate ions*. University of Waterloo.

9.8- SUGGESTED READINGS

1. Lyndon, R. F. (1998). The shoot apical meristem: its growth and development. Cambridge University Press.
2. Francis, D. (1999). The Shoot Apical Meristem. Its Growth and Development.
3. Dickison, W. C. (2000). Integrative plant anatomy. Academic press.
4. Maiti, R. (2012). Crop plant anatomy. Cabi.

9.9- TERMINAL QUESTIONS

1. What are the characteristics of root apices of *Allium*?
2. Describe the features of L.S. of shoot apices of *Triticum*.
3. Explain in detail about the L.S. of shoot apical meristem of *Datura* with well labelled diagram.
4. Describe the leaf primordial of *Saccharum*.

9.9.1- Short Answer Type Questions

1. Draw the longitudinal section of primordial of *Datura*.
2. Give a brief detail of L.S. of *Coleus* leaf primordial.
3. What are the characteristics of shoot apex of *Carex*.
4. Describe the characteristics of L.S. of *Smilax* shoot apex.

9.9.2- Long Answer Type Questions

1. Provide a detail insight of anatomy of *Triticum* shoot apex.
2. Explain the anatomy of *Allium*.
3. Differentiate between the anatomical structure of *Allium* and *Nicotiana* with well-labeled diagram.

UNIT-10 STUDY OF ALTERNATE AND DISTICHOUS, ALTERNATE AND SUPERPOSED, OPPOSITE AND SUPERPOSED OPPOSITE AND DECUSSATE LEAF ARRANGEMENTS AND EXAMINATION OF ROSETTE PLANTS AND INDUCTION OF BOLTINGS

10.1- Objectives

10.2- Introduction

10.3- Study of alternate and distichous, alternate and superposed, opposite and superposed opposite and decussate leaf arrangements

10.4- Examination of rosette plants and induction of bolting

10.5- Summary

10.6- Glossary

10.7- Self Assessment Question

10.8- References

10.9- Suggested Readings

10.10- Terminal Questions

10.1 OBJECTIVES

After reading this unit students will be able to-

- Understand the concept of phyllotaxy.
- Understand the bolting phenomenon in plants.
- Understand the alternate and distichous, alternate and superposed, opposite and superposed, opposite and decussate leaf arrangements.
- Understand the rosette plants.
- Understand the induction of bolting.

10.2 INTRODUCTION

Phyllotaxy is the mode of arrangement of leaves on the stem. It is surprising how regular and strictly mathematical this is. As a stem grows at its apex, new leaf buds form along the stem by a highly controlled developmental process. Leaf arrangement along the plant stem depends on the species and is an important identifying characteristic. The function of the arrangement of leaves (phyllotaxy) is to increase a plant's ability to carry on photosynthesis and other physiological activity like transpiration etc, it is done by positioning the leaves in such manner to maximize the surface area available to capture radiation. Leaves may be either caulescent (with obvious stems) or acaulescent (without obvious stems). Leaf is a flattened lateral appendage of stem specialized for food production. On the basis of development and origin leaves may be categorized as:

i. Seed leaves or cotyledons present on the seed.

ii. Foliage leaves: Flat, green, lateral appendages developing on stem or branch.

iii. Scaly leaves: Scale like, small, stalkless, non- green leaf. These are also called cataphylls.

iv. Bract leaves or hypsophylls: These are present at the base of flower of inflorescence.

v. Prophylls: These are bracteoles.

vi. Ligules: These are tongue like small parts present at the upper end of leaf sheath.

vii. Floral leaves: Calyx, corolla, stamens and carpels.

viii. Sporophylls: Spore bearing leaves.

Leaves are arranged on the stem in a definite manner of a particular species which are controlled genetically. The arrangement is always in regular manner by obeying order of genes and leaves are never placed on the stem in a haphazard manner. The foliage leaves are usually produced on the axis with long or short internodes between them. This arrangement is called cauline. In some

cases, however, the leaves arise in a cluster from the very short stem just on the top of the root e.g. carrot, radish etc called rosette. This arrangement is called radical although the leaves do not actually arise from the root but from a very much condensed stem as in the case of radish, turnip etc. In cauline leaves there may be one, two, three or more leaves at each node. On this manner phyllotaxy is divided in two categories: alternate or acyclic and cyclic. When there is only one leaf attached on the node the arrangement is spiral or alternate or acyclic. When there are two or more leaves arranged at each node of the stem then phyllotaxy is called cyclic.

10.3 STUDY OF ALTERNATE AND DISTICHOUS, ALTERNATE AND SUPERPOSED, OPPOSITE AND SUPERPOSED OPPOSITE AND DECUSSATE LEAF ARRANGEMENTS

Leaves arrange on the stem in two manners thus forms two types of phyllotaxy: (1) Cyclic Phyllotaxy and (2) Spiral or Alternate or Acyclic Phyllotaxy.

A. Cyclic Phyllotaxy:

In the cyclic type of phyllotaxy the leaves at each node form a whorl with the leaves placed on a circle in which the angles between adjacent leaves are the same. Cyclic phyllotaxy includes both opposite and whorled type phyllotaxy.

(I) Opposite Phyllotaxy: According to Snow and Snow in opposite phyllotaxy the two leaves at each node are arranged as opposite to one another. If the successive pairs of leaves be placed at right angles to one another, the arrangement is termed opposite decussate. In this case, when looked from the top, all the leaves will be found to be arranged along four vertical rows. This is found in *Calotropis*, *Gardenia*, *Ixora*, *Ocimum, Mentha* etc. In some other plants it is found that the successive pairs are placed exactly on top of one another so that all the leaves lie in one plane and when viewed from above all the leaves are found to lie in two vertical rows. This type of phyllotaxy is known as opposite superposed . It should be noted that while the superposed type is found in many plants like *Psidium gujava* (guava), *Combretum* etc., in many instances the phyllotaxy is initially decussate which later becomes superposed by the flattening out of the twig.

(II) Verticillate Phyllotaxy or Whorled Phyllotaxy:

Oleander plant (*Nerium indicum*) shows three leaves forming a whorl at each node while Saptparni (*Alstonia scholaris*) shows five or more leaves. These are instances of the verticillate type. Sometimes verticillate phyllotaxy is designated as whorled phyllotaxy. Thus, if there be

two leaves in a whorl, the two will be placed opposite (i.e., at an angular distance of two right angles) one another. If there be three leaves, the angle between leaves in the same whorl is 120° (i.e., one third of a circle), if four, it is 90° and so on.

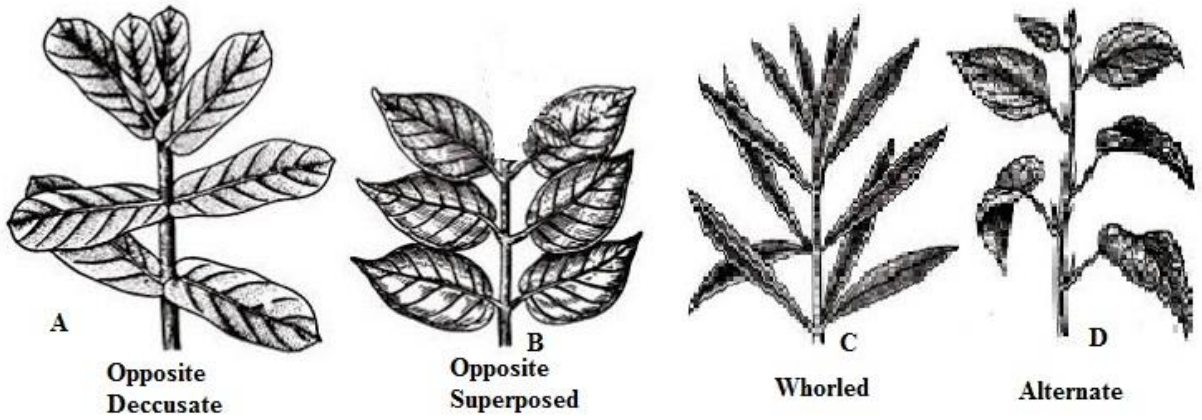


Fig.1 (A-D): Different types of phyllotaxies

B. Spiral or Alternate or Acyclic Phyllotaxy:

This is the common type phyllotaxy found in plants and the mathematical regularity of the arrangement is astonishing. One can easily verify that (1) the angular distance (angular divergence) between any two consecutive leaves is constant; (2) when looked from the top, all the leaves are found to lie in a fixed number of vertical rows or orthostichies; (3) the vertical lines (hypothetical) are evenly dispersed on a circle, the angle between adjacent orthostichies being constant; (4) although leaves look scattered, a close examination shows that leaves are evenly dispersed on all sides of the stem. In spiral phyllotaxy one has to imagine a line touching the bases of successive leaves. It will be found that this line forms a spiral (hence the name spiral phyllotaxy) on the stem. This spiral is called the genetic spiral. This genetic spiral can easily be projected on a flat surface to form a fiat spiral and the position of the leaves may be marked on this spiral. The angle subtended at the centre by two consecutive leaves is the angular divergence. In practice, the angular divergence may be denoted by finding out a leaf which is exactly above a particular leaf, then by counting the number of complete circles covered by the genetic spiral and the number of leaves beginning from the first to the one just before the last. The latter number of leaves-also gives the number of orthostichies. Thus, sometimes, only the above fraction is given without calculating the actual divergence in degrees.

(1) Distichous or ½ Phyllotaxy:

In the family Poaceae (e.g., *Cynodon, Oryza*) the leaf on the second node is just opposite of the leaf on the first node, the third leaf is above the first leaf, the fourth above the second and so on. Thus, the angular divergence is clearly 180° (straight angle) and all the leaves fall in two opposite orthostichies (hence, the name distichous). The genetic spiral covers only one circle and there are two leaves on the way in coming to the leaf exactly above the first leaf. Hence, the angular divergence = 1 (Circle)/2 (orthostichies) of 360º = 180º. As only the fraction is usually given, the phyllotaxy is also called ½ phyllotaxy.

$$Angular\ Divergence = \frac{1(Circle)}{2\ (Orthostichies)}$$

(2) Tristichous or 1/3 Phyllotaxy:

In *Cyperus rotundus* (Sedge) the leaves are three-ranked (three orthostichies); the fourth leaf is above the first leaf, fifth above second, sixth above third and so on. Three leaves lie in one circle of the genetic spiral. Hence, angular divergence = 1/3 of 360° = 120° or 1/3 phyllotaxy.

(3) Pentastichous or 2/5 Phyllotaxy:

In some plants like the *Hibiscus* and *Ficus bengalensis*, the sixth leaf is found above the first leaf, seventh leaf above the second one and so on. The genetic spiral completes two circles in passing these five leaves or five orthostichies . Hence, angular divergence = 2/5 of 360° = 144° or 2/5 phyllotaxy.

(4) Octastichous or 3/8 Phyllotaxy:

In this case the ninth leaf is found above the first leaf and the genetic spiral completes three circles in this distance as in the case of the *Carica papaya* of family caricaceae. Thus, there are eight orthostichies. The angular divergence is 3/8 of 360°= 135º.

It is rather interesting to note that in the common types of phyllotaxy the fractions of angular divergence lie in a series in which the numerator and the denominator in each case are obtained by adding up the numerators and denominators of the two preceding phyllotaxies. Thus, ½, $\frac{1}{3}, \frac{2}{5}, \frac{3}{8}, \frac{5}{13}, \frac{8}{21}$ .... This series is called the fibonacci series or the Schimper-Brown series of divergence. When the divergence becomes a big fraction it is not possible to count the orthostichies.

In plants showing these phyllotaxies, usually the internodes are very short so that the leaves are much crowded together. On date and other palms with persistent leaf bases showing such complex phyllotaxy one very close spiral of leaf bases is observed. This type of phyllotaxy is called parastichous . The sporophylls on a pine cone are also arranged in the same way and the parastichous arrangement can be seen clearly when the cone is viewed from above.

Besides the fibonacci series, which is by far the commonest, there are possibly some other series of phyllotaxy. Two such rare series 1/4, 1/5, 2/8, 3/14…. and 1/2, 2/8, 2/5, 5/8, 8/18…The laws of phyllotaxy may sometimes be disturbed by some twisting of the stem or by incomplete development as towards the apex of a twig.

10.4 EXAMINATION OF ROSETTE PLANTS AND INDUCTION OF BOLTING

A **rosette** is manner of leaf arrangement of plants in which leaves or of structures resembling leaves arrange in a circular manner. In angiospermic plants, rosettes usually sit near the soil. **Rosette** structure is an example of a modified stem in which the internodes are highly compressed and gaps between the leaves do not expand, so that all the leaves remain clustered tightly together and at a similar height. If a plant grows in a rosette form, the leaves will radiate from the center stalk either right at ground level or close to the ground. The term rosette is used because the pattern resembles the habit of a rose's flower. Many types of plant grow in a rosette pattern.

Generally rosettes are found in perennial plants whose upper foliage dies back with the remaining vegetation protecting the plant. Many perennials begin life as a rosette but progress to become more shrub like. Perennials and biennials benefit from the early rosette formation because it exposes as many leaves as possible to the sun, while maintaining a low profile to avoid being eaten by browsing animals. Most succulents that form rosettes maintain that form their entire lives. The rosette formation allows for maximum exposure to the sun while allowing the plants to capture and direct moisture toward the roots. Most succulents come from arid areas where thick leaves allow them to retain water.

Another form occurs when internodes along a stem are shortened, bringing the leaves closer together, as in *Lactuca sativa* (lettuce), ***Taraxacum officinale*** (dandelion) and some succulents. When plants such as lettuce grow too quickly, the stem lengthens instead, a condition known as

bolting. In some cases, the rosette persists at the base of the plant (such as the dandelion), and there is a taproot system.

Bolting is the elongation of internodes or production of a flowering stem. These flowering stems are usually vigorous extensions of existing leaf-bearing stems, and in order to produce them, a plant diverts resources away from producing the edible parts such as leaves or roots, resulting in flavor and texture changes, withering, and in general, a poor quality harvest. Plants that have produced flowering stems in this way are said to have **bolted**. Crops inclined to bolt include *Lactuca sativa* (lettuce), *Beta vulgaris* (beetroot), *Brassicas, Alium* (onion) etc.

According to Rood *et. al.*,(1989) bolting is induced by plant hormones of the gibberellin family, can occur as a result of several factors, including changes in Photoperiod (day length), the prevalence of high temperatures at particular stages in a plant's growth cycle and the existence of stresses such as insufficient water or minerals. These factors may interact in a complex way. According to Zeevaart (1971) photoperiod may affect the propensity to bolt thus some plants are "long day plants", some are "short day plants" and some are "day neutral", for example when a long day plant, such as spinach, experiences increasingly long days that reach a particular length, it will be inclined to bolt. Low or high temperatures can affect the propensity of some plants to bolt if they are experienced for sufficient periods at particular points in the life cycle of the plant; once these conditions have been met, plants that require such a trigger will subsequently bolt regardless of subsequent temperatures. Plants under stress may respond by bolting so that they can produce seeds before they die.

The essential cause of plant blotting is the premature production of a flowering stems on a plant before it can be harvested. Bolting is the plant's natural attempt to produce seeds so it can reproduce. It occurs when the weather heats up. The extensions are its leaf-bearing parts. Bolting is also known as going to seed. The term bolting is typically used with regard to vegetable gardening. Most of plants bolt due to hot weather. When the ground temperature goes above a certain temperature, this flips a switch in the plant to produce flowers and seeds very rapidly and to abandon leaf growth almost completely.

Gibberellins stimulate stem growth in dwarf and rosette plants. Applied gibberellin promotes elongation of internodes in a wide range of plant species. However, the most dramatic stimulations are seen in dwarf and rosette species, as well as members of the grasses. Exogenous gibberellin causes such extreme stem elongation in dwarf plants that they resemble the tallest

varieties of the same species. Accompanying this effect are a decrease in stem thickness, a decrease in leaf size, and a pale green color of the leaves. Some plants assume a rosette form in short days and undergo shoot elongation and flowering only in long days. Gibberellin application results in bolting in plants kept in short days, and normal bolting is regulated by endogenous gibberellin especially rosette species. Gibberellin is thus a component of the flowering stimulus in some plants.

10.5 SUMMARY

1. Phyllotaxy is the mode of arrangement of leaves on the stem. It is surprising how regular and strictly mathematical this is.
2. Leaf arrangement along the plant stem depends on the species and is an important identifying characteristic.
3. The function of the arrangement of leaves (phyllotaxy) is to increase a plant's ability to carry on photosynthesis and other physiological activity like transpiration, etc.
4. Leaves may be either caulescent (with obvious stems) or acaulescent (without obvious stems). Leaf is flattened lateral appendage of stem specialized.
5. In some cases, however, the leaves arise in a cluster from the very short stem just on the top of the root e.g. carrot, radish etc., called rosette.
6. Leaves arrange on the stem, two manners thus forms two types of phyllotaxies: (1) Cyclic Phyllotaxy and (2) Spiral or Alternate or Acyclic Phyllotaxy.
7. In the cyclic type of phyllotaxy the leaves at each node form a whorl with the leaves placed on a circle in which the angles between adjacent leaves are the same.
8. A **rosette** is a manner of leaf arrangement of plants in which leaves or of structures resembling leaves arrange in a circular manner.
9. R**osette** structure is an example of a modified stem in which the internodes are highly compressed and gaps between the leaves do not expand, so that all the leaves remain clustered tightly together and at a similar height.
10. Generally rosettes are found in perennial plants whose upper foliage dies back with the remaining vegetation protecting the plant.
11. When plants such as lettuce grow too quickly, the stem lengthens instead, a condition known as bolting.

12. Bolting is the elongation of internodes or production of a flowering stem
13. According to Sood et. al., bolting is induced by plant hormones of the gibberellin family and can occur due to several factors, including changes in photoperiod (day length), the prevalence of high temperatures at particular stages in a plant's growth cycle, and the existence of stresses such as insufficient water or minerals.

10.6 GLOSSARY

Phyllotaxy: Arramgement or pattern of leaf attachment on stem.

Bolting: Elongation of internode or floral axis.

Cataphyll: A simplified leaf form, as a bud scale or a scale on a cotyledon or rhizome.

Cauline: Leaves growing on a stem especially on the upper part of a stem.

Cotyledons: Primary or rudimentary leaf of the embryo of seed plants.

Gibberellins: Gibberellins are plant hormones that regulate various developmental processes, including stem elongation.

Hypsophylls: A floral leaf beneath the sporophylls.

Ligule: A thin, membranous outgrowth from the base of the blade of most grasses

Perennial: Perennial plants are those that continue to grow year after year.

Rosette: A leaf rosette is a plant growth habit in which a plant grows a cluster of leaves in a circular pattern.

10.7 SELF ASESSMENT QUESTIONS

10.7.1 Multiple Choice Questions:

1. The bolting in plants caused by

(a) Gibberellins (b) Auxins

(c) Cytokinins (d) Ethylene

2. Premature stem elongation of plants is called

(a) Vernalisation (b) Bolting

(c) Elongation (d) Flowering

3. Arrangement of leaves on stem is called

(a) Aestivation (b) Phyllotaxy

(c) Inflorescence (d) Phototaxis

4. The leaves arise in a cluster from the very short stem just on the top of the root

(a) Cyclic (b) Opposite

(c) Rosette (d) None of these

5. Photoperiod is associated with which phenomenon in plants

(a) Phototaxis (b) Bolting

(c) Phyllotaxy (d) All

6. Primary or rudimentary leaf of the embryo of seed plants

(a) Cataphylls (b) Cotyledons

(c) Sporophylls (d) None of the above

7. Spore bearing leaves

(a) Cataphylls (b) Cotyledons

(c) Sporophylls (d) None of the above

8. *Taraxacum officinale* leaves are example of

(a) Opposite phyllotaxy (b) Rosette

(c) Spiral phyllotaxy (d) Cyclic phyllotaxy

9. The other name for fibonacci series ($\frac{1}{3}, \frac{2}{5}, \frac{3}{8}, \frac{5}{13}, \frac{8}{21} \ldots$) is

(a) Schimper-Brown series (b) Goldman series

(c) Lehman series (d) Fabnori series

10. Bolting can be induced by hormone

(a) Auxin (b) Cytokinin

(c) Ethylene (d) Gibberelins

10.7.2 Fill in the blanks:

(1) Premature stem elongation in plants is called _____.

(2) Photoperiod is associated with _____ phenomenon in plants.

(3) Bolting can be induced by hormone _____.

(4) $\frac{1}{3}, \frac{2}{5}, \frac{3}{8}, \frac{5}{13}, \frac{8}{21}$ ....This series in phyllotaxy is called the 'Fibonacci Series' or the_____.

(5) Spore bearing leaves are called _____.

(6) 3/8 phyllotaxy is also called_____.

(7) Pentastichous is _____ phyllotaxy.

(8) _____structure is an example of a modified stem in which the internodes are highly compressed.

(9) Arrangement of leaves on stem is called _____.

(10) Oleander plant (*Nerium indicum*) shows three leaves forming a whorl, such type of phyllotaxy is called_____.

10.7. 3 True or False:

(1) Bolting can be induced by auxin hormone.

(2) Pentastichous is 3/8 phyllotaxy.

(3) Premature stem elongation of stems of plants is called rosette.

(4) Premature stem elongation of stems of plants is called bolting.

(5) Photoperiod is associated with bolting phenomenon in plants.

(6) *Taraxacum officinale* (Dandelion) leaves are example of verticellate phyllotaxy.

(7) Oleander plant (*Nerium indicum*) shows three leaves forming a whorl; such type of phyllotaxy is called verticellate phyllotaxy.

(8) 3/8 phyllotaxy is also called octastichous.

(9) A rosette is manner of leaf arrangement of plants in which leaves or of structures resembling leaves arrange in a circular manner.

(10) Bolting is induced by plant hormones of the gibberellin family.

10.7.4 Very short answer questions:

(1) Define bolting.

(2) Which plant hormone is called bolting hormone?

(3) What is phyllotaxy?

(4) Define Schimper- Brown series in phyllotaxy.

(5) Define rosette habit of plants.

(6) What type of phyllotaxy is found in Oleander (*Nerium*) plant?

(7) Define orthostichies.

(8) What do you mean by octastichous condition?

(9) Define sporophylls.

(10) Define cataphylls.

10.7. 1 Answer key: 1-(a), 2-(b), 3-(b), 4-(c), 5-(b), 6-(b), 7-(c), 8-(b), 9-(a), 10-(d).

10.7.2 Answer key: 1-Bolting, 2-Bolting, 3-Gibberellin, 4-Schimper-Brown series 5- Sporophylls, 6- Octastichous, 7- 2/5, 8-Rosette, 9-Phyllotaxy, 10- Verticellate.

10.7.3 Answer key: 1-False, 2-False, 3-False, 4-True, 5-True, 6-False, 7-True, 8-True, 9-True, 10-True.

10.8 REFERENCES

- Rood B. S, Pearce D, Williams P.H and Pharis R. P. (1989). A gibberellin-deficient Brassica mutant-rosette, *Plant Physiology*. 89:482-487.
- Snow, M. and Snow, R. (1934). The interpretation of Phyllotaxis. *Biological Reviews*. 9 (1): 132-137.
- Zeevaart, J. A. D. (1971). Effects of Photoperiod on Growth Rate and Endogenous Gibberellins in the Long-Day Rosette Plant Spinach. *Plant Physiology*. 47 (6): 821–827.

10.9 SUGGESTED READINGS

- Roger V. Jean. 1994. Phyllotaxis : A Systematic Study in Plant Morphogenesis. Cambridge [England]; Cambridge University Press. New York.

- Lincoln Taiz and Eduardo Zeiger 2002. Plant Physiology, 3rd ed. Sinauer Associates Sunderland.

10.10 TERMINAL QUESTIONS

10.10.1 Short answer questions:

(1) Write short note on phyllotaxy.

(2) Describe rosette habit with examples.

(3) What do you understand by bolting?

(4) Describe Brown-Schimper series.

(5) Differentiate between octastichous and pentastichous condition.

(6) Differentiate between opposite and alternate phyllotaxy.

(7) Write short note on bolting.

(8) What do you understand by pentastichous condition?

(9) Write a note on bolting.

(10) Define cataphylls**.**

10.10.2 Long answer question:

(1) Describe phyllotaxy in detail.

(2) Differentiate between cyclic phyllotaxy and spiral phyllotaxy.

(3) Write a detailed note on ostastihies.

(4) Write a note about bolting and its cause.

(5) Describe cyclic phyllotaxy and its types.

UNIT-11 MICROSCOPICAL EXAMINATION OF VERTICAL SECTION OF LEAVES

11.1- Objectives

11.2- Introduction

11.3- Microscopical examination of vertical section of leaves

11.3.1- Gymnosperms

11.3.1.1- *Cycas*

11.3.1.2- *Pinus*

11.3.2- Dicotyledons

11.3.2.1- *Ficus*

11.3.2.2- *Nerium*

11.3.2.3- *Eucalyptus*

11.3.2.4- *Pyrus*

11.3.3- Monocotyledons

11.3.3.1- *Phoenix*

11.3.3.2- *Allium*

11.3.3.3- *Musa*

11.3.3.4-*Triticum*

11.3.3.5-*Oryza*

11.3.3.6-*Zea*

11.3.3.7-*Bambusa*

11.4- Summary

11.5- Glossary

11.6- Self Assessment Question

11.7- References

11.8- Suggested Readings

11.9- Terminal Questions

11.1 OBJECTIVES

1. To examine the vertical section of leaves microscopically.
2. To know about anatomically features of gymnosperms, dicot and monocot leaves

11.2 INTRODUCTION

The leaves in collection are called foliage, are the organ of vascular plant. It provides the major site for photosynthesis. Usually, the primary photosynthetic tissue, the palisade mesophyll is present in the upper side of the blade or lamina of leaf. Leaves can be petiolated or sessile. Vertical section of leaf contains stomata openings by which oxygen and carbon dioxide can be exchanged between plant and the atmosphere. Usually, the lower surface of leaf contains highest number of stomata in its epidermal layer. These openings in the epidermal layer contribute to the process of photosynthesis by exchanging gases and to block the transpiration so that loss of water can be minimized.

11.3.1-Gymnosperms

11.3.1.1-*Cycas*

Cycas belongs to the very ancient lineage of plants. They were maximum diverse around the Jurassic and Cretaceous periods. They are evergreen perennials and contain 113 species. One of the species *C. circinalis* is an endemic species in India and it is the first cycad species to be described in western literature. The species of this genus do not belong to the palms, ferns, trees or any other modern group of plants. Most of the species of *Cycas* became extinct during the extinction of non-avian dinosaurs.

Habit and Habitat: *Cycas* is known to originate from the medieval time and hence correct region of origin is difficult to determine. Most of the species are known to become extinct with the extinction of non-avian dinosaurs. These plants are abundant in the equatorial regions of eastern and southeastern Asia including the Philippines with 10 species (out of which 9 are endemic), eastern Africa (including Madagascar), northern Australia, Polynesia and Micronesia. Australia inhabits 26 species while the Indo-Chinese area inhabits 30 species. Also, India has 9 species. The northernmost species *C. revoluta* is found at 31°N in southern Japan. The southernmost *C. megacarpa* is found at 26°S in southeast Queensland. Due to the occurrence of large number of *Cycas* species in China, Australia and India, these countries are considered as centres of *Cycas* diversity.

V.S. of *Cycas* leaf (Fig 11.1)

1. The epidermis is the outermost layer which consists of cuticularised thick-walled cells.
2. The upper epidermis is continuous but lower epidermis is interrupted by the presence of sunken stomata.
3. The stomata are haplocheilic with two guard cells and 8-10 subsidiary cells arranged in ring in the guard cells border.
4. The 1-2 layered (3-4 layered at the margins) thick hypodermis is present below the epidermis which is made up of cholrenchyma and sclerenchyma.
5. The hypodermis is followed by the parenchymatous ground tissue with many mucilage canals.
6. The mesophylls are divided into the palisade and spongy parenchyma.
7. The palisade cells are present below the hypodermal layer and below the palisade cells; spongy parenchymatous cells are placed with intercellular spaces.
8. The vascular bundles are diploxylic and they consist of triangular centripetal exarch metaxylem and centrifugal endarch primary xylem.
9. The 3-4 layers of tracheid cells are present on either side of the centripetal metaxylem, in-between the upper palisade layer and lower spongy layer.
10. The phloem is present on abaxial side below the xylem.
11. The phloem constitutes from the sieve and parenchymatous cells.

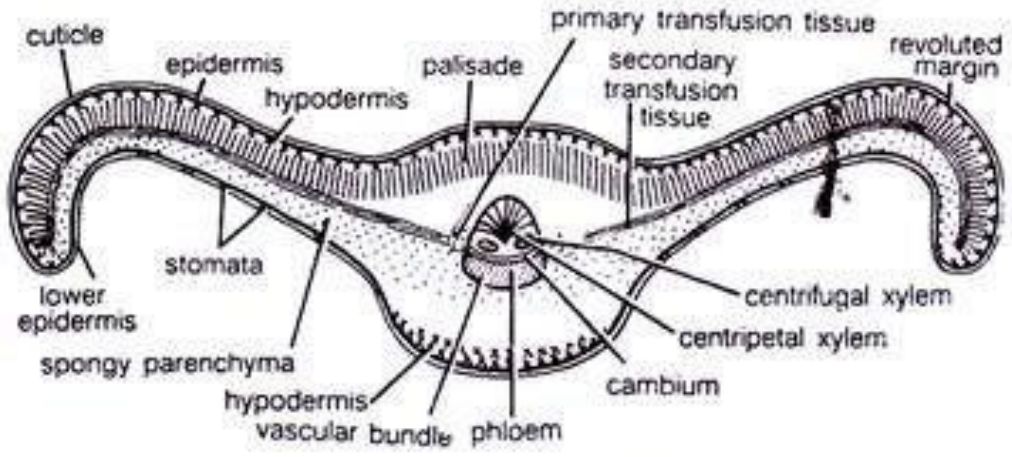


Fig.11.1: V.S. of *Cycas revoluta* leaf

11.3.1.2-*Pinus*

The *Pinus* belongs to the Pinaceae family, are conifers which contains 126 species names of pines, together with 35 unresolved species. They are also referred by lumber, derived from the pine trees. It is the sole genus which belongs to Pinoideae subfamily. All the species are listed in the Royal Botanic Gardens Kew and Missouri Botanical Garden.

Habit and Habitat:

Pines are native to the Northern Hemisphere and some parts of Southern Hemisphere. One of the species named, Sumatran pine crosses the equator in Sumatra to 2ºS. They are found in the far north as 66ºn to as far south as 12ºN, in the regions of North America. Although, pines are found in variety of environmental conditions which are ranged from semi-arid desert to rainforests, from sea level up to 5200 meters. They are dispersed from cold to hot environments on Earth. Some species are also introduced in temperate and subtropical regions of both hemispheres. They are also used as ornamental plants in parks and gardens.

V.S. of *Pinus* leaf (Fig 11.2)

1. It features a thick-walled epidermis with a single layer, dense cuticle.
2. Depressed stomata run the length of the surface (amphistomatic).
3. There are two guard cells and two subsidiary cells in each stoma.
4. It has two openings: one on the outside, termed the vestibule, and one on the interior, called the substomatal cavity.
5. Providing structural rigidity, a few layers of dense sclerenchymatous hypodermis are found beneath the epidermis.
6. Palisade and spongy parenchyma are not distinguishable.
7. Thin-walled, parenchymatous, polygonal, compactly packed cells with chloroplasts and starch grains make up one such section.
8. The inner surface has peg-like infoldings.
9. Single-layered endodermis with barrel-shaped cells and casparian strips surrounds it.
10. A multilayered pericycle with a T-shaped mass of sclerenchymatous cells amid two vascular bundles lies beneath endodermis.
11. Somewhat on side, there is transfusion tissue.
12. There are three types of bundles: collateral, open, and endarch.
13. Xylem is divided into tracheids but it lacks vessel members.

14. The parenchyma cells are modified to form albuminous cells.
15. The phloem tubes do not have direct interaction with companion cells.
16. The vessels are enclosed in transfusion tissue.
17. The vessels are consisting of parenchyma cells and tracheids.
18. The mesophyll cells are present in abundant and differentiated into the separate palisade and spongy mesophylls.
19. Casparian strips or suberin are present in endodermis layer at young stage of the leaf.
20. Suberins are arranged in radial fashion to create a barrier for water movement in the apoplast.

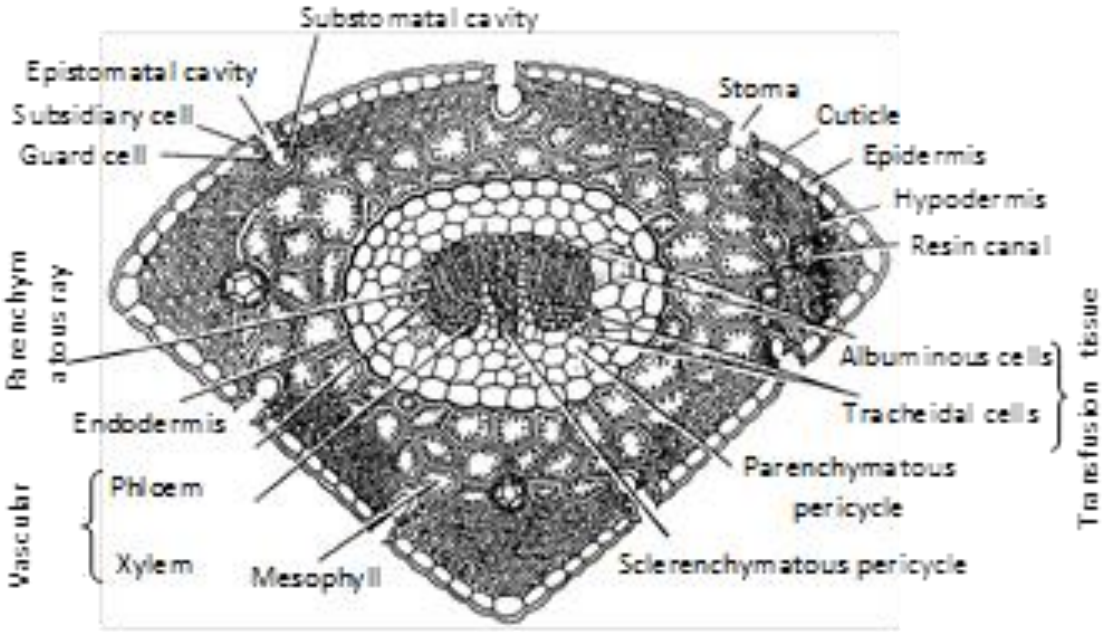


Fig.11.10: V.S. of *Pinus roxburgii* leaf (https://www.studyadda.com/notes/11th-class/biology/plant-kingdom/some-representative-rymnosperms/9569).

11.3.2-Dicot

11.3.2.1-Ficus

Ficus are the woody trees, shrubs, vines, epiphytes and hemiepiphytes. They belong to the Moraceae Family. It contains 850 species in total. They are widely known as Fig Trees or Figs. The fruits of most these species are edible but they are of low economic importance to human

and eaten as bush food. Regarding wildlife, they are considered as the extremely important food resources. They are also regarded as objects of worships and for many practical uses.

Habit and Habitat: Figs are abundant in Tropical Forest ecosystems. They are known to be native from the regions of the southwest Asia and the Mediterranean region, i.e., from Afghanistan to Portugal. They are known to carry great importance for wildlife by use by frugivores such as fruit bats and primates which include capuchin monkeys, langurs, gibbons and mangabeys. Also, birds like Asian barbets, pigeons, hornbills, fig-parrots and bulbuls are entirely dependent on figs when they are in abundant to survive.

V.S. of *Ficus* leaf (Fig 11.3)

1. The epidermis is composed of oval cells with undulating walls.
2. The mesophyll cells can be bifacil and isobilateral.
3. 3-5 rows of ordinary parenchyma cells are arranged in spongy layers.
4. The aerenchyma cells are also present in spongy layers.
5. The mid-rib cross-section is in quadrilateral shape.
6. The crescent shaped vascular bundles are present.
7. The vascular bundles are divided into regions of phloem and xylem.
8. Several layers of sclerenchyma cells are arranged around the vascular bundles.

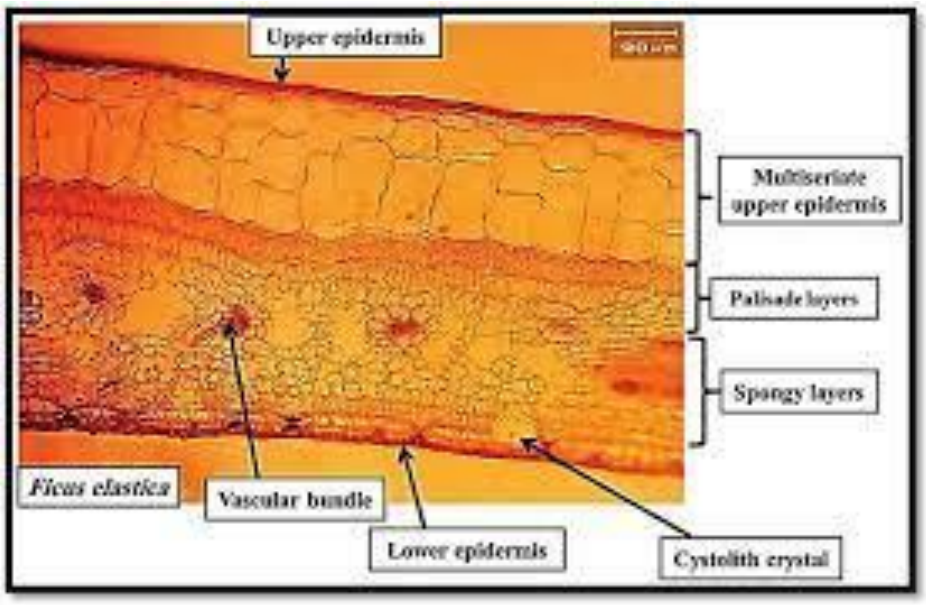


Fig.11.3: V.S. of *Ficus elastica* leaf (Shakir and Baji, 2016).

11.3.2.2- *Nerium*

Nerium contains the only single species, *N. oleander*. It is a small tree or shrub, which belongs to the Apocynaceae family. It is a widely cultivated plant. Its origin is supposed to be the regions of southwest Asia. This plant is also known for its poisonous properties but used as ornamentally grown garden plant.

Habit and Habitat: *Nerium* is native to the regions of Mauritania, Morocco and Portugal eastward through the Mediterranean region and the Sahara, to the Arabian Peninsula, Southern Asia and Southern parts of China. It is widely dispersed plant around the subtropical and tropical areas of the world. It is known to be planted around the stream beds in river valleys so that it can tolerate long seasons of drought and deluge from winter rains.

V.S. of *Nerium* leaf (Fig 11.4)

1. The leaf is dorsiventral and consists of upper and lower epidermis.
2. A thick cuticle coats the outside of leaf surface.
3. Cuticle is followed by the multi-layered (three-layered) upper epidermal cells and generally stomata are not found on this surface.
4. Two kinds of mesophyll cells are present which are called palisade and spongy cells.
5. The lower epidermis consists of a single row of barrel shaped cells, closely arranged and protected by cuticle.
6. The stomata are found in the depressions formed due to incurve of the lower epidermis.
7. Stomata are protected with trichomes.
8. Vascular system is well-developed.
9. Palisade cells are situated below the epidermal cell layers.
10. Spongy tissue is followed by palisade tissue.
11. Spongy cells are arranged in loose fashion due to which many intercellular spaces are present.
12. The rosette crystal within leaf cell is present in between the spongy tissue, called druses.
13. Stomata is placed in the pit and protected by trichomes.
14. Lower epidermal cells are lined by thin cuticle.
15. Above the lower epidermis, palisade parenchyma is present.

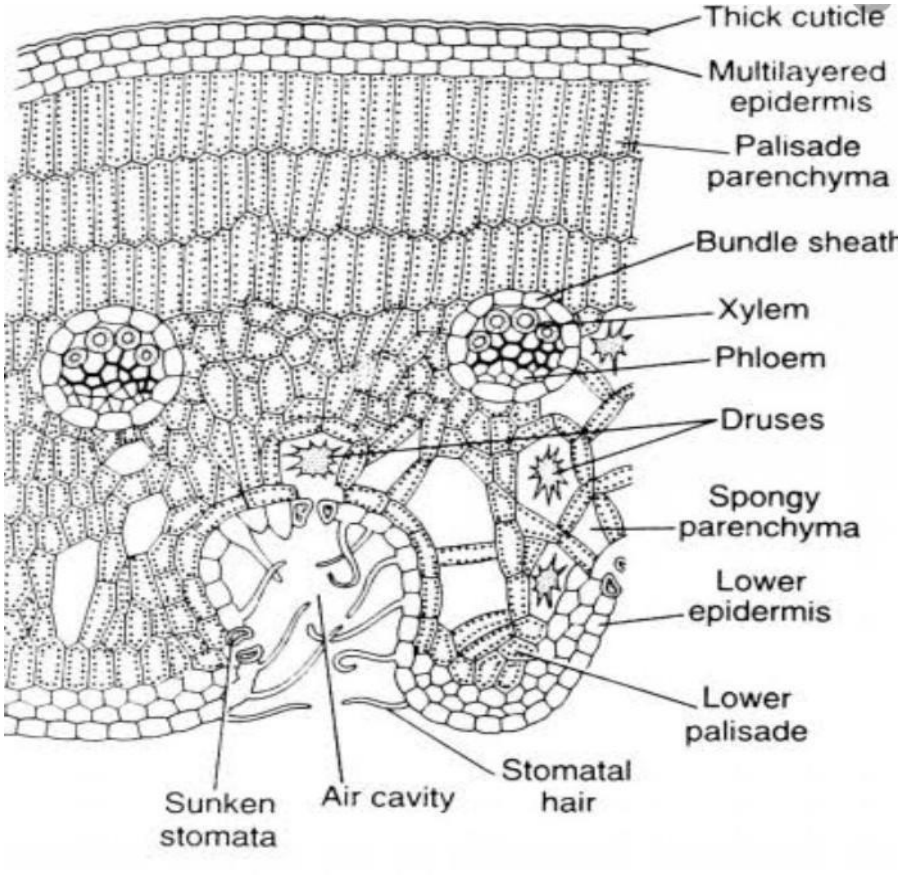


Fig.11.11: V.S. of *Nerium*leaf (https://brainly.in/question/43171596).

11.3.2.3-*Eucalyptus*

Eucalyptus is a genus of plant comprising over seven hundred species of flowering trees, shrubs or mallees. It belongs to the myrtle family, Myrtaceae. They are commonly called as Eucalypts with the other genera in the tribe Eucalypteae. This plant has much commercial importance. Its bark can either be smooth or fibrous, hard or stingy. The leaves contain oil glands, sepals and petals are fused together to form cup-like structure over the stamens, called operculum. The fruits are known as "Gumnut". They are known to be native to Australia but it is cosmopolitan in distribution.

Habit and Habitat: *Eucalyptus* are found in varying sizes. They can be diverse from different shrubs to tall trees. As a shrub, they are mature plant with 1 meter height and grow in an extreme environment. They are mainly the plants of cold tolerance.

V.S. of *Eucalyptus* leaf (Fig 11.5)

1. The single-layered epidermis covers the upper and lower surface of the leaf.

2. Mesophyll cells are arranged into two categories naming, palisade parenchyma and spongy parenchyma.
3. Palisade parenchyma cells are situated both below and above the upper and lower epidermis, respectively.
4. The layer of spongy parenchyma is placed between the palisade parenchyma.
5. The spongy parenchyma is arranged in loose fashion inside the cells due to the presence of many intercellular spaces in between.
6. The calcium oxalate crystal is placed in between the spongy parenchyma layer

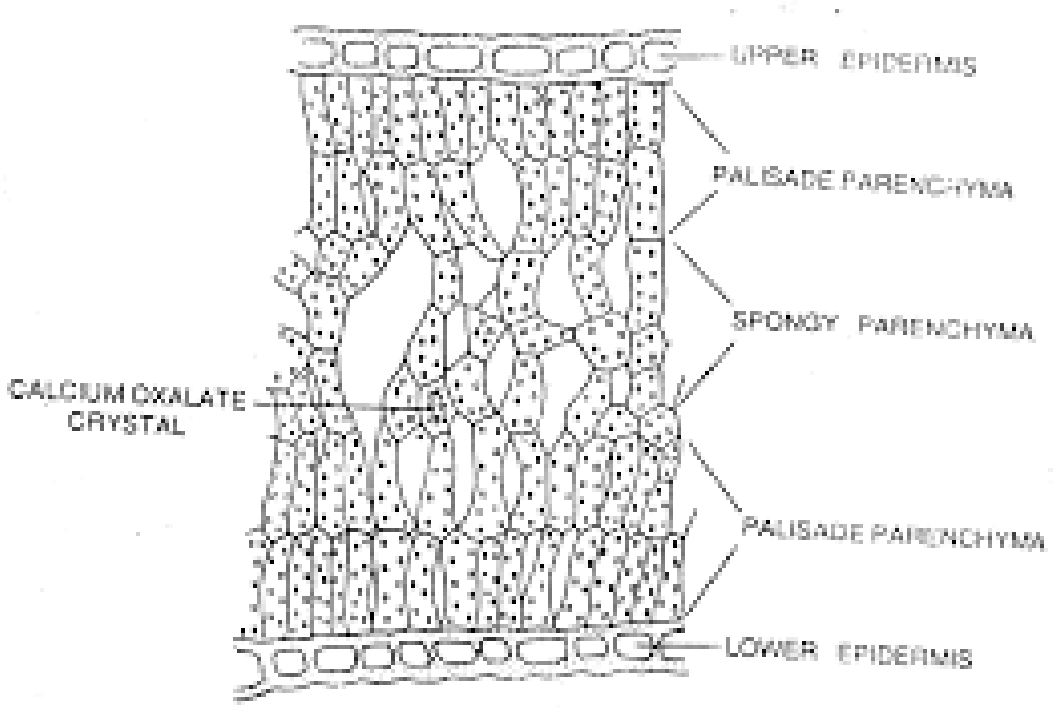


Fig.11.12: V.S. of *Eucalyptus* leaf

11.3.3.4-*Pyrus*

Pyrus belongs to the Rosaceae family which bears the pomaceous fruits. Most of the fruits belonging to this genus are edible. The tree is of medium-size and is known to be the native to coastal as well as mildly temperate regions of Europe, North Africa and Asia. This plant is known to be of great importance in manufacturing high-quality woodwind instruments and furniture. They are known to be native of central and Eastern Europe and southwest Asia. They

are mostly grown in temperate regions of Europe, North America and Australia. Two species called *P. pyrifolia* and *P. bretschneideri* are widely grown in East Asia.

Habit and Habitat: *Pyrus* is native to the coastal and temperate regions of Europe, North Africa and Asia. It is distributed across the Himalayas, from Pakistan to Vietnam and southern province of China to the northern region of India. It is also dispersed in Kashmir, Iran and Afganishtan. They are tolerant trees which grow in sandy loamy soil with well drainage. They are adaptive towards 750-1500 mm/yr or more precipitation zone and 10-35ºC temperature.

V.S. of *Pyrus* leaf (Fig 11.6)

1. The single-layered epidermis covers the upper and lower surface of the leaf.
2. Mesophyll cells are divided into two groups called as Palisade Parenchyma and Spongy Parenchyma.
3. The bundle sheath extensions are present beneath the epidermis
4. The vascular bundles are present in form of xylem and phloem with the fibers
5. The palisade parenchyma is placed beneath the upper epidermis in loose fashion
6. The spongy parenchyma is situated above the lower epidermis in a very loose manner

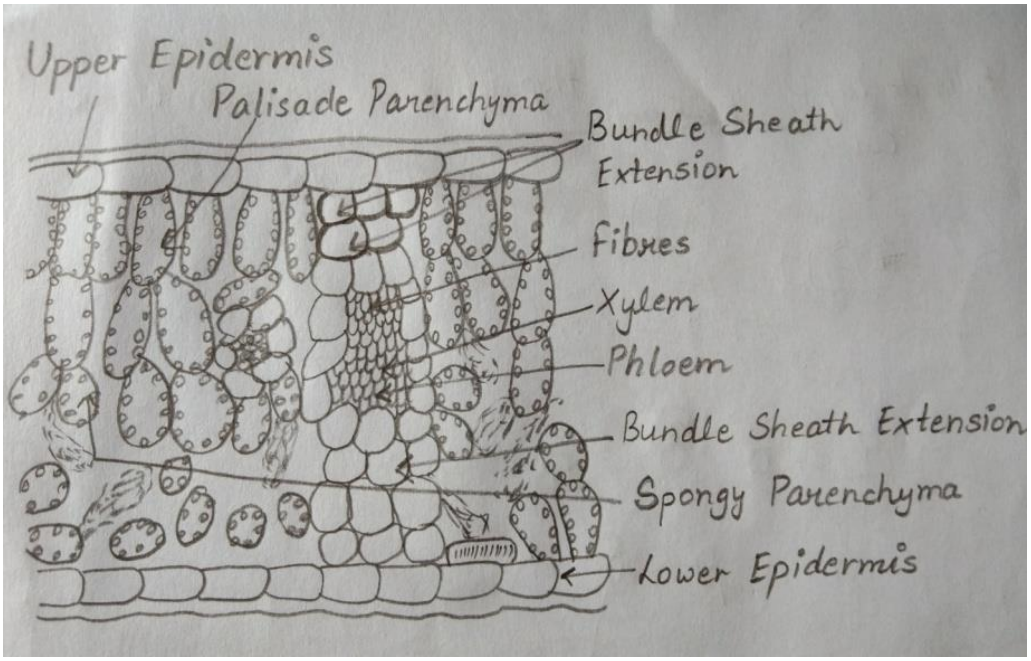


Fig.11.13: V.S. of *Pyrus* leaf

11.3.3-Monocot

11.3.3.1- *Phoenix*

It is the genus containing 14 species of palms. Genus is native to Canary Islands in the west, across northern and central Africa, to the extreme southeast of Europe (Crete) and then covering the southern Asia from Turkey east to southern China and Malaysia. They are found in swamps, deserts and mangrove sea coasts. They have been originated in semiarid region but are usually found in high groundwater levels, rivers or springs. Plants were more abundant and widespread in the past than they are now,

Habit and Habitat: In Iran, Iraq, Arabia, and North Africa west of Morocco, this genus comprises species that are important. They are also cultivated in America in southern California, Arizona and southern Florida in the United States and in Sonora and Baja California in Mexico. They are grown in variety of soils, in the degraded forest margins in grasslands, even grown under the shade of dominating forest trees along fragile hill slopes and stream courses in warm and humid conditions.

V.S. of *Phoenix* leaf (Fig 11.7)

1. The epidermis is bilayer and made up of compactly arranged tabular cells.
2. The cuticularized walls are present on them.
3. Stomata are scattered throughout the epidermal layer.
4. Just internal to both epidermal layers, a layer of parenchyma cells is present with scanty chlorophyll.
5. A special hypodermis as a subepidermal layer is observed in microscopy.
6. Mesophyll is made up of more or less isodiametric cells with small intercellular spaces.
7. Absence of palisade and spongy cells differentiation is observed.
8. Sclerenchyma patches are present in more or less parallel series towards the both upper and lower epidermis.
9. The bundles are collateral and closed and are arranged in parallel series.
10. Xylem is placed on the upper and phloem is situated on the lower side.
11. The large bundle contains the heavily thick-walled sclerenchyma patches on both the sides.
12. The small bundles are surrounded by parenchyma sheath with no chlorophyll.

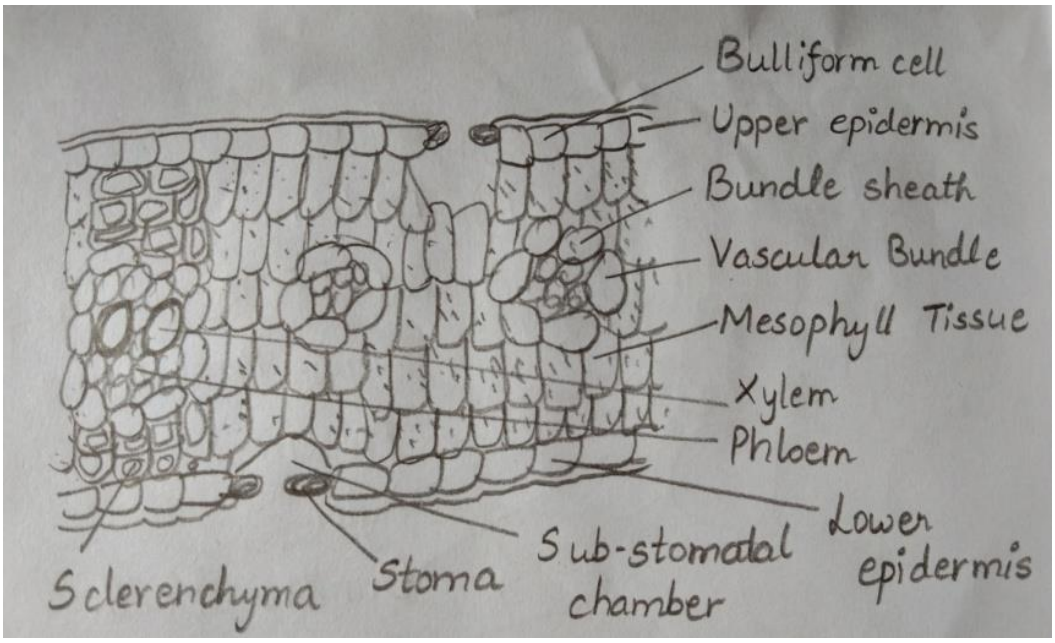


Fig.11.7: V.S. of*Phoenix* leaf

11.3.3.2- *Allium*

This genus contains common and widely cultivated vegetable onion which is also known as bulb onion or common onion. Other relative to this genus includes garlic, scallion, shallot, leek, chive and Chinese onion. They are herbaceous biennial or perennial plant but usually considered as an annual and harvested in its first growing season. Plants are about 30 cm long and a flowering scape that can be up to 100 cm tall from an underground bulb.

Habit and Habitat: Plants are native to southwestern Asia. Now are growing all around the world but mostly in temperate regions. Their original habitat is obscure; the plant probably arose in Central Asia (Turkmenistan). They are not known to grown in wild.

V.S. of *Allium* leaf (Fig 11.8)

1. The tissue of leaf is clearly differentiated into epidermal, ground and vascular tissues.
2. The epidermis is uniserate and continuous and made up of small round cells with cuticulrized outer walls.
3. Stomata are present in epidermis are slightly depressed.
4. Two types of mesophyll cells are present naming palisade and spongy parenchyma.
5. Internal to epidermis, two layers of columnar cells are present with abundant chloroplasts. They are called palisade cells.
6. A few layers of isodiametric parenchyma spongy cells are present next to palisade cells.

7. The central Leaf part is hollow.
8. Vascular bundles are scattered in lower mesophyll regions, collateral and closed.
9. Each bundle is surrounded by parenchymatous bundle sheath.

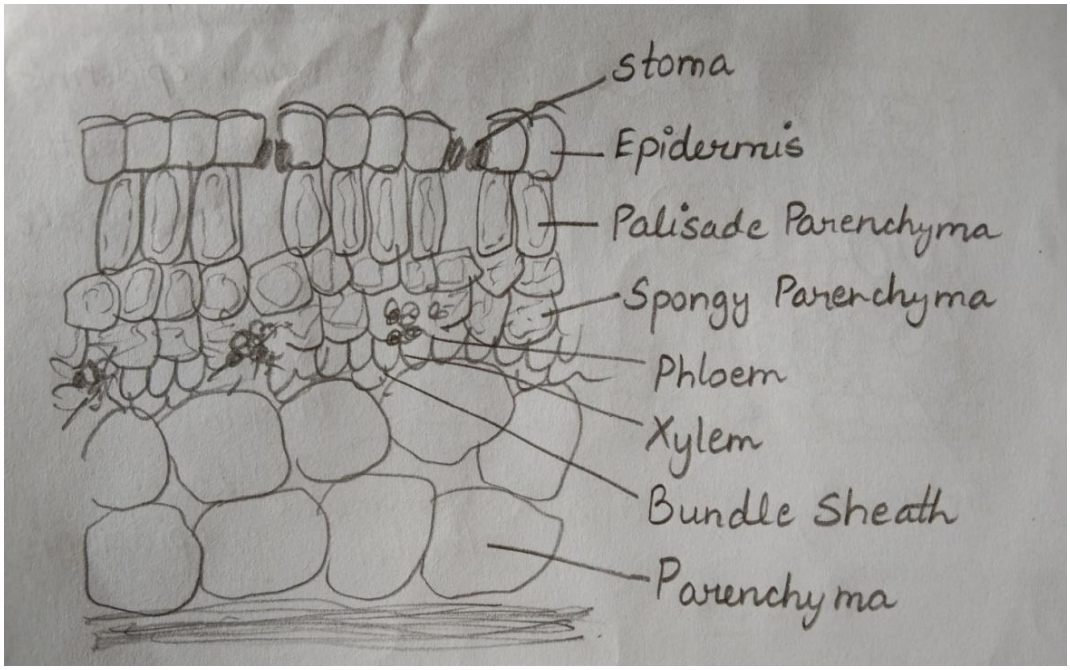


Fig.11.8: V.S. of *Allium* leaf

11.3.3.3- *Musa*

The genus *Musa* includes bananas and plantains. There are 70 species of known *Musa* which are used widely. They grow as high as trees. The banana and plantain plants are not woody. The huge leaf stalks made their apparent stem. They are considered as gigantic herbs. Their many species are used as food plants.

Habit and Habitat: They are tree-like herb with thick rhizome, pseudostem fleshy, succulent formed by the imbricate leaf sheaths. The optimal temperature for fruit production is about 27°c, and night time temperatures should not fall much below 18°c when the fruit is ripening or flavor can be impaired. The optimum pH for the soil is 6-7.5.

V.S. of *Musa* leaf (Fig 11.9)

1. The epidermis is divided into upper and lower region.
2. The epidermal cells are compactly arranged, round in shape and have cuticularized outer walls.
3. On the lower side of leaf surface, slight sunken stomata are present.

4. Both adaxial and abaxial thin-walled parenchyma cells are present next to epidermal layers.
5. Cells in upper surface are larger in size than the lower surface.
6. The hypodermal layer is marked by the sub-epidermal cells.
7. These cells contain water but lacks chlorophyll.
8. Mesophyll layer is differentiated into palisade and spongy cells.
9. Palisade cell layers are present in upper 2-3 layers and consist of long columnar cells with scanty intercellular spaces.
10. The spongy cells are smaller than palisade cells and are in isodiametric shape.
11. Numerous large air chambers are present at regular intervals towards the abaxial side.
12. The bundles are poorly developed, collateral and closed. They contain the scanty xylem.
13. Sclerenchyma patches are present in upper and lower sides of the vascular bundles.

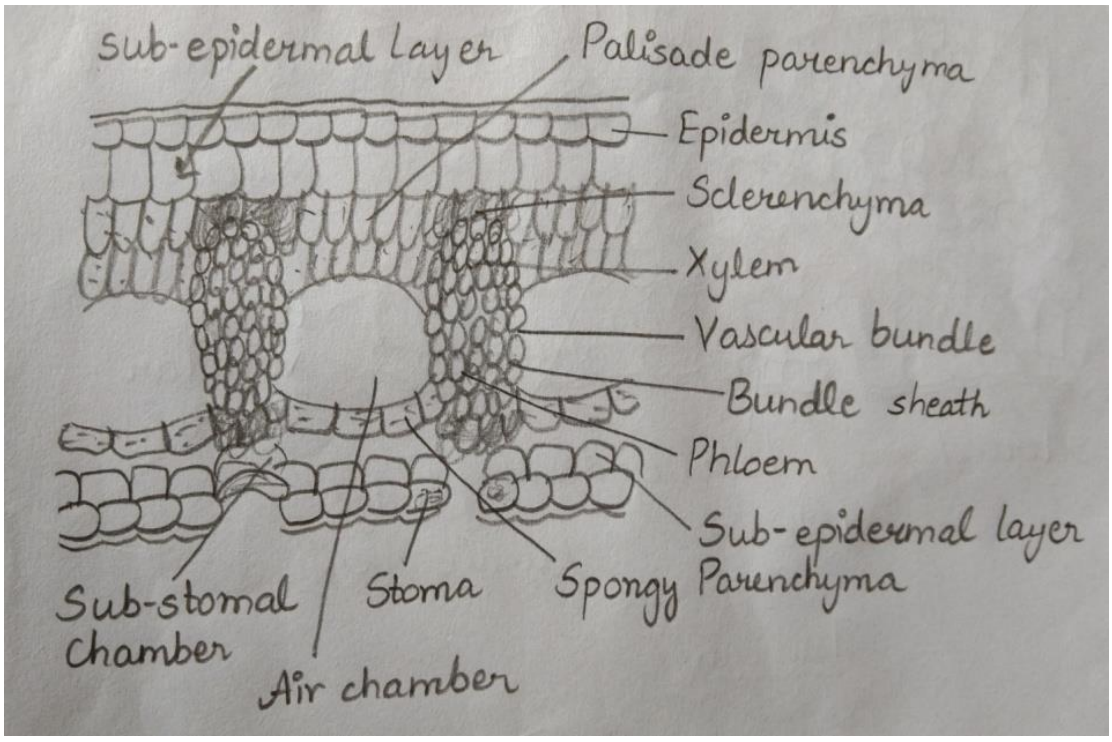


Fig.11.9: V.S. of *Musa* leaf

11.3.3.4- *Triticum*

Wheat, a worldwide staple food, is the common and mostly grown crop of this genus. They are basically grasses with economically important seeds as cereal grain. They were first cultivated in

the regions of the Fertile Crescent around 9600 BCE. The wheat kernel is called caryopsis is considered as a type of fruit.

Habit and Habitat: The common habitats include fields, roadsides, areas along railroads, area near grain elevators and open waste areas. They are known to grow in highly distributed areas with exposed topsoil.

V.S. of *Triticum* leaf (Fig 11.10)

1. The epidermis constitutes the both upper and lower surfaces of the leaf.
2. They are composed of more or less oval cells and are covered by thick cuticle.
3. Some big, motor or bulliform cells are present in the upper epidermis surface.
4. Stomata with guard cells, pore and a stomatal chamber are present in both the epidermal layers.
5. Mesophyll layer is not clearly differentiated in to palisade ad spongy parenchyma.
6. The cells adjacent to the epidermal layers are a bit longer but the cells of the central mesophyll layer are oval and arranged in irregular fashion.
7. Many chloroplasts are present in the mesophyll cells.
8. Intercellular regions are present within the mesophyll layers.
9. Sub-stomatal chambers of the stomata are also placed in the region of mesophyll.
10. Many vascular bundles are present in the leaf and are arranged in a parallel series.
11. The vascular bundles present in center are the largest in size.
12. They are conjoint, collateral and closed.
13. All the bundles are surrounded by the double layered bundle sheath.
14. The bundle sheath outer layer consists of thin-walled cells whereas inner layer consists of thick-walled cells.
15. The sclerenchyma patches are present on both the upper and lower surfaces of large vascular bundles.
16. Xylem is present towards the upper surface while phloem is placed towards the lower surface.
17. Xylem is constituted by vessels, tracheid and sometimes with the xylem parenchyma.
18. Phloem is composed of sieve tubes and companion cells.

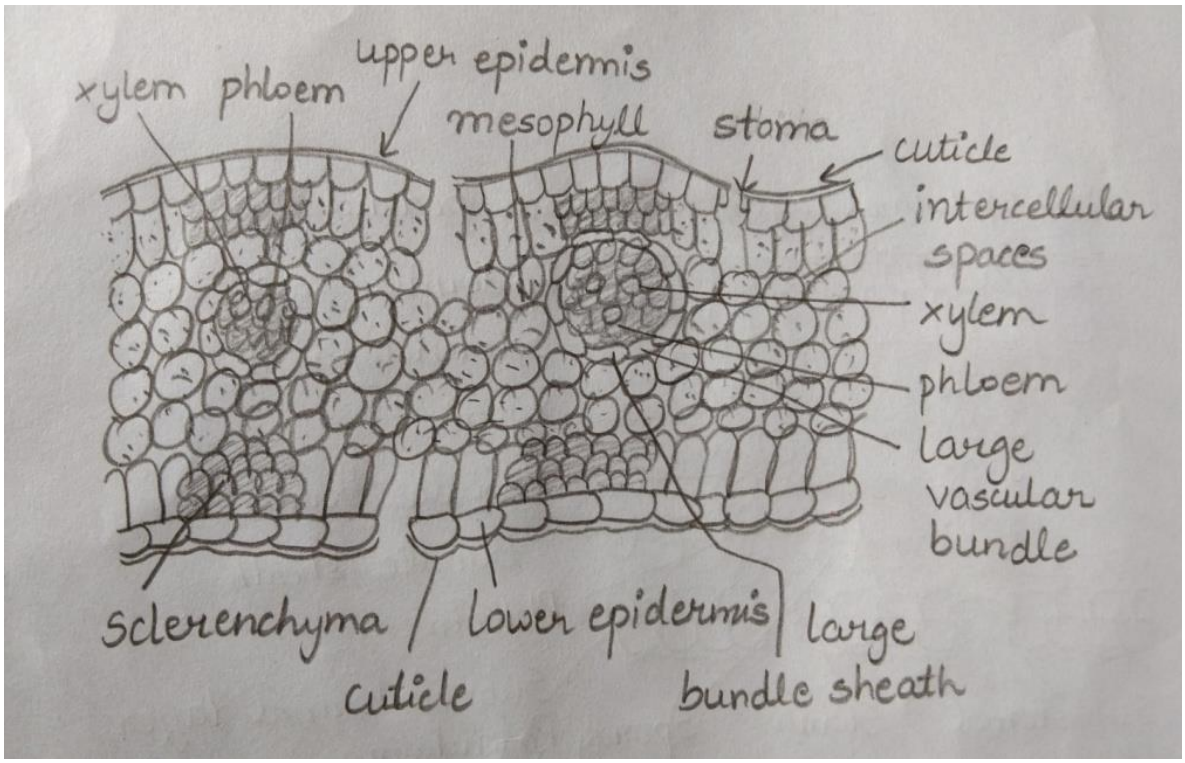


Fig.11.10: V.S. of *Triticum* leaf

11.3.3.5- *Oryza*

This genus belongs to the grass family and includes the major food crop in all over the Asia,*O. sativa* and *O. glaberrima* are most common species found. Plants are 1-2 m tall, wetland grasses both the annual and perennial species. They are morphologically characterized by the single-flowered spikelets whose glumes are completely suppressed. Species in this genus can be divided by their genome's types. Crossing is common in species of the same genome. The embryo rescue technique is used for hybridizing different types.

Habit and Habitat:

As a crop, it's grown all over the tropical world. Europe, Africa, tropical and temperate Asia, Australia, and North and South America are among the places where it is grown. Lowland swampy environments are where they are discovered.

V.S. of *Oryza* leaf (Fig 11.11)

1. The upper epidermis constitutes the topmost layer or adaxial layer of the monocot leaf.
2. The cells are of cubic or barrel shape and are arranged compactly with no intercellular spaces.
3. Thin cuticle covers the upper epidermal surface.

4. Large bulliform or motor cells are present on the upper epidermal layer of the cell.
5. Equal numbers of stomata are distributed among the both sides of epidermal layers i.e., amphistomatic distribution.
6. Air-cavities or sub-stomatal chambers are present above the stomata of the epidermal layers.
7. The mesophyll tissue is not differentiated into palisade parenchyma and spongy parenchyma.The mesophyll tissue is composed of 6-7 layers with large intercellular spaces.
8. The spongy parenchyma cells are small oval or spherical or irregular with chlorophyll and chloroplasts in them.
9. The vascular bundles are present in the mesophyll tissue.
10. Each bundle is consisting of xylem and phloem which is surrounded by the bundle sheath.
11. Large barrel shaped endodermal cells compose the bundle sheath layer of the vascular bundles.
12. These sheaths usually store starch granules and hence are called as starch sheath.
13. Xylem tissue is placed toward the upper epidermis of leaf.
14. It is consisting of xylem tracheid, xylem vessels, xylem parenchyma and xylem fibers.
15. Phloem is found towards the lower epidermal surface of the leaf.
16. Phloem is consisting of sieve tubes; sieve pores, companion cells, phloem parenchyma and phloem fibers.
17. The bundles are conjoint, collateral and closed with endarch xylem.
18. A single layer lower epidermis is present below the undifferentiated mesophyll tissue on the abaxial surface of the leaf.
19. The cells of this layer are cubical or barrel in space without the presence of intercellular spaces.

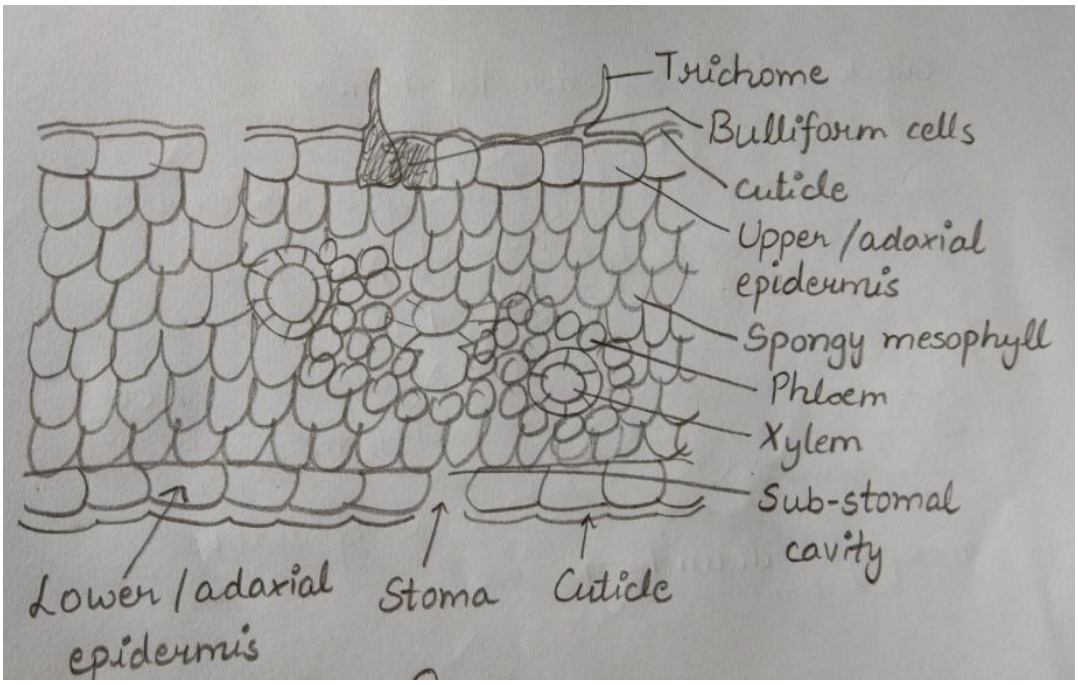


Fig.11.11: V.S. of *Oryza* leaf

11.3.3.6- *Zea*

Zea belongs to the genus of flowering plants and the grass family. One of the most important species of this genus is called *Zea mays*, which is also known as maize, corn or Indian corn. Other wild species are commonly known as Teosintes. They are known to be native to Mesoamerica.

Habit and Habitat: They are generally grown in sandy light, loamy medium and clay heavy soils.Soil is needed to be well-drained to grow *Zea*. Slightly acidic or neutral soils are optimum for some species like sweet corn. They do not grow in the shade. Nitrogen, potassium and phosphorus are the important constituents of the soil for this crop to grow.

V.S. of *Zea* leaf (Fig 11.12)

1. The epidermis is present at both the upper and lower surfaces of the leaf.
2. Both surfaces contain stomata.
3. A thick cuticle covers the epidermal layers.
4. Cells in upper epidermis are large in size and are called bulliform cells or motor cells.
5. Mesophyll layer is present between the epidermal layers and they constitute the region of chlorophyll containing cells.
6. Mesophyll layer is not differentiated into palisade and spongy parenchyma.

7. Mesophyll cells are spherical or angular and compactly arranged with few or no intercellular spaces.
8. Numerous small and larger vascular bundles are present.
9. The bundles are collateral and closed.
10. All vascular bundles are covered by bundle sheath which is made up of parenchymatous cells and contains starch and plastids.
11. Patches of sclerenchyma are present in the both ends of large vascular bundles, which are extended up to the upper and lower epidermal layers.
12. Xylem and phloem are more visible in large bundles rather than small bundles.
13. Xylem is placed towards the upper epidermis whereas phloem is situated towards the lower epidermis.
14. Xylem consists of tracheids, xylem vessels and xylem parenchyma.
15. Two large oral vessels represent metaxylem whereas a water cavity represents protoxylem. Such water cavity is called lysigenous cavity.
16. Sieve tubes and companion cells constitute the phloem.
17. Less developed xylem and phloem surrounded by a bundle sheath is present in smaller vascular bundles.

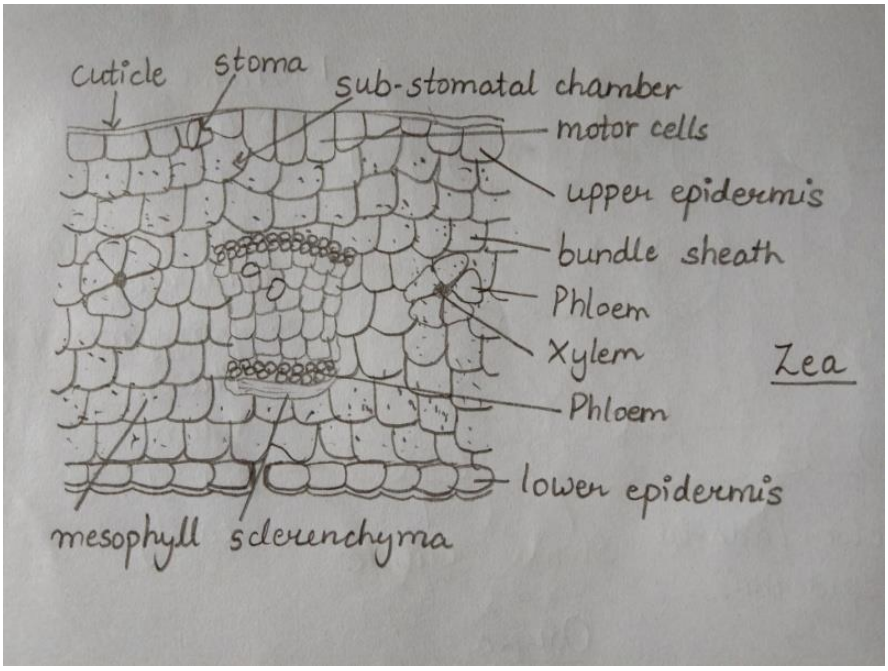


Fig.11.12: V.S. of *Zea*leaf

11.3.3.7- *Bambusa*

Bamboosare evergreen perennial grass plants. Their internodal regions of the stem are generally hollow. Absence of secondary growth is observed in the stems of monocots, including the palms and large bamboos, to be columnar rather than tapering. They are the fastest growing plants in the world because of their unique property of rhizome-dependent system. They can grow up to 910 mm (36 inch) and hence are called Giant bamboos in general. They are very important in culture and holds notable economic importance in the regions of South Asia, Southeast Asia and East Asia. They are used as food, building materials, etc.

Habit and Habitat: Plants are native to all the continents excluding, Antarctica and Europe. They are distributed from 47 S to 50 300 N and 4300 m up from the sea level. Genus is found in temperate as well as tropical regions in association with a wide variety of mostly mesic to wet forest types. Some species are adapted to grow in open grasslands or in specialized habitats.

V.S. of *Bambusa* leaf (Fig 11.13)

1. The upper and lower epidermal layers are uniserate.
2. The epidermal cells are more or less oval, large and empty bulliform cells.
3. Presence of stomata is observed in both the epidermal layers.
4. The mesophyll cells are not differentiated into palisade and spongy cells.
5. The mesophyll cells are compactly-arranged isodiametric cells with air-chambers in between them.
6. Most of the vascular bundles are small but large bundles are present at some intervals.
7. The bundles are collateral and closed, arranged in parallel series.
8. Bundle sheath covers the small bundles which have xylem on upper side and phloem on lower side.
9. The bundle sheath contains plastids with starch grains most often.
10. Xylem is constituted by trachery elements and phloem is made up of sieve tubes and companion cells.
11. Sclernchyma cells are present in patches on both sides of the bundles.

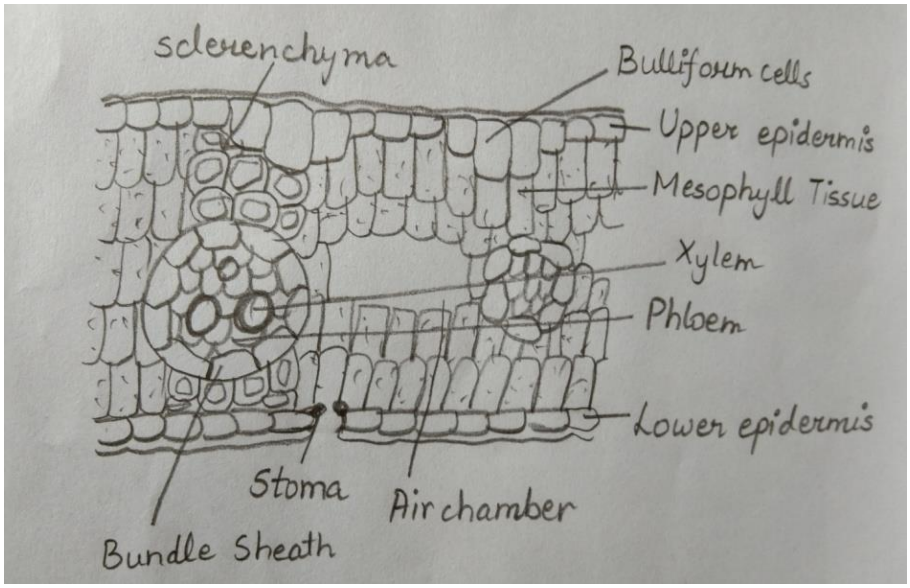


Fig.11.13: V.S. of *Bambusa* leaf

11.4- SUMMARY

Vertical section of leaf provides the insight of the presence of epidermis as the outermost layer. Cutin, a waxy substance coats the outermost surface, forming the layer of cuticle, especially on the upper adaxial surface. The epidermal layer is consisting of stomata which carries out the process of photosynthesis and helps in exchange of gases between the plant and environment. Guard cells are also present in the epidermal layer. A compact bundle sheath with adaxial xylem and adaxial phloem is also present. Trichomes, the epidermal appendages are present in the surface which can either be unicellular or multicellular. Main vein is delineated from the spongy mesophyll and a compact bundle sheath is present containing parenchymal cells. Adaxial xylem and adaxial phloem is present in the enclosed area of bundle sheath. Some sclerenchymal cells are also present in the vicinity of bundle sheath. A continuous network of vascular strand is observed throughout the leaf and this network increases with finer dimensions when originated from the main vein. The specialized parenchyma cells form the palisade mesophyll which elongates in perpendicular direction to the leaf surface.

11.5- GLOSSARY

Adaxial- It is denoting the upper surface of a leaf).

Appendages-They are the projecting part of an invertebrate or other living organism, with a distinct appearance or function.

Cosmopolitan-Something found all over the world.

Epiphytes-A plant that grows on another plant, especially one that is not parasitic.

Hemiepiphytes-It is a plant that spends part of its life cycle as an epiphyte.

Foliage- Collectively called for plant leaves.

Lamina-It is a thin, soft, pliable sheet or layer, especially of animal or vegetable tissue, serving as a covering or lining, as for an organ or cell, e.g.- leaf lamina.

Petiole- The petiole is a stalk that attaches a leaf to the plant stem.

Photosynthesis-It is a process by which green plants and some other organisms use sunlight to synthesize nutrients from carbon dioxide and water.

Precipitation-Precipitation is any product of the condensation of atmospheric water vapor that falls under gravity from clouds.

Sessile- Attached directly by its base without a stalk or peduncle.

Transpiration- Transpiration is the process of water movement through a plant and its evaporation from aerial parts, such as leaves, stems and flowers.

Trichomes- They are the small hair or other outgrowth from the epidermis of a plant, typically unicellular and glandular.

Vines- a climbing or trailing woody-stemmed plant

11.6- SELF ASSESSMENT QUESTIONS

1. Cuticle is present on the _____ surface of leaf.
2. The epidermal appendages present on the surface of leaf are called_____.
3. _____ causes the delineation of main vein on the leaf.
4. _____ cells elongate in the perpendicular direction of leaf surface.
5. _____ are the epiphytes and hemiepiphytes and commonly called as_____.
6. _____ genus is known for manufacturing high-quality woodwind instruments and furniture.
7. _____ crystal is present in the mesophyll tissue of *Eucalyptus*.
8. _____ species of *Cycas* is endemic inIndia.
9. *Eucalyptus* sepals and petals are fused together to form cup-like structure over the stamens, called _____.
10. *Cycas* stomata are _____.

11. _____are considered as gigantic herbs.
12. *Ficus* mesophyll cells can be _____and _____.
13. Stomata in*Nerium* is placed in the pit and protected by _____.
14. *Allium* is grown in _____ regions.
15. _____are evergreen perennial flowering plants.
16. In *Zea* cells in upper epidermis are large in size and are called _____ or _____.

ANSWERS TO SELF ASSESSMENT QUESTIONS

1. Adaxial
2. Trichomes
3. Mesophyll cells
4. Palisade mesophyll
5. *Ficus*, Figs
6. *Pyrus*
7. Calcium oxalate
8. *C. circinalis*
9. Operculum
10. Haplocheilic
11. *Musa*
12. Bifacil, Isobilateral
13. Trichomes
14. Temperate
15. *Bambusa*
16. Bulliform cells, Motor cells

11.7- REFERENCES

1. Liew, O. W., Chong, P. C. J., Li, B., & Asundi, A. K. (2008). Signature optical cues: emerging technologies for monitoring plant health. Sensors, 8(5), 3205-3239.
2. Evert, R. F. (2006). Esau's plant anatomy: meristems, cells, and tissues of the plant body: their structure, function, and development. John Wiley & Sons.
3. *Haupt, A. W. (1953). Plant morphology (No. 581.4 H38).*

4. *Mauseth, J. D. (2014). Botany: an introduction to plant biology. Jones & Bartlett Publishers.*
5. https://cronodon.com/BioTech/TreeTech_Pine.html
6. https://www.jagranjosh.com/general-knowledge/common-plant-species-of-india-1440146954-1
7. https://www.biologydiscussion.com/essay/gymnosperms/essay-on-cycas-top-5-essays-class-cycadopsida-gymnosperms-botany/76586
8. https://www.biologydiscussion.com/essay/essay-on-leaf-with-diagrams-botany/20581
9. http://www.nzdl.org/gsdlmod?e=d-00000-00---off-0hdl--00-0----0-10-0---0---0direct-10---4-------0-1l--11-en-50---20-about---00-0-1-00-0--4----0-0-11-10-0utfZz-8-00&cl=CL1.12&d=HASHfb83ff5b6397f7dd31ae0d.4.2.5&x=1
10. Serra, Vital Garcia-Espana (2014). "Patent Nerium Plant New Cultivar" (https://patents.google.com/patent/USPP26619).
11. Sunset Books. (1995). *Sunset Western garden book. Sunset Publishing Company.*
12. List, P. (2015). The *Plant List. Version 1.1*.
13. Group, I. S. I. S. S. The Global Invasive Species Database (GISD) and international information exchange: using global expertise to help in the fight against invasive alien species.
14. Sheikh, M. I. (1993). Trees of pakistan (Vol. 110). Islamabad: Pictorial Printers.
15. Krause, S., Hammer, K., & Buerkert, A. (2007). Morphological biodiversity and local use of the Himalayan pear (Pyrus pashia) in Central Bhutan. Genetic Resources and Crop Evolution, 54(6), 1245-1254.
16. ZAMANI, A., ATAR, F., & JOUHARCHI, M. (2009). Pyrus pashia (Rosaceae), a new record for the flora of Iran.
17. Sellers, C. H. (1910). Eucalyptus: its history, growth, and utilization. AJ Johnston, 13.
18. https://www.indiastudychannel.com/resources/168696-Anatomy-or-internal-structure-of-a-Monocotleaf.aspx#:~:text=In%20monocot%20leaf%20mesophyll%20tissue, cells%20with%20chloroplasts%20and%20chlorophyll.
19. Shakir, H. M., & Baji, S. H. (2016). Anatomical study of some characters in certain species of genus Ficus L. growing in Iraq. J Biol Agric Healthcare, 6(12), 98-105.

20. https://www.studyadda.com/notes/11th-class/biology/plant-kingdom/some-representative-rymnosperms/9569
21. https://brainly.in/question/43171596

11.8- SUGGESTIVE READINGS

1. https://biologyreader.com/difference-between-dicot-and-monocot-leaf.html#Anatomy
2. https://www.biologydiscussion.com.
3. https://www.microscopemaster.com/leaf-structure-under-the-microscope.html
4. https://garden.org/onlinecourse/Act7.htm.

11.9- TERMINAL QUESTIONS

1. What are the characteristics of a Nerium leaf when observed under microscope?
2. Draw a well labeled diagram of vertical section of Eucalyptus leaf.
3. Explain the Pine leaf anatomy.
4. Give an insight on Pyrus leaf.

UNIT-12 STUDY OF EPIDERMAL PEELS OF LEAVES, PREPARATION OF STOMATAL INDEX AND DEMONSTRATION OF THE EFFECT OF ABA ON STOMATAL CLOSURE

12.1- Objectives

12.2- Introduction

12.3- Study of epidermal peels of leaves to study the structure of stomata

12.3.1- Monocot species

12.3.1.1- *Triticum*

12.3.1.2-*Zea*

12.3.1.3- *Tradescantia*

12.3.1.4- *Saccharum*

12.3.1.5- *Cyanodon*

12.3.1.6- *Musa*

12.3.2- Dicot species

12.3.2.1-*Sedum*

12.3.2.2- *Pelargonium*

12.3.2.3- *Abelmoschus*

12.3.2.4- *Hibiscus*

12.4- Preparation of stomatal index

12.4.1- *Triticum*

12.4.2-*Carex*

12.4.3- *Avena*

12.4.4-*Gladiolus*

12.4.5-*Juncus*

12.4.6-*Allium*

12.4.7-*Orchis*

12.5- Demonstration of the effect of ABA on stomatal closure

12.6- Summary

12.7- Glossary

12.8- Self Assessment Questions

12.9- References

12.10- Suggested Readings

12.11- Terminal Questions

12.1 OBJECTIVES

After reading this unit students will be able -

1. To study the epidermal peels of leaves in order to understand the development and final structure of stomata.

2. To prepare stomatal index and demonstrate the effect of ABA on stomatal closure

12.2 INTRODUCTION

The epidermis is the outermost layer which covers the cell surface. It protects the inner tissues from adverse natural disasters like high temperature, desiccation, mechanical injury, external infection etc. The epidermis of monocotyledonous plant is originated from the periblem along with the cortex.

Stomata of monocots are tiny and are present in upper and lower epidermis of monocot leaves. They are generally surrounded by dumbbell-shaped guard cells. They are arranged in regular arrays. They are usually equally distributed in both the upper and lower epidermis. The stomata distribution in monocots is generally known as amphistomatic distribution. The frequency of transpiration can be higher than dicot leaf due to the presence of amphistomatic distribution of stomata.

Stomata of dicots are either present on the lower side of the leaf or they might be only present only on the lower side of the leaf. Such leaves having stomata present on the lower side of the leaf are given with a term, hypostomatous leaves. This special arrangement called hypostomatous distribution of stomata allows water to be conserved in the leaves.

The stomatal index is the measurement of number of stomata presents to the total number of epidermal cells, each stoma being counted as one cell in the form of percentage. The stomatal index (I) is defined as average number of stomata cells present per square millimeter of the epidermis of the leaf. The formula for calculating the stomatal index (I) is given by the formula or equation, $I = S / E + S$; where I, stomatal index; S, number of stomata cells per unit area; and E, number of epidermal cells per unit area.

12.3 STUDY OF EPIDERMAL PEELS OF LEAVES TO UNDERSTAND THE DEVELOPMENT AND FINAL STRUCTURE OF STOMATA

METHODOLOGY

Materials

- Plants with suitable leaves with smooth surface.
- Compound microscope with magnification up to 400x
- Forceps and blades
- Microscope slide
- Cover slips
- Cotton, Tissue paper
- Alcohol or ethanol
- Digital camera to capture microscopic images of structure of epidermal peel and stomata

Procedure (Fig 12.1)

a) Collect clean leaves with smooth surface.
b) Make a clean cut at one end of the leaf structure with the help of blades.
c) Clean the microscopic slides using ethanol. Place the specimen on the microscopic slide.
d) Clamp down the thick spot including the portion of epidermis along the cut using fine forceps.
e) Use forceps to pull the various layers of tissues.
f) At first thick wad of tissues will be visible.
g) After pulling sub-epidermal layers, epidermis layer is observed.
h) Place it faces up on microscopic slide and crops it so that some of the thin strip is retained.
i) Add water and place the cover slip.
j) Observe slide under the microscope.

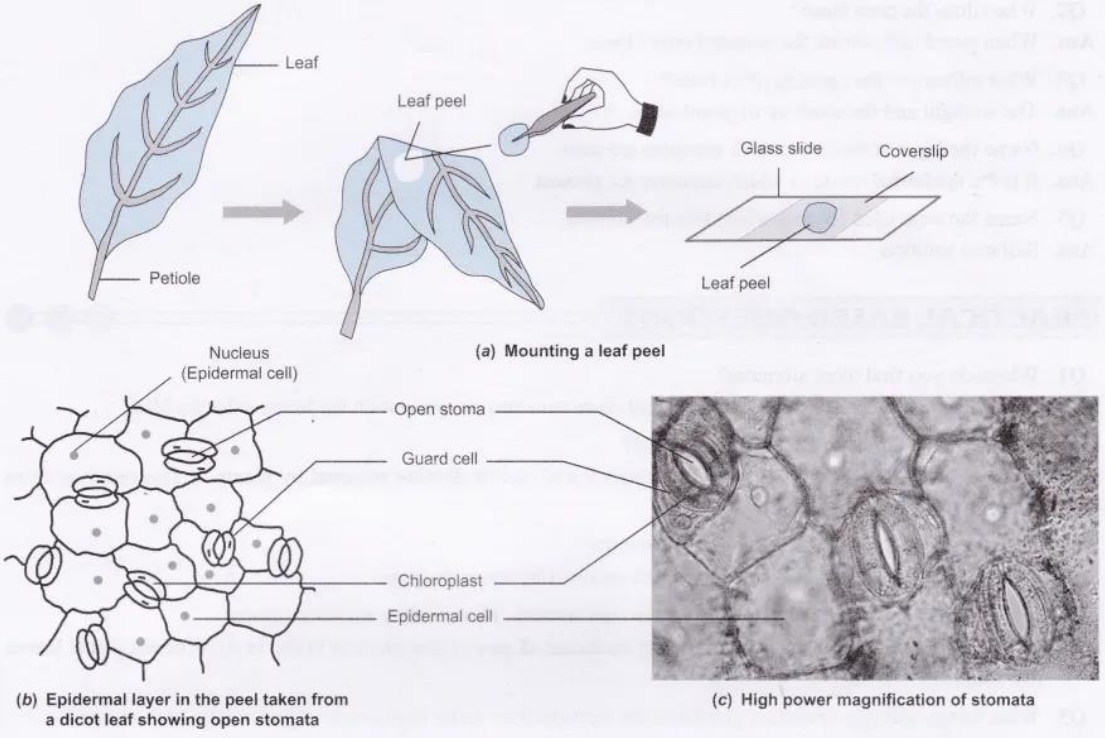


Fig.12.1: Preparation of epidermal peel.

12.3.1- Monocot species

12.3.1.1 *Triticum*

Wheat is a worldwide staple food and is the common member of this genus. It basically belongs to the grasses which has economically important seeds as cereal grain. They were first cultivated in the regions of the Fertile Crescent around 9600 BCE. The wheat kernel is called caryopsis considered as a type of fruit.

Botany of the plant

Triticum is a cultivated annual crop with herbaceous, erect, cylindrical and fistular stem. The unbranched glabrous stem comprised of distinct node and internodes and root is adventitious. Hermaphrodite, incomplete and zygomorphic flower lies between superior and inferior palea.

Morphology of leaf

Leaf is simple, alternate and green. No stipule is present and hence considered as exstipulate. Leaf has entire margin;acute apex and it has sheathing leaf base. Ligule is present at the junction of leaf-sheath and leaf-blade membranous ligule present. Parallel venation is observed.

The epidermal peel of *Triticum* leaf (Fig 12.2)

1. The upper and lower epidermal surfaces are present on the leaf surface.

2. Both the epidermal layers are uniseriate.
3. The epidermal layers are composed of more or less oval cells.
4. Some big, motor or bulliform cells are present in the epidermal surface.
5. Stomata are present on both the epidermal layers and they are consisting of a pore, guard cells and a stomatal chamber.A thick cuticle covers the outer walls of the epidermal cells.
6. The epidermal cells are thin walled, hyaline and have large vacuole.
7. The bulliform cells present in the middle are the tallest and the sides of the other cells present on sides are smaller.
8. The epidermal cells contain abundant water but are devoid of chloroplastids.
9. Dumb-bell shaped stomata are found.

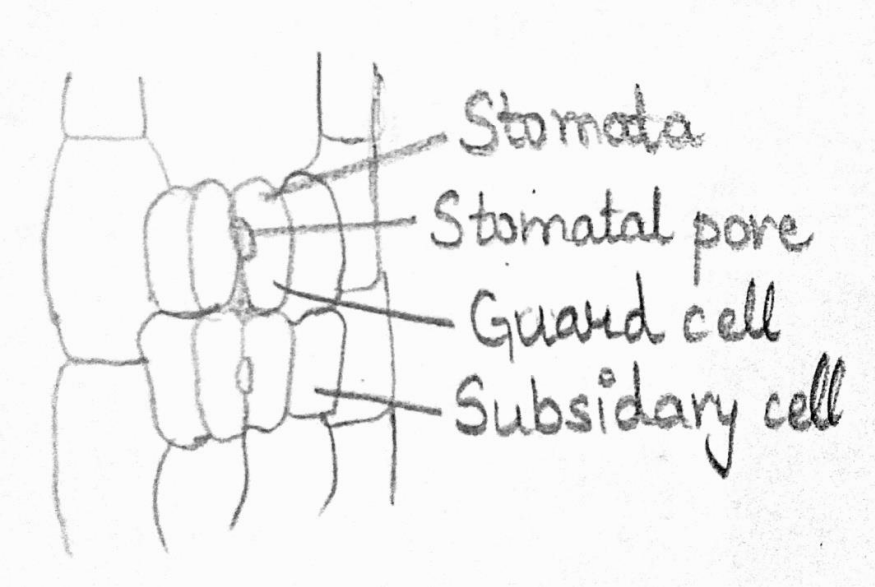


Fig.12.2: The structure of leaf epidermis of Triticum.

12.3.1.2- *Zea*

Zea belongs to the grass family. One of the most important species of this genus is*Zea mays*, which is also known as maize, corn or Indian corn. Other wild species are commonly known as Teosintes. They are known to be native to Mesoamerica.

Botany of the plant

Zea is an annual cultivated crop with erect, cylindrical, herbaceous and solid stem. Smooth stem is defined in distinct notes and internodes. Adventitious stilt roots are present. Plant is monoecious.

Morphology of leaf

Leaf is simple, alternate and linear. Long ligule is present. Venation is multicostate parallel.

The epidermal peel of *Zea* leaf (Fig 12.3)

1. The single-layered epidermis is present in both upper and lower surfaces of the leaf.
2. On the both epidermal surfaces, stomata are present.
3. Both epidermal layers are covered by thick cuticle on outer surface.
4. Some cells are larger in the upper epidermal layer and are called bulliform cells or motor cells.
5. The guard cells on the stomata are peculiarly dumb-bell-shaped in form.
6. The subsidiary cells lie adjacent to stoma mother cell and they occur on 2 sides of the guard cells.
7. Such stomata are also referred as *Zea* type.

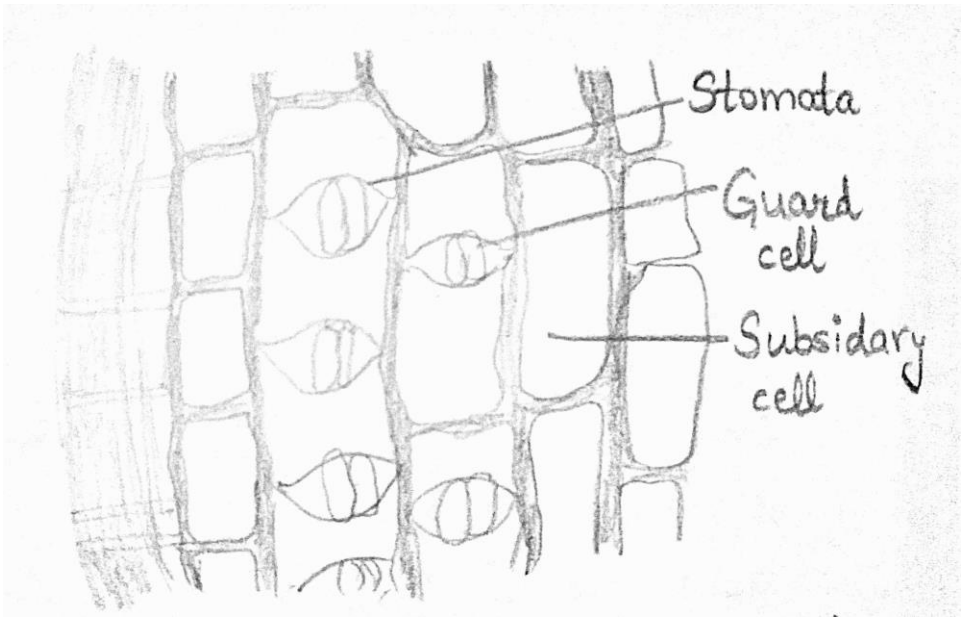


Fig. 12.3: The epidermal peel of Zea leaf

12.3.1.3- *Tradescantia*

This genus includes 75 species of herbaceous perennial wildflowers. They are native to the New World form southern Canada to northern Argentina, including West Indies. Some members of this genus are popularly known by the names such as spiderwort or Indian paint. They are used as ornamental plants in many parts of the world. They are weakly-upright to scrambling plants. They can grow up to 30-60 cm in height.

Botany of the plant

They are herbaceous annual orperennials. They have adventitious roots. Stem is like rhizome, branched jointed with swollen nodes.

Morphology of leaf

Leaves are simple, alternate with sheathing base and narrow grass-like blades. Leaves have entire margin. They are linear, oval or lanceolate parallel venation.

The epidermal peel of *Tradescantia* leaf (Fig 12.4)

1. The epidermal cells are arranged irregularly.
2. There are no intercellular spaces present between the epidermal cells.
3. Numerous small stomata are scattered in epidermal cells.
4. Each stomatal pore is guarded by 2 bean-shaped cells.
5. The guard cells contain chloroplasts and a nucleus.
6. The inner boundary of guard cell is concave and thick whereas the outer boundary is thin.
7. Both open and close stomata are present which are regulated by guard cells.
8. 4 subsidiary cells surround the stoma mother cell.

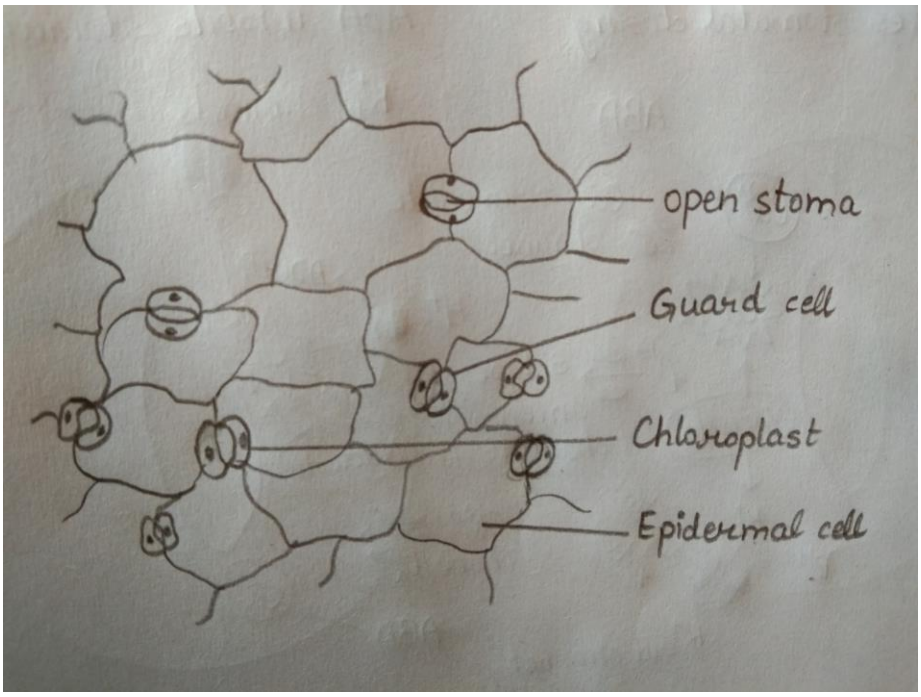


Fig.12.4: The epidermal peel of *Tradescantia* leaf

12.3.1.4- *Saccharum*

The species belonging to this genus include the tall perennial plants of the broomsedge tribe in the grass family. They are found in all around the tropical, subtropical and warm temperate

regions of Africa, Eurasia, Australia, Americas and assorted oceanic islands. Sugarcanes are the common members of this genus and also it includes Ravenna grass which is used as ornamental purposes.

Botany of the plant

They are large tropical grasses with multiple stems or culms each containing series of nodes separated by internodes. The internode length can grow up to 30 cm; consists of sucrose which is stored in parenchyma cells and vascular tissue. The stem is made up of parenchyma cells and is not hollow like other grasses. Mature stems have leaf spindles made up by enclosing the number of leaves to form the structure. Buttress roots are developed in the plants of this genus to anchor the plant. The roots penetrate and grow downwards which allows sufficient water absorption under the water or drought stress.

Morphology of leaf

The abaxial (under) surface of leaf blade is pubescent i.e. hairy and the adaxial (top) surface of the leaf is glabrous i.e. without hairs. The leaf blade terminatesin a pointed tip. The length and width of leaf blade varies from 60-150 cm long and 2-10 cm, respectively. The leaves are attached at the node from the stem and then wraps the stem to form a sheath enclosing internode from which node subtends.

The epidermal peel of *Saccharum* leaf (Fig 12.5 A,B)

1. The outermost layer is the single layered epidermis.
2. The epidermis is covered by the cuticle from outside.
3. The bulliform or motor cells are present on the epidermal layers.
4. The epidermal layer is interrupted by presence of the stomata.
5. The stomata are guarded by the guard cells.
6. The guard cells contain chloroplasts and a nucleus.
7. The guard cells regulate the opening and closing of stomata.

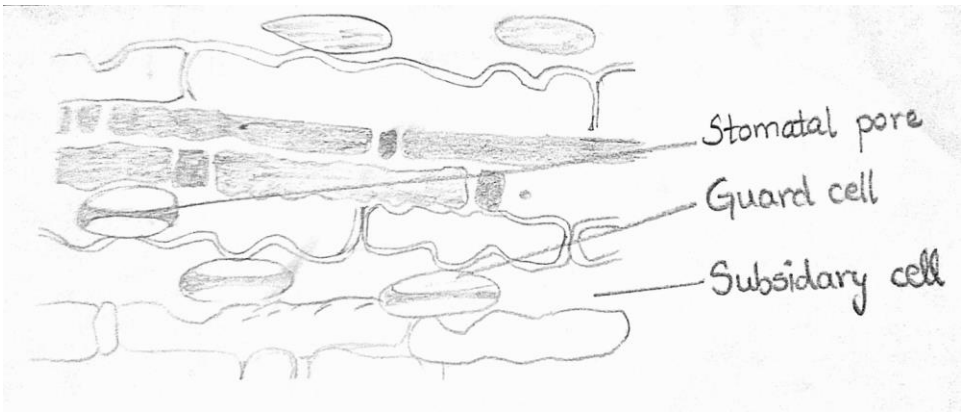


Fig.12.5 (A): The stomatal and epidermal structure at abaxial surface of *Saccharum.*

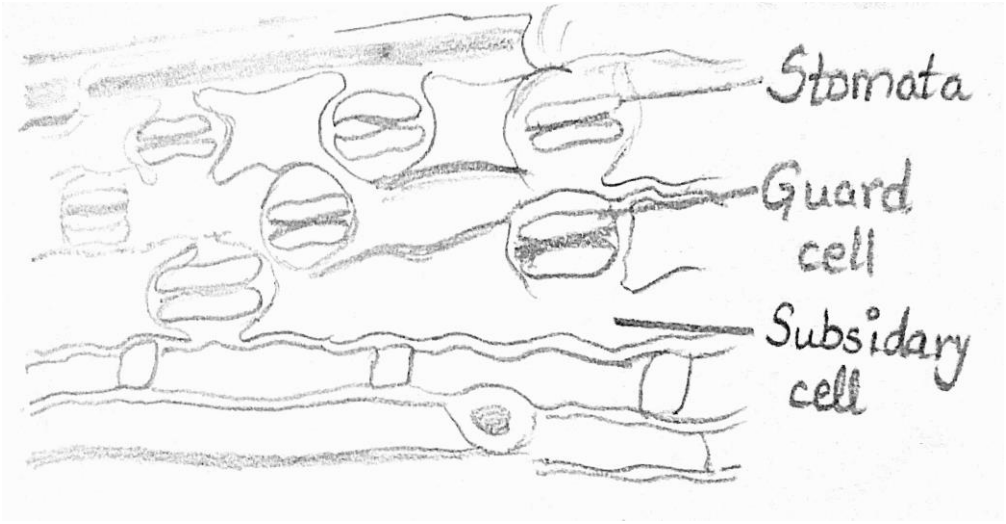


Fig.12.5 (B): The stomatal and epidermal structure at adaxial surface of *Saccharum*

12.3.1.5- *Cynodon*

This genus belongs to the large and ubiquitous family of monocotyledonous flowering plants which are known as grasses. There are around 780 genera and 12000 species of the Poaceae family. They have economic importance as they provide staple foods from cereal crops like maize, wheat, rice, barley and millets. All have hollow stems except at the nodes. The species of *Cynodon*are commonly called as Bermuda grass or dog tooth's grass. Many species of this genus have originated in southeast Africa. They are also known as ubiquitous cosmopolitan weed.

Botany of the plant

Cynodon term comes from greek word meaning 'dog-tooth'. It is a genus belonging to grass family. They best thrive in warm temperate to tropical regions. The stems are slightly flattened with a tingling of purple color in them. The stem is erect and can grow up to 1-30 cm. Deep roots

growing up to 2 meters, allows the resistance and tolerance during drought and water stress with effective ability of water absorption.

Morphology of the leaf

The short leaf blades are grey-green in color. Their length varies from 2-15 cm with rough edges.

The epidermal peel of *Cynodon* leaf (Fig 12.6)

1. Large, highly vacuolated and thin-walled cells are present in epidermis.
2. They are called bulliform i.e., bubble-like cells.
3. They are present on both surfaces of the leaf but mostly on the upper surface of leaf.
4. The cells contain water but no chlorophyll.
5. They have thin wall but the other wall is thick and cutinized mostly by silica.
6. Stomatal pore is covered by guard cells.
7. Subsidiary cells surround guard cell.

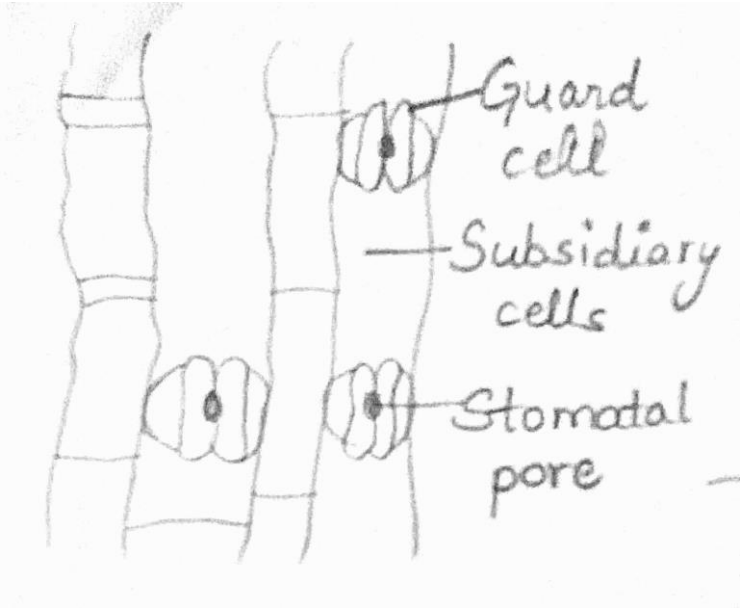


Fig.12.6: The epidermal peel of *Cynodon* leaf

12.3.1.6- *Musa*

The genus *Musa* includes bananas and plantains. There are 70 species of known *Musa* which are used widely. They grow as high as trees. The banana and plantain plants are not woody. The huge leaf stalks made their apparent stem. They are considered as gigantic herbs. Their many species are used as food plants.

Botany of the plant

The plant species are perennial, tall, tree like herbs. The stem is underground, rhizomatous, perennating sheathing leaf bases rolled upon one another to form a pseudoaerial stem. Roots are adventitious.

Morphology of the leaf

The leaves are radical, simple, alternate, exstipulate, large and broadly elliptical. The leaf base may grow up to 6 feet in length. Unicostate parallel venation is observed.

The epidermal peel of *Musa* leaf (Fig 12.7)

14. The epidermis is divided into upper and lower regions.
15. The epidermal cells are compactly arranged and round in shape and have cuticularized outer walls.
16. On the lower side of leaf surface, slight sunken stomata are present.
17. Both adaxial and abaxial thin-walled parenchyma cells are present next to epidermal layers.
18. Cells in upper surface are larger in size than the lower surface and they are markedly different from the mesophyll.
19. The stomata are Rheo type with two lateral and two-three polar cells and four subsidiary cells and are sunken.

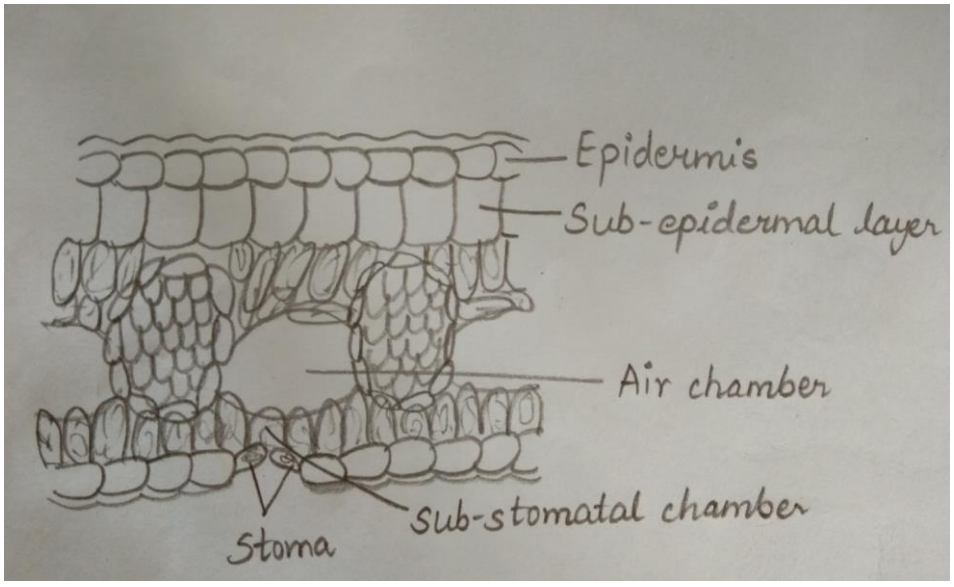


Fig.12.7: The epidermal peel of Musa leaf

12.3.2 Dicots species

12.3.2.1- *Sedum*

It is the genus of the family Crassulaceae. The genus is commonly called as stonecrop. They are succulent perennial herbs or shrubs. They plants are primarily found in the Northern Hemisphere but also found in some parts of Southern Hemisphere like in Africa and South America.

Botany of the plant

*Sedum*is an annual, biennial and perennial herb. The plant possesses succulent leaves and stems. The plants may vary from creeping herbs to shrubs. The flowers usually have five petals, seldom four or six.

Morphology of the leaf

Leaves are simple or compound, opposite, alternate or whorled, exstipulate, more or less thick and fleshy. The leaves are fleshy modified to store the water.

The epidermal peel of *Sedum* leaf (Fig 12.8)

1. Stomata are ofanisocytic cells
2. Usually, six subsidiary cells are possessed around the stomata of *Sedum*.
3. Two guard cells are formed after the spiral series of cell division from subsidiary cells.
4. Subsidiary cells act as spacers in order to give ordered arrangement of stomata.

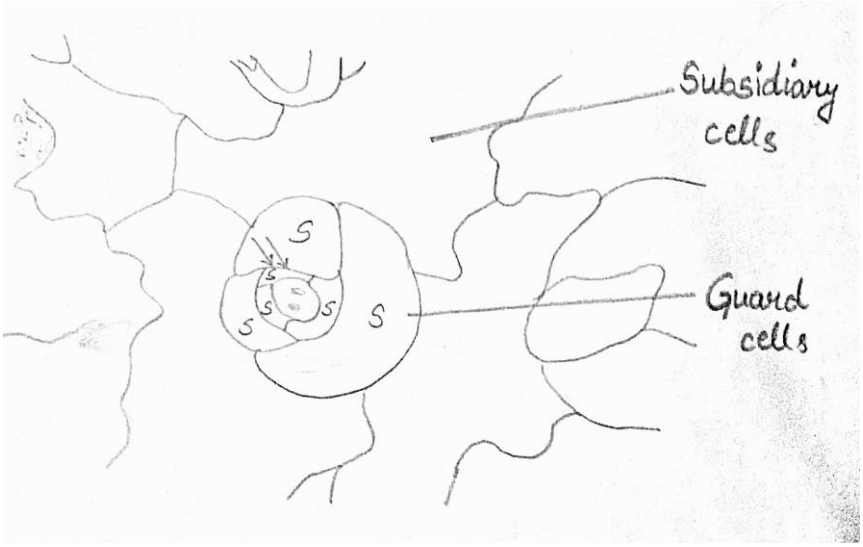


Fig.12.8: Structure of stomata and epidermis anatomy of *Sedum*.

12.3.2.2- *Pelargonium*

The plants belonging to this genus are evergreen perennials, succulents and shrubs. They are more commonly known as geraniums, pelargoniums, or storksbills. The plants thrive in warm

temperate and tropical regions throughout the world and many species are found in southern Africa. The plants are known to be tolerant of drought, heat and minor frosts.

Botany of the plant

Species belonging to this genus are herbaceous annuals, shrubs, subshurbs, stem succulents and geophytes. Stem is erect occasionally branched which on flowering bears five-petaled flowers in umbel-like clusters and are zygomorphic.Stem is fleshy and is found to be thick below and often woody.

Morphology of the leaf

Leaves are alternate, incised or palmately lobed or pinnate or incised up to the base or compound often on long stalks. The leaves are rarely entire and stipulate. Some light or dark patterns are observed on the leaves. The leaves have thick cuticle to resist drought stress.

The epidermal peel of *Pelargonium* leaf (Fig 12.9)

1. Stomata are orderly arranged on the lower surface of the epidermis.
2. Stomata are of amonocytic type and their size is 4mm long when measured by the length of central vein.
3. The space is enlarged to become substomatal cavity due to the presence of stoma.

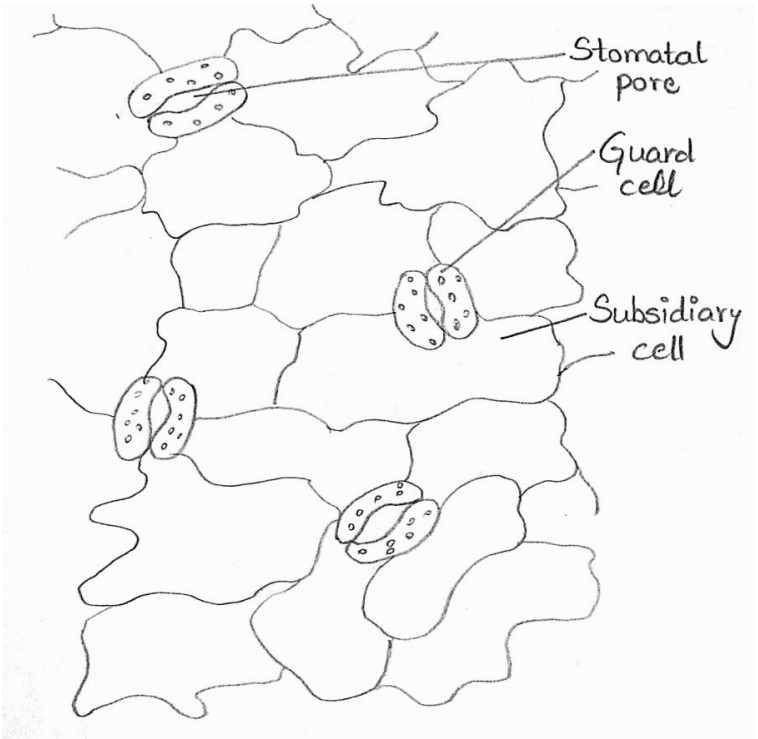


Fig.12.9: Structure of stomata and epidermal peel of *Pelargonium* leaf.

12.3.2.3- *Abelmoschus*

Abelmoschus name has derived from Abrabian language, meaning 'father of musk' or 'source of musk' related to the scented seeds. The genus belongs to the Malvaceae. It is are grown in humid, temperate and wet regions. Formerly it was included under *Hibiscus* genus. They are native to tropical Africa, Asia and northern Australia.

Botany of the plant

Species belonging to this genus comprise annual and perennial herbaceous plants. The plant height goes up to 2 m tall. It is a perennial shrub.

Morphology of the leaf

Leaf is ovate to pentagonal in shape, palmately lobed with 3-7 lobes. 5-6 bracts measuring 7-11 mm in length are present. The leaves are 10-40 cm long and broad.

The epidermal peel of *Abelmoschus* leaf (Fig 12.10 A,B,C)

1. Epidermal cells vary from polygonal to irregular in shapes and anticlinal walls are wavy on adaxial surface.
2. Epidermal cells are irregular and anticlinal wall of wavy and undulating on abaxial surface.
3. Average number of epidermal cells is 17.
4. Average number of stomata present on adaxial and abaxial surfaces are8 and 6 respectively.
5. Stomata are anomocytic, amphistomatic and often anisocytic.
6. Large irregular cells in the form of mucilaginous are present on both surfaces.

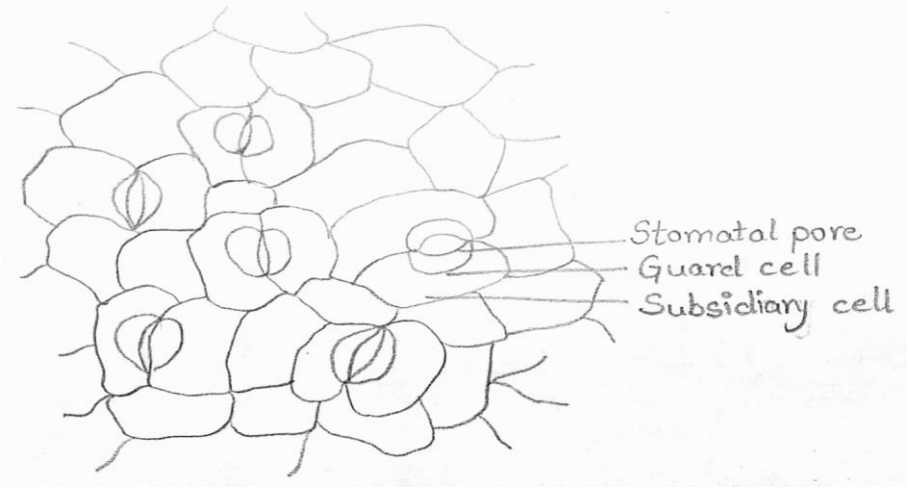


Fig.12.10 (A): The structure of polygonal epidermal cells in *Abelmoschus* leaf.

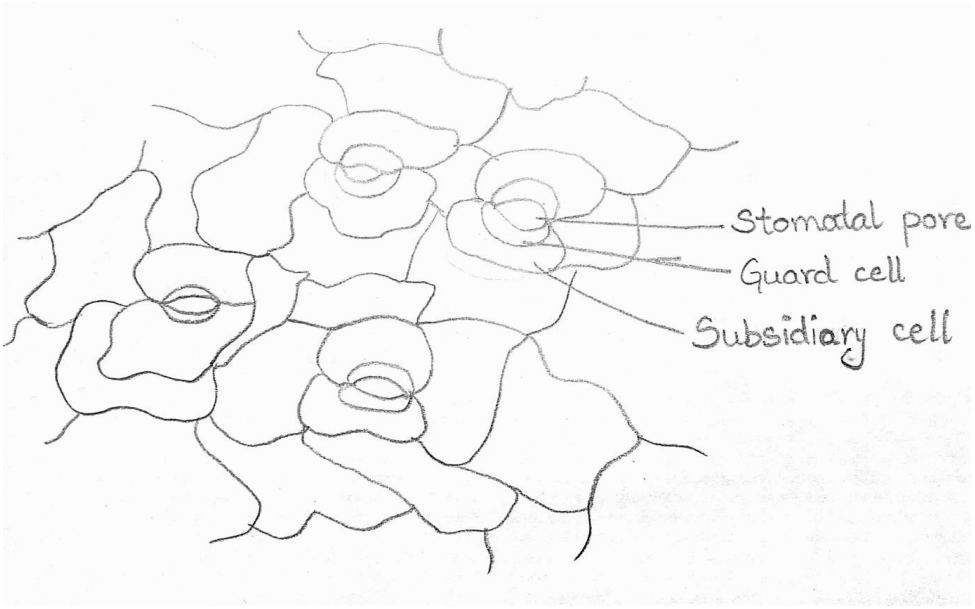


Fig.12.10 (B): The structure of isodiametric epidermal cells in *Abelmoschus* leaf.

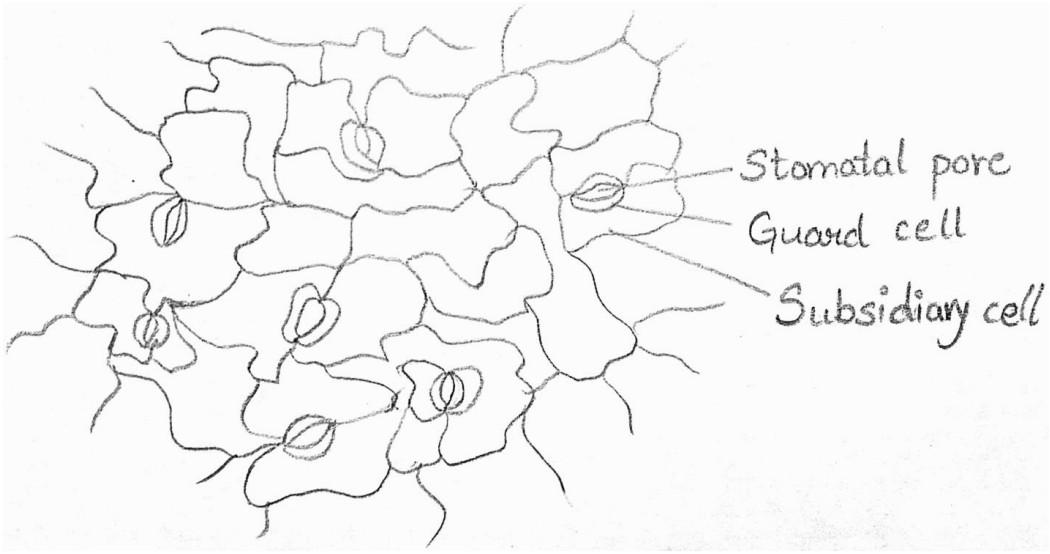


Fig.12.10 (C): The structure of irregular epidermal cells in *Abelmoschus* leaf.

12.3.2.4- *Hibiscus*

This is another genus of mallow family named as Malvaceae. Numerous species numbering to several hundred comprised of this genus. Species belonging to this genus thrive in warm temperate, subtropical and tropical regions throughout the world.

Botany of the plant

The plants belonging to this genus are used as ornamental shrubs usually cultivated in tropics. These are perennial. Stems are erect, branched, cylindrical, solid, woody and glabrous. Roots are tap, branched and deep. Inflorescence is solitary axillary.

Morphology of the leaf

Leaves are alternate, simple and possess petiole. Leaf has stipule and serrate margin. Unicostate reticulae venation is present. Leaf is ovate and glabrous.

The epidermal peel of *Hibiscus* leaf (Fig 12.11)

1. Epidermal cells vary from polygonal to irregular in shapes and anticlinal walls are straight on adaxial surface.
2. Epidermal cells are irregular and anticlinal wall of wavy and undulating on abaxial surface.
3. Average numbers of epidermal cells are 22.
4. Average number of stomata present on adaxial and abaxial surfaces are8 and 6 respectively.
5. The vascular bundles are present in a ring arc form.

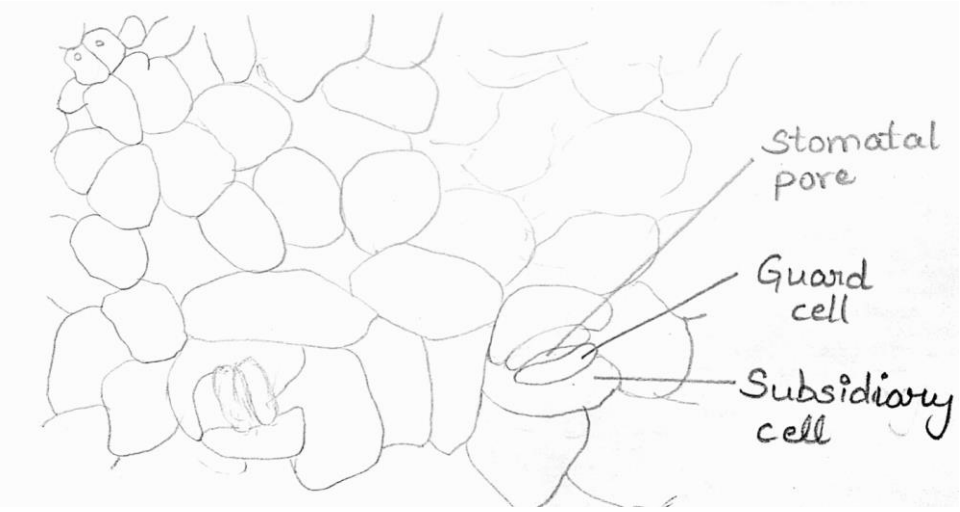


Fig.12.11: The structure of stomata and epidermis in *Hibiscus* leaf**.**

12.4- PREPARATION OF STOMATAL INDEX

The stomatal index is defined as the number of stomata presents to the total number of epidermal cells; each stoma being counted as one cell in the form of percentage. The stomatal index (I) is

the measure in terms of an average number of stomata cells present per square millimeter of the epidermis of the leaf. The formula for calculating the stomatal index (I) is given by

$I = S / E + S$

where,

I= Stomatal index

S= number of stomata cells per unit area

E= number of epidermal cells per unit area

Stomatal Distribution

Stomatal distribution refers to the distribution of stomata among monocot and dicot plants.Stomata are found on the plant surfaces like leaves, stem, etc.

Methodology

Materials required

- Compound microscope
- Microscope slides
- Cover glasses
- Forceps
- Spirit lamp
- Small watch glass
- Blade
- Cello tape
- Microscopic grid
- Dark colored pencil with sharp lead
- Chloral hydrate solution

Procedure

Preparation of lamina (Fig 12.12)

A mature leaf is taken either small or if big then cut it up to 5 mm square pieces from the middle portion between the lamina and the midrib.

Fresh leaf

a) Separate the epidermis or peeled off in thick leaves through breaking into pieces.

b) After separating the epidermis, treat with chloral hydrate.

c) Boil the leaf or leaf pieces in chloral hydrate in a test tube placed in water bath. It will allow epidermis to be separated.
d) Now place the peeled epidermis on a slide with 1-2 drops of chloral hydrate or glycerin as mounting medium.
e) Let the content be cool and then place a cover glass and then observe it under microscope.
f) Prepare separate slides for upper and lower epidermis.

Dry leaf

a) Add dry leaf in a test tube with chloral hydrate and heat it on water bath for 30 min.
b) Cut the leaf into two pieces and observe them under microscope for observing stomata.
c) Veins should be faced downwards through putting the cleared half on the microscopic slide and then upper epidermis is visible.
d) Veins facing upwards should be through putting the other half of the leaf on the microscopic slide which allows the lower epidermis to be visible.
e) Now peeled off the epidermis from both adaxial and abaxial surfaces (You will get now upper and lower epidermis)
f) Add two drops of glycerin to the cut pieces of epidermis on the slide and place a cover glass over it.
g) Observe them under microscope to trace the epidermal cells and stomata.

Tracing of cells

a) Draw an 8-10 cm square on a drawing sheet or any unit area.
b) Place the specimen after treatment in slide under the microscope.
c) Focus epidermal cells and stomata using microscope to magnify significantly.
d) Trace epidermal cells and stomata using camera lucida in the square and draw the figure with help of square grid.
e) Count the epidermal cells and stomata within the boundaries of drawn square.

To determine the stomatal index of any plant leaf:

1. Leaf pieces are cleared and mountedbetween the margin and midrib.
2. The lower surface is examined with an objective piece of microscope.
3. The sample can be observed by the eyepiece.
4. The number of epidermal cells and number of stomata are counted within a square grid.

5. The stomatal index is determined on both the leaf surfaces.

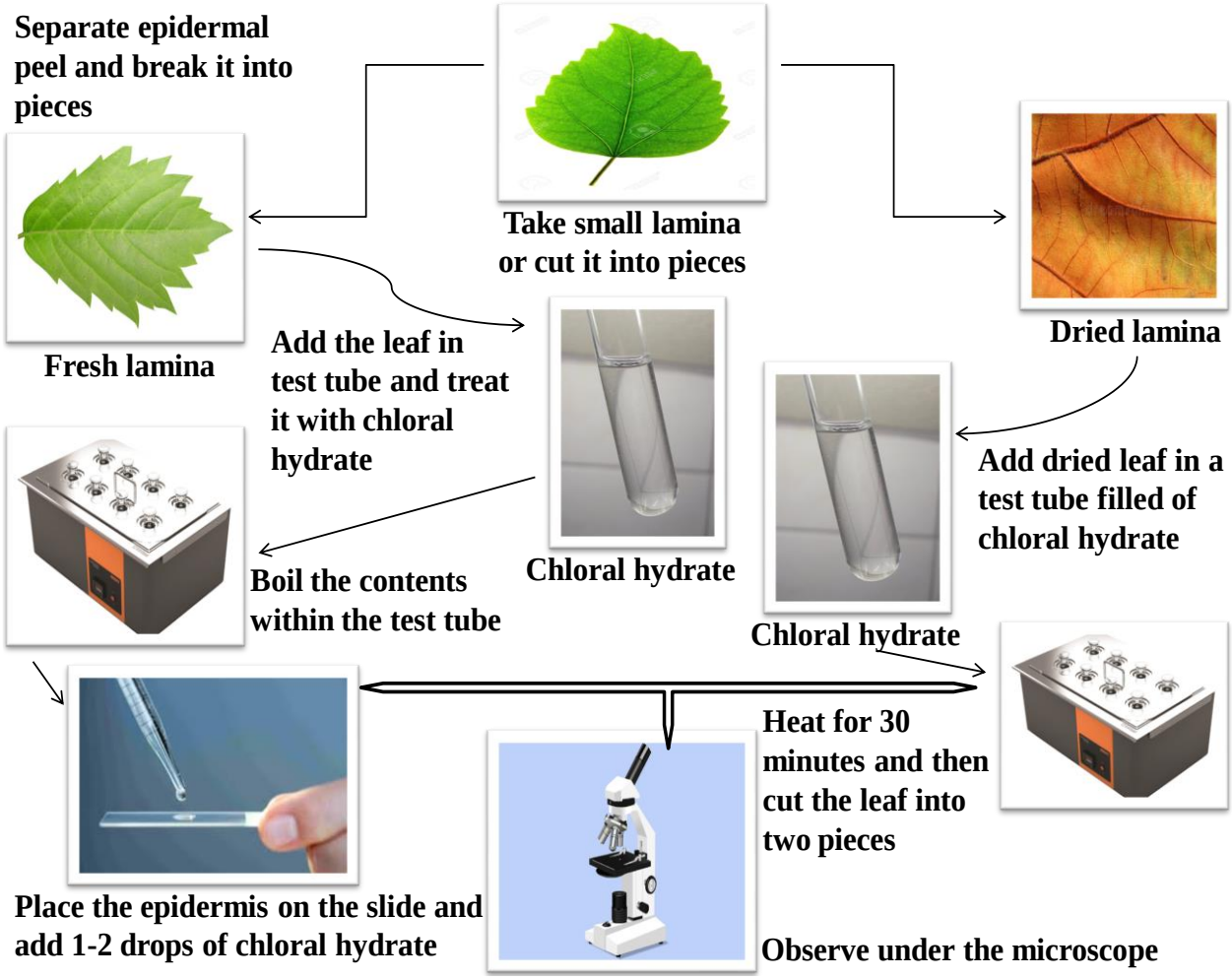


Fig.12.12: Procedure of preparing lamina for the study of stomata counting through the observance under the microscope.

Generally, on the ventral and dorsal surfaces, numbers of stomata found are 20-23 and 13-16, respectively.

Stomatal index of *Triticum*:

The stomatal frequencies vary on both the adaxial and abaxial surfaces of the leaf. It can also vary in the presence of stress. The stomatal frequency on adaxial surface of leaf is 2.77 whereas stomatal frequency on abaxial leaf surface is 2.35mm^2. The ratio of stomatal index on abaxial to adaxial surface is 0.78 and 0.81.

Stomatal index of *Carex*:

The stomata are only present in the abaxial surface. Stomata are absent on the leaf margins. The highest frequency of stomata found in *Carex nigra* is 179 mm^{-2} and the lowest frequency of stomata is found in *C. distans* is 120 mm^{-2}. The stomata are of paracytic type. The length of guard cell is in between 27.06-36.3 µm.

Stomatal index of *Avena*:

The stomata are generally found on the both surfaces of the leaf. The density of stomata on adaxial surface is higher than the abaxial surfaces but this situation is reverse in *Avenaeriantha*. Stomata are usually found in the regular rows of the lamina. The highest density of stomata is found in *A. textilis* by 200 mm^{-2} and the lowest density of stomata are found in *A. arundinaceus* by 43 mm^{-2}. The guard cells are large with average cell length of 55.86 µm on the adaxial surfaces and 30.96 µm on the abaxial surfaces.

Stomatal index of Gladiolus:

The stomata are found on both adaxial and abaxial surfaces of the leaf. The density is higher in the abaxial surface with 110 mm^{-2} and lower in adaxial surface with stomatal density of 18.69 mm^{-2}. The stomata are not arranged with regularity in whole leaves. The stomatal type found in *Gladiolus* is anomocytic. The length of abaxial guard cell is 33.17 µm in average in *Gladiolus*. Higher the stomatal frequencies, longer are the guard cells in length.

Stomatal index of *Juncus*:

The stomata are found on abaxial surfaces only. They are regularly situated in linear groups. The stoma is of anomocytic type. The stomatal density is 35-83 mm^{-2}. The length of guard cells of *Juncus* is in between 21.6-35.3 µm.

Stomatal index of *Allium*:

The stomata are present on both the surfaces. The *Allium* has greatest stomatal frequencies with an abaxial average density of 143 mm^{-2}. The stomatal frequencies onabaxial surfaces are greater than those of adaxial surfaces of the leaf. The largest guard cells are always found in the abaxial surface of the leaf. The size of stomata is equal on both the surfaces i.e. 32 µm and the length of guard cells vary from 23.64-39.48 µm.

Stomatal index of *Orchis*:

The stomata are only found in the abaxial surfaces of leaf. These are scattered or arranged without any regularity on the whole leaf. The stomata are of anomocytic type. The average length of *Orchis*guard cell is 67.4 µm. The stomatal density is 78 mm^{-2} for *Orchis*.

Table 1: Types of Stomata, Number of stomata and Stomata index of Different Species

Name of Species	**Type of stomata**	**Number of stomata per unit area(mm^2)**	**Abaxial surface Density (mm^{-2})**	**Adaxial surface Density (mm^{-2})**	**Number of epidermal cells in abaxial surface**	**Number of epidermal cells in adaxial surface**	**Stomatal index (Abaxial/Adaxial)**
Triticum aestivum	**	9859	**	**	5170	7490	0.78/0.81
Carexhirta	Paracytic	**	245	144	**	**	16.2-23.8
Avena sativa	Potamogeton	**	65	44	27-35	25-48	**
Gladiolus	Anomocytic	**	110	18.69	**	**	**
Juncus	Anomocytic	**	**	**	**	**	**
Allium	**	**	143	**	**	**	**
Oryza sativa	**	**	**	**	**	**	**
Zea mays	Paracytic	**	100±19	71±15	68-158	52-94	13.2/16.9 (0.71)
Saccharun officinarum	**	**	**	**	176-351	59-167	**

** Data was not available

12.5- DEMONSTRATION OF THE EFFECT OF ABA ON STOMATAL CLOSURE

Abscisic acid i.e., ABA is a plant hormone which is involved in plant response towards reduced water availability. It causes reduction in guard cell turgor pressure in its absence by closing the stomatal pore aperture. This is how water is conserved during periods of drought. The cytosolic Ca^{2+} mediates the reduction in turgor of guard cell through a signal transduction pathway which is elicited by ABA. ABA is responsible for Ca^{2+} mobilization pathway which involves cyclic adenosine 5'- dephosphoribose (cADPR). The cADPR microinjections in guard cells causes reduction in turgor that was preceded by increase concentration of free Ca^{2+} in the cytosol.

The effect of ABA on stomatal closure (Fig 12.13)

1. ABA controls the stomatal closure response in various environmental stresses.
2. During the period of drought or reduced water, ABA is abundant in leaves.
3. It promotes the reduction in stomatal aperture in order to reduce the extent of transpirational water loss.
4. The reduction in stomatal pore width is caused by decrease in the turgor of the two guard cells surrounding the stomatal pore.
5. ABA promotes the efflux of potassium salts from the guard cells.
6. Such operation of ABA takes place through Ca^{2+} dependent signal transduction pathways.
7. ABA increases the cytosolic free Ca^{2+} concentration.
8. A large number of Ca^{2+} permeable channels are present on both the plasma membrane and the endomembranes which are involved in the specific signal transduction pathways.
9. This Ca^{2+} releasing channelshave the dynamic properties which produce the stimulus response by which opening and closing of stomata is controlled.

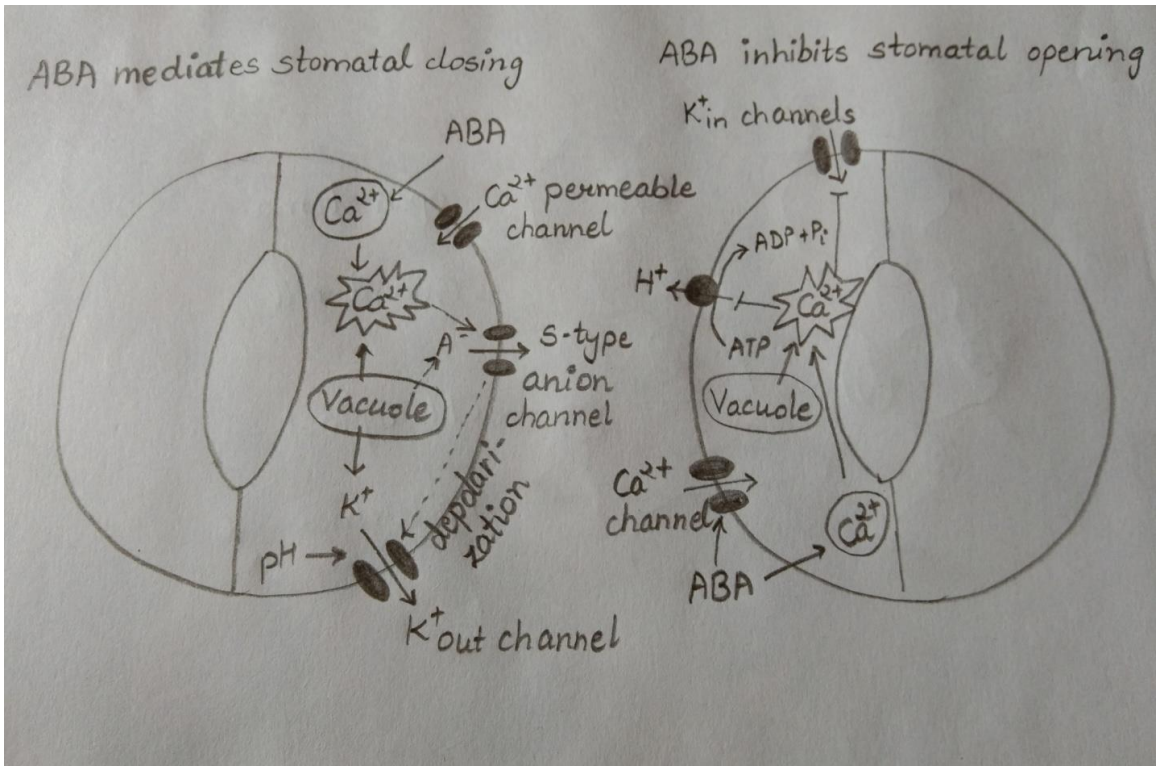


Fig.12.13: Diagrammatic representation of (A) ABA mediates stomatal closing and (B) ABA inhibits stomatal opening in guard cells.

12.6- SUMMARY

The epidermis is the outermost layer of the cell surface which protects the inner tissues from adverse natural disasters like high temperature, desiccation, and mechanical injury, external infection etc. the tiny stomata are present in upper and lower epidermis of leaves, generally surrounded by dumbbell-shaped guard cells. These stomata are arranged in regular arrays and areequally distributed in both the upper and lower epidermis. The stomatal index is the measurement of number of stomata forms to the total number of epidermal cells, each stoma being counted as one cell in the form of percentage. Abscisic acid (ABA) is plant hormone involved in plant response to reduced water availability. It does so by reduction in guard cell turgor pressure and thus closing the stomatal pore aperture.

12.7- GLOSSARY

Abaxial: Facing away from the stem of a plant or to the lower surface of a leaf.

Adaxial: Facing towards the stem of a plant or to the upper surface of a leaf.

Amphistomatic: It represents having stomata on both surfaces.

Anomocytic: It represents stomata have guard cells that are surrounded by cells that have the same size, shape and arrangement as the rest of the epidermis cells.

Cosmopolitan: Found all over the world.

Deciduous: Shedding its leaves annually.

Desiccation: The removal of moisture from something.

Gigantic: It means great size or extent; huge or enormous.

Herbaceous: It denotes to herbs.

Hyaline: Glassy and translucent in appearance.

Imbricate: Adjacent edges overlapping.

Obscure: Uncertain not unknown.

Pantropical: Occurring or distributed throughout the tropical regions of the earth.

Perennial: Living for several years.

Periblem: It is a primary meristem that gives rise to the cortex and is located between plerome and dermatogen.

Prairies: A large open area of grassland.

Scrambling: It is having a stem too weak to support itself, instead attaching to and relying on the stems or trunks of stronger plants.

Staple: They are regularly consumed in large quantities as to form the basis of a traditional diet and which serves as a major source of energy and nutrients.

Transpiration: It is the process of water movement through a plant and its evaporation from aerial parts, such as leaves

Turgor: The force within the cell that pushes the plasma membrane against the cell wall.

Ubiquitous: Found or existing everywhere.

Uniseriate: Arranged in a single row, layer, or series.

12.8- SELF ASSESSMENT QUESTIONS

1. The wheat kernel is called _____.
2. The stomatal type found in *Gladiolus* is _____.
3. The species of *Cynodon*are commonly called as _____.
4. Carex has _____ type of stomata.
5. ABA is responsible for Ca^{2+} mobilization pathway which involves _____.

6. The *Orchis*stomata are of _____ type.
7. _____ are commonly called as Rushes.
8. The ratio of*Triticum* stomatal index on abaxial to adaxial surface is _____ and _____.
9. The stomata of maize are also referred as _____.
10. _____ are herbaceous perennial.

ANSWERS TO SELF ASSESSMENT QUESTIONS

1. Caryopsis
2. Anomocytic
3. Bermuda grass
4. Paracytic
5. cyclic adenosine 5'- dephosphoribose (cADPR)
6. Anomocytic
7. *Juncus*
8. 0.78, 0.81
9. *Zea* type
10. *Tradescantia*

12.9- REFERENCES

1. Allen, G. J., & Sanders, D. (1997). Vacuolar ion channels of higher plants. In *Advances in Botanical Research*, 25: 217-252). Academic Press.
2. Aminian, R., Mohammadi, S., Hoshmand, S. A., & Khodambashi, M. (2010). The genetic analysis of stomatal frequency and size, stomatal conductance, photosynthetic rate and yield in wheat (Triticum aestivum L.) using substitution lines series. *Wheat Information Service*, *110*: 25-34.
3. Ball, P. W., & Reznicek, A. A. (2002). Carex Linnaeus, Sp. P1. 2: 972. 1753. *Flora of North America Editorial Committee*.
4. Christmann, A., Hoffmann, T., Teplova, I., Grill, E., & Müller, A. (2005). Generation of active pools of abscisic acid revealed by in vivo imaging of water-stressed Arabidopsis. *Plant physiology*, *137*(1), 209-219.

5. Yakandawala, D. M. D., Sirisena, U. M., & Dassanayake, M. D. (2005). Two New Records of Juncus Species (Rush Family-Juncaceae) In Sri Lanka. Ceylon Journal of Science (Biological Science), 33, 67-76.
6. Goldblatt, P., & Manning, J. (1998). Gladiolus in southern Africa. Fernwood Press.
7. Goldblatt, P.; De Vos, M. P. (1989). "The reduction of *Oenostachys, Homoglossum* and *Anomalesia*, putative sunbird pollinated genera, in *Gladiolus* L. (Iridaceae-Ixioideae)". *Bulletin du Muséum National d'Histoire Naturelle, Section B*. **11** (4): 417–428.
8. Hipp, A. L. (2007). Nonuniform processes of chromosome evolution in sedges (Carex: Cyperaceae). *Evolution: International Journal of Organic Evolution*, *61*(9), 2175-2194.
9. http://agropedia.iitk.ac.in/content/gladiolus-swordlily#:~:text=It%20has%20its%20natural%20habitat,of%20flowers%20known%20as%20spikes.
10. https://amrita.olabs.edu.in/?sub=79&brch=7&sim=128&cnt=1
11. https://byjus.com/biology/preparing-a-temporary-mount-of-a-leaf-peel-to-show-stomata/
12. https://www.biologydiscussion.com/experiments/experiment-to-observe-temporary-mount-of-a-leaf-peel-to-show-stomata/1733
13. https://www.biologydiscussion.com/plant-tissues/the-epidermal-tissue-system-of-plants-with-diagrams/13880
14. https://www.cabi.org/isc/datasheet/115039#:~:text=J.,Hemisphere%20as%20an%20ornamental%20plant.
15. https://www.illinoiswildflowers.info/grasses/plants/oats.html#:~:text=Habitats%20consist%20of%20cropland%2C%20abandoned,grain%2C%20forage%2C%20and%20straw.
16. https://www.rainforest-alliance.org/species/orchid#:~:text=Most%20orchid%20species%20grow%20in,lie%20along%20the%20Andes%20Mountains.
17. https://www.ukessays.com/essays/biology/determination-of-stomatal-index-biology-essay.php
18. Jermy, A. C., Simpson, D. C., Foley, M. J. Y., & Porter, M. S. (2007). General structure of Cyperaceae. Sedges of the British Isles. *Sedges of the British Isles. BSBI Handbook*, (1), 2-26.

19. Kokate, C. K. (1994). Practical Pharmacognosy. 4 [sup] th ed. *New Delhi: Vallabh Prakashan*, 107.
20. Leckie, C. P., McAinsh, M. R., Allen, G. J., Sanders, D., & Hetherington, A. M. (1998). Abscisic acid-induced stomatal closure mediated by cyclic ADP-ribose. *Proceedings of the National Academy of Sciences*, *95*(26), 15837-15842.
21. MacRobbie, E. A. C. (1981). Effects of ABA in 'isolated'guard cells of Commelina communis L. *Journal of Experimental Botany*, *32*(3), 563-572.
22. MacRobbie, E. A. C. (1997). Signalling in guard cells and regulation of ion channel activity. *Journal of Experimental Botany*, 515-528.
23. Manning, John; Goldblatt, Peter (2008). *The Iris Family: Natural History & Classification*. Portland, Oregon: Timber Press. pp. 138–42.
24. McAinsh, M. R., & Hetherington, A. M. (1998). Encoding specificity in Ca2+ signalling systems. *Trends in Plant Science*, *3*(1), 32-36.
25. McAinsh, M. R., Brownlee, C., & Hetherington, A. M. (1990). Abscisic acid-induced elevation of guard cell cytosolic Ca 2+ precedes stomatal closure. *Nature*, *343*(6254), 186-188.
26. Mittelheuser, C. J., & Van Steveninck, R. F. M. (1969). Stomatal closure and inhibition of transpiration induced by (RS)-abscisic acid. *Nature*, *221*(5177), 281-282.
27. Ralph E. Brooks; Steven E. Clemants (2000). "*Juncus*". *Magnoliophyta: Alismatidae, Arecidae, Commelinidae (in part), and Zingiberidae*. Flora of North America. **22**. Oxford University Press.
28. Scannell, M. J. (1973). Juncus planifolius R Br. in Ireland. The Irish Naturalists' Journal, 308-309..
29. Scannell, M. J., & Jebb, M. H. (2000). Flora of Connemara and the Burren: Records from 1984. Glasra, 4:7-45.
30. Watson, L. and M. J. Dallwitz. (2008). "*Avena*". *The Grass Genera of the World*. Archived from the original on 2009-08-20. Retrieved 2009-08-19.
31. Zarinkamar, F., 2005. Foliar anatomy of Gladiolus segetum and Iris acutiloba (Iridaceae). Proceedings of the 13th Iranian Biology Conference and the First International Conference of Biology, Guilan University, Rasht, Iran.

32. Zarrinkamar, F., Jalili, A., Hamzehee, B., Asri, Y., Hodgson, J. G., Thompson, K., & Shaw, S. (2002). FOLIAR ANATOMY OF CAREX IN ARASBARAN, NW. IRAN. *Iranian Journal of Botany*, *9*, 261-270.
33. Korn, R. W. (1972). Arrangement of stomata on the leaves of Pelargonium zonale and Sedum stahlii. *Annals of Botany*, *36*(2), 325-333.
34. Essiett, U. A., &Iwok, E. S. (2014). Floral and leaf anatomy of Hibiscus species. *American Journal of medical and biological research*, *2*(5), 101-117.
35. Olotuah, O. F. (2014). Anatomy of leaf epidermis and petiole of two selected species of Hibiscus. *Res. J. Agric. Environ. Manage*, *3*(8), 403-405.
36. Osawaru, M. E., Dania-Ogbe, F. M., Chime, A. O., &Ogwu, M. C. (2011). Epidermal morphology of west African okra Abelmoschus caillei (A. Chev.) Stevels from south western Nigeria. *Science World Journal*, *6*(3), 15-23.
37. https://www.gtac.edu.au/wp-content/uploads/2016/01/Leaf-Epidermal-Peel_LabPreparation.pdf
38. Taratima, W., Ritmaha, T., Jongrungklang, N., Raso, S., & Maneerattanarungroj, P. (2019). Leaf Anatomical Responses to Drought Stress Condition In Hybrid Sugarcane Leaf (Saccharum Officinarum 'Kk3').
39. Santhakumari, C., & Krishnamurthy, K. V. (1991). Development of epidermis on banana fruits. Biologia plantarum, 33(4), 325-331.
40. https://www.cbsetuts.com/ncert-class-10-science-lab-manual-stomata/

12.10- SUGGESTED READING

1. https://www.frontiersin.org/articles/10.3389/fpls.2021.716784/full#:~:text=The%20stomatal%20index%20of%20the,of%20stomata%20and%20epidermal%20cells.
2. https://www.americangeosciences.org/word/stomatal-index
3. https://www.researchgate.net/publication/284172830_Preparation_of_Epidermal_Peels_and_Guard_Cell_Protoplasts_for_Cellular_Electrophysiological_and_-Omics_Assays_of_Guard_Cell_Function
4. https://www.researchgate.net/publication/284172830_Preparation_of_Epidermal_Peels_and_Guard_Cell_Protoplasts_for_Cellular_Electrophysiological_and_-Omics_Assays_of_Guard_Cell_Function

12.11- TERMINAL QUSETIONS

1. Define stomatal index by citing wheat leaf an example.
2. Describe the epidermal peel of *Musa*in detail.
3. Give the details about the ABA exposure to the guard cells.
4. With the help of suitable diagrams, explain the epidermal peel of *Tradescantia*.
5. Describe different types of stomata found in angiosperms.

BLOCK-4 PLANT REPRODUCTION

UNIT-13-EXAMINATION OF MODES OF ANTHER DEHISCENCE AND COLLECTION OF POLLEN GRAINS FOR MICROSCOPIC EXAMINATION

13.1-Objectives

13.2-Introduction

13.3-Examination of modes of anther dehiscence and collection of pollen grains for microscopic examination

13.4-Summary

13.5-Glossary

13.6-Self Assessment Question

13.7-References

13.8-Suggested Readings

13.9-Terminal Questions

13.1-OBJECTIVES

After reading this unit students will be able to-

- Understand the male reproductive organ anther.
- Understand the modes of anther dehiscence.
- Understand the different types of anther attachment and anther dehiscence..
- Understand the techniques of pollen collection.
- Understand the techniques of pollen storage.

13.2 INTRODUCTION

An anther is a storehouse and manufacturing device of pollen, which plays structural and functional key role in the reproduction of angiosperms. It is found at the top of the androecia or more appropriately on the filament of the stamen. It produces the microspores or pollen grains, stores them, and nourishes them until the time of dispersal. Thus, the anther is a part of the male reproductive system or stamen inside the flower. Collectively, the stamens of a flower are termed as androecium, and the number androecium can vary greatly between species to species; on the basis of their numbers, plants are also classified as monandria, diandria triandria, tetrandria etc. and can be classified on the basis of their arrangement as monadelphia, diadelphia, polydelphia, tetradynamia, didynamia etc. There is also a high level of diversity associated with the position and appearance of the stamens, they also may be fused at either the filament or the anther. The male reproductive part or the structure of a flower typically consists of two elements; the slender long filament and elliptical or cylindrical distal part anther. Generally, anther is a bilobed structure which stores pollen grains in the pollen sacs (Fig. 13.1). The lobes of anther are attached together by the connective. On the basis of modes of attachment to filament, anthers are classified as, basifixed, adnate, dorsifixed and versatile (Fig. 13.2). The anther is a structure that constitutes male reproductive part or structure of the flower known as “stamen” or “androecium” by botanists (Fig.13.1). It has a distal knob-like structure, which generally consists of two lobes joined together by the connective tissue known as “connective” in palynogy**.** After anthesis, pollen grains get dispersed in the environment through dehiscence of anther. The pollen released from anther reaches the stigma of either the same flower (self pollination) or of different flower (cross pollination) during the process of pollination.

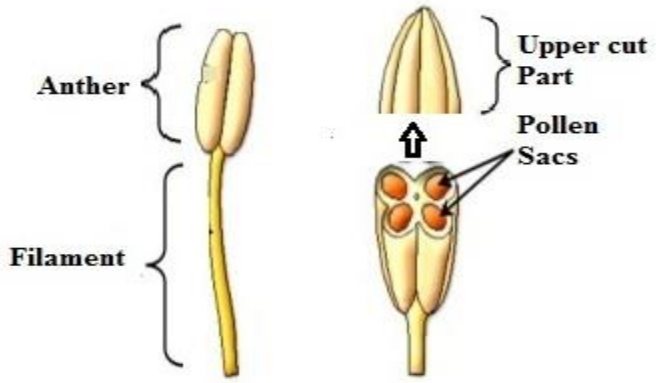
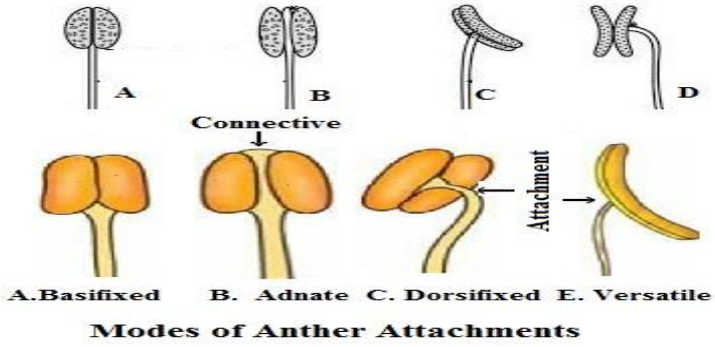


Fig. 13.1 Structure of Stamen



Modes of Anther Attachments

Fig.13.2: Modes of Anther attachment with filament.

13.3-EXAMINATION OF MODES OF ANTHER DEHISCENCE AND COLLECTION OF POLLEN GRAINS FOR MICROSCOPIC EXAMINATION

The splitting of anther at its maturity is known as anther dehiscence. Anther dehisces along the line of delicacy to release its contents, which is referred to as pollens or microspores (Fig.13.3). Anther dehiscence occurs either through detachment of a part of the anther or through special opening pore (loculicidal). The dehiscence modes may be porous, short slits, longitudinal slits, transverse, valvular and irregular (Fig.13.4). According Keijzert (1987), the pollen dispersal requires fine tuning and regulation of development of the anther in a synchronized manner to ensure that pollen release occurs at the precise time to maximize either cross or self-fertilization. Anther dehiscence is the ultimate process and final function of the anther, which causes the dispersal of pollen grains or microspores from pollen sacs. This process has finely tuned with pollen differentiation, floral development, maturation and anthesis. The walls of anther splits at a

specific site called stomium, which is developed previously for dehiscence. These sites can be seen as an indentation between the locules of each theca and runs the along the anther. In case of plant species which have poricidal anther dehiscence, it is instead a fine pore. Anthers can be classified as extrose and introse based on their facial arrangement. If it faces towards the periphery of the flower it is called extrose and when the pollen releases from the anther by splitting on the outer side, it is called extrorse dehiscence. If the face of anther lies towards stigma or axis of the flower, then it is known as introse**,** and the pollens dispersed from the inner side, is called introse dehiscence. If the pollen is released through a split that is positioned to the side, towards other anthers, rather than towards the inside or outside of the flower, this is latrorse dehiscence (Fig. 13.5).

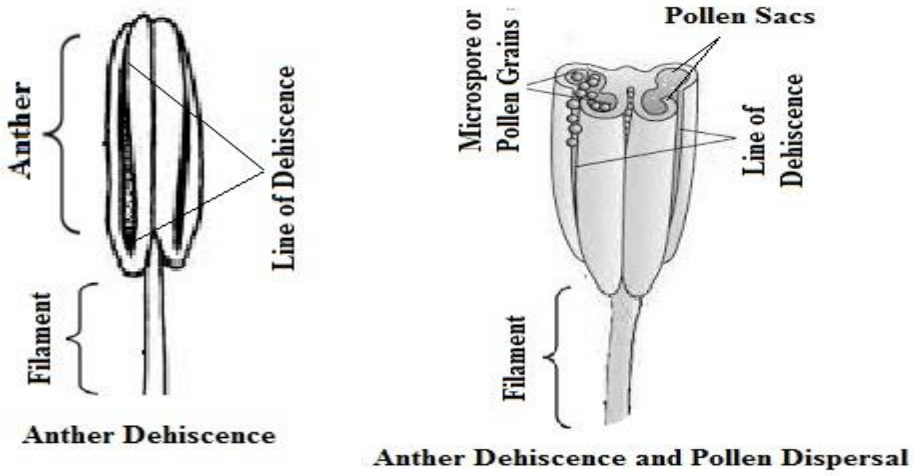


Fig.13.3: Anther Dehiscence and release of Pollen Grains

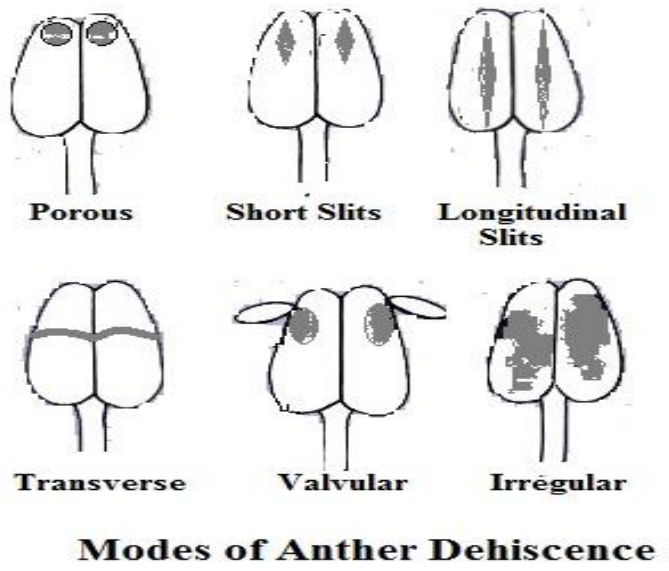


Fig. 13.4: Different Modes of Anther Dehiscence

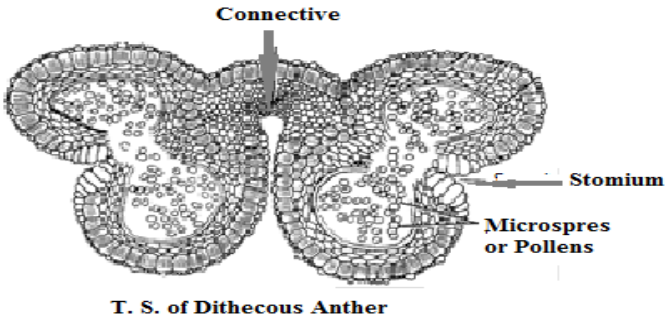


Fig.13.5: T. S. of Mature Dithecous Anther

COLLECTION OF POLLEN GRAINS

The primary requirement and crucial phase of pollen collection is to collect them in viable condition. Viability is must be determined for any experimental study on pollen. Pollens are collected soon after anther dehiscence for experimental studies to get optimal responses. In most of plant species, it is convenient to detach flowers or whole inflorescences on the previous evening and the cut end is dipped in water overnight in the laboratory. On the next day, the anthers generally would have dehisced. Gently tap flowers on a watch glass and shed the pollen grains. Another convenient method of pollen collection is to cut the mature anthers before dehiscence and dehisce them under low humidity in desiccators and remove the remaining and unnecessary part or debris with the help of pair of sterilized forceps or a brush. To prevent contamination, it is advisable to choose mature undehisced anthers for collection and surface-sterilize them with dilute chlorine water, or 10% sodium hypochlorite (NaOCl), or 0.25%

mercuric chloride ($HgCl_2$), or 70% ethyl alcohol for 2 to5 min, then dry and lay them in a sterilized petri dish for dehiscence.

Pollen storage:

Generally, pollen grains stored in glass or polythene vials (fig. 13.6) under low temperature and suitable RH (relative humidity). For pollens stored in unsealed containers or vials, suitable dehydrating agents like silica gel, sulphuric acid or solution of appropriate salts are used for maintaining relative humidity. Lycopodium powder, wheat or corn flour and talcum powder are used for preventing pollen grains from sticking together.

Non-polar organic solvents like benzene, diethyl ether and cyclohexane showed very less leaching of phospholipids, sugars, and amino acids into the solvent and found suitable for retention of viability. According to Jain and Shivanna (1988), polar organic solvents cause extensive leaching of substance and viability loss of pollen grains. For long term storage, pollens are freeze dried which involves the rapid freezing of pollen to sub-zero temperature of -60° C or -80° C by use of inert gas like helium or nitrogen, followed by gradual removable of water under vacuum sublimation. Long term preservation can also be done at ultra low temperature (Cryopreservation) by use of dry ice (solid carbon dioxide), vapour phase nitrogen and liquid nitrogen, temperature ranging between -70° C and -196° C like in pollen bank (Fig. 13.7).

Fig.13.6: Different types of vials used in pollen collection and storage.

Fig.13.7: Liquid nitrogen canes used in cryopreservation.

13.4 SUMMARY

1. The anther is a store house and manufacturing device of pollen grains which plays structural and functional key role in the reproduction of Angiosperms.
2. It produces the microspores or pollen grains, stores them, and nourishes them until the time of dispersal.
3. The anther is a part of the male reproductive system or stamen inside the flower.
4. Collectively, the stamens of a flower are termed the androecium, and the number androecium can vary greatly between species to species; on the basis of their numbers plants.
5. There is also a high level of diversity associated with the position and appearance of the stamens, they also may be fused at either the filament or the anther.
6. Generally Anther is a bilobed structure which stores pollen grains in the pollen sacs.
7. The lobes of anther attached together by connective, on the modes of attachment to filament anther are classified as, basifixed, adnate, dorsifixed and versatile.
8. The anther is a structure constitutes male reproductive part or structure of the flower known to as “Stamen” or “Androecium” by botanists.
9. It has a distal knob-like structure, generally which consists of two lobes joined together by the connective tissue called connective in palynogy.
10. The pollen released from anther reaches on the stigma of either the same flower (Self pollination) or in different flower (Cross pollination) during the process of pollination.

11. The splitting of anther at its maturity is called anther dehiscence, anther dehisces along a built in line of delicacy in order to release its contents, called pollen or microspores.
12. Sometimes this involves the detachment of upper or some other parts of anther and sometimes by special opening pore (Loculicidal).
13. The dehiscence modes may be porous, sort slits, longitudinal slits, transverse, valvular and irregular.
14. Anther dehiscence is ultimate process and the final function of the anther which causes the dispersal of pollen grains or microspores from pollen sacs.
15. The walls of anther splits at a specific site which are previously developed for dehiscence called stomium.
16. Anthers can be classified as extrose and introse on their facial arrangement.
17. The primary requirement and crucial phase of pollen collection is to get them in viable condition.
18. Generally, pollen collected soon after anther dehiscence for experimental studies to get optimal responses.
19. On the next day the anthers generally would have dehisced, gently tap flowers on a watch glass and sheds the pollen grains.
20. To prevent contamination it should be practiced to choose mature undehisced anthers for collection and surface-sterilize them with dilute chlorine water, or 10% sodium hypochlorite (NaOCl), or 0.25% mercuric chloride ($HgCl_2$), or 70% ethyl alcohol for 2 to5 min, then dry and lay them in a sterilized Petri dish for dehiscence.
21. Generally Pollen grains stored in glass or polythene vials under low temperature and suitable RH (relative humidity).
22. If pollens stored in unsealed containers or vials suitable dehydrating agents like silica gel, sulphuric acid or solution of appropriate salts are used for maintaining relative humidity.
23. Lycopodium powder, wheat or corn flour and talcum powder are used for preventing of pollen grains from sticking together.
24. Non-polar organic solvents like benzene, diethyl ether and cyclohexane showed very little leaching of phospholipids, sugars, and amino acids into the solvent and found suitable for retention of viability.

25. For long term storage pollen are freeze dried which; involves the rapid freezing of pollen to sub-zero temperature of -60° C or -80° C by use of inert gas like helium or nitrogen.
26. Long term preservation can also be done by ultra low temperature (Cryopreservation) by use of dry ice (solid carbon dioxide), vapour phase nitrogen and liquid nitrogen, temperature ranging between -70° C and -196° C like in pollen bank.

13.5 GLOSSARY

Androecium: Collective term for stamen the male reproductive organ of plants

Anther: Top fertile portion of androecium that bears pollen sacs.

Anthesis: Opening of a flower bud.

Connective: The part of tissue that connects the anther lobes.

Cryopreservation: Preservation of cells or tissue at ultra low temperature.

Dehiscence: Splitting of anther at maturity.

Extrorse: Facing outward.

Introse: Facing inward.

Leaching: Removal or washing out of chemicals under the influence of solvent.

Palynology: Science deals with the study of pollen grains.

Pollen grains: Microspore or powdery mass of male reproductive cells.

Pollination: The process of transfer of pollen from anther to stigma.

Relative humidity: The ratio of the water vapor actually present in the air to the greatest amount possible at the same temperature.

Stamen: The male reproductive part of a flower, usually with a slender filament supporting the **anther**.

Stigma: The top flat part of pistil, modified for receiving pollens.

Stomium: The thin-walled cells of anther in the region of dehiscence.

Viability: Ability or capability of germination.

Vial: A small container, typically cylindrical and made of glass, or plastic used especially for collection and storage of chemicals and cells.

13.6 SELF ASESSMENT QUESTIONS

13.6.1 Multiple Choice Questions:

1. Pollens are:

(a) Microspore cells (b) haploid cells

(c) Male cells (d) All of above

2. Pollens are produced in:

(a)Stigma (b) Anther

(c) Both (d) None of above

3. Pollen can be stored in liquid nitrogen for long term using the method of:

(a) Preservation (b) Cryopreservation

(c) Cryo-conservation (d) None of above

4. The ability of pollens to germinate is termed as:

(a) Non-viable (b) sterile

(c) Abortive (d) Viable

5. The splitting of anther at maturity is called:

(a) Cleavage (b) Splinter

(c) Fracture (d) dehiscence

6. Pollen test conducted in a test tube is called:

(a) Viability (b) fertility

(c) Sterility (d) All

7. Pollens facing inward or toward stigma are called:

(a) Introse (b) Extrose

(c) Latrose (d) None

8. Mercuric chloride ($HgCl_2$) is used in:

(a) Viability test (b) Surface sterilization

(c) Pollen culture (d) All

9. The use of organic solvents for pollen storage leads to:

(a) Leaching (b) Chelation

(c) Aggregation (d) All

10. Lycopodium powder in pollen storage is used for:

(a) Preservative (b) Prevention of aggregation

(c) Sterilizer (d) Anti leaching agent

13.6.2 Fill in the blanks:

(1) Pollens facing outward of stigma called _____.

(2) Pollens are produced inside of _____.

(3) After maturation anther undergoes in the process of_____.

(4) Generally anther consists of two lobes which are joined together _____.

(5) Dehiscence of anther occurs from specific region of anther called_____.

(6) Removal or wash out of chemicals under influence of solvent _____.

(7) Sulphuric acid or solution of appropriate salts is used for maintaining _____in pollen storage.

(8) Germination ability of pollen is called_____.

(9) Anthers can be classified as extrose and _____on their facial arrangement.

(10 Pollen sacs are found inside_____.

13.6.3 True or False:

(1) For maintaining relative humidity in stored pollen, sulphuric acid or solution of salts is used.

(2) Germination ability of pollen is called pollen sterility.

(3) Anthers are called extrose facial arrangement is outward.

(4) Germination ability of pollen is also called viablity.

(5) Pollens are collected from anther after dehiscens.

(6) Pollens are sterilized by boiling them in water.

(7) Polar organic solvents cause extensive leaching of substance and viability loss of pollen grains.

(8) Lycopodium powder, wheat or corn flour and talcum powder are used for preventing of pollen grains from sticking together.

(9) The walls of anther splits at a specific site which are previously developed for dehiscence called stomium.

(10) The temperature of liquid nitrogen is -96^0C.

13.6.4 Very short answer questions:

(1) Define microspore.

(2) What is dehiscence?

(3) Define extrose arrangement.

(4) Define viablity.

(5) What is called cryopreservation?

(6) What causes leaching of anther walls?

(7) Define stamen?

(8) Define anther.

(9) What is stomium.

(10) Define androecium.

13.6.1 Answer keys: 1-d, 2-b, 3-b, 4-d, 5-b, 6-a, 7-a, 8-a, 9-a, 10-b.

13.6.2 Answer key: 1-extrose, 2-anther, 3-dehiscence, 4-connective, 5-stomium, 6- leaching, 7- relative humidity 8-viablity, 9- introse, 10- anther.

13.6.3 Answer key: 1-True, 2-False, 3-True, 4-True, 5-True, 6-False, 7-True, 8-True, 9-True, 10-False.

13.7 REFERENCES

- Amma, M. S. P. and Kulkarni A. R. 1979. Pollen storage in organic solvents. J. Palynol. 15: 100–104.
- Iwanami Y., Nakamura N. 1972. Storage in an organic solvent as a means for preserving viability of pollen grains. Stain Technol. Vol.47(3):137-9.
- Jain A. and Shivanna K.R. 1988. Storage of Pollen Grains in Organic Solvents: Effect of Organic Solvents on Leaching of Phospholipids and its Relationship to Pollen Viability. Annals of Botany, Vol. 61(3): 325–330.
- Keijzert C. J. 1987. The processes of anther dehiscence and pollen dispersal. New Phytologist. Vol. 105(3): 499-507.

13.8 SUGGESTED READINGS

- Geitmann A. (Edt). 2020. Pollen and Pollen Tube Biology: Methods and Protocols (Methods in Molecular Biology) 2020.
- **Shivanna**, K.R. and **Rangaswamy**, N.S.1992. Pollen Biology: A Laboratory Manual. Springer.
- Stanley R. G. and Linskens H. F. 1974. Pollen: Biology Biochemistry Management. Springer.

13.9 TERMINAL QUESTIONS

13.9.1 Short answer questions:

(1) Write short note on anther.

(2) Describe microspore in brief.

(3) What do you understand by androecium?

(4) Describe cryopreservation.

(5) Differentiate between an anther and a filament.

(6) Describe the methods used for storage of pollens.

(7) Write a short note on anther attachment.

(8) What do you understand by stomium?

(9) Write a note on introse and extrose arrangement of anther.

(10) Define pollen storage techniques**.**

13.9.2 Long answer question:

(1) Write a note on stamen and its part.

(2) Describe different modes of anther dehiscence with suitable diagram.

(3) Write a detailed note on pollen collection and storage.

(4) Write a note on the use of polar and non polar solvent and their drawbacks in pollen storage.

(5) Describe the methods used in pollen storage.

UNIT-14-TESTS FOR POLLEN VIABILITY USING STAINS AND IN-VITRO GERMINATION, USING HANGING DROP AND SITTING DROP CULTURES

14.1-Objectives

14.2-Introduction

14.3-Tests for pollen viability using stains and in-vitro germination

14.4-Pollen germination using hanging drop and sitting drop cultures

14.5-Summary

14.6-Glossary

14.7-Self Assessment Questions

14.8-References

14.9-Suggested Readings

14.10-Terminal Questions

14.1-OBJECTIVES

After reading this unit students will be able to-

- Understand the concept of pollen physiology.
- Understand the pollen viability.
- Understand the methods of pollen germination.
- Understand in vitro and in-vivo pollen germination test.

14.2 INTRODUCTION

The term 'pollen' is usually used to describe male gametes of spermatophytes or powdery mass of microspores. Pollens are the male reproductive cells, which represents the male gametophytic generation of spermatophytes (seed-bearing plants) that produce male gametes (sperm cells) on germination. In gymnosperms, pollen grains or microspores are produced within microsporangium of male cones. In angiosperms, pollen grains are produced inside anthers. The process of pollen formation is called microsporogenesis.

The plant body of angiosperms is diploid and specialized organs for reproduction are present in flower. Stamens are the male reproductive organs. In most angiosperms, each stamen is composed of an anther that has anther lobes and a filament. The anther mostly contains four microsporangia which are joined by the connective. The anther wall consists of four layers: (i) the epidermis (exothecium) (ii) endothecium (iii) middle layer(s) and (iv) tapetum. In each microsporangium, the central region contains diploid microspore mother cells or pollen mother cell, which eventually forms the pollen grains. Each microspore mother cell undergoes meiosis and result in the formation of four haploid cells called tetrads. The microspores start to differentiate while associated in tetrads and give rise to four pollen grains. The second phase is micro gametogenesis, wherein pollen grain undergoes mitotic division and forms a large vegetative cell and a small generative cell. The generative cell in most of the species divides to form two sperm cells before the germination of pollen tube. Pollen grains are shed from anther and transferred or carried to the stigma by various agencies like wind, insect, water etc. After reaching on stigma, pollen germinates and produces pollen tube through the germ pore. Pollen tube grows from its tip and travels through style and reaches the embryo sac and finally enters the ovule, where it enters into embryo sac. The male nuclei of pollen tube fuse with egg and

polar nuclei, which is called fertilization. Pollen germination on stigma is a complex process, where it is identified genetically and the whole process is species specific. All pollens cannot germinate and those pollens that are able to germinate on stigma are called viable pollens. The germination ability of pollens can be tested chemically in laboratory.

13.3- TESTS FOR POLLEN VIABILITY USING STAINS AND IN-VITRO GERMINATION

Pollen Viability

Pollen viability of is described as the capability of germination and is a measure of male fertility. Viable pollen is responsible for a high fertility rate and crop yield. In crop hybridization programmes, pollen fertility and viability have a paramount importance. Pollen viability also plays a key role in germplasm or pollen bank. Pollen can be stored for germplasm conservation, conduction of hybridization experiment between plants that flower at different times or places, and for later use in hybridization programmes. For such experiment, the quality of pollens must be maintained according to Kearns and Inouye (1993).

Pollen Viability Tests:

The pollen viability can be determined by direct and indirect methods. In the direct (*in vivo*) method, pollens are transferred on receptive stigmas and to determine whether seeds are produced or not. Direct testing methods has the advantage of providing an unequivocal measure for the population of pollen grains deposited on the stigma, but it has several disadvantages as it is tedious, time-consuming, labour intensive and requires lager number of fresh samples. Pollen viability or germination percentage can also be calculated by *in vitro* techniques. Indirect methods rely on the correlation between ability to fertilize an ovule and some physiological or physical characteristics that can be determined faster. Indirect spacing methods that correlate with pollen germination include (1) the fluorochromatic procedure (FCR), (2) testing pollen for enzyme activity, and (3) testing stain ability of vegetative cells.

Pollen Staining Tests

Stains are chemical dyes that are used for particular type of cells and cell organelles. Some stains are specific to pollen components and can be used as a viability indicator or for testing pollen viability. According to Dafni *et al.* (2005), nuclear satin acetocarmine stains chromosomes; aniline blue with lactophenol stains callose, phloxin-methyl green stains cellulose and cytoplasm of cell. However, Heslop-Harrison (1985) demonstrated that immature or non-viable pollen sometimes contains enough of these elements that also shows staining properties and viable pollen of some species do not stain well. Therefore, stains provide (at best) only rough estimates of viability.

Some stains such as acetocarmine in glycerol jelly and aniline blue in lactophenol stain viable pollens but don't stain non-viable or abortive pollens. However, an improved Alexander's stain can be used as differential stain for pollens. Alexander's stain uses chloral hydrate, phenol and mercuric chloride, all of which are highly toxic. Peterson *et. al*., (2010) modified Alexander's stain (1969) technique and improved pollen staining technique by not using a regulated chemical chloral hydrate, mercuric chloride and phenol. This technique requires a much shorter time period for sample preparation and staining. This simplified method is very useful for field studies of angiosperm as well as gymnosperm pollens without high-end equipments such as fluorescence microscopes. It differentially colors abortive and non-abortive pollen; malachite green stains cellulose in pollen walls, and acid fuchsin stains protoplasm. Thus abortive (or germinated) grains appear green, and grains with protoplasm appear pink. Alexander's (1969) stain is also used to distinguish self versus outcross pollen on stigmas in species, where the incompatibility mechanism acts at the stages of pollen germination and self-sterile pollen retains cytoplasm. Following are some staining tests and protocols for pollen grains viability assessment.

Tetrazolium Test: The Tetrazolium (2,3,5 triphenyl tetrazolium chloride) test is commonly known as the TTC Test or TZ test. Norton, (1966) demonstrated that all living cells, which respire, are capable of reducing a colourless chemical TTC (2, 3, 5 triphenyl tetrazolium chloride) into a red-colored compound formation by H^+ ion transfer reactions catalysed by enzyme dehydrogenases. According to Shivanna and Johri (1989), the test has provided accurate results for many taxa. However Sedgley and Harbard, (1993) proved this method has some drawbacks like it tends to overestimate viability because sometimes it also stains non-viable

pollen grains. The concentration of tetrazolium salt, temperature and period of incubation needs to be standardizedto get optimal results in various pollen samples.

Procedure: 1% TTC was prepared by adding 0.2 g. TTC and 12 g sucrose dissolved in 20 ml dd H_2O. Drop two drops of the mixture on a slide and transfer pollen over it and place a cover glass. Count colored viable pollens after 30-40 minutes of incubation at 40^0C in a dark place. Pollen grains that stained orange or bright-red color were counted as viable.

Iodine-potassium iodide Test (I_2KI): This technique indicates viability and determines the starch content of pollen-grains. On staining with this, Iodine in a watery arrangement of potassium iodide breaks up the tri-iodide-anion edifices with starch and giving viable indicator of blue-black color to pollen grains.

Procedure: Dissolve 1 g potassium iodide and 0.5 g iodine in dd H_2O and finally prepare volume of 100 ml. Drop 1 or 2 drops of the dye over pollen sample and mix evenly in a gentle manner. Place a cover glass over it and after 5-10 minutes count the number of stained (viable) pollen grains under the microscope.

Aceto-carmine test (2%): Aceto-carmine is a nuclear stain that is used for staining nucleated live cells. The pollen nucleus is rich in chromatin material and viable pollen stains pink to deep red with aceto-carmine, whereas non-viable pollen grains do not take any stain.

Procedure: Weigh 2 g of carmine powder, dissolve it in 95 ml of glacial acetic acid. Add dd H_2O final 100 ml volume solution. Boil it and let it cool down. Filter it with the help of a sterilized filter paper and store it in a cool and dark place. Take two to three drops of stain on slide and transfer pollen grains on it and place a coverslip on it. Count stained viable pollens after 5-10 min.

Aniline blue (Cotton blue) test: Hauser and Morrison (1964) demonstrated that aniline blue stain detects the callose of the pollen walls and pollen tubes. The solution is prepared by adding 200mg/lit of aniline blue in a mixture of 10ml each of phenol, lactic acid, glycerol and distilled water. The viable pollenstained dark blue color, while dead pollen are unstained.

Modified Alexander method: Peterson et al., (2010) modified Alexander's stain (1969) which clearly differentiates between aborted and non-aborted pollens. Acid fuchsin, present in this dye, stains the protoplasm and malachite green stains cellulose in walls of pollen; dark purple pollen

was scored as viable and green as aborted. The non-aborted pollen grains stained magenta-red and aborted pollen grains stained blue-green.

Procedure: This stain is prepared by adding 10ml of 95% ethanol, 1 ml of Malachite green (1% solution in 95% alcohol), 50 ml dd H_2O, 25 ml glycerol, 5 ml acid fuchsin (1% solution in water), 0.5 ml orange G (1% solution in water), 4ml glacial acetic acid and add dd H_2O (4.5 ml) to final volume of 100 ml. Flower buds are fixed in 2 hour in Carnoy's fixative (6 alcohol:3 chloroform:1 acetic acid) before anthesis. The flower buds or pollen grains are dried and mixed with 2-4 drops of stain. Once the sample is stained, slowly heat the slide over flame until the stain solution starts boiling (~30 seconds). A more moderate rate of heating allows better penetration of the dye into the cellulose and protoplasm of the pollen. Place a cover slip over it and observe it under the microscope. The per cent pollen viability was calculated using formula:

$$\text{Pollen Viablity} = \frac{\text{No. of stained Pollen Grains}}{\text{Total no. of Pollen grain on observation}} \text{X } 100$$

***In-Vitro* Pollen Germination:**

In-vitro pollen germination technique is commonly used in physiology it provides a simple clear experimental method to study the physiology and biochemistry of pollen germination and growth of pollen tube, as well as the responses of the pollen system to physical and chemical factors. In-vitro techniques are generally accomplished within a few hours from pollen culture except in some cases. Maintenance of sterilized environment and aseptic conditions for routine exercises is not a necessity. But in case of pollens that take longer period of germination, such pollen cultures have to be free from microbial contamination, and therefore aseptic culture techniques have to be used. The culture medium can be sterilized by autoclave or sterile Millipore filter unit (pore size 0.2 μm). Antibacterial and antifungal substances such as rifampicin (antifungal) and nistatin (antibacterial) (10–15 μg/ml) can be incorporated in the germination medium. Before culturing the pollen grains, they are left in humid environment; high humidity improves the germination ability of pollen grains. This is achieved by dusting of pollen grains uniformly on a microslide, then incubating them for 15-60 minutes on a moist filter paper (>90% RH). If it is difficult to spread the pollen (in case of sticky pollen grains especially of entomophilous species), treat them with an organic solvent, such as cyclohexane or hexane for 2-3 minutes, then air-drying them for 5-l0 min prior to culturing. Culture medium plays a key role in pollen germination; composition

of a germination medium to obtain optimal responses has to be empirically formulated for each species. Generally three constituents *viz.* sucrose, boric acid, and calcium nitrate, are sufficient for pollen germination and growth. However, the optimal concentration of sucrose varies on the type of species, 100 mg/I boric acid and 300 mg/I calcium nitrate are optimal for pollens of most species. Brewbaker and Kwack's Medium, Roberts' Medium and Hodgkin and Lyon's Media are suitable and support most of pollen grains for culture and growth. Composition of different media is given as below:

Brewbaker and Kwack's Medium (Brewbaker and Kwack 1963):

Sucrose 10%

Boric acid: 100mg/l

Calcium nitrate: 300mg/l

Magnesium sulfate: 200mg/l

Potassium nitrate: 100mg/l

Roberts' Medium (Roberts et al. 1983):

Sucrose: 20%

Boric acid: l0mg/l

Calcium chloride: 362mg/l

Potassium nitrate: 100 mg/l

Tris: 60-130mg/1

Hodgkin and Lyon's Medium (Hodgkin and Lyon 1986):

Sucrose 580 mM (ca. 20%)

Boric acid 1.62 mM (ca. 100 mg/I)

Calcium nitrate 1.69 mM (ca. 400 mg/I)

Potassium nitrate 0.99mM (ca. 100mg/l)

Magnesium sulfate 0.84mM (ca. 200mg/l)

TAPS 20mM (ca. 4.86g/l)

14.4 POLLEN GERMINATION USING HANGING DROP AND SITTING DROP CULTURES

Germination Test

Pollen grains of most species germinate and grow in a tube when they are placed in appropriate solution of calcium, boron, and sucrose. Although it provides a controlled experimental system, germination *in-vitro* does not completely mimic growth in *in-vivo*. Some factors affect them such as temperature and germination media. These can also be affected by time of collection and storage conditions as well as culture media and pollen density on the media. A large numbers of pollens have been successfully germinated by palynologists/physiologists under controlled laboratory conditions on suitable media. Pollen germination in the stigma (*in-vivo*) was first observed as early as 1824 by C. B. Amici in Portulaca, who later observed the germinating pollen tube entering ovule.

There are several methods of *in-vitro* raised by palynologists/physiologists for pollen cultures according to requirement of particular species. The selection of a procedure depends on requirement of the particular species and also the nature of study and the facility available. The two mostly used methods are as follows:

Hanging Drop Culture

The hanging drop culture method is an old fashioned. In the hanging drop method of pollen germination, the growth of the pollen tube was determined by using a drop of germinating media on a cover glass; pollen grains are dusted on the media drops with the help of a sterilized and clean brush, and then the cover glass is inertly placed on the cavity slide. It is then left in an incubator under dark conditions at 25°C temperature in a culture medium with approximate composition of 5% sucrose, 5 ppm boric acid (H_3BO_3), and 1% agar for 1 day, 2 days and 3 days (24, 48, and 72 hours of time). For each incubation period, observe and record germination in three drops by counting three fields. A pollen grain will considered, if the length of germinated pollen tube was at least equal to or greater than the grain diameter. Measurements of pollen tube length are recorded directly with the help of micrometry slide or eyepiece of the microscope. The mean length of the pollen tube is calculated from each drop.

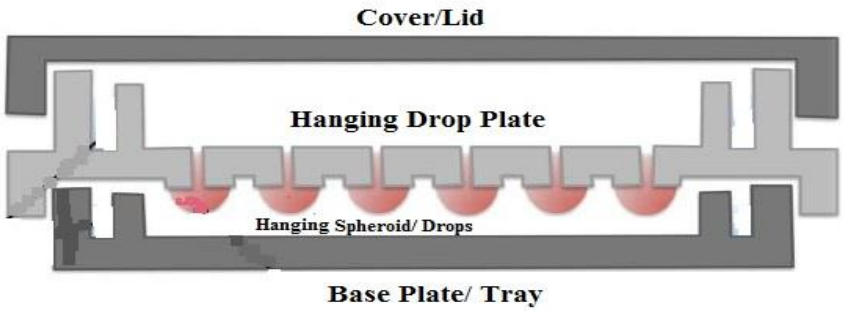


Fig.1: Hanging drop culture plate with several drop cultures

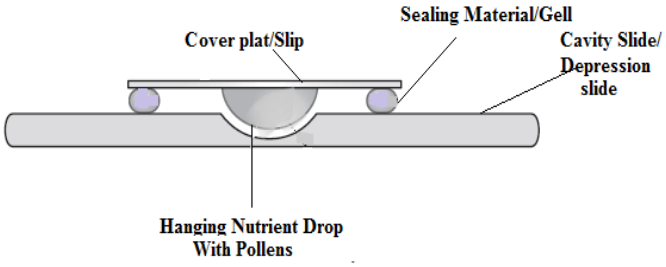


Fig.2: Hanging drop culture plate with slide

Sitting Drop Culture

The sitting drop culture method of pollen grains is a simpler one than the hanging drop method of pollen culture. The sitting drop culture method of pollen grains involves culturing of pollen grains in a drop of culture medium which is placed on a microslide. The culture is then transferred to in a humid chamber under controlled temperature to prevent evaporation. The constitution of the culture medium is the same as used in the hanging drop method.

14.5 SUMMARY

1. The term 'pollen' is usually used to describe male gametes of spermatophytes or powdery mass of pollen grains or microspores.
2. Pollen contains the male reproductive cells, which represents the male gametophytic generation of spermatophytes.
3. The process of pollen/microspore formation is called microsporogenesis.
4. The stamens are the male reproductive organs.

5. The anther mostly contains four microsporangia joined by the connective.
6. In each microsporangium, the central region contains a diploid microspore mother cell or pollen mother cell, which eventually forms the pollen grains.
7. Microspore mother cell undergoes meiosis that results in the formation of four haploid cells or tetrads.
8. Pollen grains are shed from anther and transferred or carried to the stigma by various agencies like wind, insect, water etc.
9. After reaching on the stigma, pollen germinates and produces the pollen tube through the germ pore.
10. Pollen tube grows from its tip and travels through style and reaches the embryo sac.
11. All pollens cannot germinate and the pollens that are able to germinate on stigma are called viable pollens.
12. Pollen viability is described as the capability of germination and is one of the measures to determine male fertility.
13. The germination ability of pollens can be tested chemically in laboratory.
14. Pollen viability also plays a key role in germplasm or pollen bank; pollen can be stored for germplasm conservation and for the conduction of hybridization experiment.
15. The pollen viability can be determined by direct and indirect methods.
16. Direct testing method has the advantage of providing an unequivocal measure for the population of pollen grains deposited on the stigma, but it has several disadvantages as it is tedious, time consuming and labor-intensive and requires lager number of fresh samples.
17. Pollen viability or germination percentage can also be calculated by *in-vitro* techniques.
18. Stains are chemical dyes used for a particular type of cells and cell organelles.
19. Some stains such as acetocarmine in glycerol jelly and aniline blue in lactophenol stain viable pollens but don’t stain non-viable or abortive pollens.
20. *In-vitro* pollen germination technique commonly used in physiology.
21. *In-vitro* techniques are generally accomplished in short time period i.e. within a few hours from pollen culture except in some cases.

22. Pollen germination in the stigma (*in-vivo*) was first observed as early as 1824 by C. B. Amici in *Portulaca* who later observed the germinating pollen tube entering the ovule.

23. There are several methods of *in-vitro* raised by palynologists for pollen cultures according to requirement of particular species.

24. The selection of a procedure depends on requirement of species and the nature of the study and facility available. The two most commonly used methods are **Hanging drop culture** and **sitting drop culture** method.

14.6 GLOSSARY

Anther: The part of flower that produces and contains flower.

Culture media: Any specific mixture of nutrients and other substances that supports growth.

Gametophytic: Gamete producing or haploid plant.

***In-vitro*:** In glasswares or test tube.

***In-vivo*:** In natural condition or within organism.

Incubator: A safe, controlled apparatus that provides necessary environment for growth and development of organ and organism.

Microspore: Male gamete or pollen grain in case of an angiosperm.

Palynology: Science that deals with the study of pollen grains.

Pollen: Mass of microspores in spermatophytes that usually appears in form of a fine dust.

Pollen mother cell: A diploid cell within anther or pollen sac which gives haploid pollen by meiotic division.

Pollen tube: A hollow tube that develops from a germinating pollen grain.

Protocol: A predefined procedure or method in implementation of an experiment.

Spermatophytes: Seed-producing plants.

Stain: A chemical dye used for coloration in a particular tissue or cells.

Stigma: Tip of pistil/pistils adapted for receiving pollen.

Viability: Ability to germinate or maintain itself.

14.7 SELF ASSESSMENT QUESTION

14.7.1 Multiple Choice Questions:

1. Pollen are:

(a) Microspore cells (b) haploid cells

(c) Male cells (d)All of above

2. Pollens are produced in:

(a)Stigma (b) Anther

(c) Both (d) None of above

3. Pollen tube grows from:

(a) Base (b) Tip

(c) Intercalary (d) None of above

4. Pollens that are able to germinate are called:

(a) Non-viable (b) sterile

(c) Abortive (d) Viable

5. Pollen on germination gives rise to:

(a) Root (b) Stem

(c) Cotyledons (d) Pollen tube

6. Pollen viability test in a test tube is called:

(a) *In-vitro* (b) *In-vivo*

(c) *In-situ* (d) All

7. Brewbaker and Kwack's Medium does not contain:

(a) TRIS (b) Sucrose

(c) Boric acid (d) Calcium Nitrate

8. In a viability test, acetocarmine stains:

(a) Chromosomes (b) Callose

(c) Pollen Wall (d) All

9. Tetrazolium (2,3,5 triphenyl tetrazolium chloride) is a:

(a) Stain (b) Nutrient

(c) Enzyme (d)All

10. Aniline blue stains:

(a) Callose (b) Chromosomes

(c) Cytoplasm (d) Nucleus

14.7.2 Fill in the blanks:

(1) *In-vivo* pollen germination was observed for the first time by _____.

(2) Pollen germination experiment is carried out in glassware called _____.

(3) After reaching stigma, pollen germinates and produces _____.

(4) _____ grows from its tips and travels through style and reaches the embryo sac.

(5) In _____ method of pollen germination, pollen tube growth was determined by use of a drop of germinating media on a cover glass.

(6) Tris is an additional supplement of_____ media.

(7) TAPS is an additional supplement of_____ media.

(8) Germination ability of pollen is called_____.

(9) Iodine-potassium iodide Test (I2KI) technique indicates _____ content of pollen-grains.

(10) Aceto-carmine is an_____ stain that stains nucleated live cells.

14.7.3 True or False:

(1) *In-vivo* pollen germination was observed for the first time by C. B. Amici in Portulaca.

(2) Aniline blue stains chromosomes.

(3) Experiment carried out in a test tube or a glassware called *in-vitro*.

(4) Germination ability of a pollen is also called viability.

(5) Pollen viability test can be determined with the help of staining tests.

(6) The meristem situated at the bases of internodes is known as apical meristem.

(7) In the hanging drop method of pollen germination, pollen tube growths were determined by using the drop of germinating media on a cover glass.

(8) Aniline blue with lactophenol stains callose, phloxin-methyl green stains cellulose and cytoplasm of cell.

(9) Aceto-carmine is a nuclear stain that stains nucleated live cells.

(10) Pollen tube grows from base of tube.

14.7.4 Very short answer questions:

(1) Define microspore.

(2) What is palynology?

(3) What is tetrazolium?

(4) Define viability.

(5) Define culture media.

(6) What is the name of the nuclear stain?

(7) What is *in-vitro*?

(8) Define protocol.

(9) Describe pollen tube.

(10) Define *in-vivo*.

14.7.1 Answer keys: 1-d, 2-b, 3-b, 4-d, 5-b, 6-a, 7-a, 8-a, 9-a, 10-a.

14.7.2 Answer key: 1-C.B. Amici, 2-*in-vitro*, 3-Pollen tube, 4- Pollen tube, 5- Hanging drop, 6- Roberts' Medium, 7-Hodgkin and Lyon's Medium, 8-Viablity, 9- viability and starch, 10- nuclear.

14.7.3 Answer key: 1-True, 2-False, 3-true, 4-True, 5-True, 6-False, 7-True, 8-True, 9-True, 10-False.

14.8 REFERENCES

- Alexander MP. 1969. Differential staining of aborted and nonaborted pollen. StainTechnol. 44:117-22.

- Brewbaker, J.L. and Kwack, B.H., 1963. The essential role of calcium ion in pollen germination and pollen tube growth. *American Journal of Botany*, 50, 859–865.
- Dafni, A., Kevan, P.G., and Husband, B.C., 2005. *Practical Pollination Biology*. Environ quest Ltd. Ontario.
- Hauser E.J.P. and Morrison J.H., 1964. The cytochemical reduction of nitroblue tetrazolium as an index of pollen viability. Amer. Journ. Bot., 51: 748-752.
- Heslop-Harrison, J. and Heslop-Harrison, Y., 1985. Germination of stress-tolerant Eucalyptus pollen. *Journal of cell science*, 73, 135–57.
- Hodgkin T, Lyon GD .198. The effect of Brassica oleracea stigma extracts on the germination of B. oleracea pollen in a thin layer Chromatographie bioassay. J Exp Bot 37:406–411
- Kearns, C.A. and Inouye, D.W., 1993. *Techniques for pollination biologists*. Colorado, USA.: University Press of Colorado, Niwot.
- Norton D J. Testing of plum pollen viability with tetrazolium salts. *American Society for Horticultural Science*. 1966; 89:132–134.
- Peterson R, Slovin JP, Chen C. 2010. A simplified method for differential staining of aborted and non-abortedpollen grains. International Journal of Plant Biology: 1(2): 66-69.
- Roberts AB, Lamb CC, Newton DL, Sporn MB, De Larco JE, Todaro GJ .1983. Transforming growth factors from neoplastic and non-neoplastic tissues. Fed Proc 42: 2621–2626.
- Sedgely M. and Harbard J. 1993. Pollen storage and breeding systems in relation to controlled pollination of four species of Acacia (Leguminosae: Mimosoideae) Australian Journal of Botany, 41: 601-609.
- Shivanna, K.R. and Johri, B.M., 1989. *The Angiosperm Pollen: Structure and Function*. Wiley Eastern Ltd., New Delhi.

14.9 SUGGESTED READINGS

- Shivanna K.R. and Rangaswamy N.S. 1992. Pollen Biology: A Laboratory Manual. Springer. Pp119.
- Johri B.M. and Srivastava P.S. 2001. Reproductive Biology of Plants. Springer. Pp 320.
- Shivanna K.R. 2003. Pollen Biology and Biotechnology. CRC Press. Pp 316.

- Geitmann, A. 2020. Pollen and Pollen Tube Biology: Methods and Protocols. Pp 325.

14.10 TERMINAL QUESTIONS

14.10.1 Short answer questions:

(1) Write a short note on *in-vitro* pollen germination.

(2) Describe the hanging drop method of pollen culture.

(3) What do you understand by a stain?

(4) Describe a few pollen staining techniques.

(5) Differentiate between the hanging drop and the sitting drop method of pollen culture.

(6) Differentiate between *in-vivo* and *in-vitro* methods.

(7) Write a short note on pollen viability.

(8) What do you understand by culture media?

(9) Write a note on pollen tube.

(10) Describe Brewbaker and Kwack's Medium**.**

14.10.2 Long answer question:

(1) Write a detailed note on Germination Test.

(2) Describe *in-vitro* methods of pollen germination with protocols.

(3) Write a detailed note on pollen staining viability test.

(4) Write a note about on modified Alexender’s test.

(5) Describe *in-vitro* pollen culture media.

UNIT-15-STUDY OF THE PRIMARY AND SECONDARY ABNORMAL GROWTH IN PLANTS

15.1-Objectives

15.2-Introduction

15.3- Study of the primary and secondary abnormal growth in plants

15.4-Summary

15.5-Glossary

15.6-Self Assessment Question

15.7-References

15.8-Suggested Readings

15.9-Terminal Questions

15.1-OBJECTIVES

After reading this unit students will be able to-

- Understand the phenomenon of plant growth.
- Understand the plant growth types.
- Understand the primary and secondary growth in plants.
- Understand the abnormal growth in plants.
- Understand the primary and secondary abnormal growth in plants.

15.2- INTRODUCTION

In plants increase in size and length of stems and roots is called as growth. The stems and roots of plants, continue to grow throughout life span and this is called indeterminate growth. On other hand, some plant parts, such as, spines, leaves and flowers, the growth ceased after their development, such growth pattern called determinate growth. According to Cutler *et. al*., (2008) the plant part or cells which are responsible for growth and repair are meristems and such cells are called meristematic cells. Meristem is of undifferentiated mass of cells, capable of divide continue. As plant cells divide and metamorphosed into special cells for particular function through cellular differentiation**.** Apical meristems are found at the tip or apex and responsible for growth in length. Apical meristem on division gives rise to new cells which allow growth in length or height, which is known as primary growth.

According to Evers (1982) secondary meristems on division and differentiation allow growth in diameter in woody dicots and gymnosperms. Monocots do not have cambium thus secondary growth is not found in such plant. Cork cambium and vascular cambium (Fig.15.1) are two types of secondary meristem produces which are responsible for growth to the diameter of the plant stem called secondary growth.

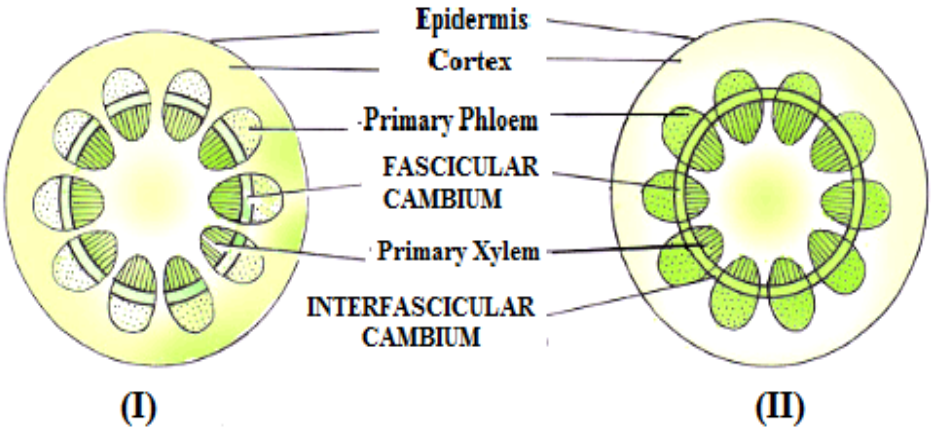


Fig.15.1: Position of Vascular Cambium in Dicots (I) Fascicular Cambium (II) Interfascicular Cambium

15.3- STUDY OF THE PRIMARY AND SECONDARY ABNORMAL GROWTH IN PLANTS

Primary growth in plants occurs at the apices, apical meristem causes the increase of stem length. Apical meristems differentiate into the three types of basic meristematic tissues the protoderm

(produces epidermal layers), ground meristem (produces ground tissue system), and procambium (produces vascular tissue). The growth caused by these tissues is considered as primary growth. Primary growth occurs when plants grow upward or toward the sunlight necessary for photosynthesis and also downward or geotropically to sink roots deep into the soil to anchor absorb water and nutrients. This 'up and down' growth is possible due to apical meristem that, upon division, produces undifferentiated cell that will become either a new root or shoot tip.

Secondary growth in plant occurs when stems or branches grow outward by activity of lateral meristem. Unlike the primary meristem which causes the plant to grow up or down, lateral meristematic tissue causes the plant to increase in girth by adding growth rings. Thus the growth in width or circumference of trees or increases in girth is termed as secondary growth and it arises from cambium. As with apical meristems, lateral meristems are regions of high cell division activity. Dicots and Gymnosperms posses cambium thus secondary growth is

possible only dicots, monocots, however, do not experience secondary growth due to lack of cambium, But exceptionally in some monocots such as Palm, *Yucca*, *Dracaena*, *Smilax*, *Agave*, Coconut etc, secondary growth takes place.

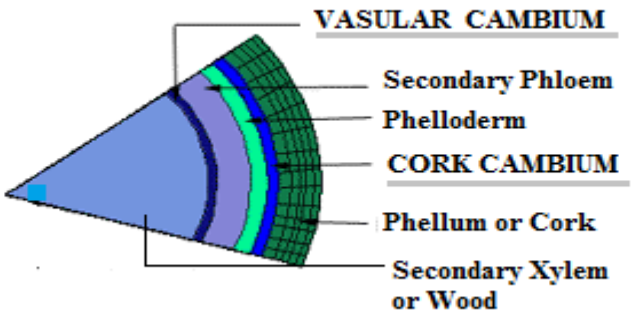


Fig. 15.2: Position of Vascular cambium and Cork Cambium

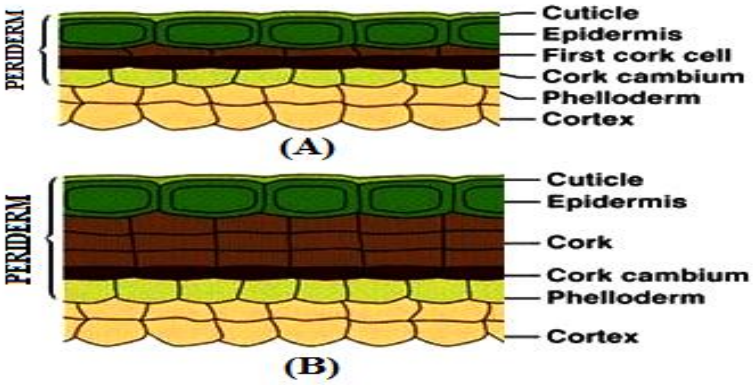


Fig15.3: Cork cambium in Dicot Stem (A) Formation of first layer of cork cells, (B) Cork formed in multiple layers by the activity of cork Cambium.

The two important factors for secondary growth are the vascular cambium and the cork cambium (Fig.15.2&15.3). The vascular cambium produces more vascular tissue i.e. xylem and phloem, which provides support for the shoot system in addition to transporting water and nutrients. Generally cambium functions normally and plant grows too normally in dicots but sometimes behavior of cambium changes and causes unequal growth pattern which is called abnormal secondary growth. There may be various factors which causes abnormal growth in plants.

Anomalous structures in Plants:

In Several dicots secondary growth deviates considerably from the normal secondary growth such secondary thickenings are called abnormal or anomalous, these anomalies or abnormalities may be as follows:

I. Following reasons are responsible for abnormal secondary growth in dicots:

- **(a)** Anomalous position of vascular cambium
- **(b)** Abnormal Activity of vascular cambium
- **(c)** Formation of accessory vascular cambium
- **(d)** Formation of extrastelar cambium
- **(e)** Formation of interxylary phloem

II. Presence of scattered vascular bundles in dicots

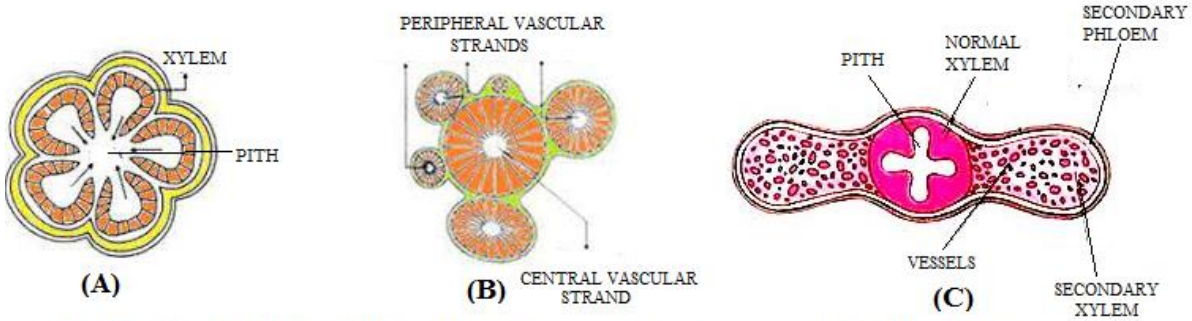
III. Presence of exclusive phloem and xylem bundles

IV. Presence of medullary bundles

V. Presence of extra stellar or cortical bundles

VI. Ring arrangement of vascular bundles in monocots

I. (a) Anomalous/abnormal position of vascular cambium: Normally vascular cambium is circular, but in stem of some plants it is folded. Later on these folds break and separate from each other. Each fold is responsible to form a complete vascular bundle. Many vascular bundles are formed in stem e.g. *Serjania, Thinouia and Bauhinia* (fig.15.4).



Anomalous secondry growth in (A) *Serjania*, (B) *Thinouia* and (C) *Bauhinia* stem
(Diagrammatic)

Fig,15.4: Abnormal position of vascular cambium showing in T.S. of (A)*Serjania,* (B) *Thinouia and* (C) *Bauhinia* Stem.

I. (b) Abnormal Activity of vascular cambium: Generally, xylem and phloem tissues are formed from the maximum part of the vascular cambium and medullary rays are formed from the few parts of vascular cambium but in some plants parenchyma (medullary rays) are formed from the maximum part of the vascular cambium and rarely in some places xylem and phloem tissues are formed *e.g. Aristolochia* (Fig15.5).

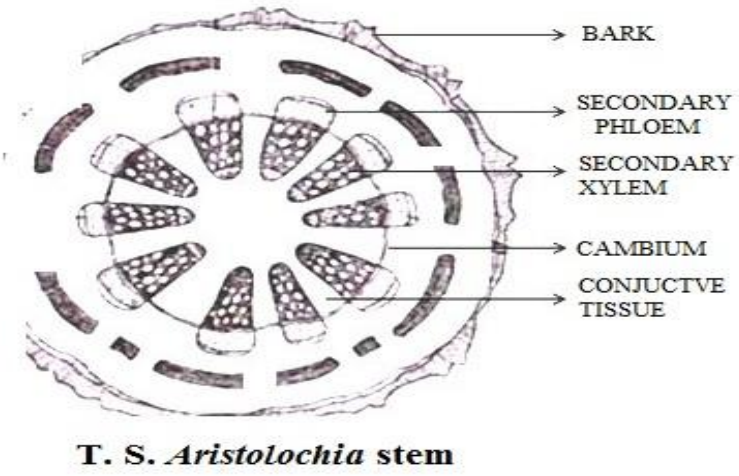


Fig15.5: T. S. of *Aristolochia* stem showing abnormal activity of vascular cambium

I. (c) Sequential (successive) ring of vascular cambium: In some of the plants, a new ring of vascular cambium is formed each year. According to Esau (1969) this is formed outside the previous ring e.g. *Mirabilis, Boerhaavia, Bougainvillea etc* (Fig15.6).

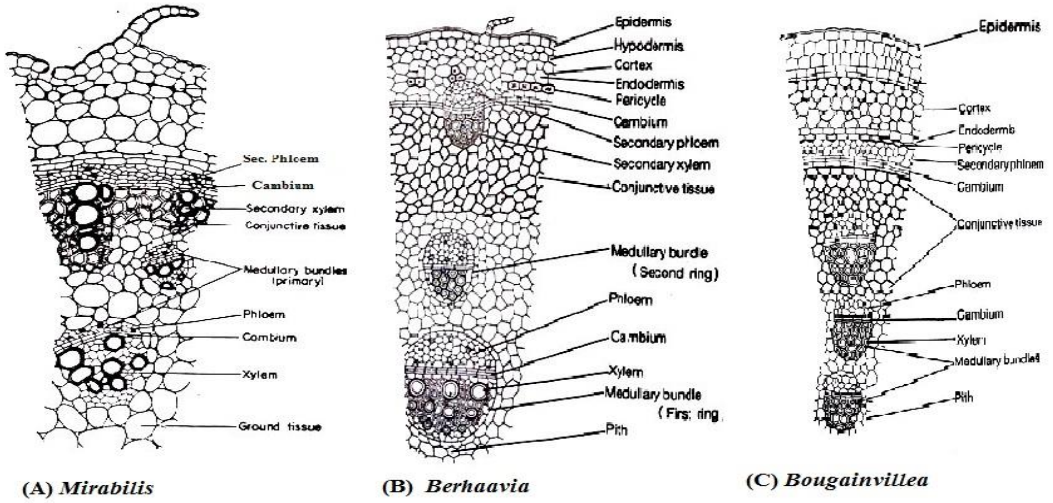


Fig.15.6: Sequential (successive) ring of vascular cambium in T.S. of *Mirabilis, Boerhaavia* and *Bougainvillea* stem.

I. (d) Formation of vascular cambium from pericycle: Vascular cambium is formed from the pericycle in plants of amaranthaceae and ahenopodiaceae families. A complete ring of vascular cambium is formed from the pericycle.

I. (e) Interxylary phloem or included phloem: This is also called internal phloem or included phloem. The origin of interxylary phloem (Fig15.7) in most plants is primary. The internal phloem develops after the development of external primary phloem. This type of development is found in plant families solanaceae, apocynaceae and lathyraceae.

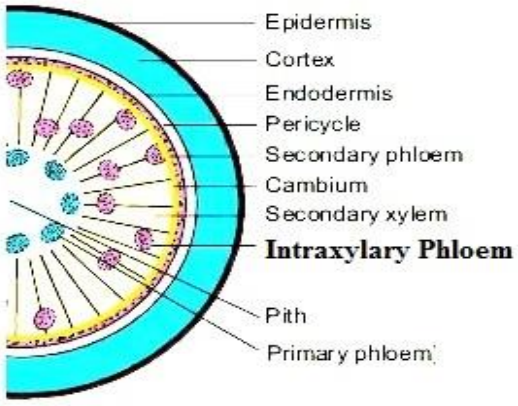


Fig.15.7: T. S. *Calotropis* (Apocynaceae) stem showing Included phloem.

II. Scattered vascular bundles in dicotyledons: In dicots vascular bundles found normally arranged in a ring but in some plants such as *Thalictrum, Piper, Peperomia, Podophyllum, Papaver* etc. vascular bundles are scattered (fig15.8).

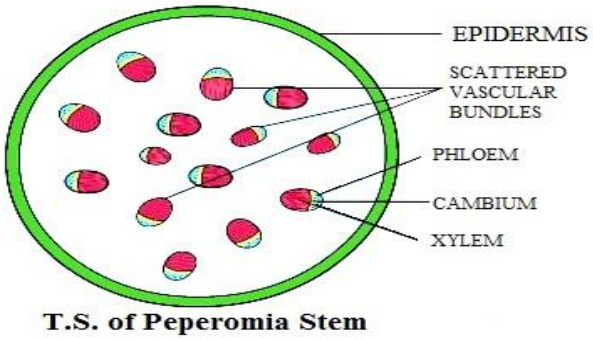


Fig.15. 8: T.S of in *Peperomia* stem showing scattered vascular bundles.

III. Presence of exclusive phloem and xylem bundles: Sometimes vascular bundles are incomplete i.e. a bundle is represented either exclusively by xylem or phloem strand. In *Paeonia*, in addition to normal vascular bundle, incomplete bundles are also present which are exclusively represented by xylem. Similarly, *in Cuscuta, Boerhaavia, Ricinus,* and *Antigonon* only phloem bundles are present.

IV. Presence of medullary bundles: In some dicots e.g. members of Ranunculaceae, Amaranthaceae, Acanthaceae, Cactaceae and Chenopodiaceae vascular bundles are present in pith (Fig15.9) and then they are known as medullary bundles and show a limited amount of secondary growth.

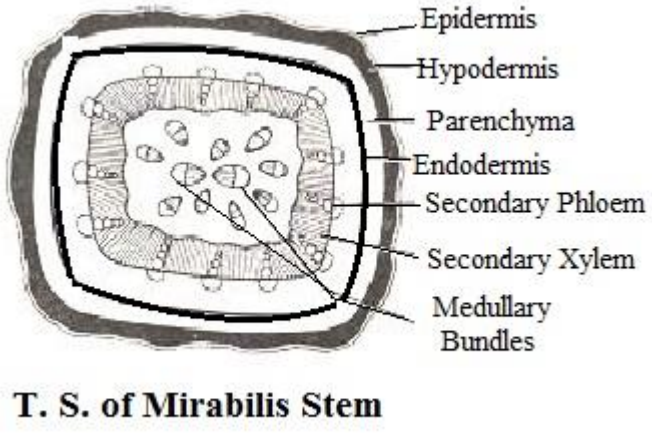


Fig.15.9:T.S.of *Mirabilis* Stem showing Medullary bundles.

V. Presence of cortical bundles: In some dicots in addition to the normal ring of stelar bundles some vascular bundles are also present in the cortex known as cortical bundles e.g. *Casuarina, Nyctanthes* (Fig15.10).

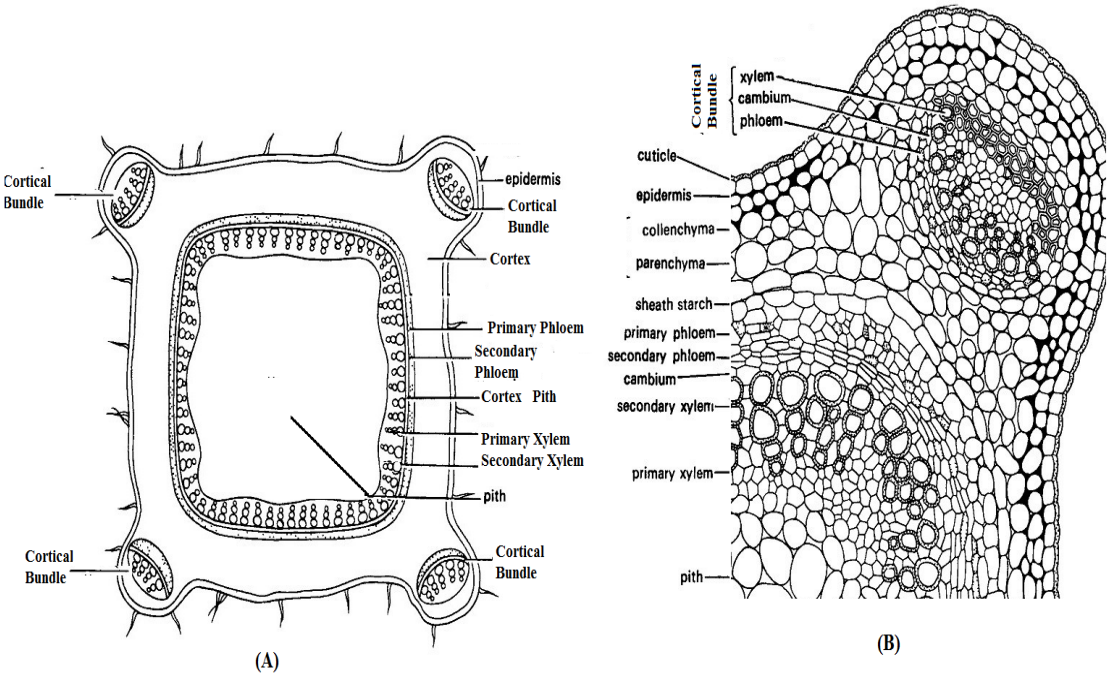


Fig.15.10: T. S. *Nyctanthes* Stem showing Cortical bundles.

VI. **Ring arrangement of Vascular bundles in monocots:** In monocots vascular bundles scattered in the ground tissue, but in some cases like *Dioscorea* vascular bundles found arranged in two rings around the pith anomously; the outer ring has only two small bundles which are embedded in the sclerenchymatous pericycle and the inner ring has several large vascular inside the pericycle.

Types of anomalous secondary growth:

The anamalous secondary growth can be divided in two types:

1. Abnormal growth from abnormal cambium in monocots
2. Abnormal growth from normal cambium in dicots

1) Abnormal growth from abnormal cambium (*e.g.* Secondary Thickening in Dracaena stem: Monocot):

The secondary thickenings normally absent in monocot plants since the vascular bundles in monocots are without cambium (closed type). However, a very few plants in monocots shows anomalous secondary growth such as *Dracaena, Yucca, Aloe, Sansevieria* and *Agave*. Here you will study this by example of anomalous secondary growth in *Dracaena* stem.

In monocotyledons normally the vascular bundles are closed. The cambium being absent the secondary growth is absent; but but later on a secondry meristem develops which behaves as cambium like *Dracaena* and *Yucca* secondary growth takes place. This abnormal cambium may either develop from cortex or pericycle and shows abnormal activity.

The young stem has typical structure i.e. epidermis is followed by sclerenchymatous hypodermis and large number of closely arranged vascular bundles scattered in ground tissue. The outer layer of cells from the ground tissue becomes meristematic and functions as cambium. The cambium formed in the region which has ceased elongation. The activity of this cambium is more on the inner side and very little on the outside where it forms only parenchyma. On the inner side it forms xylem and parenchyma in alternate patches and inner mass of parenchymatous cells form conjunctive tissue. After a short while the activity of cambium on inner side changes and above the xylem it starts forming phloem and then again xylem. The xylem formed earlier has bigger vessels. Around each vascular bundle is developed sclerenchymatous sheath. The cambium after sometime alters its activity and forms xylem on the inner side, at those places where it was previously forming the parenchyma and parenchyma in place of xylem. Similar to earlier case

again by change in activity it forms a ring of vascular bundles. Activity of cambium goes on changing regularly and more rings of vascular bundles are formed. Cork cambium is formed below hypodermis forms cork in normal fashion (fig.15.11).

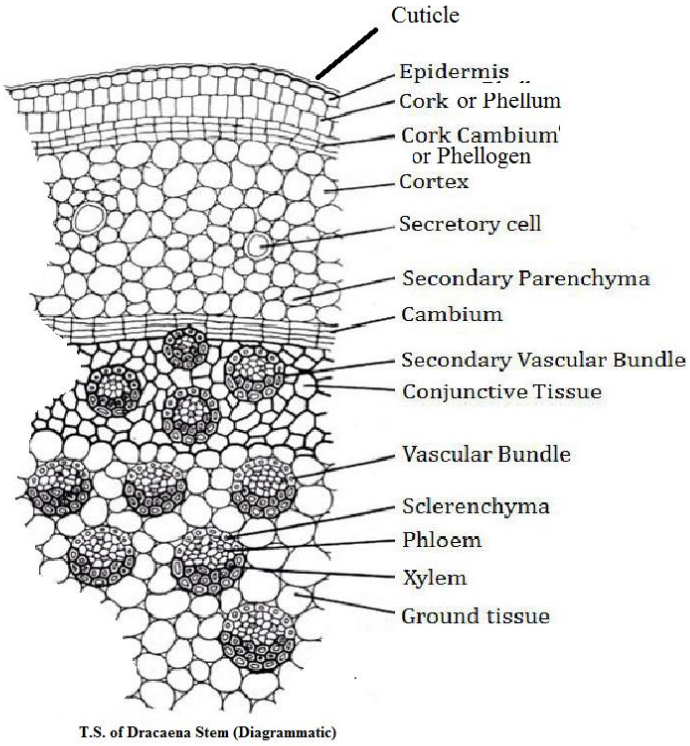


Fig.15.11: T.S. *Dracaena* stem showing abnormal secondary thickenings.

2. Abnormal growth from normal cambium (Secondary Thickening in *Boerhaavia* Stem: Dicot):

The anamolous secondary growth of dicot (*Boerhaavia*) stem showed following tissues. Single layered epidermis consists of small, radially elongated cells. Cortex is well differentiated and consists of few layered collenchymatous hypodermis followed by chlorenchyma of 3 to 4 cells deep, but generally below stomata only single layered. Chlorenchyma is present inner to collenchyma in the form of 3 to 7 layers with thin walled, oval, full of chloroplasts and enclose many intercellular spaces. Endodermis is clearly developed and made up of many, tubular, thickwalled cells. Inner to the endodermis parenchymatous pericycle is present but at some places it is represented by isolated patches of sclerenchyma. conjoint, collateral and endarch vascular bundles are present in three rings with two innermost large bundles; in the middle ring

the number ranges from 6 to 14 while the outermost ring consists of 15 to 20 vascular bundles. The innermost and middle rings are medullary bundles. Vascular bundles of inner and middle rings may show a little secondary growth. Outermost ring of the vascular bundles contains inter-fascicular cambium which is absent in other two rings. Cambium develops secondarily from the pericycle and becomes active. It cuts secondary phloem towards outer side and secondary xylem towards inner side. Due to these changes the primary phloem becomes crushed and present next to pericycle. Primary xylem is situated near the pith. Interfascicular cambium also soon becomes active and cuts internally the row of cells which become thick walled and lignified and are known as conjunctive tissue. Pith is well developed, parenchymatous and is present in the centre (fig. 15.13).

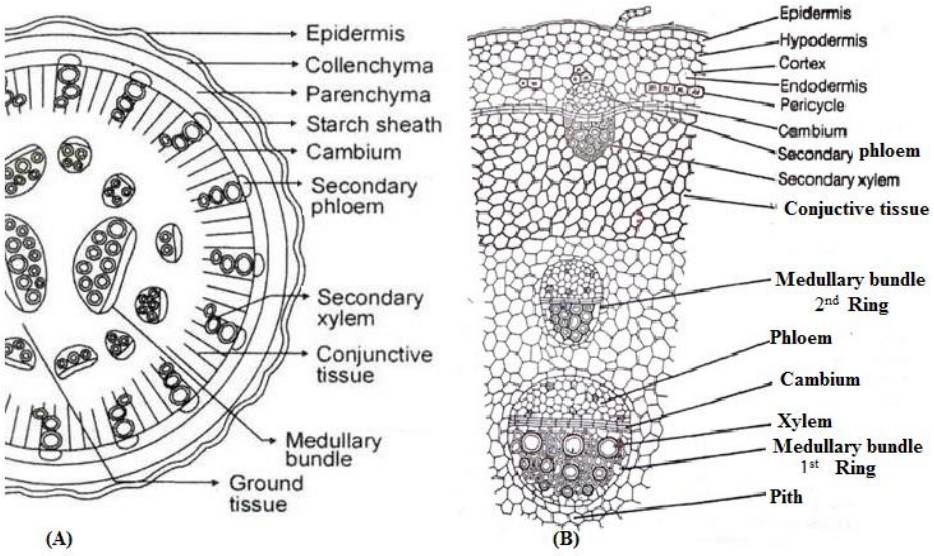


Fig. 15.13: Secondary Thickening in *Boerhaavia* Stem (A) T.S. of stem (B) Detail of a portion.

15.4 SUMMARY

1. In plants increase in size and length of stems and roots is called as growth.

2. Plants are capable to continue their growth throughout their life span.

3. The plant part or cells which are responsible for growth and repair are meristem and such cells are called meristematic cells.

4. Meristem is of undifferentiated mass of cells, capable of divide continue.

5. Apical meristems are found at the tip or apex and responsible for growth in length or height, which is known as primary growth.

6. Apical meristems differentiate into the three types of basic meristematic tissues the protoderm, ground meristem, and procambium.

7. Primary growth in plants occurs at the apical meristem which causes the increase of stem length.

8. Primary growth occurs in both directions; when plants grow upward or toward the sunlight necessary for photosynthesis and also downward or geotropically to sink roots deep into the soil to anchor absorb water and nutrients.

9. Secondary growth in plant occurs when stems or branches grow outward by activity of lateral meristem.

10. Thus the growth in width or circumference of trees or increases in girth is termed as secondary growth and it arises from cambium.

11. Dicots and Gymnosperms posses cambium thus secondary growth is possible only in these plant groups.

12. Monocots, however, do not experience secondary growth due to lack of cambium.

13. Exceptionally in some monocots such as Palm, *Yucca*, *Dracaena*, *Smilax*, *Agave*, Coconut *etc*, secondary growth takes place.

14. Generally cambium function normally and plant grows normally in dicots.
15. Sometimes behavior of cambium changes and causes unequal growth pattern which is called abnormal secondary growth.
16. There may be various factors which causes abnormal growth in plants.

17. In Several dicots secondary growth deviates considerably from the normal secondary growth such secondary thickenings are called abnormal or anomalous.
18. The reasons are responsible for abnormal secondary growth in dicots are anomalous position of vascular cambium, abnormal activity of vascular cambium, formation of accessory vascular cambium, formation of extrastelar cambium, formation of interxylary phloem etc.
19. The anamalous secondary growth can be divided in two types: 1. Abnormal growth from abnormal cambium in monocots 2. Abnormal growth from normal cambium in dicots.
20. In monocots vascular bundles scattered in the ground tissue, but in some cases like *Dioscorea* vascular bundles found arranged in two rings around the pith anomously.

15.5 GLOSSARY

Apical meristem: Embryonic, totipotent tissue in the tips of the roots and shoots of plants.

Cambium: A lateral meristem that produces secondary growth.

Conjoint: Vascular bundles which contain both xylem and phloem are called conjoint vascular bundles.

Cork: A plant tissue composed of cells whose walls are impregnated with suberin, produced by the cork cambium.

Cork cambium: A narrow cylindrical sheath of meristematic cells that produces cork cells.

Dicot: The plants, that has a pair of leaves, or cotyledons, in the embryo of the seed.

Hypodermis: The tissue immediately beneath the epidermis of a plant especially when modified to serve as a supporting and protecting layer.

Interxylary phloem: The presence of phloem strands embedded within the secondary xylem (wood).

Intraxylary phloem: Phloem situated on the inner side of the vascular bundles.

Ground tissue: A tissue consisting mostly of parenchyma cells that makes up the bulk of a young plant.

Meristem: Aformative plant tissue usually made up of small cells capable of dividing.

Monocot: The plants,that has a single cotyledon, in the embryo of the seed.

Phloem: Photosynthate conducting tissue of vascular plants.

Periderm: A tissue primarily consisting of cork cells; outer bark.

Pith: The central parenchymatous tissue in a vascular plant axis.

Primary growth: Growth in length, controlled by the apical meristem.

Sclerenchyma: Tissue, when mature, is composed of dead cells that have heavily thickened walls containing lignin and a high cellulose content.

Secondary growth: Growth in width initiated and maintained by the vascular cambium and corkcambium.

Secondary xylem: Xylem produced by the vascular cambium.

Vascular bundle: A strand of tissue composed mostly of xylem and phloem.

Vascular cambium: A lateral meristem that produces secondary vascular tissue in stems and roots.

15.6 SELF ASESSMENT QUESTIONS

15.6.1 Multiple Choice Questions:

1. Primary growth is:

(a) Increase in girth (b) Increase of stem at apex

(c) Formation of cambium (d) All of above

2. Tissue responsible for primary growth is:

(a)Cambium (b) Apical meristem

(c) Both (d) None of above

3. Cambium is:

(a) Permanent tissue (b)Meristematic tissue

(c) Apical meristem (d) None of above

4. Tissues having dividing capability is called:

(a) Permanent (b) Non Meristematic

(c) Abortive (d) Meristematic

5. Primary tissue has**:**

(a) Dividing capability (b) Differentiation capablity

(c) Both a and b (d) None of above

6. Secondary growth is the feature of:

(a) Monocots (b) Cryptogams

(c) Dicots (d) All

7. Monocotyledons shows secondary growth:

(a)Triticum, agave (b) Yucca, Dracaena

(c) Triticum, smilax (d) none of above

8. Scattered vascular bundles are found in:

(a) Hibiscus (b) Peperomia

(c) Malva (d) All

9. Cork is produced by:

(a) Cambium (b) Cork Cambium

(c) Vascular Cambium (d)All

10. Monocots in which secondary growth occurs:

(a) Hordeum, (b) *Dracaena*

(c) Both a&b (d) None

15.6.2 Fill in the blanks:

(1) The plant cells which have capability of division called_____ cells.

(2) Dicots and gymnosperms posses _____thus secondary growth is possible only plants of these two groups only.

(3) Monocots do not experience _____growth due to lack of cambium.

(4) _____growth in plants occurs at the apical meristem which causes the increase of stem length.

(5) Sequential (successive) ring of vascular cambium found in _____stem.

(6) In *Dioscorea* vascular bundles found arranged in _____rings around the pith anomously.

(7) Four cortical bundles at each corner is feature of_____Stem.

(8) In *Calotropis* phloem is found in xylem strands called _____phloem.

(9) Exceptionally in some monocots such as Palm, *Yucca*, *Dracaena*, *Smilax*, *Agave*, Coconut *etc*, _____growth takes place.

(10) _____is of undifferentiated mass of cells, capable to divide continue.

15.6.3 True or False:

(1) Secondary growth do not found in monocots.

(2) Dicots and gymnosperms lack cambium.

(3) Cambium and cork cambium are responsible for secondary growth.

(4) In *Dioscoria* ring arranged vascular bundles found.

(5) *Dracaena* stem shows abnormal secondary growth.

(6) The meristem situated at the bases of internodes is called apical meristem.

(7) Sequential (successive) ring of vascular cambium found in *Calotropis* stem.

(8) Monocots such as *Yucca*, *Dracaena*, *Smilax*, *Agave* shows secondary growth.

(9) The two important factors for secondary growth are the vascular cambium and the cork cambium.

(10) Cambium is the characteristic feature of monocots only.

15.6.4 Very short answer questions:

(1) Define growth.

(2) What is cambium?

(3) Define secondary growth.

(4) What means abnormal secondary growth?

(5) Define included phloem.

(6) What is cork cambium?

(7) What is apical meristem?

(8) Define medullary bundle.

(9) Describe cortical bundles.

(10) Define meristem.

15.6.1 Answer keys: 1-b, 2-b, 3-b, 4-d, 5-c, 6-c, 7-b, 8-b, 9-b, 10-a.

15.6.2 Answer key: 1- meristematic, 2- cambium, 3- secondary, 4- primary, 5- *Boerhaavia*, 6- two, 7-*Nyctanthes*, 8- included, 9- secondary, 10- meristem.

15.6.3 Answer key: 1-True, 2-False, 3-true, 4-True, 5-True, 6-False, 7-True, 8-True, 9-True, 10-False.

15.7 REFERENCES

- Cutler D.F., Botha, T. and Stevenson, D.W. (2008) *Plant Anatomy, An Applied Approach*. Oxford: Blackwell Publishing.
- Esau, K. & Cheadle, V.I. (1969). Secondary growth in *Bougainvillea*. *Annals of Botany* (N. S.) 33: 807-819.
- Ewers, F.W. (1982). Secondary growth in needle leaves of *Pinus longaeva* (Bristle-cone pine) and other conifers: Quantitative data. *American Journal of Botany* 69: 1552-1559.

15.8 SUGGESTED READINGS

- Cutler D.F., Botha, T. and Stevenson, D. W. (2008) *Plant Anatomy, An Applied Approach*. Oxford: Blackwell Publishing.
- Esau, K. 1977. *Anatomy of Seed Plants*. John Wiley Sons. Inc., New York.
- Evert R.F. and Eichhorn S.E. (2013) *Raven Biology of Plants* (8^{th} eds,). W.H. Freeman/Palgrave Macmillan.
- Fahn, A. (1967). *Plant Anatomy*. Pergamon Press, Oxford.
- Foster, A.S. (1951). *Practical Plant Anatomy*. Van Nostrand, Princeton.
- Singh, V., Pande, P.C. and Jain, D.K. (2013). *Structure Development and Reproduction in Angiosperms.* Rastogi Publications, Meerut.

- Vasistha, P.C. (1968). *Plant Anatomy*. Pradeep Publication & Co., Chandigarh.

15.9 TERMINAL QUESTIONS

2.11.1 Short answer questions:

(1) Write short note primary growth.

(2) Describe cambium.

(3) What do you understand by cortical bundles?

(4) Describe cork cambium.

(5) Differentiate between primary and secondary growth.

(6) Differentiate between medullary bundles and cortical bundles.

(7) Write short note incuded phloem.

(8) What do you understand by anomalous secondary growth?

(9) Write a note on abnormal structures in *Dracaena* stem.

(10) Describe meristem**.**

2.11.2 Long answer question:

(1) Write a detailed note plant growth.

(2) Describe abnormal secondary growth with suitable examples.

(3) Write a note on medullary, cortical bundles and included phloem.

(4) Write a note on abnormal structures of *Nyctanthes* stem with labeled diagrams.

(5) Describe anomalous secondary growth in *Dracaena* and *Boerhaavia* stem with labeled diagrams.

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